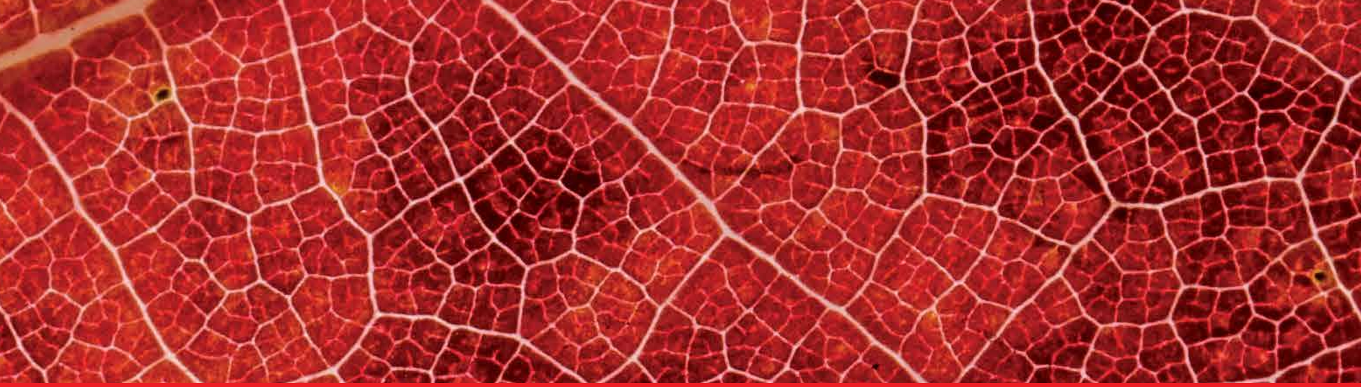

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Abiotic Stress in Plants

Edited by Shah Fahad, Shah Saud,
Yajun Chen, Chao Wu and Depeng Wang

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Published in London, United Kingdom







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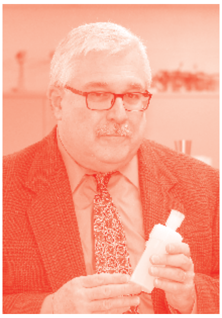

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http://dx.doi.org/10.5772/intechopen.91549
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First published in London, United Kingdom, 2021 by IntechOpen
IntechOpen is the global imprint of INTECHOPEN LIMITED, registered in England and Wales, registration number: 11086078, 5 Princes Gate Court, London, SW7 2QJ, United Kingdom
Printed in Croatia

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

Additional hard and PDF copies can be obtained from orders@intechopen.com

Abiotic Stress in Plants
Edited by Shah Fahad, Shah Saud, Yajun Chen, Chao Wu and Depeng Wang
p. cm.
Print ISBN 978-1-83881-055-9
Online ISBN 978-1-83881-062-7
eBook (PDF) ISBN 978-1-83881-063-4

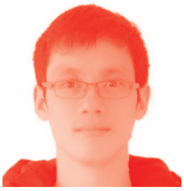
Meet the editors

Dr. Shah Fahad is an assistant professor in the Department of Agronomy, University of Haripur, Khyber Pakhtunkhwa, Pakistan. He obtained his Ph.D. in Agronomy from Huazhong Agriculture University, China, in 2015. After completing his postdoctoral research in Agronomy at Huazhong Agriculture University (2015–2017), he accepted the position of assistant professor at the University of Haripur. He has published more than 290 peer-reviewed papers (impact factor = 910.45) with more than 260 research and 30 review articles on important aspects of climate change, plant physiology and breeding, plant nutrition, plant stress responses, and tolerance mechanisms, and exogenous chemical priming-induced abiotic stress tolerance. He has also contributed fifty book chapters to various volumes published by well-renowned publishing houses. He has also edited fifteen book volumes, including this one. Dr. Fahad received the Young Rice International Scientist award and distinguished scholar award in 2014 and 2015, respectively. He won fifteen projects from international and national donor agencies. Dr. Fahad was named among the top 2 percent of scientists in a global list compiled by Stanford University, California. His areas of interest include climate change, greenhouse emission gasses, abiotic stresses tolerance, roles of phytohormones and their interactions in abiotic stress responses, heavy metals, and regulation of nutrient transport processes.

Dr. Shah Saud received his Ph.D. in Horticulture (turf grasses) from Northeast Agricultural University, Harbin, China. He is currently working as a postdoctoral researcher in the Department of Horticulture, Northeast Agricultural University. Dr. Saud has published more than 125 research publications in peer-reviewed journals. He has edited three books and written twenty-five book chapters on important aspects of plant physiology, plant stress responses, and environmental problems in relation to agricultural plants. According to Scopus, Dr. Saud's publications have received roughly 2500 citations with an h-index of 24. He is an editor and reviewer for more than twenty peer-reviewed international journals. His outstanding work has been recognized with five awards for scientific and technological progress in Heilongjiang province, China. Dr. Saud has also won five international projects.

Dr. Yajun Chen obtained a Ph.D. in Crop Cultivation and Farming System from Northeast Agricultural University, Harbin, China, in 2005. Later, she completed her postdoctoral research in biology at Northeast Forestry University, Harbin, China. Currently, she is a professor at the Horticulture College, Northeast Agricultural University. Over the course of a dozen years, Dr. Chen has studied in Australia (2003–2004), the United States (2010–2011), and Norway (2015–2016) as a visiting scholar funded by the Chinese government. Her research work encompasses ornamental plant adverse physiology and ecology, coping with ecological environment adaptation, and restoration of garden plants. Dr. Chen has published more than 110 research papers in peer-reviewed journals in her field at home and abroad. As a chief editor she has edited more than twenty books on important aspects of local flora, turf and flower culture, and plant stress physiology. She has also trained more than eighty master's students and five Ph.D. candidates in these fields. Her outstanding work has been recognized with eight awards for scientific and technological progress in Heilongjiang province, China. She has also won two international and thirteen national projects.

Dr. Depeng Wang obtained a Ph.D. in Agronomy and Crop Physiology from Huazhong Agriculture University, Wuhan, China, in 2016. He is currently a professor at the College of Life Science, Linyi University, Linyi, China. He is the principal investigator of the Crop Genetic Improvement, Physiology & Ecology Center at Linyi University. His current research focuses on agronomy and crop ecology and physiology, including characteristics associated with high-yield crops, the effect of temperature on crop grain yield and solar radiation utilization, morphological plasticity to agronomic manipulation in leaf dispersion and orientation, and optimal integrated crop management practices for maximizing crop grain yield. Dr. Wang has published more than thirty-six papers in reputed journals. He has edited one book and written four book chapters on important aspects of crop physiology, environmental stress, and crop quality formation. According to Google Scholar Citation, his publications have received about 100 citations. He is a reviewer for five peer-reviewed international journals. Dr. Wang is a provincial crop expert in green, high-quality, and efficient technology, and has participated in six national projects with more than $4 million in research funding.



Dr. Chao Wu engages in the field of crop cultivation and physiology, and plant phenomics. He received a Ph.D. from Huazhong Agricultural University, Wuhan, China, in 2016, and completed his postdoctorate at Nanjing Agricultural University, Nanjing, China, in 2019. Currently, he is an associate research fellow at Guangxi Institute of Botany, Guangxi Zhuang Autonomous Region, and the Chinese Academy of Sciences, Guilin, China. He chairs the Natural Science Foundation of Jiangsu Province and two postdoctoral science research foundations. His main research focus is physiological mechanisms of abiotic-stress tolerance (heat, drought) in crops and medicinal plants.

Contents

Preface

A biological system is an endogenic and dynamic system that always seeks a constant in-flow of energy to maintain its meta-stable condition called homeostasis. Any environmental disruption of this homeostasis may be explicated as biological stress. Biological stress limits the growth and yield potential of a crop and is broadly grouped into biotic and abiotic stresses. Biotic stress is due to interactions between organisms starting from predation to allelopathy. Abiotic stress appears due to the adverse effects of non-living environmental factors (i.e., water, temperature, light, metal, mineral nutrients, etc.) on plants, which are often sporadic and highly localized. Understanding abiotic stress responses in plants are critical for the development of new varieties of crops that are better adapted to harsh climate conditions. This book provides a comprehensive overview of the agronomical, physiological, and molecular basis of plant responses to external stress like salinity, drought, temperature, and heavy metals to aid in the engineering of stress-resistant crops.

Over twenty-two chapters, *Abiotic Stress in Plants* presents a comprehensive picture of plant responses to external stress. It is designed for researchers, technologists, policy makers, and undergraduate and postgraduate students studying sustainable crop production and crop protection. This book is a worthy addition to university libraries and research establishments where agricultural and agronomical sciences are studied and taught. We are thankful to all the authors who contributed their valuable chapters to this book. We are also extremely grateful to IntechOpen's Publishing Process Manager Ms. Sara Gojevic-Zrnic for helping us to publish this book in an excellent form in the shortest possible time. We owe our sincere thanks and irreparable gratitude to our families whose consistent encouragement and love have been a tremendous impetus for completing this book.

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Chapter 1

A Review on Kentucky Bluegrass Responses and Tolerance to Drought Stress

Jian Cui, Saud Shah, Shah Fahad and Yajun Chen

Abstract

Kentucky bluegrass (*Poa pratensis* L.) is an excellent cool-season turfgrass and is extensively used in urban green space, parks and sports fields worldwide, but it is sensitive to drought stress. Drought reduces turf quality of Kentucky bluegrass by influences on the shoot density, texture, uniformity, color, growth habit and recuperative capacity. It has been a challenge for breeding water saving cultivars and enhances water use efficiency in Kentucky bluegrass. Many studies have revealed the mechanisms of drought stress tolerance in Kentucky bluegrass via multiple approaches. The morphological and physiological attributes as well as molecular information were discovered for better understanding and improving its drought tolerance. In this chapter, we will draw a systematic literature review about Kentucky bluegrass in response to drought stress and provide future perspectives of Kentucky bluegrass drought resistance research.

Keywords: Kentucky bluegrass, drought stress, tolerance, mechanism

1. Introduction

Drought is one of the major environmental factors that affect plant growth and survival worldwide. Turfgrass as an important part of the green ecosystem in the urban area which provides many kinds of environmental functions such as beautify and green the city, cooling warm weather, and soil stabilization. As urban rapid expansion nowadays, the percentage of land converted into turfgrass has been increasing. Turfgrasses are generally classified into cool- and warm- season groups based on their adaptation to specific ranges in temperature and precipitation, which are mainly governed by latitude and altitude [1, 2]. Kentucky bluegrass (*Poa pratensis* L.) is an excellent cool-season turfgrass and is extensively used in public parks, golf courses and residential lawns in temperate and cold temperate zones and cool plateau areas. This is a highly variable, rhizomatous species. Many improved cultivars of this species have been developed and used in landscaping areas in the world. A wide range of diverse cultivars and accessions of Kentucky bluegrass have been characterized based on pedigree, common turf performance, and morphological characteristics and were grouped into different genotypes such as Common, Compact, Compact-America, Julia, Mid-Atlantic and Midnight types [3]. Although these germplasm possess prominent ornamental value, however, Kentucky bluegrass extremely limiting used by water scarcity in practice, especially in semiarid, arid regions as well as the areas with the increase demand on water for agricultural, residential and industrial use [4–6]. It has been a challenge to select water saving cultivars and reduce water input in turf management [7]. Drought reduces the turf quality of Kentucky bluegrass by influences on the shoot density, texture, uniformity, color, growth habit and recuperative capacity [8, 9], in response to drought stress, different genotypes of Kentucky bluegrass performed various adaptive mechanisms and strategies in respective to their morphology, physiology and molecular bases. Understanding these mechanisms of Kentucky bluegrass tolerance to drought stress is a key step for improving drought resistant cultivars and reduces water input in management. Here, we summarize research progress in drought stress of Kentucky bluegrass for providing an overview of the field to readers and also for providing guidelines for practical management strategies under limited water availability.

2. Responses to drought stress in Kentucky bluegrass

Kentucky bluegrass, like other agronomic, horticultural, and landscape vegetation, requires water for growth and provides esthetics functional benefits for environments. Main causes of water deficiency may result from low rainfall, inadequate irrigation, as well as summer heat, which could greatly limit growth, and turf quality of Kentucky bluegrass. Like other plants, the ability of Kentucky bluegrass to maintain growth and survive under drought stress is broadly considered as drought resistance. Three major strategies of plant drought resistance are considered as escape, avoidance, and tolerance [2, 10] and are illustrated in **Figure 1**. Drought resistance traits vary genetically by exhibiting survival strategies among Kentucky bluegrass cultivars under water limited conditions [11]. However, these strategies are not mutually exclusive, and Kentucky bluegrass may utilize more than one when facing to water shortage.

2.1 Turf performance and morphological responses

Turf quality (TQ) refers to two aspects including visual quality and functional quality [1]. Turfgrass density, texture, uniformity, color, growth habit and smoothness are the most visible factors influence turf appearance quality, while functional quality such as playability in a particular sport turf is determined not only by some of the visual determinants, but also by other characteristics as well, including rigidity, resiliency, verdure, rooting and recuperative capacity. The drought resistance characteristics of specific species in morphology, growth patterns largely determine the turf quality under drought conditions [12, 13].

Morphological traits on Kentucky bluegrass often change with soil moisture [14], and plants often exhibit a severe decline in TQ and may become dormant during extended drought conditions [15]. Although Kentucky bluegrass can escape by going into dormancy under severe drought conditions especially during summer periods [16]. People still desire to sustain a green surface during drought periods for esthetics, sports, and other eco-functions. Therefore, drought escape is only considered a viable alternative for Kentucky bluegrass in those areas where irrigation is not available and survival of the turfgrass following drought is the primary objective [17].

Water use of the turfgrass canopy is influenced by water loss via shoot transpiration and soil evaporation, and by water uptake from the soil through the root system. The differences among Kentucky bluegrass cultivars in shoot and root characteristics such as leaf orientation and canopy configuration, tiller or shoot density, rooting depth, and root density are associated with water-use rate [18].

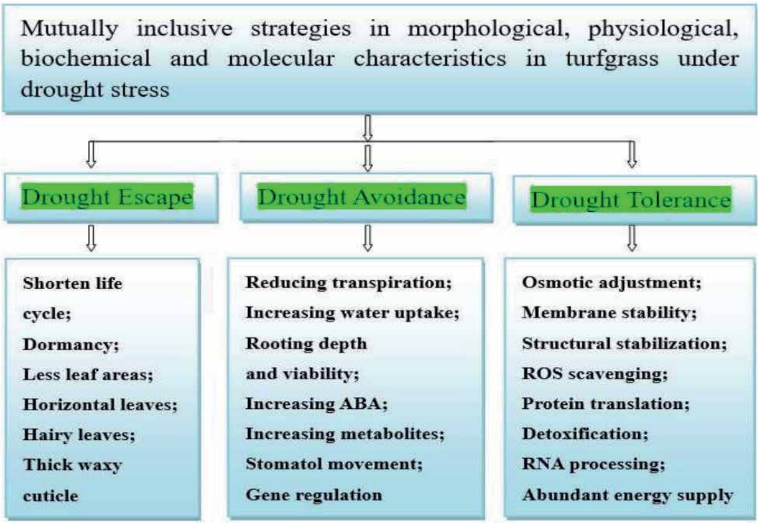

Figure 1.
Drought resistance strategies of turfgrass under drought conditions (illustrated by Jian Cui).

Lower ET rates of grass species were generally characterized with comparatively a high shoot density and relatively horizontal leaf orientation; and also with a low leaf area, including a slow vertical leaf extension rate and a narrow leaf texture [19]. Shoot vertical extension rate was positively correlated with water-use rate for Kentucky bluegrass with upright growth cultivars [20]. Based on a random spaced plant of 61 Kentucky bluegrass cultivars under untrimmed conditions, the morphological properties and comparative water use rate were strongly correlations by use discriminant analysis [21]. Low-water use cultivars had 13% more horizontal leaf orientation, 6% narrower leaf texture, 13% more lateral shoots per plant, 12% slower vertical leaf extension rate, 2% more leaves per shoot, and 7% shorter leaf blades and sheaths than the high-water use cultivar [21]. Low soil water content resulted significantly different in shoot-to-root ratio, vertical growth and survival rate among 11 Kentucky bluegrass cultivars, and more influence on tillering rate than other morphological indicators [11]. The components of leaf epidermis including stomatal apparatus, silica cells and cuticle are very important to the drought resistance of turfgrass canopy. Chen et al. found that leaf epidermis characteristics were associated with drought resistance among Kentucky bluegrass cultivars [22]. The opening or closing status of stomatal apparatus, the silica cell size and density, the thickness of the wax layer on leaf surface were related to the drought resistance and varied among Kentucky bluegrass cultivars (**Figure 2**).

Generally, an extensive deep root system of plants is important for efficient water uptake from the soil. Deep rooting enables plant to avoid water stress by taking up water from deeper in the soil profile when the surface soil is dry. The increased drought resistance of Kentucky bluegrass cultivars was correlation to the increased water uptake activity at the 15 to 30 cm soil depth [23]. Deeper root system in Kentucky bluegrass can avoid drought stress more consistently than total root mass [24]. However, other research found that not all turfgrasses with extensive root system are necessarily high water use. Hence, improvements in drought tolerance in Kentucky bluegrass may not necessarily occur by selecting germplasm with deep rooting or high root to shoot ratios due to no correlation between deep rooting and the ability to withstand long periods of water deficit [18]. Study on Kentucky bluegrass with the relation of Carbon-13 discrimination and water use efficiency (WUE) showed that turfgrass performance under drought may be

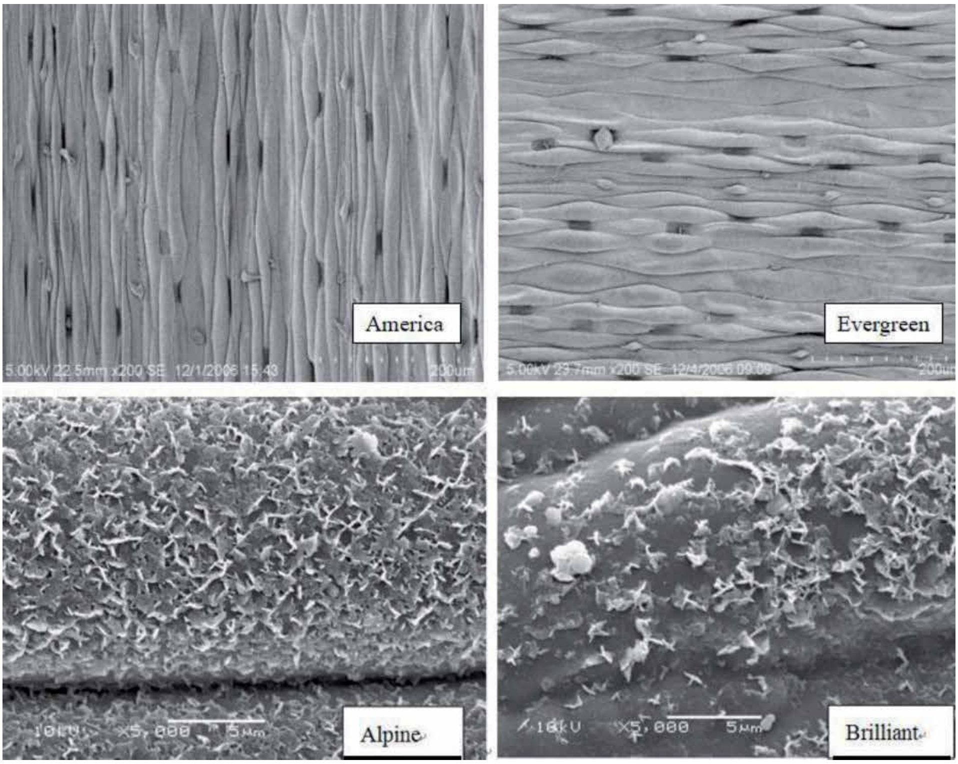

Figure 2.
Leaf epidermis characteristics among Kentucky bluegrass cultivars (photos provided by Yajun Chen).

improved for low Carbon-13 discrimination, which has been shown to be negatively correlated with WUE. Low Carbon-13 discrimination values were associated with less wilt and leaf firing, suggesting that Carbon-13 discrimination may be a useful selection criterion for superior performance under limiting soil moisture [25]. Kentucky bluegrass is a typically rhizomatous perennial, and ramets distributions in the grass clonal system connected by rhizomes largely rely on water status in soil. Drought stress severely influenced all agronomical, anatomical attributes of Kentucky bluegrass especially the seeking water behavior of rhizomes in both homogenous and heterogeneous environments [26]. Understanding these morphological characteristics respond to drought may assist to selecting low-water use Kentucky bluegrass cultivar in future for better adaptation water scarcity environment and saving water in turf management [27].

2.2 Bio-physiological and molecular in responses to drought

Severe drought can cause Kentucky bluegrass to go into dormancy and lose its greenness, which is a survival strategy as we described above, but we do not expect Kentucky bluegrass to adopt escape drought strategy to lose its landscape value during water deficiency. However, three drought resistance strategies including escape, avoidance and tolerance may be used simultaneously in different drought settings for specific turfgrass, and so does Kentucky bluegrass [2]. Drought-avoiding traits on Kentucky bluegrass can exhibit many different morphological characteristics and water absorption ability in response to drought, including previous reports as discussed before on leaf orientation and canopy configuration, tiller or shoot density, rooting system, leaf epidermis, lower ET and the like. Drought avoidance and tolerance are critical strategies for maintenance turf greenness and extending green period, fitness turf visual and functional quality under short or long drought stress. Many desirable drought resistance attributes on Kentucky bluegrass involve in physiological and biochemical metabolic activities as well as molecular regulators can serve as selection criteria for improving drought resistance cultivars using for turfgrass practical managements. For maintenance structural stabilization under drought stress, strategies on physiological responses of Kentucky bluegrass exhibited by increasing cellular osmotic potential and antioxidant enzyme activities, suppressing reactive oxygen species (ROS) and ABA production, etc.

The sucrose, fructose, glucose, starch as well as carbohydrate metabolism enzymes associated with plant tolerance to severe drought stress and post-drought recovery in two Kentucky bluegrass cultivars 'Midnight' and 'Brilliant' [28]. The differential in accumulation of different types of soluble carbohydrates could be related to the genetic variability and biological functions during drought and post-drought recovery in Kentucky bluegrass. These soluble sugars as osmolytes play critical role in maintenance cellular turgor by increasing osmotic potential and energy sources in photosynthetic process, and sucrose also plays protective roles in proteins and membranes from drought damages [29]. Sucrose, proline, as well as inorganic ions are important osmolytes contributing to in cell osmotic adjustment when turfgrass faced to drought stress [30]. In addition, another interesting result was that superior drought resistance for 'Midnight' Kentucky bluegrass could be characterized by the accumulation of sucrose in association with increased activity of sucrose-synthesizing enzymes (sucrose phosphate synthase and sucrose synthase), suggesting that increased sucrose accumulation resulting from the maintenance of active sucrose synthesis could relate to superior turf performance during water loss in Kentucky bluegrass [28]. Two Kentucky bluegrass cultivars contrasting in drought tolerance were evaluated the photosynthetic responses and underlying enzyme activities during drought stress and re-watering [31]. Compared to 'Brilliant', drought-tolerant 'Midnight' maintained significantly higher net photosynthetic rate, higher enzymatic activity and transcript level of ribulose-1,5-bisphosphate carboxylase (Rubisco), higher enzymatic activity of glyceraldehyde phosphate dehydrogenase (GADPH) during 10-d drought stress and in responses to re-watering, as well as higher Rubisco activation state upon re-watering, suggesting that carboxylation controlled by Rubisco and carbon reduction regulated by GAPDH could be the key metabolic processes imparting genetic variation in photosynthetic responses to drought stress while active Rubisco, GAPDH and Rubisco activase could all be involved in the superior post-drought recovery in Kentucky bluegrass [32]. Drought-tolerance 'midnight' and drought-sensitive 'Brilliant' as drought research model cultivars of Kentucky bluegrass have been detected 88 drought-responsive proteins by gel electrophoresis and mass spectrometry analyses. Many proteins involved in amino acid or energy metabolism were down regulated under drought stress, but most of those proteins had higher abundance in 'Midnight' than in 'Brilliant'. These proteins may serve as drought stress responsive proteins imparting Kentucky bluegrass adaptation to drought [33].

One of the major drought avoidance strategies in plant is the antioxidant physiological response by increasing antioxidant enzymes activity or by developing an effective reactive oxygen species (ROS) scavenging system to suppress ROS production which is a major cause of cellular injuries induced by drought [34]. Researches have revealed that availability of antioxidant enzyme activity and induced gene relative expression, the accumulation of abscisic acid and the level of membrane fatty acid saturation were important metabolic factors contributing to enhance drought resistance in Kentucky bluegrass although the responses in cultivars, organs and growth stages were varied [35–38]. Some studies found that the activity of superoxide dismutase (SOD) increased while that of catalase (CAT) and peroxidase (POD) decreased in leaves of Kentucky bluegrass when only surface soil drying was simulated [39]. The increased SOD activity in Kentucky bluegrass leaf cannot inhibit formation and accumulation of free radicals, but only delay accumulation of free radicals to a certain extent for alleviate active oxygen damage to cells [40, 41]. Under drought stress, the increased superoxide (O2•-) and hydrogen peroxide (H2O2) in leaves and roots of Kentucky bluegrass were associated with lipid peroxidation. In addition, the increases in the activities of ascorbate peroxidase (APX), monodehydroascorbate reductase (MHAR), dehydroascor bate reductase (DHAR) in leaves and that of CAT, glutathione reductase (GR), and MHAR in roots, but reduction in the activities of SOD and DHAR in roots [42]. After 6 days of rewatering, 'Midnight' displayed significantly higher activity levels of CAT, POD, and APX compared with 'Brilliant'. The differential responses of the activities of antioxidant enzymes to drought stress and post-drought rewatering between 'Midnight' and 'Brilliant' indicated that antioxidant enzymes including APX, SOD, GR, MR (monodehydroascorbate reductase), and DR (dehydroascorbate reductase) in the AsA-GSH (ascorbate–glutathine) cycle may play important protective roles involved in scavenging oxidant stress induced reactive oxygen species in Kentucky bluegrass for cellular survival of severe water deficit and post-drought recovery [43]. The increased content of unsaturated lipids in Kentucky bluegrass leaves under drought stress was crucial to maintain cell membrane fluidity and reduce ROS production. Leaf dehydration tolerance and postdrought recovery in Kentucky bluegrass was associated with their ability to maintain relative higher proportion and level of unsaturated fatty acids, particularly linolenic acids and linoleic acids [44, 45]. These researches on antioxidant metabolic responses revealed key information for controlling drought tolerance in turfgrass species and would facilitate the development of drought-tolerant germplasm through biotechnology.

So far, the important physiological mechanism of plant adaptation to drought resistance is ABA (Abscisic acid) accumulation, the amount of the primary chemical of ABA can reach up to more than 50-fold in plants under drought conditions [46, 47]. ABA is one of the most drastic changes observed hitherto in the concentration of a plant hormone responding to an environmental stimulus [37]. The functions and the signaling pathways of ABA in plants' responses to drought stresses have been extensively studied, and it is now well accepted that ABA plays important roles in plant including turfgrass adaptation to environmental stresses [5, 48, 49]. The relationship of ABA accumulation and drought resistance in different genotypes of Kentucky bluegrass varied. Leaf ABA content in drought susceptible cultivars increased sharply after 2 days of drought stress to as much as 34-fold the controls, while in drought resistant cultivars, the content ABA in leaves also increased with drought, but to a lesser extent than in drought sensitive cultivars. In addition, the stomatal conductance, photosynthetic rate, leaf water potential and turf quality in drought resistant cultivars performed less severe decline during drought than drought sensitive cultivars, indicating that stomatal conductance was negatively related to ABA accumulation and the ABA concentration during drought could regulate by stomatal behavior of Kentucky bluegrass [31]. The exogenous ABA application on Kentucky bluegrass demonstrated that ABA sprayed on leaves can help maintain higher turf quality and delayed the quality decline during drought stress. ABA treated grass had higher cell membrane stability indicated by less electrolyte leakage of leaves, and higher photochemical efficiency [50]. ABA effects on shoot growth and stomata, it may also facilitate osmotic adjustment and expression of specific proteins [6]. Further study suggesting that ABA accumulation in response to drought stress could be used as a metabolic factor to select for drought tolerance in Kentucky bluegrass [35]. Some studies on Kentucky bluegrass discovered that the interaction of endogenous hormones could contribute to increase drought resistance. Under drought conditions, the concentrations of ABA, JA (Jasmonate) and BR (Brassinolide) increased significantly compared to well water plants. Drought stressed Kentucky bluegrass had higher leaf ABA, lower leaf trans-zeatin riboside (ZR), isopentenyl adenosine (iPA), and indole-3-acetic acid (IAA), but similar level of leaf gibberellin A4 (GA4) when contrasted to the control, suggested that drought stress-induced injury to Kentucky bluegrass may be associated with hormonal alteration and may have better photosynthetic function and performance [5, 51].

Recently, a great progress on biological omics such as metabolomics, proteomic and transcriptomic have revealed many drought response metabolites, proteins and molecular factors in major crops [2]. However, information on Kentucky bluegrass involved in molecular mechanisms and genes underlying drought resistance is lacking. Limited researchers have identified some drought-responsive genes and gene signaling transduction pathways as described below. Under drought stress and water recovery treatments, the gene expression patterns of antioxidant enzymes of Kentucky bluegrass were differentially or cooperatively involved in the defense mechanisms in the leaves and roots. For the leaves, the expressions of iron SOD (*FeSOD*), cytosolic copper/zinc SOD (*Cu/ZnSOD*), chloroplastic *Cu/ZnSOD*, and *DHAR* were down-regulated by drought stress but recovered to control level after rewatering, while the expressions of *GR* and *MDHAR* were up-regulated and remained that levels after recovery. For the roots, the expressions of cytosolic *Cu/ZnSOD*, manganese SOD (*MnSOD*), cytosolic *APX*, *GR*, and *DHAR* were down-regulated under drought stress but recovered except for *GR* and *DHAR*, while *MDHAR* expression was up-regulated. No differences in *CAT* transcript abundance were noted among the treatments [42]. Transcriptome sequencing on cuticular wax deposition of Kentucky bluegrass have discovered that a number of genes involved in very longchain fatty acids and cuticular wax biosynthesis, transportation and regulation pathways, and these genes presented differentially expressed patterns between the leaf non-elongation zone and the emerged blade zone [52]. There were 9 candidate reference genes expression stability in the leaves and roots of Kentucky bluegrass under different stresses (drought, salt, heat, and cold), and were evaluated using the GeNorm, NormFinder, BestKeeper, and RefFinder bio-tools. Among 9 genes, *ACT* and *SAM* maintained stable expression in drought-treated leaves, and *GAPDH* combined with *ACT* was stable in drought-treated roots. The expression stability of reference genes in Kentucky bluegrass will be particularly useful in the selection of stress-tolerance genes and the identification of the molecular mechanisms conferring stress tolerance in this species [53]. A comparative transcriptomic study also found that many differentially expressed genes were enriched in 'Plant hormone signal Transduction' and 'MAPK signaling pathway-Plant'. Some key up-regulated genes, including *PYL*, *JAZ*, and *BSK*, were involved in hormone signaling transduction of abscisic acid, jasmonic acid, and brassinosteroid and possibly these genes play important roles in coping with drought stress in Kentucky bluegrass under drought stress [5]. These studies suggest importance of molecular functions and related genes for protection and improvement in Kentucky bluegrass tolerance to drought, however, molecular mechanisms underlying drought tolerance in Kentucky bluegrass are largely unknown.

3. Summary and future perspectives

Global climate change and the continuously growing population in the world, result increasingly limited and more costly in water availability. Water conservation in turf management becomes extremely important. Kentucky bluegrass is one the most popular and widely used cool-season turfgrass for amenity, sports and environmental conservation. Turf industry has released many improved Kentucky bluegrass cultivars in the world since last century. Among these germplasm, a wide range in water consumption variation represented by the daily evapotranspiration rate [20]. Hence, to identify ideal water conserving properties on Kentucky bluegrass in reducing irrigation requirements by enhance drought tolerance has been pursued by scientists. Many researchers have revealed a great deal of results in the understanding of morphological and physiological traits and mechanisms responding to drought resistance with Kentucky bluegrass. In response to drought stress, various adaptation strategies of Kentucky bluegrass were taken based on morphology, physiology and genetics. Narrow leaf texture, leaf orientation and canopy configuration, shoot and root systems are associated with Kentucky bluegrass drought resistance. Moreover, the physiological characteristics with the increased ABA and metabolites to enhance cell water potential and regulating stomatal movement, the protein translation and ROS scavenging to protect membrane stability are vital strategies for Kentucky bluegrass survive and maintain greenness under drought conditions. However, although some drought-responsive genes and gene signaling transduction pathways as well as proteins for Kentucky bluegrass tolerance to drought stress have been identified, our knowledge of the response is still limited information on how whole plants perceive and conductive these signals under long period drought. On Kentucky bluegrass, systemic study on the molecular mechanisms of drought resistance is still lagging. Identification of novel candidate genes, proteins, metabolites, and molecular markers, and integrating transcriptomics, proteomics and metabolomics to explore intercellular communication and drought resistance manipulating signals are crucial for molecular breeding and marker-assisted selection of Kentucky bluegrass with superior drought tolerance in future.

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Chapter 2

Management of Abiotic Stress in Forage Crops

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Abstract

Forage plays a key role in rearing ruminants and protecting the environment. Apart from serving as the primary source of food for domestic and wild animals, forages also contribute to human civilization in different ways like protecting soil through crop over and fertility by addition of organic matter. It also provides habitat for wild animals. A survival strategy plays a more important role than a growth strategy to improve the sustainability of forage production, especially in extreme environmental conditions . Climate change is likely to affect the forage production and nutritional food security for domestic animals. Long-term rainfall data in India indicate that rainfed areas experience 3 to 4 years of drought in every 10 years. Of these, one or two of it occur in severe form. Forage crop production is largely affected by abiotic factors related stress such as drought, salinity, etc. There is need to adopt various conventional and genetic approaches to improve stress tolerance of forage crops.

Keywords: forage crops, abiotic stress, management, breeding and micronutrients

1. Introduction

In the agricultural context, stress has been defined as the conditions in which plants are prevented from fully expressing their genetic potential for growth, development, reproduction, and, ultimately, crop productivity [1]. Abiotic stress negatively affects the livelihoods of farmers and their families, the sustainability of livestock, as well as national economies and food security. Forages are generally described as plants and its parts consumed by domestic livestock. Forage plays a key role in rearing ruminants and protecting the environment. Apart from serving as the primary source of food for domestic and wild animals, forages also contribute to human civilization in different ways like protecting soil through crop over and fertility by addition of organic matter. It also provides habitat for wild animals. In the biological soil–plant–animal system, forage is highly demanded by livestock. Escalation in the human population in the coming decades will put the higher burden on land for food crops and fiber production. As a result, we may face forced forage cultivation in those areas having poorer soils regarding fertility and management [2]. The water use for irrigation is incredibly high and this trend could increase considerably in the future leading to shortage of water availability [3]. For perennial forage and natural vegetation, the ability to survive during adverse environmental periods is a life saving feature. A survival strategy plays a more important role than a growth strategy to improve the sustainability of forage production, especially in extreme environmental conditions [4]. Forage crop production is largely affected by abiotic factors related stress such as drought, salinity, etc. There is need to adopt various conventional and genetic approaches to improve stress tolerance of forage crops.

2. Forage status

Currently, India faces a critical imbalance in its natural resource base: around 18 percent of humans and 15 percent of the world's animal population are only served by 2.4 percent of the geographical area, 1.5 percent of forests and pastures, and 4.2 percent of water resources [5]. The three main sources of forage supply in India are crop residues, cultivated forage, and forage from common property resources such as forests, permanent pastures, and pastures. Due to the multiplicity of forage crops produced in different seasons and regions, the surplus and deficit in different regions, the non-commercial nature of crops and forage production with minimal inputs from degraded and marginal land, there has been a large gap in the availability and need for forage. Currently, the country faces a net deficit of 35.6 percent of green forage, 10.95 percent of residues from dry crops, and 44 percent of concentrated ingredients for animal feed [6]. Supply and demand for the forage scenario are presented in **Figure 1**. Furthermore, in the case of forage, regional and seasonal deficiencies are more important than national deficiencies, since it is not economical to transport forage over long distances. Furthermore, the available forages are of low quality and deficient in available energy, protein, and minerals. Farmers maintain large herds of animals to compensate for low productivity, adding pressure on forage and other natural resources [7]. Almost two-thirds of the total cost of animal production is due to food and fodder. Consequently, any attempt to improve the availability of food and fodder and save the cost of food would result in better remuneration for farmers. The area under cultivated forage is only 8.4 million hectares and has been static for the past two decades. The potential for further increases seems very small due to demographic pressure for food crops. Recent crop diversification, where cash crops replace traditional cereal crops, especially coarse grains, is likely to have an impact on the availability of crop residues for animal production [8]. Likewise, the productivity of certain important cultivated forages is highly variable. Among Kharif forages, sorghum, corn, cowpea, Napier-bajra hybrid, and guinea they have a wide range. However, during rabi, the choice is limited to oats, alfalfa, and berseem. Emphasis should be placed on new area-specific crops that can break down yield barriers and meet the challenges of the food deficit.

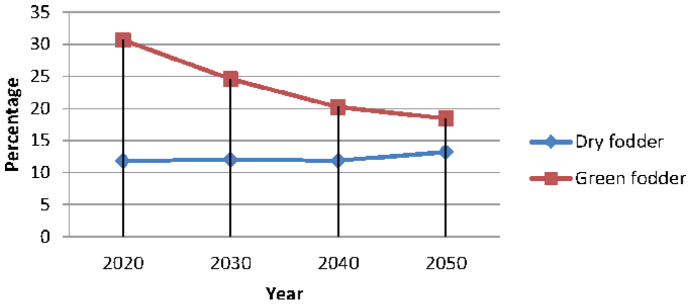

Figure 1.
*Deficient trend of fodder crop concerning future demand. *IGFRI vision, 2050.*

3. Different types of abiotic stress faced by crops

3.1 Temperature restriction

The tropical climate is cursed by higher temperatures and radiation that limit the growth and development of plants. High temperatures cause burns, sunburn, and discoloration of the leaves, reducing plant growth [9]. Limiting growth, metabolism, and performance potential due to exposure to a temperature below or above the thermal threshold for optimal biochemical, physiological, and morphological development is called thermal stress [10]. Plants are classified into psychophilic, mesophilic, and thermophilic according to their tolerance to low, medium, and high temperatures [1]. The adversity of heat stress varies with the duration, stage, and intensity of stress [11]. Increased heat stress adversely affects the spikelets number, the number of florets per plant in rice crop, and the seeds in forage crop like sorghum [12]. It also reduces quality due to reduced production of oil, starch, and protein [13]. Stress at low temperatures causes wilting, bleaching, darkening, necrosis, and death of plants [1]. Approximately 15percent of arable land is said to be affected by frost stress [14].

3.2 Moisture stress

About 28 percent of the world's land is too dry for agricultural support [15]. The estimated annual yield loss due to extraction in the tropics is almost 17 percent [16]. Increasing the draft with the changing climate scenario leads to a decrease in plant physiology, growth, and reproduction [17]. The moisture deficit causes greater transpiration and reduces the availability of water from the roots of the plants [18], which tends to balance the water on the negative side that affects growth, the relationship between nutrients and water, photosynthesis and assimilation of sharing and, ultimately, performance [19]. The stress response plan in plants varies according to the species according to its stages and other growth factors [20]. High-temperature stress affects enzyme activity, cell division in plants [21] and also changes the growth period and distribution of crops [22].

3.3 Heavy metal stress

Heavy metals are those metals that have a specific weight greater than 5 g cm^{-3} or an atomic mass greater than 20 and are generally toxic even at low concentrations [23]; some of heavy elements or metals are cadmium (Cd), lead (Pb), arsenic (As), silver (Ag), etc. Heavy metal contamination in the soil is mainly due to human activities such as mining, smelting, intensive agricultural practices, fuel production, electroplating, etc. [24] and may also be due to natural processes such as soil erosion, excessive weathering of rocks and minerals, and volcanic eruption. Among heavy metals, some have known physiological functions in the plant system called non-essential heavy metals, namely arsenic (As), lead (Pb), cadmium (Cd), mercury (Hg), and selenium. (Sc) and some are involved in different physiological functions of plants as a cofactor of enzymatic reactions [25] or role in redox reactions [26] called essential heavy metals, namely cobalt (Co), copper (Cu), manganese (Mn), zinc (Zn), iron (Fe), molybdenum (Mo) and nickel (Ni).

3.4 Salt stress

Crops are said to be subject to salt stress when they cannot express their full genetic potential in terms of growth, development, and reproduction, since the salinity of the soil exceeds the critical level [27] and dissolved salts in the soil and irrigation water vary from place to place [28]. The detrimental effect of soils affected by salt may be due to a high concentration of salt in the soil solution, i.e. osmotic effects or a high concentration of specific ions such as sodium or chloride that can damage sensitive crops, i.e. a specific ionic effect. The harmful effect of saline soil is due to the concentration of soluble salt, while the harmful effects of sodium soil are due to deterioration of the physical state of the soil [29]. The harmful effect of salt stress may be due to a specific ionic effect, that is, Na^+ and Cl^- [30] or to interact with other dynamics of mineral nutrients [31].

3.5 Nutritional stress

Several mineral elements contribute to the growth and development of a plant, 17 of which are called essential nutrients according to the essentiality criteria defined by Arnon and Stout. Since mineral nutrition is discipline independent of plant physiology [32, 33] divide essential minerals into four groups according to their biological structures and metabolic functions. There is some nutritional stress (deficiency or excess) reported by various scientists in different plants. Nitrate plays pivotal role cytokinin biosynthesis and transport, and a higher level of nitrate (NO^{3-}) inhibits root growth and the root: shoot ratio [34]. Phosphorus deficiency limits the lengthening of the primary roots and improves the formation of lateral roots, decreases the proportion of the dry weight of the roots of the shoots [35], reduces the leaves [36] and affects the reproductive organs formation [37], plants with potassium deficiency (K^+) are sensitive to lodging and airflow [38]. A sulfur deficiency decreased the net photosynthesis and the hydraulic conductivity of the roots [39], the reduction in the dry weight ratio of the roots of the shoots [40], an alteration in the metabolism of carbohydrates followed by an induced accumulation of starch [41].

4. Impact of abiotic stress on physiology of forage

4.1 Photosynthesis

Moderate stress in water deficit plants reduces photosynthesis which is accompanied by closing of stoma [42]. Measurement of the photosynthetic response and the activity of the ribosose bisphosphate carboxylase vifro (RUBISCO) in alfalfa (*Medicago sativa* L.) exposed to an increasing water deficit and found evidence of adverse osmotic effects [43].

4.2 Forage quality

The digestibility of legumes and their fiber have been largely affected by water availability [44, 45]. Drought affects the forage composition and quality by altering plant maturity and ratio of leaf mass to stem mass [46].

4.3 Establishment of seedlings in forages

Water availability highly affects the forage seedlings growth and maturity [47]. Seminal roots support seedlings for a short time. Seminal root system absorbs by the hydraulic conductivity of the suboptoptic internode. Redmann and Qi (1992) found that the diameter of the xylem vessels in warm-season grass seedlings that emerged from different planting depths and length of suboptoptic internode plays an important role in transport of water from the root to the shoot and reducing hydraulic conductivity.

5. Impact of climatic anomalies on forages in terms of stress

Climate change has become a serious threat to life on earth. There is also a global trend of increased storms on most lands. Glaciers are continuously melting, while daily high temperature with heat waves became more common [48]. Coping with climate variability is becoming a major challenge for human civilization. Higher seasonal variability regarding the distribution of precipitation, extreme events of temperature, and precipitation cause damage to crops and raise serious concerns about agricultural production. Among adverse weather events, drought is the major factor to directly affect the population. A warmer climate with increasing climatic variability will increase the risk of climatic extremes. Meteorological data analyzed over 5 decades from CRIDA's Gunegal research farm, a typical rain region, showed low precipitation. Climate change is likely to affect the forage production and nutritional food security for domestic animals. Long-term rainfall data in India indicate that rainfed areas experience 3 to 4 years of drought in every 10 years. Of these, one or two of it occur in severe form [49].

6. Abiotic stress management in major fodder crops

6.1 Sorghum

6.1.1 Water stress and its management

Sorghum with its persistent green character, well developed root system, higher water-use efficiency and epicuticular wax represents a good system for studying physiological features related to drought tolerance. Depending on stress development at any growth stage, sorghum shows a stress response before flowering and after flowering, respectively. All these different responses are affected by various genetic processes [50]. Pre-flowering stress affects plant biomass, panicle size, kernel quantity, and grain yield [51], whereas posttesthetic dryness leads to premature senescence of leaves and stems, lodging and the reduction of seed size [52]. Post-synthesis drought also increases plant sensitivity to biotic stress, such as charcoal rot (*Macrophomina phaseolina*) and fusarium stem rot (*Fusarium moniliforme*) [52]. For drought tolerance before flowering, six distinct genomic regions were Recombinant inbred sorghum lines (RIL) derived from the cross between the genotypes Tx7078 (tolerant before flowering, sensitive to post-flowering) and B35 (sensitive to pre-flowering, tolerant after flowering) [53]. The response to dryness after flowering is associated with the persistent green character of sorghum. Staying green is essentially the retention of the surface of mature green leaves (GLAM). Maintaining the remaining green character during the grain filling phase under stress conditions of soil water deficit constitutes an important element of drought tolerance [54].

6.1.2 Epicuticular wax

Epicuticular wax (EW) forms a glaucous upper coating that is visible on many cultivated plants called waxy bloom. Species, organ, stage of development, and environmental conditions are all those things that affect buildup of wax.

Composition and structure of epicuticular wax is very diverse which is considered a potential useful trait and has been related to resistance against different adverse environmental conditions [55]. Sorghum differs from other field crops in its ability to produce sufficient amount of EW that is placed on the leaf blade as well as leaf sheath generally during pre-flowering and stages of maturity. Sorghum leaf sheath bloom is composed of large amount of free fatty acids with a 16 to 33 carbon chain length [56].

6.1.3 Osmotic adjustment

Two traits named osmotic adjustment and antioxidant capacity have been related with drought tolerance mechanisms. Osmotic adjustment has been associated with sustained performance under water limiting conditions in many crops and is an inherited characteristic. Two major independent genes namely OA1 and OA2 in sorghum have been reported to control Osmotic adjustment inheritance.

6.1.4 Cold tolerance

Sorghum from the tropical and subtropical regions of Africa [57] is well adapted to warm growing conditions. Cool temperatures at the beginning of the growing season are therefore an important limitation for the growth of temperate sorghum areas [58]. Cross developed from local Chinese races, ShanQui Red (SQR, cold-tolerant), and SRN39 (cold-sensitive) was used for QTL analysis of early-season cold yields on sorghum [59].

6.2 Bajra

Bajra [*Pennisetum glaucum*] is a C4 plant with very high photosynthetic efficiency. Bajra also have high dry matter production capacity. It is generally cultivated under the most adverse agroclimatic conditions, where other crops such as sorghum and corn do not stand well.

6.2.1 Selecting genotypes is a good approach to managing abiotic stress

Pearl millet germplasm screening helped in the development of highly advanced breeding techniques, an improvement in the population, including OPVs, genetic pools and compounds, possible parental hybrids, and accessions of the high-throughput genetic material of cereals and forages, presumably with a high degree of salt tolerance (**Table 1**).

6.2.2 Low soil fertility

Soils in the areas where pearl millet is grown are often poor infertility because they contain a small amount of organic matter (0.05–0.40percent) due to low ground cover, coarse soil texture, and prevailing high temperatures [63]. Soils also contain low to moderate levels of available phosphorus (10–25 kg ha^{-1}). This problem was mainly solved through nutrient management. The possibilities of genetic improvement for the efficient use of nutrients are increasingly explored in some cultures [64]. Only recently has strategic research been launched at ICRISAT in the West and Central Africa region to identify QTL to increase the efficiency of phosphorus and examine the stability of its expression across genetic environments.

Abiotic stress	Genotypes	References
Drought	CZP 9802; 863B and PRLT 2/89-33ICMP 83,720	[60]
Heat	H77/833–2, H77/29–2 and CVJ 2–5–3-1-3, 77/371XBSECT CP1	[61]
Salinity	33, 10,876 and 10,878 (Sudan), 18,406 and 18,570 (Namibia), and ICMV93753 and ICMV 94474 (India); 863-B, CZI 98–11, CZI 9621, HTP 94/54	[62]

Table 1.
Available genotypes for abiotic stress tolerance in pearl millet.

6.3 Forage corn

Corn forage (*Zea mays* L.) has become an important component of ruminant rations in recent years. It is the only crop among non-leguminous forages that combines better nutritional quality. With a large amount of biomass [65]. Although the crop has great adaptability [66], it is the least tolerant of abiotic stress among cereals. Drought, salinity, and high temperatures are among the major abiotic stresses that negatively impact corn production in most regions of global corn production [67]. Soils with saline stress are present on all continents and in almost all climatic conditions. However, its distribution is relatively more extensive in arid and semi-arid regions than in humid regions [68]. Mohammed and Mohammed 2019 stated that the appropriate genotype based on stress selection is the inexpensive and manageable stress method based on salt, water, and heat or combined form and also concluded that the reduction in stress performance would be reduced to 20–40 parents.

6.4 Cowpea

The cowpea (*Vigna unguiculata*) is one of the most important legumes cultivated by subsistence farmers for human and animal consumption, mainly in the semi-arid regions of Africa and Brazil. In Africa, it is used for the livelihood of millions of people in the semi-arid regions of the West and Center [69] and is considered the most important grain legume crop in the sub-Saharan region.

6.4.1 Reproductive improvements

Cowpea is relatively drought tolerant. Despite this feature, however, drought can cause a considerable loss of performance. Efforts have been made to select the cowpea genetic material to identify lines with better drought tolerance than currently available varieties. According to Watanabe et al. [70], certain lines of genetic material, in particular, TVu 11,979 and TVu 14,914, were consistently very drought tolerant under real field conditions. Drought can occur at the beginning of the season, mid-season, or the crop development stage. Studies have shown that cowpea plants can show drought tolerance in the vegetative stage [71] and the reproductive stage [32]. Some cowpea lines exhibit a green persistence feature, also called delayed leaf senescence (DLS), which can help plants tolerate terminal and mid-season drought [32].

6.4.2 Gene selection

In cowpea plants, overexpression of the CPRD 8, CPRD12, CPRD14, CPRD22 and CPRD46 genes that confer tolerance to water stress [72], as well as the production of VusAPX genes connected to VucAPX, VupAPX and VutAPX of antioxidant enzymes [73], it is reported, in addition to the expression of the high level of the PvP5CS gene associated with the production of proline, an amino acid that fulfills the function of osmotic adjustment between species during drought.

6.5 Abiotic stress tolerance mechanism

Climate and soil determine many plant adaptations and the ecogeographic distribution of species and ecotypes show differences in physiology and development patterns that provide good evidence of adaptation mechanisms. Plants respond to environmental change as individuals through phenotypic plasticity and in populations through the selection and associated evolutionary processes. Determining the genetics underlying adaptation processes is not always easy because environmental factors can be complex or poorly defined. However, extreme environmental pressures, such as heavy metal contamination from the soil or harsh winter conditions [74] can produce detectable genetic changes. Multiple genes may be responsible for a response to a certain factor, or the same gene may be involved in different adaptive responses specific genetic interactions can be in a state of change or become fixed, limiting the possibilities for future evolution. Phenotypic plasticity acts as a buffer to prevent excessive gene flow in response to short-term changes.

7. Improving forages for abiotic stress response based on breeding techniques

7.1 Greater tolerance to stress through genetic transformation

Genetic improvement of forages through the selection of conventional plants is slow because most forage species are self-incompatible, limiting inbreeding to concentrate the desired genes to be used in the rapid development of new cultivars. Genetic transformation allows the direct introduction of desirable genes, thus offering new opportunities for forage molecular selection. Like many other crops, drought tolerance is an important goal in improving alfalfa. Since cuticle waxes play a central role in limiting the breathable loss of water from the plant surface, the genetic engineering of plant waxes is expected to eventually increase tolerance to environmental stress in crops such as agronomic importance [75].

7.2 Improvement of stress tolerance through intergeneric hybridization

Extensive hybridization with relative species followed by introgression of chromosomes and/or chromosome fragments has been considered an effective means of transferring salt and other stress tolerance genes to target species to extend the gene pool. Intergeneric hybrids between species of Lolium (Ryegrass) and Festuca (Fescue) have attracted much attention from forage breeders. Rye grasses are considered ideal grasses due to their fast establishment, their ability to resist intense grazing, their good palatability, and their high nutritional value [76].

Alfalfa (*Medicago sativa* L.) is widely cultivated in temperate and tropical regions for green forage, hay, silage, and grass. As a perennial forage plant, alfalfa is a fairly hardy species and has a relatively high level of drought tolerance compared too many other legume forage plants [77]. Alfalfa's increased drought tolerance is due in part to deeper roots and the ability to extract more available water from the root zone [78]. Detection of salt-sensitive proteins in two contrasting alfalfa cultivars using a comparative proteome approach revealed two new proteins, NAD synthetase, and biotin carboxylase-3, as being salt sensitive. These results provide new information on alfalfa salt stress tolerance [79]. The effects of rhizobia strains on the amino acid composition of alfalfa under salt stress indicate that proline, glutamine, arginine, GABA, and histidine accumulate significantly in salt-stressed nodules, suggesting increased production of amino acids associated with osmoregulation, nitrogen storage, or energy metabolism to counteract salt stress [80] is a widely allogeneous forage legume species distributed worldwide due to its wide range of climate adaptation [81]. But it is less drought tolerant than other temperate perennial forage legumes due to its shallow root system and its inability to effectively control transpiration [82]. Biochemical studies have indicated that when white clover was stressed by a water deficit, De novo synthesis of amino acids, including proline, has increased in both leaves and roots [83]. This phenomenon may serve as an adaptive response during the first days of drought since the transient increase in amino acid concentration has been followed by a decrease in protein synthesis that slows plant growth.

Cowpea (*Vigna unguiculata* L.) growing in a variety of environments from tropical to arid/semi-arid regions, increased tolerance to drought and heat would be desirable. The cowpeas (*Vigna marina*) that grow on sandy beaches in the tropical and subtropical regions closest to the sea have the potential to be a source of genes for breeding salt-tolerant cultivars. Chankaew et al., [84] first reported QTL mapping for salt tolerance in the *Vigna marina*, and multiple internal mapping consistently identified an important QTL that can explain 50 percent of the phenotypic variation. The flanking marker can facilitate the transfer of salt tolerance of this subspecies in related Vigna cultures.

8. Micronutrient stress management

8.1 Sorghum

Sorghum (*Sorghum bicolor*) is one of the important forage crops for high agricultural production and good nutritional value for animals. Nutrient requirements for growing sorghum are high; they are grown for forage, in part from organic sources, and are supplemented primarily with inorganic fertilizers. The growth, development, and biological yield of crops affected by balanced fertilization have shown positive effects. Micronutrients increase crop productivity and also maintain soil health. A very small amount is required. Soil application of micronutrients is preferable for what is desired. Choudhary et al. [85] observed that the combined application of micronutrients, that is, a considerably higher yield of cereals, stems, and organic, is obtained through a soil + leaf application. The results showed a significant increase in grain yield (14.15 and 12.13 percent), biological yields (11.37 and 9.31 percent), and in stem yield (10.75 and 8.60 percent) and through the combined spraying of soil and foliar on the soil and foliar application, respectively.

8.2 Pearl millet

Pearl millet (*Pennisetum glaucum* L.) is one of the main millet crops in arid and semi-arid areas. Weather. Due to the drought-tolerant nature, it grows well in poor sandy soils. Sustainable production of pearl millet can be achieved through the balanced use of nutrients in crops with the fusion of organic and inorganic sources. Intensive farming is followed in the current system, most farmers use high-yielding whole crop varieties, ultimately a significant removal of nutrients from the soil in recent years, and the consumption of fertilizers has remained well less than the elimination one. So that the qualitative and quantitative improvement of the crop yield goes through mineral fertilization and that its quality can be improved through adequate practices of nutrient management and soil cultivation [86].

8.3 Maize

The third most important cereal crop is maize (*Zea mays* L.) worldwide and India. It is cultivated in temperate and tropical regions of the world. It is the most important cereal for animal feed. In India, 45 percent of maize production is used in various forms of staple foods [87]. Corn, rice, and wheat are estimated to provide at least 30percent of food calories to more than 4.5 billion people in 94 developing countries. The demands for animal feed and biofuels can be met by increasing maize production [88]. The application of micronutrients can be carried out in several ways, such as seed treatment, soil and foliar application [89], which depends on the characteristics of the soil and the climate of the region. Corn productivity can be improved by applying Zn and B to the soil.

8.4 Cowpea

Cowpea (*Vigna unguiculata*) is a legume and is used as a forage crop that is grown during the Kharif season, requiring only an initial dose of nitrogen (15–25 kg N ha^{-1}). Most nitrogen requirements are met by symbiotic nitrogen fixation. The strong application of NPK fertilizers has led to micronutrient deficiencies in many parts of the country. To achieve high yields and maintain them over the years, it becomes highly relevant to predict emerging nutrient deficiencies and to develop appropriate breeding technologies. Balanced fertilization is inevitable to increase the productivity of the crop. Among the micronutrients, Zn, Fe, B, Mn, and Mo significantly improved yield, and micronutrient foliar spray is economical on legumes.

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Chapter 3

Responses of Neotropical Savannah Plant Species to Abiotic Stresses: A Structural and Functional Overview

Silvana Aparecida Barbosa de Castro
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Abstract

Plants under field conditions are subject to different types of abiotic stresses such as drought, salinity, and light excess that adversely affect their growth and survival. In addition, several studies have pointed out the effect of climate change such as an increase in the concentration of atmospheric CO_2, as well as an increase in global temperature on the distribution and wealth of plants. Adaptation to abiotic stress and survival occurs on different scales, at the cellular level for each individual, and requires a range of strategies, whether morphological, physiological, molecular or structural. Such strategies may be determinant in the distribution of plant species in natural habitats, depending on ecological adaptations shaped by the evolutionary history of species. In this chapter, we discuss recent information about mechanisms of plant adaptation to abiotic stress in the Neotropical savannah based on the cell and individual scales.

Keywords: ecophysiology, water stress, thermotolerance, high luminosity, soil nutrients, fire, Cerrado

1. Introduction

Plants growing under natural conditions are permanently subject to different types of environmental stresses such as drought, nutritional deficiency, salinity, heat stress and, more recently, the anthropogenic pollutants of ecosystems that negatively affect plant growth and survival [1–5]. Moreover, studies have pointed out the effect of climate changes such as the increase of atmospheric CO_2 concentration and global temperature (2 to 4°C) on the distribution and richness of plant species [6–8]. The survival of plant species under such conditions is constantly threatened, leading them to use different cellular and whole plant mechanisms in order to minimize damage and adjust growth to adverse environmental conditions [9, 10]. Plant responses to abiotic conditions have been widely investigated in different ecosystems worldwide, with this issue gaining great prominence for savannas due to the high complexity of abiotic factors that affect the native species of these systems. Among the world's savannas, the Brazilian one, also called Neotropical savannah or Cerrado *sensu lato*, is one of the most diverse in the world in terms of vegetation and plant species types, recognized as one of the biodiversity hotspots in the world [11, 12]. The Neotropical savannah flora comprises some 12,000 plant species, many of them endemic, two-thirds of which are herbaceous species or small shrubs [13]. The Neotropical savannah covers approximately 22% of the Brazilian territory (**Figure 1**) comprising several physiognomies from open grasslands to woodlands in a mosaic determined by abiotic factors such as edaphic and fire ones [14, 15]. The Campo limpo (grasslands) and Cerradão (tall woodland) are both extreme physiognomies, with great predominance of herbaceous and arboreous strata, respectively [14]. The Campo sujo (shrub savannah), Campo cerrado (wooded savannah), and Cerrado sensu stricto (short woodland) occur between them, with a gradual growth of biomass and larger plant species strata [14]. A mountain physiognomy, called rupestrian fields, also occurs and is restricted to areas of more than 900 m of altitude [16].

The diversity of life-form strategies of the Neotropical savannah plant species is associated with the response to many abiotic factors, especially the climatic seasonality with a well-defined dry and rainy seasons (**Figure 2**). The rainy season normally lasts from October to March, and the dry season from April to September, with an annual rainfall average of about 1,477 mm, a minimum temperature of 16°C and a maximum temperature of 26°C over the past thirty years (**Figure 2**) [17, 18]. High temperatures and irradiance, oligotrophic soils and high content of solubilized Al^{3+} are environmental features of the Neotropical savannah, which select local plant species and induce them to develop physiological and morphological adaptations to this harsh environment [19, 20]. Adaptation to and survival of abiotic stress occur on different scales from the cellular to the individual levels and require a range of strategies, whether morphological, physiological, molecular or structural,

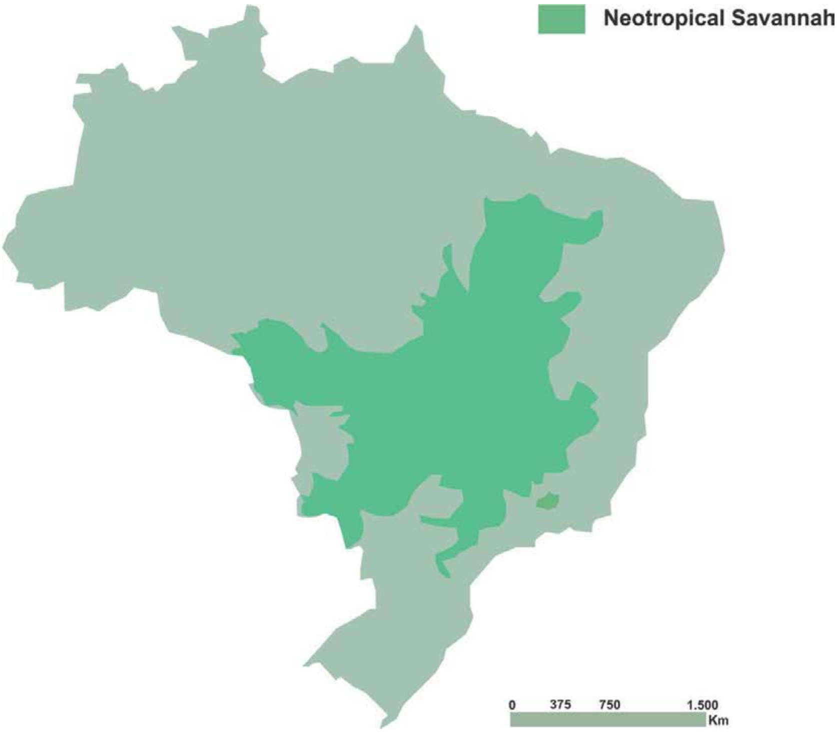

Figure 1.
Map of Brazil showing the occurrence of the Neotropical savannah, which covers approximately 22% of its surface area (Adapted from WWF Brazil, 2020).

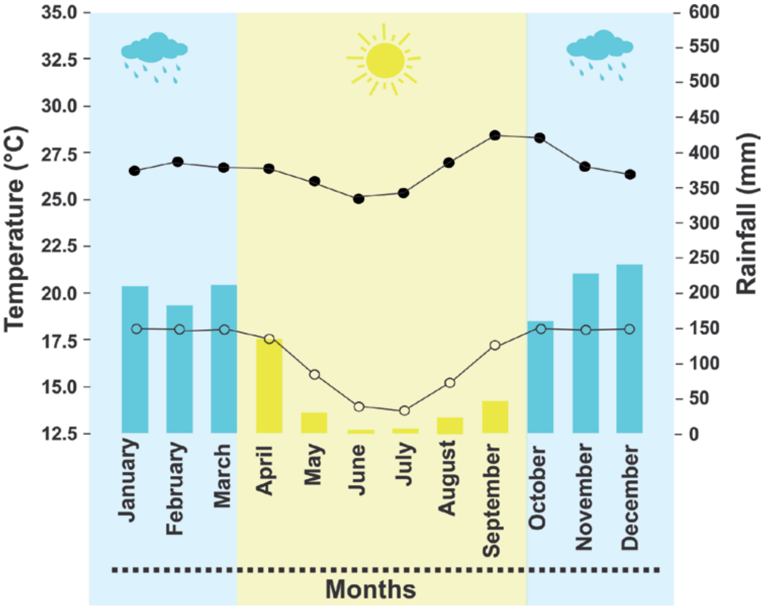

Figure 2.
Rainfall (mm) and temperature (°C) data of the Neotropical savannah during the rainy season (represented in blue area) and the dry season (represented in yellow area), over the past thirty years. Ps.: Black circles indicate the maximum temperature and white circles the minimum temperature (data obtained from Instituto Nacional de Meteorologia - INMET, 2020).

and can be determinant of species distribution in the natural habitats, depending on ecological adaptations shaped by the evolutionary history of the species.

Based on recent studies on Neotropical savannah flora, the focus of this review is on the dynamics and processes related to plant responses to the most prominent abiotic environmental conditions in this ecosystem, mainly highlighting water, temperature, light, nutrient deficiency and fire approaches. We report here information about the different adaptation mechanisms of plant species under the abiotic stress of the Neotropical savannah, from the cell to the individual scales.

2. The water stress seasonality changes the response of plant species during the seasons and along the day

Drought is considered to be one of the major abiotic stresses since it influences the distribution of plant species and limits the productivity of the ecosystems [4]. All aspects of plant development, i.e. germination, cell division, cell elongation, plant growth, and metabolic activities, are affected by a reduction in soil water content, which subsequently affects the physiological, biochemical, morphological and molecular processes of plants [21, 22]. Water flow from the soil to the roots depends of several factors, such as, potential water gradient between the soil and the plant, the hydraulic conductivity of the soil, the soil moisture content, and the atmospheric demand, that together with leaf transpiration create a tension in the xylem contributing to the creation of this water potential gradient [22, 23].

Regarding water stress, physiological and biochemical responses are more immediate in order to avoid cell dehydration, to maximize the efficiency of water use or to tolerate dehydration [1, 24]. The ability to maintain higher values of cellular water content is known as a strategy of plants sensitive to drought conditions. On the other hand, the ability to survive with lower values of cellular water content is called drought tolerance and requires the expression of molecular mechanisms since the initial growth of the seedling [25, 26]. Drought-tolerant plant species can be found in ecological niches with water seasonality, especially in the Neotropical savannah [27]. Drought-sensitive plant species also occur at the same sites as those of drought-tolerant ones; however, they use different molecular, biochemical, and physiological mechanisms to prevent desiccation, and thus deal with the lack of water in the soil. Drought-tolerant plants are able to limit damage to a repairable level in order to maintain physiological integrity due to the occurrence of repair mechanisms triggered by the dehydration/rehydration cycle [27, 28]. During long periods of drought, maintaining cell homeostasis and withstanding dehydration seems impractical for plants, directly impacting their growth rates [4, 27].

The Neotropical savannah abiotic conditions such as high irradiance, elevated air temperature, and low relative humidity impose a high evaporative demand on plants during the dry season while at the same time water is exhausted in the upper layers of the soil [29, 30]. Water use by Neotropical savannah woody plants is limited by a combination of physiological, plant architectural and tree density constraints evidencing that water use by the plant community seems to be more complex than in other savannas [30]. Even in the dry season, the water supply remains constant in the deeper soil layers, allowing access to water for tree species with long roots (in trees and shrubs) and differences between functional groups may be more related to the topography of an environmental continuum in which grass species are most subject to water restriction (**Figure 3**) [30, 31]. The hydrological niche segregation, defined as a fine scale partitioning of space for soil moisture gradients or different strategies of water acquisition, has been pointed out in different plant communities [32, 33] including the Neotropical savannah physiognomy [34, 35]. A marked decrease in water potential during the dry season has been reported for several species of the Neotropical savannah both in predawn and midday measurements [31, 35–37]. However, the water potential can vary according to the microhabitat, with plant species in mesic microhabitats showing smaller drops in the values of water potential even during the dry season (**Figure 3**). Costa et al. [38] also observed a decrease in water potential in *Hymenaea stigonocarpa* (Fabaceae) subjected to water stress, followed by recovery after rehydration. In an experimental study with *Laivosiera campos-portoana* (Melastomataceae), França et al. [39] reported that drought stress triggered a decrease of stomatal conductance and that osmotic adjustment played an important role in keeping high relative water content (RWC) values. These data suggest that the maintenance of high RWC values in plant species, despite a decrease in xylem water potential during the dry season, may be related to adjustment to stress, allowing survival in different microhabitats.

Plant responses to water deficit occur in different forms and scales, e.g. morphological, physiological, biochemical, and molecular ones, which may occur at the same time or at different times. For instance, the synthesis of cellulose microfibrils, hemicelluloses, and pectins in the cell wall may be changed under water stress, especially regarding the cell elongation pattern [40–42]. Strategies for adaptation and survival under water deficit conditions are also associated with the production and accumulation of organic solutes such as glycine-betaine, proline, and soluble sugars, which protect the integrity of the membrane and promote osmotic adjustment [43–47]. In addition, an increased content of several amino acids such as glutamic acid, glutamine, lysine, proline, serine, tryptophan, tyrosine and valine has been reported after water stress imposition in Neotropical savannah species [47, 48]. Proline is an important osmoregulator that protects plants submitted to abiotic stresses, favoring osmotic adjustment and an increase in the other osmolytes [20]. Souza et al. [48] detected increased carbohydrate content in *Hymenae courbaril* (Fabaceae) roots subjected to water deficit, which appeared to be directly linked to

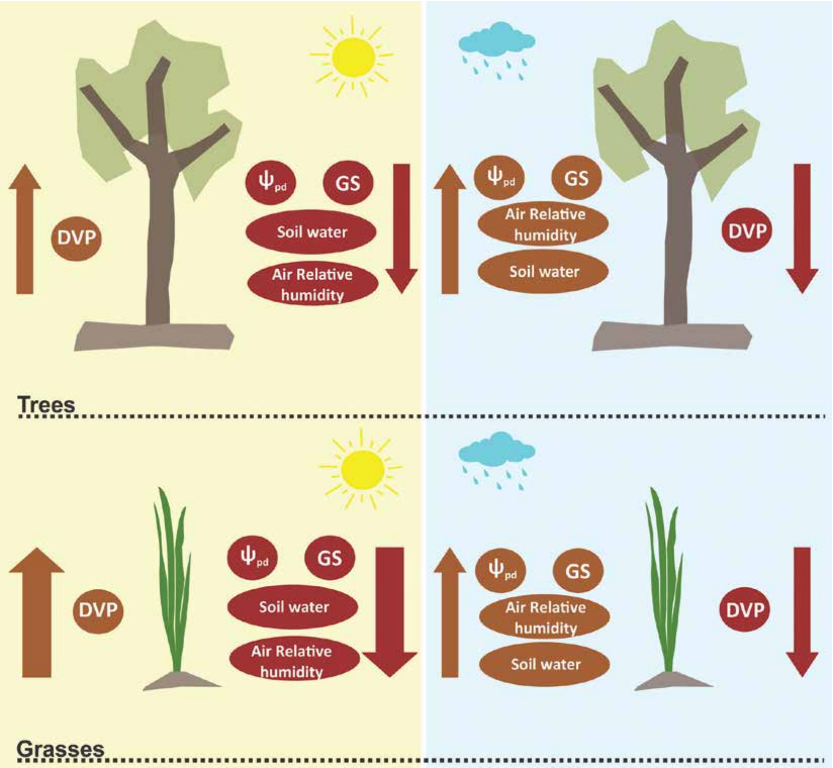

Figure 3.
Schematic presentation of the main water parameters of tree and grass species from the Neotropical savannah during the rainy (represented in blue area) and dry (represented in yellow area) seasons. Trees are able to capture water from deep layers of soil in the dry season, but there is still a certain water restriction that may affect water potential and stomatal conductance in some species. Grasses have lower water potential values and greater restriction of stomatal opening than tree species in the dry season. Brown arrows indicate an increase in rates while the red arrows show a decrease. Abbreviations: ψpd - Predawn leaf water potential; GS - Stomatal conductance; VPD - Relative vapor pressure deficit.

the osmotic adjustment of the cell. Some studies have indicated an accumulation of sucrose, raffinose and arabinose during the dry season, while glucose and fructose levels decreased with the reduction of water availability in the soil, suggesting that these are used for sucrose synthesis [49, 50].

The plant antioxidant system also plays a key role in water stress by responding with the production of enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione peroxidase (GPX), as well as non-enzymatic components such as ascorbate, glutathione, tocopherols (vitamin E) and carotenoid pigments [51, 52]. Imbalance in the production of reactive oxygen species (ROS) has been demonstrated to affect negatively cell membrane stability, with oxidative damage to carbohydrates, lipids, amino acids, proteins, and nucleic acids [51]. ROS play a key role in the process of plant acclimation to abiotic stress such as signal transduction, which regulates different pathways during plant acclimation to stress [53]. Moreover, many plants may rapidly produce and scavenge different forms of ROS, inducing fast and dynamic changes in ROS level [51]. Under normal growth conditions, ROS are continuously produced and scavenged in organelles such as chloroplasts, mitochondria, peroxisomes and apoplasts [54]. However, the balance between ROS-producing pathways and ROS-scavenging mechanisms may be disrupted when plants experience environmental stress such as drought [52, 55, 56].

Regarding the photosynthetic apparatus, Assaha et al. [57] reported a super excitation of photosynthetic pigments after ROS accumulation in plants under severe water deficit. For instance, increase in oxidative stress (i.e. increase of H_2O_2) and in SOD, CAT, and APX activity in roots was reported in two Neotropical savannah tree species subjected to water stress imposed by competitive species [58]. As well, Vieira et al. [58] also showed an increase in the activity of enzymes of the antioxidant system in *Vatairea macrocarpa* (Fabaceae) subjected to water stress. Besides, ROS detoxification systems, other strategies are essential for plant tolerance to drought, such as a reduction in chlorophyll synthesis and in the assimilatory apparatus components, as well as a balanced sugar pentose-phosphate pathway [59–61].

The seasonality of water availability can also result in dynamic photoinhibition in woody plants [62], but not in herbaceous species [63–65]. A reduction of stomatal conductance and of net CO_2 assimilation has also been reported in *Barbacenia Purpurea* (Velloziaceae) submitted to water stress, but recovery was detected hours after rehydration [50]. Decreased stomatal conductance followed by a reduction in photosynthetic rates in plants under moderate water deficit has been reported [66]. Midday depression is related to excessive sunlight exposure and has been shown for a wide range of plant species of the Neotropical savannah, with impact on photosynthesis and on CO_2 assimilation [62, 67, 68]. The reduction of the Fv/Fm ratio, of maximum variable fluorescence (Fv) and of maximum fluorescence (Fm) during the dry season seems to be a consequence of a combination of strong stomatal control, especially in shrub species, high irradiance, and low soil moisture conditions [35, 67]. Stomatal control may prevent the dissipation of excessive energy and lead to chronic photoinhibition in some Neotropical savannah plant species [68]. Chronic seasonal photoinhibition has been shown for some plant species in the xeric microhabitat of the Neotropical savannah, as reported for Melastomataceae species, although it does not occur in mesic habitats [35, 63, 69]. These data reveal the importance of the specific characteristics of the microhabitat in the physiological studies of plants despite the seasonal variation of water availability. Several factors such as microhabitat, root system, functional group and topography directly influence water use strategies by the plant community, causing the Neotropical savannah to be more complex than other savannas.

3. Thermal stress triggers morphological and physiological damage and acts as selecting factor

Plants have different temperature domains that initially determine their geographic distribution [70] but with the progressive increase in air temperature, the temperature domains become displaced to colder regions, forcing plant species to move away from their current distribution [71, 72]. The increase in temperature may cause several types of damage to plants in different stages of life, from germination to adulthood [73, 74]. The temperature range considered optimal for photosynthesis in C3 plants is between 18°C and 30°C, while temperatures above 30°C are considered to be heating zones [70, 75]. Thermotolerance acquisition occurs when a plant is exposed to low or high temperatures for a short period of time, up to a limit where no fatal injuries occur [8] being thermotolerance fundamental for the plant to deal with greater variation in temperature under certain conditions.

Morphological damage has been reported for vascular plants as a response to heat stress, with emphasis on leaf and branch burn, foliar senescence and abscission, inhibition of shoot and root growth, discoloration, and fruit damage [76]. Associated with the damage triggered by heat stress, the response to rising temperatures varies widely among plant species, functional groups and life strategies [77, 78]. For instance, fast-growing plants from tropical forests in high light environments tend to be more tolerant of high temperatures than slow-growing plant species typical of shaded places in the understory [77]. According to Franco et al. [79], there is controversy over the limiting processes that control photosynthesis at high temperatures, with different responses in the metabolism of C3 and C4 plants. In particular, under high temperatures, the photosynthetic rate (A) can increase up to a point above which it starts to be inhibited and can reach zero [77]. At higher temperatures, plants show elevated stomatal conductance as a way to dissipate excess heat on leaf surfaces. However, in combination with water stress, greater control of stomatal opening can limit heat dissipation with an impact on photosynthesis [60, 80, 81]. The reduction of photosynthesis occurs due to damage to the electron transport chain associated with increased fluidity of the thylakoid membrane and damage to photosystem II (PSII) [82, 83]. PSII is highly sensitive to temperature and damage can be observed after a few minutes to a few hours of exposure to heat [60, 82, 84, 85]. A reduction in the membrane lipid content and an increase in lipid degradation during the late period of stress with formation of ROS exposure has been reported and the balance between ROS production and elimination is rapidly disturbed, leading to an increase in ROS content [86]. Changes in chlorophyll content in plants exposed to high temperatures have also been reported, possibly due to inhibition of chlorophyll biosynthesis for the destruction of numerous enzymes involved in their biosynthesis [87].

A highly consolidated mechanism of heat acclimation and rapid response to high temperature in plants is the production of heat shock proteins (HSPs), which are key for thermotolerance acquisition [88]. HSPs protect intracellular proteins from denaturation and preserve their stability, thus acting as chaperones [88–90]. According to Ohama et al. [90], HSPs include HSP100, HSP90, HSP70, HSP60 and small HSPs that play a key role in protein quality regulation by renaturing a variety of proteins denatured by heat stress. Together, HSPs and ROS scavenging enzymes are the major functional proteins induced by heat stress [90].

Duarte et al. [91] detected increased oxidative stress at high temperatures in seedlings of the Neotropical savannah plant species *Vriesea friburgensis* and *Alcantarea imperialis* (Bromeliaceae) due to the accumulation of H_2O_2, which in turn led to a decrease in the number of leaves and in biomass and to a high mortality rate in *Vriesea friburgensis* when submitted to treatment at 35°C. Under field conditions, Neotropical savannah plant species show photoinhibition during the hottest hours of the day, especially in combination with seasonal water stress [62, 92]. Changes in morphological traits have been observed for other Neotropical savannah plant species, such as germination in *Dipteryx alata* (Fabaceae) in an experiment involving increasing temperatures [93] and in the response to zinc absorption in *Pterogyne nitens* (Fabaceae) [94]. Changes more directly related to photosynthesis, water use efficiency, growth rate, factors that can alter the competitive capacity of species, have been observed [73, 95, 96].

Model-based studies predict changes in plant species distribution and loss in areas with greater biodiversity due to increased worldwide global temperature [97–100], also affecting the Neotropical savannah [100, 101]. Chaves et al. [102], in a study of a restricted plant species from the rupestrian field, showed that although some plants were no longer sensitive to the increase in temperature, they showed lower thermal tolerance and less plasticity compared to plant species of wide distribution, with a possible impact of global climate changes on this species. A recent modeling study evaluating the effect of habitat loss and climate change on 2,354 species of restricted distribution (including the Neotropical savannah) showed that 70 to 85% of them are at high risk of extinction [103]. With the increase in global temperature it is possible that plant species may be following three paths: adapting through phenotypic plasticity, migrating to higher altitude, or becoming extinct [7, 101]. Bueno et al. [104] showed that the greatest expansion of the Neotropical savannah occurred precisely during periods of higher temperatures (Last Interglacial). However, this result was certainly based on climate stability that allowed a large number of plant species to persist over time to the detriment of areas with major climatic changes where species might not have had enough time to adapt or to move to regions with more favorable conditions [105, 106].

Multiple environmental factors vary with increasing altitude, with temperature being perhaps the most important one because it directly influences biochemical processes related to photosynthesis and water status. Therefore, studies that evaluate the physiological responses of plant species at high temperatures are important because they provide data that can generate models of future plant species distribution considering different scenarios of temperature change, and because they serve as the basis for public policies aimed at protecting areas with plant species most vulnerable to global climate change.

4. Neotropical savannah plant species must deal with spatial and temporal light heterogeneity

Light is one of the abiotic factors that influence the survival, development and growth of plants, and several traits are highly plastic in the presence of substantial variations in the amount of light received, especially traits related to light capture and photosynthesis [107–110]. Light also shows spatial and temporal heterogeneity as an important factor underlying niche partitioning among plants [111, 112], which has created plants adapted to a variety of light intensity, such as sun-adapted and shade-tolerant plant species [113]. For photosynthesis, the flux of photons in the 400–700 nm range is the most relevant variable to be considered in studies that evaluate changes in light intensity on the development and growth of plants [110]. Under high-light conditions, plants possess a variety of mechanisms that maintain the operational photosynthetic system, such as carotenoids, violaxanthin, antheraxanthin and zeaxanthin, which play an important role in the dissipation of excitation energy at times when light levels exceed the plant capacity for photosynthetic electron transport and carbon fixation [114, 115]. However, plants exposed to high radiation may suffer increased leaf temperature, resulting in photoinhibition of Photosystem II (PSII) [116, 117].

In tropical regions, the irradiance levels normally exceed those that plants use in photosynthesis, so that many plants have efficient mechanisms preventing or minimizing the damage to the photosynthetic apparatus caused by high light availability [118]. Nevertheless, different light regimes in each physiognomy can promote the establishment of different plant species or functional traits [119]. Regarding the Neotropical savannah, most plant species are well adapted to high light intensity, and at the same time they may have low plasticity for adaptation to and growth in shaded environments [120, 121]. Under contrasting light conditions, some traits such as stomatal conductance, chlorophyll and carotenoid contents have been shown to have higher rates of plasticity in plant species growth and in different seasons (i.e. dry and rainy seasons) [120, 122]. According to Franco et al. [92], a balance between the use of light absorbed for photosynthesis and the dissipation of potentially harmful excess light is essential for Neotropical savannah plant species since they still have to deal with other abiotic factors (i.e., high air temperature, low relative humidity and seasonal drought). Regarding the photosynthesis apparatus, Franco et al. [92] also reported that woody plant species are capable of maintaining the functionality of PSII under conditions of high irradiance and high evaporative demand of the atmosphere during long sunny periods at the end of the dry season. In contrast, in a study of *Hymenaea stigonocarpa* (Fabaceae) under high light intensity, Fv/Fm values decreased to 0.64, indicating the occurrence of photoinhibition as well as a lower water potential value [123]. In a study of *Copaiba langsdorffii* (Fabaceae) growing under full sun and shade conditions, Ronquim et al. [119] observed that juvenile plants under full sun conditions showed higher maximum net photosynthetic rates, light compensation and saturation points than juvenile plants growing under shade conditions. Other leaf traits associated with light have been pointed out, such as low specific leaf area (SLA) values and high carotenoid and chlorophyll content in plant species from open areas, which may represent adaptive mechanisms to high luminosity environments [36, 79, 121].

In another approach, recent studies have considered the impact of plant invasions in open areas of the Neotropical savannah caused by the widespread expansion of forest in the absence of fire, which tend to change the availability of light for native species already adapted to high radiation [121]. In areas with invasion and expansion of forest, changes in leaf area index (LAI) have been reported, as well as changes in the photosynthetically active radiation available to Neotropical savannah plant species [124]. Moreover, Rossatto et al. [121] showed that invasion of originally open savannah areas by forest trees has an impact on the persistence of savannah plant species, which need greater availability of light to maintain a positive carbon balance. Under these conditions, it is expected that grasses and herbaceous species, already adapted to high light availability, will not be able to maintain the CO_2 assimilation necessary for the maintenance of root carbon stock, which is essential for adaptation to fire and seasonal water stress.

5. Neotropical savannah plants live in nutrient-poor soils with aluminum phytotoxicity

The Neotropical savannah soils are considered to be among the most chemically infertile soils in the world [124, 125], being generally dystrophic, acidic and poor in available nutrients [126, 127], as well as having high levels of exchangeable aluminum (Al) [127, 128]. Soils are mainly represented by well-drained oxisols [129], named "latossolos" in the Brazilian soil classification system [130], followed by sandy entisol, ultisol and cambisol soil types [129, 131]. Many relevant plant nutrients such as K, Ca, Mg, N, S, and P, are deficient in the soil of most Neotropical savannah physiognomies due to the nutritional deficiency of the initial matrix rocks and the intense weathering that leads to daily loss of nutrients [127]. Although Neotropical savannah soils are generally defined as dystrophic, mesotrophic physiognomies have also been reported [132, 133], influencing the variation of floristic structure, as reported by Neri et al. [133] when differentiating dystrophic and mesotrophic Cerradão physiognomies.

In the nutritional approach to studies of the Neotropical savannah, aluminum phytotoxicity and the responses of native plants to low nutrient availability, mainly nitrogen and phosphorus, are some of the most highlighted topics, which will be discussed here. Al is one of the main metals present in the crust of the Earth [134], being a limiting factor of plant development [135, 136]. Most Al is available in soils in the form of non-phytotoxic elements such as Al oxides and Al silicates [137]. However, under acid conditions ($pH < 5$), Al is solubilized to Al^{3+} [138–140], as well as to $Al(OH)^{2+}$, $Al(OH)^{2+}$ and $Al(OH)_6{}^{3+}$ [141], all known as phytotoxic forms. The relationship between Al phytotoxicity and acidic soils stands out mainly in the

Neotropical savannah, where some physiognomies such as Neotropical savannah *sensu stricto* and associated open physiognomies contain acid soils [126]. High Al concentrations, especially Al^{3+}, occur in dystrophic soils, while soils almost free of exchangeable Al occur in mesotrophic physiognomies with pH > 6.0 [127, 133, 142].

Al^{3+} is extremely toxic for the majority of cultivated plants, but, as expected, the native Neotropical savannah plants are highly tolerant to it [133] and may be divided into Al-accumulating and non-accumulating plants [143–145]. Plants that store 1000 mg kg^{-1} Al are defined as Al-accumulating species [127, 144, 146], which can accumulate Al in roots or aerial parts [142]. For instance, *Qualea grandiflora* (Vochysiaceae), *Qualea cordata* (Vochysiaceae) and *Miconia albicans* (Melastomataceae) have been reported to be Al-accumulating species [132, 147]. Moreover, concentrations of about 20,000 mg Al kg^{-1} dry leaves have been observed for some native Al-accumulating plant species of the Neotropical savannah [132, 143] such as noted for *Vochysia tucanorum* (Vochysiaceae) [132]. Many Al-accumulating plant species not only tolerate Al but also need it for their growth and development, as is the case for some plants of the Rubiaceae, Melastomataceae, and Vochysiaceae families [148]. Thus, Al-accumulating plant species, in many cases, reduce their growth rates when subjected to the absence or a low concentration of aluminum [127, 147, 149]. On the other hand, many plant species do not accumulate more than 1000 mg/kg^{-1} Al in their leaves, as reported for 24 out of 32 plants from the central part of the Neotropical savannah, such as *Caryocar brasiliense* (Caryocaraceae), *Vellozia squamata* (Velloziaceae) and *Roupala montana* (Proteaceae) [143].

Reduced developmental rates and physiological changes may be some symptoms related to high Al concentrations in non-Al accumulating Neotropical savannah plants. The inhibition of root growth [103], the lack of lateral roots [150], and a low biomass of stems and leaves [141, 148, 151] have been demonstrated in plants growing in the presence of high Al concentrations. According to Čiamporová [152], the inhibition of root growth may be due to Al impregnation of root cell walls that promotes unequal expansion and changes in tissue organization. This process occurs in many Al-sensitive plants, while in Al-tolerant species the high Al deposition on the cell wall normally does not inhibit root elongation, as reported for soybean seedlings [153]. Organic acid exudation from the roots has been cited as an important strategy for Al exclusion [150, 154], which avoids Al uptake and the associated structural and physiological symptoms. Bittencourt et al. [155] have shown that *Styrax camporum* (Styracaceae) releases organic acids in the roots as a defense mechanism against high Al accumulation. However, this process only works when the plant is exposed to low to moderate Al levels.

Regarding the physiological responses, previous studies have demonstrated that CO_2 assimilation rate and stomatal conductance were reduced in Al non-accumulating species submitted to high Al concentrations [150, 151, 156]. The leaf gas exchange performance of Al-sensitive species has been associated with the lack of lateral roots [150, 157, 158], which are the plant's water uptake site. The lack of a water supply from the roots reduces its amount in the mesophyll, leading to low gas exchange [159]. Associated with reduced gas exchange performance, the CO_2 assimilation rate was also reduced in some plant species submitted to high Al doses [160], as reported for *Citrus* [161, 162] and for two sorghum cultivars [163]. From a photochemical perspective, quantum yield, maximum variable fluorescence ratio (Fv), minimum fluorescence (F_o), and Fv/Fm have been reduced in the presence of high rates of Al [164], an event that seems to be related to the impairment of PSII photochemistry [163] to the reduction of PSII electron transport [165], and to other changes in photochemical processes. However, substantial changes in Fv/Fm values have not been observed in Al accumulating and Al non-accumulating species subjected to high Al concentrations [147, 150].

In contrast to Al, nitrogen (N) and phosphorus (P) are reported as the limiting factors in the Neotropical savannah [166] both due to the old age of the soil and to the recurring effect of fire, which contributes to the volatility of N parts [167, 168]. N is a prevalent element in living systems and is a constituent of phytohormones, amino acids, proteins, nucleic acids, and enzymes [169]. It acts on the initial growth processes, helping with the synthesis of deoxyribonucleic acids and the replication of chromosomes [169]. P is also required for plant development since it is involved in metabolic regulation, such as amino acid synthesis and protein activation [170], and is also a constituent of ATP/ADP and phospholipids [171]. Thus, N and P are nutrients required in large amounts for initial plant development [172]. According to Reis et al. [173], both nutrients are normally present in limited amounts in Neotropical savannah soils since low N availability may be related to the presence of minimal organic matter and to the fact that P is low in soils containing iron and aluminum oxides [174], both typical conditions of most Neotropical savannah soils. N and P are constituents of photosynthetic process-related proteins such as plant pigments [141], thus representing fundamental elements for the maintenance of photosynthetic quantum yield and biochemical steps. For instance, *Eugenia dysenterica* (Myrtaceae) seedlings showed increased photosynthetic activity and growth with N fertilization at rates between 50 and 200 mg dm^{-3}, while P fertilization at rates of 100 mg dm^{-3} and 200 mg dm^{-3} increased growth and photosynthetic activity, respectively [173]. In addition, some findings suggest that the growth of many plant species is reduced in Savannah soils, although the chemical attributes *per se* do not seem to exclude the occurrence of plants in Neotropical savannah physiognomies [175]. Despite the low availability of N and P, plant species growing in Neotropical savannah soils have a variety of mechanisms dealing with shortages of these nutrients. For instance, P is allocated to photosynthetically active mesophyll cells, showing higher photosynthetic P-use efficiency (PPUE), association with mycorrhizal species, different ways of nutrient uptake, and an efficient use of nitrogen [168, 176].

From another perspective, over the last decades, the Neotropical savannah has suffered enormous losses of its original cover (more than 50%) [11, 177]. Moreover, the change in land use, such as the introduction of extensive pecuary and immense extension of soybean crops, has contributed to the growing supply of these nutrients in the Neotropical savannah soils [178]. Studies have shown that the increase in N and P favors the increase in biomass, the decrease in species richness, the establishment of invasive species and changes in plant structure (root/shoot ratio, and root structure) and in competitive relationships [179–181]. It has also been shown that ancient ecosystems with less P availability in the soil are the richest in plant species [182, 183]. Thus, it is expected that an increase in P supply in the Neotropical savannah soils may have an impact on plant species richness in the medium and long-term.

6. Fire as a selective agent for plant communities

Fire has shaped different terrestrial environments since early geological ages and has been part of Earth's system since the origin of land plants in the Silurian period [184, 185]. In the Neotropical savannah, fire is one of the determining factors that select and structure plant communities and that promote the cycling of nutrients, representing a fundamental abiotic agent for the maintenance of open ecosystems [186, 187]. In the phytophysiognomies of the Neotropical savannah, fire normally occurs at the end of the dry season [188] as a result of the highly combustible material of dry herbaceous plant organs (**Figure 4**), while during the rainy season, natural fires can be caused by lightning [187, 189]. Although fire is a natural and periodic component of this environment, anthropic activities have intensified its action and made it more frequent [190], leading to cyclic burning of the herbaceous layer and a marked reduction of the maintenance and renewal of trees and shrubs, progressively decreasing their density [191]. Thus, the main problem with fire is that it is seen as a bad event when its regime (frequency, extension, and intensity) is somehow changed [187, 192].

Given the seasonality of fire in the Neotropical savannah, plants possess physiological, morphological, ecological, chemical, and phenological adaptations to deal with it. These adaptations, as well as adaptation to drought (*see* [193]), can enable plant species to escape from fire, as is the case for plants with a short life cycle, or to resist fire, as is the case for tree species with extra protective bark [194]. As an escape strategy, many herbaceous species synchronize seed production before annual burning periods and enter a type of dormancy that only ends after high-temperature exposure [192], as reported for different plant species. For instance, the dormancy of *Sporobolus cubensis* (Poaceae), a herbaceous species of the Neotropical savannah, was broken by exposure to 200°C, indicating the need of fire temperature for its germination [195]. However, many seeds and underground plant organs may be protected against scorching since the heat from fire events does not reach deeper layers of the soil [196]. In general, a fire event is followed by a high germination rate and seedling development since the supply of nutrients increases from the ashes and more light reaches the plant from the opening of canopies [192, 197].

In a phenological approach, fire can affect vegetative and reproductive phenophases [186], leading to leaf abscission by the heat created from the fire, as well as decreasing the numbers of flowers and fruits [191, 198]. However, Silvério et al. [199] reported that the negative effects of fire on the vegetative phenophases were normally followed by an increase in sprouting and the production of leaves, revealing an epigeous recovery mechanism from fire damage. In a reproductive approach, Pilon et al. [200] reported a similar pattern, with fire triggering the production of flowers and fruits in 79% of 47 plant species such as grasses, forbs, and subshrubs. Moreover, several studies have reported flowering in several plant species after the passage of fire [201, 202] and, more recently, Fidelis et al. [203] reported massive

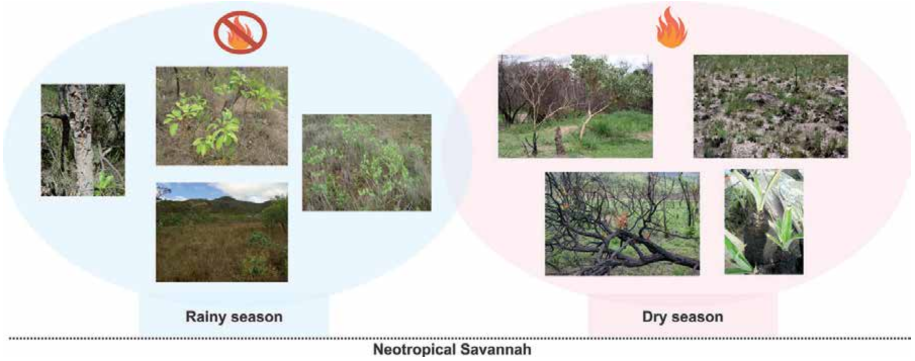

Figure 4.
Overview of the Neotropical savannah plant species in the pre-fire (blue circle) and post-fire (pink circle) periods, which coincided with the rainy and dry seasons, respectively. At a pre-fire time during the rainy season, the herbaceous stratum exhibits dry and flammable aerial organs, while tree and shrub species have green and photosynthesizes leaves. At a post-fire time during the dry season, the leaves of the herbaceous stratum regrow, while many tree species are still leafless due to leaf loss caused by heat, and with a stem blackened by the action of fire.

flowering in *Bulbostylis paradoxa* (Cyperaceae) within just 24 h after the fire. Flowering after fire certainly gives and advantage, since it allows the plant to use available resources without germination barriers and in new and fresh open areas for its establishment [204].

Many structural plant features have been proposed as important tools for fire event survival. Underground roots and stems with carbohydrate, nutrient and water storage have been reported for herbaceous and sub-shrub Neotropical savannah species, allowing them to regrow after the fire has passed [205]. For instance, diffuse underground systems, rhizophores, and xylopodium have been reported for 7 Asteraceae species from the Neotropical savannah [206], which can support the individual life during the dry season and after fire events. Xilopodium, as well as other underground systems, have been found in many species as reported for the *Brosimum gaudichaudii* (Moraceae) shrub [207], the *Ocimum nudicaule* herb (Lamiaceae) [208] and three *Erythroxylum* species (Erythroxylaceae) [209], and may indicate be a convergent trait of many plant species in this environment, often related to species dominance in physiognomies.

Regrowth after fire events requires carbon [79] for the creation of new tissues and organs; however, burning that reaches the leaves reduces the photosynthetic area of the plants [210], consequently decreasing the capture of atmospheric CO_2. The loss of leaf area can be total in herbaceous and shrub plants and partial in some sub-arboreal plants during fire events, whereas tree branches normally are not burnt but are damaged by the hot airflow [79]. In this respect, underground reserve organs may be a fundamental carbon source, mostly consisting of carbohydrates such as starch, fructan, glucose and sucrose [189, 206]. Complex carbohydrates such as starch are hydrolyzed to soluble sugars by hydrolytic enzymes such as the exoamylase β-amylase, which cleaves α-1,4 linkages, and the debranching enzyme isoamylase, which cleaves α-1,6 linkages [211] and the product used for plant development. After leaf regrowth, the new leaves of some plant species may return with higher photosynthetic rates, stomatal conductance and nitrogen concentration than during the pre-burn period [212], with high vigor and potential for growth [213]. However, these responses did not occur in *Vochysia cinnamomea* (Vochysiaceae), a native species of Neotropical savannah, since fire did not exert a direct influence on its physiological processes [214].

The bark structure and thickness of tree plants has been cited for years as one of the greatest protective traits against fire in the Neotropical savannah, preventing damage to the vascular cambium [215] and deformations of the xylem [216] and phloem. The thick corky bark may protect internal stem tissues [217], reducing mortality and maintaining water and solute transport during and after the fire episodes [218]. On this basis, the bark properties have been studied across 31 species from the Neotropical savannah, providing shreds of evidence that the outer bark acts as mechanical support and defense against pathogen attack and the inner bark as a place related to storage and transport of water [218]. Other less frequent and more specific characteristics have also been discussed regarding fire survival. For instance, *Vellozia variabilis* (Velloziaceae) has resin glands on its leaves that produce flammable substances capable of starting quick-burning on the more protected plant base, preventing damage to more sensitive upper plant portions [219]. Due to these plant specificities, as well as abiotic and biotic conditions, the Neotropical savannah is a potential environment for new structural and ecological discoveries.

Finally, the occurrence of invasive tree species in open areas of the Neotropical savannah has altered the microclimate conditions, affecting grasses and shrubs and consequently the dynamics of fire occurrence in the Neotropical savannah [124, 220]. This cascade of events will certainly have a negative impact on the plant and animal communities of the Neotropical savannah, changing the local landscape.

7. Conclusions

In the current chapter, we report the most common types of abiotic stress in Neotropical savannah plant species, which can simultaneously influence and determine the different functional types of plants. Plant species are affected and respond differently to abiotic stress in the Neotropical savannah, mostly in terms of more specific characteristics such as microhabitat, seasonality, topography and anthropic interference. Moreover, Neotropical savannah plant species have evolved over thousands of years subject to various types of abiotic stress (i.e. water seasonality, high temperature and light availability, soil with a deficiency of essential nutrients, and fire). All of them have been shaping differently the Neotropical savannah physiognomies, and recent changes in abiotic conditions are occurring in a very rapid manner, mainly due to anthropogenic actions over the last decades. Reduction of native vegetation areas, expansion of agriculture and extensive pecuary, changes in the fire regime and the introduction of nutrients, such as nitrogen and phosphorus, are examples that have been reported for Neotropical savannah in recent times and that may potentially have negative effects on biodiversity. In view of this reality, conservation strategies for the remaining area should be discussed with society and prioritized in government policies in order to conserve such areas and to encourage the sustainable use of the Savannah wealth.

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Chapter 4

Influence of Water Stress on Growth, Chlorophyll Contents and Solute Accumulation in Three Accessions of *Vicia faba* L. from Tunisian Arid Region

Hanen Enneb, Leila Ben Yahya, Mohamed Ilyas, Datta Asaram Dhale, Mohamed Bagues and Kamel Nagaz

Abstract

In this study, we aim to investigate the physiological and biochemical adaptations of *Vicia faba* plants to moderate irrigation regime (T1) and describe the effects of water stress on their growth performance and chlorophyll contents. For this reason, three Tunisia accessions (ElHamma, Mareth and Medenine) were studied. An experiment was conducted for one month. Faba bean plants were first grown in a greenhouse and then, exposed to water stress, whereby they were irrigated up to the field capacity (FC) of 0% (control, T0) and 50% of the control (moderate stress, T1). The effect of water stress on physiological parameters showed differences in relation to the accessions studied and the water regime. Relative water content (RWC) of ElHamma accession does not seem to be affected by stress as compared with the control regime. Total chlorophyll content decreases, whereas soluble sugar contents increases for all accessions studied. ElHamma has the highest content. About morphological parameters, bean growth varies according to the ascension and treatment. Hydric stress impedes the growth of the root part and caused a significant reduction in the shoot and root Dry Weight (DW) of the T1-stressed beans, compared to the optimal irrigation (T0).

Keywords: water stress, *Vicia faba* L., RWC, growth, chlorophyll, soluble sugars

1. Introduction

Environmental stresses, especially water stress, seriously limit plant growth as well as plant productivity [1–3]. Thus, we speak of water stress when the demand for water exceeds the available resources. This category includes countries where the availability of water per year and per inhabitant is less than 1700 m^3, particularly in semi-arid regions. Arid zones are characterized by a negative hydric balance, mean annual rainfall of less than 800 mm, with a mean insolation of 2800 h/year and means annual temperatures of 23–27°C. These conditions lead to high evapotranspiration rates, carrying a water deficit in nearly the entire region [4].

Abiotic stresses and inefficient production techniques are the obvious factors limiting crop productivity, especially in the southern parts characterized by severe aridity [5–7].

Faba bean (*Vicia faba* L.) is a crucial grain crop through the word; ordering as the fourth crop legume after dry beans, dry peas and chickpeas. The bean is of great importance for nutrition, it enters in human consumption and also for animal feed [8]. North Africa appears to be one of the best faba bean output global regions and especially, Tunisia.

In Tunisia, faba bean covers more than 68% (70,000 ha) of the total area annually devoted to grain legumes crops and the national average yield is around 50,000 tons [9, 10]. Although the agro-economic importance of the bean is known, its cultivation is in decline, both in some countries of the world and in Tunisia [9]. The areas reserved for legumes in Tunisia in gradual fall [11]. This mainly affected the peas and broad beans. The national average bean yield is low and fluctuates widely from year to year. This is mainly due to climatic variations [6].

The seeds of the bean are very rich in proteins (25% of the dry matter of the seeds) and especially in lysine but poor in amino acid [12]. It is also a source of carbohydrates, mineral salts, fiber, vitamins and little lipids. The seeds are the most consumed parts and like all vegetables; the bean is self-sufficient for nitrogen nutrition. The bean is used as a green manure which serves as well for the improvement of the structure of the soil as for its enrichment in mineral elements. In addition to its nutritional and fertilizing (green manure) utility, the bean also has medicinal interests [13].

The success of agriculture in arid and semi-arid zones worldwide will be tremendously dependent on the ability of agricultural systems and farmers to adjust to climate change [14]. A vast majority of these areas, despite the huge production, suffer from limiting resource of water [15]. As that resource becomes more scarce and availability more difficult to predict, water managers and farmers will be forced to implement new creative solutions to water supply challenges. Expected exposure indicates improvement and better understanding of culture adaptation to water stress in such areas is high-priority to successful development in these regions under a changing climate. This work focuses on understanding the effect of water stress on morphological, physiological and biochemical aspects of *Vicia faba* L. plant grown in Tunisian Arid Region.

2. Concept of water deficit on plants

Water is a vital element for the growth and development of plants. It generally constitutes 85–90% of the total fresh weight of plants. Water is the natural vehicle for all the substances circulating in the plant, the elements absorbed in the soil or the organic substances produced in the leaves circulating in the saps of the xylem and phloem [16]. The need for water for the plant is proven by multiple observations such as wilting and death of plants left without water, hence the importance of irrigation and the relationship between vegetation and rainfall [17]. Plants can get the water they need through their roots. These extract water from the soil to pass it through the plant; part of this water is then released into the atmosphere (a phenomenon called transpiration) [1]. Omprakash et al. [18] defines water deficit or water stress as a period of insufficient water activity in the plant. According to this same author the notion of stress implies on the one hand a more or less abrupt deviation from the normal conditions of the plant and on the other hand a sensitive reaction of the plant in the different aspects of its physiology which changes appreciably with either adaptation to the new situation, ie to the limit degradation leading to a fatal outcome. On the contrary, according to Dodd and Ryan. [19] any water restriction resulting in a drop in the plant's production potential, following a disturbance of its physiological activity caused by a deficit in water consumption is called water stress.

3. Analyses of faba bean responses to water deficit stress

The experiment was carried out during 2019/2020, in a plastic greenhouse at the IRA (Institute of Arid Regions), located 22.5 km north-east of the city of Medenine. This region is located in the lower arid bioclimatic stage characterized by a low annual rainfall of around 150 mm/year; high temperature and frequent winds. *Vicia faba* seeds used in this test is the *Vicia faba* L. originating from Medenine and Gabès (ElHamma and Mareth). The latter ascensions tested were sown in pots filled with soil in February 2019 Three seeds were planted per pot; Three repetitions for each ascension were established. Water used for irrigation is tap water, titrating ±2 g/l and a pH of 7.6. Two water treatments were tested: T0 = 100% CC (capacity in the fields 100%) and T1 = 50% CC. The quantities of water (in ml/kg of soil) added each week (220 ml/kg for T0 and 110 ml/kg for T1). The dry weight of leaves and roots was carried out separately. The above-ground and underground dry matter is obtained after passing in an oven for 48 hours at 75°C. Relative water content of faba bean for each treatment were determined by the following method: Cut the leaf blade into small squares, weigh them to determine the fresh weights (FW) and then place them in a box of petri dish at 5°C. After 24 hours, the pieces of leaves are removed and then weighed at full turgor (TW). They are then placed in an oven at 75°C and then weighed again to determine their dry weight (DW). Chlorophyll contents determined by Gross method [20] and to quantify soluble sugars, the phenol method of Dubois et al. [21] was used.

4. Morphological changes in faba bean under water stress.

4.1 Plant biomass

Three accessions (El Hamma, Mareth and Medenine) of faba bean have been analyzed for dry matter accumulation. Significant effect of treatment ($P < 0.005$) was observed and no difference has been established between ascensions (**Figure 1**). Under moderate water stress treatment (T1), faba bean (i.e., El Hamma, Mareth and Medenine) accessions were significantly decreased their shoot and root DW as compared to control plants. Total leaf and root dry masses were all two to three times greater in the well-watered treatment (T0) than those in the water-limited one (T1). The watering treatment showed significant effects among localities on apparent shoot biomass and apparent root biomass (**Figure 1**). Risk-taking by minimal reduction of shoot DW under water stress was revealed as a physiological indicator for drought resilient at the flowering stage for faba bean [22]. The capacity of populations to maintain shoot biomass in water shortage was displayed to be an important trait for drought tolerance in soybean plants [23]. Under drought faba bean decreased their root DW (**Figure 2**). Root growth reduction was also reported for pea plant [24]. According to Chavoshi et al. [25] severe drought stress reduces biomass and seed yield (by 20–90%).

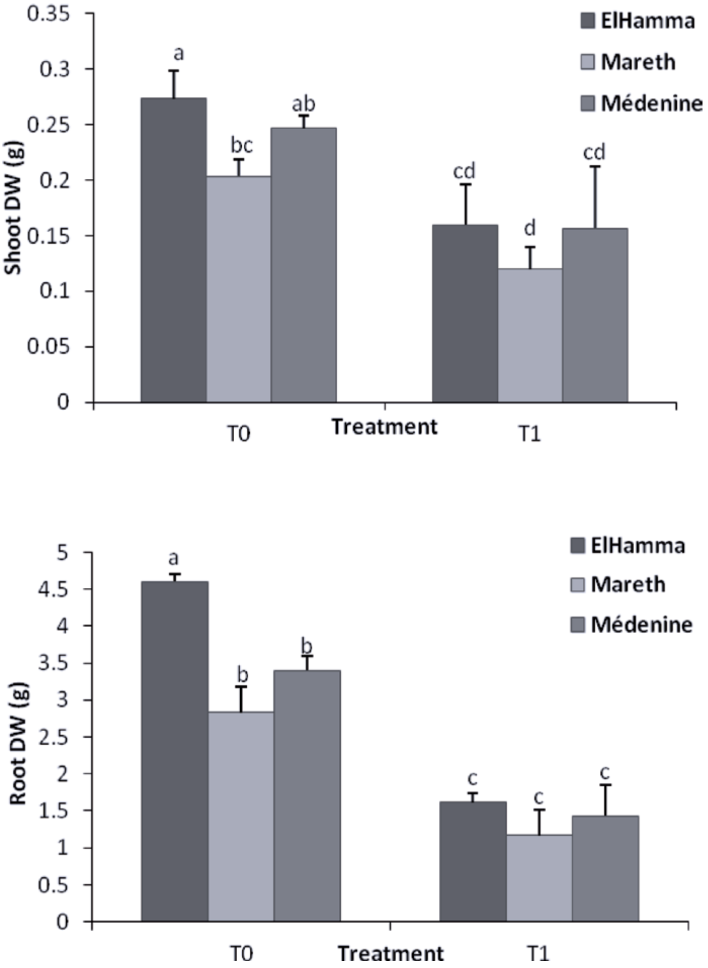

Figure 1.

Changes in (a) Shoot DW, (b) Root DW for Vicia faba *L. accensions in T0 (control) and T1 (moderate deficit irrigation (50%FC)). Means ± S.E. (n = 3). Means with different letters (a, b, c) are significantly different at P < 0.05 and values sharing a common letter are not significantly different at p < 0.05.*

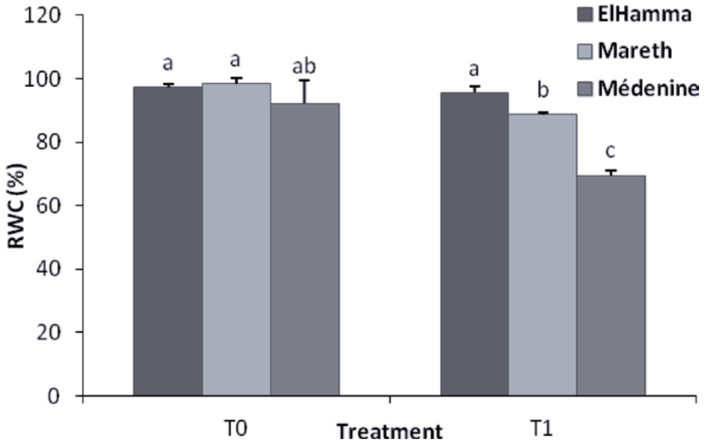

Figure 2.

Changes in relative water content (RWC) for V. faba *leaves in T0 (control) and T1 (moderate deficit irrigation (50% FC)).*

5. Physiological and biochemical responses of faba bean to water stress

5.1 Water status (RWC)

As shown in **Figure 2**, RWC varied significantly among accessions and RWC was significantly higher as expected in non-stress conditions (T0) than in

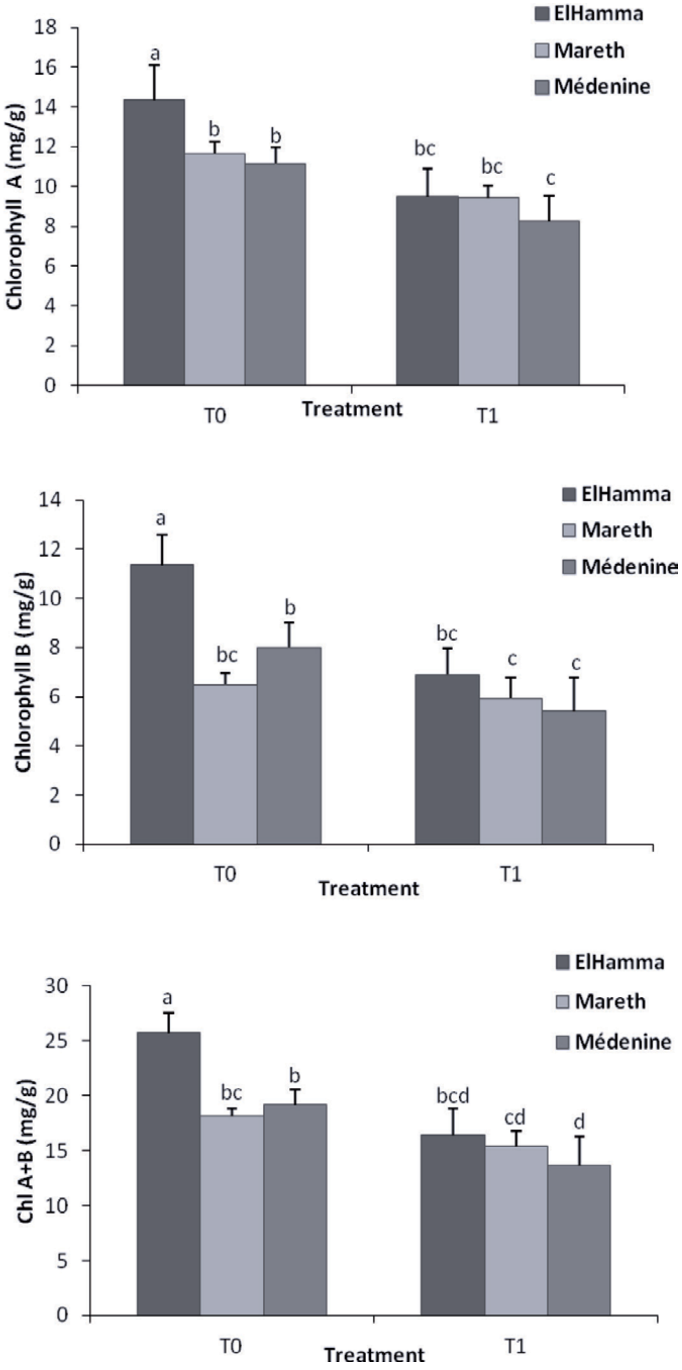

Figure 3.
Changes in Chlorophyll (a), (b) and (a+b) for V. faba *leaves in T0 (control) and T1 (moderate deficit irrigation (50% FC)).*

stressed plants (T1). This parameter is sufficiently influenced by water restriction ($P < 0.001$). Thus, leaves of the three ascensions studied reacted with a considerable drop in their relative water content (RWC). In El Hamma, the RWC is maintained at high values for irrigated plants and stressed plants, while in Mareth and Medenine, plants cultivated under T1, exhibited a significant decrease compared to the control of about 9.74% and 24.73% respectively. Repeated measures ANOVA, indicated significant interaction between accession and stress deficit ($P < 0.01$) for RWC. The analysis of the relative water content makes it possible to give a clue and describe in a global way the water status of beans in response to water stress. Decrease in RWC in plants under drought stress have been perceived in many plants and may depend on plant vigor. Likely, in this constraint, the ability to achieve good osmoregulation and to preserve cell turgor is reduced [26]. It seems that concentration of organic solutes to maintain membrane is not sufficient in this case [27, 28].

5.2 Chlorophyll contents

Chlorophyll 'A', 'B' and total chlorophyll contents, showed no significant difference among the accessions in the stress conditions, but significant difference under the non-stress conditions (**Figure 3**). The decrease in the chlorophyll contents in the three ascensions studied during the period of stress would be most likely due to the increased in catalytic activity of chlorophylls and degradation of photosynthetic pigments and this process is also the result of not providing the necessary factors for the synthesis of chlorophyll and the destruction of its structure under stress conditions [24]. Zhu [29] has been reported that a decrease in the chlorophyll content will be due to a decrease in the stomata aperture and aimed at limiting water losses by evaporation and by increased resistance to the entry of atmospheric CO2 necessary for photosynthesis [30]. These results are in agreement with Tairo et al. [31] who reported a significant increase in chlorophyll 'A', 'B' and total chlorophyll contents was seen in varieties 1(KAT B9), 4(F8 Drought line) and 5(JESCA) of common bean *Phaseolus vulgaris* (L.).

5.3 Osmoregulation (accumulation of soluble sugars)

Induced drought stress influenced ($P < 0.001$) soluble sugar contents (**Figure 4**). Untreated plants showed the lowest concentration (0.54 mg/g for Mareth accenssion).

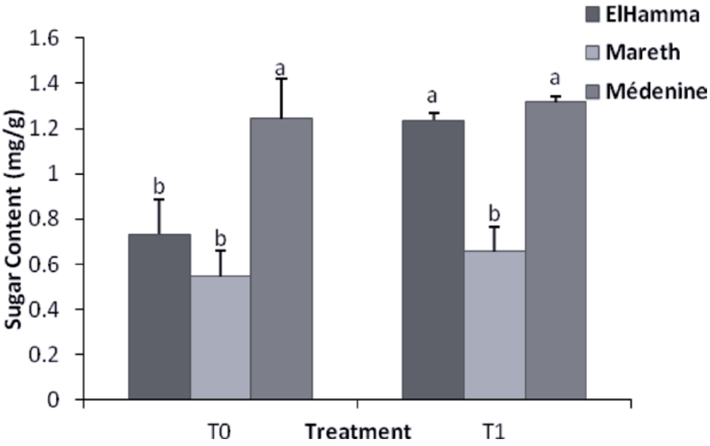

Figure 4.
Effect of water stress on soluble sugar contents of V. faba *L. leaves.*

However, the highest soluble sugar content (1.31 mg/g) was noticed under water deficit condition for ElHamma cultivar. These metabolic adaptations that improve plant tolerance to osmotic or water stress involve an increased synthesis of compatible osmolytes as soluble sugars and proline. This increase in sugar content under stress might contribute for osmotic adjustment and plays an important role in scavenging free radicals and protect DNA from damaging effects of increased reactive oxygen species (ROS) levels due to water stress [32, 33]. Moreover, soluble sugar accumulation was considered as an indicator of plant tolerance to drought conditions. It improves water absorption and reduces water loss when stress leads to slower growth [34, 35].

6. Other environmental stresses effects on *Vicia faba* L.

The effect of sodium chloride (NaCl) concentrations (0.0, 60, 120, 240 mM) on growth and chlorophyll content of (*Vicia faba* L.) seedlings caused an increase for both fresh and dry weights of the shoot. Salinity significantly reduced chlorophyll 'A' content. It also significantly reduced chlorophyll 'B', total chl., and carotenoïds contents after ten days of treatment [36]. High-temperature stress gravely reduced the photosynthesis, stomatal conductanceand water use efficiency of *V. faba* [37–39]. The effects of magnesium (Mg) supplementation on the growth performance as well as, on photosynthetic pigment synthesis, were studied in *Vicia faba* L. plants exposed to heat stress and non-heat-stress conditions. Results revealed that growth attributes and total chlorophyll decreased in plants subjected to heat stress, whereas accumulation of organic solutes had seen. These results suggest that adequate supply of Mg is not only essential for plant growth and development, but also improves plant tolerance to heat stress by suppressing cellular damage induced by ROS through the enhancement of the accumulation of Proline and glycine betaine [40].

7. Improvement of crop production under environmental stresses

Crop production is influenced by multiple environmental abiotic factors including drought, soil salinity, waterlogging and temperature interferes with the whole metabolic activities in plants [41, 42]. Plant adopted different mechanisms and modifications to cope with stresses such as plant hormones that facilitating growth, nutrient allocation and development [43, 44]. Salicylic acid (SA) significantly increased the Mn^{+2}, Co^{+3}, Fe^{+2}, Cu^{+3}, K^+ and Mg^{+2} while decreased the Na^+, Ni^{+3}, Pb^{+4}, Zn^{+2}, and Na^+/K^+ content of roots and soil content under salinity stress. SA (10-5 M) can be implicated to mitigate the adverse effects of salinity on maize plants [45, 46]. Additionally, exogenous application of gibberellic acid (GA3) reduced oxidative stress in *C. capsularis* seedlings. GA3 not only ameliorate photosynthesis, biomass and plant growth, foliar application of GA3 but also, increases metal (Cu) concentration in different organs of the plants when compared to 0 mg/L of GA3. GA3 plays a promising role in reducing ROS generation in the plant tissues [47, 48]. Furthermore, melatonin application (100 mM and 500 mM) maintaining plant growth and relative water content. More than that, improving the photosynthetic characteristics, total carbohydrate, and total phenolic content in leaf tissues of faba bean plants irrigated with saline water. Melatonin might directly eliminate ROS when produced under stressed conditions [49].

Mineral compounds play too a key role in the structural and functional integrity of the plant to better respond to abiotic stresses. Silicon application enhances drought tolerance of *Kentucky bluegrass* by improving plant water relations and morpho-physiological functions [50, 51]. Moreover, the applications of N fertilizer linearly increased kernel weight during both growing seasons [52, 53]. N deficiency reduces photosynthates production in plants while an optimum N fertilization may increase weight and kernel corn quantity resulting in higher crop growth rates [54]. In addition, a combined application of phosphorus (P) and biochar mitigates heat-induced adversities on physiological, agronomical and quality attributes of rice. The application of biochar+P recorded a grain yield of 7% (plant-1) of rice compared with control throughout different temperature treatments and cultivars. The highest grain production and better grain quality in biochar+P treatments might be due to enhanced water use efficiency, photosynthesis and grain size, which offsetting the adversities of high temperature stress [55–57]. So, fertilization enhances heat injury in rice (*Oryza sativa* L.) [58].

8. Conclusions

In conclusion, water stress seems to be the main limitation factor to growth process in *V faba* L. Under moderate stress (50%CC), the tested ascensions responded similarly on how to cope with water stress. But, El Hamma had the highest performance as compared with Medenine and Mareth accessions. *V. faba* maintains their relative water content at high values and accumulates a high concentration of soluble sugars in their tissues. Accordingly, the dehydration avoidance is considered to be an adaptive mechanism for minimizing water loss. The evaluated *V. faba* accessions responded to water stress also, by reducing shoot and root biomass. Besides, morpho-physiological attributes relative to shoots and roots play an important role in the response to water stress and contributed to drought tolerance of plants. Therefore, it is imperative to identify the specific physiological process of drought tolerance in faba bean, and to define as valuable selection criteria under stressful conditions. Thus, nutritional and economic values of *V. faba* L. can be enhanced in Tunisia.

Acknowledgements

The authors are grateful to Mr. Lachiheb Belgacem. lab technician from the Arid Land Institue for their collaboration in the laboratory analysis.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 5

Abiotic Stress Responses in Plants: Current Knowledge and Future Prospects

Deeksha Marothia, Navdeep Kaur and Pratap Kumar Pati

Abstract

Exposure to abiotic stresses has become a major threatening factor that hurdles the sustainable growth in agriculture for fulfilling the growing food demand worldwide. A significant decrease in the production of major food crops including wheat, rice, and maize is predicted in the near future due to the combined effect of abiotic stresses and climate change that will hamper global food security. Thus, desperate efforts are necessary to develop abiotic stress-resilient crops with improved agronomic traits. For this, detailed knowledge of the underlying mechanisms responsible for abiotic stress adaptation in plants is must required. Plants being sessile organisms respond to different stresses through complex and diverse responses that are integrated on various whole plants, cellular, and molecular levels. The advanced genetic and molecular tools have uncovered these complex stress adaptive processes and have provided critical inputs on their regulation. The present chapter focuses on understanding the different responses of the plants involved in abiotic stress adaptation and strategies employed to date for achieving stress resistance in plants.

Keywords: plants, abiotic stress, photosynthesis, reactive oxygen species, ion transport, osmoregulation

1. Introduction

Plants often experience unfavorable environmental conditions such as high salinity, drought, cold, heat, depletion of soil nutrients, and excess of toxic ions, etc. that hamper the plant growth and development [1–3]. These stresses not only play a major role in determining the crop yield and productivity but they also contribute to the differential distribution of plant species across different parts of the earth [4]. About 90% of the arable lands around the globe are susceptible to one or more of the above stresses causing up to 70% annual yield loss of major food crops [5]. The changing climate is further aggravating the impact of abiotic stress factors on the overall growth and development of various crops [6]. It is believed that exposure to salt stress in irrigated lands has been increased by 37% during the last 20 years [7]. Moreover, the occurrence of drought is increased due to alteration in the evapotranspiration and pattern of precipitation caused by global warming [8]. As per a recent meta-analysis study, a further increase of 2.0 to 4.9°C in the average earth temperature by 2100 is speculated which will further impose a huge challenge for sustainable agriculture in the future [9].

Plants respond to different environmental constraints through complex intricate mechanisms [1]. The ability of plants to adjust to different environmental conditions is directly or indirectly related to two major plant strategies - plant stress avoidance and plant stress tolerance. Plant's stress avoidance is a physiologically non- active phase like mature seeds, while stress tolerance is an active reversible adjustment which is generally referred to as acclimation [10]. Acclimation to stress is particularly mediated through profound changes at the level of gene expression which results in changes or modifications in the composition of plant transcriptome, proteome as well as metabolome [11]. During the last few decades, researchers have focused on recognizing and elucidating the different components and molecular partners underlying abiotic stress responses in plants [12]. Several attempts have been made to produce crops/species with improved abiotic stress adaptive traits including drought and salinity. However, one of the massive challenges in modern sustainable agriculture is the development of abiotic stress-resilient crops with new and desired agronomical traits using different approaches. For this purpose, understanding the mechanisms by which plants perceive stress signals and further transmit them to cellular machinery for activating adaptive responses is of huge importance [13–16]. In this context, marrying the various physiological, biochemical, and gene regulatory network knowledge is essential that will aid up in the development of stress-tolerant high-yielding food crop cultivars [17, 18]. Therefore, a holistic understanding of the different responses associated with abiotic stress adaptation by taking advantage of various available high throughput tools like proteomics, metabolomics, and transcriptomics is critical. Hence, the present chapter deals with the various responses associated with abiotic stress stimuli in plants and the current status, and future prospects of different approaches used to date for developing stress-resilient crops.

2. Plant's responses to abiotic stresses

Plants face several types of variations in their physical environment that hampers their growth and development. They respond to these oscillating environmental conditions through a series of external and internal changes [19, 20]. These stress-specific responses are associated with an array of molecular players that modulates the morphology, anatomy, and physiology of plants [12, 13].

2.1 Responses at the level of cellular membranes

Plant cells can sense changing environmental signals leading to significant changes in their physiology, metabolism, and gene expression [12, 13]. The stress stimuli are first perceived at the level of cellular membranes that initiates a cascade of events to transmit the signal to various organelles thus activating the appropriate molecular network [21]. In plants, the primary cell wall is composed of cellulose fibrils connected by hemicellulose tethers embedded in a pectin gel providing mechanical strength for load-bearing. It also contains several structural proteins, phenolics, and calcium [22]. These components are often modified when plants are exposed to abiotic stresses. The overall architecture of the cell wall is affected by exposure to abiotic stress depending upon the species, the stress intensity, plant phenotype, plant genotype as well as the age of plant. It appears to result in both loosening and tightening of the cell wall [23].

The viscoelastic properties of the primary cell wall are improved by elevating the levels of cell wall remodeling and biosynthetic enzymes, and by modulating the other cell wall loosening agents such as pectin, thus contributing to higher hydration status of the plant which aids up in maintaining turgor pressure necessary for growth [23]. The viscoelastic properties are also modulated by reinforcement of the secondary wall with the accumulation of cellulose and non-cellulosic components. In response to abiotic stress stimuli, the biosynthesis of xyloglucan (the most abundant non-cellulosic components of type I primary walls), and cellulose is induced [24, 25]. It is associated with an up-regulation of EXP (expansin), XTH (xyloglucan endo-β-transglucosylases/hydrolases) and Ces A (Cellulose Synthase) encoding genes [25] Moreover, the comparative analysis of changes in the cell wall of two- different drought-resistant varieties of wheat under stress showed an increase in pectin polymers RGI and RGII (rhamnogalacturonan I and II) side chains that probably leads to hydrogel formation of pectin, limiting the damage to the cells [26]. Also, methyl esterification of homogalacturonan (HG) levels regulated by PME (pectin methylesterase) reduces upon exposure to stress stimuli [27]. Such modifications in the cell wall architecture lead to relative maintenance of cell wall extensibility required to cope up with particular abiotic stress. Moreover, the genes encoding for cell wall proteins including arabinogalactan protein (AGP), glycine-rich protein (GRP), and proline-rich protein (PRP) are also induced in response to abiotic stress that could contribute to the cell wall strengthening [23].

One of the alternative responses against abiotic stress stimuli is to decrease the cell wall expansion and cell extensibility that can thus limit the water loss and prevent cell collapse due to dehydration stress [23, 28]. A decrease in cell wall extensibility or turgor pressure is often associated with the rigidification of the secondary cell wall by lignin deposition. As monolignols are the building blocks of lignin, they are synthesized from phenylalanine through the general phenylpropanoid and monolignol-specific pathways in the cytosol. The monolignols are then transported to the cell wall where they are polymerized by apoplastic peroxidase (PRX) and laccases into lignin [23].

A large number of integral plasma membrane proteins are also known to participate in stress perceptions which are the members of different receptor-like kinases RLKs (receptor-like kinases) [29]. Abiotic stresses are often responsible for alterations in wall-associated kinases (WAK) that are required for cell elongation and development [22]. In plants exposed to abiotic stresses, the expression of genes encoding for WAK proteins is up-regulated hinting towards the perception of stress at the cell wall or plasma membrane interface through the detection of released plant cell wall fragments [24, 30]. Thus, it can be concluded that modulation of the cell wall architecture is often a direct response that plays a vital role in the sensitization of the plant against abiotic stress stimuli. However, critical information on understanding this response comes from transcriptomics rather than biochemical analysis [26]. Therefore, a multidisciplinary approach is required for gaining an in-depth knowledge of this complex mechanism in the future.

2.2 Modulation of photosynthetic apparatus and gaseous parameters

Plants suffer numerous physiological reactions on exposure to environmental stress. These responses include alterations in photosynthetic rates, assimilate translocation, nutrient uptake and translocation, changes in water uptake, and evapotranspiration [31]. Among these, photosynthesis is one of the most critical plant processes affected by various abiotic stresses [31, 32]. These stresses negatively influence the photosystems (PS I and PS II) thus reducing the photosynthetic activity along with reduced chlorophyll biosynthesis, and photosynthetic electron transport. They also lead to impaired RuBp (ribulose 1,5-bisphosphate) regeneration that substantially affects the Rubisco activity. Generally, the stress-derived inhibitory effects on photosynthesis in plants may occur due to limitations in CO_2

diffusion factors and/or metabolic factors. Some reports provide evidence that stomatal closure is the key event under stress conditions resulting in a decrease in the sub-stomatal as well as chloroplast CO_2 concentration (Ci and Cc, respectively) thus producing a decline in CO_2 assimilation [32–36].

Under moderate drought stress, decreased stomatal conductance (g_s) is considered as the primary cause of photosynthetic inhibition from reduced supply of CO_2 to the intercellular space. In general, atmospheric CO_2 diffuses to the intercellular space (i.e. stomatal limitation) through stomata and then across the mesophyll (mesophyll limitations) at the carboxylation site [31]. Thus, mesophyll conductance (g_m) and biochemical limitation (b_L) (often termed as non-stomatal limitations to photosynthesis mainly under high water stress) have gained importance in the recent years, however, their relative importance to photosynthesis limitation has been a subject of debate [31, 36, 37]. Although, the function of non-stomatal limitations to photosynthesis is evident, however, controversies still exist because of the error and assumptions in the estimation of g_m and b_L under stress conditions [38].

2.3 Ion stress signaling and homeostasis

Abiotic stresses particularly salt and heavy metal stress are majorly responsible for an imbalance in ionic composition inside the plant cells [10]. For a normal metabolic function of plants, cells need to maintain high K^+ and low Na^+ levels. Thus, systematic exclusion of excess Na^+ ions from the cytoplasm or their accumulation within the vacuoles are the main adaptive mechanisms against ionic stress in plants [21]. This occurs through a highly sophisticated mechanism of ion homeostasis which involves the interplay of different molecular players. Ion homeostasis is maintained by ion pumps like symporters, antiporters, and carrier proteins located on the cell membranes [39]. At the plasma membrane of the cell, the stress signal is perceived by a sensor or a receptor which is generally regulated by the coordination of various ion pumps [40]. Exclusion of ions is typically carried out by transmembrane transport proteins excluding Na^+ from the cytosol, however, compartmentalization is carried out by H^+- pyrophosphatase proteins and vacuolar membrane H^+ -ATPase [12].

Salt Overly Sensitive also known as SOS pathway is an excellent example of intracellular ion management or homeostasis which is turned 'on' after the activation of the receptor in response to stress and transcriptional induction of genes by signaling intermediate compounds along with certain downstream interacting partners which result in the efflux of excess ions [41]. *SOS1*, *SOS2,* and *SOS3* are the three genes encoding for SOS proteins, which work in a synchronized manner and aids in the transportation of Na^+ ions from the cytoplasm by effluxing excess of Na^+ ions using a plasma membrane Na^+/H^+ antiporter. This pathway is triggered by the high concentrations of Na^+ ions perceived by the intracellular calcium (Ca^{2+}) ion signals. The high concentration of sodium chloride (NaCl) disturbs the intracellular levels of Ca^{2+} via hypothetical plasma membrane sensors. This Ca^{2+} signal is then recognized and interpreted by the SOS3 protein which belongs to the calcineurin B-like protein (CBLs) family which in association with SOS2 activates the SOS1 [42]. SOS1 encodes for a Na^+/H^+ antiporter and various studies have confirmed the functional role of SOS1 in maintaining the homeostatic balance of ions during salt stress adaptation [43]. The vacuolar Na^+/H^+ and $H^{+/}Ca^{2+}$ antiporters are also known to be differentially regulated by SOS2, thus contributing to enhanced Na^+ ions sequestration in vacuole imparting salinity tolerance. Furthermore, the SOS2/SOS3 kinase complex is responsible for the down-regulation of the activity of Na^+ ion transporters, mediating the entry of these ions into the cells of root tissue during salinity. Apart from the well-established function of ion homeostasis, SOS proteins have also been known to play novel functions during stress acclimatization including regulation of cell cytoskeleton dynamics, development of lateral roots via modulation of auxin gradients as well as maxima in roots under moderate salt stress [43].

In plants, potassium (K^+) is one of the most abundant inorganic cations involved in various aspects of plant growth and development including abiotic stress management [44]. Thus, the maintenance of K^+ homeostasis through K^+ ion transporters and channels across the plasma membrane is necessary for the survival of plants, especially during stress conditions [45]. Plants have developed a unique transport system for K^+ acquisition and release using the high-affinity K^+ uptake transporters (HKTs) [46]. There are two sub-groups of these transporters (class I and class II) which have been identified to play a critical role in selective Na^+ ion transport and cationic co-transport of Na^+/K^+, respectively [12]. They also play a significant role in the maintenance and distribution of Na^+ ions between plant shoots and roots [47]. In *Arabidopsis thaliana* (Arabidopsis) knockout mutations in the *AtHKT1* gene along with *AtSOS1* gene {induced either by T-DNA insertion or ethyl methane sulphonate (EMS) treatment} lead to over-deposition of Na^+ ions in leaves due to the decreased amount of Na^+ ions in roots under salt stress [48].

Cl^- is a plant micronutrient which regulates turgor pressure, leaf osmotic potential, and stimulates growth in plants by acting as a critical messenger in plant developmental processes [49]. Cl^- ion signaling and transporters also regulate different pathways conferring abiotic stress tolerance in plants [50]. For instance, as an early salt stress response, the Cl^- ion signal in the soil with elevated salt concentration has been connected to stomatal closure in an ABA dependent manner [21]. However, increased deposition of these ions during ionic stress is detrimental to plant growth and development [51]. Thus, plants tend to decrease the net levels of Cl^- ions during stress through reduced net Cl^- uptake by roots, decreased intracellular compartmentation, reduced net xylem loading of Cl^-, and phloem recirculation and translocation [52]. Also, inside the cytosol, threshold levels of Cl^- ions are maintained primarily through its sequestration with the help of ion transporters and voltage-gated ion channels inside the vacuole [53]. A voltage gradient is maintained between the vacuole and the cytoplasm because of a slightly positive charged vacuole and a negatively charged cytoplasm. Hence, a large number of the Cl^- ions are sequestered through voltage-gated anion channels of the CLC family which are present on the tonoplast. Different CLC proteins function as anion/H^+ exchangers or anion-selective channels. In reports, AtCLCa has been characterized as a two-anion/H^+ exchanger which drives the active uptake of anions inside the vacuoles of Arabidopsis guard cells and mesophyll with higher selectivity for NO_3^- ions over Cl^- ions [54]. Besides, CLCs play a vital role in loading anions in the vacuole of guard cells for stomatal opening in response to light and later releasing them during ABA-induced stomatal closure [55].

2.4 Intracellular osmotic adjustment and osmoprotectants

The intracellular water loss from the cell due to drought and salinity stress results in cellular dehydration thus imposing osmotic stress in plants [56]. To counteract the effects of osmotic stress, plants and bacteria accumulate certain organic solutes like quaternary ammonium compounds, polyamines, fructose, sucrose, sugar alcohols, trehalose, fructans, oxalate, malate, and many others. These metabolites are referred as osmoprotectants or compatible solutes and may accumulate in large quantities without disturbing the intracellular biochemistry [57]. Among these osmoprotectants, quaternary ammonium compounds including proline and glycine betaine (GB) abundantly accumulate in response to abiotic stresses. The imino acid proline is known to be deposited in considerable amounts in plant cells under the influence of drought, salinity, and other stresses [58]. It is synthesized inside the cytoplasm and plastids while it is degraded to glutamate (Glu) in the mitochondria. In addition to its role in osmotic adjustment, proline contributes in the stabilization of the cellular membranes and vital proteins by making clusters with water molecules that later get attached to membranes and proteins, thus, inhibiting their denaturation [59, 60]. Proline also scavenges free radicals to maintain or buffer the redox potential inside the cell under stressful conditions. It alleviates the cytoplasmic acidosis and sustains $NADP^+$/NADPH ratios at required levels for cellular metabolism, hence, supporting redox cycling [60, 61]. Researchers have observed a positive correlation between proline deposition and tolerance against various abiotic stresses in plants [58]. Furthermore, the exogenous application of proline has been used as an effective approach to improve stress tolerance in plants [62].

GB is another critical compound that plays an important role in osmoprotection, stroma adjustment as well as protection of thylakoid membranes for maintaining the photosynthetic activity during stress conditions [63, 64]. It protects the photosystem II (PS-II) complex from the impact of abiotic stresses [65]. GB also possesses a protective role for Rubisco against heat-induced destabilization [65]. The increased accumulation of GB provides abiotic stress resistance in several agronomically important crops including tobacco, potato, tomato, barley, and maize [11, 66, 67]. Moreover, the *Arabidopsis thaliana, Nicotiana tabacum,* and *Brassica napus* plants transformed with bacterial *choline oxidase* cDNA were found to show 5 to 10% increased levels of GB than the naturally found levels of GB in them that moderately improved their tolerance against different abiotic stresses [68].

The content of soluble carbohydrates also varies in response to abiotic stresses in plants. Simple and complex carbohydrates such as sugars, starch, and sugar alcohols accumulate under stress conditions in plants [68]. The major roles of these biomolecules are osmotic adjustment, carbon storage, and free radical scavenging. Their pattern of accumulation in response to stress varies under short- and long-term reactions. In short-term water stress conditions, decreased content of sucrose and starch were observed in the case of *Setaria sphacelata*, which is a naturally adapted C4 grass whereas an increased amount of soluble sugars and decreased amount of starch were reported under long term stress imposition [69]. Trehalose is a rare non-reducing sugar that occurs in some desiccation-tolerant higher plants along with various bacterial and fungal species [70]. It shows significant accumulation in plants in response to various environmental stimuli and acts as an osmolyte thus protecting the plant cells. It also protects the protein functioning by reducing the aggregation of denatured proteins and safeguards the biological molecules from the changing environmental stresses through its reversible water-absorption capacity [68, 71]. The sugar alcohols also show considerable accumulation in response to abiotic stress in plants and help in osmotic adjustment [72]. Mannitol, a sugar alcohol, accumulates upon salt and water stress conditions in plants. Wheat transgenics, expressing the *mtlD* gene (*mannitol-1-phosphate dehydrogenase*) of *Escherichia coli* showed significantly more tolerance towards salt as well as water stress. Upon analysis, increased plant height, biomass, and the number of secondary stems were observed in transgenic wheat [72].

Polyamines are small organic molecules ubiquitously present in all living organisms which play a vital role in diverse cellular processes. They are positively charged at physiological pH and are regarded as growth substances [73–75]. Under stress conditions, different plant species respond differently to polyamines levels. Some of the plants might increase the content of polyamines under stress conditions whereas others decrease their levels of endogenous polyamines when exposed to severe environmental conditions [73]. Exogenous application of polyamine and/or inhibitors of enzymes which are involved in polyamine biosynthesis also hints towards a possible role of such compounds in plant adaptation or defense process in response to environmental stresses [76]. Moreover, studies involving either transgenic overexpression or loss of function mutants support the protective, adaptive, or defensive role of polyamines in plant's response to various abiotic stresses [76, 77].

2.5 Reactive oxygen species (ROS) regulation during stress acclimation

Many evidences suggest that various environmental stresses lead to the generation of ROS in plants. Actually, in plants, each cellular compartment is equipped with its own ROS homeostasis control [78–80]. The ROS signaling is changed depending upon the cell type, developmental stage, and level of stress [81]. Under optimal growth conditions, ROS inside the cell is mainly produced at a low level in organelles like chloroplast, mitochondria, and peroxisomes [82]. It has been estimated that 1–2% of the O_2 consumed by plant tissues, leads to the ROS formation that mainly involves ${}^1O_2$, H_2O_2, $O^{\bullet-}{}_2$, and OH• [83, 84]. At this low concentration, ROS acts as a signaling molecule that triggers signal transduction pathways involved in growth and development [21, 85]. However, in response to various abiotic stresses, the generation of increased levels of ROS causes irreversible damage to cells through their strong oxidative properties [86]. They possess lethal properties and cause extensive damage to DNA, proteins, and lipids thereby affecting normal cellular functioning [82]. Plants have developed an elaborate and efficient network of ROS generating and scavenging mechanisms to overcome this ROS toxicity. The two systems interplay with each other for maintaining a steady state in plants during stress acclimation [87, 88]. The delicate balance between the generation of ROS and its scavenging is responsible for duality in its function in plants which is orchestrated by a giant network of genes known as 'ROS gene network' [84].

Plant NADPH oxidases also referred as respiratory burst oxidase homologs (RBOHs) are the most studied enzymatic source of ROS in plants [88]. These are superoxide-producing enzymes that are widely involved in various processes including abiotic stress responses in plants [89]. The superoxide radical is a short-lived ROS molecule that is characterized by moderate reactivity and can trigger a series of reactions to produce other ROS species. It is produced inside mitochondria, chloroplasts, endoplasmic reticulum, and peroxisomes as a result of their normal metabolism [90]. The activity of plant NADPH oxidase is regulated by some key regulatory components like Ca^{2+}, calcium-dependent protein kinases (CDPKs), $Ca^{2+/}$CaM-dependent protein kinase, some small GTPases, and others. The production of ROS through NADPH oxidase may result in regulating the acclimation to abiotic stresses in plants. For instance, in barley, NADPH oxidase-mediated apoplastic ROS generation (acting upstream of xylem Na^+ loading) that is linked to ROS-inducible Ca^{2+} uptake systems in the xylem parenchyma tissue is considered as a critical factor contributing to salt stress tolerance in plants [91]. In *Arabidopsis*, the double mutants of *AtRbohD* and *AtRbohF* genes with significantly inhibited ROS generation exhibited less growth and relatively higher cellular Na^+ to K^+ ratios than the wild-type (WT) as well as a single null mutant *ATrbohd* and *ATrbohf* plants under salt stress [92].

Superoxide ions generated by NADPH oxidase are converted to hydrogen peroxide (H_2O_2), catalyzed by the different isoforms of superoxide dismutase (SOD) enzyme [93]. H_2O_2 production in plant cells not only occurs under normal conditions but also by oxidative stress which is caused by different abiotic factors. The major sources of H_2O_2 production in plant cells comprises of the electron transport chain in the chloroplast, endoplasmic reticulum (ER), mitochondria, cell membrane, β-oxidation of fatty acid, and photorespiration along with various other sources including reactions comprising photo-oxidation by NADPH oxidase. The rates of H_2O_2 accumulation in peroxisomes, as well as chloroplasts, may be 30–100 times higher as compared with H_2O_2 generated in the mitochondria. It acts as a systemic signal that alerts various plant tissues to respond and adapt in response to the upcoming stress stimuli [94, 95]. H_2O_2 confer acclamatory stress tolerance by regulating osmotic adjustment, photosynthesis, ROS detoxification, and phytohormones signaling [95]. Studies have suggested that seeds pre-treated with H_2O_2, or together with the application of H_2O_2 and abiotic stress, induce an inductive pulse which aids up in protecting plants under abiotic stresses by the restoration of redox-homeostasis and mitigation of oxidative damage to membranes, lipids, and proteins by modulating the stress signaling pathways [95].

The stress-induced ROS activating responses occur rapidly with the appearance of the stress and it should decay immediately to protect the plants against their toxic effects. For this, plants are equipped with an array of ROS detoxifying proteins that mitigate the toxic effects of ROS generated as a result of different types of stresses [96]. In plants, the redox homeostasis during stressful conditions is maintained by the two arms of the antioxidant machinery—the enzymatic components consisting of the superoxide dismutase (SOD), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), catalase (CAT), glutathione-S-transferase (GST), and the non-enzymatic molecular compounds like reduced glutathione (GSH), ascorbic acid (AA), α-tocopherol, phenolics, carotenoids, flavonoids, and proline. These antioxidant enzymes are situated in different sites of the plant cells and work together to detoxify ROS. The omnipresent behavior of both arms of the antioxidant machinery explains the basic necessity of detoxification of ROS for cell survival [97].

3. Strategies to combat abiotic stresses in plants

Various strategies have been undertaken by the researchers from time to time to improve the abiotic stress tolerance in plants, particularly crop plants [98]. Plant breeding is the most traditional and widely used method for achieving the desired trait in given plants including stress adaptation [99]. However, the success of crop-breeding programs greatly depends on the availability of natural genetic variations among the germplasm resources and tedious selection procedures that are too slow and equally expensive [100]. Moreover, the various environmental factors such as plant developmental stage along with the logistical constraints of physiological screening of large breeding populations on a field-scale can affect the differential selection of a particular stress tolerant plant. Thus, plant breeding is almost always limited by the genetic complexity of the underpinning mechanisms along with the potential interaction among genetic determinants [101]. In this regard, the identification and recognition of discrete chromosomal regions having a major effect on the specific tolerance trait via quantitative trait loci (QTL) mapping and marker-assisted selection remain a valuable option for the success of many breeding programs [102]. Although, QTL mapping holds great promise, but still it remains complicated as the introgression of QTL regions in elite lines is tedious due to linkage drag that may introduce non-target regions. As an alternative, the cellular-based mutant introduction and subsequent selection under controlled *in vitro* conditions offer a method to quickly screen large populations with homogeneous backgrounds for novel fortuitous changes related to tolerance. Subsequent field screening then ensures the adequate performance of the tolerance trait under the external potentially mitigating factors [103].

In the past few decades, the genetic engineering approach has attracted the interest of the research community for producing stress-tolerant elite crops [104].

Genetic transformation with stress-inducible genes has been employed by the researchers to gain an understanding of their functional role in stress tolerance and ultimately to improve the traits in the target genotype [105]. The genetic manipulation techniques including insertional mutagenesis have largely contributed to deciphering the function of genes and thereby identifying the suitable candidates for crop improvement [106]. However, though success has been achieved in introducing desired tolerance traits into various crop varieties from wild relatives like barley and tomato, a restricted success has been reported in achieving abiotic stress tolerance with elite germplasm [107]. Moreover, the integration of transgenes into the host genome is sometimes non-specific and unstable [108]. Recently, the use of targeted genome editing using clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein9 nuclease (Cas9) (CRISPR/Cas) has generated a lot of interest in various fields of plant biology including abiotic stress management [109]. CRISPR/Cas has been adopted in the field of plant developmental biology for characterizing genes as well as to underpin the molecular mechanisms behind various plant traits [110]. It has been used in the model plants such as Arabidopsis and tobacco earlier and likewise, now it is being utilized effectively for crop plants like sorghum, rice, wheat, maize, soybean as well as woody plants. Researchers have worked on the potential use of the CRISPR/Cas9 technique for the production of abiotic stress-tolerant crops by targeting the key sensitivity (*S* genes and *cis*-regulatory sequences) and tolerance (*T* genes) players. In general, *T* genes are deployed to achieve stress tolerance in plants; however, the *S* genes negatively regulates the biological function of the *T* genes. Therefore, the silencing of *S* genes to disturb their functioning can help plants to adjust their physiological and biochemical pathways for providing tolerance in response to abiotic stress [111]. Like *S* genes, various *cis*-regulatory sequences have also been identified that negatively regulates abiotic stress tolerance mechanisms. These sequences are highly conserved and help in the regulation of gene expression by interacting with specific transcription factors [111]. Thus, editing such *cis*-regulatory sequences can also serve as a potential strategy for improving stress tolerance in plants. However, one major limitation of genome editing is the off-target mutations that are caused by Cas9 in transgenic plants. This limitation has been overcome to a considerable extent by the advent of stress-inducible CRISPR/Cas9 technique which reduces the rate of off-target mutations to negligible levels [112]. Thus, we can consider stress-inducible CRISPR/Cas as a promising tool for precise and efficient genome editing in crop plants for numerous traits, including abiotic stress tolerance.

4. Conclusion

In the last few decades, significant progress has been made in our understanding of the complex mechanisms governing abiotic stress tolerance in plants. However, still we are far from pinning the exact battery of gene activation mechanisms responsible for providing tolerance to various abiotic stresses. Our struggle to understand the complex mechanisms is ongoing and recent development of new tools for high-throughput phenotyping and genotyping gives us a new ray of hope. A complete understanding of the physiological, biochemical and molecular mechanisms especially the signaling cascades in response to abiotic stresses in tolerant plants will help to manipulate susceptible crop plants and increase agricultural productivity in the near future. Moreover, advances in genomics strategies including genetic engineering and genome editing have provided new opportunities for crop improvement by employing precise genome engineering for targeted traits in crop plants. However, the selection of the key genes is critical for the success of these approaches.

Acknowledgements

Authors are thankful to the Department of Biotechnology, GOI, and Rashtriya Uchchattar Shiksha Abhiyan (RUSA-II) Program, Ministry of Human Resource Development (MHRD), GOI.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 6

Molecular Abiotic Stress Tolerans Strategies: From Genetic Engineering to Genome Editing Era

Sinan Meriç, Alp Ayan and Çimen Atak

Abstract

In last decades, plants were increasingly subjected to multiple environmental abiotic stress factors as never before due to their stationary nature. Excess urbanization following the intense industrial applications introduced combinations of abiotic stresses as heat, drought, salinity, heavy metals etc. to plants in various intensities. Technological advancements brought novel biotechnological tools to the abiotic stress tolerance area as an alternative to time and money consuming traditional crop breeding activities as well as they brought vast majority of the problem themselves. Discoveries of single gene (as osmoprotectant, detoxyfying enzyme, transporter protein genes etc.) and multi gene (biomolecule synthesis, heat shock protein, regulatory transcription factor and signal transduction genes etc.) targets through functional genomic approaches identified abiotic stress responsive genes through EST based cDNA micro and macro arrays. In nowadays, genetic engineering and genome editing tools are present to transfer genes among different species and modify these target genes in site specific, even single nuclotide specific manner. This present chapter will evaluate genomic engineering approaches and applications targeting these abiotic stress tolerance responsive mechanisms as well as future prospects of genome editing applications in this field.

Keywords: GMO, abiotic stress, genetic engineering, genome editing

1. Introduction

Before the first examples of the crop domestication by late hunter-gatherer man approximately 10.000 years ago, plants evolved most of their traits following the most basic rule of evolution: success in reproduction and producing next generations. To achieve this goal, plants evolved various adaptation mechanisms against harsh environmental conditions described as abiotic stress factors as well as biotic stress factors like diseases and herbivores [1, 2]. The early domesticated agronomical traits desired by the primal farmers were mostly controlled by the limited number of genes. Traits as panicle size (rice, pearl millet), fruit size (tomato, eggplant), seed size (rice, sorghum, maize, common bean), seed dispersal (cereals in general) are easily controlled by small number of genes [3, 4]. Traits as bitter taste which is one of the adaptation mechanism of plants against herbivores can be controlled by single or multiple genes due to the complexity of the biochemical pathway of the compound. In the absence of genetic knowledge, first breeders domesticated almond as its bitterness is controlled by single gene, while the similar bitter taste in oak acorns is controlled my multiple genes and it was never domestication target [5]. Human intervention to natural selection of plants helped plants to achieve their reproduction goal at the cost of losing most of the gene pool diversity including abiotic stress tolerance and disease resistance genes in favor of human desired traits. Until Gregory Mendel's breakthrough findings, plant breeding was based on easily made crosses, observations and mass selections without knowing sexual recombination of alleles recombined in meiosis to produce new traits for hundreds of years. Even after the knowledge of Mendelian segregation of traits, crossing of distantly related plant species for desired traits as disease resistance became problematic due to the abnormal chromosome pairing and infertile hybrids not to mention horizontal gene transfer between different kingdoms as bacteria and viruses. Following the studies on molecular structure and function of genes during 1940s and the discovery of the structure of DNA by Watson and Crick in 1953, technical developments as PCR and use of restriction enzymes led to the first transfer of bacterial antibiotic resistance gene to plant cells in 1983. Foreign bacterial gene introduced to petunia cell cultures and plantlets derived from transformed cells retained antibiotic resistance [6]. Following these developments, tomato was the first genetically engineered plant which was intended to be used in commercial practices in 1982. Commercial use of genetically engineered crops started in 1996 in US, since four genetically engineered plants were allowed by USDA for field testing in 1985. Technical and technological research kept improving on this field. Certain trends in engineered plants divided the use of this technology in three categories. Targeted traits of the first generation genetically engineered plants were mostly oriented according to farmer's benefit as abiotic stress tolerance or herbicide resistance. Second generation of genetically engineered plants emphasized more on commercial benefits as shelf life or nutritional value. The third generation of genetically engineered plants represent the idea of functional foods which were enhanced with pharmaceutical product that are not present in the plant itself [7].

Use of genetic engineering techniques in abiotic stress tolerance of plants requires knowledge on physiology and biochemical processes to identify key gene targets. Functional genomic approaches utilize transcriptome analysis of plants under abiotic environmental stress factor by using techniques as quantitative, real-time PCR, microarray and high-throughput RNAseq. Gene expression profiles of specific tissues under development stage of choice can be searched between previously submitted Expressed Sequence Tags (ESTs) in various cDNA libraries. Abiotic stress responsive genes in particular abiotic stress tolerance mechanisms can be evaluated before progressing further in experimental design [8]. Molecular regulation type of the targeted trait is also important in genetic engineering approach. Abiotic stress tolerance is mostly regulated by multiple genes depending on stress and targeted tolerance mechanism. One of the early effects of the abiotic stresses on plants is disruption of osmotic balance in cells. Therefore, biosynthesis and accumulation of osmoprotectant molecules as proline, glycine betaine, polyamines, mannitol, trehalose can be targeted as they are regulated by single individual genes. Likewise, reactive oxygene species scavenging genes (superoxide dismutase, glutathione reductase, ascorbate peroxidase), late embryogenesis abundant (LEA) proteins, ion transport genes are some single gene targets used in genetic engineering approach for abiotic stress tolerance which will be further detailed in this chapter. Targeting a tolerance mechanism which is regulated by multiple genes is more complicated. Still, there are many successful genetic engineering applications targeting heat shock proteins (HSPs), transcription factors (TFs), signal transduction genes for abiotic stress tolerance [9, 10]. After all of these decision making process which requires intense precision, promoter type is one of the key factors since all transferred genes requires expression regulation. Calliflower Mosaic Virus 35S (CaMV35S), maize ubiquitine-1 (Ubi-1) and rice actin-1 (act-1) are some of the mostly used strongly expressed constitutive promoters which were used in abiotic stress tolerant genetically engineered plant studies. Despite their advantages to plants as strong expression in all developmental stages in all tissues without any stimulation from the environment, they are not economical for plants in contexts of energy and biochemical source consumption. Signal transduction genes as SAPK4, OsITPK2, OsCPKI2, transcription factor genes as JERF3, ZFP182, antioxidant genes as katE, CAT1, GST, OsMIOX, osmotic homeostasis genes as codA, ion homeostasis genes as AgNHX1, nhaA, OshAK5 are some examples which are regulated by CaMV35S promoter in abiotic stress tolerant genetically engineered plants while Ubi-1 promoter is utilized for DSM1, OsCPK21, OsCPK4 signal transduction genes, OsWRKY45-1, OsWRKY45-2, ZmCBF3, OsbZIP46 transcription factor genes, Sod1 antioxidant gene, otsA, otsB, adc osmotic homeostasis genes and OsKAT1 ion homeostasis genes [11]. Constitutive and avoidable use of biochemical sources in stress free periods or environments leads these genetically engineered plants to reduced growth and loss of yield in some cases. Therefore, regulation of abiotic stress induced or tissue/developmental stage specific promoters are more viable options. General aspects of desired stress induced promoters are described as: (i) having basal expression level under stress free conditions, (ii) strongly promoting expression of resistance genes, (iii) having dose dependent and dose sensitive stress induction, (iv) being stress specific and (v) having reversible induction which rapidly reduce to basal level as removal of stress factor. Arabidopsis rd29A promoter is the most frequently used stress inducible promoter in genetically engineered plant studies. Some stress inducible promoters may lack some desired features as stress induced rab16A, LIP9, OsNAC6 which have higher promoting regulation under basal conditions or some may need further characterization and knowledge as HVA1s, Dhn8s and Dhn4s from barley and DGP1 from tobacco [12].

Most of the abiotic stress tolerant genetically engineered plant trials are limited to laboratory experiments and do not have commercial uses due to the regulations or inadequate field performance. Commercial genetically engineered plants need to tolerate or resist to multiple biotic and abiotic stresses in different combination, duration and concentrations. Tolerance or resistance should not be limited only to developmental stages but also reproductive stage which plants are more vulnerable to abiotic stresses.

As an alternative to gene transfer approach, many studies utilize genome editing techniques as they allow researchers to modify the genome even in a few nucleotide level as well as they can be used to alter or replace allels, silence or insert new gene(s) to targeted sites in genome. Genome editing by using site specific endonucleases is not a new concept in developing crop plants by means of biotic and abiotic stress tolerance. Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) is the most recent site specific genome editing technique which dominated the alternatives as transcription activator-like effector nucleases (TALENs) and zinc-finger nucleases (ZFNs) due to the accuracy, cost and time efficiency and by the means of application advantages as allowing multiple site editing at the same time. Even this new approach evolved in short time due to the occurring limitations during application and new alternatives to Cas9 as Cpf1 are seriously in consideration recently. Also transgene free genome editing applications are introduced lately as the law regulations and public acceptance on this topic are crippling for researchers [13, 14].

Plant	Transformation Event / Developer	Resistance	Expressing	Common Use
Zea mays L.	MON87427 x MON87460 x MON89034 x TC1507 x MON87411 x 59122 / Monsanto	insect resistance, drought and herbicide (glyphosate and spesifically glufosinate ammonium) tolerance	cp4 epsps, cspB, cry2Ab2, cry1A.105, cry1F, pat, cry34Ab1, cry35Ab1, cry3Bb1, dvsnf	Food Feed Biofuel
	MON87460 (Genuity® DroughtGard™) / Monsanto	cold / heat drought tolerance	cspB, nptII	Food Feed Biofuel
	MON87460 x MON88017 / Monsanto	insect resistant (coleopteran and lepidoptera), drought and herbicide (glyphosate) tolerant	cspB, cp4 epsps, cry1A.105, cry2Ab2, cry3Bb1, nptII	Food Feed
	MON87460 x MON89034 x MON88017 / Monsanto	cold / heat, drought, herbicide (glyphosate) tolerance and insect resistance (lepidoptera and coleopteran)	cspB, cry1A.105, cry2Ab2, cp4epsps and cry3Bb1	Food Feed
	MON87460 x MON89034 x NK603 / Monsanto	cold / heat, drought, herbiside (glyphosate) tolerance and insect (lepidoptera) resistance	cspB, cry1A.105, cp4 epsps, cry2Ab2, nptII	Food Feed
	MON87460 x NK603 / Monsanto	drought and herbicide (glyphosate) tolerance	cspB, cp4 epsps nptII	Food Feed
	MON89034 x MON87460 / Monsanto	drought tolerance and insect (lepidoptera) resistance	Cry2Ab2, cry1A.105 and cspB	Food Feed
Glycine max L.	HB4 / Verdeca	drought and hyper salinity tolerance	Hahb-4	Food Feed
	HB4 x GTS 40-3-2 / INDEAR	drought, salinity, herbicide (glyphosate) tolerance	Hahb-4 and cp4 epsps	Food Feed
Saccharum sp.	NXI-1T / PT Perkebunan Nusantara XI (Persero)	drought tolerance	EcBetA, nptII, aph4 (hpt)	Food
	NXI-4T / PT Perkebunan Nusantara XI (Persero)	drought tolerance	RmBetA	Food Feed
	NXI-6T / PT Perkebunan Nusantara XI (Persero)	drought tolerance	RmBetA	Food

Table 1.
Commercial genetically engineered abiotic stress tolerant plant verieties [15, 16].

Consumers and farmers are interested to the traits as taste, productivity, yield, shape of commercial genetically engineered and genome edited varieties more than just survival under abiotic stresses. Therefore, most of the abiotic stress tolerant plant strategies mentioned further in this chapter are laboratory applications. **Table 1** represents successful commercial transformation events of abiotic stress tolerant maize, soybean and sugarcane developed by Monsanto, Verdeca and Persero Companies.

2. Genetic engineering applications in plant abiotic stress tolerance

One of the key points in breeding better crops under various stress conditions is understanding the cellular, biochemical and molecular changes that occur in response to stress [17]. Understanding the mechanisms underlying plants responses to abiotic stress is an important step for genetic engineering that focuses on improving or enhancing tolerance to these stresses (i.e. salinity, cold, dehydration). Identifying key genes that positively affect tolerance within these mechanisms and introduction and overexpression of them allow improvement of genetically modified plants tolerant to abiotic stress. Additionally, understanding the mechanisms by which plants perceive and transmit stress signals is another important point for genetic engineering of crops [18, 19]. Growing number of molecular and biochemical studies on this subject provide an understanding of the signal transduction network involved in the response to abiotic stress and the pathways associated with this network. The development of plants by conventional breeding methods has certain limitations as transfer of a gene requires repeated cycles of crossing and selection. Moreover, the classical breeding process is limited to species with sexual reproduction. Another disadvantage of classical breeding is that genes with undesirable characteristics are transferred together with target genes in this process. With the advent of modern biotechnology tools, it has become possible to modify genetic structure of plants using genes from other living organisms, and recombinant DNA technology has provided an effective alternative to traditional approach. In addition, it has become possible to exchange genetic material between sexually incompatible species. Genes encoding proteins known to play a role in resistance and tolerance to biotic (virus, bacteria, nematode, fungus, herbivores) and abiotic stresses (drought, salinity, temperature, cold) are widely used in the obtaining genetically modified plants. Plants have developed a signal transduction pathway that regulates various stress-response genes such as kinases, molecular chaperones, osmoprotectants, transcription factors, and thus gives an idea of how tolerance to environmental factors is achieved. Since it is known that this signal transduction and associated physiological, biochemical and molecular pathways are governed by more than one gene, it is extremely difficult for only one gene to achieve complete abiotic stress tolerance. Among the molecules known to have protective roles against abiotic stress are proline, which acts as an osmoprotectant, metal chelator, antioxdative defense molecule and signal molecule [20]; trehalose, which acts as an osmoprotectant and is involved in ROS scavenge during abiotic stress [21]; heat shock proteins (HSPs) that serve as molecular chaperones responsible for the folding, assembly, translocation and degradation of proteins [22]; Late embryogenesis abundant (LEA) proteins with antioxidant functions involved in protein protection, membrane protection, and ion binding [23]; aquaporins involved in the transport of water and neutral molecules [24]; calcineurin B-like proteins that act as calcium sensors and play a role in signal transmission. Transcription factors (NAC, WRKY, MYB, bZIP, DREB/CBF), kinases and phosphatases serve a function in stress perception and signal transduction and in the regulation of stress-inducible genes.

In addition to the identification, isolation and characterization of genes involved in all this abiotic stress response, introduction of these genes to plants is an effective molecular approach to understand the roles of genes and products in plant tolerance, behavior and phenotypes.

2.1 Late embryogenesis abundant proteins

Late embryogenesis abundant proteins, which were first discovered in the late developmental stages of plant seeds, are among the proteins most commonly used by plants in their response to abiotic stresses such as drought, high salt, extreme temperature and oxidative stress. Most of the LEA proteins and their mRNAs accumulate in high amounts in the embryo tissues in the final stages of seed development when drought initiates [23]. In addition, LEA proteins also accumulate in plant tissues exposed to dehydration, osmotic and low temperature stress. There is strong evidence that LEA proteins or their genes classified into 6 groups according to their specific domains are correlated with stress resistance [25, 26]. Many studies have reported that stress tolerance occurs as a result of the introduction of LEA genes to various plants such as Arabidopsis, tobacco, rice, barley, and corn by genetic engineering techniques (**Table 2**). Amara [30] reported that the Rab28 LEA gene was overexpressed in maize plants in which they transferred the Rab28 LEA gene with a constitutive maize promoter by particle bombardment method. With the expression of the Rab28 transcripts, the Rab28 protein accumulated in transgenic plants and the transgenic plants continued their growth in the dehydration condition in medium containing polyethyleneglycol (PEG) compared to wild-type controls. These results showed that LEA Rab28 (belonging to the 5th subgroup of the LEA protein family) protein is one of the important candidates that can be used to increase stress tolerance in maize plants. In another transformation study performed with the LEA genes, the barley (HVA1) gene encoding the late embryogenesis abundant protein was transferred to mulberry, which is an important industrial plant used in silk production, by *Agrobacterium tumafaciens*. The HVA1 gene expressing with the rd29a promoter increased the tolerance to cold, drought and salinity in transgenic mulberry [28]. Similarly, overexpression of the ZmLEA3 gene in the tobacco (*Nicotiana tabacum*) plants plays important roles in tolerance to osmotic and oxidative stresses. ZmLEA3 protein plays an active role in protecting plants from damage by preserving the protein structure and holding metals under osmotic and oxidative stress [32]. Late embryogenesis abundant genes have been isolated and characterized from many different plants. Thus, along with transgenic approaches, new gene sources that can be used in abiotic stress tolerance are discovered. The overexpression of the gene responsible for the JcLEA protein (belonging to the 5th subgroup of the LEA protein family) isolated from a tropical plant, *Jatropha curcas*, provided increased resistance to both drought and salt stress compared to the wild-type in transgenic Arabidopsis plants [31]. Transgenic *Arabidopsis thaliana* and *Setaria italica* (foxtail millet) seedlings showed higher tolerance to salt and osmotic stress than the wild-type with overexpression of the SiLEA14 gene [35]. After transformation of plants with LEA genes, chlorophyll content increases, while lipid peroxidation rates, as indicator of ROS decrease. Higher chlorophyll and low lipid peroxidation values obtained by the expression of the AdLEA (belonging to the 5th subgroup of the LEA protein family) gene isolated from the perennial *Arachis diogoi* plant in the tobacco plant (*Nicotiana tabacum*) is a representing study. These transgenic plants have been shown to tolerate dehydration, salinity and oxidative stress. Chlorophyll fluorescence measurements have shown that tobacco plants in which AdLEA is overexpressed can maintain their photosynthetic performance under drought conditions [36].

Gene Action	Gene Source	Promoter	Target Traits	Trans-formed Plant	Transfer Method	Ref.
LEA	ROB5 from Bromegrass, DHN4 from *Hordeum vulgare* SOD3.1 from *Triticum aestivum*	CaMV35S Cor78	Drought Heat Cold	*Solanum tuberosum*	Agrobacterium	[27]
LEA	Hva1 from *Hordeum vulgare*	rd29A	Salinity Drought Cold	*Morus indica*	*Agrobacterium tumefaciens* Ag11	[28]
LEA	OsLEA3-2 from *Oryza sativa*	CaMV35S	Salinity Drought	*Oryza sativa* *Arabidopsis thaliana*	Agrobacterium EHA105	[29]
LEA	LEA Rab28 gene from *Zea mays*	UBQ	Water stress	*Zea mays*	Biolistic system	[30]
LEA	JcLEA from *Jatropha curcas*	CaMV35S	Salinity	*Arabidopsis thaliana*	*Agrobacterium tumefaciens* LBA4404 floral dip	[31]
LEA	ZmLEA3 from *Zea mays*	CaMV35S	Osmotic, Oxidative stress	*Nicotiana tabacum*	*Agrobacterium tumefaciens* strain LBA4404	[32]
LEA	WCI16 from *Triticum aestivum*	CaMV35S	Freezing	*Arabidopsis thaliana*	*Agrobacterium tumefaciens* floral dip	[33]
LAE	ZmLEA5C from *Zea mays*	CaMV35S	Osmatic stress Cold	*Nicotiana benthamiana*	*Agrobacterium tumefaciens* strain LBA4404	[34]
LAE	SiLAE from *Setaria italica*	Super promoter	Osmotic stress Salinity	*Arabidopsis thaliana*	*Agrobacterium tumefaciens* strain GV1301	[35]
LEA	AdLEA from *Arachis diogoi*	CaMV35S	Salinity Oxidative stress	*Nicotiana tabacum*	*Agrobacterium tumefaciens* LBA4404	[36]

Table 2.
Summary of transgenic plants over-expressing late embryogenesis abundant (LEA) genes for abiotic stress tolerance.

2.2 Aquaporins (AQPs)

Aquaporins (AQPs) are a family of membrane water channel proteins that control the osmatic movement of water and other molecules such as carbon dioxide, glycerol, urea, and ammonia [24]. This system, which has high osmotic water permeability, operates with lower activation energy [37]. Since environmental conditions such as high temperatures and winds encountered by plants living on land cause an increase in the rate of transpiration. These plants have developed various strategies such as controlling transpiration with hormones, cellular signaling mechanisms, ion channels, and transporters to protect hemeostasis [38, 39]. However, the current situation worsens when osmotic pressure increases, water is absent and root hydraulic conductivity (Lpr) decreases [18]. In order to overcome this negative situation, the isolation of water channel genes and their introduction to the desired plant by using genetic transformation emerges as one of the most important solutions. Thus, this situation can be reorganized by transgenic expression of water channels that ensure the continuity of processes such as transcellular water transport, stomatal closure, osmoregulation, vacuolar differentiation and plant cell expansion (**Table 3**). Under saline conditions, plant growth is suppressed due to osmotic stress, and in addition, this suppression is further increased due to reduced photosynthesis and gas exchange due to salt accumulation in the leaves. Aquaporins are thought to be extremely effective in plant growth thanks to their effects on leaf gas exchange as well as root water intake. While overexpression of plasma membrane intrinsic proteins (PIP) causes an increase in leaf gas exchange under normal conditions, it increases assimilation and development under salt stress [43]. Moreover, it has been reported that shoot length and fresh weight significantly increased in PIP introduced plants under salt stress [43]. They indicated that transgenic plants developed by transferring the GmPIP1;6 genes to soybean plant (*Glycine max*) are more tolerant of salt stress than wild-type plants. In another study, overexpression of GhPIP2;7 in Arabidopsis increased the tolerance of the transgenic plant to drought stress [41]. It has also been reported that the introduced plasma membrane intrinsic gene may play a role in leaf development and in absorption in remaining water under drought stress and carry certain substrates instead of water to protect plants from drought. Additionally, seed germination rates increased in most of the transgenic plants produced by introducing plasma membrane intrinsic genes [44]. Transgenic *Arabidopsis thaliana* plants developed with introduction of BvCOLD1 gene from *Beta vulgaris* have been observed to increase in germination and early development rates at 10°C and under different abiotic stress conditions such as NaCl, LiCl, and sorbic acid [44]. Plant aquaporins function by settling in different subcellular locations. Liu [42] reported that one of the most abundant plasma membrane proteins in transgenic rice leaves and roots is OsPIP1 and that its overexpression causes changes in many physiological properties of transgenic plants depending on the stress dose. Rice seed yield, salt resistance, root hydraulic conductivity and seed germination rates increased with the moderate expression of OsPIP1 [42]. Beside plasma membranes, they can be localized in mitochondria, endoplasmic reticulum, chloroplast and vacuole [45].

2.3 Osmoprotectants

Trehalose is a non-reducing disaccharide sugar found in bacteria, fungi, invertebrates, including plants. The accumulation of trehalose, which is an important component of plant growth, affecting sugar metabolism and causes an osmoprotectant effect under abiotic stresses [46]. Trehalose is considered as an osmoprotectant as it counteracts against the effects of dehydration of plants caused by drought, salinity or low temperature [47]. Studies have shown that enzymes, proteins and lipid membranes can be stabilized by trehalose and that biological structures can be protected from damage by trehalose during abiotic stress [18]. Trehalose is an osmolite that both regulates osmosis and protects macromolecules. In higher plants, overexpression of trehalose-6-phosphate synthases (TPS) and/or trehalose-6 phosphate phosphatases genes originated from microorganisms has led to tolerance to various abiotic stresses. In addition, several genes involved in trehalose metabolism have been used together to improve the stress tolerance of some plants. The results show that the increased production of trehalose with overexpression of the bifonional fusion gene (TPSP) of the trehalose-6-phosphate synthase and trehalose-6-phosphate phosphatase genes increases the soluble sugar content and increases the tolerance to drought and salt stress in rice [48]. As a result of the expression of

Gene Action	Gene Source	Promoter	Target Traits	Trans-formed Plant	Transfer Method	Ref.
Aquaporin (plasma membrane intrinsic proteins)	TdPIP1 and TdPIP2 from *Triticum turgidum*	CaMV35S	Osmotic stress Salinity	*Nicotiana tabacum*	*Agrobacterium tumefaciens*	[40]
Aquaporin (plasma membrane intrinsic protein)	GhPIP2;7 from *Gossypium hirsutum*	GhPIP2;7	Drought	*Arabidopsis thaliana*	*Agrobacterium tumefaciens*	[41]
Aquaporin (plasma membrane intrinsic protein)	OsPIP1 from *Oryza sativa*	UBQ	Salinity	*Oryza sativa*	*Agrobacterium tumefaciens* EHA101	[42]
Aquaporin (plasma membrane intrinsic protein)	GmPIP1;6 from *Glycine max*	CaMV35S	Salinity	*Glycine max*	*Agrobacterium tumefaciens*	[43]
Aquaporin (plasma membrane intrinsic protein)	BvCOLD1 from *Beta vulgaris*	CaMV35S	Cold	*Arabidopsis thaliana*	*Agrobacterium tumefaciens* C58C1	[44]

Table 3.
Summary of transgenic plants over-expressing aquaporin genes for abiotic stress tolerance.

the TPSP fusion gene, tomatoes that accumulate high amounts of trehalose in their leaves exhibited a tolerance to drought and salt stress, and showed higher photosynthetic rates under salt stress conditions than wild-type plants [49]. Proline, another osmoprotectant, is an amino acid that plays a role in plant functions exposed to various abiotic stresses. Proline accumulates in response to many environmental stresses such as salinity, drought, heavy metals, and there is a positive correlation between plant stress and proline accumulation. Proline protects proteins and other molecules in the cell against denaturation by stabilizing them. In addition to being an excellent osmolyte, it also functions as a metal chelator, antioxidant defense molecule and signal molecule [20]. In addition, it plays an important role in the protection of subcellular structures, adjustment of cell turgor and osmatic balance, prevention of electrolyte leakage by stabilizing membranes, and balancing the concentration of reactive oxygen species (ROS). Proline synthesis in plants is carried out by two important pathways; glutamate pathway and orinithine pathway. However, among these, the glutamate pathway is the primary pathway of proline accumulation during osmatic stress [50]. This reaction is catalyzed in most plants by the enzyme Δ’-pyrroline-5 carboxylate synthetase (P5CS) encoded by two genes and the enzyme Δ’-pyrroline-5-carboxylate reductase (P5CR) encoded by a gene. In addition to the effects of proline on the physiology and biochemical processes of various plants, a high amount of endogenous proline has been shown to act as a regulator and/or signaling molecule that can alter the transcription levels of stress-related genes such as ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD) and glutathione reductase (GR) [51, 52]. A P5CS gene that can be transferred in addition to the P5CS genes present in plants by genetic transformation helps plants to adapt to osmatic conditions by regulating proline accumulation, and also supports legume plants for optimum nodule formation and maintenance of nodule function [53]. Ghanti [54] reported that when they transferred the *Vigna aconitifolia* P5CS gene to *Cicer arietinum* plant, more proline accumulation in the leaves and roots of the plants and higher chlorophyll content under salt stress, as well as increased tolerance to salinity. It has also been shown that a single copy of P5CS transferred to plants can be effective in the development of tolerance under stress, regardless of the integration position or copy number [54]. P5CS-transferred transgenic plants, when exposed to high salt stress, grew to maturity by showing good growth and flowering, but control plants died within a certain period of time under the same conditions [55]. Moreover, introduction of a mutagenized version (P5CSF129A) of the *Vigna aconitifolia* P5CS gene to pigeonpea (*Cajanus cajan*), both proline accumulation in plants increased and tolerance to salt stress enhanced. In addition to improved growth performances and higher chlorophyll and relative water content, especially transgenic plants also showed lower levels of lipid peroxidation under 200 mM NaCl.

Plants synthesize and accumulate another osmoprotectant, glycine betaine (GB), in response to environmental stresses. Glycine betaine, which also plays a role in osmoregulation, protects the activity of macromolecules and the integrity of the membranes against stresses and scavenge the ROS. Glysine betaine synthesis reactions are catalyzed in two steps by the enzymes choline monooxygenase (CMO) and NADC-linked betaine aldehyde dehydrogenase. Many studies have been carried out showing that the abiotic stress tolerance of transgenic plants obtained as a result of isolating these genes in the biosynthetic pathway and transferring them to other plants has developed. Several osmoprotectant target for gene transfer approach are listed in **Table 4**.

2.4 Calcineurin B-like proteins (CBLs), calcium-dependent protein kinases (CDPKs)

Plants perceive the stimuli from the external environment through receptors in their membranes. After transmitting this signal, they respond to this stimulus. Ca^{2+} acts as one of the secondary messengers in the ABA signaling pathway, which plays an active role in this process. Calcium acts as a central link in numerous signaling pathways, and thus external signals such as hormone, light, biotic and abiotic stresses cause changes in Ca^{2+} concentration in the cell. The fluctuation in the cytosolic calcium concentration (calcium signatures) initiates various biochemical and physiological processes in the cell. Cellular calcium sensor molecules such as calcineurin B-like proteins (CBLs), calcium-dependent protein kinases (CDPKs) and calcium-dependent protein kinases (CIPKs), calmodulin (CaM) detect Ca^{2+} signals and enable Ca^{2+} ions to be transmitted to downstream pathways. Especially, calcineurin family of B-like proteins (CBLs) interacts and activates calcium-dependent protein kinases (CIPKs). Many studies have shown that this regulatory pathway plays a role in plants in response to environmental stresses (**Table 5**). It has been reported that overexpression of GmCBL1 in Arabidopsis increases tolerance to both high salt and drought stress in transgenic plants. It has also been shown that hypocotyl elongation is promoted under light conditions by overexpression of GmCBL1. Calcineurin B-like proteins (CBLs) can regulate stress tolerance by activating stress-related genes such as DREB1A, DREB2A, RD29A and KIN1, while also controlling hypocotyl development by changing the expression of genes related to gibberellin biosynthesis [68]. BdCIPK31, a CIPK gene belonging to *Brachypodium distachyon*, decreased under polyethylene glycol, NaCl, H_2O_2 and abscisic acid (ABA) treatments. Transgenic tobacco plants overexpressing BdCIPK31 developed tolerance to drought and salinity and lost less water than control plants under dehydration conditions. BdCIPK31 affects the expression of some ion channels and transporter

Gene Action	Gene Source	Promoter	Target Traits	Trans-formed Plant	Transfer Method	Ref.
Pyrroline-5-carboxylate synthetase	P5CS from *Arabidopsis thaliana*	CaMV35S	Salinity	*Solanum tuberosum*	*Agrobacterium tumefaciens* C58C1	[53]
Pyrroline-5-carboxylate synthetase	P5CS from *Vigna aconitifolia*	CaMV35S	Salinity	*Oryza sativa*	*Agrobacterium tumefaciens* strain LBA 4404	[56]
Pyrroline-5-carboxylate synthetase	P5CS from *Vigna aconitifolia*	CaMV35S	Salinity	*Cicer arietinum*	*Agrobacterium tumefaciens* LBA4404	[57]
Pyrroline-5-carboxylate synthetase	VaP5CSF129A from *Vigna aconitifolia*	CaMV35S	Drought	*Swingle citrumelo*	-	[54]
Pyrroline-5-carboxylate synthetase	MtP5CS3 from *Medicago truncatula*	CaMV35S	Salinity	*Medicago truncatula*	-	[58]
Pyrroline-5-carboxylate synthetase	P5CSF129A from *Vigna aconitifolia*	CaMV35S	Salinity	*Cajanus cajan*	*Agrobacterium tumefaciens* LBA4404	[59]
Trehalose-6-phosphate synthase	OsTSP1 from *Oryza sativa*	CaMV35S	Salinity Drought Cold	*Oryza sativa*	*Agrobacterium tumefaciens* AGL0	[60]
Trehalose-6-phosphate synthase/ phosphatase	Recombinant fusion TPSP from *E. coli*	UBQ	Drought Salinity	*Oryza sativa*	-	[51]
Trehalose-6-phosphate synthase	TPS1 from yeast	StDS2	Drought	*Solanum tuberosum*	*Agrobacterium tumefaciens* strain C58C1	[61]
Trehalose-6-phosphate synthase/ phosphatase	Recombinant fusion TPSP from *E. coli*	CaMV35S	Drought Salinity	*Solanum lycopersicum*	*Agrobacterium tumefaciens* LBA4404	[52]
Glycine betaine	codA (Choline oxidase) from *Arthrobacter globiformis*	SWPA2	Cold Salinity	*Solanum tuberosum*	*Agrobacterium tumefaciens* strain AGL0	[62]
Glycine betaine	BADH from *Spinacia oleracea*	CaMV35S	Salinity Cold	*Ipomoea batatas*	*Agrobacterium tumefaciens* LBA4404	[63]
Glycine betaine	betaine aldehyde dehydrogenase gene (AhBADH) from *Atriplex hortensis*,	CaMV35S	Salinity	*Poncirus trifoliata*	*Agrobacterium tumefaciens* LB4404	[64]

Gene Action	Gene Source	Promoter	Target Traits	Trans-formed Plant	Transfer Method	Ref.
Glycine betaine	betA gene from *E. coli*	UBQ	Salinity	*Triticum aestivum*	*Agrobacterium tumefaciens* LBA4404	[65]
Glycine betaine	betA gene from *E. coli*	UBQ	Drought	*Triticum aestivum*	*Agrobacterium tumefaciens* LBA4404	[66]
Glycine betaine	Mpgsmt and Mpsdmt from *Methanohalophilus portucalensis*	CaMV35S	Drought Salinity	*Arabidopsis thaliana*	*Agrobacterium tumefaciens* GV3101	[67]
Glycine betaine	codA from *Arthrobacter globiformis*	-	Heat	*Solanum lycopersicum*	*Agrobacterium tumefaciens* EHA101	[68]
Glycine betain	BADH betaine aldehyde dehydrogenase from *Spinacia oleracea*	CaMV35S	Heat	*Lycopersicon esculentum*	*Agrobacterium tumefaciens* LBA4404	[69]
Glycine betaine	codA from *Arthrobacter globiformis*	CaMV35S	Salinity	Solanum *lycopersicum*	*Agrobacterium tumefaciens* EHA101	[70]

Table 4.
Summary of transgenic plants over-expressing osmoprotectant-related genes for abiotic stress tolerance.

Gene Action	Gene Source	Promoter	Target Traits	Trans-formed Plant	Transfer Method	Ref.
CIPK	OsCIPK23m from *Oryza sativa*	UBQ	Drought	*Oryza sativa*	*Agrobacterium tumefaciens* EHA105	[71]
CBL-CIPK	BnCBL1-BnCIPK6 from *Brassica napus*	CaMV35S	Salinity	*Arabidopsis thaliana*	*Agrobacterium tumefaciens* floral dip method	[72]
CBL protein	GmCBL1 from *Glycine max*	CaMV35S	Salinity Drought	*Arabidopsis thaliana*	*Agrobacterium tumefaciens* C58C1	[68]
CBL protein	PeCBL10 from *Populus euphratica*	CaMV35S	Salinity Drought Cold	*Arabidopsis thaliana*	*Agrobacterium tumefaciens* GV3101	[73]
CBL protein	PtCBL10A PtCBL10B from *Populus trichocarpa*	CaMV35S	Salinity	*P. davidiana* × *P. bolleana*	*Agrobacterium tumefaciens* EHA105	[74]
CBL protein	NsylCBL10 from *Nicotiana sylvestris*	CaMV35S	Salinity	*Arabidopsis thaliana*	*Agrobacterium tumefaciens* EHA105	[70]
CBL protein	SpCBL6 from *Stipa purpurea*	CaMV35S	Cold	*Arabidopsis thaliana*	*Agrobacterium tumefaciens* strain GV3101	[75]
CIPK	BdCIPK31 from *Brachypodium distachyon*	CaMV35S	Drought Salinity	*Nicotiana tabacum*	*Agrobacterium tumefaciens* EHA105	[69]

Table 5.
Summary of transgenic plants over-expressing calcineurin B-like (CBL) and CBL-interacting protein kinase (CIPK) genes for abiotic stress tolerance.

genes under high salinity stress. Scavenging of reactive oxygen species and osmolite accumulation has been increased with the overexpression of BdCIPK31, thereby mitigate oxidative and osmotic damages [69]. In another transformation study performed with CBL, overexpression of SpCBL6 gene isolated from *Stipa purpurea* has been shown to increase tolerance to cold stress in transgenic *Arabidopsis thaliana*. In further analysis, it was reported that SpCBL6 overexpressing plants had increased water potential and photosynthetic efficiency (Fv/Fm) and decreased ion leakage compared with wild-type plants after cold application. In another study, NsylCBL10, a CBL gene belonging to *Nicotiana sylvestris*, has increased tolerance to high salt stress by maintaining Na^+ balance. Ion content analyzes revealed that transgenic plants maintained the lower Na^+/K^+ ratio in the roots and the higher Na^+/K^+ ratio in the shoots with the overexpression of NsylCBL10 [70]. Similarly, overexpression of PtCBL10A or PtCBL10B in transgenic poplar plants under salinity stress played an important role in tolerance to salt stress by maintaining ion homeostasis in shoot tissues [74]. Overexpression of BnCIPK6, its activated form BnCIPK6M and BnCBL1 CBL genes also increased tolerance to high salinity and low phosphate conditions in transgenic Arabidopsis plants, demonstrating how important the interaction of BnCBL1 and BnCIPK6 is for signaling pathways.

2.5 Heat shock protein

Heat shock proteins (HSPs) were originally identified as proteins that respond to high temperature conditions and involve in eukaryotes and prokaryotes. However, many studies have revealed the relationship of these proteins with various abiotic stresses. Heat shock proteins are powerful chaperones produced in response to many physiological and environmental stresses. In general, heat shock proteins function in the cytoplasm, while they are also located in organelles such as the nucleus, mitochondria, chloroplasts, endoplasmic reticulum [22]. These proteins that are evolutionarily conserved in prokaryotes and eukaryotes are also abundant in plants. HSPs divided into 5 different groups including HSP100, HSP90, HSP70, HSP60 and sHSP (small heat shock protein) according to their molecular weights. Among these groups, small heat shock proteins are also induced by other stresses such as drought, salinity and cold stress, and also play an active role in processes such as seed germination, embryogenesis and fruit development [76]. Transgenic *Arabidopsis thaliana* plants introduced with the chloroplastic sHSP26 gene from wheat (*Triticum aestivum*) show tolerance to higher temperatures than wild-type plants. In addition, these plants show more photosynthetic pigment accumulation and higher biomass and seed yield [76]. Another sHSP26 gene belonging to rice (*Oryza sativa*), which encodes small heat shock protein localized in chloroplast, provided less electrolyte leakage in transgenic plants under heat stress, while it played a role in the accumulation of thiobarbituric acid reactive substances in plants. Transgenic plants showed more photosystem II (PSII) (Fv/Fm) photochemical activity under temperature stress at 42°C than control plants [77]. There is much evidence that small heat shock proteins are also associated with scavenging reactive oxygen species. Thermo tolerance of the plants increased in correlation with ROS scavenging by overexpression of the OsHSP18.6 in transgenic plants. In addition, with the overexpression of OsHSP18.6, malondialdehyde (MDA) levels decreased and CAT and SOD activities increased under heat and drought stress [78]. HSP70, another heat shock protein, stabilizes the proteins and also prevents their denaturation and aggregation. In addition, it maintains protein homeostasis by taking part in processes such as the transport of certain proteins in the cell, folding of newly synthesized proteins, denaturation of unwanted proteins, and the formation and separation of protein complexes [79]. In rice plants produced by introducing anti-apoptotic genes associated with programmed cell death (PCD) such as AtBAG4 (*Arabidopsis thaliana*), Hsp70 (Citrus tristeza virus) and p35 (Baculo virus), the salinity tolerance of rice plants has increased and along with this, ROS production and plant damage have been reduced [79]. In transgenic sugarcane (*Saccharum spp*. Hybrid) plants transformed with the HSP70 gene from *Erianthus arundinaceus*, the expression of stress-related genes increases with the overexpression of the HSP70 gene. These transgenic plants have higher germination ability and higher chlorophyll content under salt stress [80]. HSP90 is one of the proteins that have been well preserved in the evolutionary process and found in large amounts. As one of the important components of the stress response, they are actively involved in various processes such as signal transduction, protein degradation, cell cycle control and protein traffic (**Table 6**). Xu [83] reported that overexpression of HSP90 genes (GmHsp90A2, GmHsp90A4, GmHsp90B1, GmHsp90C1.1 and GmHsp90C2.1) in *Arabidopsis thaliana* plants reduced the damage caused by abiotic stress. In addition, high proline accumulation has been detected in HSP90 transgenic events and it has been reported that HSP90 genes may affect proline synthesis in relation to the AtP5CS1 (pyrroline-5-carboxylate synthases 1) gene responsible for proline synthesis [83]. Polyamine homeostasis is regulated by many different regulatory mechanisms such as transport, turnover and modification of amino groups. At the same time, these molecules affect the extent of heat shock proteins synthesis under increasing temperatures. It has been reported that silencing of HSP90 genes in *Arabidopsis thaliana* plant increased the levels of soluble spermidine (S Spd), acetylated Spd (N8-acetyl-Spd) and acetylated spermine (N1 acetyl-Spm). Moreover,

Gene Action	Gene Source	Promoter	Target Traits	Trans-formed Plant	Transfer Method	Ref.
Heat Shock	GHSP26 from *Gossypium arboreum*	CaMV35S	Drought	*Gossypium hirsutum*	*Agrobacterium tumefaciens* LBA4404	[81]
Heat Shock	OsHSP6 from *Oryza sativa*	CaMV35S	Oxidative stress Heat	*Festuca arundinacea*	*Agrobacterium tumefaciens* EHA105	[77]
Heat shock	TaHSP26 from *Triticum aestivum*	CaMV35S TaHSP26	Heat	*Arabidopsis thaliana*	*Agrobacterium tumefaciens* AGL1	[76]
Heat Shock	ZmHSP16.9 from *Zea mays*	CaMV35S	Heat	*Nicotiana tabacum*	*Agrobacterium tumefaciens* LBA4404	[82]
Heat Shock	GmHsp90s from *Glycine max*	CaMV35S	Osmotic stress Salinity	*Arabidopsis thaliana*	*Agrobacterium tumefaciens* EHA105	[83]
Heat Shock	EaHSP70 from *Erianthus arundinaceus*	Port Ubi 2.3	Salinity Water deficiency	*Saccharum spp. hybrid*	*Agrobacterium tumefaciens* LBA4404	[80]
Heat Shock	HSP70 from *Citrus tristeza* virus (CTV)	UBQ	Salinity	*Oryza sativa*	*Agrobacterium tumefaciens* AGL1	[84]
Heat Shock	sHSP18.6 from *Oryza sativa*	CaMV35S	Heat Drought Salt Cold	*Oryza sativa*	*Agrobacterium tumefaciens* EHA101	[78]

Table 6.
Summary of transgenic plants over-expressing heat shock genes for abiotic stress tolerance.

HSP90s and polyamine (PA) oxidases (PAOs) organize the acetylation, oxidation and PA/H_2O_2 homeostasis of polyamines [85].

2.6 Transcription factors

The characterization and identification of genes involved in the stress response of plants is a prerequisite for producing genetically modified plants that are tolerant of various stresses. The fact that these identified genes are regulatory genes responsible for regulation become prominent as an effective approach in terms of ensuring the efficient control of many other genes involved in stress management. The most important regulatory gene candidates that can be used in the development of stress-tolerant plants are transcription factors (TFs) that also regulate the expression of stress genes. Transcription factors belonging to many different families such as AP2/EREBP, NAC, WRKY, MYB, bZIP are involved in various abiotic and biotic stress processes.

NAC proteins stand out as one of the largest family of transcription factors in plants. In particular, they perform vital tasks in the developmental processes of plants and their response to environmental stresses. They play a role in the regulation of many processes such as cell division, flower development, lateral root development, senescence, phytohormone homeostasis, secondary cell wall formation, abiotic and biotic stress responses. There are many studies that develop tolerance to abiotic stress as a result of transformation of plants with NAC transcription factors. Huang [86] transferred the TaNAC29 gene, which they isolated from wheat

(*Triticum aestivum*), to *Arabidopsis thaliana* plants. It has been reported that plants have developed tolerance to high salt and dehydration as a result of the overexpression of this gene. Transgenic plants accumulated less MDA under stress, but showed higher amounts of SOD and CAT activities. This shows that ABA-signaling pathway and antioxidant enzyme mechanisms are involved in NAC29-mediated stress tolerance. Similarly, overexpression of the TaNAC67 gene isolated from wheat plant increased the expression of many multiple abiotic stress response genes such as DREB1A, DREB2A, RD29A, RD29B, RD22, COR15 and Rab18 simultaneously in transgenic plants. Thus, improved cell membrane stability, higher chlorophyll content and Na^+ efflux rates, and enhanced tolerance to drought, salinity and cold stress were observed in plants [87, 88].

Another important transcription factor family that is widely distributed in plants is MYB TFs. Many MYB transcription factors function in many physiological and biochemical processes, including cell development, cell cycle, hormone cycle, primary and secondary metabolism and signal transduction, biotic and abiotic stresses [89]. There are many studies reporting that transgenic plants enhance tolerance by overexpression of MYB transcription factors (**Table 7**). GmMYBJ1 introduced transgenic Arabidopsis plants have been shown to have an increased tolerance to drought and salinity compared to wild-type plants. At the same time, these plants showed higher plant height and less water loss rate when exposed to dehydration and cold stresses. Moreover, less MDA accumulation was detected in transgenic plants under stress with the overexpression of MYB [115]. TaMYB3R1 (*Triticum aestivum*) transcription factors provided drought tolerance by promoting the closure of stomata and limiting transpiration in Arabidopsis plants under stress [116].

The basic leucine zipper (bZIP) is a class of transcription factors with a conserved bZIP domain consisting of a region for DNA binding and nuclear localization at the N-terminus and a lysine-rich region for dimerization at the C-terminus. Similar to other TFs, it not only plays a role in developmental processes but also plays a regulatory role in the response to various abiotic stresses. In this context, *Nicotiana tabacum* plants in which the LrbZIP (*Nelumbo nucifera*) transcription factor was transferred have developed tolerance to salt stress by showing less electrolyte leakage and higher chlorophyll content under salt stress [137]. Another bZIP, group S transcription factor CaBZ1 (*Capsicum annuum*), reduced the rate of water loss of *Solanum tuberosum* plants under drought stress and increased the expression of ABA and stress-related genes with faster closure [110].

Large regulatory WRKY transcription factors protein family contain two conserved WRKY domains, consisting of approximately 60 amino acids containing the amino site sequence WRKYGQK at the N-terminus and zinc-finger motif at the C-terminus [138]. WRKYs manage many developmental and physiological processes including leaf senescence, regulation of biosynthetic pathways, hormone signaling, embryogenesis, trichome development. Various WRKY transcription factors are also known to be involved in abiotic stress responses. Overexpression of transcription factors, such as ZmWRKY33 (*Zea mays*), that activate various stress-induced genes plays important roles in the acquisition of stress tolerance [131]. Moreover, WRKY TFs increased physiological characteristics such as seed germination, root length and chlorophyll content in transgenic plants under stress conditions [132, 134]. Transgenic *Arobidopsis thaliana* plants developed with the transfer of TaWRKY79 (*Triticum aestivum*) gene have gained tolerance to salinity and ion stress with the improvement in their capacity to elongate their primary roots under salt stress. Transgenic plants developed by the transfer of the GhWRKY34 (*Gossypium hirsutum*) gene have similarly increased tolerance to salt stress, on the other hand, they contain a lower rate of Na^+/K^+ in their leaves and roots [134]. They also act as regulators in the ABA-related pathway [132].

Gene Action	Gene Source	Target Traits	Transformed Plant	Transfer Method	Ref.
AP2-ERFBP	GmERF3 from *Glycine max*	Salinity Drought	*Nicotiana tabacum*	Agrobacterium-mediated transformation	[90]
DREB1	OsDREB1D from *Oryza sativa*	Cold Salinity	*Arabidopsis thaliana*	*Agrobacterium tumefaciens* GV3101	[90]
AP2-ERFBP	SbDREB2 from *Sorghum bicolor*	Drought	*Oryza sativa subsp indica,*	*Agrobacterium tumefaciens* LBA4404	[91]
DREB1A	AtDREB1A from *Arabidopsis thaliana*	Drought	*Glycine max*	Particle-bombardment transformation	[92]
AP2-ERFBP	LcDREB2 from *Leymus chinensis*	Salinity	*Arabidopsis thaliana*	*Agrobacterium tumefaciens* EHA105	[93]
AP2-ERFBP	OsERF4a from *Oryza sativa*	Drought	*Oryza sativa,*	*Agrobacterium tumefaciens* LBA4404	[94]
AP2ERFB	EaDREB2 from *Erianthus arundinaceus*	Drought Salinity	*Saccharum spp. hybrid,*	*Agrobacterium tumefaciens* LBA4404	[95]
DREB1A	AtDREB1A from *Arabidopsis thaliana*	Drought	*Oryza sativa*	*Agrobacterium tumefaciens* LBA4404	[96]
AP2-ERFBP	LcERF054 from *Lotus corniculatus*	Salinity	*Arabidopsis thaliana*	*Agrobacterium tumefaciens* GV3101 floral dip	[97]
AP2-ERFBP	TaPIE1 from *Triticum aestivum*	Freezing	*Triticum aestivum*	Biolistic bombardment	[98]
AP2-ERFBP	VrDREB2A from *Vigna radiata*	DroughtSalinity	*Arabidopsis thaliana*	*Agrobacterium tumefaciens* EHA105 floral dip	[99]
AP2-ERF	OsEREBP1 from *Oryza sativa*	Drought	*Oryza sativa,*	*Agrobacterium tumefaciens* EHA105	[100]
AP2-ERFBP	SsDREB from *Suaeda salsa*	Salt Drought	*Nicotiana tabacum*	*Agrobacterium tumefaciens* EHA105	[101]
bZIP	PtrABF from *Poncirus trifoliata*	Drought	*Nicotiana tabacum*	*Agrobacterium tumefaciens* EHA105	[102]
bZIP	GmbZIP1 from *Glycine max*	DroughtSalinity Cold	*Nicotiana tabacum-Arabidopsis thaliana*	*Agrobacterium tumefaciens* EHA105	[103]
bZIP	OsbZIP39 from *Oryza sativa*	ER stress	*Oryza sativa*	*Agrobacterium tumefaciens* EHA105	[104]
bZIP	MsbZIP from *Medicago sativa*	Drought Salinity	*Nicotiana tabacum*	*Agrobacterium tumefaciens* LBA4404	[105]
bZIP	ZmbZIP72 from *Zea Mays*	Drought Salinity	*Arabidopsis thaliana*	*Agrobacterium tumefaciens* GV3101	[106]
bZIP	LrbZIP from *Nelumbo nucifera*	Salinity	*Nicotiana tabacum*	*Agrobacterium tumefaciens* GV3101 leaf disc method	[107]

Gene Action	Gene Source	Target Traits	Transformed Plant	Transfer Method	Ref.
bZIP	OsbZIP71 from *Oryza sativa*	Drought Salinity	*Oryza sativa*	*Agrobacterium tumefaciens* AGL1	[108]
bZIP	TabZIP60 from *Triticum aestivum*	DroughtSalinity Freezing	*Arabidopsis thaliana*	*Agrobacterium tumefaciens* GV3101 floral dip	[109]
bZIP	CaBZ1 from *Capsicum annuum*	Drought	*Solanum tuberosum*	*Agrobacterium tumefaciens* LBA4404	[110]
bZIP	AtTGA4 from *Arabidopsis thaliana*	Drought	*Arabidopsis thaliana*	*Agrobacterium tumefaciens* floral dip	[111]
MYB	AtMYB15 from *Arabidopsis thaliana*	Drought Salinity	*Arabidopsis thaliana*	Agrobacterium-mediated floral dip	[112]
MYB	TaPIMP1 from *Triticum aestivum*	Drought Salinity	*Nicotiana tabacum*	Agrobacterium-mediated leaf disc method	[113]
MYB	LcMYB1 from *Leymus chinensis*	Salinity	*Arabidopsis thaliana*	*Agrobacterium tumefaciens* EHA105	[114]
MYB	GmMYBJ1 from *Glycine max*	Drought Cold	*Arabidopsis thaliana*	*Agrobacterium tumefaciens* EHA105	[115]
MYB	TaMYB3R1 from *Triticum aestivum*	Drought Salinity	*Arabidopsis thaliana*	*Agrobacterium tumefaciens* EHA105	[116]
MYB	LeAN2 from *Lycopersicum esculentum*	Heat	*Lycopersicum esculentum*	*Agrobacterium tumefaciens* LBA4404 leaf disc method	[117]
MYB	SbMYB2 and SbMYB7 from *Scutellaria baicalensis*	Salinity	*Nicotiana tabacum*	-	[118]
NAC	ONAC063 from *Oryza sativa*	Salinity Osmotic stress	*Arabidopsis thaliana*	*Agrobacterium tumefaciens* GV3101 floral dip	[119]
NAC	GmNAC20 GmNAC11 from *Glycine max*	Salt Freezing	*Arabidopsis thaliana*	Agrobacterium-mediated vacuum infiltration	[120]
NAC	ZmSNAC1 from *Zea may*	Cold Salinity Drought	*Arabidopsis thaliana*	*Agrobacterium tumefaciens* GV3101 floral dip	[121]
NAC	TaNAC2a from *Triticum aestivum*	Drought	*Nicotiana tabacum*	Agrobacterium-mediated transformation	[122]
NAC	AhNAC3 from *Arachis hypogaea*	Drought	*Nicotiana tabacum*	-	[123]
NAC	SNAC1 from *Oryza sativa*	Drought Salinity	*Triticum aestivum*	Bombardment with a biolistic gun	[124]
NAC	OsNAP from *Oryza sativa*	Cold Salinity Drought	*Oryza sativa*	*Agrobacterium tumefaciens* EHA105	[125]

Gene Action	Gene Source	Target Traits	Transformed Plant	Transfer Method	Ref.
NAC	TaNAC67 from *Triticum aestivum*	Cold Salinity Drought	*Arabidopsis thaliana*	*Agrobacterium tumefaciens* floral infiltration	[87]
NAC	TaNAC29 from *Triticum aestivum*	Drought Salinity	*Arabidopsis thaliana*	*Agrobacterium tumefaciens* EHA105	[86]
NAC	MLNAC5 from *Miscanthus lutarioriparius*	Drought Cold	*Arabidopsis thaliana*	*Agrobacterium tumefaciens* EHA105 floral dip	[126]
NAC	TaNAC47 from *Triticum aestivum*	Salt Cold Drought	*Arabidopsis thaliana*	*Agrobacterium tumefaciens* GV3101	[127]
WRKY	OsWRKY45 from *Oryza sativa*	Drought Salinity	*Arabidopsis thaliana*	*Agrobacterium tumefaciens* LBA4404	[128]
WRKY	VvWRKY11 from *Vitis vinifera*	Drought	*Arabidopsis thaliana*	*Agrobacterium tumefaciens* GV3101	[129]
WRKY	AtWRKY28 from *Arabidopsis thaliana*	Salinity	*Arabidopsis thaliana*	*Agrobacterium tumefaciens* EHA105 floral dip	[130]
WRKY	ZmWRKY33 from *Zea may*	Salinity	*Arabidopsis thaliana*	*Agrobacterium tumefaciens* EHA105	[131]
WRKY	TaWRKY79 from *Triticum aestivum*	Drought	*Arabidopsis thaliana*	*Agrobacterium tumefaciens* GV3101 floral dip	[132]
WRKY	BdWRKY36 from *Brachypodium distachyon*	Drought	*Nicotiana tabacum*	*Agrobacterium tumefaciens* EHA105 leaf disc method	[133]
WRKY	GhWRKY34 from *Gossypium hirsutum*	Salinity	*Arabidopsis thaliana*	*Agrobacterium tumefaciens* LBA4404 floral dip	[134]
WRKY	MtWRKY76 from *Medicago truncatula*	Drought Salinity	*Medicago truncatula*	Agrobacterium-mediated transformation	[135]
HomeoBox 4	HaHB4 from *Helianthus annuus*	Drought	*Glycine max-Triticum vulgaris*	-	[136]

Table 7.
Summary of transgenic plants over-expressing transcription factor genes for abiotic stress tolerance.

The Apetala2/Ethylene responsive factor family is characterized by the highly conserved APETALA2 (AP2)/Ethylene Responsive Element Binding Factor (EREB) domain containing 40–70 amino acid sequences involved in DNA binding. AP2/ERFs are divided into four main groups; Apetala2 (AP2), related to abscisic acid intensive 3/Viviparous (RAV), dehydration-responsive element binding protein (DREB), ethylene responsive factor (ERF). These transcription factors play an important role in the regulation of both plant growth and the response to various stresses [139]. The AP2 family of Ethylene Responsive Element Binding Factors supports the survival of plants under stress by activating jasmonate and abscisic acid signaling pathways. The amount of α linolenate, some jasmonate derivatives and abscisic acid increased in rice plants, in which the EREB1 transcription factor gene from AP2/ERF transcription factor family was transferred. The role of this gene family in the response to both biotic and abiotic stress is very important for the regulation of multiple stress tolerance for genetic engineering [100]. Dehydration responsive element-binding proteins (DREB) cooperated with the genes involved in polyamine biosynthesis to enable plants to tolerate salt stress [93]. Thanks to the transfer of another subgroup of ERF transcription factor LcERF054 (*Leymus chinensis*) gene, an increase in relative moisture content, dissolved sugar and proline amount occurred in Arabidopsis plants. Moreover, these plants have developed salinity stress tolerance. At the same time, the expression of the hyperosmatic salinity stress response genes COR15A, LEA4-5, P5CS1 and RD29A increased with the expression of the LcERF054 gene [97]. It has been reported that DREB transcription factor is used effectively in stress tolerance like other transcription factors belonging to the Apetala2/Ethylene responsive factor family [96].

3. Genome editing applications for abiotic stress tolerance in plants on emphasis to recent CRISPR applications

Since the bacterial defense mechanism against biotic agents as viruses based on the detection and elimination of invader nucleic acids suggested as novel tool for site specific genome editing tool by Jinek [140] in 2012, CRISPR/Cas9 is used in developing crop plants for abiotic stress tolerance as well as many other plant biotechnology applications. All of the abiotic stress tolerance mechanisms mentioned earlier in this chapter which were targets for gene transfer applications can be targeted also by CRISPR/Cas9 system as it allows both induction (CRISPRactivation) or repression (CRISPRinterference) of genes. Therefore, it can be used for activation of tolerance genes (T genes) as well as suppressing sensitivity (S genes) genes [141].

Targeting hormonal regulation for abiotic stress tolerance is one of the viable options. Abscisic acid (ABA) regulates physiological processes as seed dormancy, stomatal closure, plant development, as well as plant responses to environmental stimuli and multiple stresses. 9-cis-epoxycarotenoid dioxygenase (NCED), which is responsible from regulation of ABA in rice, targeted for multiple abiotic stress tolerance including salinity, drought and subsequent H_2O_2 stress. *nced3* mutant rice plants were very susceptible to these stresses as they express very low level of ABA. Overexpression of *OsNCEB3* in rice by CRISPRa system increased ABA accumulation and tolerance to salinity and drought stresses [142]. Downstream genes of the ABA signaling pathway in response to drought stress are regulated by ABA-responsive element binding proteins/ABRE binding factors (AREB/ABFs). CRISPRa system, set by CRISPR/dCAS9 fusion with histone acetyltransferase which promotes gene expression, is used to enhance drought resistance of Arabidopsis plants through activation of endogenous promoter of *AREB1* [143].

Transcription factors (TFs) regulate the expression patterns of the genes on promoter regions. Therefore, they are important targets for both genetic engineering and genome editing applications in abiotic stress tolerance of plants. CRISPRi system designed for 696–amino acid B-type response regulator transcription factor encoding *OsRR22* gene successfully improved salinity tolerance of rice plants since its involvement in cytokinin signal transduction and metabolism [144]. In maize, ARGOS genes regulate ethylene signal transduction. It is known to be a negative regulator of ethylene responses. ARGOS genes are reported to increase ethylene sensitivity of Arabidopsis and maize when they were overexpressed through transgenic approach and enhance drought tolerance in plants [145]. Likewise, 400 inbred lines of maize were evaluated for ARGOS8 mRNA expression comparing to the transgenic ARGOS8 events. All inbred lines presented less expression than transgenic events. Therefore, overexpression of ARGOS8 also achieved by using CRISPRa approach and improved traits as drought tolerance and grain yield were generated [146]. Auxin response factors (ARFs) undertake important regulation roles in auxin response gene expression. Differential expression of ARFs were shown to involve in many physiological processes and abiotic stress responses. Downregulation and loss of function applications of *SlARF4* mediated by CRISPR/Cas9 presented salinity and osmotic stress tolerance in tomato [147].

Antioxidant scavenging is also very important in abiotic stress tolerance. Hence, elevation of antioxidant enzyme capacities in plants is a legitimate approach to improve tolerance. There are several successful transgenic application as described before. *Oryza sativa* stress-related RING Finger Protein 1 RING finger (OsSRFP1) which is a E3 ligase is responsive to cold, dehydration, salt, H_2O_2 and abscisic acid. However, overexpression of OsSRFP1 leads increased sensitivity to all of these factors. Transgenic RNAi silenced OsSRFP1 mutant plants obtained enhanced salinity and cold tolerance due to increased antioxidant capacity. It is also proposed as potential candidate target for CRISPRi applications [148, 149].

Ion homeostasis is another key factor in abiotic stress tolerance in plants especially against salinity. In Cucurbitaceae family, salt sensitive cucumber and salt tolerant pumpkin varieties were compared in context of K^+ uptake during salt stress and salt tolerant pumpkins were found superior to cucumber plants for this trait. CRISPRi knocking out of NADPH oxidase (respiratory burst oxidase homolog D; RBOHD), which was previously tested by its inhibitor diphenylene iodonium, presented decrease in salinity tolerance. On the contrary, ectopic expression of pumpkin RBOHD in Arabidopsis enhanced the salinity tolerance [150].

Along with CRISPRa and CRISPRi application to enhance tolerance to abiotic stresses, it can also be used to identify roles of particular genes in abiotic stress tolerance. Nonexpressor of pathogenesis-related gene 1 (NPR1) is well known gene in plant pathogen response. To evaluate its involvement in drought stress CRISPRi derived tomato *slnpr1* mutants presented reduced drought tolerance which presented that gene's role in tolerance [151]. Likewise, CRISPRi application on mitogen-activated protein kinases (MAPKs) presented role of these signal molecules on drought tolerance of tomato plants through generating *slmapk3* mutants with altered drought stress responsive gene expression including SlLOX, SlGST, and SlDREB [152]. Ca^{2+}-dependent phospholipid-binding proteins called annexins were targeted by CRISPRi system. *OsAnn3* knockout rice mutants generated by this method were susceptible to cold stress indicating their role in mechanism [153]. Tomato is also very susceptible plant for cold and chilling stress. C-repeat binding factors (CBFs) were tested their potential role in chilling resistance. slcbf1 mutants generated by CRISPRi system presented symptoms of chilling more severely comparing to the wild-type relatives [154].

Despite all of these present efforts and obviously more to come in near future, there are some bottlenecks on this topic beyond the genome editing technique itself. The topic of law regulations and public opinion on genetically engineered plants still have many uncertainties. In these days, when many countries regulated or starting to regulated their laws for genetically engineered plants, CRISPR/Cas9 derived products started another debate due to the lack of standardized detection methods and difficulty to distinguish these changes from naturally occurring mutations. Court of Justice of the European Union (ECJ) ruled their opinion on genome edited products in 25 July 2018 as they are to be subjected under the same stringent regulations previously determined for genetically engineered products in 2001 directives. On the other hand, United States Department of Agriculture (USDA) announced their opinion as regulation is not needed for genome edited mutations since they can already occur naturally. Likewise, some countries as Brazil, Argentina and Australia shared the same opinion. In 2016, France inquired to ECJ about reviewing 2001 directive in favor of genome editing techniques as many researchers share their opinion as this method should be evaluated similar to use of radiation in mutation breeding allowing random mutations on genome. But the deliberate and intentional nature of the genome editing mutations brings the opposition. Still, Calyxt, an agricultural biotechnology company in Minnesota, announced the first US sale of high-oleic-acid oil product generated from gene edited soybeans in February, 2019. Following this development, Intrexon Company in Maryland announced start of commercial non-browning gene edited lettuce trials [13, 14].

Woo [155] suggested a novel approach to overcome this dilemma as DNA-free genome editing. In contrary to existing CRISPR/Cas9 system which is based on delivery of RNA guided nucleases into plants either by *Agrobacterium tumefaciens* or transfecting plasmids, they suggested delivery of pre-assembled Cas9-gRNA ribonucleoprotein (RNPs) into protoplast cells of *Arabidopsis thaliana*, tobacco, lettuce and rice plants instead of plasmids encoding these required components. They modified six genes in four different plants and these targeted mutations were maintained in plants regenerated from these protoplasts. Since, this system is free from involvement of recombinant DNA, they shared their opinion as this gene editing should be exempt from current genetically engineered plant regulations. This, direct delivery of purified CRISPR/Cas9 RNPs system is also adopted for other plants. MLO-7, a susceptible gene responsible from increasing resistance to powdery mildew disease in grapevine and DIPM-1, DIPM-2, DIPM-4 which are responsible from increased resistance to fire blight disease in apple were edited through DNA-free genome editing approach [156]. Due to the fact that regeneration of whole plant from the protoplast cells can be challenging in some plants especially in monocots as hexaploid bread wheat. Liang [157] reported use of particle bombardment delivery system into immature embryo cells for CRISPR/Cas9 RNPs system. The Cpf1 protein was suggested as an alternative to type II CRISPR-Cas system in 2015 [158]. Unlike Cas9 protein which recognizes proximal 3′-G-rich PAM sequences, Cpf1 recognized 5′-T-rich PAM sequences. Cpf1 is also a ribonuclease which processes precursor crRNAs and does not require trans-acting crRNA for guidance. Merging these two approaches is also achieved as CRISPR/Cpf1-mediated DNA-free plant genome editing. Soybean and tobacco protoplast genomes were successfully edited by this approach. Researchers suggested three potential advantages of this method as: no foreign DNA insertion, larger deletions in target site comparing to the Cas9 and different cleavage pattern of Cpf1 which may assist NHEJ-mediated insertion of donor DNAs [159].

In conclusion, following the footsteps of the data generated by the genetic engineering application for abiotic stress tolerance in crop plants, the most recent developments in genome editing techniques as transgene free approach combined with superior endonucleases provides promising results on this topic from many perspectives as law regulations, public acceptance, technical issues.

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Chapter 7

Elevated CO_2 Concentration Improves Heat-Tolerant Ability in Crops

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Abstract

The rising concentration of atmospheric carbon dioxide (aCO_2) and increasing temperature are the main reasons for climate change, which are significantly affecting crop production systems in this world. However, the elevated carbon dioxide (CO_2) concentration can improve the growth and development of crop plants by increasing photosynthetic rate (higher availability of photoassimilates). The combined effects of elevated CO_2 (eCO_2) and temperature on crop growth and carbon metabolism are not adequately recognized, while both eCO_2 and temperature triggered noteworthy changes in crop production. Therefore, to increase crop yields, it is important to identify the physiological mechanisms and genetic traits of crop plants which play a vital role in stress tolerance under the prevailing conditions. The eCO_2 and temperature stress effects on physiological aspects as well as biochemical profile to characterize genotypes that differ in their response to stress conditions. The aim of this review is directed the open-top cavities to regulate the properties like physiological, biochemical, and yield of crops under increasing aCO_2, and temperature. Overall, the extent of the effect of eCO_2 and temperature response to biochemical components and antioxidants remains unclear, and therefore further studies are required to promote an unperturbed production system.

Keywords: elevated CO_2, heat stress, physio-biochemical mechanisms

1. Introduction

Climate change in the form of increasing temperature and increasingly variable rainfall patterns threatens the production of the crop [1, 2]. Therefore, elevated CO_2 (eCO_2) absorption may indorse plant growth, whereas increased temperature is repressive for C_3 plants. Both CO_2 and temperature caused significant changes in crop productivity. The collaborative properties of eCO_2 and increased temperatures on the crop growth and carbon metabolism are not well known.

The fluctuating climatic surroundings are predictable to upsurge the atmospheric CO_2 (aCO_2) meditation, temperatures and modify the rainfall outline. The aCO_2 meditation is prophesied to range 550 ppm by 2050, and possibly surpass 700 ppm by the end of the present century [3]. These fluctuations are expected to disturb the creation and output of cultivated crops, and stimulus the upcoming food safety. The influence analysis of climate alteration on worldwide food construction reveals a 0.5% failure by 2020 and 2.3% by 2050 [4]. The progress of climate arranged germplasm to counterbalance these wounded is of the highest reputation [5].

The eCO_2 is significant abiotic stress and has a noteworthy fertilization encouragement on crops. Widespread preceding educations have described that eCO_2 meaningfully enhanced the water use efficiency (WUE), reduced transpiration frequency, abridged maize growth rate, and augmented plant height, leaf number, leaf area, growth frequency, and overall yield [6]. Furthermore, the cumulative of aCO_2 disturbs precipitation equilibrium, which can alter the periodic precipitation circulation [7]. It has been predicted that this result would carry about a 10% upsurge or decline in water capitals in several areas [8]. The raised temperature, i.e., heat stress (HS) damage growth and physiological ailments, and consequentially reduce yield [9]. Increased temperature due to eCO_2 has a primary effect on the food grain invention reliant on the places. With the rising temperature by 1.0–2.0°C in tropical and subtropical states and the food grain manufacture in India is predictable to decline up to 30% [9].

The C_4 grass maize (*Zea mays* L.) is the third most vital food crop worldwide in the relation of the invention, and its claim is prophesied to rise by 45% from 1997 to 2020 [10]. Educations with maize retort to dual the ambient CO_2 presented variable possessions on growth fluctuating from no inspiration of yield [11] to 50% stimulation [12]. The growth and productivity of maize are expected to be pretentious by raised aCO_2 and temperature. Raised temperature severely disturbs the growth, and yield of maize plants [13]. There is unpredictable information on the properties of eCO_2 on the vintage of maize changing from slight positive consequence [14], no consequence [15] to rice harvest by 50% [16].

Record of the experimentations on the influences of eCO_2 and temperature on the crop yield, though, used measured atmosphere amenities like phytotron and plant growth cavities or crop growth reproduction models [13, 17]. Determination of the impact of raised CO_2 on the photosynthesis tolerance to severe HS is vital to expect the plant replies for universal warming since photosynthesis is sensitive to severe HS and aCO_2 upsurges slightly [18, 19]. Further, flowering is a critical element for plant generative achievement and seed-set. The increase in temperature and eCO_2 is the main climate revolution issues that might influence plant suitability and associated flowering actions. Resolving the influence of these ecological issues on the flowering actions like time of days to anthesis and flowering (duration from germination till flowering) is serious to appreciate the acclimatization of crops in altering climate [20].

2. Interaction of eCO_2 with high-temperature stress and other factors to climate change

The impacts of eCO_2 and stress factors on crops have been made using controlled atmosphere amenities like plant growth cavities or crop growth reproduction models in many studies [13]. The eCO_2 contributes to global warming, causing alterations in the precipitations, water scarcity and changes at temperatures in several regions affecting the growth and development of crop plants [3]. However, the interactive effects of eCO_2 and environmental stress conditions on the crop growth and carbon metabolism are not well predictable. The interactions between eCO_2 and stress factors are critical to photosynthesis performance. It has been reported how stomata react to eCO_2 levels, but the effects on photosynthesis performance of other environmental factors are poorly understood [21].

Numerous studies showed the combining impacts of eCO_2 and drought and revealed that the machines are different from singular eCO_2 and drought. The impact of combined eCO_2 and drought are varying with crop stage such as during vegetative stage, and it restricted the shoot development, decreased leaf area, diminished mobilization of nutrients due to weak root growth, reduced stomatal closure, transpiration, and relative water contents (RWC). However, it enhances the resource use efficiencies of the plant, including WUE, light use efficiency (LUE), and nutrient use efficiency (NUE) at a certain level [22, 23]. Similarly, eCO_2 and drought affect reproductive growth severely such as the impacts on the pollen abortion, pollination, flower formation, panicle length, panicle weight, seed formation, seed size and, yield potential of important agriculture crops [22].

Altering climate, counting eCO_2, increasing temperatures, changing precipitation designs have influenced terrestrial environment assembly and function, carbon and water balance, and finally production of crops [24, 25]. Several experiments have described the biological replies to CO_2 enhancement and their communication with ecological alteration at different levels [26]. The temperature has an important role in plant growth and development and regulates the several functions and enzymatic reactions in plants. Although, the increased value of temperature causes several abnormalities in plants such as reduces the chlorophyll contents, leaf growth, fresh and dry biomass, photosynthesis, and stomata limitations, inhibits the functions of several temperature-sensitive enzymes such as ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO). Impacts of eCO_2 and temperature combined stresses are very destructive at the reproductive phase. They may cause reduced pollination, spike sterility, less filling of grain, reduction of grain size and number, test weight, and yield potential of major crops [22].

It has been reported that eCO_2 promotes an initial stimulation of photosynthesis by an increase of substrate or RuBisCO carboxylation activity and self-consciousness of RuBisCO oxygenation, which might ultimately underwrite for advanced biomass in cereal crops [27, 28]. Nevertheless, growth responses over the long-standing under eCO_2 conditions include a reduction of photosynthesis measurements and several regulatory mechanisms to avoid potential damage by this condition. A regulatory mechanism to maintain the growth and expansion of plants consists of the equilibrium among manufacture and removal of reactive oxygen species (ROS) at the intracellular level. This balance is continued by both enzymatic and non-enzymatic antioxidant defense systems [29–31].

The enzymatic machinery involves numerous antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), guaiacol peroxidase (POX), peroxiredoxins (Prxs), and enzymes of the ascorbate-glutathione (AsAGSH) cycle. The AsAGSH includes different enzymes like ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) [30, 32]. Modifications of this enzymatic antioxidant component have been reported in studies of cereal crops under eCO_2 conditions with contradictory results. Thus, the response of these antioxidants components is unclear with studies that find to increase [33], decreases [34], or no consistent alterations [35].

Besides, plant cells have non-enzymatic components that involve ascorbate and glutathione (GSH) along with phenolic acids, flavonoids, carotenoids anthocyanins, and phenolic composites. The free hydroxyl groups on the phenolic rings or the chromanol rings of these non-enzymatic compounds are responsible for their antioxidant properties [36]. The ring hydrogen atom can be given to free radicals, dropping and counteracting ROS. The phenolic compounds can lose a hydrogen atom which develops a free radical that is directly non-reactive by character delocalization in the entire ring assembly [28, 37]. Several studies reported only an increase in some of the individual phenolic compounds in cereal crops under high CO_2 conditions [38].

Furthermore, crop growth responses to eCO_2 rely on the tissue category, developmental stage as well as strength and duration of these conditions which also depend on the diversity of apparatuses of construction and purification of ROS, and the result of free radicals on antioxidants [39]. Several studies have reported a high production of hydrogen peroxide (H_2O_2) after exposure of crops to eCO_2, and the concentration of H_2O_2 is dependent on the duration of these conditions. Besides, H_2O_2 production differed among various cellular compartments [40]. The outcome of elevated aCO_2 meditation on growth and various antioxidant actions is superior in C_3 plants to C_4 plants [41, 42]. For example, rice as a C_3 plant is further sensitive to the variations of the aCO_2. However, there are inconsistent studies on the impacts of eCO_2 on the antioxidant responses and yield of rice changing from the reduction of the growth responses [43] to increment of antioxidants components and enhancement of growth [44, 45].

Kumar et al. [46] reported that rice plants under eCO_2 conditions showed modifications in electrolyte leakage, leaf water potential, proline, CAT, and POD activity as compared to ambient CO_2, which assisted the plant to battle contrary effects of stressful environments. Thus, these authors suggest that undesirable possessions on rice yield subsequent from abiotic stress conditions may be moderated by the eCO_2 meditations [46]. In agreement, leaves of a susceptible wheat cultivar (*Triticum aestivum* L.*cv*. Yitpi) infected with Barley yellow dwarf virus-PAV (*PadiAvenae* virus) and grown under eCO_2 presented that the eCO_2 conditions may decrease the oxidative stress caused by virus infection [47]. Nevertheless, more evidence for direct communicating possessions of eCO_2 and biotic and abiotic stress conditions in cereal crops is necessary.

3. eCO_2 mitigates oxidative stress in plants

Various environmental stresses induce the production of ROS, which triggers oxidative stress in plants [30, 48, 49]. The most common ROS are $O_2^{\bullet -}$, $^{\bullet}OH$, and H_2O_2. In response to stressful conditions, H_2O_2 is mainly synthesized by photorespiration, beta (β)-oxidation, or due to the activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [30, 50]. The eCO_2 can efficiently reduce the ROS level by increasing RuBisCO carboxylation along with reducing photorespiratory H_2O_2 production. Several reports have provided indications regarding the eCO_2 influences on the mitigation of abiotic stress in plants [30, 51, 52]. Elevated CO_2 known to be induced plant growth by supplying additional Carbon (C) sources, subsequently alleviates abiotic stress in plants. Although the elevated CO_2 mediated particular physiological and molecular mechanisms related to abiotic stress alleviation are still to be explored. In the physiological aspect, supplying extra C by eCO_2 leads to induce stomatal closing, improves WUE that protect drought stress in plants [53]. However, abiotic stress-induced ROS (e.g., $O_2^{\bullet -}$, $^{\bullet}OH$, and H_2O_2) and cellular oxidative damages (e.g., protein oxidation, lipid peroxidation) are involved in non-stomatal factors with metabolic changes [30, 54].

A systematic study of recently published articles addressed the two major hypotheses such as enhancement of antioxidant (antioxidant hypothesis) and reduction of stress impact (relaxation hypothesis) by reducing ROS generation in the plant under stressful conditions [30, 51, 55]. Several reports have been found in favor of the relaxation hypothesis in plants in response to eCO_2 under stressful conditions. The ROS level was found to be reduced by eCO_2 in plants under drought, and heat stresses through increasing RuBisCO carboxylation as well as reducing the level of photorespiratory H_2O_2 [51]. In the same study, glycine/serine (Gly/Ser) ration, glycolate oxidase (GO), and hydroxypiruvate reductase (HPR) level were evaluated as an indicator of photorespiration, which was found to be decreased in response to eCO_2 under drought and heat stresses. In barley, these all parameters were found at a lower level in response to eCO_2 [56]. Moreover, reduced photorespiration is correlated to the decreased level of NADPH oxidase. Therefore, a combined effect of lower photorespiration and NADPH oxidase responses may lead to reduce H_2O_2 in plants.

According to the antioxidant approach, the availability of additional C by eCO_2 enhances antioxidant molecules, which increase ROS scavenging activity as well as protects grapevine and tomato plants from abiotic stress induced-oxidative damages [57, 58]. More specifically, higher C availability due to eCO_2 may enhance the supply of defense molecules, which improve protection against oxidative injury (antioxidant hypothesis) under stressful conditions in plant cells. However, changes in antioxidant levels are not specific, or C_3 or C_4 based metabolism, or not for a particular group of species. It has been reported in C_4 plants that photorespiration mildly active, in which eCO_2 reduces ROS level as well as oxidative injury without alteration of antioxidants level. It suggests a distinct non-stomatal process that except antioxidant defense or reduces photorespiration processes. Besides, several reports have provided the evidence regarding eCO_2 reduces NADPH oxidase activity and ROS formation in mitochondria and chloroplast in plants [59, 60], but the activity of beta (β)-oxidation is still to be explored.

The ascorbate-glutathione (ASC-GSH) cycle is one of the major mechanisms for stress-induced H_2O_2 regulation. However, only limited reports have been found concerning eCO_2 mediated changes of ASC-GSH cycle components in plants under stress conditions. For example, HS alleviated through enhancement of DHAR, MDHAR, APX, and GR in tomato [58]. Similarly, GR and APX were found to be increased by eCO_2 in wheat under ozone stress [61]. Also, responses of ASC-GSH cycle components varied based on the plant species and experimental set-up. Therefore, additional studies are needed concerning the eCO_2 mediated oxidative stress alleviation as well as enhancement of ASC-GSH cycle components in plants under abiotic stresses.

4. eCO_2 improves photosynthesis under high temperature

Several researchers on the consequences of eCO_2 and stress factors on crops have been made using monitored situation amenities like plant growth chambers, free-air concentration enrichment (FACE) experiment, open-top chamber (OTC) or stimulated crop growth models [13]. The eCO_2 contributes to global warming causing alterations in the precipitations patterns, water scarcity, flood, and changes at extreme temperatures in several regions affecting the growth and development of plants [3]. However, the interactive effects of eCO_2 and environmental stress conditions on the development of crops and metabolism are not well documented. It has been shown the average reduction of stomatal conductance (20–30%), stomatal density (5–7%), stomatal developments, and increment in WUE (8–18%)

under eCO_2 conditions. However, these changes vary with the crop species, developmental stages, nature of stressors and duration, surrounding environments, and plant attributes [62]. Likewise, the interactions between an eCO_2 with stress factors are crucial to understanding the photosynthesis performance. Therefore, there is a considerable deviation in the light-saturated photosynthetic assimilation rate under eCO_2 rely upon the plant type, plant functional traits, micro or surrounding environment, and resource availability. For instance, the stimulation in photosynthetic assimilation rate under eCO_2 is varied from 30 to 80% (strong stimulations in C_3 species as compared to C_4) in FACE experiment or pot conditions but diminished in field conditions because of the integration of other multiple stressors such as drought, heat, flood and nutrient deficiencies.

In this regard, several studies were conducted to observe the combining effects of eCO_2 and drought and reveals that the growth mechanisms are distinct to the singular eCO_2 or drought. The combined stress resulted in the longer retention time of dissolved organic carbon (accumulation of soil organic C), induce invertase and catalase activity in the soil, and ameliorate stress conditions via improving plant physiological traits and activates feedback mechanisms [63]. Further, it limits the activity of some antioxidant enzymes such as proline and MDA content and stimulates others such as SOD, CAT, and GPX [46, 64]. Abscisic acid, calcium-dependent protein kinase and glutathione *S*-transferase (GST) play an important role in the amelioration of drought stress responses by inducing signaling mechanisms under the combined form. Conclusively, it is suggested that drought and HS generate ROS, and affect the antioxidant defense mechanism of plants, which might be ameliorated by the eCO_2 via stimulation of antioxidant defense enzymes [51, 62]. Similarly, eCO_2 combined with drought and HS regulates the sugars (starch, sucrose) and amino acids (alanine, pyruvate, arginine, glutamate) and secondary metabolites (coumaric acid, salicylic acid) metabolism, protective proteins and readjusted the metabolic, redox, and osmotic equilibrium of plants under combined eCO_2 and drought [65, 66].

The impact of combined eCO_2 and drought are varied with crop stage such as during the vegetative phase decreased the shoot elongation and leaf area, diminished mobilization of nutrients due to weak root growth, reduced stomatal conductance by increasing stomatal resistance and stomatal movements, plant hydraulic conductance, aquaporins, and reduce transpiration and RWC. However, at certain levels, it enhances the biomass allocations to the reproductive part, improves resource use efficiencies of plants including WUE, LUE, and NUE [67]. Similarly, eCO_2 and drought affect reproductive growth severely such as its impacts on the assimilate partitioning, pollen abortion, pollination, flower formation, panicle length, panicle weight, productive tiller number, seed formation, seed size, and yield potential of important crops [22, 68]. Besides the yield potential, eCO_2 decreased the grain quality via affecting macro- and micro-nutrients content such as phosphorus (P), Sulphur (S), and Iron (Fe), Zinc (Zn) contents of dryland legumes, which further associated with yield dilutions [23].

Several findings have stated the biological responses to CO_2 enhancement and their communication with conservation alteration at diverse levels [26]. In these aspects, the temperature has an important role in plant growth and development and regulates the several functions and enzymatic reactions in plants. Although, the increased value of temperature cause several abnormalities in plants such as change the emission of volatile organic compounds, reduce nitrogen uptake, chlorophyll contents, leaf growth, fresh and dry biomass, photosynthesis, and stomata limitations (by membrane damage and photosystem II (PSII) activity), enhance the activity of mitochondrial electron transport, stress proteins and plant growth regulators, limitation of several temperature-sensitive enzymes such as RuBisCO.

The combined effects of eCO_2 and temperature stresses are very destructive at the reproductive phase. They may cause changes in flowering time, pollination, spike sterility, less filling of grain, reduction of grain size and number, test weight, grain quality, and yield potential of major crops [20, 22, 69].

Besides the drought and HS (major), other stressors also interact with the eCO_2 under field conditions and influence the plant growth and development. For instance, eCO_2 and salt stress conditions influence the nitrogen metabolism, water balance, photosynthetic inhibitions, nutrient deficiency or toxicity, stomatal conductance, carbohydrate metabolism, phenolic enrichments, and generation of secondary metabolites [63, 70]. Similarly, limited nitrogen supply under eCO_2 modifies the C/N ratio, nitrogen metabolism, protein supply, protein structure, gene expression, sugar metabolism, and decreases antioxidant enzyme, amino acid synthesis, photosynthetic pigments, and elevated ROS, which influence the redox equilibrium and leads to early senescence in plants [71]. Likewise, under N limitations, the photosynthetic rate is more affected in C_3 species (because of more N requirement for RuBisCO synthesis), and eCO_2 could help in mitigation of N limitations by reducing photorespiration, elevating starch level, increase chloroplast size, higher stomatal resistance, mitochondrial respiration, metabolites and dilution of chlorophyll concentrations [62]. Therefore, the eCO_2 and stressors impacts have differed than singular stress, and up to a certain level of eCO_2 try to recover plants via inducing defense machinery, feedback mechanisms, activating secondary messenger signaling, and expression of stress proteins.

5. eCO_2 improves yield under high temperature

By the end of the 21st century, CO_2 is expected to rise from the current level 370 $\mu mol \cdot mol^{-1}$ to 540–970 $\mu mol \cdot mol^{-1}$, and about to grasp 550 $\mu mol \cdot mol^{-1}$ near 2050 and 750 $\mu mol \cdot mol^{-1}$ in 2100. In the meantime, Earth's global temperature will rise by about 2–4°C [72]. The increased concentration of CO_2 in the atmosphere would increase the temperature of Earth that is why global heating will develop, the most important aspect of upcoming climate variation. The relations between the temperature and CO_2 will have an intense effect on global agricultural production and the Earth's environment [73]. The rise in the atmospheric temperature and CO_2 would also accelerate the procedure of growth in plants [58, 74].

Climate change impacts on crop growth are becoming global concerns. They are particularly important for food supply and sustainable agricultural development [75, 76]. CO_2 concentration and temperature are two key factors affecting crop growth, development and yield [77]. Combined or individual possessions of temperature increase and eCO_2 meditation change on crop growth and yield during the recent decades have been observed [78]. For instance, the modeled improvements in soybean absorption of CO_2 with an increase in the growing season temperature, and aCO_2 hindered the photorespiration by 23–48%, which depends on the future climatic conditions [79].

The growth and distribution of crops are reduced by environmental factors like CO_2 and temperature. The production of the biomass of modern C_3 plants was decreased by 50% when it was grown at a low concentration of CO_2 (180–220 ppm), while the other conditions were optimal. Crops need the almost dual amount of water at 2°C increase in temperatures at a higher elevation of agricultural plains. Elevated CO_2 concentration increases the yield of the crop once the substrate for the photosynthesis process of leaf and the incline of CO_2 absorption of air increases. C_3 plants are more benefitted at eCO_2 than C_4 plants [80]. However, the doubling of CO_2 does not deteriorate the adverse effects of high temperature on the reproductive growth of crops or fiber quality. Therefore, increased CO_2 concentration is associated with higher temperatures, crop yield, and quality that reduce particularly in areas where current temperatures are near to optimal [81].

Elevated CO_2 resulted in major changes in morpho-physiological restrictions. Besides, eCO_2 along with atmospheric temperatures during the phenological stages of rice cultivars showed contrasting results of the time of flowering and maturation such as eCO_2 in combination with the lower atmospheric temperature that stopped flowering g in the CR-1014 cultivar while with the higher temperature increased grain yield the in Naveen cultivar [82].

Growth and photosynthesis of C_3 crops are enhanced when it is grown at a high level of CO_2, although, the degree of stimulation differs with temperature among cultivars as well as species. The probable decline in the transpiration process due to the partial closure of stomata in the eCO_2 level is largely invalid by the energy balance between the crop and its environment, which could result in total water use in similar climate conditions. The yield of seeds is increased by an increase in the CO_2 under the ideal temperature. On the other hand, at supra-optimal temperature, the yield of seeds is decreased under both raised and ambient CO_2. The yield of kidney bean decreased in that region where temperatures are at or above optimal conditions in combination with increased CO_2 concentration [83].

The effects of HS on the grain and biomass yield of plants depend on the duration and magnitude of HS. HS at the vegetative phase decreased the grain and biomass yield mostly by increasing plant growth and dropping the time obtainable to capture possessions, and also by dropping the rate of photosynthesis [84]. At the anthesis or flowering phase, HS decreases the amount of grain due to pollen abortion. In contrast, at the grain-filling phase, HS decreases the heaviness of grain by restraining the translocation of integrating, and margarine the period of grain-filling [85, 86].

Notably, eCO_2 may lessen the harmful influence of heat stress on the grain and biomass yield by inspiration of photosynthesis, defense of the photosynthetic devices from HS injury, and improvement in the water status of plants owing to reduced transpiration. Moreover, high levels of hexoses and sucrose in plants with eCO_2 are related to increase fertile florets and dry spike mass [87], and osmotic modification [88], which can develop heat stress tolerance [89]. It is hypothesized that HS at anthesis has a drastic impact on the grain yield and plant biomass, but it has a less impact eCO_2 than ambient CO_2 [90].

6. Conclusion

It is very important to know that the impacts of climate change on crop growth are becoming global concerns. Interactive effects of eCO_2 and environmental stress conditions on crop growth and carbon metabolism are not well predictable. The influence of eCO_2 with the connection of temperature is considerable on crops under stress environments. The CO_2 concentration improves the productivity of crops because of improved carbon exchange rates, and superior vegetative and reproductive growth. In contrast, crop productivity is decreased with increased temperatures. Hence, there is a need to develop genotypes that are different intolerant to various environments or to identify genotypes that perform better under predicted climate change. In this review, cereal genotypes have been characterized by differing responses to eCO_2 and HS and identified the mechanisms of tolerance to HS. It can promote the crop potential to assist the breeding program for the development of new genotypes tolerance to HS.

Conflicts of interest

The authors declare no conflicts of interest.

Disclosure statement

Authors declare that no conflict of interest could arise.

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Chapter 8

Ecological and Economic Potential of Major Halophytes and Salt Tolerant Vegetation in India

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Abstract

Soil salinization is a global and climatic phenomenon that affects various spheres of life. The present rate of salinization is perilously fast because of global climate change and associated events leading to enhanced land degradation, loss of soil fertility and crop productivity. In this chapter, we tried to focus on the arid and semiarid regions of India along with our coastal zone which are economically fragile regions and need much closer attention. In future, India will face extreme pressure on its land resources in agriculture because of likely rapid degradation of these resources. Thus, salt affected soils must be brought under cultivation by adopting site specific strategies to ensure national food and nutritional security. In this regard, a comprehensive review of the major halophytes of these ecological zones, its mechanism of salt tolerance, ecological and economic potential is done. The potential applications of saline land vegetation including halophytes in climate change mitigation, phytoremediation, desalination, food, secondary metabolite and nutraceutical production, medicine, and saline agriculture have been discussed. Further, we tried to focus on popular farmer adopted halophytic species including edible ones, their uses, products of economic significance etc. which is highly imperative for effective utilization of these saline soils leading to improved livelihood and sustenance of resource poor farmers along with improved ecological balance.

Keywords: coastal soil, dry land, ecology, economic products, halophytes, saline vegetation, salinity stress

1. Introduction

Food security and ecological security are the two pillars of all societies. The incessant growth of human population across the world is evident from the United Nations projection of 9.7 billion people by 2050 [1]. The global food demand is expected to rise by 70–100% and to meet this additional food demand, a predicted 50% increase in yields of the major food crops will be required [2]. Moreover, our global food security is significantly affected by drastic alterations in the climate [3]. This unpredictable change in climate has resulted in to several abiotic stresses such as salinity, drought, heat and low temperature, threatening the agricultural sustenance and productivity [4]. This has also brought about a significant spike in the occurrences of extreme climatic events like flood, cyclones, storms etc. [5]. Land degradation is one of the severe consequences of global climate change and it is happening at a viciously alarming pace because of other reasons too, like vegetation loss, soil erosion, inadequate water management, excessive use of fertilizers, soil and groundwater degradation, urbanization and poor agricultural practices [6]. One of the major causes of land degradation is buildup of soil salinity as a result of natural and anthropogenic factors [7, 8]. It is estimated that agricultural soils are decreasing about 1–2% every year especially in the arid and semiarid regions of the world due to soil salinity [9].

On an average, 831 million hectares (Mha) of global soil is affected by salt (397 and 434 Mha of saline and sodic soils, respectively) which is more than 6% of the world's total land area [10]. Out of total irrigated land (230 Mha), almost 20% (45 Mha) area has already been damaged by excess soil salinity [11], whereas 14% (32 Mha) is affected by varying degrees of salt [12]. In India, the total land degraded due to soil salinity and sodicity is estimated to be 6.74 Mha [13] and it is likely to increase to 16.2 Mha by 2050 [14]. Soil salinization is grouped in to two types*viz.* Primary salinization and secondary salinization. Primary salinization occurs due to the existence of subsoil salts and by other natural processes like weathering of parent material, deposition of sea salt carried by wind and rain, inundation of coastal land by tidal water, etc. Secondary salinization is a consequence of anthropogenic or human induced activities like excessive irrigation, irrigation with salt containing water, poor drainage due to loss of natural water passages, unscientific application of inorganic fertilizers and other soil amendments [14, 15].

The arid and semiarid regions of India along with our coastal zones are economically fragile regions and need much closer attention. Low rainfall and high temperature are two specific features of the arid and semiarid regions of India which are highly conducive to excessive soil salinity [16]. India has a coastline of 7516.6 km viz. 5422.6 km of mainland coastline and 1197 km of Indian islands spread across 9 Indian states and two Union Territories [17]. Soil salinity is estimated to have affected 1.25 Mha soils in these coastal tracts of the nation [13]. The predicted rise in sea level of 1 m due to global warming may aggravate this problem to take on serious dimensions in future [14]. Moreover, there is a predicted increase in water demand by 45% in coastal areas by 2050, which will inevitably result in increased ground water depletion and induce more and more soil salinity [14]. Altogether, in both these ecological zones of the nation, soil salinity has become an important factor limiting the growth of major crops and even some halophytes [18].

Moreover, factors like global climate change, diversion of more and more productive agricultural land towards other nonagricultural purposes as a part of rapid urbanization and the alarming rate of decline in soil organic carbon will lead to further increase in soil salinity [19–24]. So we are running out of options, and the only option left is to bring these saline, sodic and other barren land in to cultivation to meet the growing food demand and more importantly to address the growing climatic and ecological concerns. Major agricultural crops and tress cannot be grown in such soils as they exhibit a low tolerance to salt [25]. So future agricultural production in these saline soils should be based on salt tolerant crops which are edible and/or of economic and ecological significance [26, 27].

Halophytes, salt mangroves, and other salt tolerant plants and trees are the options which can grow and survive in extreme environments. Many halophytes have potential agricultural value and can be grown in these highly saline areas. They not only survive in these conditions, but also produce considerable biomass coupled with various other potential/economic uses in industry, feed, medicine etc. [28]. Indian mangrove vegetation covers about 6749 km^2 along the 7516.6 km long coast line, including island territories. 82 species of mangroves distributed in 52 genera and 36 families have been identified that are spread across 12 habitats of India [29]. They along with many other associate species thrive well in coastal saline soils. Moreover, these saline soils can further be brought in to cultivation through agroforestry, silvipasture, nonconventional crops including medicinal and aromatic plants and other high value crops. These will not only utilize the saline barren land for economic growth but will also help significantly in conserving our biodiversity and improvement of ecological and social environment.

A systematic attempt has been made in bringing together critical aspects of saline agriculture, including major halophytes and other saline vegetation of India, its salt tolerance mechanisms coupled with ecological and economic potential of these plants. This is highly imperative for effective utilization of these saline soils leading to improved livelihood and sustenance of resource poor farmers along with improved ecological balance.

2. Halophytes

Halophytes are plants capable of completing their life cycle under highly saline conditions [30]. To be more scientifically concrete, halophytes are those plant species which can successfully survive, grow and reproduce in soils of salt concentration more than 200 mM of NaCl (Electrical conductivity of soil saturation extract (ECe) of 20 dS m^{-1}) [31]. Some halophytes can even grow well at higher salt concentrations than that of sea water (>500 mM Nacl) [32]).As they prosper well in severely saline soils, they are also considered to be extremophiles [33]. They constitute around 1% of the global flora [15]. The question of how they survive and complete their life cycle under such extreme conditions has led to detailed studies on the various morphological, anatomical, physiological and molecular mechanisms of high salt tolerance. The tolerance of halophytes to salts varies with species and developmental stage. In general, dicot halophytes are reported to be more tolerant (optimal growth in 100–200 mM NaCl) as compared to monocot species (optimal growth in 50–100 mM NaCl) [31].

During 1980s, James Aronson compiled a comprehensive database [34] of 1554 halophyte species, and named it as HALOPH. This database has been converted into an interactive eHALOPH repository [35] that provides online details and bibliography pertaining to halophytes. Till now 2000 to 3000 halophytic plant species are identified [32] in the world mainly belonging to angiosperms. In India, their distribution is mainly confined to arid, semiarid inlands and highly saline wetlands along the tropical and sub-tropical coasts [36]. The major halophytes/saline vegetation found across the various ecological hotspots spread across the country are summarized in **Table 1**.

A summary of classification of halophytes by researchers across the globe based on different aspects is compiled and presented below.

2.1 Based on salt tolerance

i. **Mio-halophytes**: Plants which grows in the habitats of low salinity levels (below 0.5% NaCl) [37].

ii. **Eu-halophytes**: Plants which grow in highly saline habitats. They have been further sub-divided into the following groups:

   a. **Mesohalophytes**: Plants that can tolerate salinity range of 0.5 to 1%.

   b. **Mesoeuhalophytes**: Plants that can tolerate salinity range of 5% and higher.

   c. **Eneuhalophytes**: Plants that can tolerate salinity range of 1% and above.

Scientific name	Common name	Family	Area of occurrence	Plant type
Aeluropus lagopoides	Mangrove grass, usargas, kharoga	Poaceae	Kachchh, Thar Desert	Tufted grass
Atriplex hortensis	French Spinach, Pahari Paleng	Amaranthaceae	Coastal marshes Ladakh, Kashmir	Herb
Atriplex stocksii	Khati palakh, kharo tanko	Amaranthaceae	Kachchh (Gujarat)	Bush
Avicennia marina	Whitemangrove, tavarian, tivar	Acanthaceae	Kachchh, Kerala Maharashtra, Tamilnadu,	Mangrove
Cenchrus biflorus	Indian sandbur, bhurut	Poaceae	Kachchh, Thar Desert	Grass
Chenopodium album	Lamb's quarters, bathua	Amaranthaceae	Northarn India, Kachchh	Herb
Cressa cretica	Rudravanti, machul	Convolvulaceae	Kachchh, Thar Desert	Sub-shrub
Cyperus conglomeratus	—	Cyperaceae	Kachchh, Thar Desert, Kerala	Herb
Cynodon dactylon	Bermuda grass, doob	Poaceae	Throughout India	Grass
Dactyloctenium sindicum	Tantia, ganthio	Poaceae	Kachchh	Grass
Dichanthium annulatum	Marvel grass, karad, bansi	Poaceae	Kachchh, Thar Desert	Grass
Halopyrum mucronatum	Dariyai kans	Poaceae	Kachchh	Grass
Haloxylon salicornicum	Rimth saltbush, khar, lana	Amaranthaceae	Kachchh, Thar Desert	Shrub
Heliotropium bacciferum	—	Boarginaceae	Kachchh, Southern India	Herb
Ipomoea pes-caprae	Atampa, dopatti lata	Convolvulaceae	Kachchh	Herb
Limonium stocksii	Kharia	Plumbaginaceae	Kachchh, North west India	Herb
Portulaca oleracea	Common purslane, lunia, badi-noni	Portulacaceae	Maharastra, Thar desert	Herb
Prosopis juliflora	Mesquite, vilaiti keekar	Fabaceae	Throughout India	Shrub
Salicornia brachiata	Glasswort, umari keerai	Amaranthaceae	Kachchh, Thar desert	Herb
Salsola baryosma	Loonuk, lani	Amaranthaceae	Kachchh, Thar desert	Shrub
Salvadora oleoides	Bada peelu, meethi jaal	Salvadoraceae	Kachchh, Thar desert	Shrub
Salvadora persica	Meswak, piludi, khari jaal		Kachchh, Thar desert	Shrub/Tree
Sesuvium portulacastrum	Sea purslane, lunio, dhapa	Aizoaceae	Kachchh, Rajasthan,	Herb
Sporobolus marginatus	poolongi, khevai	Poaceae	Kachchh	Grass

Scientific name	Common name	Family	Area of occurrence	Plant type
Suaeda fruticosa	lunaki, moras	Amaranthaceae	Kachchh, Thar desert	Shrub
Suaeda maritima	Annual sea blite, alur		Maharastra	
Suaeda nudiflora	Muchole		Kachchh	
Tamarix dioica	Red tamarisk, lai, arseli	Tamaricaceae	Kachchh	Shrub
Urochondra setulosus	Kkariyu	Poaceae	Kachchh	Grass
Ziziphus nummularia	Jhar beri, chanibor		Kachchh, Thar desert	Shrub
Zygophyllum simplex	Bean-Caper, pat lani	Zygophyllaceae	Kachchh, Thar desert	Herb

Table 1.
Major halophytic species and other saline vegetation spread across different ecological zones of India.

2.2 Based on mechanism of tolerance

i. **Salt excluding**: The root architecture of this category of plants is embraced by an ultrafiltration mechanism which leads to establishment of such species in saline conditions. Mangrove vegetation shows such type of tolerance. e.*G. Rhizophora mucronata*, *Bruguiera gymnorrhiza* [38].

ii. **Salt excreting**: This category of plants release excess salts in their internal tissues to outside via. Specialized structures called as salt glands. e.g. *Avicennia officinalis*, *Avicennia alba*, *Avicennia marina*.

iii. **Salt accumulating**: These plants are able to maintain very high levels of salt in their tissues either by virtue of succulence or by compartmentation of excess salts in to comparatively safer cellular locations like vacuole. e.g. *Salvadora persica*, *Sesuvium portulacastrum*, *Suaeda nudiflora*.

2.3 Based on ecological aspect

i. **Obligate halophytes**: They grow only in salty habitats and show satisfactory growth and development under high saline condition. Many plant species belonging to chenopodiceae family comes under this category. e.g. *Salicornia bigelovii* [39].

ii. **Facultative halophytes**: Plants of this group are able to establish themselves on salty soils, but their optimum growth is observed in a salt free or low salt condition. Most poaceae, cyperaceae, and brassicaceae species as well as a large number of dicotyledons like *Aster tripolium*, *Glaux maritima, Plantagomaritima* belong to this group.

iii. **Habitat-indifferent halophytes**: They normally grow on salt free soils but can thrive better than sensitive species under saline conditions. Plants like *Chenopodium glaucum, Myosurus minimus,* and *Potentillaanserina* are categorized as habitat insensitive plants.

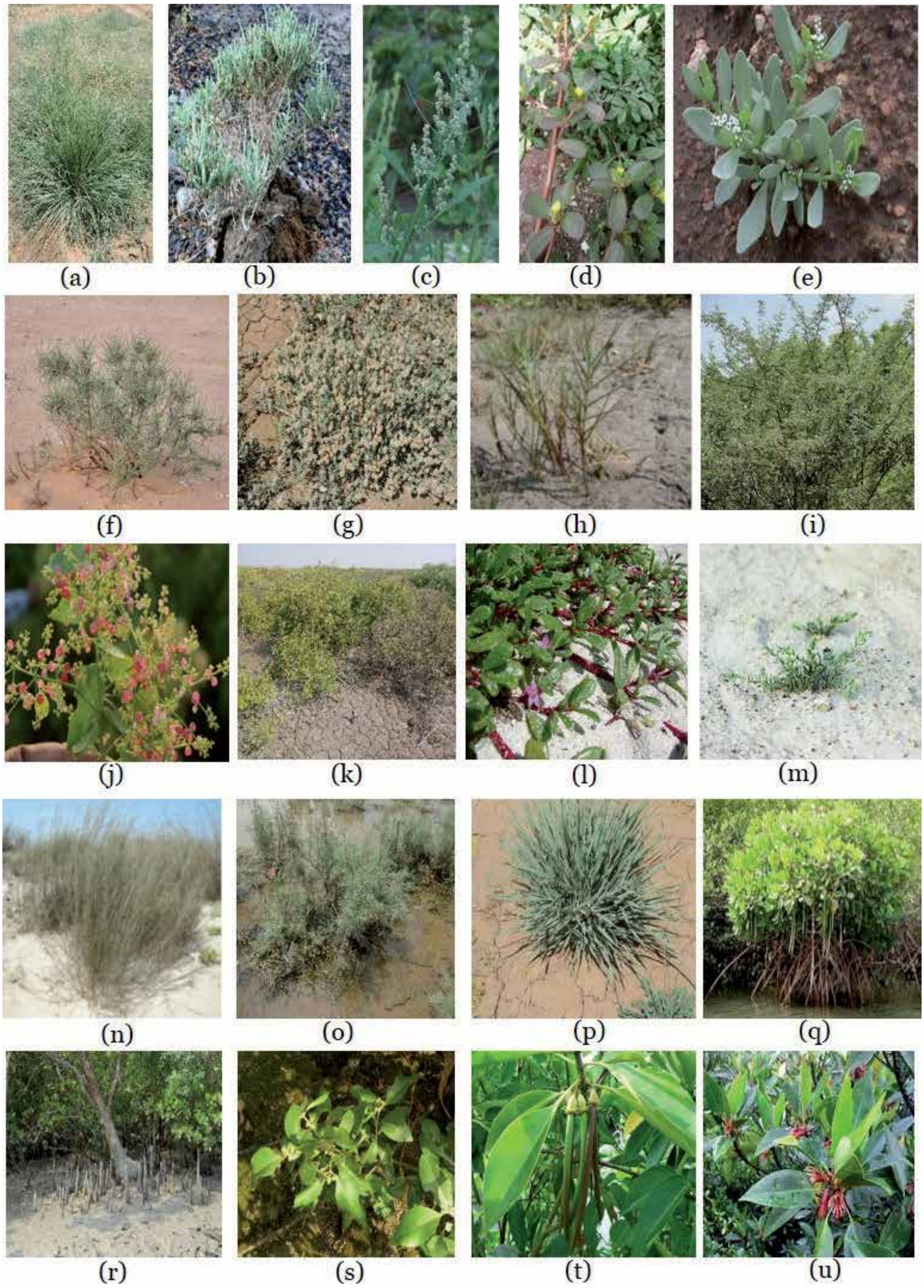


Figure 1.
Major halophytes and mangroves found in India. (a) Sporobolus marginatus, *(b)* Salicornia brachiate, *(c)* Chenopodium album, *(d)* Portulaca oleracea, *(e)* Heliotropium curassavicum, *(f)* Haloxylon salicornicum, *(g)* Cresa cretica, *(h)* Aeluropus lagopoides, *(i)* Prosopis juliflora, *(j)* Salvadora oleoides, *(k)* Salvodera persica, *(l)* Sesuvium portulacastrum, *(m)* Suaeda fruticosa, *(n)* Halopyrum mucronatum, *(o)* Tamarix spp, *(p)* Urochondra setulosa, *(q)* Rhizophora mucronata, *(r)* Heritera fomes, *(s)* Avicennia marina, *(t)* Bruguiera cylindrical, *(u)* Bruguiera gymnorrhiza.

2.4 Based on habitat

i. **Hydro-halophytes**: These are halophytic plants which grow in aquatic conditions. Most of the mangroves and salt marsh species along costal lines belong to this group [40].

ii. **Xero-halophytes**: They grow in environment, where the soil is saline and the soil moisture content is very low due to high evaporation. Most plant varieties in desert areas and succulents belong to this group.

There is an enormous diversity among halophytes with regard to its ecological hotspots like coastal saline soils, arid and semiarid saline and sodic soils, soils of mangrove forests, wet lands, marshy lands and even agricultural fields [15]. A picturesque view of the major halophytes and mangroves found in India is given in **Figure 1**.

3. Mechanism of salt tolerance

Any review on halophytes/extremophiles is incomplete, without touching on the mechanisms by which these extremophiles survive under saline conditions. Indian Council of Agricultural Research- Central Soil Salinity Research Institute (ICAR-CSSRI) initiated such basic studies long back to critically understand the mechanism of salt tolerance in the local, native dry land saline vegetation [41]. This precise knowledge is highly imperative in developing other crops by a combination of improved salt tolerance and high yield. Another mandate was to document such species and, to the maximum extend, popularize them among the resource poor dry land farmers for better livelihood and sustenance.

3.1 Salt stress and halophytes

Any plant species initially suffers from osmotic stress as a result of increased soluble salts in the soil solution and later gets subjected to ionic stress due to specific accumulation of toxic ions. The osmotic phase of salt stress is characterized by disruption of water potential gradient and there by leads to reduced water uptake and inhibition of cell expansion [42]. Most plant species tries to adapt to this osmotic stress by accumulation of compatible solutes and thereby lowering water potential of cells, but this process consumes lot of energy and hence growth is heavily compromised.

Another major deleterious effect of salt stress is nutrient imbalance where in which high Na^+ ions in the soil solution reduces the availability of other cations like K^+ Ca^{++} and Mg^{++} [43]. This second phase of salt induced injury (specific ion toxicity) results from very high levels of Na^+ and Cl^- in the plant cells. In normal soils, plants maintain around 100–200 mM of K^+ and 1–10 mM of Na^+ in cellular cytosol for optimum cellular functions. Any salt concentration above this threshold level disrupts enzyme activity, protein synthesis, photosynthesis and other metabolic activities [44]. The light reactions of photosynthesis are comparatively less affected by salt stress as compared to the carboxylation reactions. A summary of salinity mediated effects and responses of plant cells are presented in **Table 2**.

The cell membrane permeability, composition and integrity gets affected as excess Na^+ replaces Ca^{2+} from its surface [46]. A lot of salt tolerant crop varieties have been developed by various research institutes which can survive up to moderate levels of salt stress. Under these circumstances, it is highly imperative to identify and propagate halophytic species in the arid and semiarid, high salt affected regions of the country, so that the vast tract of unproductive land can be put to cultivation for realization of sustainable income to resource poor farmers.

3.2 Salt induced responses and adaptations in halophytes

Halophytes and glycophytes have similar components in the stress tolerance network, but certain additional characteristics help these halophytes tolerate very high levels of salinity. Similar to glycophytes, halophytes also use osmoprotective and

Causes	Effect	Response/adaptation
Osmotic stress	Reduced water uptake, inhibition of cell elongation and expansion and leaf bud development	Compatible solute accumulation (ions/organic compounds)
Ion specific stress (high levels of Na and Cl in plant cell)	Inhibition of enzyme activity, protein synthesis, photosynthesis and leaf senescence and necrosis	Ion homeostasis through ion accumulation or ion exclusion
Imbalanced ion uptake	Nutritional deficiencies, reduced availability of other cations like K, Ca and Mg	Ion reabsorption

[a] *Adopted and modified from [45].*

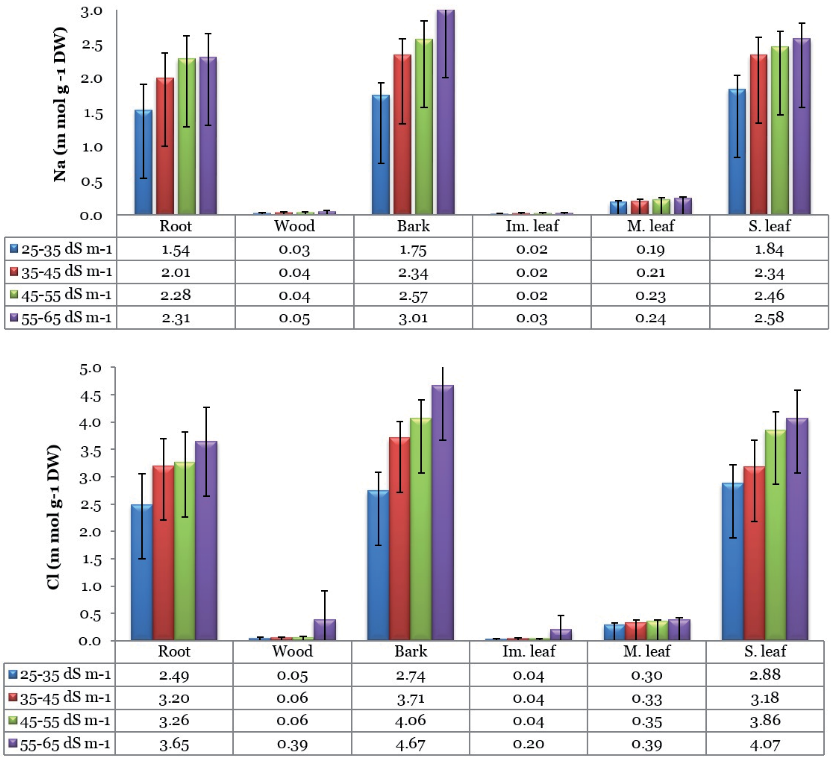
Table 2.
Salt stress mediated effects and adaptations in plants.[a]

ion-detoxification strategies consisting of Na^+ removal from cytosol, Na^+ transport from root cells to xylem, and ion compartmentation in the vacuoles, involving salt overly sensitive (SOS1), high affinity potassium transporter (HKT1) and Na^+ H^+ antiporter (NHX) ion transporters, respectively. Under salt stress, most halophytes accumulate more Na^+ in their shoots than in their roots while retaining higher levels of K^+ and, thus, a more optimal K^+/Na^+ ratio [47, 48].

Salt stress induced accumulation of specific osmolytes like proline, glycine betaine and sugar alcohols have also been reported in both halophytes and glycophytes [47]. Nevertheless, halophytes exhibit a greater capacity to accumulate very high levels of such osmolytes, even under normal conditions which explains its preparedness to stress [49]. In line with this, over accumulation of proteins involved in carbohydrate metabolism have been reported in the leaves of many halophytes [50]. Specific transporter mediated intracellular compartmentalization of excess ions in to vacuole is another key mechanism used by halophytes to maintain a moderate cytosolic K^+/Na^+ ratio in the cytosol. Thus, membrane ATPases and ion transporters play vital roles in salt tolerance of halophytes [51].

Salvadorapersica, a very common inhabitant of dry saline tracts was grown at different in situ salinities, and the partitioning of sodium and chloride ions in to different plant parts were studied by [52] (**Figure 2**). The results showed very high content of these ions in the bark and senescing leaf tissue and a comparatively low content in photosynthetically active leaves [53]. The capacity of these sink tissues (bark and senescing leaves) to accumulate more and more salts increased with increasing salinity as well as with age of plant, which indicates a well established salt compartmentation mechanism in this halophyte species [41].

However, recent physiological and molecular observations across the globe indicate that halophytes may employ different mechanisms in ion transportation and homeostasis under salt stress. Latest studies showed that over expression of *SOS 1* and *HKT 1* in the halophytes *Eutrema salsugineum* and *Schrenkiella parvula* conferred much stronger salinity tolerance than that of their glycophytic counterpart [54]. Many halophytic species have now been understood to be constitutively higher expressers of the component genes of salt overly sensitive (SOS) pathway [55]. Genome studies of *Schrenkiella parvula* via. next generation sequencing (NGS) platform revealed the presence of three tandem duplicates of *nhx8* and two copies of *hkt1* which clearly explains the higher transcript abundance of these genes as compared to their glycophytic counterpart [56]. Thus genomic variation may be another important factor leading to enhanced salt tolerance in these halophytes which needs further confirmation. Moreover, the presence of specific Na^+/Ca^{2+} converse transportation mechanism also facilitates better adaptation to salt stress in halophytes [57].



	Root	Wood	Bark	Im. leaf	M. leaf	S. leaf
🟦 25-35 dS m-1	1.54	0.03	1.75	0.02	0.19	1.84
🟥 35-45 dS m-1	2.01	0.04	2.34	0.02	0.21	2.34
🟩 45-55 dS m-1	2.28	0.04	2.57	0.02	0.23	2.46
🟪 55-65 dS m-1	2.31	0.05	3.01	0.03	0.24	2.58

	Root	Wood	Bark	Im. leaf	M. leaf	S. leaf
🟦 25-35 dS m-1	2.49	0.05	2.74	0.04	0.30	2.88
🟥 35-45 dS m-1	3.20	0.06	3.71	0.04	0.33	3.18
🟩 45-55 dS m-1	3.26	0.06	4.06	0.04	0.35	3.86
🟪 55-65 dS m-1	3.65	0.39	4.67	0.20	0.39	4.07

Figure 2.
Sodium (Na^+) and chloride (Cl^-) ion partitioning in Salvadora persica grown at different in situ salinities. Data represents mean of 2nd, 3rd and 4th years [43]. DW = dry weight, Im. Leaf = immature leaf, M. leaf = mature leaf & S. leaf = senescing leaf.

Epidermal bladder cells (EBCs) is another important feature of certain halophytes by which they secrete excess salt. 50% of the existing halophytes have been reported to contain these EBCs [31]. Removal of these EBCs resulted in enhanced salt sensitivity in a facultative halophyte called quinoa (*Chenopodium quinoa*) [58]. These EBCs are 1000-fold bigger than the general epidermic cells in volume, indicating its enhanced sodium excretion ability [59]. EBCs are similar to the trichome cells of *Arabidopsis* and transcriptomic analysis of quinoa EBCs showed enhanced expression of salt stress responsive genes, genes belonging to transporter family, sugar transporters, while photosynthesis-related genes showed reduced expression [60]. Nevertheless, the genetic determinants of EBCs coupled with the molecular mechanism of sodium pumping in to the EBCs is far from clear except a few studies in *Arabidopsis* [61]. This EBCs mediated strategy to tolerate high salt stress is not present in all halophytes. So certain other mechanisms are adopted by those halophytes which do not rely on EBCs. One category of halophytes called as succulent halophytes, further classified in to stem succulent and leaf succulent, accumulate large quantities of salt in its cells and tissues instead of secretion or compartmentation [40].

Succulence aids them in resisting the salt induced toxicity which can even reach up to 60% of leaf dry weight [62]. *Suaeda* is a classic example of this category of halophytes which can tolerate very high levels of salt without external secretion [31, 63]. Several other succulent halophytes have been reported belonging to amaranthaceae family [26, 64]. *Sesuvium portulacastrum* is another typical example of succulent halophyte often called as euhalophyte which accumulates lot of salt in its succulent leaf tissue [65].

3.3 Photosynthetic adaptation in halophytes

Photosynthesis is the most vital physiological process in plants, which if affected by any kind of stress, leads to significant yield reduction. Under salt stress, stomatal conductance gets reduced in order to save water, so internal CO_2 also gets reduced leading to reduced photosynthesis [66]. This reduced CO_2 limits the dark reactions of photosynthesis and leads to accumulation of reducing powers in grana thylakoid, thereby damaging the photosystem [67]. The differential effect of salt stress on photosystem II activity of halophytes has been studied in *Spartina versicolor* as compared to the glycophyte *Cyperus longus. Cyperus* displayed a significant reduction in the activity of photosystem II (PSII) under salt stress, but on the contrary, *Spartina* did not show distinct reduction [68]. Moreover, a deeper mechanism oriented study showed that halophytes were able to absorb light even under high salt stress, indicating a much stronger PSII complex [69]. Proteomic study showed that the halophytic relative of wheat was able to accumulate more chlorophyll a-b binding protein (CP24) protein under high salt stress, which in turn stabilized the PSII complex [70]. The ability of halophytes to change their carbon assimilation pathways from C3 to C4 and even crassulacean acid metabolism (CAM) according to stress levels is highly unique and vital trait for tolerance to high salt stress [57]. Altogether, the ability to protect PSII from oxidative damage coupled with situation specific shift of carbon assimilation pathways is the photosynthetic adaptive mechanism in halophytes under salt stress.

3.4 Molecular signatures of halophytes

The unique molecular signatures of halophytes place them very high on the salt tolerance hierarchy as compared to glycophytes [71]. A concentrated effort is made to bring together diverse research attempts on the various signaling aspects under salt stress conditions in halophytes, starting from sensing, activation of downstream signaling elements and all other potential candidates in the salt stress signaling network. A much stringent regulation of gene expression has been reported in halophytes as compared to its glycophytic relatives. Research attempts to exploit the rich source of salt responsive genes and promoter regions of diverse halophytes have also yielded significant achievements in the recent past [72]. Moreover, halophytic gene sequences are more complex with the presence of transposons and intergenic sequences [73]. The copy number of genes related to salt tolerance is also high in halophytes as depicted by the presence of three copies of calcineurin B-like 10 (*CBL 10)* in *Thellungiella parvula* as compared to one copy in *Arabidopsis* [74]. *SOS1,NHX1* and many other salt stress related genes have constitutive expression in halophytesas compared to salt induced expression in *Arabidopsis* [75]. Further, the transcript abundance of H^+ ATPase gene was low in the halophyte *Chenopodium quinoa* as compared to *Arabidopsis*, but the activity of the transporter protein was much higher, indicating some kind of post translational modification in halophytes [76].

4. Ecological and economic potential of halophytes and other saline vegetation

Harnessing the huge economic potential of the diverse halophytic plants and other saline vegetation is highly imperative to reduce the damage caused by soil and water salinization with special reference to the poor rural agrarian sector. This section of the chapter deals with the wide range of utilization of halophytes in various ecological zones of India as food, fodder, bio fuel, medicine and industrial raw materials for mass production of various compounds. The commercially untapped economic potential of certain abundantly found halophytes are also discussed as a possible livelihood source to the resource poor farmers of these saline dry lands. The oldest known attempt to use halophytic plants was the utilization of *Alhaji maurorum* as a soil ameliorant. However, Israel has to be credited for the prolific rediscovery and utilization of halophytes in saline tracts [77]. The first half of this section deals with the prime potential of halophytes that has to be harnessed from a national point of view, relating to replanting and ecological recovery of barren saline dry tracts, cheap biomass for renewable energy, climate change mitigation, CO2 sequestration and biological reclamation. The second part consists of the economic potential of halophytes related to use as food, fodder, medicine, chemicals, sea weed, mangrove based aquacultures etc. which can be a possible livelihood source to the resource poor farmers of these saline lands.

4.1 Halophyte mediated climate change mitigation

The climate foot print of present agricultural practices is univocally accepted to be similar to that of fossil fuel burning. The sustainability of current agricultural practices is questioned as they squander rich resources and acts as priming agents to global climate change [78]. So there has to be an inevitable change in the ethos of research and policy making to focus more on crops that grow well on limiting resources. Next generation agriculture should also be based on alternative crops like halophytes and mangroves which are water and nutrient use efficient and have sustainable yields across varying environmental conditions. The climate change mitigation potential of halophytes can be explained by just one example ie. *Suaeda fruticosa* thatcan survive and complete its life cycle under soil salinity of 65 dS m^{-1}, pH of 10.5 and under little or no water [79]. Such halophytes and other saline vegetation has tremendous potential in saving water, preventing soil erosion and replanting barren saline and sodic soils which is highly imperative in the current scenario of global change. Moreover, halophytes are suited to our brackish or saline water resources there by helping in fresh water conservation and replenishment. Scientific management of halophytic fields has tremendous potential to prevent further salinization of aquifers and groundwater of adjacent landscape. Due to climate change, global flood has become quite common and it is accompanied by post flood rise in water table and waterlogging. Under such situation, halophytes possess the ability to reduce saline water table and reclaim the affected land [53].

Mangroves species play a pivotal role in protection of our coastal ecosystem. The abundant aerial roots of these species are home to hundreds of creatures which are mostly endangered. Many species are true to sandy beaches and hence prevent soil erosion. More importantly, the role of mangroves as a livelihood to resource poor farmers cannot be underestimated [28, 80, 81]. As per National cyclone risk mitigation project (NCRMP), 308 cyclones of varying intensity impacted the eastern coast of India between 1981 and 2000. Extreme wave conditions are very common after effect of such cyclones originating in the Bay of Bengal [82]. As a result thousands of fertile agricultural land gets transformed in to wastelands. To cope with this, ICAR-CSSRI, regional research station (RRS), canning town, West Bengal developed a land shaping technology [83]. In this technique, land was converted in to medium ridge and shallow furrows where suitable halophytic tress along with paddy cum fish cultivation was practiced in *kharif* and in *rabi* season, furrows were used for rice cultivation [83]. Deep furrow and high ridge shaping technique was also practiced on coastal land of Sundarbans to overcome post cyclone stress [84]. Altogether, this technology plays a major role in rain water harvest, improved drainage and there by reduction in soil salinity and reduces environmental foot print by increased carbon sequestration.

4.2 Carbon sequestration potential of halophytes

Carbon sequestration is defined as the process of increasing organic carbon reserves by appropriate scientific land management interventions [85]. A much up scaled effort has to be put forth to harness more and more atmospheric CO_2 and store it in our soil. This has been reported to have enormous potential to reduce greenhouse gas (GHG) emissions [86, 87]. Halophytes helps in restoration of barren saline and sodic soils and sequester more and more carbon to enrich the organic carbon status of our infertile soils. ICAR-CSSRI has worked on the carbon sequestration potential of various agroforestry components in saline sodic soils. Results showed that these trees acts as carbon sink by virtue of their high growth rate, attractive wood and bio drainage properties. *Eucalyptus tereticornis* plantation was able to sequester 9.5 to 22.5 Mg ha^{-1} carbon in different spacing and 90.6 Mg ha^{-1} in block plantations along the canal after 6 years of planting in waterlogged saline soils of northwestern India [88–90]. Six years old *Eucalyptus tereticornis* plantation in sodic land showed a cumulative carbon stock (above ground biomass C+ below ground biomass C + soil carbon) of 122.6 Mg ha^{-1} with CO_2 mitigation capability of 369.2 Mg ha^{-1}. Moreover, carbon storage in soil was found to be 44.4% higher in agri-silviculture as compared to rice wheat cropping system in partially saline and sodic soils [91]. Several other studies by ICAR-CSSRI research groups showed very high carbon sequestration potential of tree species like *Eucalyptus tereticornis*, *Syzygium cumini*, *Pongamia pinnata* and *Populus deltoides* [92, 93].

4.3 Source of bioenergy

The dream of producing bioenergy can only be conceptualized if we can identify alternate species that can grow and survive in barren saline and sodic soils and therefore, would not compete with our conventional agricultural components. Halophytes are potential candidates as bioenergy crops as they can be watered even with sea water without any significant reduction in biomass or seed yield. Second generation biofuels are the topic of discussion, where nonfood biomass is used as raw material for biofuel production. Four prominent raw materials used for second generation biofuel production in India are the lignocellulosic biomass of four halophytes namely *Pongamia*, *Jatropha, Panicum virgatum* and *Miscanthus* [94]. Two significant attributes of such fuels are reduced environmental foot print and improvement in soil quality [95]. The salt excluder category of halophytes is better suited to biofuel production as fouling is a common problem in fuel developed from salt accumulating halophytes as salt is non-combustible. The latest review on major halophytes used for second generation biofuel production in China is the benchmark reference [96]. *Salicornia* (glasswort), *Suaeda* (sea-blite), *Atriplex* (saltbush), *Distichlis* (arid salt grass) and *Batis* spp. are another set of promising halophytes rich in lignocelluloses content [26, 97]. Abundantly found halophytic species like *Salicornia*, sea grass along with two mangrove species, *Avicenia berminans* (black mangrove) and *Rhizospora mangle* (red mangrove) has found a place in the green lab of National Aeronautics and Space Administration (NASA), USA as a viable alternative energy resource [98]. Another innovative and attractive concept that has emerged is to develop an integrated seawater energy and agriculture system (ISEAS) where there will be a coupling of biofuel feed stock, aquaculture and mangrove silviculture for the ultimate aim of producing sustainable aviation fuel [99].

4.4 Phytoremediation potential of halophytes

Recently, large tracts of agricultural lands in arid and semiarid regions have been subjected to heavy salinization and heavy metal pollution, arousing serious health and environmental concerns. Phytoremediation is defined as the use of plants to remove pollutants from soil and (or) render them harmless [15, 100, 101]. Deep rooted, high biomass producing accumulator category halophytes are potential candidates for phytoremediation of saline heavy metal contaminated soils [102]. It is cost effective and provides additional output in terms of forage [103]. The added advantage of halophytes in phytoremediation is its high tolerance to heavy metals and increased uptake of these heavy metals [104, 105]. Recent research attempts on halophytes mediated phytoremediation of heavy metal contaminated saline soils across the world are more in number indicating its environmental significance [106, 107]. Functional biology or potential of halophytes based studies also took place but are few in number [105, 108, 109]. Halophytes such as *Atriplex halimus* [110], *Spartina alterniflora* [111], *Sesuviumportulacastrum* [112] and *Tamarix africana* [113] are well proven examples in phytoremediation of heavy metal contaminated saline soils.

4.5 Halophytes: potential source of nutrition and value addition

It is next to impossible to grow conventional crops in the barren salt affected soils spread across the country which develop the gap of demand and supply of quality food in such areas. It ultimately creates barrier to food and nutritional security of the resource poor farmers living in such harsh areas. So halophytes have a huge potential as an edible source of nutrition there by securing livelihood of farmers. Such concept is gaining popularity very quickly and has spread across the world [101]. Among other salt tolerant crops, beetroot (*Beta vulgaris*), date palm (*Phoenix dactylifera*), amla (*Emblica officinalis*), karonda (*Carissa carandas*), guava (*Psidium guajava*), jamun (*Syzygium cumini*), pomegranate (*Punica granatum*), ber (*Ziziphus mauritiana*), bael (*Aegle marmelos*) and clusterbean (*Cyamopsis tetragonoloba)* are well known for their food value, and these are successfully grown in such saline soils using saline water irrigation. Also in India there is an extensive traditional use of non-conventional halophyte crops and naturally occurring halophytic vegetation as a nutrition source and further value addition (**Table 3**).

"Saji" or "barilla" is anindigenous value addition of locally available halophytes. It is the soda ash obtained from the air dried foliage materials of chenopod shrubs like *Haloxylon*, *Salsola* and *Suadea*. It is an essential ingredient of papad, and contributes to organoleptic qualities in terms of crispness and expansion of fried papad. The local Rajasthani *Banwaria* community has expertise in making *Saji.* The *Saji* produced from *Khara lana* (*Haloxylon recurvum*) is of the best quality, whereas*Saji* produced from *Pichki lana* or *luni* (*Suaeda fruticosa*) is of medium quality, and that produced from *lani* (*Salsola baryosma*) is of inferior quality [121]. Another aspect on which ICAR-CSSRI has extended its research is edible cactus (*Opuntiaficus indica*) and its growth and yield potential under saline soils. Certain specific moderately salt tolerant clones have been identified for raised bed planting in saline tracts [122].

Moreover, around 50 species of seed producing halophytes are potential sources of edible oil and proteins. The best part of their use for oilseed is that they generally do not accumulate salts in their seeds. Prominent halophytes such as *Suaeda fruticosa*, *Arthrocnemummacrostachyum*, *Salicornia* spp., *Halogeton glomeratus* and *Haloxylon stocksii* have been reported to produce high grade edible oil with an unsaturation content of 70–80% [123]. Seeds of *Salvadora oleoides* and *S. persica* contain 40–50% fat and are a good source of industrially important lauric acid and myristic acids. Its purified fat is used for soap and candle making. *Terminalia catappa* (seed oil 52%) is another tree species widely used for edible oil production at industrial level. *Salicornia bigelovii* is another halophytic species used for

Plant scientific name/ common name	Plant parts	Nutritional aspects and uses
Anthrocnemum indicum	Phylloclades	Highly nutritious, used in pickles
Amaranthus spinosus (Kanta chaulai, kante bhaji)	Tender shoots, young leaves	High in Ca content, leaves cooked as vegetable, mature stems cooked with small fishes
Aster tripolium (Salt bush)	Leaves	Fresh salads, cooked vegetable
Atriplex hortensis (Pahari paleng)	Leaves	High nutritive value as a green leafy vegetable
Balanites aegyptiaca (Hingor, hingod)	Fruits	Both fresh and dried fruits are edible
Beta maritime (Palak)	Young shoots	Highly nutritious, consumed like spinach
Borassus flabellifer (Palmirah palm)	Edible radicles, fruits	Consumed as toddy, jaggery and vinegar
Capparis decidua (Kair)	Raw Fruits	Used as pickles and high medicinal values
Carissa carandas (Karaunda)	Fruits	Rich in iron, vitamin C, A, Ca and P, immature fruits are pickled and eaten raw.
Centella asiatica (Brahma manduki)	Leaves, young shoots	Widely used in Indian regional cuisines as a culinary vegetable.
Chenopodium album (Bathua)	Leaves, young shoots	High in protein, vitamin A, Ca, P, and K, cooked as vegetable, curries, raita and paratha-stuffed breads in northern India
Corchorus capsularis (Chanachedi, mora-pat)	Leaves, shoots, fruits	Rich in vitamins and minerals, used in salads, leafy vegetable
Eleocharis dulcis (Neerchelli)	Tubers	Rich in vitamin B_6, Mn, and K, tubers cooked
Grewia tenax (Gondni, kanger, kaladi)	Fruit, leaves, seeds	Fe-rich fruit, consumed raw or use as refreshing drink or porridge, leaves eaten as a green vegetable
Haloxylon salicornicum (Lana, khar)	Leaves, stem, seed	Fatty acids, vitamin C & A, used as salad and pickles, seeds mixed with bajra for roti making
Hemidesmus indicus (Anantbel, nannari)	Roots	Roots used for pickle, “nannari sharbat” also made from roots
Leptadenia pyrotechnica (Khimp, jivanti)	Pods	Pods are of medicinal value and used as vegetables in Rajasthan
Morinda citrifolia (Noni)	Fruit	Starvation fruit during famine, consumed as raw, pickled and used for extracting juice
Nypa fruticans (Nipa palm)	Young shoots, Fruits	Eaten as a green vegetable and the immature fruit is used in deserts
Oxalis corniculata (Amrit sak)	Young shoots, Leaves	Rich in vitamin C, Ca, beta-carotene and P, consumed as vegetables
Pisonia alba (Chandu, muruval)	Leaves	Leaves are eaten as a green vegetable
Portulaca oleracea (Lunia, badi-Noni)	Above ground part	Vitamin-rich with high omega-3 fatty acids, consume as a salad or with yoghurt
Prosopis cineraria (Khejri)	Pods	Pod called as Singhri or Sangri used in various types of bhaaji and kadhi in Thar desert of India
Salicornia brachiata (Umari keerai)	Biomass	High protein edible oil similar to safflower, value-added by-products like vegetable salt
Sesuvium portulacastrum (Lunio, dhapa)	Leaves, stems	Rich in Ca, Fe, and carotene, consumed as vegetables by local peoples in arid region

Plant scientific name/ common name	Plant parts	Nutritional aspects and uses
Suaeda fruticosa (Khario luno)	Leaves, shoots	Protein rich, eaten by local people as vegetables
Suaeda maritimum (Luno, lano)	Young leaves	Leafy vegetable, can be used in combination foods as seasoning
Suaeda nudiflora (Morus)	Leaves	Pickles, salad, and as a vegetable
Terminalia catappa (Deshi badam)	Fruits	Sweet fruit and edible kernel
Ziziphus nummularia (Jhar beri, chanibor)	Fruits	Fruits eaten raw

Source: [114–120].

Table 3.
List of halophytes, other salt tolerant crops, their edible portion and nutritional properties.

commercial oil production in the western states of India. The seed (31% protein) is pressed for its high quality edible oil (28%) which is an alternative source of omega-3 polyunsaturated fatty acids [124].

Quinoa (*Chenopodium quinoa*) is another annual facultative halophyte which has every potential to become a highly economic crop in saline areas of India. The exceptional tolerance of this crop to soil salinity has already been reported [125]. Protein content of quinoa ranges from 12 to 17% depending on variety, environment and crop management practices [126]. This is higher than our conventional cereal crops like rice (6–7%), wheat (10.5–14%) and barley (8–14%). More importantly, it is rich in lysine and methionine which are the two amino acids absent in cereals and pulses respectively [127]. ICAR-CSSRI has initiated a network project on morpho physiological characterization and standardization of agronomic practices of quinoa (*Chenopodium quinoa*) for salt affected agro-ecosystems. Nineteen germplasm lines were evaluated under four levels of irrigation water salinity ie. best available water, 8 dS m^{-1}, 16 dS m^{-1} and 24 dS m^{-1}. Germplasm EC 507740 gave maximum grain yield of 9.20 g per plant at highest levels of salinity (24 dS m^{-1}) [128].

"Kharchia 65" is a wheat race native to Pali district of Rajasthan which is commonly called as red wheat. It is universally recognized as highest salt tolerant genotype. This genotype has been extensively used in the development of salt tolerant wheat varieties by ICAR-CSSRI, Karnal, namely KRL1-4, KRL 19 and KRL 39. It is a universal donor to salinity breeding in wheat coupled with high yield and has been registered in ICAR- National Bureau of Plant Genetic Resources (NBPGR), New Delhi (registration number: INGR99020). The main reason for growing this wheat variety in the arid and semiarid regions of the country is that it is highly salt tolerant and requires very less water as compared to other hybrids and can also be grown as rain fed crop purely on conserved soil moisture. Another useful trait of this genotype is that the plants are tall with high straw content which can be effectively used as a fodder to cattle as it is much preferred by animals as compared to other wheat straw [129].

4.6 Silvipastoral system for ecological restoration of saline sodic soils

Biodrainage is a term that is getting more and more popularized which signify the use of salt tolerant trees and grasses for reducing salinity and waterlogging [130].

Apart from reclamation of these saline lands, silvipastural system aids in improved carbon sequestration [131], increased soil rhizosperic activity [20], reduced greenhouse gas emissions [132] and long term adaptation to changing climates [133]. Mesquite (*Prosopis juliflora*) combined with Kallar grass (*Leptochloa fusca*) was reported to be a promising silvipastoral system in sodic soils [134]. Other successful silvipastural combinations *are Acacia nilotica + Desmostachya bipinnata*, *Dalbergia sissoo + Desmostachya bipinnata* and *Prosopis juliflora + Desmostachya bipinnata* [134]. *Tamarix articulate* is another very productive halophytic tree species with a biomass production of 93 Mg ha^{-1} in 7 years [135]. Aromatic grasses like lemon grass (*Cymbopogon flexuosus*) and palmarosa (*Cymbopogon martinii*) were studied by ICAR-CSSRI and found to be suitable to moderate alkali soils up to pH 9.2. Moreover, the most popular aromatic grass called vetiver (*Vetiveria zizanioides*) was found to be dual tolerant to high pH and waterlogged soils [136]. Licorice (*Glycyrrhiza glabra*) was reported by ICAR-CSSRI to be highly tolerant to sodicity level up to 9.8 [81]. Other promising grasses suitable to saline sodic soils are *Aeluropus lagopoides*, *Dichanthium annulatum*, *Chlorisgayana*, *Bothriochloa pertusa, Eragrostis spp.*, *Sporobolus spp.* and *Panicum spp.* [130]. Other farmer preferred halophytes in saline sodic soils are *Ziziphus*, *Atriplex*, *Kochia*, *Suaeda*, *Salsola*, *Haloxylon* and *Salvadora* as they are preferably browsed by camel sheep and goat [137].

Salvadora persica is an oil yielding salt bush that has been extensively studied by ICAR-CSSRI. A *Salvadora persica* based silvipastural system was developed and popularized with forage grasses like *Leptochloa fusca*, *Eragrostis sp.* and *Dichanthium annulatum* on the saline *Vertisols* of Gujarat, India [138]. This model was successful in saline soils of electrical conductivity ranging from 25 to 70 dS m^{-1}.Moreover, high biomass producing halophytic trees like *Acacia tortilis* (hybrid), *Ziziphus mauritiana*, *Pithecellobium dulce*, *Melia azedarach*, *Cassia fistula*, *C. javanica*, *Callistemon lanceolatus*, and *Acacia farnesiana* were popularized among the resource poor farmers of the saline and sodic soil tracts of northern India. The waterlogged soils of semiarid regions of northern India were also subjected to reclamation via. *Eucalyptus* based agroforestry [81, 139]. *Eucalyptus tereticornis* was the preferred species. *Elaeagnus angustifolia* is another tree species recently found to be effective for bio drainage based on its water use efficiency, salt tolerance and growth rate [89].

4.7 *Salicornia*: a case study in western coasts of India

Salicornia is an obligate halophyte belonging to chenopodeaceae family commonly found at the edges of wetlands, marshes, sea shores, and mudflats [140]. It is commonly called as pickleweed, glasswort, sea beans and sea asparagus across India. Some species of *Salicornia* can even tolerate and complete its life cycle under 3% Nacl [141]. In India, this halophyte has been used as an edible crop as well as for non-edible purposes. Use of this plant as a source of soda for glass manufacture is time immemorial. *Salicornia brachiata,* a leafless shrub, was indeed the first source of salt produced from plants in 2003 by Council of Scientific and Industrial Research (CSIR)- Central Salt and Marine Chemicals Research Institute (CSMCRI), Gujarat, India. This vegetable salt, unlike common salt contains salts of potassium, calcium, magnesium and iron. On farm trials have shown that it has the potential to produce 3–4 tons of vegetable salt/hectare which can fetch a market of Rs 10–12 per Kg to the resource poor farmers of these barren saline tracts [142]. Further, recurrent selection mediated germplasm improvement was also carried out for better yield. In order to minimize the cost of cultivation, this species (improved variety: SOS 10) were sown in monsoon along the western coast of India in a large scale. Public private partnership based salt product named "*Saloni*" was also developed on a commercial scale in Gujarat [142].

Low content of seed sodium makes it a very good source for human heart, apart from its other medicinal properties against diabetes, asthma, hepatitis, gastroenteritis and cancer [143]. Moreover, the edible oil from its seeds is rich in poly unsaturated fatty acids and similar to safflower in fatty acid composition. It is also used as a green salad in the western areas of India. Antibacterial, antitubercular and antioxidant activities of *Salicornia brachiata* has been previously reported [144]. It is very popular and commonly used by villagers of western and eastern coast as an animal fodder, herbal salt and as a source of oil, while the ash of the whole plant has been reported to be useful in itch treatment [145, 146]. Most recently, prolific study on its polysaccharides and other phytochemical profile for phenolic compounds, oils, proteins, flavanoids, sterols, saponins, alkaloids and tannins are under way and shows promising results [147]. The oligosaccharide profiling of Indian species *Salicornia brachiata* was performed and the results showed this plant to be rich source of dietary supplements [148]. The ecological benefits of large scale cultivation of this plant along the coasts of India may be summarized as utilization of barren saline lands, upscaling of green belt, coastal development and protection and biodiversity conservation [89]. Finally, the equity benefits are vast export income and private industrial and institutional collaboration. So, this plant is a plant of future which needs special mention.

4.8 Medicinal halophytes: a formidable source of medicine, neutraceuticals and other products

One of the major physiological adaptations of halophytes to saline stress is the production of different biomolecules which possess highly useful biological activities like antioxidant, antimicrobial, anti-inflammatory and antitumoral [149]. So, if introduced in to our diet, it has the potential to prevent a lot of diseases like cancer and cardiac disorders [36]. These biomolecules also enhances the neutracutical value of halophytes as the concentration is very high as compared to their glycophytic counterparts [150]. Moreover, certain biomolecules are specific to halophytes and hence have great agri food, pharmaceutical and cosmetic value [151]. Presence of a wide array of compounds like alkanes, fatty acids, carbohydrates, aminoacids, alcohols, terpenoids, flavanoids etc. have been reported from major halophytes [152]. Halophytes have long been used as folklore medicine by villagers and very little documentation is done in this aspect. A few glimpses off current use of halophytes as medicine in India is discussed here. *Salicornia* sp. is widely used as a folk medicine for constipation, diabetes and cancer [153]. *Suadea* sp. and *Atriplex* sp. are widely known to possess hypoglycaemic and hypolipidaemic activities [154].

Catharanthus withstands EC of 12 dS m^{-1} and produces about 130 *catharanthus* alkaloids compounds, including vinblastine and vincristine, two drugs used to treat cancer. Mangroves are also good source of biomolecules. For instance, the mangrove *Cynometra ramiflora* oil has antibiotic properties and is used in skin diseases [155]. Among other notable use of halophytes as medicine, use of *Pandanus odoratissimus* in leprosy, scabies, heart diseases, *Salvadora persica,* in cough, rheumatism, toothache and piles, *Salsola baryosma, S. kali* as anthelmintic, emmenagogue, diuretic, *Tamarix articulata* in eczema, ulcers, piles, sore throat, diarrhea, liver disorders and*Cress cretica* as tonic, aphordisiac, stomachic deserves special mention [28]. Among other products, biopetrol/diesel from *Jatropha curcas* and *Euphorbia antisyphilitica*, multiple bioactive compounds from *Salsola baryosma,* essential oil from *Pandanus* sp., *Terminalis catappa,* aromatic oil from *Grindelia camporum, Larrea tridentate* and *Simmondsia chinensis,* rubber from *Chrysothamus nauseousus* and *Parthenium argentatum,* multiple beverages from the mangrove palm *Nypa fruiticans,* pulp and fiber from *Phragmitesaustralis, P. karka, Juncus rigidus* and *J. acutus* deserves special mention [28].

4.9 Seaweeds: valuable resource pool

Sea weeds are an integral component of coastal ecosystems that lend invaluable support to the diverse marine life. The economic value of these sea weeds is of significant importance to the resource poor farmers of saline coastal areas. It is used as a food, but more importantly the phycocolloids derived from it is of significant export value [156]. In India, apart from phycocolloid production, sea weeds are being used for the commercial production of crop growth stimulating agents. Moreover, the agar and alginate industry full depends on this valuable coastal resource. Off late, they are extensively being explored for biofuel, neutracuticals, medicines and food additives [157]. In Indian context, carrageenophytes (red sea weeds), *Gracilaria* spp. and *Gelidiella* spp. for agar production, *Sargassum* spp. and *Turbinaria* spp. for alginate production, *Kappaphycus alvarezii*, *Hypnea musciformis* and *Sarconema filiforme* for phycocolloid kappacarrageenan production is of major economic importance [156]. Another matter of pride for Indian Council of Agricultural Research (ICAR) is the endorsement of ICAR-CIFT (Central Institute of Fisheries Technology) by world health organization (WHO) to fight COVID-19 pandemic. The research group proposed that sulphated polysaccharide from seaweed can be a potent molecule to fight against the COVID-19 Pandemic, hence, is a candidate molecule to be studied against SARS-CoV-2 [158].

5. Conclusion and future ideas

Tangible evidences of global climate change and land degradation due to salinization are quite evident and so it is highly imperative to bring more and more salt affected land into cultivation to satisfy the food and nutritional security of our burgeoning population. In this context, halophytes and other saline vegetation has paramount importance to ensure economic returns and maintain ecological balance. India has a very rich source of halophytic, mangrove and other saline vegetation which has huge potential in monetary as well as environmental terms. It can reduce the gap between demand and supply of food and fodder for livestock, besides being a source to numerous other products of economic significance. The role of halophytes in climate change mitigation, carbon foot print reduction, renewable energy source and greening of our barren saline lands has to be conceptualized on field basis. Moreover, deteriorating water resources and lower availability of good quality water for agriculture demands a paramount shift to edible halophytic crops like quinoa, already being termed as super food, highly tolerant to drought and salinity stress.

Identification and documentation of region specific halophytes, access to seeds, establishment of halophytic nurseries, optimizing package of practice and development of processing plants are immediate requirements to spread the concept of halophyte based biosaline agriculture. Long term experiments are the need of the hour to prove the sustainability of halophyte based cultivation and economic security. More importantly, breeding programs should be initiated to improve traits such as yield, taste, biomolecule quantity and quality for faster adoption of halophyte based production. Another major issue to be tackled is the low or nonexistent demand on the market and a lack of value chain. For this consumer awareness need to be created and a value chain which consists of different players need to be established. Let us all hope that the ultimate dream of greening our barren saline lands and replenishing soils with saline water table in to sites of bio saline agriculture gets fulfilled in coming years as a boon to mankind.

Acknowledgements

We acknowledge the funding and support from Indian Council of Agricultural Research (ICAR), Ministry of Agriculture and Farmers Welfare, Government of India. Vineeth TV acknowledges Dr. Ashwani Kumar, ICAR-CSSRI for providing photographs of major halophytes.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 9

Protagonist of Mineral Nutrients in Drought Stress Tolerance of Field Crops

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Abstract

The food demand is increasing hastily, that is inducing continuous pressure on agriculture sector and industries to fulfill rising dietary needs. To meet with increasing demand, the food production must be elevated up to 70% until the year 2050. On the other hand, changing climate is disturbing crop production around the World. Crops grown under field conditions are affected by more than one abiotic stress. It is continuous task and challenge for agronomists to make crops environment hardy to obtain maximum yield. It is considered that different agronomic managements, if done appropriately, could be beneficial for increasing crop production. The optimal provision of plant nutrients can assist the crops to fight in better way with environmental stress like drought; it can help them to continue their normal metabolism even under hostile abiotic circumstances. The regions that have reduced availability of water for crop production, a balanced nutrient management can assist crops to give adequate production. Some of nutrients have potential of not only maintaining plant metabolism but also to enhance the quality of product. This chapter highlights the protagonist of plant nutrients in alleviation of drought stress in field crops.

Keywords: drought, physiology, consequences, alleviation, macronutrients, micronutrients, mechanisms

1. Introduction

Water shortage is an emerging limitation to crop production due to climate change. It critically influences development and growth of crops and results in significant production loss. It is important to recognize morphological, physiological and bio-chemical effects of drought in relation to nutrient uptake in crops [1]. Drought impairs mineral transport and effects stomatal conductance. By considering nutrients role in plants growth, negative consequences of drought can be avoided by management strategies [2, 3]. Previously, many scientists have worked to understand the role of mineral nutrients in alleviation of drought stress, but more is to be done. Among minerals that are essential for plant growth, macronutrients has significant importance because their shortage lead to quick response and plants become more susceptible to other abiotic and biotic stresses. On the other hand, micronutrients deficiency effect at molecular level and results in altered enzymatic activity and blockage in signal transduction pathways [4]. Those plants that have capability to attain and retain water in large amount, as well as better water usage efficiency, are more tolerant to drought stress. Response in the direction of water stress depends upon crop growth stage, intensity and severity of drought [5, 6].

There are many reports available previously that addresses the consequences of drought on different physiological parameters like photosynthesis, respiration, homeostasis and assimilates transportation but very few discourses the drought effects on mineral in crops. Albeit, if crops are grown on mineral-rich soils, water limitations can be the reason of disruption in nutrient uptake. Minerals are taken up by plants in inorganic ionic forms. When a plant is subject to drought, due to low soil moisture, the diffusion of minerals is disrupted and ultimately transport is affected [3, 7, 8].

2. Effect of drought stress in crops

Field crops are simultaneously subjected to more than one abiotic stress during their complete life cycle. Drought and high temperature are the most detrimental abiotic stresses. It is continuous task for scientists to make crops hardy against biotic and more importantly abiotic stresses to increase food productivity. The simulation model predicts that to cope with rising food demand, supply must be increased to 70% till the year 2050 [9–12].

Drought stress influences crops by disturbing their physiological and biochemical functioning [13–16]. Previously, work is done making crops vigorous to deal with climatic challenges [9, 16–18] but more is still to be done.

Early droughts due to changing climate can reduce crop productivity [19]. The struggle of water use among domestic, industrial and agricultural sector is making situation worse for irrigated agriculture [20]. This problematic situation is shifting agriculture from irrigated to rainfed areas where periodic drought events are occurring due to disturbed rainfall pattern [21, 22].

2.1 Impact of drought on morphological traits

Crops when subjected to drought stress show different behavior. Some crops are resistant to drought while others are susceptible [23]. Those crops that have taproot system are more tolerant to short term drought events. They can stand with mild to moderate drought condition. On the other hand, prolonged drought can affect all crops likewise and can cause significant yield loss [24].

2.1.1 Effect of drought on seedling emergence

Seed germination is the most critical stage in complete life cycle; it is influenced by water availability for imbibition [25]. Drought stress at this stage can results in irregular germination and deprived seedlings [26, 27]. In rainfed areas, absence of shower at seedling establishment stage critically reduces field emergence [28, 29].

2.1.2 Growth phase affected by drought

Water shortage at vegetative stage disturbs growth and development through impaired turgor and stomatal conductance [30]. The reduction of water potential inside cytosol increases solute level. This leads to damage of cell structure and functioning. Cell division and expansion is also inhibited [31]. Under drought stress, nutrient uptake is also exaggerated that primes to reduction in leaf area and photosynthesis [32, 33]. Several traits of crops that are affected by drought at vegetative stage include leaf area, assimilation rate, total dry matter and chlorophyll [34, 35]. Root length and dry weight of leaves and stem is also reduced [36].

2.1.3 Effect of drought on crop yield

The loss of crop yield due to drought stress is decided by many factors like intensity, duration and ability of crop to tolerate drought stress. In higher plants, anthesis is the most drought susceptible stage [37]. Water shortage at that stage can results in substantial yield loss [30].

In oilseed crops, almost all yield related traits are affected by drought [38–40]. Severity of drought is also an important aspect; it distresses all growth stages regardless of crop, eventually results in considerable yield loss [41–44].

2.1.4 Effect of drought on crop quality

Among oilseed crops, sunflower has significant importance because it is rich in linoleic acid. Drought stress at reproductive stage reduces oil quality in oilseed crops and deteriorates its texture [45]. Drought stress also reduces quality of end products. It disturbs biochemical enzymes [46] and gene regulation that are responsible for oil constituents in sunflower [47].

2.2 Effect of drought on physio-biochemical traits

Crops are responsive to abiotic stresses from molecular to morphological level. Those crops that are tolerant to drought stress modify their cells at molecular level like increasing concentration of osmolytes in cytosol under harsh environment [48–52]. However, in susceptible crops, drought can affect at biochemical level [53–56].

2.2.1 Water relation disturbance

The key phenotypic adoption in drought tolerant crops is tap root system. They can extract water from deeper soil layer even under severe environment. Those plants that have shallow root system, when subjected to drought, it affects their water potential inside cell [57]. The low water potential leads to turgor loss and interrupted stomatal conductance [36, 41]. Transport of nutrients through xylem is concerned under drought [58, 59].

2.2.2 Photosynthesis reduction

The metabolic process of carbon fixation that occurs in leaves in the presence of light is called as photosynthesis. This is the main energy harvesting phenomenon that is accountable for growth and development. It is affected by different environmental factors like, availability of moisture, sunshine, humidity and temperature [60].

The plants that have C4 carbon fixation pathway are more efficient in carbon harvesting [61], but under drought, they perform in the same way as C_3 plants. Stomatal closure is triggered by water deficit condition that eventually restricts CO_2 diffusion [62], thus diminishes photosynthesis [36]. Ribulose bisphosphate is a vital enzyme in carbon fixation. The activity of RuBP is affected under drought stress. Those crops that can maintain RuBP production are more resistant to drought stress [63–68].

2.2.3 Disrupted uptake of nutrients

Under drought, absorption capacity of roots is affected that condenses nutrient uptake. Nitrogen, being a vital constituent of plants, is required in high quantity. The reduction of soil moisture reduces ability of roots to absorb adequate moisture. Phosphorus uptake, transport and translocation are also affected in drought conditions [6]. It lessens NPK uptake in sunflower [41].

2.2.4 Drought induced oxidative stress

Free radicals of oxygen, that are also known as reactive oxygen species has significant role in cell signaling. Their production remains continue unceasingly inside cell in controlled amount. When a plant is subjected to any environmental stress, its production increases. This augmented concentration induces oxidative stress to crops. They are highly reactive in action; they can cause injury to cellular structure [69]. In oilseeds like sunflower, drought overproduces ROS [70]. Malondialdehyde is an indicator of cell membrane damage in plants. Water deficiency increases MDA production that specifies increment in cellular injury [71–73].

3. Role of nutrients in drought stress alleviation

Optimum nutrient supply not only improves growth of crops but is also helpful for plants under adversative climatic conditions. There are seventeen nutrients that are crucial for plant growth [74]. Upon their requirement, these are grouped as macronutrient and micronutrient. This review deals with role of essential nutrients in drought stress mitigation.

3.1 Macronutrients

3.1.1 Nitrogen

Under dry climatic conditions, water use efficiency and growth of crops is restricted due to less accessibility of water. Efficient nitrogen application can serve the purpose under drought stress [75, 76]. Plants facing drought stress are more susceptible to heat tremors as well. Nitrogen deficiency in drought stress outcomes as biomass reduction in crops [77, 78]. Previous studies have suggested that shoot biomass is more affected under drought-cum-nitrogen stress, while root biomass is not much exaggerated primarily [79]. On the other hand, plants become drought hardy under sufficient soil nitrogen availability [75, 80, 81]. Increasing nitrogen significantly improved crop performance under drought stress. Nitrogen also play significant role in prevention of plasma membrane damage and osmotic adjustment. Application of N under water deficiency also enhances other major nutrient uptake like potassium and calcium [82].

Nitrogen availability diminishes malondialdehyde content that alleviates in drought stress [80]. It recovers photosynthetic contents and improves cell division that lead to leaf area increment [83]. At molecular level, drought stress greatly influences photosystem-II efficiency that is recovered by optimum nitrogen accessibility [51, 84–93].

3.1.2 Phosphorus

Previously, many researchers have testified that phosphorus application under water deficiency in many crops significantly enhance their water usage ability and helps in drought resistance [74, 94, 95]. It is also well known that optimum phosphorus in crops improves root growth and stomatal activity [96, 97]. Phosphorus availability also optimizes leaf area [98], plasma membrane stability and water use efficiency [99–102]. It was observed that phosphorus in leaves was relatively higher under drought condition as compared to optimum water availability which suggests that phosphorus has contribution in drought tolerance [94, 96].

Phosphorus also improves nitrogen mobility under water deficiency [103]. Morphological and physiological parameters were also improved when phosphorus was applied at high rate in drought such as, plant height, leaf area, dry weight and water use efficiency [102, 104]. Application method of phosphorus also influences crop growth in drought, deep phosphorus placement (DPP) method works excellently for drought affected areas that ultimately promotes root growth [101, 105].

3.1.3 Potassium

Potassium is well-known for its osmoregulatory functions in crops. It regulates stomatal conductance and water uptake; the optimum K application increases WUE [106, 107]. Potassium soothes aquaporins and osmotic pressure that regulates water uptake, stomatal regulation, carbon intake, cell elongation and ROS detoxification [108, 109]. In grasses like sorghum, K application under drought improves photosynthesis which leads to growth and yield [106, 110]. In maize, potassium plays role photosynthates assimilation [111]. Potassium availability is correlated with aquaporins activity and stem cell expansion [112].

The hydraulic conductivity of root and anatomical traits has great influence on crop performance. The increment in hydraulic conductivity is associated with drought tolerance [113]. In higher plants, reduction in K influences aforementioned traits, hence compromised yield. Drought simulates ethylene production that in return hinders abscisic acid activity. The starvation of K further worsen the situation, it delays stomatal conductance [109]. Potassium also play role in ROS detoxification and promotes photosynthesis process [114, 115].

3.1.4 Magnesium

Magnesium has central place in chlorophyll molecule, thus has significant importance. It has great role in dry matter partitioning from sink to source. Passable Mg is required at reproductive stage to avoid flower sterility. Foliage application also improves nutrient mobility and helps in growth maintenance under stressful environment [116, 117]. Magnesium is highly mobile nutrient. It has positive correlation with nitrogen and potassium. Adequate magnesium increases their mobility; they are helpful in stress tolerance [118].

Drought stress in field crops affects magnesium uptake from soil. This deficiency can be fulfilled by foliar Mg application [119]. Earlier, it is known that foliage applied Mg can satisfy plant's need [120]. The mechanisms of Mg that are responsible for drought stress induction include growth of root, NPK uptake and improvement of WUE [74].

3.1.5 Calcium

Drought stress leads to overgeneration of ROS that result in cell damage [121–124]. Calcium has its role in detoxification of ROS [125]. It is known that in the activity of aquaporins, pH and calcium are of significance importance [126, 127]. Exogenous application of Ca induces drought resistance in wheat cultivars. Calcium has cell signaling mechanism, which simulates proline accumulation.

Calcium, when it is applied under drought stress, it improves chlorophyll and catalase activity and decreases plasma membrane damage. It also maintains osmolytes like proline and other soluble antioxidants [128, 129]. Foliage applied Ca under drought stress helps to improves drought stress alleviation by refining catalase, peroxidase and superoxide dismutase activity [130].

3.1.6 Sulfur

The role of sulfur application in mitigation of drought stress is very little known previously. It has a substantial role in stress signaling pathway. It improves crop growth, morphological parameters and nutrient contents [131]. In counter stress mechanism, increment in glutathione also has significant importance. It aids in ROS detoxification [132]. The uptake of sulfur in adequate amount helps crops to stand with drought events. Its transport and assimilation is among one of the drought stress responses [133, 134].

3.2 Micronutrients

3.2.1 Zinc

Zinc has role in various physiological processes like activity of catalytic, carboxypeptidase, superoxide dismutase, RNA polymerase and alkaline phosphates [4, 118, 135, 136]. Under water shortage, zinc has been known to improve drought resistance by improving WUE and water activity [4, 137, 138]. The reduction in zinc uptake, that is caused by water shortage, leads plants toward stress condition. Under limited soil moisture, zinc is immobile [118].

In cereals like wheat, when drought is subjected at anthesis and grain filling, it constrains nutrient uptake which become cause of stunted growth [139]. The process of photosynthesis and water activity is affected under zinc-cum-drought stress, however, when zinc is present in optimum amount, it helps crop to stand with drought. It aids in deactivation of ROS [4, 140]. At reproductive stage, plants are highly susceptible to Zn shortage [141]. When plants are subjected to prolonged drought, it impairs activity of different cell metabolic contents like NADPH. Zinc application inhibits photooxidative damage, reduces ROS generation, and promoting osmolytes concentration like SOD [74, 142–145].

3.2.2 Manganese

It is vital micronutrient that has several functions in plants. It assists in activation of various metabolic enzymes of tricarboxylic cycle. It is the part of photosystem-II, also aids in ATP synthesis and RuBP carboxylase activity. It helps to maintain balance among superoxide dismutase activity and chlorophyll contents, even under water stress [130].

The role of manganese is well known for detoxification of ROS like superoxide and hydrogen peroxide [146]. On the other hand, manganese shortage leads to oxidative stress in plants that causes chlorophyll damage thus stunted photosynthetic activity [4]. Water shortage can also be responsible for manganese deficiency. Low soil availability of manganese as it occurs under dry conditions makes it unavailable for plants [147]. The starvation of manganese leads to WUE reduction. In cereals like barley, lower WUE is correlated with abrupt stomatal control during the day and imperfection in stomatal closure during night. This leads to degradation of waxy layer of plasma membrane that is consequence of ROS activity [148].

3.2.3 Iron

It is involved in chlorophyll pigments production. It is the part of enzymes that are involved in transfer of energy, reduction of nitrogen and formation of lignin. It creates compounds along with sulfur that are the catalysts for other vital biochemical procedures in plants. The iron deficiency results in chlorosis which is the consequent of low chlorophyll concentration. Severe deficiency of iron turns leaf color from yellow to white that is sign of leaf death. Under high soil pH, iron uptake is affected. It also has antagonistic effects with phosphorus and manganese [149].

The moisture in soil greatly inhibits iron uptake [150]. The iron has vital protagonist in oxidative damage protection of leaves under stress. Its deficiency is highly dreadful for plants growth [4]. Sufficient iron amount in plant is essential for activities of antioxidants [151].

3.2.4 Boron

Boron is unavailable in soil barring basic pH and low moisture. It is highly immobile in pedosphere as well as plant. The continuous supply of boron can prevent crops from its deficiency and detrimental effects [152].

Low soil moisture greatly hampers boron uptake from rhizosphere. Its uptake via roots involve passive uptake frequently that is maintained by water uptake. As the water decreases in soil, its uptake is compromised [153]. Main function of boron is to take part in synthesis of cell wall and its extension. It also recovers biosynthesis of lignin and differentiation of xylem. It increases photosynthetic activity and plasma-membrane integrity. It facilitates assimilate transportation [4, 74].

It is necessarily required for H-ATPase activity and the coding involved for it. It also influences uptake of other nutrients like K and deteriorate cell expansion [4]. Boron is also involved in lessening of photochemical damage of cell. Among reasons for low photoinhibition, boron deficiency and drought are well known [153].

3.2.5 Copper

Among micronutrients, copper is essential for growth of plants. It has vital role in electron transport chain and cell wall loosening. It also involves in sensing ethylene, metabolism of cell wall and oxidative stress protection [154, 155]. The well-known function of copper is its involvement in formation of pollens and upholding their viability [4, 155].

There are many enzymes in which this metal acts as cofactor like ascorbic oxidase, laccase, amino oxidase and polyphenols. At molecular level, copper is also involved in cell signaling, trafficking of proteins, mobilization of iron and oxidative phosphorylation. The reproductive parts of plants are more susceptible to cooper deficiency [155, 156].

4. Conclusion

The changing climate is making situation worse for field crop production. Abrupt variations in rainfall and temperature is limiting crop yield. Under field condition, more than one abiotic stresses are disturbing plant growth simultaneously. Drought stress is among the major agricultural yield limiting factor worldwide. Different agronomic practices like optimum plant nutrition management are greatly obliging for crops under drought stress. It can alleviate drought consequences affectively. Drought stress greatly inhibits different physiological functions and biochemical processes. It leads to ROS over-generation that significantly damages cell structure. Optimal nutrients supply like NPK and Ca be accommodating for ROS detoxification and maintenance of cell functions. Under drought stress, they also facilitate in antioxidant generation like catalase, superoxide dismutase and peroxidase. They inhibit photooxidation of vital cell molecules and maintain cell membrane integrity. Likewise, micronutrients such as Zn and Mg also play role in antioxidant generation. Other mechanisms that are maintained by nutrients to induce drought stress are water uptake and stomatal conduction regulation. Optimum supply of K and Ca helps to regulate water activity and aquaporin function. In a nutshell, efficient nutrient management will be helpful in mitigation of drought stress in field crops. The best practice should be adopted to increase their availability to plants. Effective nutrient utilization cultivars need to be focused on.

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Chapter 10

An Insight into the Responses of Early-Maturing *Brassica napus* to Different Low-Temperature Stresses

Xin He

Abstract

Rapeseed (*Brassica napus* L.) is an important oil crop worldwide, responds to vernalization, and shows an excellent tolerance to cold stresses during vegetative stage. The winter-type and semi-winter-type rapeseed were typical winter biennial plants in Europe and China. In recent years, more and more early-maturing semi-winter rapeseed varieties were planted across China. Unfortunately, the early-maturing rapeseed varieties with low cold tolerance have higher risk of freeze injury in cold winter and spring. The molecular mechanisms for coping with different low-temperature stress conditions in rapeseed recently had gained more attention and development. The present review gives an insight into the responses of early-maturing *B. napus* to different low-temperature stresses (chilling, freezing, cold-acclimation, and vernalization), and the strategies to improve tolerance against low-temperature stresses are also discussed.

Keywords: *Brassica napus*, low-temperature, early-maturing

1. Introduction

Low-temperature is a major environmental stress that adversely affects plant growth and development, limiting the productivity and regional distribution of crops [1, 2]. Rapeseed is an important oil crop worldwide, with planting area of 37.58 million hectares producing 75.00 million tons of oilseeds in 2018 (http://www.fao.org/faostat/). Based on vernalization requirement, rapeseed is divided into three main ecotypes, i.e., winter, semi-winter and spring types [3–5]. The winter type rapeseed is mainly grown in Europe and is sown in late summer, which requires strong vernalization and flowerings in spring, exhibiting a classical winter annual and with excellent cold tolerance during vegetative stage [3, 6]. The semi-winter type rapeseed is mainly grown in China only needs moderate or weak vernalization to promote flowering in spring, and with week cold tolerance [3, 7]. The semi-winter type rapeseed excessive exposure to low temperature stress in winter will lead to plant damage at vegetative stage and finally cause yield loss [8]. Yangtze River basin is the major region for planting semi-winter rapeseed in China, which accounts for at least 90% of the nation's total production [9]. The semi-winter rapeseed is usually sown in late September and early October shortly after the harvest of rice, and harvested in May before the cropping of rice in this area [10].

However, in recent years, due to the delay of rice harvest which leads to the postpone of rapeseed sowing until late October or early November, therefore, more and more early-maturing semi-winter rapeseed varieties were planted across Yangtze River basin. Unfortunately, the early-maturing rapeseed varieties with low cold tolerance have higher risk of freeze injury in cold winter and spring [11]. Hence, it is vital to compare early-maturing rapeseed varieties tolerant to cold and evaluate molecular mechanisms that adapt to different low-temperature stress conditions.

2. Morphophysiological mechanism of rapeseed in responses to low-temperature stress

Cold (low-temperature) stress included chilling stress (>0°C) and freezing stress (<0°C) [12]. Chilling stress (0–15°C) causes the membrane to rigidify, destabilizes protein complexes and impairs photosynthesis, eventually made plant stop growing, whereas freezing stress (<0°C) causes intracellular and extracellular ice crystal formation, and results in mechanical injury, and plant death [13–15].

Despite the fact that winter and semi-winter rapeseed is an overwintering oil crop, cold stress can still affect rapeseed development and ultimately lead to a decrease in production [8, 11]. The suitable temperature scope is 10 ~ 20°C for the growth of winter and semi-winter rapeseed. The rapeseed flower number was reduced below 10°C and the rapeseed flowering was arrested when the temperature decreased to 5°C. The rapeseed growth was arrested below 3°C and rapeseed leaves was injured below 0°C [8]. The delay of rapeseed sowing results in poor germination [16], decreased seedling biomass [17, 18], delay of floral initiation and floral bud differentiation processes [17, 19], and decreased flower number, effective pod number, pod length, and seed yield [17, 20, 21] due to low-temperature stress. In January 2008, South China was exposed to an extremely ice-frozen weather, which caused serious injuries to winter rape, affected 77.8% of the overall winter rape area in China and resulted in 10.9% yield losses [22]. Due more and more early-maturing semi-winter rapeseed varieties were planted across Yangtze River basin, rapeseed faces increased risks from continuous low temperature overcast and rainy weather in March. Continuous low temperature overcast and rainy weather during the rapeseed flowering stage or after flowering decreased the ratio of effectual silique, seeds per silique and oil content [23]. In March and April 2010, the middle and lower reaches of the Yangtze River region were exposed to continuous low temperature overcast and rainy weather, which resulted in 10–20% yield losses [23].

To date, many studies have investigated the morphological and physiological changes of low-temperature stressed rapeseeds. Leaves are the main organ to perceive low temperature stress and transmit stress signal in plants [24]. The morphological changes (dehydrated and wilting) of leaves became increasingly evident with the decrease of temperature, due to the total water content in leaves of rapeseed decreased [25, 26].

In winter rapeseed, prolonged cold acclimation led to increased thickness of young leaf blades and leaf cell walls, modified dimensions of mesophyll cells, numerous invaginations of plasma membranes and large phenolic deposits in chloroplasts, large vesicles or cytoplasm/tonoplast interfaces [27, 28]. Unlike cold acclimation, transient freezing treatment reduced the thickness of leaf cell walls and phenolic aggregates, caused reversible disorganization of the cytoplasm and chloroplasts swelling [27, 28]. Obvious gaps existed in the chloroplast grana and starch grains increased in quantity and volume [25]. In general, cold-tolerant winter rapeseed usually grows slowly, having small thick creeping deep-green waxy leaves and large root system.

Low temperature-induced thermodynamic constraints on carbon metabolism was the primary reason for lower photosynthetic activity in plants [24]. Photosynthetic efficiency is a good indicator for Low temperature tolerance in plants [10]. Just like in other crop plants, a marked reduction of photosynthetic activity is observed in rapeseed leaves when treated with low temperature [24, 29]. Tough the photosynthetic activities were reduced both in the cold-stressed leaves of cold-tolerant and cold-sensitive rapeseed cultivars, the chlorophyll a, chlorophyll b and photosynthetic activities in the young leaves of cold-tolerant cultivar all were higher than that in cold-sensitive cultivar [24].

Simultaneously, low-temperature stress caused the overproduction of reactive oxygen species (ROS), elevated H_2O_2 level and increased malondialdehyde (MDA) content in plants, which leads to a necrosis of plants. Plants possess an effective antioxidant system includes superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POD) and catalase (CAT) enzymes, whose combined activities play an important role in elimination of destructive effects of ROS [24, 30]. Furthermore, under natural cold stress in field, the proline, soluble sugar, soluble protein, MDA contents and SOD, POD, CAT activities changed obviously in functional leaves of rapeseed. CAT and SOD activity reached the highest when temperature dropped to 5 and 3°C, respectively. The proline and soluble sugar contents increased when mean daily temperature decreased to 5°C and reached the maximum when temperature was below 0°C. The contents of soluble protein and MDA showed a trend to decrease at first and then increase when mean daily temperature dropped to 10, 5 and 0°C [30]. The SOD and APX activities were both increased by low temperature in the young leaves of cold-tolerant rapeseed cultivar. However, the APX activity was decreased by low temperature in the young leaves of cold-sensitive rapeseed cultivar. While, in the cold-stressed mature leaves, both cold-tolerant and cold-sensitive rapeseed cultivars represented similar antioxidant capacities [24].

Under chilling and freezing stress, the increment of proline accumulation, soluble sugar and protein contents were enhanced in cold-tolerant cultivar compared with cold-sensitive cultivar [24, 26]. Leaf abscisic acid (ABA) was enhanced in cold-tolerant cultivar under chilling and freezing stress [26].

3. Molecular mechanisms influencing responses to different low-temperature stresses in rapeseed

Plants showed increased freezing tolerance during exposure to chilling and low nonfreezing temperatures in a phenomenon known as cold acclimation [31]. The molecular mechanism of cold acclimation and cold tolerance in *Arabidopsis* and winter cereals has been extensively studied. Cold acclimation is a very complex trait involving an array of physiological and biochemical modifications, and these altered processes involved changes in gene expression patterns via phytohormone and the ICE (Inducer of CBF Expressions)-CBF (C-repeat binding factors)-COR (cold-responsive) signaling pathway [13, 32, 33].

3.1 ICE-CBF-COR signaling

In most plant species, CBF transcription factors could bind directly to the promoters of *COR* genes and induce the expression of *COR* genes [34–36]. The *COR* genes protected plant cells against cold-induced damage, repaired cold-rigidified membranes and stabilized cellular osmotic potential by encoding cryoprotective proteins and key enzymes for osmolyte biosynthesis [37]. In Arabidopsis, the basic helix-loop-helix transcription factor ICE1/2 were induced by cold stress, could bind directly to the promoters of CBF and induced CBF expression under low-temperature stress [38–40].

Similar as other plants, the expression of CBF and COR genes were induced by chilling and freezing stresses in different ecotypes rapeseed with different cold tolerance [11, 36, 41–45]. CBFs (BnaAnng34260D/BnaCnng49280D/BnaC03g71900D/BnaC07g39680D), *Kin1* and COR15 all were upregulated in both winter and semi-winter ecotype rapeseeds after cold stress (4°C for 7 days), while *BnaA08g30910D* (a CBF-like gene) and *BnCOR25* were not varied in expression in any cold stressed rapeseeds [41]. Furthermore, COR15A/B, COR413-PM1 and nine CBF1/2/4 genes all were strongly upregulated in winter and spring ecotype rapeseeds after cold stress (4°C for 12 h) [42]. While *ICE1* and COR413-PM2 was downregulated in both winter and spring ecotypes after cold stress, two *CBF3* genes were not induced by cold stress [42]. Most of *COR15A* and *COR15B* were significantly induced in both cold-tolerant and cold-sensitive semi-winter early-maturing rapeseeds after chilling (4°C for 12 h) and freezing (−4°C for 12 h) stress, regardless of cold acclimation (4°C for 14 days) [11]. Ten of 12 *CBF* genes were strongly induced by freezing stress more than chilling stress, regardless of cold acclimation [11]. Unlike other CBFs, BnaC03g71900D was induced by chilling stress more than freezing stress and BnaA03g13620D was induced by freezing stress but suppressed by cold acclimation [11]. However, two *ICE1* genes were not induced by chilling and freezing stress [11], as opposed to *AtICE1* [38]. Inexplicably, no CBF genes were identified in responsive to freezing stress in freezing-tolerant rapeseed as reported by Pu [25].

BnCOR25 were significantly induced by cold and osmotic stress treatment in rapeseed, overexpression of BnCOR25 in Arabidopsis enhances plant tolerance to cold stress [46]. Overexpression of two rapeseed CBF-like transcription factors BnCBF5 and BnCBF17 in spring rapeseed resulted in increased constitutive freezing tolerance, increased photochemical efficiency and photosynthetic capacity [29]. However, constitutively overexpressing *BNCBF5/17* in rapeseed resulted in various degrees of dwarf habit and longer time to flower [29]. The multi-gene (NCED3, ABAR, CBF3, LOS5, and ICE1) transgenic rapeseed plants exhibited pronounced growth advantage under both normal growth and stress conditions [47].

3.2 ABA signaling

Abscisic acid (ABA) is a vital plant hormone that plays a key role in stress resistance during plant growth and development [48–50]. It was reported that ABA levels are increased after cold stress in plants and exogenous application of ABA can induce plant cold tolerance [11, 51, 52]. OST1/SnRK2E, a serine-threonine protein kinase in ABA core signaling pathway, acted upstream of CBFs to positively regulate freezing tolerance via phosphorylating ICE1 to prevent its 26S proteasome-mediated degradation by HOS1 [53]. OST1 phosphorylated basic transcription factors 3 (BTF3) and BTF3-like factors, and facilitated their interactions with CBFs to promote CBF stability under cold stress [54].

27 ABA biosynthesis genes (nine-*cis*-epoxycarotenoid dioxygenase (NCED3/4/5/9), ABA DEFICIENT 1/2 (ABA1/2), abscisic aldehyde oxidase 1/2/3 (AAO1/2/3) and carotenoid cleavage dioxygenase 1 (CCD1)) were regulated by cold stress in winter and/or spring rapeseed. Additionally, many genes involved in ABA signal transduction, such as ABA INSENSITIVE 1/5 (ABI1/5), ABA-responsive element binding protein 3 (AREB3), ABA responsive element-binding factor 1/2/3 (ABF1/2/3), highly ABA-induced PP2C gene 1/2/3 (HAI1/2/3), OPEN STOMATA 1 (OST1), PYR1-like 4/6/7/10 (PYL4/6/7/10), regulatory component of ABA receptor 1/3 (RCAR1/3), SNF1-related protein kinase 2.2/2.5/2.7/2.10 (SnRK2.2/2.5/2.7/2.10),

all were differentially expressed in winter and/or spring rapeseed after cold stress [42]. Furthermore, in freezing-treated (−2°C) leaves of cold-tolerant winter rapeseed line 2016TS(G)10, one PYL gene and one ABI5-like gene were up-regulated, while 13 PP2C and 4 ABI5-like genes were down-regulated [25]. 72.8% ABA signaling genes (94/129) were regulated by chilling and/or freezing in both cold-tolerant and cold-sensitive rapeseed plants but they presented different expression profiles [11]. The ABA receptors *PYL5*/*7* genes were both induced by cold and freezing stress, while the *PYL1*/*9* genes were suppressed. The *PYR1*/*4* genes were only induced by chilling stress but not by freezing stress. The *PYL6* were induced by chilling stress and freezing stress following cold acclimation. ABA co-receptor *ABI1* and *HAB1* were suppressed by all low-temperature treatments, while *HAB2* was upregulated. The *SnRK2B* and *SnRK2D* were induced by all low-temperature treatments, while *SnRK2C* was suppressed. *SnRK2F* and one ABI5 were both induced only by freezing treatment. The *OST1* was induced only in cold-tolerant but not in cold-sensitive rapeseed [11]. While 13 ABI5-like genes have a complex expression pattern in response to different low-temperature conditions, including up-regulated, down-regulated and no changed. Exogenous application of ABA significantly improved the rapeseed seedlings freezing tolerance [11]. Overexpression of *BnaABI3* leads to improved embryo degreening following frost exposure and enhanced pod strength in rapeseed [55].

3.3 Ca^{2+} signaling

Calcium (Ca^{2+}) is an important second messenger of signal transduction in the plant stress responses, plant growth and development. Ca^{2+} signaling were detected and transmitted by calmodulin/calmodulin-like proteins (CaM/CML), calcium-dependent protein kinase (CDPK) and calcineurin B-like proteins (CBLs) [56, 57]. The level of cytosolic Ca^{2+} was transiently increased in plants under cold stress [57–59]. In rice, COLD1 interacts with the G-protein α subunit and activates the Ca^{2+} channel, results the increment of expression of CBF under low-temperature stress [60]. In *Arabidopsis*, overexpression of CaM3 repressed the expression of *COR* genes (*RD29A*, *KIN1* and *KIN2*) [61]. CaM-binding transcription activators (CAMTAs) bind to the promotor of CBF2, promoting CBF2 expression and plant freezing tolerance [62, 63].

88 of 129 *CaM*/*CML* genes were regulated by cold stress in semi-winter rapeseed cultivar ZS11 [64]. 91 of 129 *CaM*/*CML* were regulated by chilling and/or freezing stress in semi-winter early-maturing rapeseed, and most of them were strongly induced by freezing stress [11, 64]. Additionally, 22 rapeseed CDPK genes were up-regulated by freezing treatments [11]. Furthermore, there were 91 genes involved in Ca^{2+} signaling (35 *CDPK*, 38 *CaM*, 16 *CIPK* and 2 Ca^{2+}-ATPase) were differentially expressed in winter rapeseed after cold stress, while 79 genes (44 *CDPK*, 19 *CaM*, 15 *CIPK* and 1 Ca^{2+}-ATPase) were differentially expressed in spring rapeseed after cold stress [42]. In Zhang's report, there were 5 CBL, 7 CIPK and 7 CDPK genes were regulated by cold treatment [65, 66].

4. Future directions

Rapeseed is one of the most important oil crops in the world and China and is affected by chilling and freezing stress. In recently years, several studies have tried to identify the main signaling pathways and genes responsible for low-temperature stress (chilling and/or freezing; cold acclimation and/or cold shock) in different rapeseeds (winter, semi-winter and spring type; cold-sensitive and cold-tolerant;

late maturing and early maturing) based on transcriptomics, metabolomics, lipidomics, and QTL analyses [11, 25, 41, 42, 45, 67–69]. Tough there were so many candidate genes involved in the response to low-temperature stress have been identified, only few genes' functions in cold tolerant have been tested and verified in rapeseed [10, 29, 55, 70, 71]. It is a pity that constitutive overexpression of rapeseed BnCBF5 and BnCBF17 resulted in various degrees of dwarf habit and longer time to flower, tough which resulted in increased freezing tolerance remarkably in spring rapeseed "Westar" [29]. There is still much work to be performed to understand rapeseed plants' responses to low-temperature stress and breed cold-tolerant rapeseed.

Genome editing is an efficient approach for crop improvement either by loss or gain of gene function and several different strategies have been developed [72]. Tough there were a few studies using CRISPR/Cas9 system for editing genes associated with plant/pod development, fatty acid synthesis and biotic stress response [72], no application of CRISPR-Cas9 for editing genes involved in chilling and freezing tolerant in rapeseed. It is expected that the newly emerging genome editing system will make a contribution to future gene function research and molecular design breeding in cold-tolerant rapeseed.

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Chapter 11

Effects of Salinity on Seed Germination and Early Seedling Stage

Cüneyt Uçarlı

Abstract

Salinity is the major environmental stress source that restricts on agricultural productivity and sustainability in arid and semiarid regions by a reduction in the germination rate and a delay in the initiation of germination and subsequent seedling establishment. Salt negatively effects the crop production worldwide. Because most of the cultivated plants are salt-sensitive glycophytes. Salt stress affects the seed germination and seedling establishment through osmotic stress, ion toxicity, and oxidative stress. Salinity may adversely influence seed germination by decreasing the amounts of seed germination stimulants such as GAs, enhancing ABA amounts, and altering membrane permeability and water behavior in the seed. Rapid seed germination and subsequent seedling establishment are important factors affecting crop production under salinity conditions. Seed priming is one of the useful physiological approaches for adaptation of glycophyte species to saline conditions during germination and subsequent seedling establishment. In seed priming, seeds are exposed to an eliciting solution for a certain period that allows partial hydration without radicle protrusion. Seed priming is a simple, low cost, and powerful biotechnological tool used to overcome the salinity problem in agricultural lands.

Keywords: salinity, germination, glycophyte, halophyte, seed priming, plant hormones

1. Introduction

Seed dormancy and germination are distinct physiological processes, and the transition from dormancy to germination is not only a critical developmental step in the life cycle of higher plants but also determines the failure or success of the subsequent seedling establishment and plant growth [1]. Seed germination begins with the water uptake of dry seed (imbibition) and ends with radicle protrusion. Seed germination is affected by adverse environmental conditions including salinity, high temperature, and drought [2].

It is estimated that about approximately 7% of world land is affected by salinity and approximately 20% of 230 million ha irrigated land is salt-affected [3]. This number could be increased in the future due to increased land salinization as a consequence of contaminated artificial irrigation, climate change, and unsuitable land management. Salinity is a major stress responsible for the inhibition of seed germination or reduction in germination percentage and a delay in germination time in crops. At present, around 30 crop plants provide 90% of plant-based human food and the majority of these crops are not salt tolerant, even salt-sensitive, called glycophytes [4]. There have been high yield losses in these crops under moderate salinity (EC 4–8 dS m^{-1}, approximately 40–80 mM NaCl) [5].

High salinity leads a decrease in osmotic potential of ambient soil water, resulting with a decrease in water uptake by dry seeds (imbibition). Besides, the absorption of excess Na^+ and Cl^- ions from soils creates ionic stress and cause toxicity which contributing to disruption in biochemical processes including nucleic and protein metabolism, energy production, and respiration [6]. Salinity also damages the nutrient and hormone balances, especially gibberellin (GA)/abscisic acid (ABA), during germination. As a result, high salinity level causes a delay in germination, even inhibition of seed germination depending on salt tolerance of plants. Dynamic balance between the generation and scavenging of reactive oxygen species (ROS) such as hydroxyl radicals, superoxide, and hydrogen peroxide could be disturbed by high salinity stress. ROS damage the macromolecules including proteins, carbohydrates, nucleic acids, and lipids, or cellular structures like membranes, resulting with inhibition of seed germination [7].

Germination has been found to be under strict regulation of plant hormones, especially GA and ABA [8]. ABA promotes seed dormancy and inhibits germination of seed, whereas GAs release dormancy and stimulate germination. Plant hormones ethylene (ET), and brassinosteroids (BRs) also have positive effect on seed germination by controlling the inhibitory effects of ABA on germination and rupturing testa and endosperm [9, 10]. The plant hormones widely took part in determining the physiological state of a seed and regulating the germination process by interacting each other [11]. Hormones are regulated by distinct transcription factors and signaling components including NO and H_2O_2, showing the complexity of seed germination regulation. While some plant genes control the activity of plant hormones, and the other plant genes are activated by plant hormones [10]. Signaling molecules, such as NO and H_2O_2, also promotes germination and reduce the dormancy by enhancing ABA catabolism and GA biosynthesis [12].

Rapid seed germination and subsequent seedling establishment are important factors determining crop production and yield under salinity stress. One of the useful physiological approaches for glycophytes to adapt saline condition is seed priming [7]. Seed priming is an easy, low cost and low risk technique. The seeds are hydrated in specific solutions including plant hormones (GA3, ET, auxins, kinetin), antioxidant compounds (ascorbic acid, glutathione, tocopherone) organic solutes (proline, glycine betaine), inorganic salts (KNO_3, $CaCl_2$, and KCl), and particular bacteria and fungi species for a certain time to allow metabolic process of germination, followed by drying the seed to inhibit occurring of radicle protrusion [13].

2. Soil salinity and salinity stress

Plants, being sessile nature, are simultaneously subjected to various adverse conditions including salinity, drought, cold, heat, excess water, and heavy metals, which limit their development and growth. Salinity is the major environmental stress source that restricts on agricultural productivity and sustainability in arid and semiarid regions [14]. Salinity is a global issue that affects about 7% of the world's total land area, including 20% total cultivated lands and 33% of irrigated land, causing estimated yield losses of 20% worldwide [15, 16]. Besides, it is estimated that every year 10 million ha of agricultural land destroyed by salinized soil [17]. This rate can be increased by global climate change, use of contaminated irrigation water, intensive farming and poor drainage [18–56]. Without proper and sustainable control, salinity-affected areas will increase to more than 50% of the world's total arable land by 2050 [15]. This rate can be accelerated by increase in sea water level by climate change, excessive use of groundwater for irrigation, increasing use of low-quality water for irrigation and massive introduction of irrigation associated with intensive farming and poor drainage [57].

Soil salinity is a measure of the concentration of all the soluble salts in soil water, and is usually expressed as electrical conductivity (EC) of the saturation extract (ECe) with units of deci siemens per meter (1 dS m^{-1}) [58]. The soils were classified as saline, sodic or saline-sodic based on the total concentration of salt and the ratio of Na^+ to Ca^{2+} and Mg^{2+} in the saturated extract of the soil [59]. When the ECe exceeds 4 dS m^{-1} (approximately 40 mM/L NaCl) and exchangeable sodium percentage is less than 15 with sodium adsorption ratio (SAR) < 13, the soil is saline. The major problem with saline soils is the presence of soluble salts, primarily Cl^-, SO_4^{2-}, and sometimes NO_3^-. The pH of saline soils is usually below 8.5. Sodic (alkali) soils have an ECe < 4 dS m^{-1}, ESP > 15, and SAR > 13. Therefore, Na^+ is the major problem in these soils. Sodic soils have a pH between 8.5 and 10. Saline-sodic soils have an ECe > 4 dS m^{-1}, SAR > 13, and an ESP > 15. Thus, both soluble salts and exchangeable Na^+ are high in these soils. Saline-sodic soils have similar salt and pH levels as saline soils. USSL Staff [59] has described the general relationship of ECe and plant growth as the following:

- non-saline (ECe ≤ 2 dS m^{-1}): salinity effects mostly negligible;
- very slightly saline (ECe = 2–4 dS m^{-1}): yields of very sensitive crops may be restricted;
- slightly saline (ECe = 4–8 dS m^{-1}): yields of many crops are restricted;
- moderately saline (ECe = 8–16 dS m^{-1}): only salt tolerant crops yield satisfactorily; and
- strongly saline (ECe ≥ 16 dS m^{-1}): only a few very salt tolerant crops yield satisfactorily.

3. Seed germination

Seed germination is a complex multi-stage developmental process and regulated by internal and external factors. Internal factors include proteins, plant hormones (gibberellins/ABA balance, ethylene, and auxin), chromatin-related factors such as methylation, acetylation, histone ubiquitination, related genes (maturating genes and hormonal and epigenetics-regulating genes), non-enzymatic processes, seed age, seed size, and structural components of seed including (endosperm and seed coat). Besides, external factors containing moisture, light, salinity, temperature, acidity, and nutrient also affect the seed germination [60, 61].

Seed germination begins with imbibition, the uptake of water by the dry mature seed, and ends with visible protrusion of radicle through testa [62]. Successful germination requires optimum environmental conditions, including water, oxygen, and temperature to initiate this process. Germination/sprouting is regulated by plant hormones such as gibberellic acid (GA), abscisic acid (ABA), ethylene, auxins, cytokinins, and brassinosteroids [63]. Among them, ABA and GA are two important regulators, which play antagonistic roles in seed dormancy and germination [64].

The process of seed germination can be divided into three phases (**Figure 1**) [65]. Phase I begins with imbibition of dry seeds and ends with the early plateau phase of water uptake. Phase II includes reactivation of metabolisms, significant induction of hormonal and enzyme activity using surviving structures and components in the desiccated cells, genes involved in amino acid and nucleic acid synthesis, restarting of cellular respiration with genesis of mitochondria, mobilization of reserved, RNA and protein synthesis machinery [66, 67]. Phase III is post-germination stage involves establishment of seedling and the induction of genes for photosynthetic metabolism after radicle cells elongate and divide [68].

Gibberellins and ABA are two key phytohormones regulating seed germination and seedling growth [69]. While GA breaks dormancy and enhances the seed germination and seedling, ABA inhibits germination and enhances seed dormancy [10]. However, the ratio of the two hormones, rather than the absolute level of each hormone, plays a key role in regulating the breaking of seed dormancy and the onset of germination [70]. GA/ABA balance determines fate of the seed; germination or dormancy. Gibberellins induce the synthesis and production of α-amylase, proteases, and β-glucanases, resulting in the germination of seeds [71]. GAs also stimulate the genes involved in weakening of endosperm and expansion of embryo cell [10]. On the other hand, ABA suppresses expression of many hydrolytic enzyme genes to prevent viviparous germination and inhibits promoting effect of GA on radicle growth and embryo expansion by inhibiting water uptake and hence cell-wall loosening, which is a key step to start germination [72].

Ethylene is a gaseous hormone involved in various processes, including positive regulation of seed germination. Ethylene breaks the primary and secondary dormancy and promotes seed germination by reducing ABA levels or sensitivity [73]. Brassinosteroids (BRs) and auxin induce the secretion of ethylene which works in conjunction with GAs to induce germination [10]. Auxins reduce seed sensitivity to ABA by overexpressing microRNAs and interacting with GAs to counteract ABA suppression during germination [74, 75].

Low temperature decreases seed dormancy and enhances germination in many species, while high temperature has the negative effect on germination and induces secondary dormancy [70]. High temperature down-regulates the genes involved in synthesis of GA synthesis and deactivation of ABA, whereas genes involved in ABA synthesis are up-regulated by high temperature. Therefore, transcriptional changes in ABA and GA metabolism and signal pathways results with inhibition of germination or a delay in germination [76]. Light has been considered both to stimulate

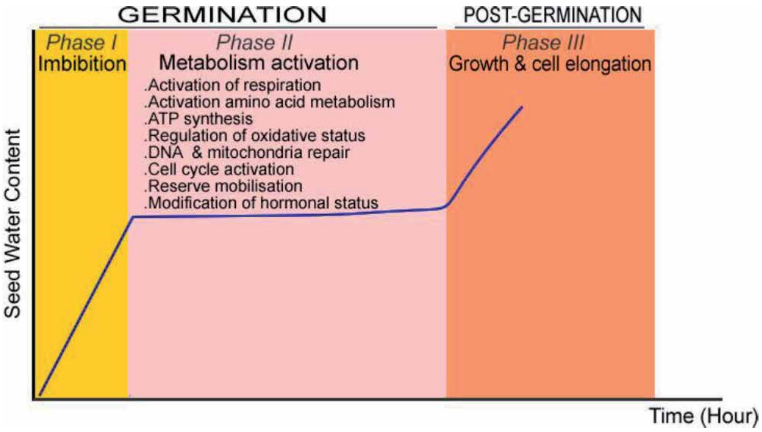

Figure 1.
Major events associated with germination and subsequent post-germinative growth (based on [13, 65]).

germination and to terminate dormancy by increasing the expression of GA anabolic genes, GA3ox1 and GA3ox2, and repressing expression of GA catabolism gene GA2ox2 [77].

In addition to phytohormones, several signal molecules, including as nitric oxide (NO) and reactive oxygen species (ROS), also regulate seed dormancy and germination [68]. ROS is an important regulator during seed germination because of the interaction with lipids, DNA, and protein molecules, as well as phytohormones including ABA and GA in the cell [78]. The biochemical and cellular reactions stimulated by water uptake are accompanied by the generation of ROS [79]. Hydrogen peroxide (H_2O_2) serves as a signaling hub for the regulation of seed dormancy and germination; the accurate regulation of H_2O_2 accumulation by the cell antioxidant mechanism is important to achieve a balance between oxidative signaling that enhances germination and oxidative damage that inhibits germination or delays in germination time [80]. N compounds, including NO, promotes seed germination through increasing amylase activities, adjusting K^+/Na^+ balance, and enhancing seed respiration and ATP production [81].

4. Effect of salinity on seed germination and early seedling stage

Salinity affects seed germination process through osmotic stress, ion-specific effects and oxidative stress, shown by decreasing germination rate and extended germination time [82]. Salinity increases external osmotic potential that reduces water uptake during imbibition [83]. Salinity may affect the germination of seeds by the toxic effects of excess sodium and chloride ions on embryo viability [84, 85]. The toxic effects include disruption to the structure of enzymes and other macromolecules, damage to cell organelles and the plasma membrane, the disruption of respiration, photosynthesis and protein synthesis [85–87].

In general, seed germination progresses in three phases under normal conditions. Seed germination begins with the rapid water uptake by dry seed (imbibition) (Phase I). A plateau phase, known as phase II, follows this phase. The cellular metabolisms are reactivated, and water uptake is restricted in phase II. This is followed by phase III, a post-germination phase, which is characterized by continuous water uptake until germination is complete (**Figure 1**). Based on these three phases, the inhibition of seed germination or delaying in germination time under salinity stress may be generally ascribed to osmotic stress in the phase I and ionic stress in the phase II. Osmotic stress and ionic stress interact together to inhibit or delay germination of seed during the phase III [88].

Salinity may adversely influence seed germination by decreasing the amounts of seed germination stimulants such as GAs, enhancing ABA amounts, and altering membrane permeability and water behavior in the seed [89]. In higher plants, salinity has been demonstrated to change expression profiles of the genes encoding GA metabolic enzymes, including copalyl diphosphate synthase (CPS), ent-kaurene synthase (KS), ent-kaurene oxidase (KO), ent-kaurenoic acid oxidase (KOA), GA 20-oxidase (GA20ox), GA 3-oxidase (GA3ox) and GA 2-oxidase (GA2ox), resulting with change in endogenous GA levels during germination [12].

The germination of seeds is characterized by transcriptional induction of hydrolytic enzymes such as α-amylase [90]. The α-amylase is excreted into the endosperm to break the stored starch to metabolizable sugars that provide ready energy and nutrients for the growing embryo and radicle. Salinity stress may have much effect on delayed germination time than on final germination percentage for most crops. A delay of water uptake and a decrease in the activity of α-amylase with an increase in the concentration of NaCl may be main reasons for delaying of the germination time [91].

The decrease in the α-amylase activity have been reported to be higher in the salt-sensitive genotypes than in the salt-tolerant genotypes. This reduction in the α-amylase activity results with a significant reduction in the translocation of sugars, essential for the developing embryo. Besides, decreasing sugar concentrations also change the osmotic potential of growing cells, resulting in a decrease in water uptake [88].

Both osmotic and ionic effects of salt stress leads to generation of excess reactive oxygen species (ROS) and oxidative damage, which disrupts proteins, lipids, and nucleic acids or the cellular structure including lipid membrane [83].

Plants can be divided into two main groups based on their response to saline stress; salt-tolerant halophytes and salt-sensitive glycophytes (non-halophytes) [6]. The halophytes are plants that are able to grow in the presence of high salt concentrations that generate a low water potential of the soil and kill 99% of other

Plant species	***Maximum salt tolerance**	**Salt tolerance type**	**Reference**
Salicornia herbacea	1.7 M NaCl	Halophyte	[93]
Suaeda aralocapsica	1.5 M NaCl	Halophyte	[94]
Limonium vulgare	1.5 M NaCl	Halophyte	[95]
Sarcocornia perennis	1.3 M NaCl	Halophyte	[96]
Haloxylon ammodendron	1.3 M NaCl	Halophyte	[97]
Kochia scoparia	1.0 M NaCl	Halophyte	[98]
Kochia prostrata	0.85 M NaCl	Halophyte	[99]
Haloxylon salicornicum	0.8 M NaCl	Halophyte	[100]
Prosopis juliflora	0.6 M NaCl	Halophyte	[100]
Limonium mansanetianum	0.5 M NaCl	Halophyte	[101]
Limonium stocksi	0.4 M NaCl	Halophyte	[102]
Limonium lilacinum	0.3 M NaCl	Halophyte	[103]
Tanacetum cinerariifolium	0.26 M NaCl	Halophyte	[104]
Quinoa (*Chenopodium quinoa* Willd.)	0.3 M NaCl	Halophyte	[105]
Barley (*Hordeum vulgare* L.).	0.25 M NaCl	Glycophyte	[106]
Maize (Zea mays)	0.24 M NaCl	Glycophyte	[107]
Chicory (*Cichorium intybus* L.)	0.21 M NaCl	Glycophyte	[108]
Lentil (*Lens culinaris* Medik.)	0.2 M NaCl	Glycophyte	[14]
Brassica napus	0.2 M NaCl	Glycophyte	[109]
Peanut (*Arachis hypogaea*)	0.2 M NaCl	Glycophyte	[110]
Rice (*Oryza sativa*)	0.16 M NaCl	Glycophyte	[111]
Fig (*Ficus carica* L.)	0.17 M NaCl	Glycophyte	[112]
Button grass (*Dactyloctenium radulans*)	0.1 M NaCl	Glycophyte	[113]
Sorghum (*Sorghum bicolor* Moench)	0.1 M NaCl	Glycophyte	[114]
Ryegrass (*Lolium rigidum*)	0.1 M NaCl	Glycophyte	[115]
Chickpea (*Cicer arietinum* L.)	0.09 M NaCl	Glycophyte	[116]
Tomato (*Solanum lycopersicum*)	0.05 M NaCl	Glycophyte	[117]

**Maximum NaCl concentration at which seed germination percentage reduced to 10–20%.*

Table 1.
Maximum salt tolerance of halophytes and glycophytes at the germination stage.

species. They are adapted to survive and complete their life cycle under saline levels of higher than 200 mM NaCl. However, seed germination was also affected under salt stress and germination percentage was reduced to less than 10% under 1.7 M NaCl [92, 93]. In halophytes, maximum salt tolerance for seed germination has been reported to vary from 1.7 to 0.26 M NaCl depending on halophyte species and other environment conditions such as temperature, moisture, and light (**Table 1**).

A majority of the common crops, such as tomato, bean, rice, corn, etc., are salinity sensitive or even hypersensitive and they are described as glycophytes [5]. The glycophytes contain 99% of the world's flora and are susceptible to even low levels of salinity (ECe < 4 dS m^{-1}, approximately 40 mM NaCl) [92]. Under conditions of moderate salinity (EC 4–8 dS m^{-1}), all important glycophytic crops reduce average yields by 50–80% [118]. Seed germination in glycophytes is severely inhibited under salinity due to both osmotic stress and ionic toxicity stress, while halophytes are less affected by osmotic stress during germination [12].

5. Alleviation salinity stress on germination by seed priming

Most crops are highly susceptible to saline soil, even when soil has electrical conductivity (ECe) as low as 3 dS m^{-1} [119]. Therefore, salinity stress appears to be a major limitation factor for crop productivity. Seed germination and seedling establishments are the two critical stages in plant growth. These stages are the most sensitive to environmental conditions including salinity [120]. Plants are usually seeded within the top layer of the soil which is more saline than lower layers [121]. Salinity stress may delay or prevent germination of germination of high quality seeds, resulting with crop loss. Rapid seed germination and subsequent seedling establishment are important factors affecting crop production under salinity conditions. Therefore, to decrease the negative effects of salinity stress on seed germination, it is important to know to what extent the genotypic variation in the water uptake pattern during these phases is associated with the salt tolerance of genotypes at the germination stage.

Seed priming is one of the useful physiological approaches for adaptation of glycophyte species to saline conditions during germination and subsequent seedling establishment. Seed priming is a simple, low cost and powerful biotechnological tool used to overcome the salinity problem by promoting seed germination and seedling establishment in agricultural lands [122]. Seed are exposed to an eliciting solution for a constant period that allows partial hydration, but radicle emergence does not occur by re-drying of seed. Seed germination occurs three distinct phases: (i) imbibition, (ii) lag phase (reactivation of metabolisms) and (iii) protrusion of the radicle through the testa. The goal of seed priming is to extend the lag phase, which allows pre-germinative physiological and biochemical processes, but prevent the seed transition towards full germination [123]. Enhanced and uniformed germination of primed seeds occurs by reduction in the lag time of imbibition, activation of enzyme involved in seed germination, initiation of biochemical mechanisms of cell repair, increase in the RNA content and DNA replication, decrease in ROS and lipid peroxidation with increased activity of antioxidant enzymes including as superoxide dismutase, catalase, and glutathione reductase, and increase in osmotic adjustment and starch metabolism [124, 125].

Several methods of seed priming have been developed in order to revive seeds under salt stress conditions. Some of these methods are hydro-priming, osmopriming, solid matrix priming, hormonal-priming, bio-priming, chemical priming, and nutripriming [13]. In recent years, many studies have been reported to exhibit the positive effects of seed priming on germination under salinity conditions in many crops (**Table 2**).

Hydro-priming is the simplest and one of the mostly used seed priming method. Hydro-priming depends on seed soaking in pure water without chemical substances for 6–24 h and re-drying to original moisture content prior to sowing without emergence of radicle [144]. This method is a low-cost and environmentally friendly due to no use of additional chemicals. The uncontrolled water uptake by seeds is major disadvantage of this technique. Rapid hydration may cause leakage of

Plant	**Treatment**	**Alleviating effect**	**Reference**
Barley (*Hordeum vulgare* cv. Bülbül 89)	Priming with aqueous solution of 30 μM H_2O_2 for 24 h at room temperature	H_2O_2 increased the germination index from 16.71 to 25.07%, and from 8.19 to 14.65% under 250 mM and 300 mM NaCl, respectively	[126]
Tomato (*Solanum lycopersicum* cv. Hezuo 903)	Priming with 100 μM Epigallocatechin-3-Gallate (EGCG) at 28 ± 3°C	EGCG increased germination rate and index from 84.7 to 97.0%, and from 29.4 to 35.2%, respectively	[127]
Wheat (*Triticum aestivum* cv. Chamran)	Priming with 0.5 mM spermidine for 24 h, 25 mM proline for 2 days, or 1.5 mM silicon (K_2SiO_3) for 6 h	Spermidine, proline, and K_2SiO_3 enhanced the germination rate by 32, 18, and 17%, respectively, under salinity stress (20 dS m^{-1})	[128]
Zea mays, *Pisum sativum, Lathyrus sativus*	Priming with 0.2 g/L GA3 solution for 12 h at room temperature without light.	GA3 enhanced germination percentage from 16.67, 26.67, and 50 to 60, 73.3, and 86.67% in *Z. mays*, *P. sativum,* and *L. sativus,* respectively, and resulted in 20% reduction in mean germination time under salinity stress (12 dS m^{-1})	[129]
Pakchoi (*Brassica chinensis* L. cv Tiancuiqing)	Priming with sodium nitroprusside (SNP) for 2 h in dark at 25 ± 1°C	Germination potential, germination index, and vitality index were increased by 7.67%, 14.20% and 74.51% after 10 μM SNP pre-treatment under 100 mM NaCl	[130]
Melilotus officinalis	Soaking with 10 mM Ca^{2+}	Ca^{2+} significantly increased the germination percentage and recovery germination percentage under 200 mM NaCl	[131]
Melon (*Cucumis melo*)	Priming with 10–50 μM melatonin for 6 h	Melatonin increase the germination percentage from 50 to 80% under salinity stress (14 dS m^{-1})	[132]
Wheat (*Triticum aestivum* cv. Khirman)	Priming with 50 mg L^{-1} ascorbate, 50 mM proline, 25 μM triacontanol, or 100 μM indole acetic acid for 12 h	Priming treatments significantly enhanced germination index and final germination percentage, and reduced mean germination time under salinity stress (12 dS m^{-1})	[133]
Grain sorghum (*Sorghum bicolor* Moench)	Priming with 100–500 mg L^{-1} nano-iron oxide (n-Fe_2O_3) for 10 h and soaking with 10 mg L^{-1} n-Fe_2O_3 for 3 days	Treatments improved the speed and percent of germination under 150 mM NaCl	[134]

Plant	Treatment	Alleviating effect	Reference
Lentil (*Lens culinaris* cv. Ncir)	Soaking with 0.5 mM salicylic acid or 0.1 mM H_2O_2 at 25°C in the dark	Salicylic acid and H_2O_2 enhanced the germination percentage from 71 to 86 and 87%, respectively	[135]
Limonium bicolor	Priming with 80 μM salicylic acid (SA)	SA significantly increased germination rate, germination potential, and germination index of the seeds under 200 mM NaCl	[136]
Sweet sorghum (*Sorghum bicolor* cv. Chuntian 1)	Priming with 288 μM Gibberellin (GA3) for 32–48 h	GA3 significantly increased the water uptake, resulting with increased cumulative germination percentage and germination index under 100 mM NaCl	[122]
Maize (*Zea mays*)	Priming with 2 mM silicon (K_2SiO_3) for 7 days at 25°C in the dark	Silicon significantly enhanced the germination rate and percentage, as well as vitality index under 90 mM NaCl	[137]
Oat (*Avena sativa* cv. NDO-2)	Priming with 150 ppm gibberellin (GA3) for 24 h	GA3 enhanced the germination percentage from 56.64 to 76.03% under 100 mM NaCl	[138]
Cucumber (*Cucumis sativus* cv. Jinyou 1)	Priming with 0.3 mM silicon (NaSi) for 36 h	Silicon enhanced the germination percentage and index, and seedling vigor index under 200 mM NaCl	[139]
Limonium bicolor	Priming with 200 μM melatonin	Melatonin significantly increased germination rate, potential and index under 200 mM NaCl	[140].
Ceratoides lanata	Priming with 10 mM ethephon, 5 μM fusicoccin or 50 μM kinetin	Fusicoccin, kinetin, and ethephon increased the germination percentage from 10 to 40, 50, and 84%, respectively under 900 mM NaCl	[141]
Leymus chinensis cv. Jisheng 3	Priming with 200 μM gibberellins (GA4 + 7), 200 μM fluridone (FLU), 200 μM cytokinin (CK), 100 μM sodium nitroprusside (SNP), or 100 μM thiourea (TH) in the dark or light	GA and FLU significantly increased the germination percentage from 7 to 23 and 59% in the light, respectively, while SNP, CK and TH increased the germination percentage from 9 to 54, 55, and 30%, respectively, in the dark under 200 mM NaCl	[142]
Salicornia ramosissima	Inoculation with *Bacillus aryabhattai* SP1016-20	Inoculation with *B. aryabhattai* enhanced the final germination percentage and mean daily germination from 21.3 to 46.7%, and from 1.6 to 4.5%, respectively, under 510 mM NaCl	[143]

Table 2.
The functions of seed priming in plant at the germination stage under salinity condition.

essential nutrients out of the seed during germination, resulting in seed damage in some species [145].

Osmo-priming, also known as osmotic conditioning, involves soaking seeds in aerated low water potential solution including sugar, polyethylene glycol (PEG), glycerol, sorbitol, or mannitol with low water potential instead of pure water, followed by air drying before sowing. Due to low water potential of osmotic solutions, water is absorbed slowly by dry seed, which allows gradual seed imbibition [146]. While osmo-priming promotes activation of early phases of germination, inhibiting radicle emergence. Osmo-priming improves seed germination and enhances general crop performance under salt conditions. Water potential of osmotic agent is critical factor since main purpose is to restrict oxidative damage caused by ROS by inhibiting excess water from entering [147]. If inorganic salts such as NaCl, KCl, KNO_3, K_3PO_4, $MgSO_4$, and $CaCl_2$ are used as an osmo-priming agent, the method is generally referred as halopriming.

In hormonal priming, seed imbibition occurs in the presence of plan hormones such as GA3, ethylene, auxins, and salicylic acid, which can gave effect on seed metabolism. Chemical priming is a promising seed priming technique to enhance germination under high salinity stress. Seeds were pre-treated with different chemical solutions used as priming agents. Chemical agents includes a wide range of both natural and synthetic compounds such as antioxidants (ascorbic acid, glutathione, tocopherol, and melatonin), sodium hydrosulfide, polyamines hydrogen peroxide, sodium nitroprusside, urea, selenium, chitosan, fungicide, etc. [13].

Biopriming involves seed imbibition together with particular bacteria or fungi. These microorganisms are able to create endophytic connections with the plant. As other priming method, this treatment increases rate and uniformity of germination under salt conditions, as well as protects seeds against the soil and seed-borne pathogens [147]. The most frequently used biopriming species are *Bacillus* spp., *Enterobacter* spp., *Pseudomonas* spp., and *Trichoderma* spp. [148].

Seed priming efficiency is influence by many factors and strongly depends on treated plant species and chosen priming technique. Physical and chemical factors including osmotica and water potential, priming agent, duration, temperature, presence or absence of light, aeration, and seed condition also influence priming success and determine germination rate and time, seedling vigor, and further plant development [13, 144].

Conflict of interest

No conflict of interest.

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Chapter 12

Abiotic Stress Tolerance in Crop Plants: Role of Phytohormones

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Abstract

Crop plants are encountered by various abiotic pressures which limit their growth and development. Stresses such as drought, heat, pathogen attack, heavy metal, salinity, and radiations impose negative effect on crop plants. The reduction in crop productivity in the current era of climate change is compromising the efforts/strategies used for sustainable agricultural practices. Therefore, plant stress physiologists are engineering plants with suitable exogenous signaling elicitors to engineer tolerance to various stresses. In the present chapter, an appraisal has been made in the "Introduction" section to first assess the damages caused by various abiotic stresses in crop plants. In the second section, we attempt to summarize the role of various plant hormones, namely, salicylic acid (SA), brassinosteroids (BRs), ethylene (ET), and methyl jasmonate (MJ) in enhancing abiotic stress tolerance. The current concept may lead to the development of strategies for unraveling the underlying mechanisms of plant hormone-mediated abiotic stress tolerance in crop plants.

Keywords: abiotic stress, plant hormones, crop plants, stress tolerance

1. Introduction

Due to overexploitation of natural resources for catering to basic human needs coupled with hunger, the aim of Food and Agricultural Organization's [1] of making world agriculture for enhancing 70% food production by 2050 for 2.3 billion newborn individual is facing a lot of challenges. As a result of changing climate and global warming, these threats are now called as "global warning" due to their effects on plant productivity [2]. According to a report published by IPCC [3], global climate change will increase the mean temperature of the Earth by 2–4°C. Due to increased anthropogenic activities, the change in climate will further increase and will definitely affect the agriculture sector as this is being more prone to climate changes [4]. A change in climate scenarios is the main reason of biotic and abiotic pressures of a particular region. Various abiotic stresses all over the world have now been adjudged as the most potential threat for sustainable agricultural productivity [5, 6]. Myriads of anthropogenic emissions in the developmental era have further intensified the abiotic stress-induced negative impacts on agricultural productivity. The major abiotic stresses such as metals/metalloids (hereafter termed as "metal/s"), salt, ozone, UV-B radiation, temperature extremes, nutrient (deficiency and excess), flooding, and water deficit are jeopardizing the agriculture system worldwide [7–19]. These abiotic stresses can modulate almost all plant physiological, biochemical, and molecular processes starting from the seedling to maturity stage and can ultimately cause severe negative impacts on economic yield of various crop plants. Nevertheless, abiotic stresses have been reported to adversely affect 70% yield of crops [20]. The stress of metal/s is known to affect the photosynthetic process to a great extent [19, 21]. According to an estimate of U.S. Environmental Action Group, various metals pose negative effects on health in more than 10 million of human population in eight countries (such as China, India, Russia, Dominican Republic, Kyrgyzstan, Peru, Ukraine, and Zambia) and the effect imposed by them is regarded as one of the major threats to the agriculture [22]. Wang et al. [23] have reports to show that the salinization of arable land could accelerate to a land loss of 30% by the year end of 2028 and up to 50% by 2050.In current times, an increase in atmospheric CO_2 concentration has been reported to reach by 407.4 ppm, and its levels today are more than at any point recorded for at least the past 800,000 years [24]. In addition, the data accessed on NOAA website [25] show that the average global temperature in June 2020 was found to be 1.66 °F (0.92 of a °C) above the twentieth century's average of 59.9 °F (15.5 °C) and the average temperature of June across the U.S. was found to be 70.3 °F (which is 1.8° above average), which ranks it the warmest third month in the history of the 126-year record. Hence, all these abiotic stress factors under the effect of climate change could be expected to significantly pose a potential threat to agriculture [11, 26–30]. Therefore, the knowledge of crop productivity improvement techniques under various abiotic stresses is one of the biggest challenges encountered by the scientific community across the world. Despite much and extensive efforts being utilized in the current field, the underlying quest for obtaining abiotic stress-resistant crops is very low and receives little attention. Various efforts are being utilized to decipher the potential mechanisms behind plant responses to abiotic stress conditions. Plants are sessile and are exposed to various environmental regimes and have to tolerate these abiotic stress-induced adverse impacts for performing their growth and development in an optimal manner. As a result of an exponential increase in the global human population, the demand for enhanced food is also accelerating and in order to meet these challenges under a challenging environment, it has become necessary to increase crop productivity. Nevertheless, the exogenous use of phytohormones in these adaptive mechanisms for engineering abiotic stress tolerance in diverse crop plants can yield better results.

Phytohormones are small chemicals messengers that play important roles in regulating the promotion of growth, development, and metabolism of the plants in a wide range of biotic and abiotic stresses. These are biodegradable and highly promote the growth and crop yield under optimal as well as stressed conditions. The knowledge of engineering in plants could be exploited to impart tolerance against a wide range of abiotic stresses. Thus, the present chapter focuses on the potent role of various phytohormones in enhancing the morphological and physiological properties of crop plants and also sheds light on the alleviative role in providing plant tolerance to various environmental stresses.

2. Regulatory role played by various phytohormones in abiotic stress tolerance in crop plants

In the following sections, we will be discussing the potential role of different phytohormones like SA-, BRs-, ET- and MJ-mediated mechanisms in countering diverse abiotic pressures like salinity, drought, heat, and metal/s stress in diverse crop species. The role of these phytohormones in eliciting various responses in different crop plants has also been represented in **Table 1**.

Name of plant	Abiotic stress, type, and doses	Phytohormone doses	Consequent response	Reference
Mentha arvensis L.	Metal stress Cd (150 mg/Kg)	Methyl jasmonate 1 μM	A significant decrease in length of root and shoot, fresh and dry masses of shoot and leaf area; decreased chlorophyll and leaf gas exchange traits, and leaf N, P, and K content; increase in the activities of APX, GR, SOD, CAT, ROS production, osmolytes; and closure in stomatal movement under Cd. Improvement in these traits and alleviation of stress by MJ application	Zaid and Mohammad [31]
Mentha piperita	Cd 30, 60, and 120 mg/kg	Salicylic acid 10^{-4} M	Decreased growth and photosynthetic traits and enzymes like NR and CA activities; increased phenolics, activities of SOD, POX, and proline content under Cd. SA improved photosynthesis; activity of RuBisCo, CA, and GSH pool, EO content; and yield of peppermint plants	Ahmad et al. [32]
Glycine max L.	Salt 100 mM	24-epibrassinolide 10^{-7} M	EBL application decreased Na^+ accumulation and promoted the uptake of the mineral nutrients, modulated osmolytes, activities of key antioxidant enzymes, and the levels of nonenzymatic antioxidants thus improves growth and photosynthesis	Alam et al. [33].
Linum usitatissimum	Salt 150 mM	24-epibrassinolide 10^{-8} M	NaCl decreased methylation of CCGG sequences. EBL application induced the total methylation under salinity stress conditions	Amraee et al. [34].
Brassica juncea	Cr 1.2 mM	200 μL/L ethephon application	ET was found to be involved in reversal of Cr-inhibited growth and photosynthesis by increasing PSII efficiency and enhanced N and S assimilation	Asgher et al. [35]
Brassica juncea L.	As 50 and 200 μM	Methyl jasmonate 0.1 and 1.00 μM	MJ application effectively mitigated the adverse effects of As stress by increasing the biomass, chlorophyll fluorescence, and the biosynthesis of secondary metabolites, reducing the oxidative stress biomarkers and maintaining antioxidant system	Farooq et al. [36]
Phaseolus coccineus	Metal stress Cu (50 μM)	Methyl jasmonate 10 μM	The enzymatic activities SOD, CAT, APX, POX, as well as MDA, GSH, and proline increased under Cu stress. MJ also elevated the activities CAT, APX, and POX activities in the roots. Final results showed that MJ was partially effective in modulating the anti-oxidative enzymes' activities and accumulation of metabolites, in both normal and Cu-stressed plants	Hanaka et al. [37]

Name of plant	Abiotic stress, type, and doses	Phytohormone doses	Consequent response	Reference
Mentha piperita	Salt (0, 50, 100, or 150 mM NaCl)	Salicylic acid, gibberellic acid, and triacontanol, each with 10^{-6} M	The salinity stress decreased growth, photosynthesis, activity of CA, contents of N, P, K, peltate glandular trichome density, EO and menthol content, and herbage yield but increased CAT, POX, and SOD activities and proline. The foliar spray of SA surpassed the other two phytohormones and maximally improved all parameters under both salt stress and salt free conditions	Khanam and Mohammad [38]
Brassica juncea	Ni 200 mg/kg	Ethylene 200 μL/L	The results showed that Ni decreased growth and photosynthesis but increased oxidative stress. Supply of ET ameliorated the stress by increasing the proline synthesis, antioxidant defense, and glyoxalase systems to promote the photosynthetic effectiveness under Ni stress	Khan et al. [39]
Artemisia annua	Metalloid stress (Boron) 1.00 and 2.00 mM	Methyl jasmonate 300 μM	B decreased the stem height, fresh and dry masses of the plant, the net photosynthetic rate, stomatal conductance, internal CO_2 concentration, and total chlorophyll content. MJ supply alleviates changes in growth and photosynthetic efficiency by increasing the activities of antioxidant enzymes and yield of secondary metabolites	Aftab et al. [40]

Table 1.
Various studies in diverse crop plants under different abiotic stresses conditions and the consequent response shown by these plants under various phytohormone application.

3. Role of salicylic acid

The name of "salicylic acid SA" is known to be derived from the Latin word *Salix* (willow tree). A German scientist, namely, Johann Andreas Buchner in 1828, isolated a quantity of glucoside of salicylic alcohol "salicin." Later on it was found that salicin, an important salicylate, is present in 36 other plants in addition to willow tree [41]. Chemically, SA is a simple phenolic compound ($C_7H_6O_3$) and consists of an aromatic ring to which one carboxylic and a hydroxyl group are attached. SA participates in several important plant processes either in stress-free or stressful environmental cues. It is now regarded as an important protector and growth regulating molecules in plants [42]. SA has an evidenced role in improving abiotic stress tolerance in various crop plants. Nonetheless, a report by Li et al. [43] suggested that SA acts upstream of NO under high concentration of carbon dioxide (CO_2) to induce enhanced flavonoid biosynthesis in tea plants. The results advocated the cross talk of SA for enhanced biosynthesis of secondary metabolites in tea plants under the era of climate change. In watermelon plants, resistance against root-knot nematode by red light is regulated by the coordination of SA with jasmonate signaling. This shows the potentiality of SA in enhancing tolerance against biotic stress. In Indian mustard plants, SA (10^{-5} M) alleviated the toxicity of Ni (50, 100 and 150 μM) by upregulating the activities of enzymes associated with antioxidant defense and glyoxalase systems [44]. In yet a recent study, Zaid et al. [45] tested the efficacy of foliar supplementation of SA (10^{-6} M) in two menthol mint cultivars, namely, Kosi and Kushal grown under Cd (50 μM) stress conditions. A reduction in growth, photosynthesis, and mineral nutrients with a concomitant increase in electrolyte leakage (EL), malondialdehyde (MDA), and hydrogen peroxide (H_2O_2) contents was observed under Cd stress in both cultivars. Nevertheless, a differential upregulation of proline content and the activities of antioxidant enzymes under Cd stress were also noticed in both cultivars. In contrast, out of three foliar-applied PGRs, the application of SA at three different growth stages proved best in alleviating Cd toxicity that too in both the cultivars but the effect of SA in Kushal was much more pronounced and better than Kosi. Application of SA (10^{-6} and 10^{-5} M) was also found to restrain As toxicity in two varieties of Artemisia annua L., namely, "CIM-Arogya" and "Jeevan Raksha" at 90 and 120 days after plantation [46]. The results suggested that the minimum reduction in growth and yield occurs with enhanced artemisinin production in "CIM Arogya" compared to "Jeevan Raksha" under As toxicity (45 mg kg^{-1} of soil). The application of SA via leaf significantly increased the content and yield of artemisinin in both varieties. El Dakak and Hassan [47] in a study evaluated the underlying physiological and biochemical mechanisms through which SA (1.0 mM) applied as a soil drench treatment alleviated the toxicity of Cd (5 and 10 μM) in maize plants. The results showed that exposure of maize plants to Cd reduced the chlorophyll content, chlorophyll fluorescence traits, photosynthesis, and catalase (CAT) activity, but, however, enhanced non-photochemical quenching (NPQ), MDA content and activities of superoxide dismutase (SOD), ascorbate peroxidase (APX) and guaiacol peroxidase (GPOX). Application of SA through soil drenching had an additive effect on the activities of antioxidant enzymes, but an inverse effect was found in case of MDA content. Es-sbihi et al. [48] studied the effects of SA (0.5 and 1 mM) on physiological traits, distribution of glandular hairs and essential oil (EO) composition in Salvia officinalis L. grown under Cu contamination (40 mM). The Cu stress decreased the growth of stem and root, Ca, P and K content in leaves as well as in roots. Interestingly, Cu increased EO yield. SA spray more prominently at 0.5 mM significantly improved stem and root growth, Ca, P, K and EO content and increased the density of peltate glands in presence of Cu stress. The study concluded with the observation that foliar spray of SA showed the remediating effect on growth traits, biosynthesis, and composition of EO of S. officinalis plants grown under Cu-challenged environment. This shows the key role of SA in enhancing tolerance to metal stress. In case of salt stress, SA also plays a mitigating role in minimizing the salt-induced damages in diverse crop plants. Miao et al. [49] in a hydroponic study applied exogenous SA (0.3 mM) in alleviating salt stress (50 mM NaCl) in cucumber seedlings. The results showed that 50 mM NaCl toxicity reduced relative growth rate and leaf photosynthetic traits like Fv/Fm, ΦII, and Pn. However, plants receiving NaCl + SA treatment showed a significant increment in relative growth rate, leaf photosynthetic traits (mentioned above) and an increase in case of total root length, root surface area, number and total lengths of primary and secondary roots. Both NaCl and SA downregulated and upregulated the expression of *GL2* and *RHD2* gene expression, while the application of SA treatment to NaCl-treated plants showed a notable upregulation in the expression of *NAC1*, *NAC2*, *GL2*, *EXP*, and downregulation of *RHD2* gene expression. Taken together the exogenous application of SA alleviated the NaCl toxicity by enhancing photosynthesis and architecture of root system in cucumber seedlings. Hussain et al. [50, 51] worked out the mechanistic elucidation of SA (0.5 mM) interplay with S (2.0 mM) under salt stress (50 mM NaCl) in mung bean plants. Salt-exposed plants showed an elevation in reactive oxygen species (ROS) content, lipid peroxidation, glucose and enzymatic activities like APX, GR, SOD, and reduced glutathione (GSH) and proline but marked inhibitions in the nitrate reductase (NR) and nitrite reductase (NiR) activities, N content, photosynthesis, photosynthetic N-use-efficiency (NUE), and growth traits. The supplementation of SA and S strengthened the antioxidant machinery, improved NR and NiR activities, N content, GSH, APX, GR and also decrease in ROS, MDA and glucose (a photosynthesis repressor) accumulation. These observations suggested that fine-tuning of SA with S diminished the NaCl-mediated changes in tested plants. Fahad and Bano [52] investigated the effect of foliar spray of SA (10^{-5} M) on maize (Zea mays L.) hybrid cv. 3025 grown in saline environments (pH 8.4 and EC 4.2 ds/m). The salinity treatment was found to significantly increased sugar contents, protein, proline, and activities of SOD, POD, CAT, and APX but decreased the pigment contents like chlorophyll and carotenoid and osmotic potential and membrane stability index. The application of SA to plants grown under salt-stressed plants further incremented the osmolytes, antioxidant enzymes, contents of endogenous abscisic acid (ABA), indole acetic acid (IAA), root length, and fresh and dry weights of roots. The results indicated that foliar application of SA proved to be effective in ameliorating the ill effects of salinity stress on maize plants. Pan et al. [53] worked out the involvement of hydrogen sulfide (H_2S) in SA-induced chilling stress tolerance in cucumber seedlings by using specific scavenger and inhibitor of H_2S. They found that under optimal conditions, the donor of H_2S or removing of endogenous H_2S by its scavenger (hypotaurine) or inhibitor (DL-propargylglycine) showed no impact on the level of endogenous SA, but SA induced H_2S endogenously and triggered the activities and mRNA transcript levels of L-/D-cysteine desulfhydrase (L-/D-CD). In addition, inhibiting endogenous SA with paclobutrazol or 2-aminoindan-2-phosphonic acid inhibited the effects of SA on H_2S. Nevertheless, both SA and H_2S donor NaHS minimized chilling-induced injury by lowering EL, MDA content, and ROS accumulation, improving the activities and mRNA levels of SOD, POD, CAT, APX, and GR, contents of ascorbate (AsA) and GSH contents and enhanced the expression of the chilling responsive genes (*ICE*, *CBF1*, and *COR*). It was postulated from this study that SA acts as an up-streaming signaling molecule under chilling tolerance of cucumber plants via increasing antioxidant defense system and modulating the expression of chilling stress-responsive genes. In a comparative study involving three deficit irrigation regimes, viz., 100, 80, and 60%

of crop evapo-transpiration and four levels of salinity, that is, 2, 4, 8, and 12 dS m^{-1}, the effect of SA in pearl millet and wheat plants was studied [54, 55]. Water and salinity stress caused significant reductions in grain yields, plant height, water and proline content, and Fv/Fm and Na/K^+ ratios in both pearl millet and wheat plants. Supply of SA caused a significant enhancement in plant height and grain yield and significantly improved all physiological, biochemical traits and consequently grain yield in both crop plants. In order to evaluate the participation of NR in SA-mediated improvements in water deficit tolerance in pepper plants, Kaya [56] applied 0.5 mM SA as a foliar spray. The results revealed that water stress caused decrement in total plant dry weight, Fv/Fm, contents of chlorophyll *a* and *b*, relative water content, leaf water potential but increment in MDA, H_2O_2, EL, methylglyoxal (MG), proline, and (nitric oxide) NO, NR and antioxidant enzymes' activities. The supply of SA reduced oxidative stress biomarkers, but triggered a marked increment in antioxidant defense system, enzymes of ascorbate-glutathione (AsA-GSH) cycle and glyoxalase system-glyoxalase I (Gly I) and glyoxalase II (Gly II), growth and photosynthetic traits, activities of NO, NR and proline content. In a recent study, the effect of foliar SA (0, 0.5, and 1 mM) under drought stress (90, 60, and 30% field capacity) in Portulaca oleracea L. was studied [57]. Imposition of drought stress showed a decrement in the contents of photosynthetic pigments, gas exchanges attributes, growth, and biomass production, soluble sugars, total phenolic, flavonoids and unsaturated fatty acids (FA) like oleic, linoleic and linolenic acid, and stearic and behenic acid but increased the contents of H_2O_2, MDA, and palmitic and arachidonic acid, respectively. Application of SA improved the growth, photosynthetic pigment contents, gas exchanges traits, FA contents, compatible solutes, and secondary metabolites, while simultaneously decreasing drought-induced oxidative stress biomarkers. With regard to heat stress, SA is effective in alleviating the damages on plants' physiology. Karpets et al. [58] studied the interplay of hydrogen sulfide (H_2S) in SA-mediated induction of heat resistance via the performance of antioxidant system in wheat plantlets. The data showed that H_2S actively participates under the protective influence of SA application in wheat heat stress tolerance. Khan et al. [59] exposed wheat plants to heat stress (40°C for 6 h) and studied the potential of 0.5 mM SA in alleviating the negative effects of heat stress on photosynthesis. The net photosynthesis (Pn) and activity of ribulose 1,5-bisphosphate carboxylase (RuBisCo) and photosynthetic nitrogen use efficiency (NUE) decreased under heat stress, but metabolism of proline was found to be increased. The application of SA alleviated heat stress by further increasing proline metabolism via the enhancement in the activity of γ-glutamyl kinase (GK) and decrement in proline oxidase (PROX), which maintained optimum osmotic and water potential for optimally maintaining the photosynthesis. Nonetheless, SA restricted the production of stress ethylene under heat-stressed plants to optimal range. The results suggested that SA supplementation alleviates heat stress effects by interacting with proline metabolism and ethylene formation to improve photosynthesis in wheat plants. In yet another related work, Kumar et al. [60] by using MALDI-TOF–TOF/MS analysis showed that spraying 100 mM SA alleviates the heat-induced (38°C) oxidative stress damage in wheat plants via modulation of the expression of heat-stable genes and proteins. Thus, SA application plays a pivotal role in alleviation the damages of myriads of abiotic stress in diverse crop plants.

4. Role of brassinosteroids

Brassinosteroids (BRs) are an important class of plant steroid-based phytohormone that plays multiple roles in regulating plant growth, development, and responses to environmental stresses [7, 8, 50, 51, 61–64]. 28-homobrassinolide (HBL) and 24-epibrassinolide (EBL) are the two isomers of BRs which regulate an array of plant physiological processes [65, 66]. BRs have structural resemblance with androgens, estrogens, corticoids, and ecdysteroids. The discovery of brassinolide (BL) dates back to 1979, and now about 70 compounds which are naturally occurring have been reported as free molecules or form conjugates with glucose and FA [67]. The isomers of BRs play a significant role in imparting abiotic stress tolerance in crop plants. In potato plants, Kolomeichuk et al. [68] studied the EBL-mediated (10^{-10} M) alleviation of NaCl toxicity (root application 100 mM) on photosynthetic processes. Salinity inhibited the contents of photosynthetic pigments, photosynthetic electron transport and photosystem II (PSII) maximal and effective quantum yields, oxidation of the plastoquinone (PQ). The treatment with 24-EBL under salinity stress maintained the efficiency of PSII photochemistry, PQ pool oxidation, and decrease in leaf osmotic potential. Amraee et al. [34] reported the 24-EBL-mediated (10^{-8} M) alteration on the extent and pattern of DNA cytosine methylation in Linum usitatissimum L. plants under salinity stress (150 mM) by using methylation-sensitive amplified polymorphisms (MSAP) technique. In conclusion, they found that the seed priming with 24-EBL play an important regulatory role via the epigenetic modification and induction of methylation in response to salinity toxicity. 24-EBL (10^{-8} mM) also confers tolerance against NaCl (100 mM) stress in soybean plants [33]. Exposure of soybean plants to 100 mM salt stress decreased growth, biomass yield, gas exchange traits, photosynthetic pigment contents, and chlorophyll fluorescence but increased H_2O_2, MDA, EL, and the antioxidant activities like SOD, CAT, accumulation of proline, and glycinebetaine (GB), total phenols, and total flavonoids. Application of 24-EBL (10^{-8} mM) to salt-stressed plants' upregulated the growth, biomass, yield, gas exchange and photosynthetic pigment contents, and chlorophyll fluorescence parameters but concomitantly reduced the accumulation of oxidative stress biomarkers. In a factorial experiment, Kaya et al. [69] studied the effect of 24-EBL (1.5 and 2.0 μM) application in regulating key physio-biochemical traits and oxidative defense system in two cultivars of maize, namely, PR32T83 and PR34N24 under two salinity levels (1.1 and 8.0 dS/m). Salinity levels decreased the fresh and dry weights of plant, yield of PS-II, chlorophyll contents, leaf water potential, and leaf K and Ca levels, but triggered an increase in membrane permeability, lipid peroxidation, H_2O_2, SOD, POD, CAT enzymes' activities, leaf Na and Cl, proline and GB, and leaf sap osmotic pressure. However, 24-EBL supply via both seed treatment and foliar application to the maize cultivars alleviated the detrimental effects of salt stress. In Cucumis sativus L. cv. Jinyan No. 4, plants grown under vermiculite and in hydroponic culture, Ahammed et al. [70] tested the efficacy of 24-EBL in alleviating organic pollutants (OPs), namely, 2,4,6-trichlorophenol, chlorpyrifos and oxytetracycline-retarded root elongation by unraveling the redox homeostasis and secondary metabolism-mediated underlying mechanisms. The results revealed that all the three tested OPs retarded elongation of roots, increased MDA, H_2O_2 and NO, while exogenous 24-EBL markedly decreased the accumulations of H_2O_2, NO and MDA *vis-a-vis* upregulating the expression of genes associated with antioxidant and detoxification systems. In case of pesticide stress, 24-EBL maintained the optimum synthesis of proteins and amino acids in Brassica juncea L. plants [71]. They applied 24-EBL (100 nM) via the seed presoaking treatment to study the changes in the synthesis of protein and amino acid contents grown under im-idacloprid (IMI)-challenged environment. Plants grown under IMI stress (300 mg/kg soil) showed a decrease in protein and 21 amino acid contents. However, application of 24-EBL restored the total protein as well as amino acid contents which was decreased under IMI amended soils. In a recent study laid out in a randomized block design under greenhouse, Jan et al. [72] applied 24-EBL (10^{-7} M) for 8 h as seed priming method to alleviate the toxic effects of Cr (10 mg/kg soil) in tomato plants. The results suggested that that 24-EBL application effectively stimulate growth of tomato plants exposed to Cr stress via the remodulation of the physiological, metabolic, and defense mechanisms and by maintained accumulation of Cr. Alam et al. [33] applied 24-EBL (10^{-7} M) in countering NaCl (100 mM) stress in soybean plants. Salinity decreased growth, gas exchange parameters, pigment contents, and chlorophyll fluorescence, Ca^{2+}, K^+, and P and biomass yield but H_2O_2, MDA, EL, MG content, the activities of SOD and CAT, accumulation of proline, GB, total phenols, Na^+ uptake in root and shoot, and total flavonoids were found to be increased. Overall, 24-EBL supplementation provided tolerance to soybean plants by upregulating the antioxidant system, AsA-GSH cycle, and glyoxalase system. Yusuf et al. [73] under completely randomized block design studied the BRs-mediated protection of growth and N metabolism and the potentiality of antioxidant system under Ni stress (0, 50, 100, or 150 mg/kg) in two cultivars of Vigna radiata, namely, PDM-139 and T-44. The role of two doses (10^{-8} or 10^{-6} M) of HBL in alleviating the Ni-induced damages via the ability of antioxidant enzymes and proline content was investigated. It was found that the Ni imposes toxic impact on the plants in a concentration dependent manner. The supply of HBL was effective in countering Ni stress as its application conferred tolerance up to 100 mg/kg in improving growth, nodulation, photosynthesis and yield attributes consequently. Fariduddin et al. [74] exposed Cucumis sativus L. plants to chilling stress to study the ameliorative role of HBL through studying the growth, photosynthesis, enzymes, and biochemical parameters. Cucumber seedlings were exposed to two chilling regimes, that is, 10/8 and 5/3°C for 18 h. The spray treatment consisted 10^{-8} or 10^{-6} M of HBL applied at 30-day stage to plants. Chilling stress imposes marked reductions in growth, chlorophyll, net photosynthesis, efficiency of photosystem II and alterations in NR and carbonic anhydrase (CA) activities. In contrast, the activities of antioxidant enzymes viz-CAT, POD, and SOD along with the proline content increased in response to chilling stress. HBL supply exerted a protective role via the maintained higher values of antioxidant enzymes and proline content under chilling stress. In order to assess the role of HBL (10^{-8} M) under two Cd doses (3 and 9 mg/kg sand) in Solanum lycopersicum plants, Singh and Prasad [75] designed an experiment in plant growth chamber. Treatment of plants to Cd stress resulted in a significant decrease in enzymes associate with N metabolism. The application of exogenous HBL supply ameliorated the ill effects of Cd stress by triggering an increase in growth, photosynthesis, protein and carbohydrate content and inorganic N and N assimilating enzymes. Kaur et al. [76] proposed the model of HBL-mediated regulation of antioxidant enzyme activities and gene expression in Brassica juncea plants to counteract the combined salt-and temperature-induced oxidative stress. HBL treatments (0, 10^{-6}, 10^{-9}, and 10^{-12} M) was applied as seed priming on plants exposed to the low (4°C) or high (44°C) temperature and salinity (180 mM) stresses. The combined treatments of salt and temperature significantly reduced length but increased H_2O_2 content and the activities of SOD, CAT, APX, GR, DHAR, and MDHAR. Application of HBL improved growth traits, reduced H_2O_2 and the response of plants was dose-dependent.

5. Role of ethylene

Phytohormone "ethylene" (ET) is a small gaseous plant growth regulator which is responsible for ripening of fruits and is known for inducing triple response in plants [77]. The growth-regulating effects of ET were first observed by a Russian physiologist Dimitry K. Neljubov in 1901, however almost 60 years later in 1965 ET was established as a plant hormone [78]. Plant growth and senescence are chiefly regulated by the signaling mechanisms of ET [79–81]. However, it exerts its multifunctional role as one of the diversified signaling molecules to confer acclimatization to crop plants under various abiotic stresses. The signal perception of ET takes place at the membrane of endoplasmic reticulum and is bacterial two-component type which initiates a cascade signaling events through transcriptional regulation in the nucleus, thus eventually controlling a myriad of plant developmental events. Meanwhile, the interplay of ET with other signaling molecules provides adaptation to plants under various abiotic pressures [7, 8, 82–88]. Now, we will focus our attention on various reports on the functions of ET on tolerance toward major abiotic stresses in crop plants. Indian mustard plants were grown under 200 mg Zn/kg soil and the effects of 200 μL/L spray of ethephon at 20 days after sowing (DAS) was tested to alleviate the Zn toxicity. The results after 30 DAS revealed that exposure to Zn stress causes toxicity by inhibiting photosynthesis and growth, while increasing the oxidative stress biomarkers. ET application (as ethephon) to Zn-stressed plants restored photosynthesis and growth of mustard plants by decreasing oxidative stress via augmenting antioxidant activity, the metabolism of proline, glyoxalase system, and optimum nutrient homoeostasis [89]. In tomato plants, Alves et al. [90] provided new insights into the interactive role of ET on selenium-mediated antioxidant enzymes under Cd stress. Overall, the results suggested that cross talk of ET with Se manipulated the ET regulated expression of genes to induce Cd tolerance. The effect of Cr toxicity at 1.2 mM concentration given as $K_2Cr_2O_7$ after 10 DAS through nutrient solution on four cultivars of Brassica juncea, namely, Varuna, Pusa Bold, Rohini and SS2 were studied under ET supplementation [35]. Plants grown under Cr stress showed an enhanced accumulation of H_2O_2, MDA content, and superoxide production and a marked reduction in photosynthesis and growth traits. Ethylene (2-chloroethyl phosphonic acid, 200 μL/L) boosted the activities of enzymes associated with S assimilation, proline accumulation, anti-oxidant defense system, ATP-sulfurylase and serine acetyl transferase, and GSH level and resulted in inhibition of stress ET levels to the optimal range under Cr stress, thus increasing the photosynthesis and growth. The key role of ET was further substantiated by using ET action inhibitor, silver nitrate ($AgNO_3$) in the reversal of Cr-induced changes. In Brassica juncea plants, alleviatory role of ET (200 μL/L as ethephon) in the presence of 200 mg/kg soil each of Ni and Zn stress was investigated [91]. Application of ET reverses photosynthetic inhibition and promotes growth by inducing changes in PS II photochemistry, photosynthetic NUE, and modulation of antioxidant enzymes. The study got further confirmation by using ET action inhibitor-norbornadiene which reversed ET-induced changes. In Arabidopsis thaliana, Hartman et al. [92] studied the changes in NO and oxygen in controlling the stability of TFs in regulating of N-degron pathway and adaptation to flooding-induced hypoxia. They concluded that ET-mediated decrease in NO conferred tolerance to plants against hypoxia stress. The pretreatment of rice plants with the ET precursor under submergence stress plays a key role in the survival of rice plants [93]. ET signaling helped in the survival of rice seedlings under submergence stress by affecting intracellular redox homeostasis and antioxidant systems. Jegadeesan et al. [94] unraveled the proteomics involved in pollen of Solanum lycopersicum thermotolerance mechanisms mediated by ET. The quantitative analysis showed that heat stress-affected protein homeostasis and metabolism of carbohydrate and energy. ET (ethephon) increased pollen quality prior to heat stress exposure. According to gel LC-MS fractionation a total of 1355 proteins were identified. ET application shifted pollen proteome exposed to the heat stress closer to the proteome under non-stressful conditions and caused an upregulation into the protective mechanisms against the heat-induced oxidative stress by causing a significant higher abundance of glutathione-disulfide reductase, glutaredoxin, and protein disulfide isomerase. Iqbal et al. [95] studied the potential of exogenously sourced ET (200 μL/L) under nutrient deficiency (40 mg N/kg soil) at 40 DAS. ET supply increased the stomatal conductance, photosynthesis, and promotes growth under low nutrient availability in mustard plants. Application ET also alleviated salt stress response in various crop plants. In fact, the alteration of ET signaling mechanism significantly affects the response of plants to salt stress [96]. Freitas et al. [97] worked out the capacity of ET in triggering salinity stress tolerance in maize plants. During NaCl exposure, a biphasic ET production (at 5.5 and 12.5 h) and decrease in total polyamine content was noticed in salt-sensitive plants. In the salt-tolerant genotype, there was lack of stress ET biosynthesis and this was attributed to the 1-aminocyclopropane-1-carboxylic acid oxidase (ACO) and *ZmACO5b* gene downregulation activity and expression. The ET was involved in promoting salt stress tolerance by activating pathways of signaling by H_2O_2 that is dependent upon enzymes of polyamine catabolism. Application of ET also improved the tolerance to salt tolerance in Arabidopsis thaliana by maintaining K^+ content in shoots and roots to an appreciable extent [98]. Gharbi et al. [99] showed that inhibition of ET biosynthesis minimizes salt (125 mM) tolerance in Solanum chilense. The involvement of ET was also ascertained in the alleviation of salt-induced photosynthesis and growth reductions in Vigna radiata L. [100]. A central role of ET in BRs-induced alternative respiratory pathway which played an important role in abiotic stress tolerance in Cucumis sativus L. has also been reported [88]. A critical role of ET for the maintenance of root growth and mineral homeostasis under excess Fe stress in Arabidopsis thaliana has also been reported [101].

6. Role of methyl jasmonate

Methyl jasmonate (MJ) is one of the conjugates of jasmonic acids (JAs) and are important as well as ubiquitous phytohormone distributed in plants [102]. MJ is implicated in plant responses to salt, drought, metal/s, and heat stresses. MJ exert its alleviatory role under abiotic stresses by modulating plant morphology and physiology to a great extent. Taheri et al. [103] studied the effects of exogenous MJ-mediated (0, 60, and 120 mM) improvements in physiological and biochemical traits of *Anchusa italic* plants under salinity (5, 10, and 15 dS m^{-1}). Salinity stress significantly decreased growth characters, RWC, chlorophyll a, total chlorophyll, soluble sugars, protein, and K^+ contents but at the same time induced a marked increase in proline, Na^+, and Ca^{2+} contents. The application of MJ increases the aforementioned traits in plants grown under salt stress. In salt-tolerant rice genotypes, a higher concentration of MJ has been reported [104]. In wheat plants, application of 2.0 mM MJ ameliorated salt induced changes by boosting the expression of antioxidant enzymes, such as CAT, SOD and APX, and enhancing the GSH, chlorophyll b, and carotenoids contents [105]. In Hordeum vulgare, MJ-mediated (12 μM) regulation of genes of enzymes such as arginine decarboxylase, apoplastic invertase, and RuBisCo activase were found to be involved in imparting salt stress resistance [106]. The signaling of JAs promotes salt stress tolerance via the wheat allene oxide cyclase gene [107]. The wheat plants were exposed to saline stress conditions (12 dS m^{-1}) and the results showed that height of plant, leaf number per plant, tiller number, fresh and dry weight per plant, spike length, grain number per spike, grain weight per spike, and yield were reduced. MJ application mitigated the ill effects of salt stress by increasing the abovementioned traits in wheat plants [108]. In a polyethylene applied pot study, Tavallali and Karimi [109] studied the effect of foliar application of MJ (0, 0.025, 0.050, and 0.075 mM) in enhancing salinity stress (0, 50, 100, and 150 mM) mitigation of almond root stocks. The MJ-mediated improvement in salt stress tolerance was attributed to the restriction of gene expression and activity of cytokinin oxidase which results in the inhibition of cytokinin decrease. MJ imparted salt tolerance by regulating the endogenous biosynthesis of phytohormones, expression of antioxidant activity, and gas-exchange traits of almond rootstocks. The possible role of MJ (0.25 mM) on the physiological responses and growth of *Fragaria × ananassa* cv. "camarosa" under salinity stress (0, 30, and, 60 mM NaCl) was investigated. Application of 0.25 mM MJ boosted the activities of antioxidant enzymes that ameliorated the salt-induced salinity stress effects [110]. Priming rice plants with MJ was reported to ameliorate the polyethylene glycol-induced osmotic stress [111]. In a recent study involving wheat plants, Allagulova et al. [112] applied MJ (0.1 μM) to unravel its alleviatory role under water-induced stress by investigating the potential participation of dehydrins. The stress was imposed by increasing the concentration of mannitol from 3 to 5% in the growth medium. Wheat seedlings pretreated with 0.1 μM MJ found to reduce the level of drought-induced retardations and enhanced the accumulation of the transcripts of *TADHN* dehydrin and proteins as well. In order to evaluate the effects of drought under exogenously applied MJ in wheat cultivars (Yu-Mai-1, Lu-Nong-116, HuaiMai-19, Yan-Nong-19, Bai-Maizi), a simple randomized complete design experiment was executed [113]. The drought was imposed at three stages: jointing, heading, or grain filling. The application of MJ was accomplished one week after the exposure of plants to the imposition of drought stress. The results showed that drought stress substantially decreased the growth and yield of all wheat cultivars reflected in the form of plant height, peduncle length, dry biomass of different plant parts, and also in yield. The application of MJ improved the drought resistance mainly by maintaining growth and yield traits of wheat plants. A pot-culture experiment in order to investigate the role of MJ presoaking (50 μM) for 8 h treatment on maize plants under water stress in the form of water field capacity (WFC) 65, 55, and 45% was setup. MJ application lead to an increase in plant height, fresh and dry weight, the pigment levels total carbohydrates, total soluble sugar, polysaccharides, free amino acids, proline, total protein, activities of CAT, POX and SOD and contents of N, P, and K and endogenous IAA but a decrease in ABA levels of wheat plants under stress condition [114]. In Solanum lycopersicum, the role of JA accumulation in the modification of water deficit-induced stomatal closure was studied. The drought stress-induced the closure of stomata that was correlated with the accumulation of JA, but in grafts of WT and *def-1* mutant which does not accumulate JA, the stomata was found to be insensitive [115]. Improvement of drought stress tolerance by the application of MJ has also been reported in soybean genotypes (Giza 22 and 35) [116], pear [117], sunflower seedlings [118], and *Cistus albidus* L. [119] plants. Nonetheless, application of MJ (10 μM) has been found to reduce chilling injury in cucumber by inhibiting the production of H_2O_2 [120]. MJ-mediated improvements in chilling stress tolerance in cold-stored lemon fruits has also been reported [121]. In case of metal/s stress, MJ application played a significant role in ameliorating the damages in physiology and morphology of diverse crop plants. The effect of exogenous MJ in two Brassica napus L. cultivars (ZS 758—a black seed type, and Zheda 622—a yellow seed type) was investigated against exposure to arsenic toxicity. MJ alleviates As-induced oxidative damages by significantly increasing chlorophyll fluorescence, biomass production and concomitant reduction in MDA and ROS contents. MJ application also maintained the high pools of GSH and AsA along with enhanced transcript expression SOD, APX, CAT, POD, and secondary metabolites (*PAL, PPO, CAD*) and reduction in As uptake [36]. Zaid and Mohammad [31] applied 1 μM MJ to test its alleviating capacity against Cd (150 mg/kg) stress in Mentha arvensis plants. Cd applied through soil imposes restrictions in growth, physio-biochemical traits. The enhanced ROS accumulation and stomatal closure were also observed in plants grown under Cd stress. Application of MJ alleviated the Cd stress by reducing the damages in physio-biochemical traits along with reduced ROS detoxification. Pre-incubating Phaseolus coccineus plants with 10^{-5} M MJ has been found to minimize the Cu toxicity [122]. The identification of differentially expressed protein spots suggested the putative role of MJ in diverse physiological processes including oxidative tolerance and defense-related metabolism [123]. Thus, the above discussion clearly suggests that MJ alleviates the metal/s-induced oxidative stress by modulating the key physio-morphological traits in diverse crop plant to impart stress tolerance.

7. Conclusion and future outlook

Crop plants are exposed to myriads of abiotic stress conditions and undergo a significant retardation in growth and development, thus reducing crop productivity. Stresses are perceived, and modulation in the expression of genes encoding diverse proteins are induced in the signal transduction pathways in plants. Application of discussed phytohormones in the present chapter showed that the cross talk of phytohormones with various signaling agents under different stress conditions in diverse crop plants impart stress tolerance under stress pressures. A schematic representation on phytohormones-mediated abiotic stress tolerance in given in **Figure 1**.

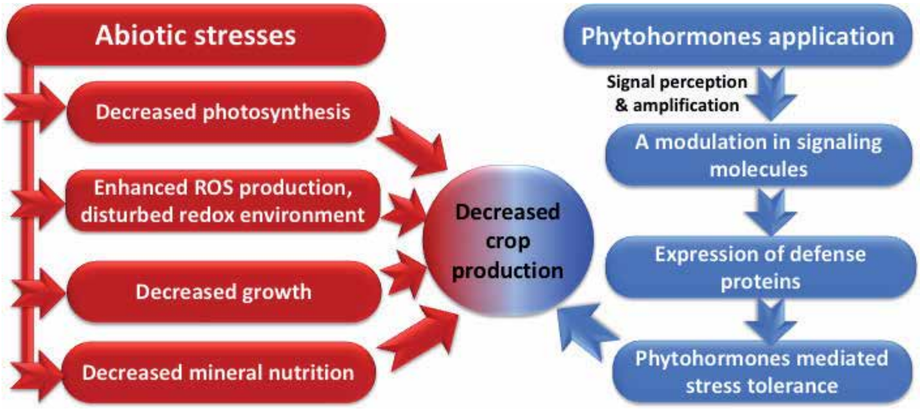

Figure 1.
Abiotic stress and phytohormones' signal transduction pathway in plants.

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Chapter 13

Morphophysiological Traits, Biochemical Characteristic and Productivity of Wheat under Water and Nitrogen-Colimitation: Pathways to Improve Water and N Uptake

Nawab Ali and Mohammad Akmal

Abstract

Drought stress is the most prominent limiting factor and abiotic stress that manipulates the physiological pathway, biochemical traits and hence negatively affects wheat crop productivity. The global nitrogen (N) recovery indicated that about two-fifths of N inputs are lost in the ecosystems through emission, denitrification, gaseous loss, leaching, surface runoff and volatilization etc. Farmers are using higher rates of N to harvest maximum yield but about 50–60% of applied N to crop field is not utilized by the plants and are lost to environment causing environmental pollution. These deleterious environmental consequences need to be reduced by efficient management of N and/or water. N-availability is often regulated by soil water; hence crop is experiencing N- and water-limitation simultaneously. There is great impetus to optimize their uptake through interconnectedness of water and N for yield determination of wheat because of the water scarcity and N losses. It is further advocate that there is need to investigate the intricate role of economizing N rate and water simultaneously for wheat crop growth, yield and backing quality may be beneficial to be investigate.

Keywords: drought, water-N co-limitation, physio biochemical traits of wheat, N losses, NUE and WUE

1. Introduction

Wheat is one of the most important staple food crops supporting the world population. It ranks the second most important cereal crop of the world grown on about 220 million hectares (mha) area with 716 million tons food grain production and 3.2 tons per hectare productivity globally [1]. The current wheat production meets the demand for the existing population, but we will have to increase the production with time keeping in view to achieve the target of 858 million tons by 2050 against the current production globally [2]. Hence about 1.5% annual increase in next three decades should be kept under consideration to feed the overwhelming population. Similarly, about 37% of the wheat is cultivated as rainfed where drought is the most dominant constraint to limit wheat production [3]. Wheat is extensively grown in Asia, where Pakistan ranks eight in term of production (25 million tons) in the world [4]. The productivity of wheat is less than the 2.89 million tons per hectare for population with annual increment of 1.8% [5]. Wheat production affected by various biotic and abiotic stresses. Among the abiotic stress, water stress is affecting the vegetative growth and ultimately affecting yield of the wheat crop [6] (**Figure 1**).

Drought stress is the most prominent factor in crop production that affects the yield stability in the world [6]. Likewise, in rainfed areas the erratic rainfall is also the prime factor that limits crop productivity in in semiarid areas [7]. The occurrence of insufficient rainfall which does not meet the water requirement of the crop life cycle in certain areas occasionally cause the total crop failure [8]. The use of ground water is practice for crop production, and this ground water is declining with time with significant adverse effect on the water table due to the non-judicious use of the ground water resources [9]. Therefore, certain water conservation measures and water saving strategies and techniques should be adopted to cope with this adverse condition thereby conserving the precipitation water in the rhizosphere for the optimum crop production [10, 11].

As an essential macronutrient of plants, N is necessary for the vegetative growth, biomass, protein synthesis and the photosynthetic machinery of the crops [12]. This N is available in various forms in the rhizosphere, i.e. organic, such as in the form of amino acid and proteins, and inorganic forms, urea, nitrate ions ($NO3^-$), ammonium ions (NH4+) and is also found in the form of other nitrogenous compounds [13]. Of them, only NO3- and NH4+ are the forms of soil N that are easily taken by the plants through roots from the rhizosphere [14]. The aforesaid two forms need enough soil moisture for efficient absorption through the plant roots because of their high mobility in water [15]. The soil water is extracted by the plants through its roots and thereby using it for various physiological processes, maintaining internal temperature and for the process of transpiration [16]. Plant uptake various nutrients from soil solution including N for the optimum growth and development. In drought prone areas or areas where there is less and/or no eater availability because of the shortage of water or undependable nature of the

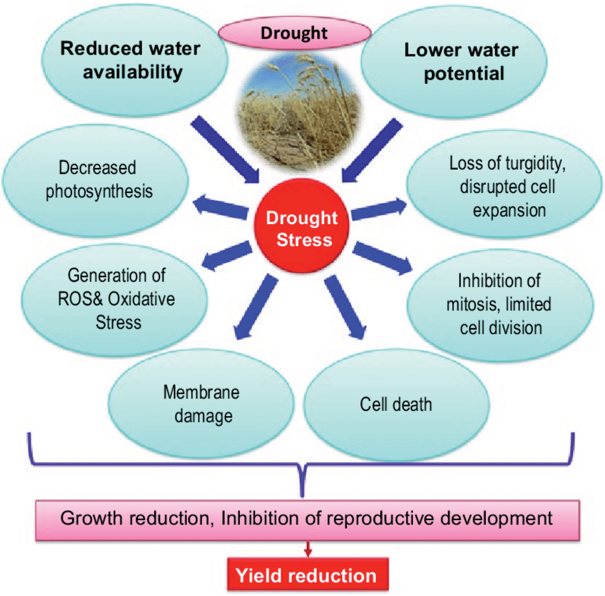


Figure 1.
Schematic representation of the drought effect on physiological processes, growth and yield.

rainfall, plants are with lower N uptake and hence the overall physiological process and activity of N assimilation enzyme is also retarded [17, 18]. Therefore, the plants ability to uptake N from the soil is not only the function of N concentration and availability in soil but also the soil moisture in the root zone [19].

Soil water and Nitrogen (N) are considered as the key limiting factors for maximizing plant growth, yield and quality [20]. The effect of these limiting factors is most prominent in the growing area where water stress is prevalent [21]. All the metabolic processes, biomass assimilation, growth functions and nutrients allocation in the plants are controlled by the interaction of N and water [22]. It is therefore kept in mind that wheat yield might be increased when crop ins not restricted with both N and water supply. For the availability of N optimum moisture in soil is needed. Contrary to this, enough amount of N needs to be in soil to make use of water available. In most areas, wheat yield is affected by the drought induced N deficit. Wheat morphology, physiology and biochemical character is also affected by water stress and nitrogen dynamics in the rhizosphere.

2. Morphophysiological, biochemicals and yield of wheat under water and N-colimitation

Water and N availability, the most limiting crop growth factors affecting adversely the morphological traits, physiology of the crop, alter the biochemical changes in the grains and shoots and final yield of the crop [23]. The resource use efficiency of N and water for the crop is the need of the day. Water stress adversely affects the vegetative growth, physiological processes, biochemical properties of the grains and shoots [24] (**Figure 2**). The amount of N applied to the crop is determined by the soil moisture status, amount and frequency and frequency of precipitation during the crop growth season [25]. The higher photosynthesis, water use efficiency (NUE) and water uptake could be achieved with optimum N supply under optimum water supply [26]. Leaf anatomy, leaf rolling, leaf hairs, stomatal positions and opening/closing, are the traits of wheat crop to withstand with under various environmental stresses especially water

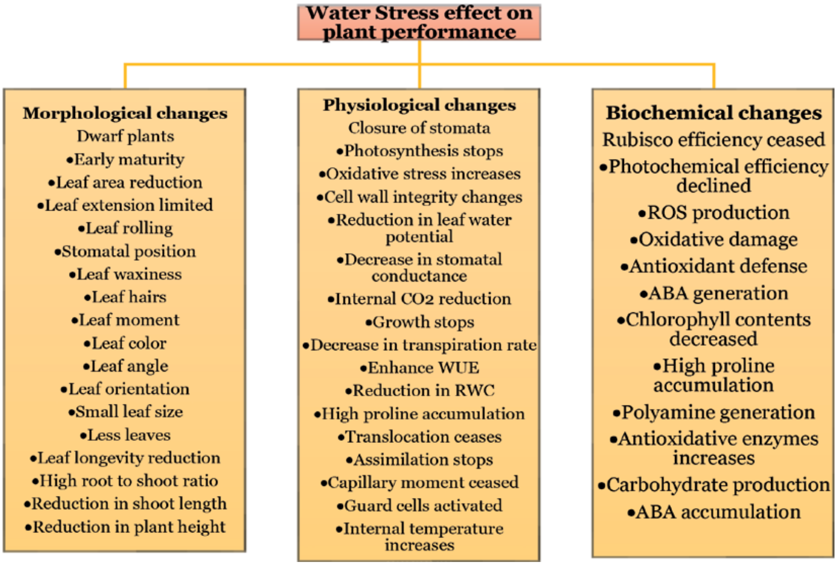


Figure 2.
Morphological, physiological and biochemical dynamics of plants affected by water stress.

deficit [27]. Likewise, the photosynthesis rate, transpiration, osmotic potential, relative water contents, stomatal conductance are also affected by water and N depletion [28]. Rubisco efficiency, photochemical efficiency, reactive oxygen species, antioxidants defense system, abscisic acid generation, proline accumulation, polyamines generation, increase in antioxidative enzymes, carbohydrate and protein dynamics, amylose and amylopectin contents are affected by under water and N colimitation [29].

2.1 Morphological traits of wheat under water and N colimitation

The strong association of WUE and NUE are observed commonly in different agriculture systems including crop husbandry [29]. Certain management practices which are in the way to enhance N and/or WUE at the same time are considered beneficial in term of production cost for the crops especially cereals thereby economizing water and N inputs separately [30]. The morphological attributes of the wheat crop especially the leaf traits i.e. leaf shape, size, expansion, leaf area, senescence, waxiness, leaf hairs and pubescence and the cuticle tolerance of the wheat is affected under water limited conditions [31, 32]. Similarly, the root characteristics i.e. dry weight, density and root length are adversely affected by soil moisture limitation in the rhizosphere [33]. Despite this, the vegetative and reproductive stages of the wheat crop are also affected by water deficit and limited N supply [34]. The better strategies adopted by the plants by modifying its life cycles to theses abiotic stresses (i.e. water and N deficit) are early maturation, small plant size and reduced leaf area for the drought tolerance [35]. It is observed that under water limitation, the flag leaf length is increased, and no significant change was observed in leaf width [36]. Hence, leaf expansion is the function of water extracted from the rhizosphere through rood and its abundance in plant tissues [37]. A decrease in leaf number, size and longevity may be observed under water and N limitation subsequently and their non availability alone as well [37]. In wheat crop, the leaf development is significantly affected by soil moisture depletion thereby decreasing overall biomass [38]. Similarly, N is considered as the integral part of the plant and plays a key role in increasing the leaf area, plant height and finally biomass of the plant under optimum supply [39]. Therefore, both water and N are equally important, their distribution and prevalence should keep in mind as there is a strong linkage between water and N limited supply [40]. Better morphological attributes with less production cost could be achieved under N and water colimitation.

2.2 Physiological response of wheat under water and N colimitation

In regulating plant growth, N plays vital role with a prominent impact on the crop physiology under drought induced N deficit [41]. The biomass of the wheat crop is not only affected under low N availability, but does significantly increased under optimum water supply, mainly because of the higher N content in the rhizosphere which clarifies that with the optimum N supply the presence of adequate availability of soil moisture is therefore essential for biomass, yield and physiological attributes of the wheat crop [42]. The N deficiency the sensitivity of the stomata under water deficit condition in drought prone areas [43]. The leaf photosynthesis rate is significantly affected by the optimum supply of both N and water to the crop [44]. Increase in the photosynthetic activity and biomass accumulation is observed under optimum water and limited N supply [45]. Water use efficiency (WUE) is enhanced through photosynthesis, stomatal conductance and mesophyll conductance in wheat crop and thus ultimately increasing N uptake and nitrogen use efficiency (NUE) [46].

Non availability of soil moisture will not only affect N supply but also have bad effect on N cycling [47]. Thus, water plays vital role in the availability of N in the soil solution to crop and thereby enhancing the photosynthesis process, translocation of assimilates, metabolism and conversion efficiency of the crop [48].

2.3 Biochemical traits of wheat crop under water stress and N limitation

Certain different compounds and metabolites that are accumulated in the crop under water deficit conditions to cope with the drought stress imposition [49]. These metabolites are of key importance because of their peculiar biochemical routes and thereby enhancing the tolerance mechanism in crop plants [50]. A cross talk between various biochemical substances and metabolites are initiated through drought signaling [51]. Various substances are accumulated in the plants body under abiotic stresses especially water stress. Under water limited conditions, proline a key metabolite, that is accumulated in higher concentration under dehydration [52]. The antioxidation enzymes that scavenge the ROS (reactive oxygen species) through various osmo-protection processes that includes stability of the membrane, osmotic adjustment and gene signaling are strongly associated [52]. The vital role in drought tolerance and strong defensive antioxidant mechanism in wheat genotypes are strongly correlated with high proline concentration [53]. The genotypes grown in rainfed areas having high proline concentration compared with the ones grown in irrigated and/or humid areas [54]. Various internal physiological processes are taking place under the crop plants to cope with the drought stress [55]. Plant growth is inhibited, water loss and stomatal closure are regulated, guard cell ion translocation is alternated under water stress through the translocation of ABA from roots to leaves [56]. The wheat crop having less ABA accumulation in leaves are considered to be susceptible to drought stress compared to those which accumulate high proline contents in leaves are drought tolerant [57]. Similarly, the wheat genotypes with high proline concentration in the leaves exhibited better water use efficiency [58]. In wheat crop auxin is considered as negative regulator for drought stress. Similarly, the indole acetic acid (IAA) contents also decreasing under water limitation [59]. The antioxidant enzymes (POD), H2O2, glutathionse (GSH), proline, and malondialdehyde (MDA) concentrations are measured maximum in the wheat plants subjected to water stress [60].

2.4 Yield and yield contributing traits

Most of the yield traits that are associated with the improved productivity in water limited areas are complex and polygenic in nature [61]. Drought stress significantly affected the yield and yield attributes of wheat compared with well water conditions [62]. Thousand kernel weight decreased by 16% under water limited condition thereby affecting overall grain production of wheat crop under drought prone environments [63]. Similarly, the most important yield contributing trait i.e. grain number were also affected by the water stress with less grain number in the crop under drought stress imposition [64]. All the yield components are affected by the drought stress, but the effect was more prominent on grain weight and grain number that contribute to the economic yield of the crop [65]. Water stress causes reduction in grain number to a greater degree that size of the grain, hence grain number exhibiting more plasticity that rest of the components [66]. The narrow variability and low plasticity of the grain size under water stress environments resulting in efficient transport of the pre anthesis assimilates to sink [67]. The reproduction process in the plants is severely affected by water stress, cereal crops exhibits a strong adaptive mechanism up to some extent through the accumulation of the photosynthates in various parts i.e. stem and roots in early growth phase and having the ability to reallocate the assimilates to the sink in reproductive phase of development [68]. The efficient use of the resources like limited water and N rates are the better strategy for wheat production [69]. For primary productivity photosynthetic capacity of the crop plants is the key to higher yield [70]. Water use efficiency provides a simplest mean of assessing whether yield is limited by water supply or other factors and is considered an important component of adaptation to water deficit conditions [71]. As the amount of water used is strongly affected by crop management, a good understanding of crop management effects on WUE may provide researchers opportunities to identify and select appropriate crop management practices for improved water use efficiency [72]. Limited supplemental irrigation and fertilization during the growth season can significantly increase WUE and wheat yield [73].

3. Pathways to improve water and N use efficiency

3.1 Approaches for enhancing water use efficiency (WUE)

Certain agronomic, soil management strategies that may improve the WUE are discussed as under.

3.1.1 Agronomic perspectives

The water use efficiency is the function of the agronomic practices that includes tillage practices, drought resistant and adaptive cultivars, soil fertility and pest management [74]. The crop growth and yield tends to increase under no till system or incorporation of the crop residues which directly influences the availability of soil moisture [75]. Similarly, WUE of the wheat crop enhanced under optimum sowing window, row spacing and seed rate which ultimately increments in wheat production [76]. Decreasing row spacing and increasing seed rate could enhance water soil moisture and thereby increasing water use efficiency [77]. Under optimum row spacing weeds growth is discouraged and hence crop may uptake nutrients and water efficiently [78]. Adjustment in the crop growth season through alteration of the sowing dates keeping in view the seasonal transpiration demand [79]. Early sown crop under conservation agriculture could also increase WUE of grain crops. Drought resistant cultivars resulting in high WUE than the cultivars which are drought susceptible [80]. A well-prepared sowing beds and leveled fields are the prerequisites for water saving and greater yield harvesting. The laser leveling is considered as the effective method responsible for various benefits i.e. germination uniformity, irrigation time reduction, decrease in pest and decreasing production cost [80]. The exogeneous application of certain compounds like silicon, selenium, potassium, hydrogel etc. increase the water use efficiency of the wheat crop under stressful environments, decreasing the adverse effects of biotic and abiotic stresses, enhance bioavailability of nutrients, photosynthetic efficiency and better light harvesting [81].

3.1.2 Soil management perspective

The crop yield and WUE could be significantly increased under better soil management practices through increasing water holding capacity (WHC), ease in soil moisture extraction and by decreasing the water losses through leaching [82]. Increment in WUE could be observed under good soil management practices, that causes soil moisture dynamics by evaporation and infiltration to soil that tends to increase soil water retention capacity and organic matter contents [83]. It is observed over various types and classes of soils, increment in water content at field capacity (FC) is greater than that at permanent wilting point (PWP) when soil organic matter contents are increased [84]. There is also a chance to conserve soil moisture through albedo under high plant density and narrow row spacing [85]. Compared to no tillage, disturbance taken place through tillage practices are more prominent in moisture loss through evaporation. The soil evaporation could be reduced up to 50% by crop residue incorporation [86]. This shows the gaps related to soil management that are responsible and having potential to improve WUE.

3.1.3 Physiological and anatomical perspective

3.1.3.1 Stomatal physiology and biochemistry dynamics

High stomatal conductance in needs more water to fix per unit leaf area for increasing the soil and water use during transpiration [87]. For increasing WUE, during peak hours of the day when transpiration is high by preventing the loss of water from the leaves through prevention by sensitivity of the stomata. Through the process of transpiration water is lost in the form vapor which facilitates CO2 uptake [88]. Thus stomata plays a vital role in increasing WUE, thereby controlling the water loss in the form of vapor and CO2 gain [89]. The opening and closing of stomata is also regulated by the environment determinants [90]. Stomatal moment is controlled by the transport and osmotically active solute accumulation and the guard cells lateral moments [91]. In most of the plant leaves, stomata are separated through an epidermal cell creating space for opening and closing [92]. In certain plant species, the clustering of stomata is also considered a better strategy for lowering evaporation demand and this phenomenon in the crop plants is considered better adaptation in drought prone areas [93].

3.1.3.2 Crop environment, root architecture and harvest index enhancement

Growing of the crops in environments where there is less transpiration will result in higher WUE. The identification and allocation of such areas having less transpiration could be identified through spatial and temporal scale [94]. Enhancement in harvest index increases the water use efficiency, this provides a new research window for increasing WUE in drought prone environments [95]. The harvest index is decreased by the water stress imposition at reproductive stage of the wheat crop, hence certain strategies need to be defined to conserve the soil moisture for the future use in reproductive stage of plant growth and development [96]. Similarly, root canopy and architecture are playing important role in extracting soil moisture [96]. Rood density, root angle, root hairs, root type and root length are the characteristics that determines the water uptake from various depths of the rhizosphere [97]. In wheat crop stele and xylem number are considered to be more responsive in wheat crop in water stress conditions because of the more xylem developmental plasticity in wheat crop [98]. In cereal crops, the metaxylem diameter and density are responsible for high WUE [99].

3.2 Approaches for enhancing nitrogen use efficiency (NUE)

The grain production per unit of soil available N is termed as nitrogen use efficiency. Certain approaches that should be kept in mind while enhancing the NUE of grain crop are described as under.

3.2.1 Agronomic perspective

Nitrogen is accepted universally as an essential component of crop production. The climatic condition is different for every crop. There are some of the main factors i.e. rainfall, solar radiation and temperature that significantly influence the demand for nitrogen, influence nitrogen use efficiency (NUE) and crop growth [100]. The demand of N to plant increases as plant grows showing a sigmoid curve (**Figure 3**). It has generally observed that nitrogen element significantly enhanced the biomass of the plant/crop if it absorbed by plant properly because maximum biomass and plant growth totally determined by the uptake and loss of nitrogen [101]. Yet, due to leaching and volatilization of nitrogen the crop cannot uptake nitrogen easily from the soil because the due to leaching the nitrogen moves down from the below root zone. Thus, efficient nitrogen management could significantly improve the nitrogen use efficiency [102].

Water availability is the essential factor that affected the NUE under limited water availability which is crucial from an environmental and economic standpoint [103]. Maximum nitrogen use efficiency (NUE) in drought tolerant varieties of winter wheat as compared with drought sensitive lines of winter wheat. Drought resistant varieties of wheat crop consistently produced higher yield as compare with drought sensitive varieties [104]. Higher nitrogen use efficiency (NUE) and water use efficiency (WUE) in drought resistant wheat varieties. Nitrogen rate parabolically increased with rainfall, while WUE and NUE were linearly related [105]. Simultaneous enhancement of NUE and WUE were more beneficial than optimization of nitrogen and water individually. It is also stated that low nitrogen application could enhanced the nitrogen use efficiency when supplied it from organic sources rather than commercial. Excess application of nitrogen to the crop could significantly reduce the crop yield and drastically decreased the nitrogen use efficiency. It is therefore, important to obtain the higher nitrogen use efficiency through adaptation of nitrogen management strategies and agronomic practices under deficit irrigation or water stress condition.

3.2.2 Rotation of crops

It is an effective strategy to enhance the nitrogen use efficiency. Effective use of soil resources i.e. nutrient and water and appropriate crop sequences could

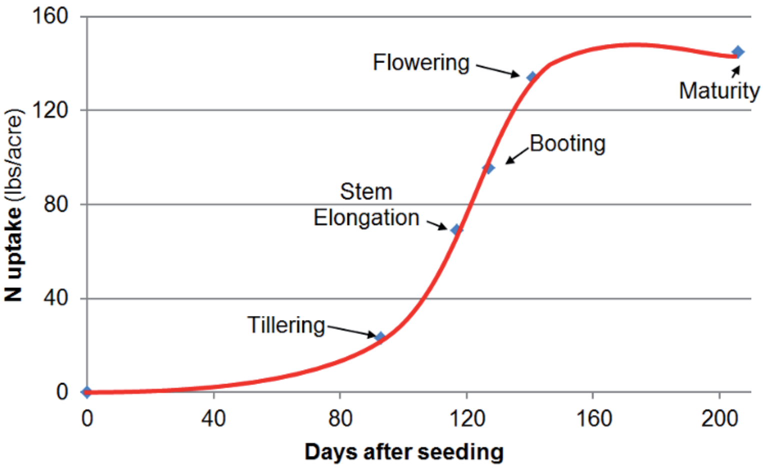


Figure 3.
Graphical representation of N needed for plant growth/N uptake pattern during life cycle the plant.

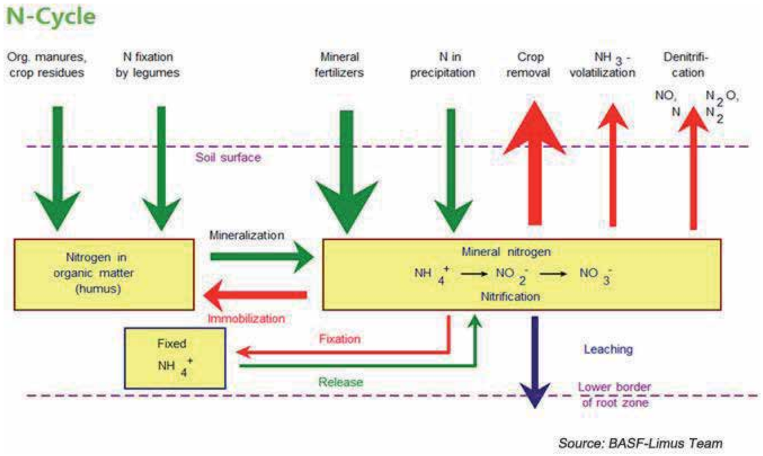
maximize the crop yield. Increase in crop yield is associated with crop rotation is known as rotation effect while monoculture practice significantly decreased the crop yield [106]. There is an effective management practice of legumes and non-leguminous crop which linearly enhanced the crop yield and other yield components. Monoculture practice in field significantly declined the cop yield and degrade the soil fertility, while crop rotation is an effective strategy to obtain the higher crop yield [107]. Leguminous crops should be included to enhance the crop yield and also suppress the weeds and decreased the risk of disease infestation. There are the following benefits of crop rotation which enhanced the nutrient use efficiency, water use efficiency, reduced allelopathy and improved soil quality [108]. Cereals are the largest consumers of nitrogen use efficiency as compared with leguminous crop. Crop rotation could reduce the immobilization and residual soil nitrate available for leaching. Leguminous crops increased the soil fertility, enhanced the soil microbial activity and improve the soil structure. Leguminous crop also add nitrogen to the soil from the atmosphere due to presence of nodules in the roots [109]. Rotation practices also enhance the nitrogen use efficiency (NUE). As compare to cereals crop, leguminous crops enhanced the soil fertility, improve soil texture and structure and enhance microbial activity. Rotation practice enhance the nitrogen use efficiency (NUE), However, they also stated from their study that nitrogen supplied to the crop following a legume have been inconsistent [110]. Several studies have been documented that NUE could enhance the crop yield when supplied it from organic sources as compared with inorganic sources. Crop rotation practice significantly increased the nitrogen use efficiency (NUE) and other components.

3.2.3 Irrigation management

Irrigation management is an appropriate strategy to improve the water use efficiency and fertilizer use efficiency. Border and furrow irrigation are traditional method used for vegetable and field crops [111]. These two practices are generally associated with higher percolation losses which resulted in low nitrogen use efficiency (NUE) and water use efficiency (WUE). These traditional methods are increasingly becoming difficult in the changing climate scenario. Adoption of water efficient irrigation and other modern irrigation techniques are the best strategies to obtain the higher NUE and WUE. Many researchers confirmed that deficit irrigation strategy enhance the WUE and NUE (water use and nitrogen use efficiency) [112]. According the several authors, maximum recovery efficiency, of nitrogen applied is due to high irrigation of overhead sprinklers. Strategy of water management is linked with the nitrogen nutrition and significantly affects the NUE and WUE [113]. Water application and their adjustment is to match the actual crop demand which is an effective management technique for obtaining higher NUE and WUE.

3.2.4 Nitrogen source and placement method

The uptake efficiency of most of the crops is dependent on the N source and application method. Nitrogen is lost (**Figure 4**) through urease hydrolysis to atmosphere when applied to the soil surface from urea based N source, this is because of the increased soil pH resulting its volatilization [114]. Farmers are increasing the application rate to their field for optimum productivity but in vain because this have a deleterious effect on soil, atmosphere and aquatic bodies as well [115]. Certain slow N release fertilizers are manufactured with the aim to reduce the losses [116]. The NUE could be maximized and losses are reduced under proper N application strategies [117]. Side dressing or band application, mixing in soil, injecting to soil sub surface of N fertilizers are the different strategies that could enhance the N



Source: BASF-Limus Team

Figure 4.
Schematic diagram showing various processes of N cycle in soil plant atmosphere system.

efficiency than the broadcast application. Surface broadcast N application resulting in greater loss as ammonia-N to atmosphere, this could be minimized if banded, side dressed, placed with or below seed, or injected to soil [118].

3.2.5 Physiological perspective

The vital factor which is responsible for growth and development of the crop is nitrogen, increases various traits of the crop thereby improving final yield [119]. The N availability affects the carbon and N partitioning between roots and shoots of the crop [120]. Under high N availability, the root shoot ratio decreases because of the prominent growth of shoots [121], this varies among varieties and cultivars. The timing of N availability is of utmost importance in wheat crop where it affects all the yield parameters especially tiller density, spike compactness, grain number, kernel weight and grain N ad protein concentration [122]. Wheat crop store and sequester the N in vegetative stage, this stored N is re allocated to the grains during reproductive stage, of them some become the part of cell wall. In grain crops, about 50–70% of the N is translocated and accumulated at maturity [123]. The N harvest index could be improved by the sequestration of N in shoots and grains. Alteration in leaf area index and specific leaf N can enhance the NUE. Understanding these physiological characteristics that regulate plant N uptake, grain N uptake, root N uptake, assimilatory process, redistribution of N within the cell, and leaf structure and architecture can help improve NUE physiologically [124]. N uptake and LAI were found directly proportional for NUE and wheat productivity [125]. Under N- and water-limited conditions the overexpressing a downstream N assimilation gene called alanine amino transferase has increased N uptake and use efficiency [126]. Another way of increasing N uptake in plants is enhancing larger and deeper roots that can capture more available N in the soil profile. Vigorous root and shoot growth are necessary for higher N recovery [127].

4. The intricate relationship between nitrogen and water colimitation

Various literatures are present and research work has been done on the nitrogen and water stress individually but there is need to explore the intricate effect of both

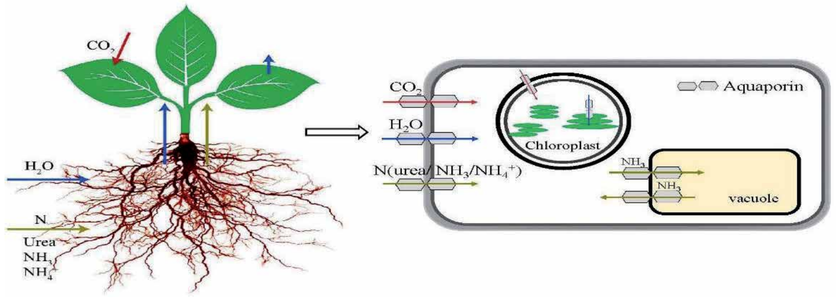


Figure 5.
The intricate relationship of water and N for optimum N uptake affecting plant physiological processes.

N and/or water (**Figure 5**) thereby decreasing cost of production of wheat crop and to enhance the grain and biomass yield [128]. Both the water and N rates need to be economized for optimum wheat production under high WUE and NUE simultaneously [129]. The effect of N and water on the process of photosynthesis is present in literature, claims the positive effect of N rate on photosynthesis. Similarly, with increase in soil moisture the physiological processes are positively influenced and results in optimum wheat production [130]. In fact, there are diminishing return that with the increase of N and water, the photosynthesis rate increases, but the rate of photosynthesis tends to saturate with further increment of water and N resulting in less WUE and NUE for the wheat crop and increasing cost of production [131]. Under certain environments, it is reported that NUE may increase under maximum supply of water and contrary to this N increment may also increase WUE [132]. Therefore, the interaction of N and water is the interplay of management, environment and cultivar collectively [133]. Application of irrigation water at higher rate may cause N losses through leaching resulting in N shortage in the rhizosphere [134], this condition also leads to stomatal closure thereby affecting the crop physiological processes. Under low N availability, photosynthesis process will be less leading to reduction in wheat productivity with less WUE [135]. On the other hand, the crop susceptibility to drought, temperature stress and may increase under high N application. Plant species differs in demand for N and water that affects the photosynthesis process. The studies also suggest that increasing both N and water has positive effect on yield rather than increment in either factor. This increment in both N and water leads to higher production cost, water loss, affecting water quality, aquatic flora and fauna and ultimately causes pollution of water and air as well [136]. It is thought that N input effect will be more efficient at low water input that the higher water supply. Similarly, the NUE decreases with higher N rate and increment in water input may increase NUE at lower N rate.

5. Nitrogen (N)-climate interaction; fix N to fix climate change

The wide spread use of synthetic fertilizers in agriculture [137, 138], fossil fuel use from the industry, leguminous crops cultivation on large scale has therefore increased the reactive nitrogen in the atmosphere in the last five decades [139–141]. Globally, a great threat is there to impair the water and air quality [142], biodiversity and human health due to unpredictable alteration in biogeochemical cycle especially N [143]. The considerable amount of N is lost through leaching in the form of NO3-N [144, 145]. This NO3-N leaching varies due to the N status of the soil and greatly depends on soil properties [146–149]. Thus, the shallow ground water and aquifers are contaminated through the soluble nature of the NO3 in soil water [150, 151].

Worldwide, NO3-N is considered as the major concern as it deteriorates the ground water and surface water quality since few decades [152–154]. Increment in the NO3-N contents in drinking water have bad effect on human and animals health as well [155, 156], also the aquatic bodies are contaminated through its higher concentration accumulation [157–160] thereby favoring the aquatic plants to grow and nourish well [161, 162] by making a surface layer thereby impeding the solar radiation to reach the aquatic fauna [163–166] and ultimately causing the death of the aquatic fauna [167] which creates eutrophication [168]. Besides this, blue baby syndrome in infants (methemoglobinemia) and human birth defects [169] are the major serious problems linked with NO3 contamination [170–171]. Therefore, it is of prime importance to estimate the NO3 leaching from cropland [172] and to evaluate the environmentally compatible and economically suitable agronomic practices [173] that could maintain and/or decrease this phenomenon [174].

The increment in the NO3 leaching loss is strongly correlated with the rainfall occurrence, intensity and irrigation volume and methods [175]. The excessive fertilization increases the risk of N loss through leaching in agriculture system [176, 177]. To feed the overwhelming population of the world, higher fertilizer application to crop field [178] is under practice have rapidly raised the NO2 emission of the atmosphere [179, 180] and is expected to increase linearly in coming decades [188], aggravating the environmental quality [181]. Through the anthropogenic rise in NO2 emission from agriculture sector to atmosphere thereby offset the efforts to decrease the CO2 sully from other sectors [182].

Global warming is directly affected by NO2 emission [183] which is considered as a major greenhouse gas [192], having 300 times more potent than CO2 because of its greater resident time in the atmosphere usually greater than 100 years [184]. The unregulated and increasing trend of the NO2 defines the global warming [185]. This increment in N availability in atmosphere affects the sinks and sources of NO2, CH4 and CO2 thereby potential increase in CO2 sequestration [186].

Climate change will certainly increase the N emission, which in turn will affect the agriculture system, aquatic water bodies, humans and animals' health [187]. Thus, climate N cross talk will expose the crops to elevated O3 [188]. Greater emission of the N will be observed from livestock and agriculture production systems as well [189]. Drought spell, erratic and unpredictable rainfall, temperature events taken place because of climate change will ultimately affects the farmers decisions and practices thereby enhancing C and N release from agriculture ecosystem [190] when higher N rates are practiced keeping in mind leaching losses [191]. N availability to crops impedes and its losses will increase under drought induction, irregularity in rainfall, high temperature events and flooding [192].

Nitrogen, the essential macronutrient responsible for plant growth and development is fundamental for farming [191]. Climate change impact may be taken under consideration for minimizing N losses [193, 194] and to manage N in better way [195–197]. Perturbation to both N and climate needs to be fixed to reduce its adverse effects environmentally compatible and economically suitable agronomic practices [198]. Firstly, irrigation water availability to field crops is a great question and this the situation become worsen in each passing day. Then the application volume to field crops in higher rates decreasing the WUE on one way and depleting the available nutrients in the soil on other hand because farmers are applying higher fertilizers to harvest maximum yield thereby ultimately increasing their cost of production. Therefore, a gate way for future research is to economize the N rates/levels for the crop growth, yield and quality by monitoring the rhizosphere moisture for irrigation water optimization till moisture allowable depletion (MAD) of the respective crops that may not affect its growth and development but also enhance the availability and utilization of the nutrients in soil solution.

6. Conclusion

This chapter provides the information regarding water and N rates on wheat physiological, morphological, biochemical, qualitative changes and yield traits of wheat crop focusing on the NUE and WUE. Drought stress is the most prominent limiting factor that manipulates the physiological pathway, biochemical traits and hence negatively affects wheat crop productivity. The global nitrogen (N) recovery indicated that about two-fifths of N inputs are lost in the ecosystems through leaching, gaseous emission, denitrification, surface runoff and volatilization. About 50–60% of the applied N fertilizer to the field are lost to environment and thus not available to the plants. These deleterious environmental consequences need to be reduced by efficient management of N and/or water focusing on their intricate relationship. N-availability is often regulated by soil water; hence crop is experiencing N- and water-limitation simultaneously. There is great impetus to optimize their uptake through interconnectedness of water and N for yield determination of wheat because of the water scarcity and N losses for enhancing wheat productivity and thereby reducing the production cost. There is need to optimize the N and water colimitation simultaneously to determine the minimum N and water to harvest optimum yield with better baking quality and hence safeguarding the environment, soil micro fauna and water bodies.

Therefore, a gate way for future research is to economize the N rates/levels for the crop growth, yield and quality by monitoring the rhizosphere moisture for irrigation water optimization till moisture allowable depletion (MAD) of the respective crops that may not affect its growth and development but also enhance the availability and utilization of the nutrients in soil solution.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 14

Adapting Cereal Grain Crops to Drought Stress: 2020 and Beyond

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Abstract

Among other environmental instabilities, drought stress is the primary limitation to cereal crops growth, development and productivity. In the context of continuing global climate change, breeding of drought resistant crop cultivars is the most economical, effective and sustainable strategy for adapting the crop production system and ensuring food security for the growing human population. Additionally, there is need for improving management practices. Whereas conventional breeding has sustained crop productivity gains in the past century, modern technological advancements have revolutionized our identification of important drought tolerance genes and underlying mechanisms, and accelerated new cultivar development. Large-scale high throughput sequencing, phenotyping, 'omics' and systems biology, as well as marker assisted and quantitative trait loci mapping based breeding approaches have offered significant insights into crop drought stress tolerance and provided some new tools for crop improvement. Despite this significant progress in elucidating the mechanisms underlying drought tolerance, considerable challenges remain and our understanding of the crop drought tolerance mechanisms is still abstract. In this chapter, therefore, we highlight current progress in the identification of drought tolerance genes and underlying mechanisms, as well as their practical applications. We then offer a holistic approach for cereal crops adaptation to future climate change exacerbated drought stress.

Keywords: drought stress, drought tolerance, cereal crops, omics approaches, phenotyping, genetic engineering, climate change, conservation agriculture

1. Introduction

Drought stress is the primary environmental factor influencing the growth, development and productivity of crops and its significance is expected to increase in the wake of global climate change [1–4]. This presents a serious challenge to the food and nutrition security of an ever-rising world human population. Moreover, the current transition from carbon/fossil intensive fuel driven economies to modern climate-smart low-carbon economies further strains our crop production systems [5]. Adapting field crops to drought stress therefore becomes critical for sustainable agricultural production under such climate change scenario [6]. To achieve that goal, breeding drought resilient crop cultivars that maintain yield stability under such conditions befits the most economical, effective and sustainable strategy. This is particularly relevant for cereal grain crops.

Cereal grain crops, chiefly rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), sorghum [*Sorghum bicolor* (L.) Moench], barley (*Hordeum* *vulgare* L.), and pearl millet (*Pennisetum glaucum* L.) provides above 50% of the total food calorie requirements of billions of people in developing countries across the Sub-Saharan Africa, South Asia and Latin America [7–9]. For instance, the top three cereals; maize, rice, and wheat jointly provide 48% of the total calories and 42% of total protein consumed in developing countries. Additionally, as staple foods, maize and wheat contribute approximately two thirds of the global food energy intake [8]. Further, these cereal crops are important raw materials in the animal feed and bio-fuel manufacturing industries [10]. However, most of these crops are grown across arid and semi-arid regions of the world, where they often endure exposure to recurrent drought episodes throughout their growth cycles. Therefore, understanding how cereal crop plants respond to drought stress is critical for guiding drought tolerance breeding.

In the last century, conventional breeding approach has proven itself capable of sustaining productivity growth in various crops [11]. Meanwhile, modern technological advancements have accelerated the pace and impact of new cultivar development. Such technologies include high throughput omics approaches, identification of quantitative trait loci (QTL) underlying abiotic and biotic stress resistances, marker assisted selection (MAS) and gene cloning [12–15]. Despite this significant progress in elucidating the mechanisms underlying drought tolerance, considerable challenges remain and our understanding of the crop drought tolerance mechanisms is still abstract.

In this chapter, therefore, we look at various aspects of drought stress in major cereal grain crops such as maize, sorghum, wheat, rice and finger millet. We also discuss the current approaches in identifying drought tolerance genes and metabolic pathways. Further, we highlight the progress made to date on elucidation of key drought stress responses, phenotyping and QTL mapping for drought tolerance, genetic engineering of drought tolerant crops and management of crops. We conclude by offering an integrated strategy for adapting cereal grain crops to drought stress in the context of climate change.

2. Drought stress effects in cereal grain crops

A decrease in water inputs into an agro/ecosystem over time that is sufficient to cause soil water deficit (SWD) is often termed drought, and this encompasses various forms such as rainfall anomalies, irrigation failure, seasonal or annual dry spells [16]. In agricultural context, drought signifies a period of below-average precipitation when the available soil water in the plant rhizosphere drops beyond the thresholds for efficient growth and biomass production [17]. The resultant oxidative stress emanating from such SWD is dubbed drought stress.

Numerous research reports have reflected on the effects of drought stress on cereal crops. Drought stress effects span from morphological to molecular levels, and are exhibited at all phenological growth stages at whatever stage the water deficit takes place. Generally, drought stress impairs seed germination resulting in poor crop stand establishment [1, 18]. Drought stress reduces the plant cellular water potential and turgor pressure, thereby increasing the cytosolic and extracellular matrices solute concentrations. Resultantly, cell growth is diminished due to the reduction in turgor pressure [19]. Additionally, abscisic acid (ABA) and compatible osmolytes such as proline are excessively accumulated, causing plant wilting. Simultaneously, reactive oxygen species (ROS) such as H_2O_2 are overly produced. Although they function as signal transduction molecules, over-accumulation of ROS could result in extensive cellular oxidative damage and inhibition of photosynthesis [20].

Moreover, when moisture deficit becomes severe, cell elongation becomes inhibited by the interruption of water flow from the xylem vessels to the surrounding elongating cells [21]. Consequently, vegetative growth, dry matter partitioning, reproductive organ development and reproductive processes, grain filling and grain quality are disrupted [22]. In cereal grain crops, reproductive processes and grain filling are more susceptible to water deficit stress, with optimum and ceiling temperatures that are relatively lower than those for seedling and vegetative growth stages [21, 23–25]. Moisture deficit stress reduces yield by delaying silking, thus increasing the anthesis-to-silking interval [11]. Drought stress at flowering period is critical as it can increase pollen sterility resulting in hampered grain set [26].

Drought stress induced yield reduction in cereal crops depends upon the severity and duration of the stress period. The reduction in photosynthesis, emanating from the decrease in leaf expansion, impaired photosynthesis machinery, premature leaf senescence and related food production decreases, is the major contributing factor on yield reduction [21, 23]. Drought stress induced yield reductions have been reported and reviewed in maize [1, 21, 26–29], wheat [26, 27, 30, 31], rice [1], sorghum [32–33] and pearl millet [25].

3. Plants drought stress responses and resistance mechanisms

Plants have evolved numerous dynamic acclimation and adaptive ways of responding to and surviving short-term and long-term drought stresses [34]. The physiology of plants' drought response at the whole plant level is complicated as it encompasses lethal and adaptive alterations. Moreover, how plants respond to drought stress differ significantly at various organizational levels, and this is generally dependent on plant species; the nature, duration and intensity of the drought stress; plant growth and phenological state at the time of stress exposure [19].

Drought stress triggers a wide range of plant structural changes which are essential for plants to respond to such drought stress conditions. These adjustments include morphological adaptations such as reduced growth rate, deepened rooting system, and root-to-shoot ratio modifications. The increased root-to-shoot ratio under drought stress conditions enables water and nutrient uptake and maintenance of osmotic pressure [19, 26, 35]. Additionally, in their response to moisture fluctuations in the soil rhizosphere, plants alter their physiology, modify their root growth and architecture, and regulate the closure of stomata on their aboveground structures. Such tissue-specific responses adjust the cell signals flux, consequently inducing stunted growth or premature flowering, and generally reduced yield [36]. Thus, drought stress is associated with alterations in leaf anatomy and ultrastructure. Reduced leaf size, decreased number of stomata; thickened leaf cell walls and induced premature senescence are some of those morphological changes [19].

Plants resist drought stress through a combination of strategies, which have been widely classified as drought escape, drought avoidance and drought tolerance [18, 21, 37, 38]. Drought escape is achieved by matching the duration of the crop cycle to water supply through genetic variation in phenology [39]. Plants prioritize early flowering and completing their life cycles before the effects of drought cause harm [21].

Drought avoidance denotes plant's ability to maintain high tissue water potential under drought conditions. Usually, plants achieve drought avoidance through morphological and physiological alterations, including reduced stomatal conductance, decreased leaf area, promotion of extensive rooting systems and increased root to shoot ratios [38]. Drought avoidance mechanisms help in maintaining favorable cellular water balance, by enhancing water absorption, decreasing water loss, or allowing desiccation tolerance at low leaf water potential [34]. Stomatal closure, reduction of leaf growth and increased root length and density all contribute to increased water use efficiency under drought stress conditions. Further, water flux into the plant is reduced or water uptake enhanced to achieve drought avoidance [19].

On the other hand, plants attain a state of drought tolerance by cell and tissue specific physiological, biochemical, and molecular mechanisms. Drought tolerance is a complex trait which refers to the capacity of the plant to be more productive under drought stress [40]. In other words, it denotes the potential of crop plants to maintain their growth and development under drought stress [21]. The main aspects of plant drought tolerance mechanism include homeostasis maintenance, via ionic balance and osmotic adjustment; ROS scavenging and antioxidant enzyme activation; growth regulation and recovery by way of phytohormones; specific gene expression; and accumulation of specific stress responsive proteins [1, 2, 19, 21, 26].

To protect themselves against ROS induced oxidative stress and photo inhibition, plants activate an efficient antioxidant (enzymatic and non-enzymatic) defense system [17, 18, 21, 41]. Enzymatic antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase (GPX) and glutathione S-transferase (GST) among others. SOD acts as the first line of defense by converting singlet oxygen into H_2O_2. APX and GSTs then detoxify H_2O_2 [42]. Non-enzymatic antioxidants including glutathione (GSH), ascorbic acid (AsA), carotenoids, tocopherols and flavonoids are also crucial for ROS homeostasis in plants [43].

At the molecular level, our current knowledge thus far regarding drought stress response pathway suggest that, sequentially, the stress is relayed through the following chain: signal perception, signal transduction, transcriptional control by way of transcription factors (TFs), stress responsive genes activation, and in-turn activation of physiological and metabolic responses [1, 19, 37, 44].

Stress signal perception in plants has revealed the role of plasma membrane in perceiving and transmitting signals to the cell interior, where molecules such as receptor-like kinases and G-proteins function as primary receptor molecules [45]. The roles of Ca^+ and ROS as secondary messengers in stress perception and signaling have also been well acknowledged [4]. These secondary messengers adjust the calcium levels and activate protein phosphorylation. Then, phosphorylation of inactive proteins may be directly involved in cellular protection by protein folding or activation of stress specific genes. The dominant plant signal transduction pathways involve the mitogen activated protein kinase (MAPK) and calcium dependent protein kinases (CDPK) cascades, and their role in abiotic stress response have been well reviewed [38, 42, 46].

Upon drought exposure, the resulting signal transduction triggers the production of several biochemicals, including phytohormones, to respond and adapt to the ensuing drought stress [47]. Phytohormones are molecules produced in low concentrations but are critical in regulating plant growth, development, response to biotic and abiotic stresses, and other physiological processes [48]. These phytohormones include ABA, salicylic acid (SA), ethylene (ET), cytokinins (CKs), gibberellin acid (GA), jasmonic acid (JA) and brassinosteroids (BRs) among others. Among these, ABA is the key and most extensively studied hormone that regulates drought resistance in plants [38, 49]. ABA acts as the second messenger coordinating hormonal cross-talk between several stresses signaling cascades, thereby leading to adaptations to changing physiological and environmental conditions [50]. Additionally, SA, ET, JA, CKs, GA, and BRs play vital roles in regulating various phenomena in plants acclimatization to drought stress [51].

At the molecular level, plants institute stress responsive proteins, TFs and signaling pathways among other strategies. Several studies [52, 53] have identified conserved and species-specific drought responsive genes, including membrane stabilizing proteins and late embryogenic abundant (LEA) proteins, which increase cells' water binding capacity [1, 14]. Several heat shock proteins (HSPs), which play a major role in stabilizing protein structure, were also identified [54, 55]. The HSPs are chiefly involved in unwinding some folded proteins and averting protein denaturation under abiotic stress conditions. The membrane stabilizing proteins, LEA proteins, detoxification enzymes, water channel proteins and ion transporter genes all constitute a group of functional proteins, that is, a group of important enzymes and metabolic proteins which directly function to protect cells from stress [37, 56]. Besides functional genes, regulatory genes are also critical for drought tolerance. These encodes various regulatory proteins such as transcriptional factors (TFs), protein kinases and protein phosphatases – involved in regulating signal transduction and gene expression in response to stress [57, 58]. Several TFs that regulate a large spectrum of downstream stress-responsive genes and provide adaptive response under drought stress have been identified and reviewed, including myeloblastosis (MYB), abscisic acid responsive elements binding factor (ABF), ABRE binding (AREB), dehydration responsive element binding (DREB), C-repeat binding factor (CBF), [NAM, ATAF1/2, and CUC2 containing proteins] (NAC) and WRKYs [59–63].

Additionally, protein kinases and protein phosphatases mediate phosphorylation and dephosphorylation of proteins, respectively. In several signal transduction pathways; they are vital and an effective mechanism for stress signal relaying [19, 56, 63]. At the phosphorylation cascade terminals, protein kinases or phosphatases activate or suppress TFs, respectively. The TFs further specifically bind to cis-elements in the promoters of stress responsive genes, thereby modulating their transcription [64]. The TFs are further subjected to post translational modifications (PTMs), including ubiquitination and sumoylation, thereby forming an intricate regulatory network to modulate stress responsive genes, which consequently trigger the activation of appropriate physiological and metabolic responses [62, 65].

4. Approaches for deciphering drought stress responsive genes, proteins and metabolic pathways: where are we?

The recent convergence of crop physiology, next generation sequencing and molecular biology approaches has offered us convenience in deciphering mechanisms underlying plants' response to various abiotic stresses [13, 15, 66]. Whereas plant physiology enhances our understanding of the complex network of traits related to drought tolerance and improving selection efficiency, genomics and molecular biology methods identify the candidate genes and quantitative trait loci (QTLs) underlying these traits [11, 13]. The classical cDNA and oligonucleotide microarrays have been widely employed to identify candidate genes for drought tolerance in several cereal grain crops including maize [67, 68], rice [69, 70] and barley [71]. Additionally, the use of tilling microarrays has allowed for the identification of differentially expressed DNA sequences at the whole genome level [72]. Other techniques such as differential display; cDNA amplified fragment length polymorphism (cDNA-AFLP); and serial analysis of gene expression (SAGE) have been essential in analyzing global gene expression profiles in functional genomics studies [56, 73–74].

Analysis of large scale, high throughput sequencing data is now facilitating the identification and cloning of important genes at target QTLs. Additionally, the 'omics' analysis approaches are showing monumental capacity to quicken and broaden our understanding of the molecular, genetic and functional basis of crop drought stress tolerance [12, 75]. Encouragingly, some novel insights meant to help us develop new drought tolerant cultivars are being generated [76]. Due to its low cost, high-throughput, and high sensitivity, RNA sequencing (RNA-seq) has offered us breakthrough in performing transcriptome analysis of plants' drought stress responses [77]. Resultantly, we have obtained transcripts from RNA in a tissue- or cell-specific manner, and transcribed at a different developmental stage or functional state; this has been fundamental to fishing out functional genes [78]. Therefore, our knowledge pertaining to gene expression networks modulating drought stress tolerance has been significantly improved. RNA-seq technology has been used in several drought stress response studies in cereal crops and numerous genes have been identified [79–81].

Recently, large scale, high-throughput proteomics has become a very powerful tool for performing comprehensive analysis of crop proteins and identification of stress responsive proteins in comparative abiotic stress studies [82, 83]. Proteomic approaches, particularly gel free methods, that is, those involving digestion of intact proteins into peptides prior to separation, have now become very popular in proteome profiling, comparative expression analysis of two or more protein samples, localization and identification of post translational modifications (PTMs) [14]. For instance, isobaric tags for relative and absolute quantitation (iTRAQ) and isotope-coded affinity tags (ICAT) based methods have become widely used in descriptive and comparative drought stress proteomic studies in cereals [84–87]. The iTRAQ-based method allows for the time-dependent analysis of plant stress responses or biological replicates in a single experiment [88]. Besides, proteomics offers complementarity to genomics; providing clues on the molecular mechanisms underlying plant growth and stress responses, as well as being a crucial link between transcriptomics and metabolomics [82]. Moreover, genomics based methods offer access to agronomically desirable alleles localized at QTLs that affect particular physiological responses. This helps us to effectively improve the drought resilience and yield of crops. Additionally, MAS has aided us in improving drought-related traits [12, 89].

Further, a systems biology approach, premised on the advancement and integration of omics (genomics, transcriptomics, metabolomics, phonomics and proteomics) methods to establish a meaningful relationship between the genotype, phenotype and subsequent abiotic stress tolerance, has also become prominent [76]. Integrated knowledge of the morphological, physiological, biochemical, genetic and molecular events in plants allows for in depth understanding of the complex physiological and cellular processes involved in drought stress adaptation. Thus, meaningful interpretations from complex networks and component integrations can be developed from voluminous omics data, which helps us better decipher the mechanisms underlying cereal crops drought tolerance [63].

Meanwhile, the physiological analysis on contrasting genotypes provides information on the mechanisms underlying drought tolerance and aids as a useful screening strategy for drought tolerance [41]. Therefore, it is important to realize that physiological analysis remains essential in corroborating the molecular analyses in abiotic stress response studies. Thus, it would be essential that, going forward, we build on the progress made to date by harnessing the full potential of genomics-assisted breeding, and integrating our knowledge on the physiological and molecular basis of drought tolerance. This calls for crop physiologists, molecular geneticists, breeders and cytogeneticists to collaborate in a multidisciplinary manner [12].

5. Some identified key drought tolerance mechanisms, genes and metabolic pathways

By applying genetic, biochemical and molecular approaches, we have identified essential genes central in plant responses to drought stress. For instance, several physiological responses contributing to drought tolerance in cereal crops have been identified including thermal dissipation of light energy, stomatal closure, decreased hydraulic conductance, altered source-sink relations and carbon partitioning, ABA biosynthesis, among others (**Table 1**).

No.	Physiological response	Purpose	Reference
1	Thermal dissipation of light energy	Uncoupling of photophosphorylation and electron transport	[41, 90]
2	Activation of photosynthesis (PSII) proteins	Preventing photoinhibition of the PSII and improving leaf photosynthetic capacity	[81, 91]
3	Altered source–sink relations and carbon partitioning	Induction of root growth	[92]
4	Prioritized supply of CHOs to rapidly growing or metabolically hyperactive cells or tissues	Promotion of early seedling/hypoctyle growth	[80]
5	Cell wall biosynthesis	Enhancing cellular contents preservation	[81, 93, 94]
6	Cell wall remodeling	Increasing cell wall elasticity to maintain tissue turgidity	[80]
7	Amino acid biosynthesis	Enhanced protein biogenesis	[80]
8	Osmotic adjustment through increased synthesis of soluble solutes (proline, soluble sugars, etc.)	Increased cellular homeostasis maintenance	[11, 40, 95, 96]
9	Changes in ROS scavenging and enzyme activities	Prevention of cellular oxidative damage	[18, 81]
10	ABA biosynthesis	Stomatal closure regulation and improved stress signaling	[11, 40]
11	Stomatal closure and reduced hydraulic conductance	Prevention of water loss through transpiration	[97]
12	Alteration in root morphology and physiology	Increased water and nutrient absorption under drought conditions	[98, 99]
13	Reduced stomatal density and enhanced control of stomatal opening and closure	Improved cellular water conservation	[100]
14	Alternative oxidase pathway, uncoupling proteins, NADPH dehydrogenases down-regulated	Uncoupling of oxidative phosphorylation and electron transport	[101]

Table 1.
Some key drought stress tolerance mechanisms identified in cereals.

Besides the mechanisms highlighted in **Table 1**, several drought responsive genes have been identified and validated in different crop species. For instance, recent excellent reviews [4, 26, 102–106] provide highly informative details about some crop drought tolerance conferring genes that have been functionally validated to date. Some of these genes are listed in **Table 2**.

Further, several metabolic pathways implicated in drought stress tolerance in cereal grain crops have been identified through comparative physiological and omic analysis approaches. Chief among these pathways are those related to photosynthesis, secondary metabolites biosynthesis, plant hormone signaling, starch and sucrose metabolism, and nitrogen metabolism. Chloroplasts, particularly the thylakoid membranes—PSII reaction centers, are one of the organelles most influenced by drought stress [123, 124]. Photosynthesis (antenna protein) pathway related genes *lhcb5-1* and *lhcb5-2* are part of the light harvesting complexes (LHCs) and the electron transport components of the PSII of the plant photosynthesis machinery, where they participate

No.	Gene name	Donor	Host	Physiological change	References
1	*ZmVPP1*	*Arabidopsis thaliana* L. (Arabidopsis)	Maize	Enhanced photosynthetic efficiency and root development	[81, 107]
2	*ZmPP2C-A10*	Arabidopsis	Maize	ABA signaling	[108]
3	*Zeaxanthin epoxidase (ZEP)*	Arabidopsis	Maize	Improved ABA biosynthesis	[104, 109]
4	Mannitol-1-phosphate dehydrogenase (*mtlD*)	*Escherichia coli*	Wheat	Improved fresh and dry weights, plant height, and flag leaf length	[110]
5	*AtNF-YB1*	Arabidopsis	Maize	Higher photosynthesis capacity	[102, 111]
6	*AtABF3*	Arabidopsis	Rice	Higher Fv/fm	[112]
7	*OsDREB1A*	Arabidopsis	Rice, wheat	Shoot growth retardation at the expense of root growth	[102]
8	*AtHARDY*	Arabidopsis	Rice	Enhanced WUE and photosynthesis efficiency	[113]
9	*OsWRKY11*	Rice	Rice	Sluggish water loss and lessened leaf wilting	[114]
10	*AtLOS5 (LOS5/ABA3)*	Arabidopsis	Maize	Increased ABA biosynthesis	[115]
11	*HVA1*	Barley	Rice	Higher WUE	[116]
12	*HVA1*	Barley	Wheat	Enhanced biomass accumulation and WUE	[26, 117]
13	*Beta*	Escherichia coli	Wheat	Accumulation of glycine betaine	[118]
14	*Nicotiana protein kinase (NPK1)*	Tobacco	Maize	Preventing dehydration damage to the photosynthesis machinery	[119]
15	*AtSNAC1*	Arabidopsis	Rice	ABA-hypersensitive, stomatal shutdown	[120, 121]
16	*SbER2–1*	Sorghum	Maize	Increased Pn rate and higher WUE	[122]
17	Light harvest complex related genes (*LHCA1, LHCB, LHCA3, LHCA2*)	Maize	Maize	Balancing light capture in the PSII	[91, 93]

Table 2.
Examples of drought tolerance conferring genes that have been functionally validated in cereal crops.

as peripheral antenna systems enabling more efficient absorption of light energy [125, 126]. Further, Lhch5-1 is involved in the intracellular non-photochemical quenching and the cysteine biosynthesis processes [91]. Previously, the photosynthesis pathway has been significantly enriched in drought stress response in maize, with chlorophyll a-b binding proteins being up-regulated in an ABA-dependent manner [87, 91], and pearl millet [127]. This photosynthesis related pathway plays a critical role in balancing light capture and utilization to avoid photoinhibition of the PSII [87].

Phenylpropanoid metabolism is the first step of the secondary metabolites (flavonoids, phenylpropanoids, phenolic compounds and lignin) biosynthesis and phenylpropanoids act as antioxidants to protect plants against oxidative damage [128, 129]. Flavonoids play different molecular functions in plants, including stress protection. All these compounds are widely synthetized in response to several abiotic stresses, including drought [130]. In wheat leaves, an increase in flavonoid and phenolic acids content was shown together with stimulation of genes involved in flavonoid biosynthesis pathway in response to drought stress [131]. Moreover, secondary metabolites biosynthesis related pathways were found to be significantly enriched in response to drought stress in maize [80] and sorghum [37], suggesting their involvement in plant protection. A coordinated reaction of the genes and pathways involved in secondary metabolite biosynthesis is therefore vital for improved drought stress tolerance in plants [80]. Plant hormone signaling pathway participates in drought stress response via either ubiquitin-mediated proteolysis or ABA-mediated response [132], and was observed to be significantly enriched in pearl millet response to drought stress [127].

Sucrose is widely acknowledged to play a crucial role as a key molecule in energy transduction and as a regulator of cellular metabolism [130]. Additionally, sucrose and other sugars are energy and carbon sources required for defense response and are necessary for plant survival under drought stress conditions [133]. Further, sucrose can act as primary messenger controlling the expression of several sugar metabolism related genes [134]. Nitrogen metabolism pathway, being the most basic and central physiological metabolic process during plants' growth period, directly influences the formation of cellular components and regulation of cellular activities, as well as the transformation of photosynthetic products, mineral nutrient absorption and protein synthesis. It therefore follows that the nitrogen pathway is directly affected by drought, hence its significant enrichment under such conditions [80, 127]. These key identified drought responsive pathways should be used as targets for future genetic engineering of drought stress tolerant genotypes.

6. QTL mapping for drought tolerance in cereals

Most yield-related traits in cereal crops are quantitative. Therefore, cloning of the causal genes and deciphering the underlying mechanisms influencing these traits remains critical for continuous genetic improvement [135]. Precisely, drought tolerance is a complex quantitative trait that is multi-genic in its expression and one of the most challenging traits to study and characterize [11]. In comparison to conventional approaches, genomics offers unparalleled opportunities for dissecting quantitative traits into their single genetic determinants, known as QTL, thereby facilitating MAS, gene cloning and their direct manipulation via genetic engineering [12]. Through advances in next generation sequencing, identification of major QTLs regulating specific drought responses has been made possible, via the development of large numbers of genetic markers such as single nucleotide polymorphisms (SNPs) and insertion-deletions (InDels), thereby opening the doors for an efficient way to improving drought tolerance in cereal crops [89]. Additionally, large-scale genome-wide association studies (GWAS) have been conducted to detect genomic regions and candidate genes for various agronomic traits, including drought tolerance in cereals [13, 136, 137]. Resultantly, hundreds of studies reporting thousands of major drought-responsive genes and QTLs in cereal grain crops can be found in the literature, including those for maize [13, 138–140], rice [12, 13, 105, 138, 141, 142], wheat [13, 31, 137, 138, 143], sorghum [138, 144, 145], barley [138, 146], and pearl millet [136, 147, 148]. The high number of studies on QTL mapping suggests that for the past decade, QTL has been the focal target of research to identify the genetic loci regulating the adaptive response of crops to drought stress. Although several QTLs for drought tolerance have already been mapped in these cereal crops, there has been little success in introgression of those QTLs and the number of causal genes that have been confirmed within these QTL regions remains relatively small as compared to Arabidopsis and rice [11, 106].

Going forward, MAS remains a useful tool for major QTL, whereas QTL cloning is increasingly becoming a more routine activity. This has been necessitated by increased use of high-throughput sequencing, precise phenotyping and identification of appropriate candidate genes through omics approaches [89, 136, 137]. Cloned QTL facilitate a more targeted search for novel alleles and will offer novel insights for genetic engineering of drought resilient cereal crops [13]. Moreover, compared to other crops, research in millets is still lagging behind. However, with millets considered predominantly climate resilient crops, millets could serve as valuable source of novel genes, alleles and QTLs for drought tolerance. Therefore, the identification and functional characterization of these genes, alleles and QTLs in millets is critical for their introgression and drought tolerance improvement in cereal grain crops [89].

7. Phenotyping for drought tolerance in cereal grain crops

Phenotyping has become an integral component of the crop improvement programme by contributing towards understanding of the genetics behind crop drought tolerance [105, 149]. Since many component traits of drought tolerance are controlled quantitatively, improving the accuracy of phenotyping has become more important to improve the heritability of the traits, and the target traits would require rapid and precise measurement [106]. High throughput phenotyping now provides an essential link in translating laboratory research to the field. This is vital in developing novel genotypes that incorporate gene(s) expressing promising trait (s) into breeding lines adapted to target field environments [150].

Auspiciously, the recent advances in phenotyping technology and robotics for measuring large number of plants means that large numbers of genotypes could be readily phenotyped [34, 151–153]. More promising approaches that target complex traits tailored to specific requirements at the different main crop growth stages are now available [150]. Precise phenotyping of drought-related physiological traits often requires the utilization of sophisticated and expensive techniques. These include magnetic resonance imaging (MRI) and positron emission tomography (PET), near-infrared (NIR) spectroscopy on agricultural harvesters, canopy spectral reflectance (SR) and infrared thermography (IRT), nuclear magnetic resonance, hyperspectral imaging, laser imaging, 3D imaging and geographical information systems (GIS), among others [34, 138, 154, 155]. For example, 3D visual modeling can be used to determine the plasticity of the canopy architecture, and to evaluate the architectural and physiological characteristics that contribute to the higher productivity of the super rice varieties under drought stress conditions [156]. Though currently expensive, up scaling the use of these phenotyping platforms will eventually enhance our understanding of crop growth kinetics and aid us improve crop models for systems biology and drought tolerance breeding programs.

Selection of primary (grain yield and yield contributing traits) and secondary agronomically important traits (ASI, root architecture, stay green, etc.) is the way to achieve drought tolerance in cereal grain crops [31, 35, 106, 152]. Yield and yield attributing factors are targeted for direct selection whilst secondary traits are vital in conferring drought tolerance and contributing to final yield indirectly [157]. Crucially, considering that under drought stress conditions, the genetic correlation between grain yield and some secondary traits increases meaningfully, and the heritability of some secondary traits remains high, identification and selection of those highly heritable secondary traits that are positively correlated with yield related traits in the target environment, and responsive to high throughput phenotyping, will be critical in achieving the desired drought tolerance goals [11, 152, 158]. Managed stress screening approaches through the utilization of phenomics offers an opportunity to keep heritability high and phenotyping under controlled environments can be helpful in large-scale characterization studies such as trait mapping experiments [13]. However, great caution needs to be taken when phenotyping for drought stress tolerance since controlled environments may fail to mimic the real field conditions, thereby becoming less useful to study the genotype × environment interactions which are very essential to dissect the drought tolerance mechanisms [106].

8. Genetic engineering of drought tolerant cereal crops

The development of tolerant crops by genetic engineering requires the identification of key genetic determinants underlying stress tolerance in plants, and introducing these genes into crops [159, 160]. The momentous progress garnered in abiotic stress response research in the model plant Arabidopsis has created an avenue for the identification of drought tolerance conferring genes and the development of transgenics carrying these genes in other crop species. Therefore, genetic engineering approach has opened the door to the development of new crop genotypes with improved drought tolerance [103]. Over the past decade, numerous articles and reviews on drought stress tolerant transgenic crop species have been gathered [4, 26, 102–106, 160]. A selected list of transgenic cereal grain crops, which includes information on transgenes used for the transformations and the resultant drought stress tolerance mechanisms, has already been provided above in **Table 2.** Despite the availability of numerous reports in the scientific literature of transgenic approaches to improving drought tolerance, restrictions on the establishment of transgenic plants in the field presents a bottleneck in true testing of the effectiveness of transgenic approaches to improve crop drought tolerance [161].

In recent years, transgenesis has taken center stage in our crop improvement efforts. Advances in genome engineering has made it possible to precisely alter DNA sequences in living cells, providing unprecedented control over a plant's genetic material [162]. The genome engineering approaches, also known as gene editing or genome editing techniques, involve the use of programmable site-directed nucleases (SDNs) engineered to modify target genes at desirable locations on the genome [163]. These SDNs cleave the double-stranded DNA at a particular location by means of clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9 (CRISPR/Cas9), zinc finger nucleases (ZFNs), or transcriptional activator-like effector nucleases (TALENs). The double-stranded DNA break then undergoes natural reparation either via homologous recombination or non-homologous end joining [105, 164]. The restoration of the DNA break can be directed to create a variety of targeted DNA sequence modifications such as DNA deletions or insertions of large arrays of transgenes [162].

Among the several genome editing methods developed to date, CRISPR-Cas9 is the most advanced and has received much attention because of its great accuracy, quickness, adaptability and simplicity [165, 166]. This technique has been successfully used in major food crops [167–169]. Whereas convectional genomics and breeding approaches alone cannot resolve the global food security challenge [170], genetic engineering approaches have great potential to improve crops that feed the burgeoning populations of developing countries [162, 166, 171]. However, the extent of regulation imposed upon crop cultivars generated through genetic engineering will have a huge impact on the cost of their development itself and how rapidly they will be deployed into the food supply chain. Linked to that, the readiness with which the public will accept food products made from genetically engineered crops will also play a role in the extent to which this new technology will be fully utilized for crop improvement, particularly in the developing countries where cereal grain crops are staple diets [162].

9. Field management of crops in the context of climate change

Genetic and management strategies that are aimed at improving grain yields under water constrained environments target three variables, which are the amount of water captured by the plant (W), the efficiency with which that water is converted to biomass (water use efficiency, WUE), and the harvest index (HI) or the proportion of biomass forming grain. Interestingly, each of these variables can be altered [172]. For instance, WUE can be maximized by early planting of crops, and by maintaining healthy leaves with high levels of nutrients [173]. Additionally, extending leaves` longevity through selection for delayed leaf senescence is commonly regarded important for maintaining WUE and root health, as well as increasing the kernel filling duration [172]. Supplementation of irrigation, where available, would be the major means for combating drought stress condition, besides being a prime approach to the intensification of agriculture and the generation of stable income for farmers [174]. However, its uptake will depend on various environmental, economic and social factors on both micro and macro levels [175].

Additionally, agronomic interventions, that is, improved crop management methods can complement the use of drought tolerant cultivars, contributing meaningfully to enhanced and stabilized yields under water constrained environments. Conservation agriculture (CA), a collection of practices embodying the use of reduced tillage and mulch to reduce evaporation of soil water, is an obvious means of increasing water available to the plants [172, 176]. Scaling up CA, which has recently gained wider acceptance in developing countries, offers a great potential of increasing drought resilience and sustainability of cropping systems and ensuring food security. The use of plastic mulch in semi-arid cooler areas on the Loess Plateau of China has significantly increased WUE in maize [177]. Moreover, CA has become the cornerstone of dryland systems in some regions of USA, Canada and Australia [174]. Recently, the application of melatonin with date (*Phoenix dactylifera* L.) residue and wheat straw biochars has enhanced biochar efficiency for drought tolerance in maize cultivars [178]. This can be a game changer in CA farming systems where biochar is a key component for ground cover. Therefore, the management of a sustainable dryland farming system would require that farmers apply good agronomic practices (GAPs) such as improved soil and water conservation and the associated reduced tillage practices, holistic weed and pathogen control, soil fertility management with respect to water regimes, optimized plant population densities and effective control of soil biotic stress factors that may inhibit root development, as well as practicing crop diversification in order to reduce the risks associated with farming in unpredictable environments. Further, governments should increase investments in weather forecasting and cloud seeding.

All these approaches and decision support systems, when integrated, would birth a more holistic strategy for adapting cereal grain crops to future climate change induced drought stress as summarized in **Figure 1.**

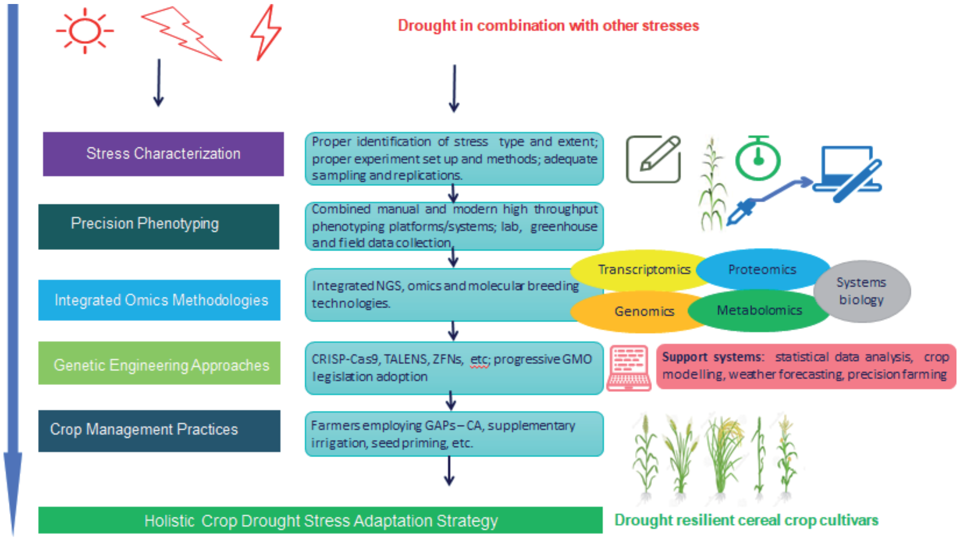

Figure 1.
Proposed holistic approach to cereal crop drought stress adaptation.

10. Future outlook

Currently, crop improvement efforts are shifting focus from solely yield, quality, or abiotic stress resistance to a holistic approach integrating breeding for a combination of these factors. For example, targeting breeding for combined drought and heat stress tolerance [179, 180], drought tolerance and nutritional quality [161] or drought tolerance and disease resistance [3, 181] are being underlined. Drought-tolerant micro-nutrient dense cereal crop cultivars have been developed [8]. In that regard, future-proofing of global food security would call for double-pronged transformation aimed at developing high yielding cultivars possessing both adaptability to abiotic or biotic stresses and higher nutritional quality. Thus, going forward, it is necessary that more breeding programs use high-priority abiotic stresses in their portfolios [11]. In the wake of addressing malnutrition challenges in developing countries, breeding for bio-fortified drought tolerant cultivars should be strengthened [8, 182]. In addition, promising genotypes in trials for fall armyworm (*Spodoptera frugiperda* L.) resistance, once approved, should be screened for drought tolerance and improved cultivars harboring both traits be developed. Though seemingly a daunting task, combined efforts from crop physiologists, molecular geneticists, breeders and pathologists would make this a reality.

In our 'omics research', future proteomics studies should intensify the identification, quantification and analysis of stress-responsive proteins PTMs, since PTMs can affect protein functions, interactions, subcellular targeting and stability [183]. Moreover, breeding for resource use efficiency, for instance, nitrogen use efficiency (NUE) should be integrated in future drought tolerance breeding programs. Previously, researchers have revealed that improving NUE in crops such as paddy rice and cotton will be critical in greenhouse gas emission management [184–188]. Additionally, application of biochar, either singly [189] or in combination with P [190] has been shown to alleviate heat-induced oxidative stress damage on the cellular physiological processes in rice plants. Thus, as we navigate the future, breeding for NUE and its proper management in the field remains essential in helping adapting crop plants to abiotic stresses such as drought.

Another area of focus will be crop physiology. Since photosynthesis is the basis of plant growth, improving photosynthesis can significantly contribute towards greater food security in the future. Multiple targets for manipulation of crop photosynthesis have been extensively reviewed. These include improving Rubisco kinetic properties and improving canopy architecture to enhance light penetration [191]. Therefore, harnessing the benefits of improved photosynthesis for greater yield potential will require that we intensify manipulation of these targets.

Furthermore, the microbes resident in the rhizosphere can potentially improve plant growth and enhance crop resilience to abiotic stresses [192]. For instance, phosphate-solubilizing bacteria can nullify the antagonistic effect of soil calcification on bioavailable phosphorus in alkaline soils, and thus, can be one of the best options for improving soil P nutrition [193]. It would be crucial to investigate, understand and quantify the complex feedback mechanisms occurring between root and microbial responses to drought stress, particularly in cereal crops. Integrating crop ecology, physiology and molecular methodologies in a multidisciplinary approach would be central [194].

In the short to medium term, exogenous application of plant growth regulators at different crop growth phases would be an important strategy in inducing drought resistance. In a very short term, seed priming will be of value [3]. The crucial roles of plant phytohormones and growth-promoting rhizobacteria in abiotic stress responses have been extensively reviewed [195–197]. Particularly, the exogenously applied plant growth regulators can enhance morpho-physiological, growth and abiotic stress responses of crops such as rice [198–203]. Recently, Saleem et al. [204] have shown that exogenously applied gibberrellic acid (GA3) can reduce metal toxicity induced oxidative stress in jute (*C. capsularis* L.) seedlings. Moreover, exogenous application of salicicic acid (SA) has been shown to ameliorate the adverse effects of salinity on maize plants [205]. Further, silicon (Si) application improved plant water relations, photosynthesis and drought tolerance in Kentucky bluegrass [206, 207], and Si enhanced germination, growth, P and arsenic uptake in rice [208]. Therefore, targeting the modification of hormone biosynthetic pathways may be a gateway to the development of drought tolerant transgenic plants.

Meanwhile, the potential of transgenic technologies across developing countries, particularly in Sub-Saharan Africa, is being hampered by uncoordinated over-regulation by authorities. Unfortunately, the biosafety framework leading to ordered testing and deregulation in such countries is being developed on individual countries basis, instead of a more efficient resource-use regional approach. Moreso, present systems are modeled on overestimated, not science-evidence-based risks [172]. There is need for policy makers to revisit their stance on genetically modified organisms (GMOs) with science guaranteed evidence, not political grand-standing, guiding formulation, adoption and utilization of GMO related polices for food and nutritional security.

11. Conclusion

Though a daunting task, breeding for drought tolerance in cereal crops remains the most economical, effective and sustainable strategy for ensuring food security for the ever increasing human population. While a significant progress has been made to date towards achieving that goal, our understanding of the mechanisms underpinning plant drought stress tolerance remains fragmentary. In the face of global climate change, a multi-disciplinary research strategy becomes obligatory to integrate physiological, genotypic, omics and epigenetics data essential to dissect the complex networks regulating plant drought tolerance, which can then be manipulated through genetic engineering to develop drought resilient crops. Additionally, cereal crop breeding programs should integrate high-priority abiotic stresses, particularly drought and heat, with other goals such as resource use efficiency, biotic resistance, and nutritional improvement. Further, formulation and effective deployment of efficient screening and precise phenotyping approaches at both laboratory and field levels to identify drought tolerant genotypes or mutants remains critical. Promisingly, our new cultivar development thrusts are now endowed with some innovative methodologies that include high-throughput phenotyping, doubled haploidy, mutation and speed breeding, as well as CRISP-Cas 9 technologies. Going forward, we should harness the potential of these technologies. Eventually, our understanding of the crop drought tolerance mechanisms will be quickened and broadened, greatly assisting our development of new drought-resilient cereal crop cultivars. This should be supported by robust, science-evidence-based and progressive policy frameworks that recognize the centrality of GMOs and modern biotechnology in increasing food production. Consequently, this would lead to improved sustainable crop productivity and global food security.

Acknowledgements

We acknowledge the funding received from the National Key Research and Development Project of China (Selection and Efficient Combination Model of Wheat and Maize Water Saving, High Yield and High Quality Varieties; Grant No. 2017YFD0300901).

Conflict of interest

Authors declare that they have no conflict of interests.

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Chapter 15

Abiotic Stress-Induced Molecular and Physiological Changes and Adaptive Mechanisms in Plants

Sivaji Mathivanan

Abstract

Abiotic stress is the primary cause of crop loss worldwide, reducing average yields for most major crop plants by more than 50%. Among abiotic stress, drought, salinity, high temperature, and cold are major adverse environmental factors that limit the crop production and productivity by inhibiting the genetic potential of the plant. So, it leads to complete change of morphological, physiological, biochemical, and molecular behavior of the plants and modifies regular metabolism of life, thereby adversely affecting plant productivity. Major effects of the drought, salinity, extreme temperatures, and cold stress are often interconnected and form similar cellular damage. To adopt plants with various abiotic stresses, plants can initiate a number of molecular, cellular, and physiological changes in its system. Sensors are molecules that perceive the initial stress signal from the outside of the plant system and initiate a signaling cascade to transmit the signal and activate nuclear transcription factors to induce the expression of specific sets of genes. Understanding this molecular and physiological basis of plant responses produced because of abiotic stress will help in molecular and modern breeding applications toward developing improved stress-tolerant crops. This review presents an overview and implications of physiological and molecular aspects of main abiotic stress, i.e., drought, heat, salt, and cold. Potential strategies to improve abiotic tolerance in crops are discussed.

Keywords: abiotic stress, signal transduction, stress-inducible genes, gene expression, mitigation process, genetic engineering and genome editing

1. Introduction

Plants live in constantly changing environments that are often unfavorable or stressful for growth and development. These unfavorable environmental conditions for plant growth are drought or water stress, high temperature or heat stress, low temperature or cold stress, excessive salt or salinity stress, and heavy metals toxicity like aluminum, arsenate, and cadmium in the soil. These adverse abiotic stresses are major threat that limits agriculture production and productivity, thereby creating great food insecurity. In the near future, it is predicted that because of climate change, abiotic stresses may become more intense and frequent. Drought and salinity are becoming drastically increased in many regions and may cause serious salinization of more than 50% of all arable lands by the year 2050. Consequently, because of rising temperatures and frequent flooding events for several decades, fertile agricultural land and crop yields may decrease rapidly, especially in the mid-latitudes [1, 2]. In addition to these factors, anthropogenic activities may lead to an increased abundance of soil, water, and air pollutants, factors that plants must cope with. Moderate estimates propose that more than 90% of the land in rural areas is affected by abiotic stress factors at some point during the growing season [3]. On the other hand, the population explosion has resulted in a higher demand for food and other natural resources.

Thus, understanding stress responses is essential when attempting to develop stress-resistant cultivars that can withstand abiotic stressors and in order to feed the growing population. Plants, which undergo various abiotic stresses, sense the enormous stress signal in order to respond to the stress condition. The primary signal caused by drought is hyperosmotic stress, which is often referred to simply as osmotic stress, and salt stress has both osmotic and ion toxicity effects in cells. The secondary effects of drought and salt stresses are complex and include oxidative stress; damage to cellular components such as membrane lipids, proteins, and nucleic acids; and metabolic dysfunction. Thus, drought and salt have unique and overlapping signals. Salt and drought stress disrupts homeostasis in water potential (osmotic homeostasis) and ion distribution (ionic homeostasis). This disruption of homeostasis occurs at both the cellular and the whole plant levels. Drastic changes in ion and water homeostasis lead to molecular damage, growth arrest, and even death. While some cellular responses are induced from primary stress signals, others arise mainly from secondary signals. An important feature of drought and salt stress is that the hyperosmotic signal causes the accumulation of the phytohormone abscisic acid (ABA), which in turn elicits many adaptive responses in plants [4]. Cold or chilling stress affects plant growth and development, by changing the cell structure. First, symptom of cold stress is changing the cell membrane structure in plants; this event initiates primary cold stress responses in plants [5]. Second, chilling stress disturbs the stability of proteins or protein complexes and reduces the activities of enzymes such as ROS scavenging enzymes. These processes result in photo-inhibition and impaired photosynthesis, as well as considerable membrane damage [6, 7]. Third, chilling stress affects gene expression and protein synthesis, as it favors the formation of secondary structures in RNA [8].

To achieve stress tolerance, three interconnected aspects of plant activities are important. First, damage must be prevented or alleviated. Second, homeostatic conditions must be reestablished in the new, stressful environment. Third, growth must resume, even though at a reduced rate [9]. Significant progress has been made in understanding the physiological, cellular, and molecular mechanisms of plant responses to environmental stress factors [10]. The detection of a stressful condition results in variations in gene expression, causing changes in the composition of plant transcriptome, proteome, and metabolome. Responses to stress are not linear pathways, but are complicated integrated circuits involving multiple pathways and specific cellular compartments, tissues, and the interaction of additional cofactors and/or signaling molecules to coordinate a specified response to a given stimulus [11]. With advancement of omic technologies, i.e., genomics, transcriptomics, proteomics, and metabolomics, now it is possible to analyze and identify the most complicated interlink between various stress response, signal transduction, gene expression, and metabolites production in plants with respective to the abiotic stress [12, 13].

2. Crop plants and abiotic stresses

Generally, many stress factors act at same time, such as the frequently combined, heat, water, and high-light stress [14]. Abiotic stress changes the expression pattern of the various genes in crop plants. So, this modification affects the regular function of plant metabolism, and source-sink relationship in turn reduces the growth, production, and productivity.

2.1 Drought

Distribution of rainfall is uneven due to the change in climate, which acts as an important stress as drought. Drought is the main abiotic stressor around the world and drastically reduces grain yields. It devastatingly influences the capability to meet the food demands of an ever-increasing global population. Drought stress is associated with water deficit and cellular dehydration. Plant adaptation to drought is a trait involving morphological, physiological, and biochemical changes. Plants reduce their growth of shoots under drought conditions and reduce their metabolic demands. Reduction in yield by as much as 40% was observed for maize and 21% for wheat at approximately a 40% water reduction [15]. In the case of cowpea, yield reduction can vary between 34 and 68% depending on the developmental timing of the drought stress [16].

2.2 Heat or higher temperature

Plants are more sensitive to the temperature conditions; in extreme cases, the unfavorable temperature condition leads to plant death. Normally plant growth and function would be better at optimum temperature level; both conditions below and higher temperature than optimum temperature severely affect the plant growth and production. The rate of most biochemical, enzymatic reactions rises two-fold for every 10°C increase between 20 and 30°C. Temperatures outside this range reduce the reaction rate because enzymes become either inactivated gradually or denatured.

Change of a few degrees considerably affects the plants' growth and development, especially reproduction. Abiotic stresses, specifically high and low temperatures, have a harmful effect on the early stage of male gametophyte in several agricultural crops such as rice, wheat, maize, barley, sorghum, and chickpea [17]. Male sterility and abnormalities in the spikelets' production were induced by heat stress in rice and wheat [18]. In both wheat and rice, heat and cold stresses caused tapetum degradation, microspore callose wall and exine formation, and changes in carbohydrate metabolism, eventually resulting in male sterility [19]. By contrast, temperature stress has no negative effect on female gametophyte development [20].

2.3 Salt

Soil salinization is a major threat to agriculture in arid and semi-arid regions, where water scarcity and inadequate drainage of irrigated lands severely reduce crop yield. More than 6% of the world's total land area and out of 230 M ha of irrigated land 45 M ha (19.5%) is already affected by salt [21]. Salt accumulation inhibits plant growth and reduces the ability to uptake water and nutrients, leading to osmotic or water-deficit stress. Salt stress tolerance level varies from one species to another. For cereal crops, barley (*Hordeum vulgare*), the most tolerant cereal, can tolerate up to 250 mM NaCl (equivalent to 50% seawater) and bread wheat is a moderately salt-tolerant crop, whereas rice, durum wheat (*Triticum turgidum* ssp.), maize (*Zea mays*), and sorghum (*Sorghum bicolor*) are less tolerant to salinity [22]. The reduction in plant growth following salt exposure is due to two phases, osmotic stress and ionic toxicity [23].

2.4 Cold

Cold stress has proved to be the main abiotic stress that decreases productivity of agricultural crops by affecting the quality of crops and their postharvest life. Cold stress, including chilling (0–15°C) and freezing (<0°C), is an abiotic stress that adversely affects the growth and agricultural productivity of plants [24, 25]. Freezing stress is highly detrimental to plants when compared with chilling stress. Usually, freezing damage will start with formation of ice nucleation in between the cell, then slowly grow and form ice crystals, and induce water leakage, leading to cell dehydration [26, 27]. However, many important crops are still incompetent to the process of cold acclimation. Rice (*Oryza sativa*), maize (*Zea mays*), tomato (*Solanum lycopersicum*), soybean (*Glycine max*), and cotton (*Gossypium hirsutum*) lack the ability to acclimate to cold temperatures and can only grow in tropical or subtropical regions [28]. Thus, cold stress adversely affects plant growth and development, limits the geographical distribution of plant species, and decreases crop yields worldwide [26].

3. Stress and crops

Stress refers to any substance or stimulus that restricts plant metabolism, growth, development, and crop productivity, including biotic and abiotic stresses [29]. Once this threshold is surpassed, an organism is stressed and mechanisms are activated at molecular, biochemical, physiological, and morphological levels. The activation of the mechanisms can result in the establishment of a new physiological state and homeostasis is reestablished [30]. Stress-related alterations in plant development, growth, and productivity reduce yield and cause unacceptable economic losses in agriculture. It has been assessed that abiotic stresses may reduce up to 70% of crop production of many economically important crops and perform at only 30% of their genetic potential with respect to yield [31].

4. Abiotic stress sensing and responding mechanism in plants

Sensors are biological molecules that recognize the adverse environmental modification and evoke the immediate response to the particular environment change by initiating the signal molecules in the system. Drought, salt, and cold stresses are inducing more amount of Ca^{2+} entry into the cell cytoplasm from internal stores or apoplastic source. Passages controlling Ca^{2+} entry are considered as one type of sensor for the stress signals [32–34]. Other than Ca^{2+}, ROS and nitric oxide (NO) are other messenger molecules involved in inducing plant response to cold stress. Reactive oxygen species (ROS) like superoxide ($O_2^{\cdot -}$), hydroxyl radicals (OH^-), and hydrogen peroxide (H_2O_2) are produced in plants in order to face various stresses [35]. Receptor-like kinases (RLKs) have an extracellular domain in which ligand is binding or protein-protein interaction will occur, a transmembrane domain, and an intracellular kinase domain. When the ligand or signal binds extracellular domain, histidine residue present in the intracellular kinase domain is auto-phosphorylated and the phosphoryl moiety is received by aspartate receiver part of the sensor protein or a separate protein. Then, the activated sensor protein(s) may induce cellular responses specific to signal through the mitogen-activated protein kinase (MAPK) cascade or directly phosphorylate specific targets. Intracellular signaling mode, i.e., protein

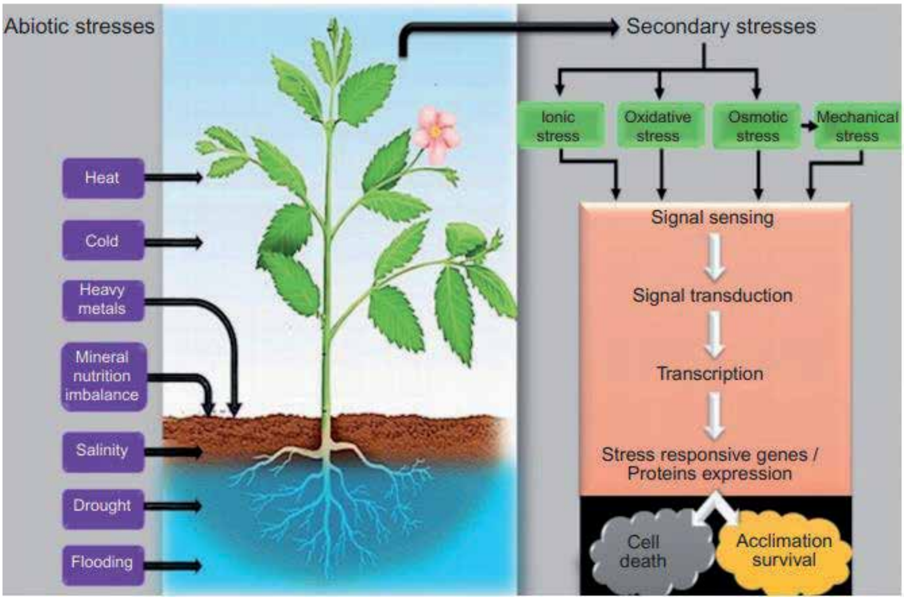

Figure 1.
Plant responses to abiotic stress.

phosphorylation and dephosphorylation regulate a wide range of cellular processes such as enzyme activation, assembly of macromolecules, protein localization, and degradation [36]. Upon sensing of abiotic stress by plants, signaling cascades are induced that activate ion channels, kinase cascades, assembly of reactive oxygen species (ROS), and accumulation of plant hormones leads to induce expression of specific subsets of genes that responsible to combat the abiotic stress (**Figure 1**) [37].

5. Similar and variable features of drought, salt, and cold stress

All the three stresses such as drought, salt, and cold stresses cause a primary loss of cell water, which leads to decrease in cell osmotic potential but the reason of cell water loss varies among stresses: (i) the decrease of the cell water content under drought stress is due to water shortage in soil or/and in the atmosphere. (ii) In salt stress, osmotic or water potential of surrounding root zone is decreased by Na^+ and Cl^- solutes, which in turn create more difficulty in uptake by roots and water translocation to metabolically active cells; (iii) osmotic stress is created in cold stress mainly because of inability to transport the water available from the soil to the living cell of leaf mesophyll. This condition is called as physiological drought. Anyhow, water loss in cell increases abscisic acid (ABA) biosynthesis, and it is well-known fact that it is involved in activation of various drought, salt, and cold stresses, responsive genes in plant system [38]. In plant system losing of cell water and increasing of solute concentration (especially Na^+) by these three stress cause lower osmatic potential and it creates harmful effects to the protein and enzymes. This effect can be avoided by producing more amounts of low molecular osmolytes (carbohydrates [39], betaine [40], and proline [41]) that can counteract cellular dehydration and turgor loss [42]. Production of low molecular osmolytes in higher quantity is a common stress alleviating process for drought, cold, and salt stress. While main cause of drought stress is osmotic, Na^+, ion toxicity, for salt stress, and physiological drought for cold stress, all stresses have an influence on most biochemical reactions such as photosynthesis, carbon metabolism reactions, and enzyme activities.

6. Gene expression and regulation under abiotic stress

Expression of a variety of genes in plants is induced by environmental stresses such as drought, high salinity, and low temperature. Upon expression, various proteins are produced in various parts of the plants, which not only protect the cell but also initiate humpty number of genes which are responsible for inducing various abiotic resistance mechanisms in plants. Different types of proteins, i.e., chaperones or late embryogenesis abundant (LEA) proteins are produced mainly involved to create tolerance, whereas stress-responsive genes are all involved in to generate stress response [43]. The regulation of stress-responsive plant genes at three levels: transcriptional, posttranscriptional, and posttranslational.

6.1 Gene regulation at transcriptional level

Transcriptional regulation involves (i) chromatin and its alteration and remodeling; (ii) cis-regulatory elements such as enhancers and promoters, which are often binding sites, located upstream and downstream the coding region; and trans-regulatory elements, usually transcription factors. Different environmental stresses create altered methylation pattern of DNA and changing histones protein in order to suppress or increase the transcription of the gene.

Promoters are specific sequences involved in regulatory function, where they bind RNA polymerase and different transcription factors to start the transcription [44]. Dehydration-responsive element-binding (DREB) or C-repeat binding factor (CBF), MYB, basic-leucine zipper (bZIP), and zinc-finger families are some of the trans-regulatory elements involved in the regulation of plant defense and stress-responsive genes upon binding in cis element of the respective gene promoters [45]. Overexpression of the *Oryza sativa* WRKY11 (transcription factor or trans-regulatory elements) under the control of heat shock protein 101 (HSP101) promoter led to enhanced drought tolerance [46, 47]. The important discovery of a novel *cis*-acting element, C-repeat/dehydration response element (CRT/DRE), is responsive to drought, cold, and high-salt stress [48]. Since this discovery, CBF proteins have been isolated sequentially by screening for DNA-binding proteins that bind to the CRT/DRE motif [49, 50]. *Arabidopsis* contains three cold-induced *CBF* genes, *CBF1–3* (*CBF1/DREB1B*, *CBF2/DREB1C*, and *CBF3/DREB1A*), which are arranged in tandem on chromosome IV. CBF 1–3 are APETALA2/ETHYLENE-RESPONSIVE (AP2/ERF1)-type transcription factors that directly bind to the conserved CRT/DRE motifs in the promoters of *COR* genes (known as CBF regulons) and activate their expression under cold conditions [50–52]. Transgenic *Arabidopsis* plants overexpressing *CBF1* display increased *COR* expression and enhanced freezing tolerance [53]. *CBF* orthologs have been isolated in many plant species, including rice, tomato, wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), and maize [54]. Heterologous expression of *Arabidopsis CBFs* enhances freezing tolerance in various species, and heterologous expression of *CBFs* from other plant species enhances freezing tolerance in *Arabidopsis* [55–57]. Cold-sensitive tomato (*Lycopersicon esculentum*) become freezing tolerant upon overexpression of its own *CBF* genes, i.e., *LeCBF1*; however, overexpression of cold-tolerant *Arabidopsis CBF3* in tomato plants do not exhibit freezing tolerance; this proves that there are different CBF regulons in tomato and *Arabidopsis* [56]. It also indicates that the biological function of *CBF1–3* in modulating freezing tolerance is not only highly conserved among plants but also species specific.

6.2 Gene regulation at posttranscriptional level

Regulation that occurs in the stage of pre-mRNA till translation of mRNA is called posttranscriptional gene regulation. It occurs in four stages: (i) pre-messenger (mRNA) processing (capping, splicing, and polyadenylation), (ii) mRNA nucleocytoplasmic trafficking, (iii) mRNA turnover and stability, and (iv) mRNA translation [58]. One more strategy, i.e., alternative splicing (AS) is regulating the gene under cold and heat stress. STABILIZED1 (STA1), a gene coding for a nuclear pre-mRNA, is one of the best examples for alternate splicing factor which is involved in cold stress resistance in *A. thaliana* [28, 59]. Posttranscriptional regulation also is important for *COR* gene function. *REGULATOR OF CBF GENE EXPRESSION1* (*RCF1*), encoding a DEAD-box RNA helicase, helps ensure the proper pre-mRNA splicing of many *COR* genes under cold stress [60]. *STABILIZED1* (*STA1*) encodes a pre-mRNA splicing factor that controls the pre-mRNA splicing and mRNA turnover of *COR* genes [59]. Genome-wide AS profiling analysis revealed that hundreds of genes such as *RCF1* and *STA1* that have highly altered AS in the first few hours of cold treatment. This study showed that plant using AS pathway to change the gene expression in order to respond the temperature stress [61, 62]. Small RNAs (20–25 nucleotides) are processed from noncoding double-stranded RNA precursors by RNAses of the DICER-LIKE (DCL) family and mediate a series of gene silencing mechanisms at posttranscriptional level. One of these mechanisms cleaves mRNAs or prevents their translation through the mediation of 21 nucleotide microRNAs [63–65].

6.3 Posttranslational level regulation

Phosphorylation, sumoylation, and ubiquitination of proteins are posttranslational-level processes that play vital roles in the changing of plant response to various abiotic stresses. Mitogen-activated protein kinases (MAPKs) and SNF-1-relatedprotein kinases (SnRKs) are formed numerous signal transduction cascades, induced by dehydration and osmotic stress through the phosphorylation of specificresidues [66]. XERICO controls the level of ABA by enhancing the transcription of the key ABA biosynthetic gene AtNCED3. SnRK2 proteins and XERICO gene, encoding a H2-type zinc-finger E3 ubiquitin ligase, are involved in ABA-dependent responses to water deficit, like stomata closure [67–69].

Posttranslational histone modifications, along with DNA methylation, are associated with gene expression levels in response to cold stress. Histone acetylation/deacetylation catalyzed by histone acetyltransferases (HATs) and histone deacetylases (HDAs) plays a role in cold responses in plants [70]. *Arabidopsis HISTONE DEACETYLASE6* (*HDA6*) is upregulated by cold stress and positively regulates freezing tolerance [71]. *HDAs* also are upregulated by cold stress in maize, leading to global deacetylation at H3 and H4. Under cold stress, HDAs appear to directly activate maize *DREB1* (*ZmDREB1*) expression and histone hyperacetylation. Histone acetylation of *OsDREB1b* in rice and *ZmDREB1A* and *ZmCOR413* in maize is induced by cold stress [72, 73]. RNA-DIRECTED METHYLATION4 (RDM4) protein was reported to function in RNA-directed DNA methylation (RdDM) by working with RNA polymerases Pol V and Pol II in *Arabidopsis* [74]. Under cold stress, RDM4 is important for Pol II occupancy at the promoters of *CBF2* and *CBF3* genes [75].

7. Important signal transduction pathway for drought cold and salt

Abiotic stresses such as cold, drought, and salt inducing signal transduction networks are divided into three types: (I) osmotic/oxidative stress signaling that uses MAPK modules to generate ROS scavenging enzymes, antioxidant compounds, and osmolytes; (II) Ca^{2+}-dependent signaling helps to activate late embryogenesis abundant (LEA)-type genes (such as the DRE/CRT class of genes), and (III) Ca^{2+}-dependent salt overlay sensitive (SOS) signaling that regulates ion homeostasis (**Figure 2**) [14, 76].

7.1 Oxidative or osmatic stress signaling

Formation of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, and hydroxyl radicals is common for all the stresses, particularly drought, heat, salt, cold, and oxidative stress. Reactive oxygen species which are produced by the environmental stresses cause major plant damage (oxidative stress) [77]. High amount of ROS acts as a signal, and synthesizing ROS scavengers is one of the protective mechanisms in plants. Osmotic stress stimulates many protein kinases; among them, one is mitogen-activated kinases, which are involved in

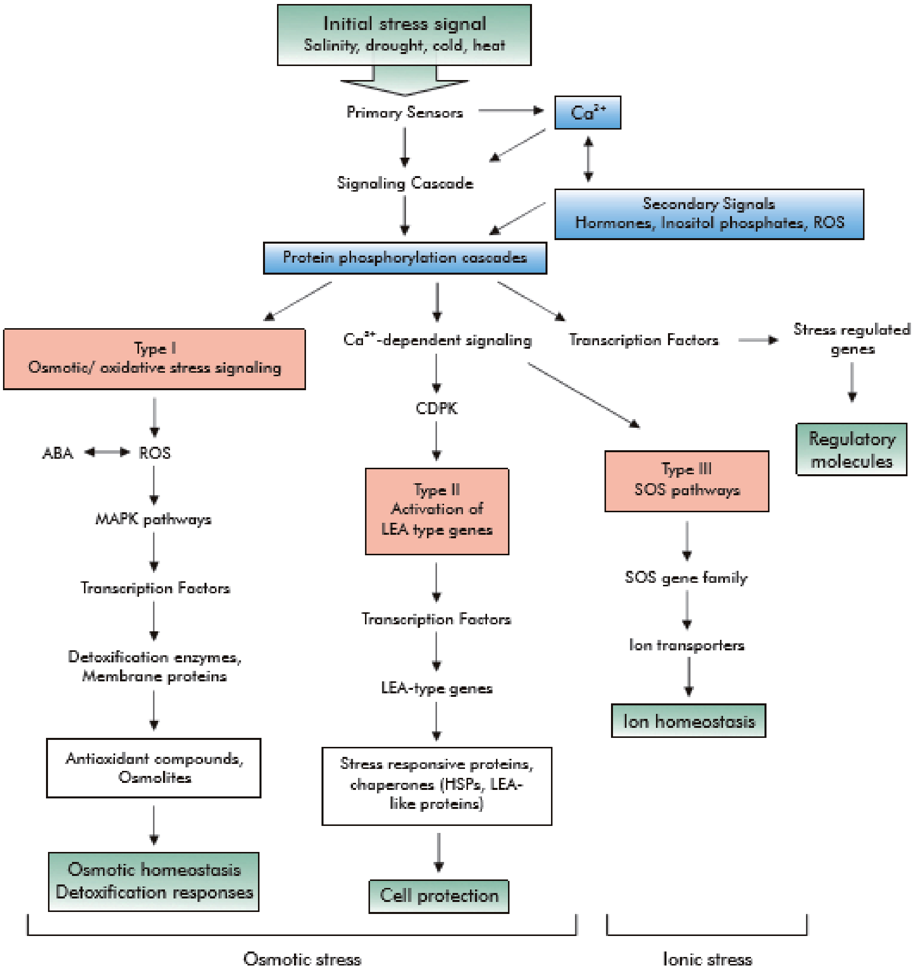

Figure 2.
Schematic pathway for the transduction of osmotic and ionic stress in plants.

reestablishing the osmotic homeostasis. Plant cells which undergo osmatic stress should produce more osmolytes in order to mitigate the negative effect of the ROS and maintain the osmotic homeostasis. So, the osmotic stress activate the receptors/sensors proteins such as protein tyrosine kinases, G-protein-coupled receptors, and two-component histidine kinases; this will trigger MAPK pathway and signal cascade, which is responsible for production of more amount of osmolytes that are necessary for osmotic adjustment. The main purpose of osmolytes is to maintain cell turgor, thus the driving gradient for water uptake. Compatible solutes such as amino acids (e.g., proline), quaternary amines (e.g., glycine betaine and dimethyl sulfoniopropionate), and polyol/sugars (e.g., mannitol and trehalose) will act as free radical scavengers or chemical chaperones by directly stabilizing membranes and proteins [78].

The MAP kinase pathways are intracellular signal modules that mediate signal transduction from the cell surface to the nucleus. The core MAPK cascades consist of three kinases that are activated sequentially by an upstream kinase. The MAP kinase kinase kinase (MAPKKK), upon activation, phosphorylates a MAP kinase kinase (MAPKK) on serine and threonine residues. This dual-specificity MAPKK in turn phosphorylates a MAP kinase (MAPK) on conserved tyrosine and threonine residues. The stimulated MAPK either travels to the nucleus to stimulate the transcription factor directly, or activates additional signal components to regulate gene expression, cytoskeleton-associated proteins or enzyme activities, or targets certain signal proteins for degradation [14].

7.2 Ca^{2+}-dependent signaling to activate late embryogenesis abundant (LEA) genes

Abiotic stress induces more Ca^{2+} entry into the cell cytoplasm; channels which control the Ca^{2+} entry act as a sensor for these abiotic stress signals. Ca^{2+} activates the calcium-dependent protein kinases (CDPKs), and CDPKs are serine/threonine protein kinases with a C-terminal calmodulin-like domain with four EF-hand motifs that can directly bind Ca^{2+}. CDPKs are encoded by multigene families, and the expression levels of these genes are spatially and temporally controlled throughout development. CDPK pathway is involved in production of high amount of anti-desiccation protection protein (LEA proteins) by activation of LEA-type genes including the dehydration-responsive element (DRE)/C-repeat (CRT) class of stress-responsive genes. Activation of LEA-type genes actually represents damage repair pathways but this is completely different from the pathways regulating osmolyte production [79, 80]. At the time of seed maturation, naturally it undergoes desiccation; to avoid desiccation shock during seed germination, seeds accumulate more transcripts and relatively high concentration proteins; for this reason, these proteins were named as late embryogenesis abundant (LEA) proteins [81]. Water deficit, high osmolarity, and low temperature stress induce the accumulation of an LEA protein in crop plants. Such proteins are used to prevent protein denaturation or renaturing unfolded proteins, realm protein structure and membrane integrity, and sequestering ions in stressed tissues. Many scientific reports suggest that LEA proteins and chaperones are involved in protecting macromolecules such as enzymes, lipids, and mRNAs from dehydration, and these proteins have been grouped into at least six families on the basis of sequence similarity [12, 82, 83]. LEA proteins are specialized in desiccation protection of membranes; and antioxidant enzymes and molecules are involved to achieve desiccation tolerance. Both osmolytes and LEA proteins work together in stabilization of membrane and protein structures by conferring preferential hydration at moderate desiccation and replacing water at extreme desiccation [84].

7.3 Ca^{2+}-dependent salt overlay sensitive (SOS) signaling

Calcium-dependent SOS signaling regulates ion homeostasis relatively specific to the salt stress. Ion transporters are main target for this type of signaling that controls ion homeostasis under salt stress. Excess extracellular or intracellular Na^+ acts as an input for the SOS pathway and mainly increases a cytoplasmic Ca^{2+} signal, and this signal changes expression and activity of transporters for ions such as Na^+, K^+, and H^+. The input for osmotic stress signaling is like a change in turgor. Salt stress signal transduction comprises of ionic and osmotic homeostasis signaling pathways, detoxification (e.g., damage control and repair) response pathways, and pathways for growth regulation [66]. This signaling pathway mediates salt induction of the SOS1 gene in *Arabidopsis*. In addition, the SOS2-SOS3 kinase directly phosphorylates and activates the SOS1 transporter [14, 85]. Studies comparing the growth of wild-type and mutant plants in response to NaCl and sequence analysis of the predicted SOS1 protein suggested that SOS1 encodes an Na^+/H^+ exchanger (antiporter) on the plasma membrane [86]. Because the SOS pathway operates during ionic stress, it is thought that homologs of SOS3 and SOS2 may also function in the transduction of other stress or hormonal signals [87]. Transient increases in cytosolic Ca^{2+} are perceived by various Ca^{2+}-binding proteins. In the case of abiotic stress signaling, evidence suggests that Ca^{2+}-dependent protein kinases (CDPKs) and the SOS3 family of Ca^{2+} sensors are major players in coupling this universal inorganic signal to specific protein phosphorylation cascades. It seems that calcium signaling is crucial for salt tolerance in plants [14].

Second messengers can control intracellular Ca^{2+} levels, often initiating a protein phosphorylation cascade that finally targets proteins directly involved in cellular protection or transcription factors controlling specific sets of stress-regulated genes. The products of stress-regulated genes like the plant hormones ABA, ethylene, and salicylic acid (SA) are mainly involved in the induction of stress-tolerant mechanism in plants. Salt and water-deficit stress participates in the production and activation of regulatory molecules, and to some extent, cold stress causes an increased biosynthesis and accumulation of ABA by activating genes coding for ABA biosynthetic enzymes, which can be quickly catabolized after the stress. Most of the abiotic stress-responsive genes are upregulated by ABA [88].

8. Managing abiotic stress by genetic engineering or genetic manipulation or genome editing

All over the world, abiotic stress significantly affects the production and yield potential of the crops and creates a major challenge for crop improvement sectors such as plant breeders and biotechnologists. Genetic manipulation of crops can be done to generate desirable character by so many ways. Among them, transgenic technology or genetic engineering and genome editing are the best strategies to develop abiotic resistant crops. Abiotic stress resistance in crops is possible by transgenic approach through boosting endogenous defense mechanisms by overexpressing of genes, which normally involves the synthesis of compatible osmolytes, antioxidants, polyamines, maintenance of hormone homeostasis, and modification of transporters and/or regulatory proteins, including transcription factors and alternative splicing events. Sometimes overexpression of some genes and thereby synthesizing of specific protein and metabolites will affect the normal metabolism and reducing the yield. Transgenic crop with abiotic tolerance but diminished yield potential and reduced growth is undesirable. So, it is important to analyze the functions of stress-inducible genes not only to understand the molecular mechanisms of stress tolerance and responses of higher plants but also to improve the stress tolerance without reducing the yield potential of crops by gene manipulation. Hundreds of genes are thought to be involved in abiotic stress responses [89].

8.1 Transgenic crop technology for abiotic tolerance

Transgenic crop or genetic engineering technology has ample opportunity in development of crops with specific objectives by overexpression of responsible genes or suppression of undesirable genes. Present engineering approaches rely on the transfer of one or several genes that are either involved in signaling and regulatory pathways or that encode enzymes present in pathways leading to the synthesis of functional and structural protectants, such as osmolytes and antioxidants, or that encode stress-tolerance-conferring proteins [78]. Phytohormones such as ABA are major targets for genetic manipulation to obtain abiotic stress-tolerant crops. Overexpression of ABA biosynthetic pathway-related TFs imparts an ABA-hypersensitive response and also improves the osmotic stress tolerance in transgenic plants [90, 91]. Under moderate drought stress, during the flowering period, the yields of transgenic canola overexpressing a farnesyltransferase protein were significantly higher comparatively to the control [92]. The overexpression of TFs that control root architecture induced drought tolerance in rice and transgenic *Arabidopsis* plants by promoting root growth and thus enhancing WUE [93, 94]. Other TFs linked to WUE, such as those stimulating wax deposition in cuticle and suberin deposition [95]. Many scientific researches revealed that glyoxalase pathway is involved in enhancing tolerance to abiotic stress; so, overexpression of glyoxalase I and glyoxalase II genes enhances the various abiotic stress tolerance in plants [96–103]. Transgenic rice with overexpression of choline oxidase (codA), D-pyrroline-5-corboxylate synthase (P5CS), LEA protein group 3 (HVA1), alcohol dehydrogenase (ADH), and pyruvate decarboxylase (PDC) genes have shown improved tolerance to abiotic stress [104, 105]. Usually rice does not accumulate glycine betaine but transgenic rice with overexpression of codA gene in the chloroplast and the cytosol accumulate more amount of glycine betaine, which recovered to normal growth at a faster rate give comparatively more yield under salt and cold stress [106]. Overexpression of *Escherichia coli* trehalose biosynthetic genes (otsA and otsB) with stress-inducible promoter enhances abiotic stress tolerance in rice [107–109]. Transgenic tobacco plants overexpressing chloroplastic Cu/Zn-SOD showed increased resistance to oxidative stress caused by high light and low temperatures. Transgenic tobacco plants expressing alfalfa aldose aldehyde reductase, a stress-activated enzyme, showed reduced damage when exposed to oxidative stress and increased tolerance to heavy metals, salt and dehydration stress. Targeting detoxification pathways are an appropriate approach for obtaining plants with multiple stress-tolerant traits [26, 110–113].

Water stress increases the formation of ROS through membrane perturbation of electron transport chains. The loss of catalase and gain of the glutathioneS-transferase/peroxidase functions in plants associate defenses against oxidative damage,which are more important in plant salt tolerance [79]. Loss of osmotic homeostasis is the important process in the abiotic stress, which affects the cell ion concentration; so, to achieve abiotic tolerance especially salt, plants should re-establish homeostasis (ionic and osmotic homeostasis) under stressful conditions. More amount of Na^+ within the cell inhibits enzyme activity and is harmful to the proteins; so, either compartmentalization of Na^+ in the vacuole or controlling of Na^+ accumulation in the cytoplasm is more important [9]. To revert ionic and osmotic homeostasis, many ion transporters act as terminal determinants. Protein AtNHX1 of *Arabidopsis thaliana*, one type of transporter, is located in cell vacuolar membranes involving transport of excessive Na^+ ions from cytoplasm to vacuole; thereby protecting the plant from the drying effect of salt. Overexpression of the AtNHX1 gene in transgenic tomato increased the salt tolerance and also produced good quality fruit containing less Na^+ ions because the plants store the sodium in the leaf vacuoles [114]. Plant salt tolerance genes include defenses against osmotic and oxidative stresses. The genetic analysis indicates that enzymes involved in osmolyte synthesis, osmoprotecting LEA proteins, and antioxidant enzymes such as catalases and glutathione S-transferase/peroxidase are important for plant salt tolerance [79].

8.2 Genome editing technology

Despite the benefits of commercial genetically engineered plants [115] and successfully addressing abiotic stresses, still this technology is not accepted unanimously because of the negative perception of the public; so, it limits the usage of this technique to develop the abiotic resistant crop varieties. The major concern in transgenic technology might be in many cases; the source of the gene to generate transgenic crop is taken from non-related organism, i.e., microorganism and non-related plant and animal; so, this issue can be addressed in a better manner by genome editing technology. In this technique, genetic modifications are accomplished by creating minor genome changes that are comparable with changes generated through mutation breeding (conventional crop improvement method) by using chemical and physical mutagenic agents. Mutational breeding produce changes in the genome at random manner; so, the success rate or chance of obtaining desirable genotype is quite low. But in genome editing, modification is done at targeted site, by application of sequence-specific nucleases that create double-stranded breaks in the target genomic loci selected for editing; so, the success rate of obtaining desirable genotype is very high. The major genome editing tools are zinc finger (ZF) nucleases, transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR) [116]. Many strategies or options are there to improve agronomic traits by using genome editing tools. Introduction of a premature stop codon to terminate the functional protein production or modify to a gene promoter motif to control gene expression is one of the best examples of genome editing. Genome editing techniques such as TALEN and CRISPR-Cas9 are used to introduce targeted mutations in MILDEW-RESISTANCE LOCUS (MLO) proteins in hexaploid bread wheat [117]. On the other hand, Piatek et al. used synthetic transcriptional repressor and activator to increase gene expression [118]. C-repeat binding factors (CBFs) are responsible for cold acclimation in plants. Since *CBF1–3* loci all are on the same chromosome, by traditional genetic crossing it is highly difficult to generate *cbf1,2,3* triple mutant lines. So by using genome editing tool CRISPR/Cas9, generating single, double, and triple mutants of *CBF* genes was achieved successfully. The *cbfs* triple mutants are the most sensitive to freezing stress of these different mutants under cold-acclimation treatment. RNA-seq analysis of the triple mutants revealed that the expression of *c*. 10–20% of *COR* genes is CBF dependent [119, 120]. These findings support the notion that CBFs are key regulators that play redundant roles in cold acclimation in plants.

9. Conclusion

Plants are developed with inherent adaptive mechanisms to cope up with varied and composite abiotic stresses. Now it is possible with the help of science and technological advancement to understand function of gene, gene manipulation strategy, and plant traits development to overcome the abiotic stress. Signaling pathways have to be regarded as complex networks. Molecular analyses of the signaling factors provide a better understanding of the signal-transduction cascades during abiotic stress. A notable improvement in crop genome characterization and functional annotation of the gene will advance our knowledge in genetic manipulation (transgenic technology) and optimization of genome editing technology toward development of abiotic stress-tolerant crops. In due course, to accomplish the desired improved varieties, genome editing and transgenic approaches both should be combined with conventional and marker-assisted breeding activities. Further, identification of new adapted germplasm is also most important to guide breeding programs in target trait identification for changing scenario of climate. These combined efforts will make notable progress to face effect of climate change, especially stress such as drought, heat, and cold stress and will contribute to enhanced crop production, productivity, and thereby food security.

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Chapter 16

HSPs under Abiotic Stresses

Noor ul Haq and Samina N. Shakeel

Abstract

Different organisms respond to the altered environmental conditions by different ways. Heat shock proteins' (HSPs) production is one among the different defense mechanisms which defend the photosystem II and thylokoid membrane in plants. There are different types of HSPs based on their size, that is, high molecular weight (60–100 kDa) and low molecular weight heat shock proteins (15–30 kDa). Small HSPs are further classified based on their localization and role in different sub-cellular organelles. Cp-sHSPs are the chloroplast-specific small HSPs that protect the photosystem II and thylokoid membrane. A model to control the Cp-sHSPs in *Chenopodium album* has been put forward in this chapter. According to this model, Cp-sHSPs of *Chenopodium album* are created in cytoplasm and are moved toward chloroplast. The transit peptide is removed on reaching to the target sub-cellular organelle, that is, chloroplast and the premature Cp-sHSPs are converted into mature ones which have multiple roles under different abiotic stress conditions.

Keywords: plants HSPs, abiotic stresses, HSPs model, *Chenopodium album*

1. Introduction

Organisms respond to the changed growth conditions through heat shock proteins' (HSPs) production [1] and that is the way of survival for the cell which responds differentially [2]. Different environmental conditions including abiotic and biotic stress conditions influence the plants' development and production [3]. Different stress conditions like heat, salt, and low water conditions may majorly influence the plants' physiology and production [4–8], but plants response to the changed environmental conditions may vary depending upon duration, intensity, and combination of different environmental growth conditions [9]. Different processes in the plants including biochemistry, development, and physiology may affected by stress conditions and so the expression of different genes may be turned off or on in response to the changed environmental conditions, which may lead to the creation of different proteins and metabolites that protect the cells against such conditions [10].

2. Stress types

Stresses due to living and non-living things can affect the plants' development and production. Different organisms like viruses, bacteria, and fungi may cause stress conditions for the plants [8] which may activate different defense pathways of the plants [9]. There are reports that abiotic stress conditions are responsible to make mostly changes in plant biochemistry and physiology [10]. So plant growth may negatively be affected by abiotic stress conditions also known as non-living factors [6], and any kind of change in environmental conditions may lead the plants toward adaptation under altered growth conditions [11]. Below are the details of different abiotic stress conditions which may affect the plants.

2.1 Types of abiotic stresses and their effects on plants

2.1.1 High temperature or heat stress

Heat stress is the main factor among abiotic stress conditions that affects the plants yield [12] and so different factors in the plants like metabolite concentration, osmolytes, membrane fluidity, proteins structure, and nucleic acids are seriously changed by temperature [13]. Additionally, high-temperature stress affects the chloroplast photochemical activity [14]. Photosystem II is considered as the most sensitive part of thylokoid membrane [15] and heat stress conditions may influence the photosystem II (PS II) reaction center and the light harvesting complexes [16].

Plants adapt their system to the changed growth conditions through complex mechanisms [17]. Thus, different processes at cellular level are reprogrammed under high- and low-temperature growth conditions and many changes in transcription may happen in different parts of the plants, that is, seedlings, roots, pollens, and leaves [18, 19]. Effect on plants may vary with intensity and duration of temperature [20]. One of the plants responses is the reactive oxygen species (ROS) production which is increased by low- and high-temperature stress conditions, while oxidative damage and cell death have also been reported as a result of high-temperature stress conditions [21]. Photosynthesis inhibition has also been reported by researchers under high-temperature conditions [17], additional to the damage of the oxygen evolving complex (OEC) of photosystem II caused by heat stress [22].

Plants adapt to the high-temperature conditions through heat shock proteins (HSPs) production, which are found to be produced in all organisms from prokaryotes to eukaryotes and have role in cell protection under harsh conditions [2]. Establishment of defense mechanism under high-temperature growth conditions is necessary for cells survival which is not specifically occurred only under high temperature but it is also significant under different stress conditions [23].

2.1.2 Low temperature or cold stress

Low temperature represses the plants development without stopping the cell functions and may cause problems to different processes at cellular level [3]. Temperature is the main factor to control the growth changes from vegetative till reproductive level [24]. Low temperature may increase the ROS production additional to the reduction of cellular respiration [25] as well as damages the cell membrane [26].

Low-temperature stress conditions may reduce photosystem I and this effect has been reported to be increased under low light conditions [27]. The same effects have also been observed by different researchers in different plants like winter rye and barley [28, 29].

Expression of different genes and proteins has been reported to be up- or down-regulated by low-temperature stress conditions [30]. Researchers have reported the up-regulation of the defensive genes under cold stress [24]. For example, almost 300 genes have been reported to get up-regulated under cold stress conditions, while 88 genes (27%) were down-regulated in *Arabidopsis thaliana* [31].

2.1.3 Metal stress

Development of the plants is badly affected by heavy metals [32] and roots are usually damaged by heavy metals which lead to build up different defensive mechanisms for normal growth [33]. Membrane potential and permeability are changed by interactions of heavy metals with membrane components [32]. Plants take up the heavy metals as essential nutrients and are passed to the upper parts of the plants following the pathways of the essential elements transport [34].

Plants respond differentially to the heavy metal toxicity [35] and that is the reason that some plants do not show any phytotoxicity symptoms on heavy metals accumulation [36]. But heavy metals restrict the plants growth and cause cell death due to interruption in different physiological and biochemical pathways [37]. Different essential ions are replaced by heavy metals, for example, Ni replaces Mg ion that results in the changed activity of ribulose-1,5-biphosphate carboxylated oxygenase [38]. Chlorophyll activity is altered [39], while heavy metals break the disulfide bridges of the proteins, which leads to the destabilization of proteins [37]. Besides the formerly mentioned adverse roles in plants, heavy metals interact with the hydroxyl and carboxyl groups of proteins and thus interrupt in the proteins functions [40].

Plants adopt different defense mechanisms while get exposure to heavy metals. These mechanisms include the synthesis of cystein-rich polypeptides phytochelatins and metallothioneins [32]. Researchers have also reported the up-regulation of HSP70 gene and chaperonin 60 family members under different heavy metals, that is, Cd and Ni [41, 42]. Additional to the former HSP families, chloroplast small heat shock proteins (Cp-sHSPs) are also reported to be up-regulated by heavy metals [43].

2.1.4 Salt stress

Based on the response to salt stress, plants may be two types either glycophytes or halophytes. The former kind of plants has no tolerance to the saline environment, while the latter group plants covering are natively grown in saline environment [44]. Halophytes cover almost 1% of the world flora [45]. Salt stress adversely affects the plants growth and productivity by different ways; for example, sodium chloride salt can cause the ionic toxicity and osmotic stress to the plants [46]. Researchers have also reported the adverse effect of salt on growth and photosynthesis of the plants [47] by lowering the intra-cellular CO_2 availability [48] or by changed photosynthetic metabolism [49].

2.1.5 Drought stress

Crops yield and quality are adversely affected by drought conditions. Drought conditions may affect the macro- and micromolecules in a cell including minerals, lipids, proteins, hormones, carbohydrates, or even DNA or RNA [50]. The combination of drought with salt, high- or low-temperature stress conditions becomes more severe for the plants, which affects the plants' growth, development, and signal transduction [51, 52]. Besides the abovementioned macro−/micromolecules, photosynthesis that needs water is adversely affected by environmental stress conditions [53, 54]. Additional to the above, drought conditions may affect the metabolism of the plants because catabolism is enhanced due to hydrolytic enzymatic activity while anabolism is decreased due to lowering synthase activity [52]. In short, drought stress conditions adversely affect the photosynthesis in the chloroplast by decreased nutrient uptake and ion transport [55, 56].

2.2 Effect of stress conditions on gene expression

Stress conditions may activate the defense mechanism of the plants and result the change in different gene expression. The expression of heat shock proteins has been reported to be changed due to heat stress [57]. Heat shock proteins function as chaperones and safeguard the heat sensitive organelles and intra-cellular processes [2].

Proteins other than HSPs have also been reported to get produced and their expression is regulated differentially under heat stress conditions [58]. Besides the HSPs expression under heat stress conditions, these proteins have also been up-regulated under different stress conditions including heavy metal, cold, salt, drought, and oxidative stress conditions [43, 59–62].

3. Heat shock proteins (HSPs)

Heat shock response has been characterized in salivary glands of *Drosophila* [63]. Heat shock proteins have been studied in the result of transcription and translation in chromosomal puffs with active sites [64]. HSPs are produced in all organisms, that is, from bacteria to humans under changed environmental conditions [2] and have chaperone activity that protects the proteins from damage [65].

3.1 Role of heat shock proteins

Genes encoding HSPs respond to abiotic stress factors like high temperature, drought, salt, and low-temperature stress conditions [66]. HSPs having low expression under normal environmental conditions may have different function like chaperone function, prevention of proteins aggregation and folding, as well as to target the miss-folded proteins toward the specific pathways or for degradation [67]. Additional to the HSPs expression under abiotic stress conditions, these proteins have differential expression in different tissues and organelles. Taking all together, HSPs production is to protect the metabolic apparatus for adaptation under different environmental conditions and survival [68].

3.2 Types of HSPs

HSPs are divided into two classes based on their molecular weight, that is, high molecular weight heat shock proteins (HSP100, HSP90, HSP70, HSP60, and HSP40) and low molecular weight heat shock proteins (sHSPs), the weight of which is ranging from 15 to 30 kDa [69].

3.2.1 High molecular weight heat shock proteins

High molecular weight heat shock proteins are further divided into different classes based on molecular weight, that is, HSP100, HSP90, HSP70, and HSP60, the details of which are as below.

3.2.1.1 HSP100

HSP100 (protein family), found in all organisms from prokaryotes to eukaryotes [70], possess two subunits and are reported primarily in prokaryotes, that is, bacteria: (1) large-subunit (ClpA) which is ATP-dependent unfoldase and (2) protease which is a small-subunit ClpP [71]. Nucleotide-binding domain 1 & 2 (NBD1 & NBD2), carboxyl domains, middle domain, and amino and are the five parts of HSP100 proteins family members [72].

HSP100 genes have been reported to be up-regulated under heat stress conditions while the same pattern of expression has not been observed [73] but earlier than these findings, researchers have reported the expression of a member of HSP100 family under abscisic acid (ABA), cold and salt stresses additional to the high-temperature stress conditions [74]. Differential expression of one gene or this family member has been suggested under different abiotic stress conditions [75]. HSP100 family members have been reported with up-regulation under heat stress conditions in different plants like wheat and tobacco [75], rice [74], *Arabidopsis thaliana* [76], soybean [77] and maize [78]. Besides the above, HSP100 family members have also been reported with differential expression at different developmental stages [79] which may be the reason that HSP100 family members have been reported with high concentration in mature seeds of different plants [80].

3.2.1.2 HSP90

All organisms from prokaryotes to eukaryotes have HSP90 [81] and are involved to activate the component proteins involved in proteins transportation, assembling, folding and signal transduction [82]. Seven different isoforms of HSP90 have been identified in *Arabidopsis* and are classified based on sub-cellular localization, that is, three have been reported to be localized in endoplasmic reticulum, chloroplast, and mitochondria while the remaining four are localized in cytosole [83]. Three among the four cytosolic isoforms are expressed constitutively while fourth one is expressed under heat stress conditions [84].

3.2.1.3 HSP70

HSP70 are expressed under normal conditions in plants so these are also named as heat shock cognates [85]. HSP70 are having important role under different environmental conditions including heat stress [86, 87]. This class of proteins may function to stabilize the unstable proteins [82] additional to the proteins transport among sub-cellular compartments and proteins folding [88].

HSP70 family proteins may be classified into four classes based on the sub-cellular localization and thus are localized in four different compartments (cytosol, mitochondria, plastids, and endoplasmic reticulum) of the cell [89].

3.2.1.4 HSP60

HSP60 family members encoded by nuclear DNA [90] are present in prokaryotes to eukaryotes and have function in cells under stress and normal conditions [91]. Bacterial HSP60 plays role in proteins assembling to form complexes (oligomeric) and movement through cell membrane [91] but the same family proteins are involved in organelle (chloroplast and mitochondria)-specific proteins folding [91].

3.2.2 Small heat shock proteins (sHSPs)

Plants' small heat shock proteins having molecular weight from 15 to 30 kDa are encoded by nuclear DNA and are classified into further six classes based on sub-cellular localization [92]. Researchers have classified the abovementioned proteins as per the localization in different cellular organelles, that is, first two are localized in cytosol and the next three classes (III, IV, and V) are localized in endoplasmic reticulum, mitochondria, and plastids, respectively [93]. Additional to the above, class VI has been reported to be localized in endoplasmic reticulum [94].

C-terminal region, N-terminal region, and α-crystallin domain are the three main parts of small heat shock proteins. Small HSPs are characterized by 100 amino acids sequence having α-crystalline domain [95] as well as N-terminal region on one side and C-terminal region on the other side of the formerly mentioned domain [96]. The abovementioned three domains are the conserved regions of small heat shock proteins [97].

Small HSPs expression has been reported in different plants, for example, *Chenopodium album* [43, 62], carrot [98], sugarcane [99], Agave [100], *Arabidopsis* [101], cotton [102], tomato [18], maize [103], tobacco [104], etc. The abovementioned studies of sHSPs in different plants show the importance of this class of HSPs in adaptation under different environmental conditions [92].

3.2.3 Chloroplast small heat shock proteins (Cp-sHSPs) and their role

Cp-sHSPs are produced in cytoplasm followed by its import toward chloroplast [105]. As the name shows, these kinds of proteins are located in chloroplast and have consensus-III or methionine rich region at the N-terminal region additional to the other sHSPs-specific regions [106].

These proteins protect photosynthesis of the plants under heat and oxidative stress conditions [107]. There are different mechanisms to protect photosynthesis, for example, chloroplast membrane stabilization or avoiding everlasting proteins aggregation [108] but the role of Cp-sHSPs is very important in this case [109]. Different researchers have shown the relation of sHSPs with the adaptation of the plants under environmental stress conditions [43, 60–62, 109, 110].

It has been established in vitro by researchers that these chloroplast-specific proteins may protect photosynthetic electron transport under high-temperature stress conditions [59]. Cp-sHSPs associate with photosystem II (PS II) through oxygen-evolving complex (OEC) proteins under high-temperature conditions. It has been confirmed by researchers that these proteins protect PS II from inactivation under heat stress conditions by the protection of oxygen evolution and OEC proteins but have no capability to repair inactivated PS II [107].

4. HSP gene expression and promoters

Promoters regulate gene expression quantitatively and qualitatively [111]. There are three types of promoters that regulate the gene expression, that is, inducible, spatiotemporal, and constitutive promoters. Constitutive promoters promote the gene expression throughout the tissues irrespective to the environmental and developmental conditions, while spatiotemporal promoters direct the target gene expression in specific tissues, but inducible promoters are independent of the endogenous factors but dependent upon the external stimuli and environmental conditions [112]. Almost all kinds of promoters have the same core sequence with TATA-box, initiator, and the TF binding-specific cis-acting motifs specific to the target genes [113].

There are very less reports about the regulation of organelle-localized sHSPs under specific stress conditions or even under combination of stresses though it has been known that these genes are mainly regulated at transcriptional level. Researchers have reported the use of soybean promoter (GmHSP17.3B) to induce the sHSPs expression in *Physcomitrella patens* [114]. Additional to the above, researchers have also reported the rice promoter (Oshsp16.9A) to induce the expression of sHSPs under high-temperature stress conditions [115]. Small heat shock proteins have also been reported to get expressed under different abiotic stress conditions additional to the sHSPs expression at different developmental stages [43, 61, 62, 110, 116].

Heat shock transcription factors (HSFs) and heat shock elements (HSEs) may control the HSPs expression in the result of complex network of interaction [117]. HSFs (more than 20 in number) [118] may control the heat shock response both in vitro and in vivo [119]. Thermotolerance is increased in the result of higher expression of HSPs that is resulted by binding the HSFs to HSEs [120, 121]. Differential expression of HSPs is resulted by the variations in HSEs of HSPs. These HSEs have difference in the location and arrangements of its basic units (nGAAn), for example, AtHsp90–1 gene promoter has heat shock element 1 (HSE1) (tGAAgcTTCtgGAAt), heat shock element 2 (HSE2) (agTCtcGAAacGAAaaGAActTTCtgGAAt), and heat shock element 3 (HSE3) (gGAAgaaTCcaGAAt) [122]. Additional to the above elements, other motifs to regulate HSPs (gap-type 1, gap-type 2, and gap-type 3 with the sequences nTTCnnGAAn[5bp]nGAAn, nTTCn[1bp]nGAAn[5bp]nGAAn and nTTCn[2bp]nGAAn[5bp]nGAAn respectively) have also been reported. Researchers have also reported TTC-rich type regulatory elements with 2–4 units of nTTCn with 0–8 bp gap {e.g., TTC-rich 1 (nTTCn[1bp]nTTCn[6bp]nTTCn) and 3 (nTTCnnTTCn[8bp]nTTCn[1bp]nTTCn)} that have binding capability with HsfA1a of *Arabidopsis*. But some TTC-rich regions are also present with no binding potential with HsfA1a, for example, TTC-rich 2 (nTTCn[5bp]nTTCn[4bp]nTTCn) and TTC-rich 4 (nTTCn[3bp]nTTCn) [119]. Besides the above, other cis-regulatory elements are also present in HSPs promoter to regulate their expression under different growth conditions, for example, stress response elements (STREs), metal response elements (MREs), and CAAT boxes C/EBP [123–125]. Metalothionein gene of animals and plants has also been reported to get activated by heavy metal stress conditions because of the presence of MRE in promoter region of this gene [126–128]. Similarly, another stress-related element, that is, STRE (AGGGG) is also regulated by different abiotic stress conditions in yeast [129].

5. Model to express the Cp-sHSPs under different environmental conditions

There is no model put forward by researchers to control the expression of chloroplast-specific small heat shock proteins (Cp-sHSPs), but a model (**Figure 1**) to control the formerly mentioned genes has been proposed by Haq et al. [62]. According to this model, the presence of different cis-regulatory elements in Cp-sHSPs promoter shows the role of Cp-sHSPs under different abiotic stress

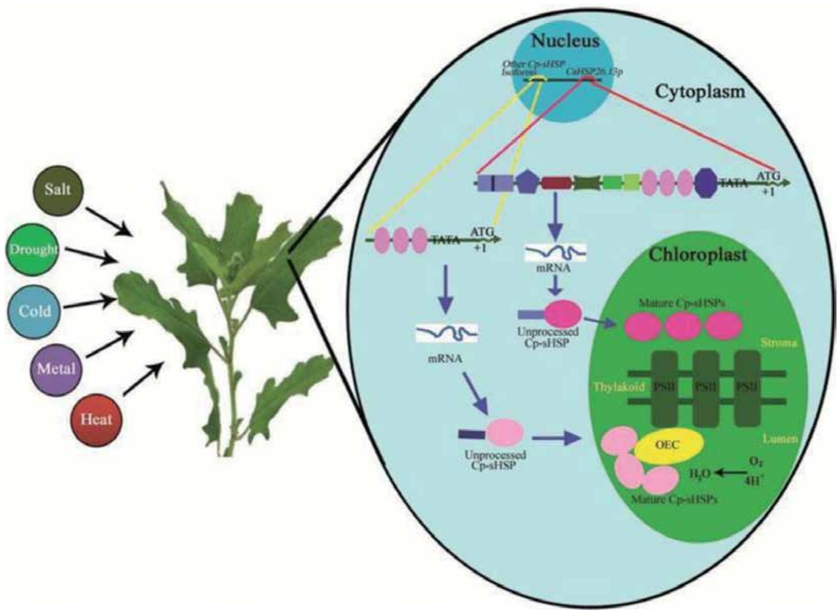

Figure 1.
Proposed model of expression and role of Cp-sHSPs [65].

conditions, that is, salt, drought, cold, metal, and high-temperature stress conditions. Cp-sHSPs in *Chenopodium album* have been shown to protect thylakoid membranes and photosystem II under different abiotic stress conditions. Different abiotic stress conditions, that is, heat, cold, heavy metal, drought, and salt stress conditions may regulate the single Cp-sHSP transcript in *C. album* which produces the precursor proteins that have transit peptide which directs that toward chloroplast. The transit peptide is detached from the proteins while reaching toward chloroplast in the result of which these proteins are matured that have the function in chloroplast. As per this proposed model, differential regulation of the same Cp-sHSP family member in *C. album* makes it able to play multiple roles under different abiotic stress conditions, that is, salt, drought, heavy metal, cold, and heat stress conditions [62].

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Chapter 17

Plant Growth and Morphophysiological Modifications in Perennial Ryegrass under Environmental Stress

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Abstract

Perennial ryegrass (*Lolium perenne* L.) is a popular and important cool-season turfgrass used in parks, landscapes, sports fields, and golf courses, and it has significant ecological, environmental, and economic values. It is also widely used as forage and pasture grass for animals around the world. However, the growth of perennial ryegrass is often affected by various abiotic stresses, which cause declines in turf quality and forage production. Among abiotic stresses, drought, salinity, temperature, and heavy metal are the most detrimental factors for perennial ryegrass growth in different regions, which result in growth inhibition, cell structure damage, and metabolic dysfunction. Many researches have revealed a lot useful information for understanding the mechanism of tolerance to adverse stresses at morphophysiological level. In this chapter, we will give a systematic literature review about morphological and physiological changes of perennial ryegrass in response to main stress factors and provide detail aspects of improving perennial ryegrass resistance based on research progress. Understanding morphophysiological response in perennial ryegrass under stress will contribute to improving further insights on fundamental mechanisms of perennial ryegrass stress tolerance and providing valuable information for breeding resistance cultivars of perennial ryegrass.

Keywords: perennial ryegrass, morphology, physiology, abiotic stress, stress resistance

1. Introduction

Urban green areas have important various functions contributing to the quality of human health. Well-kept lawns enhance the esthetic value of the entire city and are involved in phytoremediation, leading to an improvement in the quality of the air and soil [1–4]. Perennial ryegrass (*Lolium perenne* L.) is an important and widespread perennial cool-season grass cultivated in temperate climates, originating in Europe, temperate Asia, and North Africa [5]. Perennial ryegrass is commonly used in home lawns, sport fields, and parks with rapid growth and establishing rate, and other elements for ecosystem service due to its massive root system, superior regeneration, and tillering ability. It is also widely used as nutritive forage and pasture grass for animals around the world [6–8]. Moreover, numerous perennial ryegrass genotypes and hybrids are now released by commercial utilities [9, 10].

In fields, the growth and development process of plants needs to counteract various environmental stresses such as salinity, drought, cold, heat, and heavy metal [11–13]. Harsh environmental conditions may result in growth inhibition, cell structure damage, and metabolic dysfunction [14–20]. Moreover, stresses will further be intensified for the potential impact of climate change in future. Thus, maintaining proper growth of turfgrass with minimal inputs under abiotic stress conditions is a great challenge for turfgrass industry. This challenge could be addressed through improving the stress tolerance of turfgrass [14, 21]. Understanding morphological and physiological mechanisms of turfgrass adaptation to various abiotic stresses is a key step for the development of stress-tolerant ability and cost-effective and efficient management practices [13]. Morphophysiological mechanisms of turfgrass in abiotic stresses tolerance involve phenotypic changes, multiple physiological and biochemical response, and complex metabolic processes, such as water and nutrient relations, carbohydrate metabolism, protein metabolism, hormone metabolism, as well as antioxidant defenses [22, 23]. Current studies on morphophysiological mechanism controlling turfgrass adaptations to various growth conditions have provided important information for production of abiotic stress-tolerant germplasms and the further understanding of regulation mechanism of turfgrass response to abiotic stresses [13, 24, 25]. However, the mechanisms of the adaptive responses are integrated but are not necessarily the same [14]; thus, studies on how perennial ryegrass adapts to stress conditions will become more important with the increasing pressure of utilizing both ecological and economical strategies in the turf management. Furthermore, insights into mechanisms of stress resistance in perennial ryegrass will aid in identifying important characteristics for selecting the criteria of improving stress tolerance and will ultimately lead to better selection of new cultivars adapted to adverse environments. This chapter, therefore, focuses on an extensive overview of the current understanding of changes in physiology and growth/development of perennial ryegrass under various abiotic stresses. In addition, strategies for improving the stress tolerance of perennial ryegrass are also presented. This review can contribute to the better understanding of the mechanisms of perennial ryegrass response to environmental stresses and can provide valuable information for improving resistance characteristics of perennial ryegrass by breeding. Moreover, enhancing our understanding of physiological effects of abiotic stresses can provide guidelines for the practical management strategies of the maintenance of high-quality turf under limited resource availability.

2. Abiotic stresses

Abiotic stresses are major environmental conditions that reduce plant growth, productivity, and quality. Plants have evolved mechanisms to perceive these environmental challenges, transmit the stress signals within cells as well as between cells and tissues, and make appropriate adjustments in their growth and development for survive and reproduce [26–29]. The morphological and physiological changes of perennial ryegrass under abiotic stress will be discussed in this chapter.

2.1 Responses of drought stress in perennial ryegrass

Growth and development processes are inhibited when plant is exposed to drought stress [30–36]. Morphological adjustments, such as biomass allocation and leave changes, have been proposed as the key mechanisms used by turfgrass to enhance survival under drought [37]. There is a series of morphology changes in perennial ryegrass under drought stress. Drought stress reduced the turf quality (TQ), number of live tillers and dry-matter yield [38–40]. Moreover, drought significantly enhanced root to shoot ratio (R/S) in perennial ryegrass to an less extents, depending on the intensity, the reason may be that perennial ryegrass in drought stress develop a large R/S to maintain water and nutrient uptake [39]. The leaves of perennial ryegrass under drought stress were also dramatically different from that of nonstressed perennial ryegrass, for example, under drought stress, the diurnal variation in the rate of leaf extension was smaller but the leaves tended to grow faster at night compared to normal irrigation controls; however, water stress ultimately reduced the rate of leaf extension and leaf area in perennial ryegrass [40]. Furthermore, the leaves' epidermis of perennial ryegrass under drought reduced the stomatal size and increased the numbers per unit leaf area. Drought also resulted deeper ridging on leaf ad-axial surface, smaller epidermal cells and bigger ridge angle [40]. Under drought stress, leave stomata of perennial ryegrass began to close to reduce their evapotranspiration rate (ET), at leaf water potentials below—13 bars [40].

Drought stress causes significant physiological changes, including photosynthesis, osmotic adjustment substances, proteins, and antioxidant metabolism, in perennial ryegrass. For instance, the content of leaf total nitrogen and leaf relative water content (RWC) were tested to decrease, on the contrary, antioxidant activity including ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione S-transferase (GST), amino acids such as aspartic acid, threonine, serine, glutamic acid, abscisic acid (ABA) concentration, and proline content increased under drought stress [41]. Photosynthesis is the primary process controlling plant growth and adaption to drought stress [42]. The canopy photosynthesis of perennial ryegrass at saturating light intensity was reduced by about half in the stressed field swards and by more than 80% in the stressed simulated swards [38]. Drought stress inhibits photosynthesis, which may be the result of low CO_2 availability caused by stomatal closure and/or the inhibition of photochemical reactions and carbon assimilation metabolism [43]. In addition to the photosynthesis, starchis also considered as a buffer for imbalance between acquisition by photosynthesis and C-sink activities such as growth and respiration resulted from drought, also stress due to the excessive use of inorganic fertilizer [44]. However, use of green manure also has risk of xenobiotic contamination [45–47], and the soluble sugars, including sucrose, fructose and glucose, are involved in multiple physiological functions such as respiration, turgor maintenance, signaling and defense. Under drought conditions, starch of perennial ryegrass significantly decreased in shoots, but did not change in roots, which indicated that perennial ryegrass in drought condition preferentially allocates carbon not only to root growth, but also to root storage, while soluble sugars were enhanced in both shoots and roots. Accumulation of soluble sugars has been widely reported for plants upon water stress as a means to provide osmotic protection [39], which suggested that increasing of soluble sugars was benefit to plants to maintain growth and active metabolic activities under water deficit.

It is generally accepted that there is a noticeable genotypic variation in perennial ryegrass for drought stress responses. The research showed that one self-pollinating genotype "S10" showed higher RWC, shoot dry weight (SDW), proline, ABA, nitrogen and amino acid contents, and antioxidant enzymes activities in comparison with two commercial genotypes of "Vigor" and "Speedy" [41]. Proteins involved in carbon and energy metabolism, photosynthesis, tricarboxylic acid cycle (TCA) cycle, redox, and transport categories were upregulated in the two commercial genotypes of "Vigo" and "Speedy," while the protein profile of the "S10" changed slightly under drought stress, and the reason may be that self-pollination in the genetic background of the "S10" genotype may have a lower variation in response to drought stress conditions [41]. Additionally, other research indicated that tetraploid perennial ryegrass exhibited a greater biomass under severe drought, whereas diploids had a greater biomass under the current rainfall [48, 49]. Moreover, tetraploid perennial ryegrass populations were able to develop more shoot and root dry matter than diploid populations in following the application of drought stress [50].

The above researches showed that drought stress caused significantly physiological and morphological changes in perennial ryegrass (**Table 1**) [55, 56]. Thus, the growth of perennial ryegrass is severely restricted by soil water deficits [57]. Increasing drought tolerance of perennial ryegrass via strategies is importance for both water conservation and maintaining growth in water limiting environments. For example, the grass-Epichloë endophytic improved water utilization and drought tolerance in perennial ryegrass [58]. Moreover, arbuscular mycorrhizal fungi (AMF) + Epichloë treatments increased phosphorus (P) uptake, net photosynthetic rate (Pn), root activity, soluble sugar concentration, peroxidase (POD) activity, and decreased malonyldialdehyde (MDA) concentration in perennial ryegrass under drought stress, the reason may be that Plant-AMF-Epichloë symbiosis alleviated the damage caused by drought stress by promoting P uptake, photosynthesis, and the accumulation of osmoregulatory substances [59]. Additionally, application of plant growth regulators (PGRs) have been reported to be a promising way of reducing drought stress impacts [60]. The study manifested that trinexapac ethyl (TE) treatment increased chlorophyll content, proline content, the RWC, soluble sugar content, antioxidant enzymes activities, decreased MDA and hydrogen peroxide (H_2O_2) contents in perennial ryegrass under drought stress, while Paclobutrazol (PAC)- and ABA-treated perennial ryegrasses were all effective in mitigating physiological damages resulting from drought stress [52]. Furthermore, overexpression of some drought-related genes has been shown to effectively improve drought tolerance of plants [61]. According to Patel et al. [53], overexpression of *LpHUB1* gene conferred drought tolerance in perennial ryegrass.

2.2 Responses of temperature stress in perennial ryegrass

Perennial ryegrass can grow throughout the year, and the major constraint on growth is temperature [51, 62]. Perennial ryegrass has an optimal growth temperature of about 20°C, and it is sensitive to high (30–40°C) and low (−20 to 0°C) temperatures [63, 64]. Common perennial ryegrass germinates quickly and can be used as a temporary ground cover while the slower growing bluegrass plants take

Morphological responses	Physiological responses	Strategies
• Decreased turf quality • Enhanced R/S • Decreased leaf area • Reduce the number of live tillers • Had smaller stomata and epidermal cells • Had bigger ridge angle • Controlled stomatal opening [38–40]	• Decline biomass • Decline photosynthetic rate • Increased osmotic adjustment substances • Increased antioxidant activity • Increased amino acid content [39, 41, 51]	• Application of plant growth regulators (PGRs) • Selected drought resistance cultivars from different cultivars • Using endophytes • Using transgenic technology [52–54]

Table 1.
Morphophysiological response of perennial ryegrass under drought stress.

hold in cool temperate region. In warm climates, it is used as an overseed to maintain winter green in the lawn after the warm season grasses go dormant. However, populations of perennial ryegrass will not survive the summer heat. Severe heat stress (40/35°C day/night) caused significant physiological damages, including declining in TQ, RWC, CAT activity, and enhancing in electrolyte leakage (EL) of leaves and MDA content, in perennial ryegrass [65]. Moreover, heat stress decreased plant height (HT), leaf fresh weight (LFW) and leaf fresh dry (LFD), and increased cytokinin and auxin at 35/30°C (day/night) of temperature [66]. Moreover, low temperature is one of the main factors that limit the persistence of perennial ryegrass-dominated grasslands in northern regions. Cold stress decreased TQ, regrowth, dry weight, and tiller density in perennial ryegrass when the winters were mild with short (2–6 weeks) periods of lower than −10°C temperatures and no permanent snow cover [67]. Furthermore, cold stress decreased RWC and increase EL in leaves and roots when perennial ryegrass was exposed to −15 or −25°C [68]. To resolve these problems and maintain high visual quality of perennial ryegrass through the year, it is important to found new cultivars to adapt to temperature stress. Variations of heat- or cold- resistance were also found among different perennial ryegrass cultivars. Thus, selection cold- or heat-tolerant cultivars during perennial ryegrass genotypes can be an effective method for temperature tolerance improvement in perennial ryegrass. The research tested the heat tolerance of 58 cultivars collected from seed companies and research centers in U.S.A., New Zealand, and Europe, the result showed that distinct heat tolerance was found among the cultivars at all the temperature regimes, and the least and most tolerant cultivars were “JPR005” and “JPR178,” respectively [69]. The other research indicated that changes of morphology and physiology were different for heat-tolerant accession “PI265351” and sensitive accession “PI225825” [66]. Similarly, the heat-tolerant populations of perennial grass showed significantly lower degree damage in efficiency of photosystem II and cell membrane stability than the sensitive ones at different levels of stress [70]. Additionally, the study showed that 21 accessions sampled from a larger set of 300 accessions with known winter hardiness, the result showed that the degree of semi-lethal temperature in 21 ryegrass varieties varies from −10.31 to −13.95°C, with 3 accessions possessing significantly greater freezing tolerance than the most freeze-tolerant check “NK200” [71]. Moreover, tetraploid genotypes of perennial ryegrass demonstrated higher tolerance to cold stress conditions, better spring growth, and regrowth after cuts, and higher dry matter yield compared to diploid genotypes [67].

The studies indicate that temperature stress caused the morphological and physiological damage in plant, and the response of genotypes to temperature stress was different [72–80]. Therefore, founding some strategies which could improve cold- or heat-tolerant of perennial ryegrass is important. It was also reported that 24-epibrassinolide promoted carbohydrates accumulation in crowns of perennial ryegrass during cold acclimation by regulation of gene expression and enzyme activities, and which resulted in increased frost tolerance [81]. Moreover, drought preconditioning increased in crown fructans, proline, and total soluble protein content for “Buccaneer” and “Sunkissed” during cold acclimation, which suggested a synergistic effect between drought exposure and low temperature, and drought preconditioning resulted in an improvement in freezing tolerance of perennial ryegrass [82]. Additionally, previous studies have shown that the enzyme activity level and gene expression of antioxidants are associated with cold and heat tolerance in a cool-season perennial grass species [83, 84]. For instance, *LpHOX21* was positively associated with heat tolerance of perennial ryegrass [85]. Similarly, P450 gene (*LpCYP72A161*) showed remarkable upregulation in perennial ryegrass under heat and cold treatment. Therefore, transferring key genes into perennial ryegrass will be beneficial to improve heat or freezing tolerance [86].

2.3 Responses of salt stress in perennial ryegrass

Salinity stress has become a more significant problem in turfgrass management in many areas [13]. Responses of plants to salinity stress occur mainly through two distinct phases over time: osmotic-changing and ion specific phases [87–89]. Like other turfgrasses, salt stress caused morphology, physiology, molecular changes in growth and development of perennial ryegrass, such as TQ LFW, LED, and RWC of perennial ryegrass decreasing after exposure to salinity [89, 90]. The alterations of morphological characteristics of turfgrass under salt stress are derived from the changes of physiological traits such as cell membrane stability [14]. It was reported that MDA content and EL enhanced by NaCl concentration in perennial ryegrass [54]. Simultaneously, superoxide radical (O^{2-}), H_2O_2, and singlet oxygen (O_2) concentration increased observably in perennial ryegrass after salt stress treatment [54, 91]. To scavenge reactive oxygen species (ROS), salt-stressed leaves of perennial ryegrass exhibited greater activities of SOD, APX, and CAT at the initial stage of salt stress, but lower levels of enzyme with the extension of salt stress [89]. Salt stress also negatively affected on the total chlorophyll (Chl), Chl a and Chl b, in perennial ryegrass [89], which showed that salt stress induced Chl decomposition in leaves. Moreover, a further research of PSII changes in perennial ryegrass discovered that quantum yields, efficiencies, and energy fluxes were impacted after salt stress treatment [92, 93]. Additionally, a vast amount of Na^+ accumulated in plants could induce ionic imbalance in the cells. It was reported that Na^+ concentration accumulated rapidly and other ion concentrations including K^+, Ca^{2+} and Mg^{2+} were decreased in response to salt stress in perennial ryegrass [89].

Salt stress causes dramatically changes in morphology and physiology of perennial ryegrass as showed above and summaries in **Figure 1**. However, these responses varied greatly among different genotypes. The research compared the salt tolerance in 10 accessions of perennial ryegrass, and determined that "PI275660"

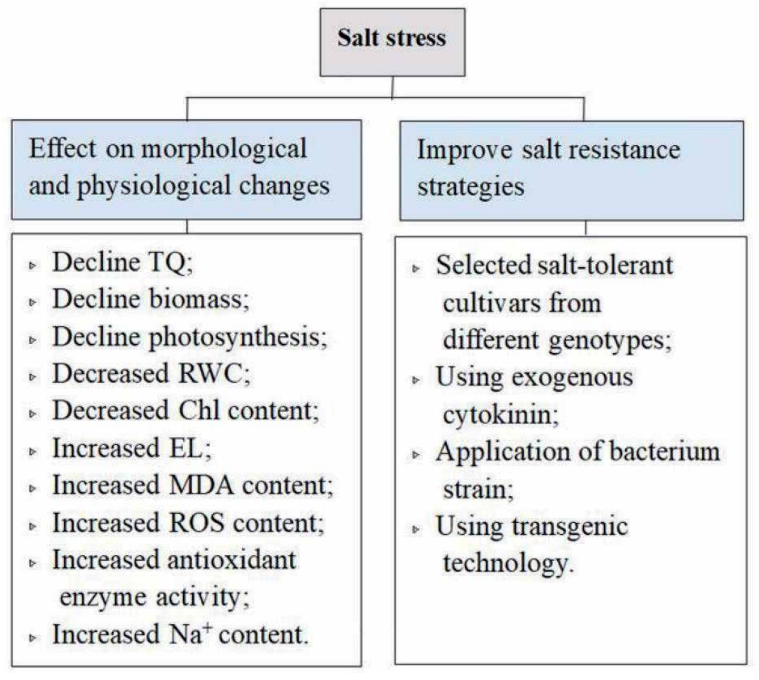

Figure 1.
Morphophysiological response and strategies for salt stress in perennial ryegrass.

and "BrightStar" showed the best tolerance to salt stress, while "PI231595" and "PI251141" were the most sensitive accessions [5]. The other research reported that the effect on parameters of photosynthetic efficiency in perennial ryegrass "Roadrunner" was less than that in "Nira" under salt stress condition [6]. Moreover, the highest salt tolerance accessions were from the European group, wild accessions and exhibited more variation in functional traits and salt tolerance than commercial cultivars [90]. Some other strategies can also improve the salt-tolerance in perennial ryegrass. Salt-tolerant transgenic perennial ryegrass could be obtained by *Agrobacterium tumefaciens*-mediated transformation of the vacuolar Na^+/H^+ antiporter gene [94]. Additionally, exogenous cytokinin applications alleviated salt-induced leaf senescence in perennial ryegrass [8]. Furthermore, salt tolerance of perennial ryegrass can increase by a novel bacterium strain from the rhizosphere of a desert shrub *Haloxylon ammodendron* induced [95].

2.4 Responses of heavy metals stress in perennial ryegrass

The continuing industrialization has led to extensive environmental problems worldwide [96–98]. Heavy metals produced from industry are released to soil. Thus, high accumulation of heavy metal in soil can induce environmental stress on plants [14]. Research on the response of perennial ryegrass to heavy metal stress has also progressed in recent years. It has been proved that heavy metals can induce damage and affect metabolic processes in perennial ryegrass [98–100]. For example, perennial ryegrass had characters in yield reduction and visible symptoms of phytotoxicity under cadmium (Cd) and zinc (Zn) stress [98]. Moreover, the cellular membrane system was damaged because of elevated MDA and EL contents when perennial ryegrass was exposed to salt condition [101]. According to studies, a dramatic inhibition of root and shoot growth was detected in perennial ryegrass after heavy metals treatment [101–103]. Moreover, the composition of the leaves of perennial ryegrass, including apparently opposite effects on the calcium (Ca), potassium (K) and P levels, was changed under the aluminum (Al) stress [104]. Additionally, ROS bursts occurred in perennial ryegrass under heavy mental stress conditions. For instance, H_2O_2 and O^{2-} were significantly accumulated in perennial ryegrass under Cd stress [105]. Hence, the protection mechanisms in perennial ryegrass such as the antioxidant system were triggered under heavy stress, resulting in the increase of SOD, CAT, and POD activities and their corresponding genes [106]. Moreover, content of fructan, sugar, and starch showed an increasing trend in perennial ryegrass after heavy metal stress [98]. However, certain concentrations of heavy metal were beneficial for the growth of perennial ryegrass [107]. Heavy metal stresses not only induce physiological damage, but also inhibit germination and growth of perennial ryegrass [108].

To improve the heavy metal stress tolerance of perennial ryegrass, several investigations were conducted in recent years. It was reported that signal messengers such as nitricoxide (NO) and glycine betaine (GB) play crucial roles in alleviating Cd and Cu-induced damages in perennial ryegrass [109, 110]. Moreover, the exogenous P was testified to improve the Cd tolerance of perennial ryegrass, the reason may be that exogenous P facilitates chelation-mediated Cd detoxification processes [105]. Similarly, a high dose of P amendment alleviated Mn-toxicity in Mn-sensitive genotype in perennial ryegrass [102]. Furthermore, the addition of biochar to a contaminated mine soil improved the nutrient status of this mine soil and contributed to a better establishment of perennial ryegrass [100]. Additionally, AMF enhance both absorption and stabilization of Cd by perennial ryegrass in a Cd-contaminated acidic soil [96], and ethylene diamine tetra acetate (EDTA) enhanced phytoremediation of heavy metals from municipal waste compost and sludge soil by perennial ryegrass [99, 111, 112].

3. Conclusions and future research perspectives

Significant progress has been made in the understanding of morphological and physiological mechanisms associated with perennial ryegrass tolerance to drought, salinity, temperature, and heavy mental stresses. Harsh stress conditions inhibit the growth and development and decrease TQ, root length, and dry weight in perennial ryegrass. Moreover, physiological response to abiotic stress in perennial ryegrass displays changes of the cell membrane, photosystem, metabolites, and antioxidant system. The contents of MDA and EL are increased, while Chl content and photosynthesis are decreased under stress conditions. To regulate the osmotic potential of the cell after stress treatment, some metabolites such as proline, soluble sugars, and proteins accumulate. Meanwhile, antioxidant enzymes' activities increase in perennial ryegrass for scavenging ROS. Perennial ryegrass has protective responses against unfavorable conditions, but there is a threshold to these physiological changes. To understand the response to abiotic stress and resistance attributes in perennial ryegrass will be beneficial to breeding in future.

For improving the stress tolerance of perennial ryegrass, some practical strategies are exploited currently, such as application of phytohormones, endophytes, and chemical compounds. Further research on increasing perennial ryegrass stress tolerance should pay more attention to transgenic technology to identify effective genes for modifying stress-tolerance ability.

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Chapter 18

Advances in Developing Multigene Abiotic and Biotic Stress-Tolerant Rice Varieties

Nitika Sandhu, Shailesh Yadav and Arvind Kumar

Abstract

Increasing incidences of multiple abiotic stresses together with increasing population are the major constraints to attain the global food security. Rice, the major staple food crop is very much prone to various abiotic and biotic stresses, which can occur one at a time or two or more together in a single crop growing season and adversely affects the rice production and productivity. The devastating effect of multiple stresses on rice crop is much more erratic and complex leading to higher losses in the crop grain yield. The concurrent occurrence of multiple streeses can destroy rice production in many of the rainfed areas of South and Southeast-Asia. Genomics-assisted breeding strategies have been instrumental in introgression of various major effect QTLs/genes into rice mega varieties and have proven successful in achieving the desired level of tolerance/resistance to various abiotic stresses in diffferent crop species. Keeping the present scenario of changing climate in mind, the chapter discusses the recent past success in combining tolerance to two or more abiotic stresses in mega rice varieties applying genomics-assisted breeding and development of high-yielding climate resilient rice through stacking of multiple genes/QTLs, which can withstand in a cascade of multiple stresses occurring regularly in rainfed environments.

Keywords: abiotic stress, biotic stress, genomic-assisted breeding, pyramiding, QTLs, rice, yield

1. Introduction

Global warming and the changing climatic conditions lead to the concurrence of multiple abiotic and biotic stresses individually/or in combination [1, 2] thus adversely affecting the rice crop growth and yield [3]. The changing climate, more and more extreme weather events are increasing the probability of simultaneous multiple abiotic stresses, including extra pressure from biotic stresses. Abiotic and biotic stresses reported to have significant negative impact on rice crop survival, growth, development and yield in most parts of the world, especially the Asia and Africa [4, 5]. The abiotic stresses such as drought, salinity, cold, high temperature and heavy metals are known to influence the occurrence of biotic stresses [6–8]. The combined effect of multiple stresses may resulted the minor pests to become the potential threats in the coming future [1, 9, 10].

The rice farming is practiced in various diverse ecological zones. The rice cultivation system in diiferent growing areas are mainly depends upon various factors such as available water, soil type, and the prevailing monsoon. Rice production faces various constraints in various ecology of rice cultivation (**Table 1**). Rice crop faces multiple stresses during different stages of its growth and development and around 70% reduction in yield was reported due to the occurance of abiotic stresses at different stages of growth and development [5]. Similarly, the major biotic stresses such as bacterial leaf blight, blast, brown plant hopper, brown spot, sheath blight and gall midge reported to impart severe crop yield losses or even complete crop failure during infestation [4]. The growth of rice yield has deteriorated from 2.3% per year during 1970s–1980s to about 1.5% during 1990s, and to <1% in the first decads of this present century [11]. Although the rice production has improved considerably over time but it is not sufficient to cope with the increasing demand globally [12]. The annual shortage of rice is expected to rise from 400,000 tons in 2016 to around 800,000 tons by 2030 [13].

As crop plants are immobile, they have to respond to the different abiotic and biotic stresses in the field itself. Breeding efforts in developing tolerance for single stress such as drought, heat, salinity, cold, insect and pathogen or a single stress type viz. abiotic or biotic may be tricky because plants may respond differentially to different or simultaneous occurance of stresses. The increase in resistance/tolerance to one type of stress may be at the cost of resistance/tolerance to another stress [14]. Breeding of high yielding multiple stress tolerant/resistant rice varieties with better grain quality is the urgent need of the hour since many decades [15]. Improvement of germplasm involving improved donors free from undersirable linkage, identification and introgression of genomic regions after validation involving recent advances in genomics-assisted breeding has provided opportunity to combat the challenges arising due the occurance of multiple stresses [16]. An integrated genomics-assisted breeding approach to introgress desirable genes/QTLs conferring tolerance/resistance to major abiotic and biotic stresses in addition to improved yield and quality will help to combat the present situation [16–20]. The commercial use of QTLs/genes-conferred multiple stress tolerant/resistant rice varieties provides an effective, economical and environment friendly approach to protect the crop yield and productivity. In past few years, the identification of genomic regions associated with drought, submergence and heat tolerance and introgression and pyramiding of these regions applying markers assisted selection/backcross approach have successfully led to the development of

Ecosystem	Source of water	Constraints
Upland	Rainfall	Drought, blast, weeds, low soil fertility, Fe toxicity, soil nematode problem, lodging
Rainfed shallow lowland	Rainfall, water table	Lack of assurred irrigation, frequent drought, blast, bacterial leaf blight
Rainfed medium lowland	Rainfall, water table	Lack of assurred irrigation, drought, flood, drought and flood in same or different season, bacterial leaf blight, brown plant hopper, gall midge
Rainfed deep lowland	Rainfall, water table, flood water	Lack of assured irrigation, fragile and low productivity, Prevailing abiotic stresses such as flood, salinity, Biotic stresses such as bacterial leaf blight, gall midge, brown plant hopper
Irrigated	Irrigation	Salinity, bacterial leaf blight, brown plant hopper

Table 1.
Rice production constraints in various ecologies of rice cultivation.

drought or flood tolerant version of some of the mega varieties such as Swarna, IR64 and Sambha Mahsuri. Some of these developed genomics-assisted derived breeding lines have been released as varieties in various countries of South Asia and South East Asia for cultivation.

2. Biotic stresses

2.1 Bacterial blight

Rice crop is most vulnerable to bacterial blight (BLB) caused by *Xanthomonas oryzae* pv. *Oryzae* (*Xoo*). The incidences of BLB reported yield losses of 20–30% and as high 80–100% in moderate and severe infection conditions, respectively [21, 22]. This might be due to the partial grain filling resulted from low photosynthetic activity [23]. Out of already identified 45 BLB resistant (R) genes, 11 genes have been fine mapped and cloned till date applying modern biotechnological [24–27]. The marker-assisted pyramiding approach was applied to pyramid four BLB resistant genes; *Xa4, xa5, xa13* and *Xa21* in background of high yielding rice varieties to achieve wider and durable resistance [28–31]. The combination of these four BLB genes reported as most stable and showed resistance to most of the pathogen isolates [32, 33].

2.2 Blast

Rice blast (*Magnaporthe oryzae*) is another crucial threat to the rice production caused by fungus *Pyricularia oryzae*. It is affecting leaves, collar, nodes, panicles and panicle neck during vegetative to reproductive stage causing 10–30% under mild infestation [34–36] to 70–80% [37] to 100% [38] yield losses under severe infestation conditions. Till now, more than 100 blast resistant genes have been identified, however only 30 of them has been cloned and functionally characterized [17, 39]. The identified linked markers can be used effectively to provide resistance against this devastating fungal disease. The broad-spectrum blast resistant genes *viz. Pi9* which encodes the NBS–LRR gene clusters and *Pita2* which was mapped on the short arm of chromosome 12 confer resistant to many of the blast races in different countries [40, 41]. Transgenic rice lines carrying *Pi-d2* blast resistance gene transformed involving vectors pCB6.3 kb, pZH01–2.72 kb, and pCB5.3 kb showed various levels of resistance (~92%) against 39 strains of rice blast [42]. The durability of rice blast resistance can be further improved by the hybridization of rice varieties carrying complementary genes to attain multi-genic resistance against broad spectrum pathogen races [43], thereby reducing the selection pressure on a single isolate.

2.3 Brown plant hopper

Brown plant hopper (*Nilaparvata lugens*) is one of the most notorious insect-pest of rice causing large scale destructions across Asia amounting to around 60% crop loss [44]. In addition, it is responsible for the transmission of the virus diseases such as rice grassy stunt virus and rice ragged stunt (RRSV) viruses [45, 46]. To date, 37 BPH resistant genes on six of the total twelve rice chromosomes have been reported from cultivated rice and wild *Oryza* species [17, 47, 48]. Out of these 34 genes, 20 genes were fine mapped and only 8 genes (*Bph3*, *Bph14, Bph9, Bph17, Bph26, Bph18, Bph29* and *Bph32*) have been cloned and functionally characterized [49–56]. These resistance genes encode NBS-LRR protein family that are being widely used in marker-assisted breeding programs to develop rice varieties resistance to BPH. However, notable achievements have been made in identification and introgression of BPH resistance genes, rapid evolution of the virulent populations of BPH poses a prime concern. The Srilankan rice cultivar Rathu Heenati was first reported as the potential donor providing resistance against four BPH biotypes [57]. Even after 30 years of deployment in the Philippines, Rathu Heenati still reported to provide resistance to BPH [58]. The cloning and functional characterization of BPH resistant genes provides unique opportunity to effectively use these genes in marker-assisted gene introgression program [53].

2.4 Gall midge

The Asian rice gall midge (*Orseolia oryzae*) is another serious insect pest of rice prevailing mainly in wet season in the South-East Asia, China, and India, while *Orseolia oryzivora*, a closely related species is prevalent in the Africa. The infestation of gall midge (GM) amounting an annual yield loss of $550 million in different countries in Asia [59]. Till date, various genetic studies have identified 11 major resistance (R) genes providing resistance to 7 biotypes of the gall midge of rice which are prevailing mostly in South Asian countries [60–63]. Out of 11, eight of GM resistance genes (*Gm1, Gm2, Gm4, gm3, Gm6, Gm8, Gm7, and Gm11*) have been mapped successfully [64, 65]. Interestingly, none of the identified GM resistant genes confers resistance to all the biotypes of gall midge, while none of the GM biotype is virulent against all the identified resistance genes. Four gall midge resistance genes designated as *Gm1, gm3(NB-ARC), Gm2(NB-ARC),* and *Gm4 (NB-LRR)* have been functionally validated and linked markers can be used for the marker-assisted introgression program [63, 66–68]. Marker-assisted introgression/pyramiding of gall midge resistant genes (*Gm1* + *Gm4*) and (*Gm4* + *Gm8*) in background of improved Samba Mahsuri and an elite rice hybrid DRRH3 respectively was attempted by Divya et al. [62] and Kumar et al. [69]. Further, marker assisted pyramiding of multi-genes conferring to bacterial blight, gall midge, blast along with Saltol QTLs for salinity tolerance was reported [18, 33].

3. Abiotic stresses

3.1 Drought

Among the abiotic stresses, drought is one of the most disruptive, and risky events of the ongoing climate change that affect millions of people every year across the world. Depending upon the intensity and pattern of rainfall, drought can occur from few days to few months or even to years [70]. The development of high yielding drought-tolerant rice varieties is the final goal of rice breeders to reduce the yield losses due to drought and to ensure the projected world food production. However, the development of drought-tolerant rice varieties is immensely tough due to the complex quantitative nature of trait [71, 72]. The selection of lines under differential level of drought and due to the occurrence of drought at different stages [73–75] is again not an easy task. In addition, the strong GxE interactions and low heritability of traits such as grain yield also add to the difficulty of the task [76]. Cost-effective modified breeding strategy involving combined phenotyping and genotyping selection approaches in the development and screening of large segregating populations covering high genetic variation have led to the successful identification of 12 major effect QTLs ($qDTY_{1.1}$ on chromosome 1; $qDTY_{2.1}$, $qDTY_{2.2}$ and $qDTY_{2.3}$ on chromosome 2; $qDTY_{3.1}$ and $qDTY_{3.2}$ on chromosome 3, $qDTY_{4.1}$ on chromosome 4; $qDTY_{6.1}$ and $qDTY_{6.2}$ on chromosome 6, $qDTY_{9.1}$ on chromosome 9, $qDTY_{10.1}$ on chromosome 10, and $qDTY_{12.1}$ on chromosome 12) with consistent effects in background of widely cultivated, popular, high-yielding but drought-susceptible rice varieties, such as IR64, Swarna, Sabitri, MTU1010, Vandana and TDK1 [77–85].

3.2 Salinity

Salinization of soil is an another important crises the world is facing nowadays. Salty soil which is widely distributed across the world is major factor of rice yield reduction. The salt affected land in India accounts for 6.73 mha (million heactare) which is predicted to increase to 16.2 mha by 2050 [86, 87]. The complexity of salt tolerance mechanisms limits the development of high yielding salt tolerance rice varieties [88]. Salinity stress reported to affect rice grain yield from 20 to 100% depends on the severity of the stress and the duration of stress exposed to the rice crop [89]. Fortunately, the exiting wide genetic variability in rice germplasm in response to soil salinity stress makes possible to develop salt tolerant rice varieties [90–92]. The identification and introgression of the trait (s)/genomic regions of interest are the well-known approaches for the development of salinity tolerant varieties [93, 94]. Marker assisted breeding approaches have been proven successful in developing new improved, high yielding salt tolerance rice varieties [95–100].

3.3 High and low temperature

Global changes in the climate conditions and increasing greenhouse gas emission led to a rise in earth's surface temperature in some past decades, and the temperature is predicted to rise by 2 to 4°C by 2050 [101]. The high temperature duration of 3–5 days, 5–7 days and above 8 days is generally considered as mild, moderate and severe heat injury, respectively [102] while low temperature ranged from 0 to 15°C and <0°C categorized as chilling and freezing stress, respectively. Over the past few decades, extensive efforts have been made in identification of genes/QTLs improving heat [103–105] and cold tolerance [106, 107] in rice, which are very complex trait.

4. Marker-assisted pyramiding of multiple QTLs/genes for abiotic/biotic stresses

The challenges from the climate change scenario require the development of climate-adapted rice varieties that combine the tolerance of various abiotic and biotic stresses to better sustain yield losses from unpredicted climate-related events. Recent developments in the identification of major QTLs/genes for drought, submergence, salinity, bacterial blight, brown plant hopper, gall midge, and blast and the successful introgression of identified QTLs to develop improved varieties tolerant of different individual stresses indicate that, with the advent of new marker technology, the development of varieties that combine tolerance of the various abiotic and biotic stresses prevalent in any region is feasible. Such varieties once developed can help farmers overcome yield losses and better farm income under the changed climatic conditions.

The identification of major effect QTLs for the grain yield under drought $qDTY_{12.1}$ [77], $qDTY_{3.1}$ [80], and $qDTY_{1.1}$ [78]; *Sub1*, the gene for submergence [108, 109]; and Saltol, the QTL for salinity [110, 111] using modern breeding tools has provided novel opportunities to the breeders to develop the rice varieties

Variety	QTLs/gene combinations	Targeted trait	Targeted country	Year of release
DRR dhan-42	$qDTY_{2.2}$ + $qDTY_{4.1}$	Drought	India	2014
Yaenelo 4	$qDTY_{2.2}$ + $qDTY_{4.1}$	Drought	Myanmar	2015
Yaenelo 5	$qDTY_{2.2}$ + $qDTY_{4.1}$	Drought	Myanmar	2016
Yaenelo 7	$qDTY_{2.2}$ + $qDTY_{4.1}$	Drought	Myanmar	2016
CR dhan-801	$qDTY_{1.1}$ + $qDTY_{2.1}$ + $qDTY_{3.1}$ + *Sub 1*	Drought + flood	India	2017
Bahuguni dhan-2	$qDTY_{3.1}$ + *Sub 1*	Drought + flood	Nepal	2017
Bahuguni dhan-1	$qDTY_{1.1}$ + $qDTY_{3.1}$ + *Sub 1*	Drought + flood	Nepal	2017

Table 2.
Marker-assisted derived variety released in various countries of South Asia for tolerance to drought and flood in rice.

tolerant of abiotic stresses. Marker-assisted backcrossing of *Sub1* to improve popular variety Swarna had been successfully demonstrated to provide a yield advantage under submerged conditions for up to 18 days [112]. Similarly, the development of drought-tolerant versions of IR64 and Vandna [84, 113] at IRRI that are shown to possess a 1.0 and 0.5 t/ha yield advantage under drought over IR64 and Vandna, respectively, and successful introgression of Saltol into BR28 [114] are some of the recent successes using marker-assisted introgression.

Marker assisted gene pyramiding is an effective breeding strategy to transfer more than one tolerance/resistance genes into a single rice line in order to achieve durable and broader resistance level which can prevent the breakdown of tolerance/resistance against specific races/pathogens [115]. Pyramiding of BLB resistant genes such as *xa5* + *xa13* + *Xa21* [30, 31], *Xa4* + *xa5* + *Xa21* [29] and *Xa4* + *xa5* + *xa13* + *Xa21* [70] had been reported to provide durable resistance in rice against bacterial blight disease. Pyramiding of blast resistance genes *Pi9* and *Pita* has proven effective to combat the blast incidence and increase the durability of blast resistance genes [116].

Rice lines pyramided with multiple disease resistance genes (*Xa4, Xa21*, *xa5, Bph18* and *Pi40,*) has conferred resistance against BLB, blast, and BPH disease [29]. Among abiotic stresses, recently, drought and flood tolerance were combined using marker assisted pyramiding of the drought QTLs ($qDTY_{1.1}$ + $qDTY_{2.1}$ + $qDTY_{3.1}$) and submergence gene (*Sub1)* together in a popular rice variety, Swarna [19]. The marker-assisted derived rice varieties have been released in different countries (**Table 2**).

5. QTLs/gene pyramiding through multiple parents crossing

To tackle the multiple problems of rice cultivation under ongoing climate change, a high yielding climate smart new rice lines with superior grain quality is the urgent need to intensify the sustainable rice production. Genomics-assisted breeding (GAB) was attempted to introgress and assemble multiple QTL/genes- $qDTY_{1.1}$, $qDTY_{2.1}$, $qDTY_{3.1}$, $qDTY_{12.1}$, *Sub1, Gm4, Pi9, Pita2, Bph3, Bph17, Xa4, xa5, xa13, Xa21* and *Xa23* into the background of a high yielding breeding line suited for lowland ecosystem of rice under Stress Tolerant Rice for Africa and South

Asia(STRASA) project at IRRI. Developed climate resilient rice lines carrying 6–10 QTLs/genes combinations for tolerance to multiple biotic (BLB-*Xa4, xa5, Xa21, xa13* and *Xa23*; Blast-*Pita2*, *Pi9*; BPH-*Bph3 & Bph17* and gall midge-*Gm4*) and abiotic stresses (yield under drought stress- $qDTY_{1.1}$, $qDTY_{2.1}$, $qDTY_{3.1}$, $qDTY_{12.1}$ and submergence- *Sub1*) as well as superior grain quality traits are free from undesirable linkage drags and can be released as varieties on different countries after evaluation in the national system or can be used as an elite parental lines for making diverse crosses targeted to achieve high genetic gain [117]. Recently, introgression lines with 7 to 10 QTLs/genes for multiple-biotic stresses (blast, BLB, BPH and GM) with drought QTLs in background of Swarna has been reported [14]. A MAGIC (multiparent advanced generation intercross) population developmental strategy has also been proposed to examine the effect of multiple alleles to provide high grain yield, better grain quality, and tolerance to a wide range of multiple biotic and abiotic stresses [118].

An increase in rice productivity through introgression of multiple traits which can improve rice adaptability under dry direct seeded (DSR) and additionally carrying traits for abiotic/biotic stresses looks a promising breeding strategy to adapt with changing climate, limited water and labor resources and increase rice yield under mechanized DSR conditions. QTLs for traits that increase adaptability to direct seeded rice conditions such as root traits [nodal root number ($qNR_{4.1}$, $qNR_{5.1}$) and root hair density ($qRHD_{1.1}$, $qRHD_{5.1}$, $qRHD_{8.1}$)], early vegetative vigor ($qEVV_{9.1}$), early uniform emergence ($qEUE_{1.1}$, $qEUE_{11.1}$), grain yield under DSR conditions ($qGY_{1.1}$, $qGY_{8.1}$, $qGY_{10.1}$), and lodging resistance ($qLDG_{4.1}$) had been pyramided with abiotic stresses (drought QTLs-$qDTY_{1.1,}$ $qDTY_{2.1,}$ $qDTY_{3.1}$ and $qDTY_{12.1}$) as well as biotic resistance (gall midge-*Gm4*, blast- *Pi9*, *Pita 2*, bacterial leaf blight- *Xa4*, *Xa21, xa5*, *xa13*, and brown plant hoppers-*Bph3* and *Bph17*) using MAS approach [119, 120].

5.1 Steps in multiple-trait breeding

Three steps involved in multiple traits introgression scheme, (a) assemble first (b) line fixation and (c) line evaluation. In assemble first step, a complex crossing scheme utilized in transferring the desirable alleles/traits from all the targeted parents aimed to accumulate one copy of all targeted genes/QTLs in a single genotype. In line fixation step, gene based/SSRs/linked markers were utilized in each generation from F_2 to F_6 generation for tracking the presence of desirable alleles of targeted QTLs/in order to find homozygous plants carrying all the targeted QTLs/genes. Phenotyping of the homozygous lines for the targeted traits were performed in line evaluation step and proceed further for multilocation testing of promising lines in the targeted environments. The detailed description on traits, donors, QTLs/genes and markers associated that were used in genomic-assisted breeding program for the development of climate resilient lines at IRRI, Philippines (**Table 3**).

5.2 Challenges in multiple-trait breeding

The complex breeding program that targets combining tolerance of various abiotic and biotic stresses together in the various genetic backgrounds is unpredictable and more research is needed as such, little is known about the effect that each gene/QTL on the others. However, it is highly assumed that most of the QTLs/genes should work in an additive manner, as far as the targeted QTLs/genes are either located on different chromosomes or in different regions of the same chromosomes. Genomic interactions play a significant role in deciding the performance

Trait	Donor	QTLs/genes	Markers (SNPs/Indels/SSRs/gene based markers)	Reference
Biotic stress				
Blast	WHD-1S-75-1-127, Tadukan, IRBL9	*Pi9*, *Pita2*	*Pi9: Pi9*STS2, Pi9-659T, Pi9-1477G, MSU7_6_10381500 (M492 + M493), M891 (C), Pi9-659T, Pi9-1477G *Pita2:* MSU7_12_9177624 (M535 + M536), SnpOS00488(G), YL155/YL87, YL153/YL154	[40, 117, 121]
Bacterial leaf blight	IRBB60,	*Xa4, xa5, xa13, Xa21, Xa23*	*Xa4:* snpOS0054 (AG), RM224, MP1 + MP2 *xa5: xa5S*, *xa5R, xa5*DRR *xa13: xa13*-promoter (M478Lm + M479Lm + M480Lm), *xa13*F_130-147/*xa13* R_1678-1662 *Xa21:* Xa21s_exon (M769 + M770), snpOS0061 (C), U1/I1, M1207 (T), pTA248	[117, 122]
Brown plant hopper	Rathu Heenati	*Bph3, Bph17*	RM589,RM586,RM190, RM8213, RM16556, RM586, RM589, RM190, RM7639, RM19311(linked markers)	[14, 117, 123]
Gall midge	Abhaya	*Gm4*	GM4_LRR-del_F, GM4_LRR-del_R	[49, 117, 124]
Abiotic stress				
Drought + submergence	IR96322-34-223-B	$qDTY_{1.1}$ + $qDTY_{2.1}$ + $qDTY_{3.1}$ + *Sub1*	$qDTY_{1.1:}$ RM431, RM11943, RM12233(linked markers), snpOS0071 (A), snpOS0074 (G) $qDTY_{2.1:}$ RM324, RM3549, RM12868, RM12987, RM12995(linked markers), snpOS0078 (A), snpOS0079 (A) $qDTY_{3.1:}$ RM520, RM16030, RM416(linked markers), snpOS0085 (G), snpOS0089 (C) *Sub1:* ART5, snpOS0040 (T)	[19, 117]
Drought	IR74371-46-1-1	$qDTY_{12.1}$	RM28099, RM28166, Indel 8, SnpOS00483(G), SnpOS00484(A)	[77, 117]
Cold	IR 83222-8-1-1-1-1-1-1-1, IR 66160-121-4-4-2, HGKN	*qCTS4a, qCTS11.1*	*qCTS4a:* RM349, RM17604, RM17623, RM3648, RM2799 *qCTS11:* RM26889, RM21, RM206	[125, 126]
Heat	N22/IR64	*qHTSF4.1, qHTSF4.2*,	id4005120, id4011562	[104]
Salinity	Pokkali/IR29	*Saltol*	G11A, AP3206, RM3412, RM493	[110, 127]

Table 3.
List of traits, donors, QTLs/genes, and markers associated used in crossing program for the development of climate resilient lines at IRRI, Philippines.

of introgression lines pyramided with various drought grain yield QTLs in rice [128–131]. In some cases, epistatic interactions between different loci can enhance or reduce the effect of some of the genes/QTLs. Under such a situation, as an alternative strategy of identifying and advancing lines with different combinations of genes/QTLs- six, seven, or eight for different stresses and showing higher grain yield under nonstress conditions will also be selected and the best combinations that will show tolerance of a maximum number of abiotic and biotic stresses and the highest yield advantage will be advanced for testing. Plants carrying maximum number QTLs/gene but having negative interaction with grain yield and showing inferior plant type can be rejected for further advancement.

Maintenance of larger population size could also be a feasible strategy which can allow to select rare recombinants having maximum number of targeted QTLs/genes and also free from undesirable linkages. In previous studies, drought tolerant rice lines were developed through successful breakage of the linkages between loci for tolerance to drought and undesirable traits by fine mapping and maintain huge population size [132].

6. Role of genomic selection in multiple-trait breeding

Genomic selection (GS) in crop plants facilitates the rapid selection of superior accessions/genotypes and accelerate the breeding cycle targeted for higher genetic gain. It aims to use the genome-wide markers to predict the effects of all associated loci. The developed best prediction model is applied to the tested breeding material which has been charactized only genotypically but not phenotypically. The breeding estimated value called as GEBV (genomic estimated breeding value). The parental lines with higher GEBV can be selected as the candidate lines for future breeding programs. Most of the previous studied in cereal crops has shown great potential for GS to enhance the selection for grain yield and yield related traits [133, 134]. Multi trait genomic selection can be also implemented on phenotypic data of multiple traits *viz.* grain quality traits, grain yield and yield components, and reaction to the biotic and abiotic stresses, however it is important that a favorable genetic correlation exists between traits to implement genomic prediction model effectively [135, 136].

7. Conclusions

To solve the global issue of food security in the era of changing climate, novel approaches involving successful stacking of multiple genes/QTLs in a single rice line utilizing strategic phenotypic-genotypic selection could provide opportunity targeting genetic gain in rice. New advances in hybridization stratgies, genomics, marker development, and sequencing permitted the opportunity to create muti-gene carrying high-yielding rice varieties to combat multiple stresses. The development of rice varieties carrying multiple QTLs/genes in homozygous conditions can address the production constraints faced due to both biotic and abiotic stresses simultaneously. These stress-tolerant rice varieties with desired grain quality can greatly help farmers in improving productivity under multiple stress conditions.

Conflict of interest

The authors declare that they have no conflict of interest.

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Chapter 19

Salt Stress in Plants and Amelioration Strategies: A Critical Review

Sajal Roy and Nasrin Chowdhury

Abstract

High salt concentration in soil is a major abiotic stress, which adversely influences the growth, overall development, and productivity of crops. More than 20% of the land of the world used for crop production is adversely affected by high salt concentration. The problem of salt stress becomes a major concern when previously fertile, productive agricultural lands are salinized more profoundly as a result of anthropogenic activities along with natural causes. Therefore, this review is focused on various aspects of salt-affected soils (SAS), their effects on plants, and different approaches for reclamation of SAS to enhance the potentiality for crop production. Salt-affected soils are categorized into saline, saline-sodic, and sodic soils based on the amount of total soluble salts as expressed by electrical conductivity (EC), sodium adsorption ratio (SAR), exchangeable sodium percentage (ESP), and soil pH. The inhibition of plant growth in saline soils is mainly induced by osmotic stress; reduced uptake of essential macro- and micronutrients, including nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu); and specific toxicities of sodium (Na) and chloride (Cl). Sodic soils adversely affect the plant through high soil pH and poor physical condition resulting from an excessive amount of exchangeable Na. Different plants respond to salt stress in different extents. Salt-affected soils must be reclaimed to restore their productivity for increasing food production. The approaches for the management of SAS include leaching, incorporation of different organic and inorganic amendments, mulching, and development of salt-tolerant crops. The suitability of approaches depends on several considerations such as cost of reclamation, the time required, the extent of the salt stress, soil properties, availability of technology, and other environmental factors. Among different strategies, the incorporation of organic amendments is beneficial, cost-effective, environment friendly, and sustainable for amelioration of salt stress and enhancement of crop production due to the extensive roles of organic amendments in improving the soil's physical (structural stability, porosity, and permeability), chemical [pH, EC, ESP, organic matter, cation exchange capacity (CEC), and Na leaching], and biological and/or biochemical (microbial abundance, microbial activity, biomass carbon, and enzymatic activities) properties.

Keywords: abiotic stress, nutrient uptake, organic amendments, reclamation, salinity, sodicity

1. Introduction

The term stress in plants is defined as the environmental constraint that leads to the inhibition of morphological, physiological, and biochemical functioning of plants adversely affecting their growth and development [1–4]. The stresses may be biotic (pest, diseases, weed, etc.) or abiotic (soil salinity, radiation, water lodging, drought, extreme temperature, organic and inorganic pollutants, etc.) [4–12] that may act alone or in combinations, limiting the productivity of crops and food security worldwide. Salt-affected soils (SAS) cause greatest environmental abiotic stresses to plants [13–15] and cover more than 20% of the cultivated lands throughout the world [16, 17].

Salt-affected soils are grouped into saline, sodic, and saline-sodic and exhibit stresses to plants differently through various mechanisms. The principle mechanisms of salt stress in plants include osmotic effect, ionic toxicity, and nutritional imbalances [4, 15]. The increase in the uptake of Na and Cl caused by the salt constraint is attributed to the reduction in N and P concentrations that may be due to the antagonistic relations of Na and Cl with ammonium (NH_4^+), nitrate (NO_3^-), and phosphate ($H_2PO_4^-$) [18, 19]. The salt stress adversely affects plant growth by inhibiting various steps of N metabolism such as uptake, assimilation, and amino acid and protein synthesis [20]. High concentrations of Na ions at the root surface have detrimental effects on the uptake of K [21]. Because of the similar chemical nature of Na and K ions, Na has an adverse effect on K uptake by the root specifically through high-affinity potassium transporters (HKTs) and nonselective cation channels (NSCCs) [22, 23]. Similar to K, the uptake and transport of Ca and Mg can also be adversely affected by the high concentrations of Na commonly found in saline soils, resulting in lower Ca:Na and Mg:Na ratios in plants [24]. The concentration of micronutrients in plants may be increased, decreased, or remain unaffected under salt stress depending on the plant type, tolerance of plants to salinity, macro- and micronutrient concentrations in soil, pH of the soil solution, the adsorption phenomena on the surface complexes of mineral and organic particles, and different environmental conditions [1, 25].

The approaches for the management of SAS include removal of salts from the root zone through leaching, incorporation of organic and inorganic amendments, mulching, maintaining groundwater table, and cultivating crops tolerant to salt stress [26, 27]. In salt-affected areas, different methods are applied to remove excess soluble salts from the root zone of plants to improve crop growth and production. Scraping, flushing, and leaching are most commonly used, but these methods are found to be very expensive [28]. The application of organic amendments is effectively being practiced to ameliorate physical, chemical, and microbial complications associated with SAS. Organic amendments help to flocculate mineral particles to organic polymers because of their bonding or adhesion properties [29], resulting in a good structural stability, which is a precondition to maintain an appropriate soil structure. The application of organic matter to SAS can improve the aggregate stability and porosity, resulting in increased Na leaching and decreased exchangeable sodium percentage (ESP) and electrical conductivity (EC) values [30]. Several studies reported a significant increase in the soil microbial and enzymatic activities in SAS as a result of organic matter incorporation [31–33]. Salt-affected areas are increasing globally with the intervention of human activities and natural events. With the increase in salt-affected areas and population worldwide, the net cultivable land is decreasing, pushing enormous pressure on food security. It is pertinent to study how SAS harms plant growth and productivity as well as the strategies to ameliorate SAS by improving their physical, chemical, and biological conditions for the sustainable production of crops. Therefore, this chapter is arranged by compiling the existing knowledge in the literature in such a way that will demonstrate plant responses to salt stress, especially the uptake and accumulation of essential nutrient elements. Besides, different approaches that can be practiced for the amelioration of SAS have been discussed with the aim to improve soil health and boost up crop production in sustainable ways for ensuring food security worldwide.

2. Salt-affected soils: a brief preview

Understanding the differences in properties among SAS is important for proper reclamation and management. Salt-affected soils are categorized into saline, sodic, and saline-sodic groups based on the amount of total soluble salts (TSS) (measured by EC), sodium adsorption ratio (SAR; the ratio of Na^+ to Ca^{2+} and Mg^{2+} on the exchange sites of soil), exchangeable sodium percentage [ESP; the relative amount of the Na^+ ion expressed as a percentage (%) to the cation exchange capacity (CEC) or the sum of exchangeable bases], and soil pH. Based on these criteria, the classification of SAS is given in **Table 1**.

The pH of sodic soils is usually greater than 8.5, which may rise as high as 10.5. The high pH of sodic soils may be due to a greater extent of hydrolysis of exchangeable Na compared to more strongly held ions such as Ca and Mg. Upon hydrolysis, exchangeable Na contributes to high soil pH according to the following phenomenon [26]:

$$\mathrm{Na-clay-Na+H-OH} \leftrightarrow \mathrm{Na-clay-H+Na++OH-} \tag{1}$$

The increase in the concentration of OH^- ion contributes to the increase in soil pH. The limited hydrolysis of $CaCO_3$ and $MgCO_3$ causes low solubility of these salts, resulting in soil pH no higher than 8.5, while soils containing Na_2CO_3 have a pH of more than 8.5 or even 10 to 10.5 due to their higher solubility [26, 34, 35].

The processes by which saline soils are formed are known as salinization, whereas the processes that are responsible for the formation of sodic soils are called as sodification. The causes of salinization and sodification are multifactorial, and one factor that affects the salinization and/or sodification may influence the others [36]. Soil salinization and sodification are interrelated with such factors as soil characteristics; the amount and composition of salts in the soil; and the quantity, quality, and methods of irrigation [37]. Salt-affected soils are formed either by natural processes or anthropogenic activities. Natural processes are known as primary salinization/sodification, whereas human-induced processes are known as secondary salinization/sodification [38]. The main sources of soluble salts under natural and anthropogenic factors involving the development of SAS are summarized in **Figure 1**.

Types of SAS	pH	EC (dS/m)	SAR	ESP	Major cations and anions and their relative concentrations
Saline	<8.5	>4.0	<13	<15	$Cl^- > SO_4^{2-} > HCO_3^- > CO_3^{2-}$ $Na^+ > Ca^{2+} + g^{2+} > K^+$
Saline-sodic	<8.5	>4.0	>13	>15	High content of Na^+ on exchange sites as well as in soil solution
Sodic	<8.5	>4.0	>13	>15	High Na^+ on exchange site of soil particles with little amount in soil solution

Table 1.
Classes of SAS.

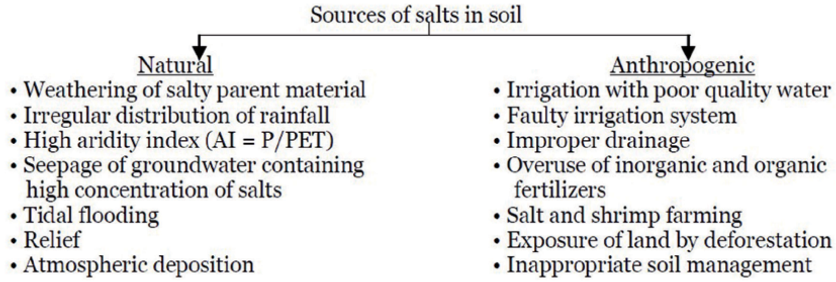

Figure 1.
Natural and anthropogenic sources of salts in soils.

Soil salinity and sodicity have contributed to the changes in land use/land cover features over the years, which are directly related to land degradation and results in many changes in the environment [39]. More than 20% of the total agricultural lands are affected by high salinity [38, 40–42], which accounts for more than 7% of the world's total land area [23, 43]. It has been reported that the majority of the SAS occur mainly in the arid and semiarid regions of Asia, Australia, and South America, covering an estimated 1 billion ha [36]. Data summarized from Abrol et al. [34] and Szabolcs [44] give an account that globally about 932 million ha areas are occupied by SAS. There are sporadic studies on the estimates in the global distribution of salt-affected areas in recent years. For the establishment of better management strategies of SAS, it is important to identify their extent and distribution in systematic ways. However, different countries assess the extent and severity of their SAS at national levels.

Soil salinity can be determined by measuring either the total soluble salts (TSS) by evaporation of a soil-water extract or by determining the EC of a soil-water suspension. In the laboratory, EC as an indicator of soil salinity can be measured by analyzing the soil suspension either in a 1:5 soil:distilled water dilution ($EC_{1:5}$) or in a saturated paste extract (EC_e). The measurement of $EC_{1:5}$ is most commonly employed from an unfiltered 1:5 soil-water suspension prepared by taking a unit of 2-mm sieved soil (usually, 5 g) and 5 units of distilled water (25 ml). The suspension is shaken for 30 min to dissolve the soluble salts and left for 15 min to settle down the soil particles, followed by the EC measurement using EC meter after necessary calibration [45].

3. Mechanisms of salt stress in plants

Though all soils invariably contain soluble salts, under saline and sodic conditions, excess salts in the root zone deteriorate the physical, chemical, and biological properties of soils to such an extent that the crop growth is adversely affected [46]. The adverse effects of salinity on plant growth depend on plant factors such as growth stage, species, and variety; soil factors such as temperature and moisture, degree of salinity, and presence of heavy metals; and environmental factors such as growing season, temperature, humidity, light, pollutants in the atmosphere, etc. [47–52]. Based on the degree of salinity and associated effects on the plant growth, the saline soils can be classified from nonsaline to very strongly saline (**Table 2**).

The mechanisms of how the SAS distress plant growth are shown in **Figure 2**. Under saline environment, the inhibition of growth is mainly induced by the physiological and biochemical disturbances resulting from osmotic stress; changes in the uptake of mineral nutrients such as N, P, K, Ca, and Mg; and specific ion toxicities

Classes of saline soils	EC_e (dS/m)	Salinity effects on plant	Yield loss (%)
Nonsaline	0–2	Salt effects are negligible	0
Slightly saline	2–4	Yields of only sensitive crops may be restricted, and the yields of most of the crops are not likely to be affected	20–30
Moderately saline	4–8	The yields of most of the crops are likely to be hampered	30–60
Strongly saline	8–16	Most of the crops are likely to be affected, while the yields of only tolerant crops are satisfactorily	60–100
Very strongly saline	>16	Only limited tolerant plants can sustain	100

Adapted and modified from Shin et al. [53].

Table 2.
Soil salinity classes and associated effects on plants.

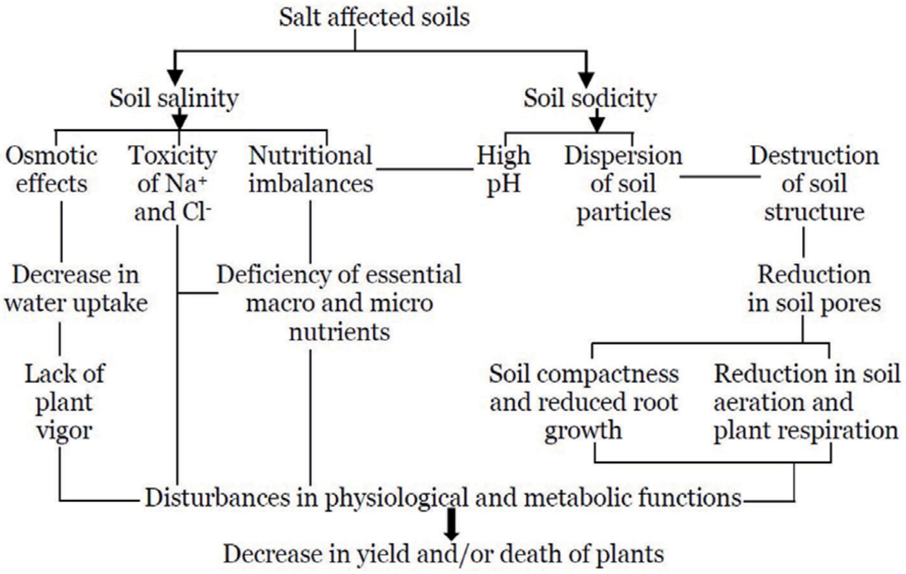

Figure 2.
Mechanism of salt stress in plants.

such as Na and Cl [54–57]. Plants containing approximately 0.25–0.5% Na in leaf tissues on dry weight basis may exhibit toxicity symptoms such as necrosis and burns of leaf edges [1, 58]. When the salt concentration in the soil is equal to that of the plant, there is no net movement of water. But when the salt concentration in the soil solution is greater than that of the plant, water moves from the plant into the soil causing physiological drought [59, 60]. To counteract the low osmotic potential of the soil solution, plants accumulate organic and inorganic solutes to reduce the osmotic potential inside the cell. Maintenance of such osmotic adjustment requires a considerable expenditure of energy, resulting in reduced growth [35]. Besides direct effects of SAS through osmotic stress and nutritional imbalances, the growth of plants may be adversely affected by a high content of exchangeable Na relative to Ca and Mg on soil particles which leads to the breakdown of soil structure, resulting in decreased porosity, permeability, hydraulic conductivity, and aeration in the vicinity of plant root [35, 41].

The adverse effects of salts are first expressed by the inhibition of growth and other physiological symptoms. Salt stress has been found to negatively affect the biomass of cabbage, *Brassica oleracea* [19]; cotton, *Gossypium hirsutum* [61]; Jerusalem artichoke, *Helianthus tuberosus* [62]; pistachio, *Pistacia vera* [63]; poacea, *Catapodium rigidum* [15]; rice, *Oryza sativa* [64]; saltmarsh grass, *Spartina alterniflora* [65]; strawberry, *Fragaria ananassa* [66]; tomato, *Lycopersicon esculentum* [64]; and wheat, *Triticum aestivum* [67].

The capacity of roots to transport nutrients and water to shoots is restricted due to abiotic stress, adversely affecting the functional balance between roots and shoots, which results in lower shoot:root ratio [68]. The leaves of plants are the most sensitive organs and greatly affected, while the roots are reasonably less affected when exposed to high salinity [69]. However, all plants do not respond to salinity to the same extent. Different crops exhibit a broad spectrum of responses to salt stress.

4. Salt stress on nutrient uptake of plants

Though the most common cations found in SAS are Na^+, Ca^{2+}, and Mg^{2+}, accompanied by anions Cl^-, SO_4^{2-}, and HCO_3^-, these soils are mainly dominated by Na^+ and Cl^- [35, 45, 70]. Nitrogen, P, K, Ca, and Mg play important physiological functions in plants, and their replacement by Na and Cl may lead to nutritional imbalances [47]. For the optimum growth and yield of crops, an adequate and balanced supply of mineral nutrients is essential [42]. High contents of Na and Cl in the rhizosphere can interfere with the uptake of essential elements, leading to their deficiencies or imbalances [71, 72] through the processes of fixation, adsorption, and transformation in soil [18]. The effects of high concentrations of Na and Cl on the uptake of essential elements by plants are outlined in the following subsections.

4.1 Nitrogen

Nitrogen is a major constituent of the nucleotides and proteins of all micro- and macroorganisms [9, 73]. It is considered as one of the limiting factors and is required in large quantities compared to other essential nutrients for the growth, yield, and quality of crops [74–77]. In general, the total N content in the plow layer of mineral soils ranges from 0.05 to 0.2% corresponding to approximately 1750 to 7000 kg N/ha, and in plants, it ranges from 1 to 4% on dry matter basis [77]. Nitrogen rarely exists in the lithosphere in rock deposits, and more than 90% of the total N comes from the soil organic matter [35]. It is principally taken up by plants in the forms of NO_3^- and NH_4^+ [61]. As a consequence of high salinity, the uptake and assimilation of NH_4^+ and NO_3^- are inhabited by excessive concentrations of Na and Cl. The uptake of other essential nutrients may be disturbed due to the inhibition of N metabolism as the presence of N in the growth medium stimulates the uptake and assimilation of other essential nutrients [77]. Decreases in N uptake may be a physiological response of plants under salt stress [78].

Salt stress was found to strongly inhibit the uptake and accumulation of N in different plant parts of green chireta, *Andrographis paniculata* [14]; cabbage, *Brassica oleracea* [19]; canola, *Brassica napus* [78]; cotton, *Gossypium hirsutum* [61]; gray poplar, *Populus × canescens* [20]; saltmarsh grass, *Spartina alterniflora* [65]; sesame, *Sesamum indicum* [79]; and wheat, *Triticum aestivum* [67]. However, the uptake and accumulation of N in response to salt stress depend on different plant and soil factors [80]. Kanagaraj and Desingh [79] observed variations in foliar N among different varieties of sesame, where the maximum reduction was found at the highest dose of NaCl.

The decline in the uptake of N by plants could be due to the increased uptake and accumulation of Cl. The antagonistic relationship between the uptake of NO_3^- and Cl^- was found in rice, *Oryza sativa* [64]; cabbage, *Brassica oleracea* [19]; and barley, *Hordeum vulgare* [81, 82]. High soil salinity was found to increase the concentration of Cl in the different plant parts of cotton, *Gossypium hirsutum* [47]; cowpea, *Vigna unguiculata* [72]; and poacea, *Catapodium rigidum* [15]. However, the accumulation of Cl is regulated by the duration of salt stress as well as the phase of the plant life cycle [83]. As a consequence of high salinity, the uptake of NH_4^+ can also be reduced. It was observed that the decreased uptake of NH_4^+ in plants was accompanied by an increased $Na^+:NH_4^+$ ratio in the growth medium [20].

Several studies reported inhibited accumulation of nutrients with decreasing water uptake under salt stress [15, 64]. The effect of salt stress on N metabolism varies depending on the forms of N applied [20]. Botella et al. [67] reported that the increased concentration of NaCl in the root medium decreased the net uptake of N more profoundly in NO_3^- compared to the NH_4^+ form when their compounds were incorporated as the source of N, which was assumed to be a reason for the greater affinity for NH_4^+ compared to NO_3^- under the saline environment. Similarly, Saud et al. [84] found higher N content, N isotope abundance, and relative water content in both roots and leaves of Kentucky bluegrass, *Poa pratensis*, in $^{15}NH_4^+$ compared to the $^{15}NO_3$ treatment under abiotic stress. By contrast, Dluzniewska et al. [20] observed that the net uptake of NH_4^+ decreased in poplars when exposed to increased NaCl concentrations, resulting in decreased whole-plant N content in comparison to control. Hofman and Cleemput [77] stated that NH_4^+ is more preferably taken up by plants in comparison to other forms as it does not require to be reduced before incorporation into plant compounds. On the other hand, Dai et al. [61] found better root growth and low Na content in NO_3-fed compared to NH_4-fed cotton seedlings, and they reported the superiority of NO_3-N relative to the NH_4-N source in the uptake of N under salt stress. The high salt content in soil may also interrupt the synthesis of protein in plants. Chakraborty et al. [85] observed that the uptake of N of the *Brassica* spp. decreased due to the high salt content, which reflected in the low protein levels in seeds. The physiological drought of plants, which is caused by the low osmotic potential of soil solution, is attributed to the reduced metabolism of N [18] that may result in the low protein content under saline condition.

4.2 Phosphorus

Phosphorus is the second most essential nutrient requiring 0.3–0.5% of the dry matter for the optimal growth of plants [86]. It is an integral part of nucleic acids and membrane lipids [73]. In contrast to N, the main sources of P in the lithosphere are the rock deposits [77]. Phosphorus exists in the soil solution as orthophosphate ions such as $H_2PO_4^-$, HPO_4^{2-}, and PO_4^{3-} [87] and plants take up P from the soil solution in the form of $H_2PO_4^-$ and HPO_4^{2-}, although $H_2PO_4^-$ is taken up to a greater extent [88, 89]. Exposure to high salinity resulted in significant decrease in P levels in green chireta, *Andrographis paniculata* [14]; cabbage, *Brassica oleracea* [19]; canola, *Brassica napus* [78]; pistachio, *Pistacia vera* [63]; saltmarsh grass, *Spartina alterniflora* [65]; and spinach, *Ipomoea aquatica* [90]. The salt stress had been found to magnify the adverse effects on the uptake of P by plants in several studies when plants were exposed simultaneously to both salinity and drought [19, 65]. The reduction in P concentration under high salinity may be due to the competition between $H_2PO_4^-$ and Cl^- ions [18]. The accumulation of P in plants exposed to salt stress varies with the plant organs. Silva et al. [72] found a greater P content in leaves compared to roots. Conversely, Shahriaripour et al. [63] reported that the translocation of P from root to shoot was inhibited under a high saline environment.

The evidence of increased P under high salinity had also been reported in several studies. The presence of NaCl in nutrient solution resulted in an increased concentration of P content in leaves [72, 83]. Zribi et al. [15], however, demonstrated that salinity had no significant effect on both P concentration and acquisition efficiency (PAE), and they also found a higher concentration of Cl in the shoot when subjected to both salt stress and P treatment compared to salt stress only.

The uptake of P by plants under salt stress is influenced by the plant species, plant stage, degree and extent of stress, temperature, moisture, soil pH, and the prevailing soil P level [55, 83, 89]. The solubility of P may also be influenced by strong sorption-desorption processes with divalent cations such as Ca^{2+}, Mg^{2+}, and Fe^{3+} at different pH ranges because of their high sorption capacity regulating the uptake by plants [5, 87, 91].

4.3 Potassium

Potassium is an important and abundant mineral nutrient that comprises 2.6% of the earth's crust [92] and 1–10% of the plant tissue on a dry weight basis [92, 93]. It contributes to important physiological, biochemical, and biophysical roles such as photosynthesis, osmotic adjustment, and turgor maintenance [94, 95], regulating the growth and development of plants. The concentration of K in the growth medium determines the net uptake of K [13]. The seldom deficiency of K in most soils is believed due to comparatively high concentration and greater mobility [96, 97]. However, in the condition of salt stress, the plants may suffer from K deficiency as a result of Na toxicity. Both Na and K are monovalent cations with similar physicochemical properties. The hydrated ion radius of Na ion is 0.358 nm, while that of K ion is 0.331 nm [23]. Due to similar ionic radius and cationic competition for entry into the plant cells, high concentrations of Na can adversely affect the uptake and accumulation of K in plants.

Salt-affected soils led to a significant influence on K in plant tissues, either increasing or decreasing its concentration. The reduction in K content in plant tissues is one of the primary responses of plants to high Na, which ultimately causes nutrient imbalances [98]. High salt stress with increased concentrations of NaCl was found to decrease the total K and cause an increase in the Na content in *Aloe vera*, *Aloe vera* [99]; barley, *Hordeum vulgare* [100]; bean, *Phaseolus vulgaris* and *P. acutifolius* [54]; cabbage, *Brassica oleracea* [19]; cotton, *Gossypium hirsutum* [47]; green chireta, *Andrographis paniculata* [14]; maize, *Zea mays* [101]; pistachio, *Pistacia vera* [63]; poacea, *Catapodium rigidum* [15]; rice, *Oryza sativa* [102]; saltmarsh grass, *Spartina alterniflora* [65]; strawberry, *Fragaria ananassa* [66]; and tomato, *Lycopersicon esculentum* [13, 103, 104]. A positive relation of salt-tolerance capacity with Na and a negative relationship with that of K was found in saltbush, *Atriplex canescens* [105]. Besides, there is evidence that both the contents of Na and K in plants increased with the increase of salinity. The concentrations of Na and K in the above-ground and below-ground portions of Jerusalem artichoke, *Helianthus tuberosus*, were found to increase with the increasing amount of seawater application in a greenhouse experiment [62]. Similarly, the concentrations of Na and K in the leaves of cowpea, *Vigna unguiculata*, increased in salt-stressed condition and the responses differed depending on the duration of stress and leaf age [83]. Al-karaki [13] found higher translocation of K from root to shoot in tomato plants to a greater extent in the saline environment in comparison to nonsaline conditions, with the increase of K concentration in medium.

The higher concentration of Na restricts the transport of K by impairing the route AKT1 (hyperpolarization-activated inward-rectifying K channel) used for the uptake of K and, in this way, reduce the uptake of K [106]. The inhibitory effect of Na on the transport of K through channels in the membranes is probably more important in the phase of uptake of K from the soil solution than in the phase of K transport to the xylem [107]. Besides, the inhibitory effect of Na on K translocation depends on the concentration of K in solution. The effect is lower with low Na and high K levels in the medium [13, 108, 109]. However, the effects of Na on the uptake and accumulation of K can vary among species and even between varieties within such same species of *Aloe vera*, *Aloe vera* [99]; bean, *Phaseolus vulgaris* [110]; cotton, *Gossypium hirsutum* [111]; green chireta, *Andrographis paniculata* [14]; mustard, *Brassica nigra* [112]; tomato, *Lycopersicon esculentum* [104, 113]; and wheat, *Triticum aestivum* [109]. Salt-tolerant species were found to maintain a high concentration of K and low concentration of Na. High concentration of K and low concentration of Na in plants under saline environment can be considered as a good indicator for salinity tolerance [114]. The plant parts also differently respond to salt stress. Generally, a higher concentration of Na in roots relative to leaves in various plant species was explained by the high tolerance in roots in addition to reduced translocation of Na from root to leaf [1]. Different authors reported lower content of K as a result of salt stress to a greater extent in stems in comparison to leaves and roots [99, 110]. A high concentration of K in leaves is important to maintain constant photosynthesis and leaf stomatal conductance [54].

4.4 Calcium and magnesium

Calcium is the fifth most plentiful element, whereas Na is the sixth most abundant element comprising, respectively, about 3 and 2.6% of the earth's crust. Because of the greater charge density of Ca^{2+} compared to Na^{+}, it attracts more water, resulting in a greater hydrated ionic radius of 0.44 nm [48]. Calcium is an essential inorganic nutrient and plays a vital role in maintaining the structural and functional integrity of cell wall and membrane because of its ability to form intermolecular linkages [98, 113]. Considering the importance of external Ca in enduring K transport and K:Na selectivity in plants under Na stress condition, a large number of research was carried out on Na:Ca interactions. The deficiency of Ca in plants is a common indication of Na toxicity [115]. The Na:Ca ratio of plants is a good indication used to express the extent to which a plant can survive under salt stress [69]. A high concentration of Na can decrease the uptake of Ca and Mg by inhabiting their influx through roots, decreasing the extracellular binding sites of Ca and Mg in the plasma membranes [69, 116], decreasing the osmotic potential [19] under saline condition.

Increased level of Na was found to significantly decrease the concentrations of Ca and Mg in saltmarsh grass, *Spartina alterniflora* [65]. Significant negative correlations were also found between salt stress and the contents of Ca and Mg, where the ratios of Ca:Na and Mg:Na in the leaves and roots of cabbage, *Brassica oleracea*, decreased with the increasing salinity levels [19]. Similar results were reported by other authors where the salt stress resulted in an increased accumulation of Na and Cl and decreased the contents of Ca and Mg in plants [18, 117]. On the other hand, Chen et al. [47] found both higher Na and Ca in cotton leaf with increasing salt stress. Rahimi and Biglarifard [66] observed that the Ca and Mg concentrations decreased in the shoot and increased in the root of strawberry, *Fragaria ananassa*, with the increase in salinity. There were evidences from several studies that the supplemental addition of Ca increased the concentration of Ca in rapeseed, *Brassica napus* [114], and in tomato, *Lycopersicon esculentum* [113] under high salinity levels. However, the effects of salinity had been found more detrimental in conditions having different levels of drought stress [19, 65, 118].

The influx of Ca and Mg in the root and transport to the shoot can vary with the amount of Na as well as genotypes. The uptake of Ca had been found to be higher in a salt-sensitive variety of wheat, *Triticum aestivum*, at a low level of Na and the transport became lower as the concentration increased [115]. Furthermore, tolerant varieties of green chireta, *Andrographis paniculata*, contained high concentrations of Ca and Mg and low concentration of Na in comparison to sensitive accessions under high salinity [14].

4.5 Micronutrients

Micronutrients are involved in various important physiological and biochemical functioning of plants, including enzyme activation, chlorophyll formation, protein synthesis, carbohydrates, and lipids and nucleic acids metabolism [119]. Different authors reported differently in the uptake and accumulation of micronutrients such as Fe, Mn, Zn, and Cu in salt-stressed condition. Though the exact mechanism in the literature is scarce, different soil, plant, and environmental factors are believed to influence the uptake and accumulation of micronutrients in different plant parts under salt stress. The response of micronutrient concentrations in plants under salt stress was found to be variable in previous studies. The plants grown in saline soils often showed the deficiency of micronutrients such as Fe, Zn, Mn, and Cu, which was assumed to be the result of their low solubility and availability [120]. A negative relationship was found between the salt content and Cu, Mn, and Fe concentration in tissues of *Avicennia marina* (Forssk.) Vierh [49]. Chakraborty et al. [85] found a reduced accumulation of Fe, Mn, and Zn in different parts of *Brassica* spp. at the flowering and post-flowering stages when exposed to high salt concentration. Similarly, salt stress had been found to decrease the concentrations of Fe and Zn in the roots and leaves of cabbage, *Brassica oleracea* [19]. On the other hand, the concentrations of Fe, Zn, and Cu in green chireta, *Andrographis paniculata*, significantly increased though the Mn content decreased under high salinity [14]. The concentrations of Fe, Mn, and Zn increased in the above-ground part of strawberry, *Fragaria ananassa*, while their contents did not change in the root when plants were exposed to salt stress. On the other hand, while Cu content did not change in the aerial part, the concentration increased in the root as a result of salt stress [121]. Significant reduction in the Fe content in both root and shoot of strawberry was observed, while Zn, Cu, and Mn concentrations remained unaffected under salt stress [66]. The change in concentrations of Fe, Zn, and Mn was not found as a limiting factor for the growth of wheat, *Triticum aestivum*, and their contents in plants were not much affected by salt stress [25]. The response of micronutrient concentrations under salt stress differed among varieties in green chireta, *Andrographis paniculata* [14]; *Brassica* spp. [85]; and strawberry, *Fragaria ananassa* [121].

5. Reclamation of salt-affected soils

To make SAS productive, integrative soil, water and agronomic reclamation, and management approaches can be practiced. Saline and sodic soils differ in their reclamation and management practices because of their unlike complications. The problem of saline soils is mainly oriented with high soluble salt content, whereas sodic soils are associated with high exchangeable Na. The suitability of approaches depends on such considerations as cost of reclamation, the time required, the extent of the salinity or sodicity problem, soil properties, availability of technology, and other environmental factors [26]. This section will mainly focus on the existing information in the literature on various aspects, including the effectiveness and downsides of the leaching approach for removing salts as well as the application of organic and inorganic amendments as ameliorative for the restoration of SAS.

5.1 Removal of salts

Removing salts from the root zone is the first requirement to restore productivity of saline areas. However, saline-sodic and sodic soils cannot be reclaimed only by the leaching approach. Thus, to improve the growth and yield performance of crops in saline soils, the harmful concentration of salts must first be washed out from the root zone, which can be achieved by leaching, the most effective procedure for removing excess soluble salts. The efficiency of leaching to remove the salts from the soil profile depends on such factors as the initial salt content, nature of soluble salts, desired EC of soil after leaching, properties of soil, quality of water to be used for leaching, etc. [34]. The key to leaching of soluble salts is to provide an appropriate amount of water at the proper time with adequate drainage. A reliable estimate of the favorable soil moisture content is essential to alleviate the harmful effects of salinity by leaching. In general, depth of water equal to the depth of soil removes 70–80% of the soluble salts for continuous ponding, that is, 15 cm of water is required to reduce the salt content by 70–80% in the upper 15 cm of soil. However, as continuous ponding leads to reduced soil aeration and quick loss of water in coarse-textured soils, intermediate ponding or sprinkler irrigation is preferred for more efficient leaching of salts through increasing contact time of salts with water [122]. On the other hand, prolonged drying may increase the concentration of salts in soil solution, resulting in lower osmotic potential of the soil solution. Due to high permeability and less workability, a large volume of water can be leached over a shorter period in coarse-textured soils. Fine-textured soils having high CEC and organic matter require more water to remove the salts from the soil profile [123]. The desalinization of soils through leaching also depends upon the drainage condition of the soil. The application of leaching with surface drainage at shallow groundwater levels may further exacerbate salinity problems, while the subsurface drainage can sustain the groundwater depth and prevent additional salinization [124]. The role of organic amendments on the reclamation of SAS through improvements in physical properties found in the literature has been presented later in the organic amendment section. Soils having a good structure and internal drainage favor the leaching process [125]. Therefore, a judicious application of water having proper drainage at the right time is important to prevent irrigated lands from becoming saline. The leaching process is accomplished by either natural precipitation and/or artificial irrigation water containing minimum salt content and allowing the water to drain out. The salinity problem of the agricultural land may become worse if saline water is used for leaching purpose because of considerable quantities of such cations as Na^+, Ca^{2+}, Mg^{2+}, and K^+ and such anions as Cl^-, HCO_3^-, and SO_4^{2-} in saline water [126, 127].

5.2 Reclamation with organic amendments

Appropriate nutritional management is often considered feasible and cost-effective, which can lead to the better performance of plants grown in contrasting environments by reducing the adverse effects [128–130]. The application of organic manures as amendments to reclaim SAS is considered highly sustainable and commonly practiced over the last few years [29, 131]. Due to the high cost and quick release of nutrients involved in chemical fertilizers, the application of organic amendments as the substitute of chemical fertilizers or in a combination with chemical fertilizer has gained worldwide acceptance from the farmers [132]. Besides, the extensive application of inorganic fertilizer contributes to groundwater pollution due to leaching loss, and the global climate change is caused due to the emission of potent greenhouse gas such as nitrous oxide from agricultural lands [133, 134]. Though inorganic fertilizers provides easily available nutrients for plant, these are quickly lost from the soil. On the other hand, organic manures contribute to the physical and biological improvements of soil with a gradual release of nutrients. The incorporation of organic manures along with the chemical fertilizers is considered as an effective and sustainable approach for enhancing the resistance of crops to abiotic stress [135–138]. Different sources of organic materials originating from plant and animal residues such as green manure, cattle manure, poultry manure, food processing wastes, etc. are used to augment the organic matter content and nutrient status. The role of organic amendments on physical, chemical, and biological properties of soils having salinity and sodicity problems is shown in **Table 3**.

Sources of organic amendments	Rate	Soil condition (pH, EC, and ESP/SAR)	Effects on soil properties	Reference
Farmyard manure, and sewage sludge	5 and 10 t/ha	Different sites having variable PH and EC	Decreased bulk density, increased available water and hydraulic conductivity, decrease in pH, and increased organic matter and available macronutrients	[139]
Biochar, green waste compost (GWC), and municipal sewage sludge	1.0, 2.5 and 5.0%	pH: 4.04 $EC_{1:10}$: 4.91 ESP: 67.62	Increase in pH, total organic carbon, and CEC; decrease in EC, ESP, and SAR; and increase in catalase activity and acid-and alkaline phosphatase activity	[140]
Vermicompost and compost	5 and 10 Mg/ha	pH: 7.3 EC_e: 4.26	Decrease in pH and EC, exchangeable Na^+; increase in organic carbon, CEC, exchangeable K^+, Ca^{2+} and Mg^{2+}; and increased microbial C and N, and basal soil respiration	[32]
Municipal solid waste and palm waste	50, 100, and 150 t/ha	pH: 7.97 ECe: 5.13	Increased organic carbon and N; increased microbial biomass and enzymatic activities	[141]
Cow dung, and paddy husk	1 g/kg	pH: 7.86 EC_e: 24.35 SAR: 26.53	Decreased bulk density; decreased EC and SAR; and increased exchangeable Ca^{2+}	[142]
Cotton gin crushed compost and poultry manure	5 and 10 t/ha	pH: 8.0 $EC_{1:5}$: 9.1 ESP: 15.7	Decreased bulk density and ESP; increased soil microbial biomass, respiration, carbohydrate, and enzymatic activities	[33]
Green waste compost (GWC), sedge peat (SP), furfural residue (FR), and a mixture of GWC, SP, and FR (GSF) (1:1:1 by volume).	4.5 kg/m^3	pH: 7.75 $EC_{1:5}$: 3.69 ESP: 15.8	Decreased bulk density; increased total porosity; increased CEC, organic carbon, and available nutrients (N, P, and K); and decreased EC and ESP	[30]

Sources of organic amendments	Rate	Soil condition (pH, EC, and ESP/SAR)	Effects on soil properties	Reference
Cattle dung, vermicompost, biofertilizer, and their combinations	1.8, 3.7, and 5 t/ha	pH: 7.39 $EC_{1:5}$: 7.44	Decreased EC; increased organic matter and available nutrients; and increased microbial biomass carbon	[143]
Pig manure, cattle dung, chicken manure, rapeseed meal, and biochar	50 g/kg	pH: 8.29 $EC_{1:5}$: 19.35 ESP: 67.62	Decrease in pH; increase in organic carbon, K, Ca, and Mg; and increased enzyme activities such as catalase, urease, alkaline phosphatase, and saccharase	[144]
Poultry manure, commercially available organic fertilizer, reed straw, and fresh reed straw with green leaves	15 g/kg	pH: 8.44 $EC_{1:5}$: 11.61	Decreased bulk density; increased available N, water-soluble organic carbon; decreased SAR; and increased soil respiration	[145]
Straw, composted straw, fresh reed, and chicken manure	15 g/kg	pH: 7.82 $EC_{1:5}$: 6.59 ESP: 36.17	Decreased pH, ESP; increased organic carbon, CEC, macronutrient concentration; and increased soil respiration	[146]

Table 3.
Effects of organic amendments on the physical, chemical, and biological properties of SAS.

Organic amendments have profound influences on soil's physical properties. Several studies revealed that the application of organic manures decreased the bulk density [33, 147–149] and penetration resistance [149], whereas it increased the aggregate stability [150–152], total porosity [152, 153], hydraulic conductivity, and permeability [150, 154]. Soil organic matter is an important attribute of soil quality and aggregate stability, which is influenced by the inherent properties of soil such as soil type and texture [155, 156] as well as agronomic factors such as management, inputs, and nature of the organic matter [157]. Improvement in the aggregate stability is related to increased soil porosity and decreased bulk density. As a consequence of good aggregation with a concomitant increase in porosity and decrease in bulk density, soil water infiltration is facilitated causing the soluble salts to leach down, and an adequate oxygen supply is maintained in the root zone which is necessary for crop production in SAS.

The incorporation of organic amendments had also been found to improve chemical properties such as decrease in pH [27, 147, 158, 159], EC [27, 158, 160], ESP [33, 158], and SAR [27], while there is an increase in the soil organic matter [153, 161], organic carbon [132, 147, 151, 152, 159, 162–164], CEC [152, 162], total nutrients [32, 151, 161], and available nutrients [32, 132, 147, 149, 153, 159, 162, 164, 165].

The decline in soil pH resulting from the incorporation of organic amendments reported in several studies can be explained by the acidic nature of the amendments. Microbial activities resulting from the incorporation of organic materials release carbon dioxide that reacts with water to form carbonic acid, and thus contribute to lowering of the soil pH [166]. The pH of soils was also found to be increased by the addition of organic amendments in several studies [30, 33, 141, 143, 167]. Roy and Kashem [132] found that the addition of organic amendments slightly increased the soil pH initially which thereafter declined significantly with time. However, the change in pH with the addition of organic amendments depends on the initial pH of the original soil, nature of organic materials, and rate of organic matter application [32, 141]. The increase in pH is explained by the mineralization of carbon (C) and the subsequent production of OH^- ions by ligand exchange and release of such basic ions such as K, Ca, and Mg [167]. The decrease in EC due to the application of organic amendments might be a result of Na displacement from the exchange sites and washing out with soluble salts through the leaching process. Organic amendments substantially contain Ca, Mg, and K [30, 131]. The presence of Ca can contribute to the low ESP of SAS due to increased exchange of Na by Ca at the cation exchange sites of soil particles, allowing greater leaching of exchanged Na with percolating water [32, 168]. Moreover, the presence of soluble and exchangeable K can limit the entry of Na into the exchange complex due to a similar ionic balance resulting in lower ESP [169]. Furthermore, Ca improves the aggregation of soil by cationic bridges between the soil organic matter and clay particles, and thus increases the soil porosity. The greater the total porosity, the greater the leaching of the soluble and exchangeable Na ions, and the greater the subsequent reduction in soil sodicity and salinity as expressed by the ESP and EC values, respectively [30]. Salt adsorbing ability of organic amendments is also known to be considered in lowering the EC of soils [140]. The incorporation of different organic amendments in soils had often contributed to a slight increase in EC depending on the rate and duration of incorporation [139, 146]. The increase in EC after the application of organic manures could be attributed to the presence of high amounts of K and Ca [141]. The role of organic matter in increasing the CEC has already been established. Hue [170] reported the decrease in Na in soil solution due to greater CEC resulting from incorporation of organic matter. The removal of organic matter had also been found to increase the CEC of a specific soil, which could be due to the exposure of the permanent charge of the montmorillonitic clay that was blocked by the interaction of the organic matter with the clay [171].

Several studies reported a significant increase in the soil microbial and enzymatic activities as a result of organic matter incorporation. The incorporation of organic amendments resulted in enhanced enzymatic activities [31, 33, 140, 161, 164, 172], microbial biomass C [32, 143, 161, 164, 173], microbial biomass N [32, 161], soil microbial activity as expressed by basal respiration [32, 174], and nematode abundance [143]. However, the response of microbial and enzymatic activities to organic amendments differs depending on the kinds of amendments, rates of incorporation, the types of crops grown, etc. The rate of microbial respiration was found to be highest in poultry manure-amended soils compared to reed-, composted straw-, and straw-treated soils, and all the amended soils resulted in a rapid increase in respiration rate in the beginning of incorporation that decreased gradually with time [163]. Tejada et al. [33] also observed the highest soil microbial biomass C and cumulative C-CO_2 in soils amended with a maximum dose of poultry manure that was 37% higher compared to cotton gin crushed compost-amended soils. Similarly, the urease, protease, b-glucosidase, phosphatase, arylsulfatase, and dehydrogenase activities were found to be increased by 34, 18, 37, 39, 40, and 30%, respectively, in poultry manure-amended soils compared to the cotton gin crushed compost-amended soils. In another study, Liang et al. [31] found that the urease activity increased by 62.3 and 117.4%, respectively, in pig manure- and rice straw plus pig manure-amended soils in comparison to rice straw treatment alone. The author also observed that the addition of rice straw, poultry manure, and their combination increased the urease activity by 21, 96, 163%, respectively, in rice, whereas by 57.4, 93.1, and 152.5%, respectively, in barley compared to the control. On the other hand, Zhang et al. [164] found dual effects where the application of vermicompost increased the activities of dehydrogenase, urease, and phosphatases by 37–68%, 22–107%, and 3.4–56%, respectively, while vermicompost addition decreased the activities of β-1,4-glucosidase and β-1,4-N-acetylglucosaminidase by 17–53% and 24–42%, respectively. Easily decomposable organic substances such as biosolids, swine manure, and chicken manure may likely be retained in the soil over short periods, resulting in an intense and short effect, while recalcitrant, lignin-rich amendments such as woody biomass have a smaller but long-lasting effect on these soil properties [131, 175].

Several studies reported the beneficial effects of phytohormones and plant growth-promoting rhizobacteria in modifying the physiological and metabolic responses of plants to salt stress, enhancing their tolerance as well as growth and yield [2, 176–178]. Egamberdieva [179] found that the indoleacetic acid producing bacterial strains significantly increased the seedling root growth of wheat up to 52% compared to control under conditions of soil salinity. However, in different studies, the morphological and physiological growth and yield attributes of crops were found to be increased under abiotic stress when several plant growth regulators were applied in combination compared to their single dose [180, 181].

The beneficial effects of the organic amendments on physical, chemical, and biological properties of SAS greatly influence the growth, nutrient uptake, and accumulation of plants under salt stress. The application of organic amendments in SAS is considered a useful and effective way to increase soil fertility and enhance crop growth [31, 157]. The application of organic amendments in SAS increased the biomass yield of alfalfa, *Medicago sativa* [182]; barley, *Hordeum vulgare* [31]; cotton, *Gossypium hirsutum* [143]; maize, *Zea mays* [32, 159, 183]; onion, *Allium cepa* [142]; rice, *Oryza sativa* [31, 153, 184]; seepweed, *Suaeda salsa* [185]; sweet fennel, *Foeniculum vulgare* [186]; tomato, *Solanum lycopersicum* [187]; and wheat, *Triticum aestivum* [139]. The quantitative and qualitative improvements in the growth and yield attributes of crops as affected by abiotic stresses in the presence of different additives might be due to the enhanced photosynthesis, cholorophyll contents, stomatal conductance, water-use efficiency, and synthesis of metabolites [137, 188–191].

Organic manure incorporation into the SAS also increased the N, P, and K contents in rice, *Oryza sativa*, barley, *Hordeum vulgare* [31], and sweet fennel, *Foeniculum vulgare* [186]; K content in rice, *Oryza sativa* [184, 192]; and K and Ca contents in tomato, *Solanum lycopersicum* [187], while it decreased the Na uptake [31, 184, 186, 187]. Improved soil physical conditions, availability of macro- and micronutrients, and enhanced microbial activities in soil resulting from the incorporation of organic amendments lead to better growth and yield of crops under salt stress [30, 175]. Maintaining a high K:Na ratio as a result of organic manure incorporation is an important mechanism of plants to resist the harmful effects of salts and perform better growth [31, 186]. Decreased uptake of Na may be due to the action of organic matter which acts as salt-ion chelating agents detoxifying the toxic ions, especially Na and Cl [184]. The C:N ratio also determines the growth of plants by influencing the availability of nutrients, especially N. Incorporation of organic amendments having a lower C:N ratio attributes to higher N availability [147].

5.3 Reclamation with inorganic amendments

As saline soils are usually good in structure, removal of excess salts can be obtained merely by the leaching process, and in most cases, the application of inorganic amendments is unnecessary. However, in the case of saline-sodic and sodic soils, exchangeable Na must first be removed from the exchange sites of soil particles and then leached to wash out from the root zone. As sodic soils are characterized by poor soil structure and limited infiltration rate, in addition to organic materials, various inorganic amendments are used to improve the soil structure and facilitate the leaching process. The application of Ca containing salt especially gypsum along with the organic amendments in SAS, in order to replace exchangeable Na, improves the physical condition of the soil, facilitates leaching of salts, and increases crop yield, which has been previously been reported in several studies [142, 184]. The application of gypsum followed by leaching of soils enhanced the reclamation and decreased the salinity as well as the sodicity levels [193]. Khattak et al. [194] observed a decrease in the pH, EC, and SAR of leached soils and an increase in the yield of rice and wheat by 9.8–25.3% and 10–80%, respectively, in salt-affected soil as a result of gypsum application. Khosla et al. [195] reported that the use of additional quantities of water can be minimized by the application of gypsum to achieve a reduced SAR value to a greater extent. The amount of gypsum required to reclaim saline-sodic and sodic soils is based on the amount of exchangeable Na, soil texture, leaching rate, crop to be sown, solubility, and reaction rates of the amendments [34, 125]. Abdel-Fattah et al. [193] studied the effects of different size fractions of gypsum (<0.5, 0.5–1, and 1.0–2.0 mm) on the efficiency of the reclamation of SAS and found that the salinity and sodicity decreased with the increasing fineness of gypsum. Gypsum is usually required to spread uniformly in the field and is incorporated into the upper 10–15 cm of soil by 2–3 shallow plowings at least 10–15 days before planting [34].

Zeolite ($CaAl_2Si_4O_{12}{\cdot}nH_2O$), an aluminosilicate, had also been studied as an inorganic amendment with the aims to reclaim SAS and enhance plant growth. Zeolite can enhance plant growth and nutrient uptake by mitigating salt stress. In an experiment, Al-Busaidi [196] studied 1 and 5% rates of zeolite and found that the application of zeolite resulted in a significant increase in the biomass of barley, *Hordeum vulgare*, and increased concentration of Ca, Mg, and K in postharvest soils. Application of zeolite in the soil also increased fresh and dry weights of shoots and roots, fruit weight, and the number of achenes of strawberry, *Fragaria ananassa*, as well as the available N, P, K, Ca, and Mg of the medium [197]. Milosevic and Milosevic [198] found higher amounts of humus, total N, and available P and K in soil along with a significant increase in the shoot length and trunk cross-sectional area by the application of zeolite in combination with cattle manure and inorganic fertilizer. Zeolite is characterized by large sorption and ion-exchange capacity. As a sorbent, it has an important effect on the mobilization of heavy metals as well as micronutrients and macronutrients [199]. In the structure of zeolite, the negative charges developed through the replacement of quadri-charged silicon cations by triply charged aluminum can be balanced by the adsorption of Na under salt stress conditions. Besides, the three-dimensional framework of zeolite is made up of $[SiO_4]^{4-}$ and $[AlO_4]^{5-}$ tetrahedra, which are bonded together by sharing the oxygen atoms located at the corner of each tetrahedron in such a way that the framework develops voids or pores in the form of cages and channels between the tetrahedra [200]. The incorporation of zeolite into SAS thus can lead to the adsorption of Na on the surfaces or entrapment in the void spaces, resulting in decreased uptake by plants. Moreover, zeolite plays an important source of Ca (CaO, 16.0%) to the soil-plant system. The release of Ca in the root media from the Ca-type zeolite can maintain a high Ca:Na ratio in the shoot and root by decreasing the Na while increasing the Ca uptake [201]. While using zeolite as an amendment to reclaim SAS, the concentration of Na should be taken into consideration. The application of zeolite contributed to a substantial increase of Na in soil and plant [196]. Besides, in soils having low pH (below 4.2), decomposition of zeolite and concomitant placement of Al^{3+} and Mn^{2+} ions in the sorption complex may lead to increased leaching of Mg and Ca, root damage, deficiency of Mg and P, toxicity of Mn and Fe, and restricted plant growth [199].

6. Conclusion

Plants are subjected to various abiotic and biotic stresses due to natural or human interferences. Among the abiotic factors, the problems of soil salinity and sodicity occurring in arid, humid, coastal, and even in irrigated agricultural lands possess great threats to sustainable food security worldwide. Due to differences in properties of saline, sodic, and saline-sodic soils, their ways of stresses to plants as well as amelioration approaches are different. The adverse effects of salt stress on the uptake of essential nutrients by plants vary depending on the genotype, growth stage, concentration of salts in medium, etc. While salinity can be reclaimed by leaching of salts through good quality water together with a proper drainage system, saline-sodic and sodic soils cannot be reclaimed merely by leaching. The application of inorganic and organic amendments is often required to reclaim the saline-sodic and sodic soils. The incorporation of organic amendments is beneficial to reclaim saline as well as saline-sodic and sodic soils. Organic amendments contribute to the physical, chemical, and biological improvements of saline, saline-sodic, and sodic soils, enhancing the magnitude of their reclamation. Besides, organic amendments act as important sources of essential plant nutrients. Therefore, application of organic amendments in combination with the judicious application of inorganic amendments can be a better approach to improve the properties of SAS and the plant's response to salt stress for sustainable crop production and food security.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 20

TIP Aquaporins in Plants: Role in Abiotic Stress Tolerance

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Abstract

Tonoplast Intrinsic Proteins (TIP) are one of five subfamilies of aquaporins in higher plants. Plants typically contain a large number of TIP genes, ranging from 6 to 35 compared to humans. The molecular weight of the TIP subfamily members ranges from 25 to 28 kDa. Despite their sequence diversity, all TIP monomers have the same structure, which consists of six transmembrane helices and five inter-helical loops that form an hourglass shape with a central pore. Four monomers form tetramers, which are functional units in the membrane. TIPs form channels in the tonoplast that basically function as regulators of the intracellular water flow, which implies that they have a role in regulating cell turgor. TIPs are responsible for precisely regulating the movement of not only water, but also some small neutral molecules such as glycerol, urea, ammonia, hydrogen peroxide and formamide. The expression of TIPs may be affected by different environmental stresses, including drought, salinity and cold. TIPs expression is also altered by phytohormones and the appropriate *cis*-regulatory motifs are identified in the promotor region of the genes encoding TIPs in different plant species. It was shown that manipulating TIP-encoding genes expression in plants could have the potential to improve abiotic stress tolerance.

Keywords: aquaporins, tonoplast intrinsic proteins, abiotic stress, drought, salinity, cold, phytohormones, gene expression analysis, *cis*-regulatory motifs, plants

1. Introduction

Aquaporins (aqua-pore, AQP), which are members of the major intrinsic proteins (MIPs) superfamily, facilitate the bidirectional flux of water and non-aqua substrates across the cell membranes [1–4]. These transmembrane proteins form pores in the lipid bilayers of archaea, bacteria, fungi, plants, non-mammalian metazoans and mammals including humans. In plants, the first AQP Noduline-26 (GmNOD26) was identified in soybean (*Glycina max* L.) [5], whereas their ability to conduct water in plants was demonstrated for the first time for an Arabidopsis tonoplast intrinsic protein homologue (AtTIP1; 1, gamma-TIP) *via* its heterologous expression in *Xenopus* oocytes [6]. In higher plants, MIPs are divided into five evolutionarily distinct subfamilies in terms of the similarities of their amino acid sequence and intercellular localisation: plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), nodulin-26 like intrinsic proteins (NIPs), small basic intrinsic proteins (SIPs) and X intrinsic proteins / uncharacterised-intrinsic proteins (XIPs) [7]. In turn, the TIP subfamily can be further divided into different subgroups. In Arabidopsis (*Arabidopsis thaliana*), the TIP subfamily has been divided into five subgroups from TIP1 to TIP5. Each subgroup is then again divided into different isoforms. In Arabidopsis, the TIP subfamily consists of 11 isoforms. Some of these have synonymous name such as TIP1;1 (gamma-TIP, γ-TIP, RB7), TIP1;2 (gamma-TIP2, γ-TIP2), TIP2;1 (delta-TIP, δ-TIP), TIP3;1 (alpha-TIP, α-TIP) and TIP3;2 (beta-TIP, β-TIP) [8].

Water, whose transport is facilitated by aquaporins and, among them TIPs, is their most important substrate. TIPs are responsible for precisely regulating the movement of not only water, but also some small neutral molecules that have great physiological significance such as glycerol, urea, ammonia, hydrogen peroxide (H_2O_2) and formamide [7]. The transport of these substrates was predicted using a bioinformatic analysis and some of them have also been proven experimentally by using their *in vitro* expression in yeast or in a *Xenopus* oocyte system in which the expression of gene of interest led to, for example, an increase water permeability (**Table 1**).

Species	**TIP name**	**Substrate**					**References**
		Water (H_2O)	**Hydrogen peroxide (H_2O_2)**	**Urea**	**Ammonia (NH_3)**	**Glycerol (GLY)**	
Arabidopsis (*Arabidopsis thaliana*)	AtTIP1;1	+	+	+	+		[6, 9–13]
	AtTIP1;2	+	+	+	+		
	AtTIP2;1	+		+			
	AtTIP2;3			+			
	AtTIP4;1						
	AtTIP5;1						
Barley (*Hordeum vulgare* L.)	HvTIP1;1	+					[14]
	HvTIP1;2	+					
	HvTIP2;3	+					
Bread wheat (*Triticum aestivum* L.)	TaTIP2;1				+		[15–17]
	TaTIP2;2				+		
Maize (*Zea mays* L.)	ZmTIP1;1	+	+	+	+	-	[18–20]
	ZmTIP1;2	+	+	+	+	-	
	ZmTIP2;3	+		+			
	ZmTIP4;4						
Rice (*Oryza sativa* L.)	OsTIP1;2	+				+	[21]
	OsTIP2;2	+				+	
	OsTIP3;2	+				+	
	OsTIP4;1	+					
	OsTIP5;1						
Radish (*Raphanus sativus*)	RsTIP1;1	+					[22]
	RsTIP2;1	+					
Sunflower (*Helianthus annuus* L.)	TIP7	+					[23]
	TIP20	+					
Tobacco (*Nicotiana tabacum* L.)	NtTIPa	+		+		+	[24]

+ *transport of the substrate has been confirmed,* – *transport of the substrate has been excluded.*

Table 1.
The transport of different substrates by TIPs, which was demonstrated via their in vitro expression in yeast or oocytes.

The central vacuole occupies most of the intracellular space of most mature plant cells [25]. The plant vacuole plays important roles not only in space filling, but also in osmotic adjustment, storage and digestion [26, 27]. TIPs form channels in the vacuolar membrane (tonoplast) that basically function as regulators of the intracellular water flow, which implies that they have a role in regulating cell turgor. It has been proposed that the presence of a specific TIP isoform may define the function of the vacuole and that specific TIP proteins could be used as markers for the different types of vacuoles that occur in different tissue types or in response to developmental and environmental stimuli [28, 29]. Vacuoles whose tonoplast contains TIP2;1 (delta-TIP, δ-TIP) are used by plant cells to store pigments and vegetative storage proteins (VSPs) [28]. Moreover, in vegetative tissues, the lytic or degradative vacuoles (LVs) are marked by the presence of TIP1;2 (gamma-TIP, γ-TIP) [26, 28, 30]. TIP1;2 may be expressed primarily at the time when the large central vacuoles are being formed during cell enlargement [30], while the protein storage vacuoles (PSVs) in mature seeds are marked by the presence of TIP3;1 (alpha-TIP, α-TIP) [26, 31, 32].

TIP proteins are most abundant in the tonoplast, but some isoforms have also been detected in the chloroplast, e.g. on the luminal site of the thylakoid, the chloroplast membranes or the envelope fraction using mass-spectrometry-based proteomics. Such a location was detected for TIP1;1, TIP1;2 and TIP2;1 in Arabidopsis [33–35].

2. Structure and diversity of TIPs

Although it has been suggested that AQPs and, among them, TIPs are multifunctional proteins, their structure is unique across all kingdoms of life. Despite their sequence diversity, all TIPs have a similar structure that consists of six transmembrane helices (TM1 to TM6) with five connecting inter-helical loops (LA to LE) and two half helices that contain one highly conserved Asn-Pro-Ala (NPA) motif each of which forms its functional pore (**Figure 1**). The hydrophobic NPA motif is located at the first intracellular (loop B) and the third extracellular loop (loop E) and the overlap in the middle of the lipid bilayer ('hourglass' model) and forms two hemipores that generate a narrow part of the channel [7, 36]. A second filter region is the aromatic/Arginine (ar/R) selectivity filter, which includes four amino acids (aa), which are located at the non-cytosolic end of the pore [37]. These two constriction regions are very important for the transport selectivity of the channel, which have been predicted based on structural knowledge combined with

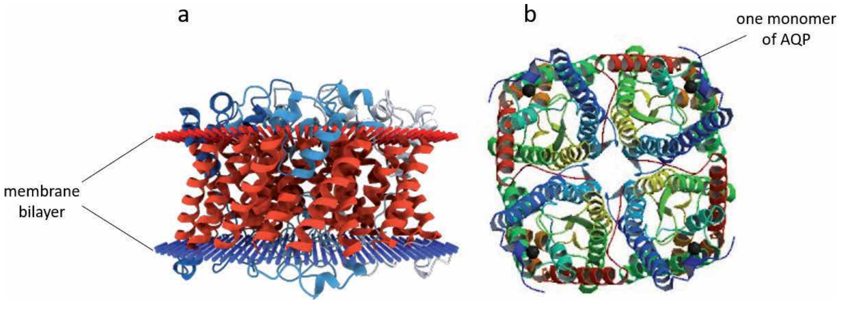

Figure 1.
The structure of aquaporin as represented by the crystal structure of spinach SoPIP2;1 (PDB 1Z98). The view from different sides of the holoprotein, which consists of four monomers. (a) the side view, (b) the tetrameric assembly from the cytoplasmic side. Each monomer functions as a single channel pore.

Species	Genome size/ ploidy x/no. of chromosomes*	AQP	PIP	TIP	NIP	SIP	XIP	References
Arabidopsis (*Arabidopsis thaliana*)	~135 Mbp 2n = 2x = 10	38	13	**11**	11	3	0	[8]
Barley (*Hordeum vulgare* L.)	~5.3 Gbp; 2n = 2x = 14	39	18	**11**	8	2	0	[51]
Bread wheat (*Triticum aestivum* L.)	~17 Gb 2n = 6x = 42, AABBDD, hexaploid	113 (65-A, 42-B, 36-D)	51	**29**	29	4	0	[52]
Cucumber (*Cucumis sativus* L.)	~350 Mbp 2n = 2x = 14	39	19	**8**	9	2	1	[53]
Foxtail millet (*Setaria italica* L.)	~490 Mb 2n = 2x = 18	39	12	**11**	13	3	0	[54]
Maize (*Zea mays* L.)	2.4 Gb 2n = 2x = 20	41	12	**18**	8	3	0	[55]
Potato (*Solanum tuberosum* L.)	~840 Mb 2n = 4x = 48	41	15	**11**	10	3	8	[56]
Rice Japonica (*Oryza sativa* L.)	500 Mb 2n = 2x = 24	33	11	**10**	10	2	0	[57]
Sorghum (*Sorghum bicolor*)	~730 Mb 2n = 2x = 20	37	13	**11**	11	2	0	[58]
Tomato (*Solanum lycopersicum* L.)	~828 Mb 2n = 2x = 24	47	14	11	12	4	6	[59]

*Based on the Ensemble Plants Database.
The number of Tonoplast Intrinsic Proteins (TIP) has been marked in bold.

Table 2.
The number of AQP isoforms in the selected plant species.

simulation studies [38, 39]. Both filters at the pore "mouth" constitute barriers to the passage of inorganic cations (such as Na^+ and K^+) and protons [39]. The NPA motif not only plays a role in regulating membrane transport but also in protein localisation [40]. The substrate selectivity of AQPs is controlled by the amino acid residues of the NPA and ar/R filters as well as another conserved region, which is known as the Froger's residues, which consist of five conserved residues [41]. This region enables the aquaglyceroporins that transport glycerol from the AQPs that facilitate the transport of water to be distinguished [39].

All MIP sequences take on a typical hour-glass MIP helical fold with six transmembrane helices and two half-helices [42]. This fold has been maintained during evolution via its conservation around 40 positions within the transmembrane region [42]. The stability of the functionally important half-helix is modulated by a stabilising intra-helical salt-bridge interaction and/or two helix destabilising residues, glycine and proline, which was demonstrated by an analysis of the loop E region of 1468 MIP sequences and their structural models from six different groups of organisms [43]. In the cell membrane, MIPs are grouped as homotetramers that are located in the lipid bilayer (**Figure 1**). Each monomer functions independently as a single channel pore [44]. In plants, the only the crystal structure of the MIPs for two species is currently available in the Protein Data Bank (PDB: http://www.pdb.org), the spinach (*Spinacia oleracea*) aquaporin SoPIP2;1 was in its closed confirmation at a 2.1 Å resolution and its open confirmation at a 3.9 Å resolution [45] and the Arabidopsis aquaporins

AtPIP2;4 at a 3.7 Å resolution [46] all of which were determined using X-ray diffraction, which showed their structural identity. However, the functions and regulation of the majority of the MIP channels have not yet been fully characterised [42].

A large number of *MIP* genes and, among them *TIP* genes, have been identified in the genomes of different plant species. In plants, the MIP family contains around 2008 members while the TIP subfamily contains 585 members from different plant species, which have been deposited in the MIPDB [47, 48]. The length of the TIP sequences ranges from 55 to 780 aa. Plants typically contain a large number of *AQPs* genes and among them the *TIP* genes, when are compared with for example humans, ranges from 6 to 35 in moso bamboo (*Phyllostachys edulis*) and oilseed rape (*Brassica napus* L.), respectively [49, 50] (**Table 2**). The molecular weights of the TIP subfamily members ranges from 25 to 28 kDa [47].

3. Study of the tissue- and developmental-dependent expression of TIPs

Aquaporins are postulated to be the main water transporter protein in plants. However their presence and expression may vary among plant species, tissues and time during development. In plants, AQPs are present in almost all organs, including the roots, leaves, stems, flowers, fruits, seeds, dry seeds [60], pollen [13, 61–63], anther and specific cells such as the guard cells [64, 65]. Moreover, some *TIPs* are expressed in specific tissues, e.g. *AtTIP5;1* was localised in the pollen mitochondria, which are probably involved in the remobilisation of N via the transport of mitochondrial urea to the cytoplasm [61] as well as in a time-specific manner, e.g. the *RsTip1;1* (*gamma-VM23*) gene expression was high not only in the hypocotyls but also in the developing tap roots and young growing leaves and the pattern of its expression was connected with cell elongation [22] (**Table 3**).

Species	TIP name	Organ/tissue/process	References
Arabidopsis (*Arabidopsis thaliana*)	*AtTIP1;3, AtTIP5;1*	Pollen mitochondria	[61, 66, 67]
	AtTIP3;2	Senescence	
	AtTIP3;1	Seed-specific	
	AtTIP1;1	Entire vegetative body	
	AtTIP2;1	Developing vascular tissue, primary shoot	
Barley (*Hordeum vulgare* L.)	*HvTIP1;1, HvTIP2;3, HvTIP1;2, HvTIP3;1*	Leaf	[14]
	HvTIP1;1, HvTIP2;3, HvTIP1;2	Root	
Bread wheat (*Triticum aestivum* L.)	*TaTIP1;12*	Root-specific	[52]
	TaTIP2;22	Root-specific	
Eucalyptus (*Eucalyptus grandis* L.)	*EgTIP1;1, EgTIP2;1*	Flower bud	[68]
	EgTIP1;1, EgTIP1;4, EgTIP2;1, EgTIP2;2	Flower	
	EgTIP1;4	Root	
	EgTIP1;1, EgTIP1;2, EgTIP4;1	Stem	
	EgTIP1;3	Cambium	
	EgTIP1;2, EgTIP3;2	Leaf	
	EgTIP3;1, EgTIP3;2	Fruit	

Species	TIP name	Organ/tissue/process	References
Maize (*Zea mays* L.)	*ZmTIP2;3*	Root-specific	[20]
Radish (*Raphanus sativus*)	*RsTip1;1 (gamma-VM23)*	Hypocotyl of seedlings, developing tap roots and young growing leaves	[22]
	RsTIP2;1 (delta-VM23)	Not expressed in young roots or leaf mesophyll	
Rice (*Oryza sativa* L.)	*OsTIP3;2*	Mature seed, spikelet, callus	[21, 69]
	OsTIP1;1	Shoots, roots	
Sunflower (*Helianthus annuus* L.)	*TIP7 (delta-TIP)*	Guard cells	[23]
	TIP27 (delta-TIP)	Guard cells	
Tobacco (*Nicotiana tabacum* L.)	*NtTIP1;1 (TobRB7)*	Root-specific	[70]

Table 3.
The expression of the selected TIP genes in different plant tissues.

4. Study of the expression of *TIPs* under abiotic stress or phytohormone treatment, and their role in maintaining cell homeostasis under stress conditions

The gene expression is the process in which gene information is converted into a protein [7]. Analysing the gene expression profiles is a prerequisite for determining the physiological function of a gene [71]. The function of AQPs can generally be modified in two ways: by regulating the gene expression, which leads to changes in protein abundance or by changing its permeability – gating. However, it should be kept in mind that changes in the mRNA expression level often reflect the protein abundance, but that this is not necessarily strictly connected in any cell or tissue [37]. In term, AQP trafficking and activity could be affected by the post-translational modifications (PTMs), like deamination, phosphorylation, methylation, ubiquitination and acetylation [37]. Water homeostasis is fundamental for cell survival [72]. AQP facilitate the transport of water and play roles in response to abiotic stress conditions during which the cell homeostasis is disturbed. Different studies have suggested that a low abundance of the AQP proteins reduces the water permeability of membranes and that a high abundance of them increases it. Although it can be assumed that all AQPs are able to transport water, an experimental study was performed for only a few members of the TIP subfamily in different plant species (**Table 1**). The expression of TIPs may be affected by different environmental conditions, including drought, salinity and cold and this has been investigated in many plant species. Comparative transcriptome studies using RNA sequencing (RNA-seq), which is a next generation sequencing technology, could lead to the discovery of a differential expression of multiple aquaporin homologues in different tissues and/or in different conditions of growth, e.g. under abiotic stress. The transcriptome defines as a set of coding and non-coding RNA molecules that are present in a single cell or a population of cells [73]. Nowadays, RNA-seq technology is used more and more frequently [74, 75]. On the other hand, the reverse-transcription quantitative PCR (RT-qPCR) technique still serves as the method of choice to perform the expression analysis of specific genes in many researches.

Abiotic stresses, which have a significant impact on the plants, are involved in changes at the transcriptome, cellular and physiological levels. When the stress is prolonged, plant growth and productivity are severely diminished [76, 77]. The response of plants is very complicated and many mechanisms are initiated simultaneously to restore cellular homeostasis and promote survival [78]. Aquaporins are thought to be responsive to the stress signalling pathways and are also thought to be involved in the stress-coping mechanisms such as altering the tissue hydraulic conductivity [79, 80]. Drought and salinity are two major factors that significantly limit plant growth and productivity [81, 82]. The huge yield losses caused by drought stress ranging 45–92 (%) have been reported in major field crops like maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), chickpea (*Cicer arietinum* L.), soybean (*Glycine max* L.), sunflower (*Helianthus annuus* L.) [83]. Cold is also often associated with climate change [84]. Drought, salinity and freezing stresses cause a changing water status in plant cells and all of these result in cellular dehydration [85]. In order to improve the tolerance of crop plants to unfavourable environmental conditions, a detailed understanding of their stress responses is required.

4.1 Drought, salinity and cold stress

Aquaporins are responsible for precisely regulating the movement of water and therefore may play a crucial role in the drought-stress response as well as in drought-stress tolerance [78]. A transcriptome analysis of the leaf and root tissues in plant species revealed significant changes in the expression of the *TIP* genes in response to drought stress. Under drought stress, when water is limited, the down-regulation of the *TIP* genes can lead to a decrease in the water permeability of the tonoplast in order to avoid water loss and to minimise the water flow through the cell membranes in order to prevent the further loss of leaf turgor. This expression pattern has been shown in many studies. In *Nicotiana glauca*, the *NgMIP2* and *NgMIP3* genes, which are homologous to *TIP*, were down-regulated in leaves under drought stress when the leaves were wilting, usually three to four days after water was withheld [86] and in Arabidopsis, the levels of *AtTIP1;1, AtTIP1;2, AtTIP2;1* and *AtTIP2;2* were more than four-fold down-regulated after 12 days of drought [87]. In contrast to these results, the expression level of *AtTIP1;1* and *AtTIP2;1* under drought treatment was up-regulated, but the time that the stress was applied was only 24 hours [88]. Moreover, in barley (*Hordeum vulgare* L.), the expression of five *HvTIP* aquaporins in leaves: *HvTIP1;1, HvTIP1;2, HvTIP2;1, HvTIP2;2* and *HvTIP2;3* was down-regulated after ten days of severe drought treatment [89]. In turn, in *Festuca* species, the transcript level of the *TIP1;1* aquaporin decreased in leaves after 11 days of a water deficit [90]. The possible functional role of coffee (*Coffea arabica* L.) TIPs in in regulating the water balance has been explored by measuring the *TIP* gene expression in leaf and root tissues that had been subjected to drought [91]. In root tissue, the expression of three *CsTIP* genes (*CaTIP1;1, CsTIP2;1, CsTIP4;1*) decreased as the severity of the drought stress increased. In the leaf, a similar expression profile of their expression was obtained only for of *CaTIP1;1* [91]. By contrast, the level of *TIP* expression increased in response to drought in some plant species. In soybean (*Glycine max*), transcriptome analysis data revealed that under dehydration stress, the expression of *GmTIP2;1, GmTIP1;7* and *GmTIP1;8* was up-regulated. The authors suggested that these three genes are probably the major AQPs that are involved in the stress response [75].

Interestingly, the expression profile of *TIP* genes can be compared for cultivars that have contrasting responses to drought stress under control and stress conditions. In two cultivars of strawberry (*Fragaria* x *ananassa*) that have contrasting drought stress phenotypes, the gene expression of *FaTIPs* in the roots was analysed [79]. During severe drought stress, the *FaTIPs* were only up-regulated in the drought-tolerant cultivar. This was connected with a gradual stomatal closure in this cultivar, which was further associated with conservative water use [79]. In turn, in soybean (*Glycine max*), the difference in gene expression was investigated using RNA-seq and it was found that the expression level of seven *TIP* genes (*GmTIP2;1, 2;2, 2;3, 2;4, 2;5, 3;2* and *5;1*) in the leaves was lower in the slow-wilting soybean lines, but that it increased in the fast-wilting soybean lines after drought stress [74]. Slow wilting is an important physiological parameter that is used in stress tolerance studies of plants. A cultivar-specific *TIP* expression under drought has also been observed in the common bean (*Phaseolus vulgaris* L.) [92]. In this study, the expression of *PvTIP1;1* and *PvTIP4;1* decreased under drought compared to the control conditions in two cultivars, which has different phenotypes in terms of their tolerance to drought and yield at harvest, which were determined by time that was required for the plants to wilt after withholding water. Interestingly, the gene expression of *PvTIP4;1* during drought was cultivar specific with greater down-regulation of these gene in the drought-tolerant cultivar [92]. The differences in the relative water content (RWC) and water potential of leaves between these cultivars indicate that there is greater prevention of water loss in the tolerant cultivar during drought, which may be associated with the rapid and adequate down-regulation of the *AQPs* [92]. However, it should be kept in mind that the adaptive mechanism for reducing the effects of water deficit stress in which the *TIP* or other *AQP* expression is changed should be further analysed by a functional characterisation of these candidate genes or proteins [52].

The regulation of water transport has been postulated as being the most important and the best recognised function of all of the AQPs including TIPs under drought stress. A second important function could be their involvement in the movement of hydrogen peroxide (H_2O_2), which is one of the reactive oxygen species (ROS), which include the unstable, highly reactive molecules that are produced during abiotic stress, e.g. drought [83, 93]. This activity makes the AQPs important players in both the redox signalling network and in H_2O_2 detoxification [94]. TIPs can facilitate the movement of H_2O_2 across the membranes that have already been shown by their *in vitro* expression in the yeast or oocyte (**Table 1**). In barley under drought stress, the expression of the *HvTIP3;1* gene increase 5000-fold compared to their expression under optimal water conditions [89].

Soil salination and a high salt accumulation affects the growth, development and metabolism and yield of plants [95]. A wide range of physiological and biochemical alterations in plants are induced by salinity, which causes a lower water potential in the soil solution, ionic disequilibrium and also a higher accumulation of reactive oxygen species (ROS) [96]. Salinity stress leads to changes in the expression profiles of the *TIP* genes, which was shown in many plant species. In Arabidopsis (*Arabidopsis thaliana*), the plants were treated with 100 mm NaCl, which led to osmotic stress. Macroarray experiments with gene-specific tags were performed to investigate the expression of all of the *AQPs* including the *TIP* subfamily in roots that had been under salt treatment for 24 hours [97]. The *AtTIP1;1, AtTIP1;2, AtTIP2;2* and *AtTIP2;3* genes were highly expressed in the roots and their expression was maximally reduced at 6 h of treatment and remained low until 24 h of treatment, except for *AtTIP1;1*. In turn, in *Festuca arundinacea,* the plants were exposed to salt stress by irrigating them with 250 mM NaCl solution over 21 days, which resulted in an increase in the abundance of the *FaTIP1;1* transcript in two contrasting salt tolerance genotypes compared to the control conditions. This increase was noticed earlier on day six of the NaCl treatment in the high-salt tolerant (HST) genotypes and later on day 11 in the low-salt tolerant (LST) genotype [90].

Similar to water and salt stresses, cold stress is an important abiotic stress factor that significantly limits plant growth and development [98]. In *Festuca pratensis*, cold acclimation was associated with a lower transcript level of the *FpTIP1;1* gene in the leaves in both the high and low frost-tolerant genotypes. The authors concluded that the down-regulation of these isoforms during the exposure to cold may be one of the cold-acclimation components that prevents frost-induced cellular dehydration [90]. A similar pattern of expression was observed in roots in rice (*Oryza sativa* L.) under cold treatment. A 4°C treatment caused a decrease in the transcript level of the *OsTIP1;1* and *OsTIP2;2* genes. The mRNA levels of these genes started to decrease after 48 hours and continued to decrease after 72 hours of a chilling treatment [57]. One more example of a change in the expression of the *TIP* member genes comes from the cold treatment of cotton (*Gossypium hirsutum*). The *GhTIP1;1* transcripts mainly accumulated in the roots and hypocotyls under normal conditions, but were dramatically up-regulated in the cotyledons and down-regulated in the roots within a few hours after the cotton seedlings had been cold treated [99].

4.2 Phytohormone treatment

Phytohormones play vital roles in the ability of plants to acclimatise to varying environments, by mediating growth, development, and nutrient allocation [100]. Interestingly, it has been shown that the *TIPs* expression is also altered by phytohormones, including abscisic acid (ABA), cytokinin (CK), gibberellins (GA), and jasmonates (JA). Abscisic acid (ABA) is a plant growth regulator that plays an essential role in the abiotic stress response mainly during water stress [101]. It regulates the movement of the stomatal guard cells [102]. Jasmonates act as stress hormones that play an important role in the plant response to biotic and abiotic stresses [103]. In general, JA inhibits plant growth and modulate many growth and developmental processes of plants including root, shoot and leaf growth, trichome and tuber formation, fruit ripening and leaf senescence [104]. The gibberellins stimulate the growth of most organs via cell elongation and also respond to abiotic stresses [105]. Cytokinins play a role in regulating plant growth, development and their acclimation to environmental stresses [106, 107].

Cytokinin has been reported to influence the expression of the aquaporin genes. The expression of *PgTIP1* from ginseng (*Panax ginseng*) was positively regulated by applying cytokinin in suspension-cultured hormone-autotrophic ginseng cells [108]. Methyl jasmonate treatment caused a decrease in the nitrogen content in barley leaves, which was associated with an increased expression of the four tonoplast aquaporin genes (*HvTIP1;2, HvTIP2;2, HvTIP4;1* and *HvTIP4;2*), which are predicted to transport the nitrogen compounds from the vacuole to the cytosol. The up-regulation of the nitrogen-transporting *HvTIPs* may lead to a vacuolar unloading of ammonia and urea, which could both be remobilised when the nitrogen content in barley leaves decreases [109]. In barley, the changes in the expression of all of the investigated *HvTIPs* in response to MeJA treatment were associated with the presence of the *cis*-regulatory elements in their promotor regions, which are recognised by the jasmonate-related transcription factors [109]. Other types of elements that possibly participate in the response to phytohormones in the *HvTIP* promotor regions such as abscisic acid (ABA), gibberellins (GA) and auxin were also identified [89]. In barley, the effect of ABA and GA on the expression of the *HvTIP1;2, HvTIP2;1, HvTIP2;2, HvTIP2;3* and *HvTIP4;1* genes after 24 hours of treatment was studied in both the shoots and roots using quantitative real-time RT-PCR [110]. Interestingly, the exogenous application of ABA increased the expression of *HvTIP2;1* and *HvTIP4;1,* while the GA-treatment led to an increase of *HvTIP1;2* and *HvTIP4;1* in the roots compared to the control conditions. However, in the shoots, the ABA treatment decreased the expression of *HvTIP1;2*, while the GA treatment led to the up-regulation of *HvTIP4;1* [110].

5. Genetic manipulation of the TIP-encoding genes

The tonoplast intrinsic proteins genes have been reported to be involved in the increasing the abiotic stress tolerance in several plant species using genetic modification technology (**Table 4**). It was shown that manipulations of the expression of

TIP name/species	Method/expression in species	Phenotype	References
AQUA1 (TIP) *Populus alba* L.	OX *Populus alba*	Increased plant growth rate and water use efficiency under excess Zn conditions	[111]
SlTIP2;2 *Solanum lycopersicum* L.	OX *Solanum lycopersicum*	Increased drought tolerance due to the ability of plant to regulate its transpiration under drought stress conditions, improved CO_2 uptake and a balanced nutrient supply	[112]
SlTIP2;2 *Solanum lycopersicum* L.	OX *Arabidopsis thaliana*	Enhances the tolerance to salt stress	[113]
PgTIP1 *Panax ginseng*	OX *Glycine max*	Higher tolerance to salinity	[114]
PgTIP1 *Panax ginseng*	OX *Arabidopsis thaliana*	Acceleration of plant development, with faster growth, precocious flowering and a higher accumulation of biomass, increased seed size and seed mass, greatly increased growth rate	[85]
PgTIP1 *Panax ginseng*	OX *Arabidopsis thaliana*	Accumulated more Na + under salt stress, and exhibited superior performance under drought stress	[108]
CsTIP2;1 *Citrus spp.*	OX *Nicotiana tabacum*	Increased plant growth and tolerance to drought and salinity	[115]
JcTIP1;3 *Jatropha curcas*	OX *Arabidopsis thaliana*	Increased tolerance to drought and salinity, improved germination under high salt and mannitol stress	[116]
AtTIP5;1 *Arabidopsis thaliana*	OX *Arabidopsis thaliana*	Increased tolerance to boron toxicity	[117]
AtTIP1;1 *Arabidopsis thaliana*	Down-regulation /RNAi technology	Early senescence and plant death	[118]
AtTIP1;1 -mutant AtTIP1;2 and AtTIP1;2 -double mutant *Arabidopsis thaliana*	KO Transposon insertion	Single mutant -no significant effect on the metabolism or elemental composition of the plants Double mutant -minor increase in the anthocyanin content, and a decrease in catalase activity, no changes in the water status, mutant alive and thriving	[119]
AtTIP2;2 *Arabidopsis thaliana*	KO T-DNA insertion	Less sensitive to abiotic stresses (mannitol, NaCl and PEG)	[120]

OX-overexpression, KO- knockout, RNAi-RNA interference, PEG- polyethylene glycol.

Table 4.
Genetic manipulation of the TIP genes in different plant species and its effect on the phenotype.

the *TIP*-encoding genes in plants could have the potential to improve their abiotic stress tolerance (**Table 4**). In tomato (*Solanum lycopersicum* L.), overexpression of *SlTIP2;2* has been shown to substantially improve the water homeostasis under drought stress. Conversely, this overexpression also promoted more prodigal water use [112]. Another gene, *TIP1;1*, was artificially manipulated in a few studies *via* genetic engineering (**Table 4**). It is not only believed to be play an important role during plant growth and development, but also to be beneficial for growth under stress when it is overexpressed. The overexpression of the *PgTIP1;1* gene from *Panax ginseng* in Arabidopsis led to a general acceleration of plant development, which included a faster growth, precocious flowering and a higher accumulation of biomass compared to the wild type (wt), or the accumulation of more Na^+ under salt stress and also caused a superior performance under drought stress compared to wt [85, 108]. The authors concluded that PgTIP1;1 plays an important role in the growth and development of plant cells and suggested that regulating it in the water movement across the tonoplast has a great impact on plant vigour under favourable growth conditions and also in the responses of plants to drought, salt and cold stresses [85]. Therefore, *TIP1;1* might be a potential target in biotechnology and agriculture. It was interesting to determine whether silencing this gene would have the opposite effect on growth in control and stress condition. First, it was shown that the down-regulation of *AtTIP1;1* using RNA interference (RNAi) resulted in early senescence and plant death [118]. However, later research using transposon insertion did not confirm these results [119]. A single mutant (tip1;1–1) that had been generated using transposon insertion showed any significant effect on the metabolism or elemental composition of the plants. Additionally, a double mutant in the *AtTIP1;1* and *AtTIP1;2* genes (tip1;1–1 tip1;2–1) had a small increase in its anthocyanin content and a decrease in its catalase activity, but showed no changes in its water status. Moreover, the mutant was alive and thriving [119].

6. Conclusions

It is believed that aquaporins and, among them, the tonoplast intrinsic proteins may be important players in the plant water relations at the cell, tissue, organ and whole plant levels. TIP proteins could have a vast impact on the acquisition of knowledge about plant tolerance against abiotic stress and could serve as a target sequence for genetic modifications. A drawback in this approach is that in the genome of each plant species, many genes encode the same subgroup of isoforms. It is possible that some of these have a redundant function. Taken together, TIPs probably play complex and diversified roles in the response of plants to abiotic stresses depending on TIP isoforms and the type and degree of stress treatment.

Acknowledgements

This work was supported by the National Science Centre, Poland, SONATA project 2015/19/D/NZ1/03441.

Conflict of interest

The author declares no conflict of interest.

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Chapter 21

Drought Responses on Physiological Attributes of *Zea mays* in Relation to Nitrogen and Source-Sink Relationships

Suphia Rafique

Abstract

Maize is the staple food crop and essential for world food security. Maize plants' water requirement is high for proper growth and development at vegetative stage and grain formation at reproductive stage. Drought is the major abiotic stress that affects almost all the growth stages of maize crop and it has a strong impact on all the physiological process of maize plants. Similarly, N metabolism is of central importance during drought stress. Nitrogen (N) is one of the macronutrients; it is a major requirement for crop growth and grain yield of maize. Therefore, nitrogen and water separately or in combination are the two most critical factors in maize production. Drought modifies source-sink relations and weakens the source and sink strength, which disturbs plant's growth, plant's adaptation to stress, and consequently affects crop productivity.

Keywords: maize, drought, nitrogen, source-sink relationship

1. Introduction

Plants are nonmotile, and they protect themselves from various biotic and abiotic stresses through structural and metabolic changes like altering leaf orientation, transpirational cooling, or adjustment of membrane lipid compositions [1]. Maize is a staple food crop that grows under optimal environmental conditions and throughout their life span is often exposed to various abiotic stresses [2] like either combined stresses like drought and heat, drought and salinity, drought and low-N stresses, or singly drought. In combined stress, plants show a wide range of responses that brings changes in growth and morphology, and the plants' ability to withstand these stresses greatly varies from species to species [3]. However, drought along with high frequencies, longer durations, and large ranges occur almost yearly across the globe, even in wet and humid regions [4, 5]. Water deficit is the most detrimental environmental stress that adversely affects the maize productivity. The effect of drought is manifested at cellular, physiological biochemical, metabolic, proteins, and gene levels. Water is an essential component for plants; it maintains cell turgidity and keeps the structure intact (by keeping the pressure inside cells high) so that they are fully expanded (i.e., turgid). In wilted plants, when turgor pressure approaches to zero, the cell membranes collapses, damage and becomes leaky, the key enzymes proteins denatured and their structure altered. To ensure high survival rates and production under drought conditions, maize plants rely on several strategies, including drought avoidance, escape, and tolerance [6–8]. Nitrogen plays a major role in plant nutrition and also combats with several abiotic stresses like salinity, drought, heat stress, etc. [9–13], but this mineral element is most often deficient in arable soils. Though the requirement of nitrogen is greatest among all mineral elements, its deficiency limits the plant's growth and development [14–16]. There is a tight regulation of carbon and nitrogen metabolism in photosynthesis and N uptake [14, 17–19]. In a review, Aziz Khan et al. [20] outline the nitrogen (N) responses in crop production and to ameliorate abiotic stresses for better crop production. Further they emphasize that nitrate and ammonium nitrogen are essential nutrients for successful crop production [21]. Moreover, shortage and N excess affect assimilate partitioning between vegetative and reproductive organs [22]. Mineral nutrition influences the physiological response of plants to water stress [23]. While Waraich et al. [24] discussed the role of macro- and micronutrients to decrease the adverse effect of drought in crop plants. Therefore, water and nitrogen affects the crop growth, development, and production either separately or in combination. Humbert et al. [25] observed the physio-molecular changes in response to water and nitrogen and finally concluded that the responses of plants to the combination of these two stresses may cause additional effect that was different from the individual effects, and hence, cannot be inferred from the end results obtained from different stresses applied individually. Drought affects maize grain yield to some degree at almost all growth stages, but the crop is the most susceptible during flowering [26, 27]. At the reproductive phase, N availability affects assimilate partitioning between vegetative and reproductive organs and N metabolism in young ear shoots [28]. Therefore, the timing and intensity of stress determine the extent to limits yield either due to source or sink limitations.

2. Maize response under drought stress

2.1 Effect of drought on morpho-physiological parameters

The water deficit conditions Boyer [29] showed the decrease in leaf area attributed to decrease rate leaf initiation and expansion or increase leaf senescence or shedding. Though the number of leaves per plant did not reduce in early vegetative maize growth in severe nonlethal water deficit conditions, Abrecht and Carberry [30] also demonstrated the gradual reduction in plant height in maize with increasing water deficit. Further supported by Moss and Downey [31] showed the significant reduction in plant height with increase in water stress. While, dry matter yield accumulation depends on the leaf area and leaf dry weight because leaf area is the major assimilatory surface for most crop any factor that affects the leaf area also affects dry matter [32]. In similar way, the short term responses of corn to a pre-anthesis water deficit, delayed leaf tip emergence and reduction in leaf area. While long-term consequences of water deficits have reduced final sizes of the leaves and internodes [33]. Drought stress significantly repressed relative leaf water content, leaf size, and photosynthesis-related parameters in maize seedling [34]. Drought affects various morpho-physiological processes including plant biomass, root length, shoot length, photosynthesis, water use efficiency (WUE) and leaf water content [35, 36]. At early growth stage 50 maize genotypes for drought tolerance was assessed. Principal component analysis revealed important morpho-physiological traits (FSL, FRL, DSW, DRW, RWC, and TDM) plays key role in drought tolerance. Later, Ali et al. [37] identified various morpho-physiological traits that have higher heritability, genetic advancement, and correlation and which contribute for improved maize grain yield. A set of 24 genotypes bred at different centers in India as well as in CIMMYT showing variability for drought tolerance were selected for molecular and morpho-physiological characterization. Phenotyping of inbred by morpho-physiological traits revealed that there was a positive relationship among root length, chlorophyll content, relative water content while anthesis-silking interval (ASI) have negative relationship with all these traits [38]. Water stress occurring during vegetative and tasseling stages reduced plant height, as well as leaf area development [39].

2.2 Photosynthesis under drought stress

In a study Anjum et al. [40] showed that drought stress in maize led to considerable decline in net photosynthesis (33.22%), transpiration rate (37.84%), stomatal conductance (25.54%), water use efficiency (50.87%), intrinsic water use efficiency (11.58%) and intercellular CO_2 (5.86%) as compared to well water (WW) control. The main cause of reduced photosynthesis under drought stress is due to decreases in both assimilation (A) and internal CO_2 concentration, that finally inhibits total photosynthetic metabolism [41]. Besides there are other several non-stomatal effects during drought which are responsible for stomatal closure. These include photophosphorylation, ribulose-1,5-bisphosphate (RuBP) regeneration [42, 43], rubisco activity, and ATP synthesis [44, 45]. The response of growth and some physiological characteristics were compared in two maize *(Zea mays* L.) cultivars, one drought-resistant (PNR473) and the other drought-sensitive (SR52). Drought-resistant (PNR473) had a higher growth rate and deeper rooting than the drought-sensitive cultivar under water stress treatment. But the drought-resistant cultivar had a higher transpiration rate and lower diffusive resistance during the onset of water stress, and higher relative water content and levels of abscisic acid and proline throughout the period of water stress [46]. While, Jama and Ottaman [47] reported delay in the irrigation during early growth stages of a corn plant (including anthesis) decreased plant dry weight. The responses of maize (*Zea mays* L.) from the third leaf stage to maturity for different soil water levels (well watered, moderately stressed, and severely stressed) indicated that drought stress relied on drought intensity and duration, with more severe drought stress creating more serious effects on maize. It is well known that leaf water status always interacts with stomatal conductance through plant hormone abscisic acid, increased production of ABA would help the plants to survive under drought stress [48]. According to Bray [49], dry soil induced and increased ABA concentrations in the roots to maintain root growth, increase root hydraulic conductivity, through these process shoot water uptake increases thus drought induced root-to-leaf signaling. Also direct correlation between the xylem ABA content and stomatal conductance has been demonstrated [50]. The role of other phytohormones like Brassinosteroid is for plant growth, as they are involved in main plant antioxidant processes encompassing the regulation and enhancement of plant tolerance against different environmental stresses [51].

2.3 Metabolic changes under drought stress

To maintain cell turgor and to lower the osmotic potential plants accumulate different types of organic and inorganic solutes in the cytosol [52]. Primary metabolites, such as sugars and amino acids, have also been reported to accumulate during water stress [53]. Two maize (*Zea mays* L.) cultivars, 704 and 301, accumulate soluble sugars, starch, and proline content in shoots and roots in response to drought stress. This suggests role of sugars in osmotic adjustment whereas, proline minimized the damage caused by dehydration [54]. The high salt concentration of soil causes a water deficit in crops [55], exogenous application of SA mitigated the adverse effects of salinity on maize plants by osmoregulation which is possibly mediated by increased production of sugar as well as proline [9]. Besides, the ROS (including oxygen ions, free radicals, and peroxidases) forms major by-product of normal metabolism and in abiotic stress conditions. However, during environmental stress such as drought, ROS levels increase dramatically resulting in oxidative damage to proteins, DNA, and lipids [56]. Among six maize genotypes (A638, B73, Grace-E5, Lo964, Lo1016, and Va35) studied, the drought-sensitive maize genotypes accumulated significantly more reactive oxygen species (ROS) and reactive nitrogen species (RNS). In addition, they showed rapid increases in enzyme activities involved in ROS and RNS metabolism compared to tolerant genotypes. However, antioxidant enzyme activities were higher of tolerant genotypes than in the sensitive genotypes [57]. In a study, the protective role of biochar (date and wheat) in combination with Me and P against water stress in two maize varieties were examined. The maize variety Mallika performed better and was more drought-tolerant with its increased osmolyte production and an efficient antioxidant defense system that eliminated ROS. Hence, ROS elimination further alleviated the damage to the photosynthesis system induced by water stress. On other hand, Azam variety was more sensitivity to drought [58]. Plants tolerate the water deficit conditions by adjusting their physiological and biochemical approaches. Maize leaves subjected to water deficit rigorously down-regulated NR activity and photosynthesis, also a correlation between maximal extractable foliar nitrate reductase (NR) activity and the rate of CO_2 assimilation was observed [59]. The assimilatory sulfate reduction in roots and leaves of the staple crop maize demonstrated the organ specific impact of drought upon sulfate metabolism. Under drought stress, the allocation of sulfate was significantly shifted to the roots allowed for significant increase of thiols derived from sulfate assimilation in roots. This enabled roots to produce biomass, while leaf growth was stopped. Therefore, sulfur metabolism related alterations at the transcriptional, metabolic and enzyme activity level are consistent with a promotion of root growth to search for water at the expense of leaf growth. The results provide evidence for the importance of antagonistic regulation of sulfur metabolism in leaves and roots to enable successful drought stress response at the whole plant level [60]. During the silking and blister stages moderate stress significantly change the relative water content (RWC) and also change the relative conductivity (RC) ($P < 0.05$) of the leaves; however, severe stress significantly decrease ($P < 0.01$) the leaf RWC and increase ($P < 0.01$) membrane permeability (leaf relative conductivity). Furthermore, under severe drought stress antioxidant enzyme activities declined ($P < 0.01$) in later stages, namely for superoxide dismutase (SOD) the tasseling and blister stages, for peroxidase (POD) the milk stage, and for catalase (CAT) during the tasseling, blister, and milk stages. Meanwhile, membrane lipid peroxidation (measured as malondialdehyde content) significantly increased ($P < 0.01$) in all stages [61].

2.4 Drought-tolerance mechanism

Phytohormones like, ABA, IAA, CKs, GA, SA, BRs, JA, ethylene, and triazole are directly or indirectly involved in plant responses to a wide range of stresses. They play critical roles in regulating plant growth and development, and stress tolerance to promote survival and acclimatize to varying environments [10]. Abscisic acid accumulates in response to drought stress and displays its well characterized effect on stomatal closure and promotes root growth in maize seedlings at low water potential [62]. Another possible role of endogenous ABA is to modulate glycinebetaine (GB) metabolism in maize particularly at the seedling stage in drought stress. The leaves of two maize cultivars, Zhengdan 958 (ZD958; drought-tolerant), and Jundan 20 (JD20; drought-sensitive) after exposure to integrated root-zone drought stress (IR-DS) shows increased betaine aldehyde dehydrogenase (BADH) activity and choline content the key enzyme and initial substrate, in GB biosynthesis. The peak of ABA content reached earlier than that of GB in the leaves of drought-stressed maize plants. Therefore, endogenous ABA seemed to be involved in modulating GB accumulation by enhancing BADH activity, thereby improving leaf RWC and enhancing shoot DM in drought-stressed maize plants, especially in the drought-sensitive cultivar (JD20) [63]. Similarly, Si application (400 mg L^{-1}) had positive effect on photosynthesis, water use efficiency, stomatal conductance, cholorophyll contents, Rubisco activity, and Rubisco activation state at 20 days of drought. Possibly Si may have direct or indirect role in maintenance of more active Rubisco enzyme and Rubisco activase and more stable proteins for carbon assimilation under stress conditions [64]. Maize was subjected to drought at the start of tasseling (6 days) followed by foliar spray of BR (0.1 mg l). Exogenous application of BR remarkably improved the gas exchange attributes, plant height, leaf area, cobs per plant, seedling dry weight both under drought and well-watered conditions [40]. The formation of cortical aerenchyma (RCA) in maize roots is associated with drought tolerance. RCA reduces root respiration by converting living cortical tissue to air volume. It has been hypothesized that RCA or large cortical cell size (CCS) increases drought tolerance by reducing root metabolic costs, permitting greater root growth and water acquisition from drying soil [65, 66]. Maize growth and yield responses were related to ROS production, osmolyte accumulation, and activation of anti-oxidative defense system under drought conditions. The regulation of these physio-biochemical responses of plants can be used as markers for drought stress tolerance [67]. Drought-tolerance mechanism is difficult in maize this may be due to complex genetic makeup. Metabolic traits provides better substitute to unravel the genetic mechanism of drought tolerance. The physiological status of plants can be monitored by metabolites that interconnect the visible phenotype with core genome, so that gene or gene loci could be identified and selected which are less affected by environmental factors [68–71]. However, the negative effects of abiotic stresses can be minimized by adopting the genetic approaches or by inducing resistance through transgenic approaches [3]. The importance of QTLs-based approach is associated to improve the maize crop performance under various abiotic stresses and in achieving increase in maize yield. Besides, QTLs with large effect and linked with stress-tolerance traits provides several modes to investigate the various components affecting source sink relationships of maize plants under abiotic stress [72]. Chen et al. [73] compares the role of drought resistance and drought recovery in drought adaptation in maize seedlings. After recovery most of the physiological parameters (like leaf water content, water potential, osmotic potential, gas exchange parameters, chlorophyll content, Fv/Fm and nitrogen content, and increased H_2O_2 accumulation and lipid peroxidation) rapidly return to normal level. Correlation analysis shows that physiological bases of drought adaptability closely related to drought recovery (r = 0.332) and not to drought resistance and ($r = 0.714^{**}$) both are definitely different. Under drought stress higher chlorophyll content ($r = 0.874^{***}$) and Fv/Fm ($r = 0.626^{*}$) contributes to drought recovery. In addition, leaf water potential, chlorophyll content, and Fv/Fm could be used as efficient reference indicators in the selection of drought-adaptive genotypes.

3. Nutrient status in drought

The role of N metabolism regulation in maize drought tolerance was comprehensively studied [74]. Drought have strong impact on all the physiological process of plants and N metabolism is of central importance during drought stress because to survive drought and to maintain growth plants adaptive strategies includes such as improved nutrient uptake and transport, photosynthesis regulation, and to produced solutes and proteins that contains N compounds such as amino acids, amides and betaines. While Kant et al. [75] reported that relationship between drought and N nutrition consists of a complex network of regulatory interactions that affects almost all physiological processes in plants. Nitrogen (N) is one of the macronutrients, its major requirement for crop growth and grain yield of maize, whereas maize water requirement is highest in the reproductive stage [76]. Therefore, nitrogen and water separately or in combination are two are most critical factors in maize production. According to Saud et al. [77] the use of nutritional soil with a proper nitrogen rate remained effective in ameliorating the adverse influence of drought stress. However, a contrast study showed low field capacity irrigation and low nitrogen fertilizer rates application improves water use efficiency and nitrogen recovery efficiency (NRE) simultaneously [78]. Water and nutrient availability influence root system architecture and development [79, 80]. During vegetative stages of corn, under moderate drought stress rooting depth typically increases, allowing more efficient uptake of water and nutrients from deeper within the soil profile [81]. The increase in plant height related with increasing N fertilizer application under drought stress [82, 83]. Andrade [84] reported that N deprivation reduced leaf area index, leaf area duration, radiation interception, and radiation use efficiency. Improved tolerance to various abiotic stresses alternative breeding strategies are adopted, one of them is to select and inbreed maize plants under high population density to improved tolerance for low-N and drought [85]. Although under dense plant population maize prone to lodging due to increase in plant height and reduced culum diameters makes the stem weaker [86]. The mechanism underlying tolerance to high population density and low-N diverse tropical germplasm were grown under optimal, high plant population density and low-N. The association between ASI and grain yield, delayed senescence (expressed as chlorophyll concentration or number of green leaves above the ear) and ear/tassel weight ratio was observed in low-N. Also, grain yield negatively correlated with abortion rate. While under optimal and high population density, a positive association was reported between ovule number and abortion rate, suggesting a source limitation for C products [87]. In the same way selection of hybrid progenies for mid-season drought tolerance was due to improvements in morpho-physiological secondary traits such as reduced anthesis-silking interval, increased ears per plant, delayed senescence and relatively high leaf chlorophyll during late grain filling [88]. Drought at one of the sensitive growth stages, caused up to 40% grain yield losses. However, prolonged water stress during tasseling and ear formation stages leads to yield loss of 66–93% greater magnitude [39].

3.1 Effect of nitrogen at vegetative stage under drought stress

Variation in N supply affects both growth and development of maize plants [89]. Uhart and Andrade [90] reported that N deprivation reduced leaf area index, leaf area duration, radiation interception, and radiation use efficiency. However, nitrogen fertilizer changes canopy size which has an effect on the radiation use efficiency (RUE) of the crop thus affect plant growth and productivity [91]. According to Bänziger et al. [92], about 50% of all N in the leaf is directly involved in photosynthesis as either enzymes or chlorophyll. Thus, if the N supply is insufficient, photosynthesis is decreased by reducing the leaf area and photosynthesis rate as well as accelerating leaf senescence. Longer duration of green leaf area is one of the most important ways to improve maize yields. Bertin and Gallais [93] found that leaf senescence was highly correlated with a nitrogen nutrition index, mainly at low levels of nitrogen input. Using a comparative proteomic analysis of *Zea mays*, Prinsi et al. [94] found that the nitrogen status of plants may affect the post-translational modification of phosphoenolpyruvate carboxylase (PEPCase) that plays a role in phosphorylation in leaves. Several studies have reported that drought stress decreases N uptake and assimilation in plants [24, 95, 96]. Maize growth and many physiological processes associated with it are enhanced by N supply [97]. Nitrogen efficiency is a complex trait, to identify for N-efficiency 16 tropical maize cultivars, studied for leaf senescence under N deficiency (short-term nutrient solution experiment). Leaf chlorophyll contents and photosynthesis rates were used as measures for leaf senescence. The results show that the photosynthetic capacity of senescing leaves correlated with the N efficiency of the cultivars, rather than leaf chlorophyll content [98]. The effect of nitrogen on maize leaves at three level of drought stress (normal, mild, and severe stress) was examined, The NO content and nitrate reductase (NR) activity of maize leaves were significantly reduced under drought stress, while moderate nitrogen supply promoted the accumulation of NO and an increase in the Nitrate Reductase activity. Also, abscisic acid content increased and was positively correlated with the nitrogen concentration under drought stress. Together, these results indicate that moderate nitrogen supply increases plant resistance to drought stress, while high or low nitrogen concentrations increase the sensitivity of maize to drought stress [99]. Maize (*Zea mays* L.) crop was subjected to different periods of deficit irrigation and N rates in the field. The results indicated that optimum use of both water supply and N application will maximize the maize production. Deficit irrigation during early vegetative growth modestly reduced LAI, plant height, CGR, N uptake and total biomass production as compared to reproductive stage [100] also N uptake decreased with greater water and N deficits. Two maize hybrids differencing resistance to drought were fertilized with two different forms of nitrogen fertilizers, $Ca(NO_3)_2$ and $(NH_4)_2$ SO_4, and after fourth leaf stage they were exposed to drought stress. The results shows that two maize hybrids different in adaptability to two nitrogen treatment. NH_4 treated plants maintain the high turgor by improved osmotic adjustment under drought stress. Whereas, chlorophyll a, b values significantly higher in NH_4 treated plants compared to NH_3 treated plants in which chlorophyll content decreased throughout the drought stress [23]. Similar work Zhang et al. [101] indicated increased NO_3 nutrition played a favored anti-oxidative metabolic role, as compared with NH_4 nutrition, in the plants thereby increasing tolerance to drought stress. Further, two maize genotypes were accessed for effect of nitrogen rates and water stress. Under water stress, Shaadan 9 accumulates higher dry matter, grain yield, anti-oxidative enzyme activity, and lower MDA content than Shaadan 911. However, the addition of nitrogen increased dry matter and grain yield as well as activities of SOD, POD, and CAT to different levels and significantly decreased MDA content under water stress, higher for Shaandan 911 than for Shaandan 9. Thus, drought-sensitive variety showed its full potential after adding nitrogen in water stress condition [102]. The dry matter accumulation and nitrogen uptake were compared in sorghum and maize. Drought reduced nitrogen availability in soil for both the crops, although sensitivity of maize crop to nitrogen is more. Directly, maize crop shows less accumulation of dry matter in water deficit condition and indirectly because of nitrogen nutrition [103]. Twenty maize inbred lines were phenotype in response to two levels of water and nitrogen supply (control and stress) and combined nitrogen and water deficit. Image analyses study provide the opportunity for new traits, identified several color-related traits and kinetic chlorophyll fluorescence (PSII). For biomass production ability in maize, kinetic chlorophyll fluorescence (PSII) is relevant traits particularly under severe stress conditions. While architectural traits, like greater leaf area which provide good discrimination of resistant cultivars to abiotic stresses under climate change scenarios [104].

3.2 Effect of nitrogen at reproductive stage under water deficit condition

Maize crop tolerant to N-deficit conditions at early vegetative stage than later reproductive phase [105]. There is a synergistic relationship between water availability and N use efficiency in maize [106]. When N supply is limited, grain yield is more associated with N deficiency than drought stress, but with adequate N supply, drought stress is the main yield-limiting factor [97]. Similarly Halvorson et al. [107] described maize yield as a function of available water and nitrogen. Maize plants selected for tolerance to mid-season drought also provide tolerance toward nitrogen stress. This may be due to an increase in both the number of kernels per plant and kernel weight. Hence consistently increases grain yield across N-level [108]. The pre-anthesis drought significantly reduced the number of kernel rows, the number of kernels per row, as well as the 1000-kernel weight. The 80 kg N ha^{-1} was sufficient to achieve maximum grain yield under pre-anthesis drought. It is hypothesized that the adverse effects of pre-anthesis drought on grain yield can be mitigated if varieties are selected for roots which rapidly penetrate the soil and exploit the water resources in deep soil layers [109]. The effects of water stress imposed at low-sensitive growth stages (vegetative, reproductive, and both vegetative and reproductive) and level of nitrogen (N) supply (100 and 200 kg ha^{-1}) on the physiological and agronomic characteristics of the two hybrids of maize (*Zea mays* L.) were studied. The results showed that proline content increased and the relative water content, leaf greenness, 100-kernel weight, and grain yield decreased under conditions of water deficits. The limited irrigation imposed on maize during reproductive stage resulted in more yield reduction than that during vegetative stage. The 100-kernel weight was the most sensitive yield component to determine the yield variation in maize plant when the WD treatments were imposed in low-sensitive growth stages. The increase of N supply improved yield and IWUE when maize plant endured once irrigation shortage at vegetative stage. But, the performance of high N fertilizer reduced and eliminated when water deficit imposed once at reproductive stage and twice at vegetative and reproductive stages, respectively [110]. Further, the hybrids B73 × LH38, FS854, B73 × Mol7, and US13 were subjected to drought stress from the seventh leaf stage to evaluate differences in carbon and nitrogen accumulation and partitioning under drought. The results indicate that the greater drought tolerance of B73 × LH38 and FS854 to stress imposed during vegetative and early reproductive development resulted from their more active N uptake and assimilation and sugar production during the later portion of grain fill and from their more efficient partitioning of assimilate to the developing kernels [111]. Drought severity decreased grain yield sharply, but grain yield increased with nitrogen fertilizer. The time taken for pollination, grain weight, and total number of grains/ear were also affected. Increased proline content significantly under drought stress conditions shows activation of osmotic adjustment mechanism [112]. In a field study (carried out from 1995 to 1997) the effect of irrigation and water stress imposed at different development stages on vegetative growth, grain yield and other yield components of corn (*Zea mays* L.) was determined. The results of this 3-year study show that water stress occurring during vegetative and tasseling stages reduced plant height, and also leaf area development. Short-duration water deficits during the rapid vegetative growth period caused 28–32% loss of final dry matter weight. Whereas, single irrigation omission during one of the sensitive growth stages, caused up to 40% grain yield losses. Although prolonged water stress during tasseling and ear formation stages the predictable loss could be much greater (66–93%) [39]. Though, stay-green and kernel numbers are affected by nitrogen uptake and use efficiency, and by nitrogen remobilization [113]. Whereas, insufficient N supply combined with water deficit reduces per-plant kernel number and mass, total aboveground dry matter (DM) yield, and harvest index (HI) [114, 115].

4. Source-sink relationship under drought

Higher plants are heterotrophic, leaves are the major organs that are photosynthetically active sources with tissues that synthesize sugars and translocate mainly sucrose to other parts of the plants, which are photosynthetically less active or inactive sinks such as roots, fruits kernel, and tubers. This physiological dynamics is not static and changes with each different phonological stages. The plant life cycle begin from embryo (sink) that mobilize nutrient from storage organ seed (source). During vegetative stage the source organ are photosynthesizing leaves (fixed CO_2 + H_2O = sugars) that export carbohydrates to various developing sink organs, like, emerging leaves, roots for their utilization in growth and development. During reproductive stage, sugars and other nutrients are mobilized from mature leaves to developing seeds, kernel, fruits, and tubers. This sink transition and various competing sinks changes with respect to sink strength and all compete for carbohydrates allocation. This carbohydrate production (source) and carbohydrate partitioning (sink utilization of carbon) is also influence with various other factors such as nutrients, hormones, environmental factors. This coordinate regulation of source activity and sink strength determine the carbon © allocation throughout the plant and has been crucial for defining yield of the crop [116]. The balance between source-sink is disturbed due to insufficient sink strength or slow carbohydrates export leading to accumulation of carbohydrates in source organ causes feedback inhibition which in turn, down regulate the photosynthesis in leaves [117]. Drought, modify source-sink relations that disturb plant growth, also adaptation to stress and consequently affect crop productivity. Source-sink altered under water deficit condition because the growth primacies of plant changes. Since the source organ unable to supply assimilates to various competitive vegetative organ growth, to maintain reproductive structures and adaptation to stress. This decrease in photo assimilates reduces sink number and size [118]. They also emphasize the role of several metabolic and hormonal factors influencing not only the source strength, but especially the sink activity and their interrelations and their potential to improve yield stability under drought and salinity stresses. Further, Fahad et al. [10] highlighted the regulatory circuits of different phytohormones and cross talks among ABA, indole ABA, CKs, GA, SA, BRs, JA, ETHY, and TR at physiological and molecular levels on exposure to salinity. The decline in source and sink strengths during water deficit leads to important reductions in crop yield [3]. In the present climate change model [119], drought periods are becoming more frequent and severe [120, 121], and future crop varieties have to be more resilient to this stress. Maize is the third most important crop after wheat and rice, mainly grown for food, feed, and fodder. Plant breeding and agronomic practices have major role in genetic contribution to improved grain yield. Two main important physiological processes involved are sustained leaf photosynthesis during grain filling, which contributes to increases in dry matter accumulation, and second, an increase in kernel number due to higher partitioning to the kernels during the sensitive period of kernel number determination. Water use in corn is greatest during the late vegetative through early grain filling stages [76]. There are several factors that contribute to high plant performance under drought stress, like better portioning of biomass to the developing ear that results in faster spikelet growth which may results reduction in the number spikelet formed on the ear thus, facilitate overall seed set by reducing water and carbon stress per spikelet [122].

4.1 Source limitation and strength under drought stress

4.1.1 Source limitation

Growth conditions are favorable during pre-flowering and flowering, and a certain maize crop therefore establishes a large leaf area and many kernels and ears. Drought occurs after flowering causing the leaves to senesce early. The supply of assimilates will limit grain yield in this crop, and the plant will have many small kernels, thus limiting the source.

4.1.2 Regulation of source activity

Leaves are the main source organs; in maize, leaves ranged from 8 to 20 and these are present alternatively on nodes, and drought stress reduced the leaf size and number of leaves. Alternatively, reduced leaf areas under drought stress consider as an adaptive strategy. This may reduce the plant water requirement by reducing the leaf area and probability of plant survival is increased under limited water availability [123] but decrease in chlorophyll contents, chloroplast contents and photosynthetic activity reduced the grain yield [124, 125]. The important characteristics of superior germplasm include, more biomass allocation to leaf relative to stem weight, more leaf area, longer active leaf area duration [126] under different growing conditions. However features like morpho-physiological attributes such as leaf area, chlorophyll content, the rate and duration of photosynthesis, time of flowering, dry matter partitioning during silking, and leaf stay green plays key role in dry matter accumulation, harvest index and grain yield of corn [127]. Selection for stress tolerance has improved the grain yield in maize and it depends on higher leaf area per plant and higher harvest index (HI). Moreover, "stay green trait" or leaf greenness or reduction in the rate of leaf senescence during grain filling was one of the distinctions between older and newer hybrids [128]. Lafitte and Edmeades [129] selected maize cultivars in low and high N and secondary traits (Such as increased plant height, leaf area, chlorophyll concentrations, and delayed senescence) to improved grain yields. However, selection based in low-N to increase grain yield disturbs the balance of source and sink. Various secondary traits like water depletion pattern, leaf rolling, canopy temperature, reveals root potential and water extraction capacity. Whereas chlorophyll concentration quantifies the stay green trait of leaf. Flowering traits are associated with specific developmental stage, while, photosynthetic rate indicates the plant growth rate throughout the plant life cycle. Thus, these secondary traits are a related to specific mechanism and they contribute to enhance grain yield under drought stress [130]. Monneveux et al. [131] indicate that the traits of source organs contribute marginally to drought tolerance; variation of leaf or root traits seems to be less important than variation in tassel parameters for increasing drought tolerance. Further progress in drought tolerance in maize, the solution might reside in the manipulation of sink organs. It is therefore suggested that selection for even greater number of ears, bigger grains and smaller tassels may help to increase grain yield under water limited environments in the near future. Selection based on crop ideotype by Bolaños and Edmeades [132] was successful in producing drought-tolerant cultivars that were able to partition the assimilates to ear at flowering. However, Gambin et al. [133] indicated maize reproductive efficiency in kernel set is not constant across different plant growth rates (PGR) around flowering, therefore PGR per kernel used during this period as an indicator of source availability per kernel. The curvilinear response relating kernel number per plant and PGR around flowering, increased PGRs resulted in higher PGR per kernel around this period ($r2 = 0.86$; $p < 0.001$). Grain filling duration was partially explained ($r2 = 0.27$; $p < 0.01$) by the ratio between PGR per kernel during the effective grain filling period and kernel growth rate. Together, these results support the importance of source availability per kernel during early grain filling on the determination of maize potential sink capacity and final KW.

4.2 Sink limitation and strength under drought stress

4.2.1 Sink limitation

Growth conditions are favorable during pre-flowering and a certain maize crop therefore establishes a large leaf area. There is stress during flowering time and therefore the crop can establish only few ears and kernels. After flowering, the growing conditions may be favorable again, but the demand for assimilates by the kernels and their capacity to absorb the available assimilate will limit grain yield, thus limiting the sink.

4.2.2 Regulation of sink activity

Flowering is the most crucial stage for silk growth, pollination, and kernel setting in maize [134] and is the utmost susceptible stage to drought stress. An asynchrony between silk emergence and pollen shedding under drought stress before and during flowering increases the anthesis-silking interval (ASI) and primarily causes yield loss [135–138]. High-temperature stress also hampered the pollen characteristics. However, exogenous application of various PGRs significantly reduced the damaging effects of high temperature, compared with control. Further, pollen characteristics can be exploited as screening tools for varietal development but selection must focus for those germplasm sources which can tolerate temperature above 38°C [1, 11, 12]. Under drought stress, a positive correlation was observed between flowering and kernel number per plant ($r > 0.8$), barrenness ($r > 0.7$), and ASI ($r = \_0.4$ to $\_0.7$) in tropical maize [136]. In maize, increased abiotic stress tolerance increased yield and yield stability in some tropical and temperate maize germplasm [139, 140]. Canadian maize hybrids show increased tolerance toward various stress factors like high population density, weed interference, low night temperature, low soil moisture, and low-N stress due to the features such as increased leaf longevity, increased nutrient and water uptake, and greater assimilates supply during grain filling [140]. Similarly, tropical maize shows decreased kernel and ear abortion, selected for mid-season drought [141, 142]. Therefore, constitutive stress-tolerance mechanism may be operative in maize germplasm, which may be related to the creation of sink size [92]. The ovule, ear and kernels are the reproductive sinks, for their proper development assimilates supply above threshold level are necessary. In unstressed condition, ear abortion may occurs due to insufficient assimilates supply that led to barren plants. Thus, ovule fails to extrude silk due to slow growth, whereas kernel aborted following pollination [141, 143, 144]. The corn was subjected to low water potential (Cw) for 5 days at the time of pollination [145]. Zinselmeier et al. [145] observed that embryos formed but abortion occurred and kernel number decreased markedly. Besides, during this abortion, all of the intermediates in starch synthesis were depleted and the starch contained in the ovary almost disappeared. The period for ovule, kernel, and ear abortion falls 1 week before until 2 weeks after silking; during this period, stress factors like drought, shade, high density, and low-N stress accelerate these processes [27, 90, 146, 147]. Physiological reasons for abortion of reproductive structures under various stresses have shown that concurrent photosynthesis is required to maintain the flux of carbohydrates to the young ear around flowering but under drought it not possible to remobilize carbohydrate reserve for the support of ear development [148–150]. Drought also reduces invertase activity in ovaries and this will reduce the flux of hexose sugars; starch reserves of ovaries are depleted and it leads to the ovaries abortion [151]. In tropics, maize yield could be increased by improving the balance between the source supply and sink demand [152]. The main focus of maize breeding program is to increase the grain yield under high N; this practice resulted in an increase in both the source and sink in size and efficiency [153]. However, in N-limited conditions, not only the importance of C but also for N associates with source and sink [154] the N required during the exponential phase of grain growth to sustain the kernel number per plant and grain yield. The role of glutamine synthetase (GS) and asparagine synthetase (AS) (their main function in plant nitrogen remobilization) was determined in two maize varieties (ZD958 and NH101) in relation to post-silking drought stress (PD) nitrogen partitioning. The results indicate the PD stress increased nitrogen remobilization, and in ear leaves, the expression of *ZmGln1-3* was enhanced for both varieties. While under PD treatment, three AS genes (*ZmAS1, ZmAS2*, and 10 *ZmAS3*) were differentially regulated, of which the expression of *ZmAS3* was stimulated at the late stage of leaf senescence. In developing kernels, there were no significant differences in expression patterns of GS and AS genes between the well water (WW) and PD plants. Therefore, at the whole plant level, PD stress showed more influence on leaf nitrogen status, and the upregulation of GS and AS genes may contribute to the higher leaf nitrogen remobilization when exposed to PD treatments [155]. Genotypes selected for high grain yield under normal growing conditions also had a high level of dry-matter accumulation and partitioned more assimilates to the grain under water deficit conditions. The improved genotypes produced more numbers of ears/plant and kernels/ear [156]. Leaf growth and ASI are the main determinants of source and sink strengths of maize.

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Chapter 22

Ecofriendly Marigold Dye as Natural Colourant for Fabric

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Abstract

This chapter highlights on the applications of marigold plant extracts as an antibacterial and antimicrobial best dyer for textiles. *Tagetes erecta* usually known as Marigold is a vital wellspring of carotenoids and lutein, developed as a nursery plant. Marigold blossoms are yellow to orange red in colour. Now a days, lutein is transforming into an unquestionably common powerful fixing, used as a part of the medicines, food industry and textile coatings. This has increased more noticeable vitality of marigold and its exceptional concealing properties. Regardless of the way that marigold blooms; its extract has been used as a measure of veterinary supports. The examination was directed to contemplate the usage of a concentrate of marigold as a trademark shading, which is antibacterial and antimicrobial. The marigold extract ability was focused on colouring of the cotton fabrics. Investigations of the dye ability, wash fastness, light fastness, antibacterial tests and antimicrobial tests can be endeavoured. Studies have exhibited that surface concealing was not impacted by washing and drying in the shadow/sunlight. These surprises reveal that the concentrate of marigold extract can be used for cotton fabrics.

Keywords: dyeing, marigold, colour fastness, fabric, antibacterial, antimicrobial

1. Introduction

Marigold is a common name of Tagetes species, a genus of herbs and the member of the Asteraceae family. It is a native of Mexico and other warmer parts of America and naturalised elsewhere within tropics and sub tropics. In India it is cultivated as garden plant. The flowers which are yellow to orange in colour with corymbose clusters are much used for garlands and functions and also used to decorate households during celebration of festivals.

These plants do not require any additional care. They grow in all conditions of the environment. Marigold plants bare sun, heat, drought and grow in any well-drained soil. Marigolds are easy to grow even from transplants also. Marigold requires a mild climate for smooth growth and flowering. Mild climate is required during the growing period is 14.5° - 28.6°C and it improves the production of flowers at higher temperature (26.2° - 36.4°C). Depending on the climatic conditions, the marigold grows three times in a year – rainy, winter, and summer seasons. The different coloured flowers are shown in **Figure 1**.

Figure 1.
The types of marigold flowers available in India.

The earliest use of marigolds was by the Aztec people who attributed magical, religious and medicinal properties to marigolds. The first recorded use of marigolds is in the De La Crus-Badiano Aztec Herbal of 1552. The Herbal records the use of marigolds for treatment of hiccups.

The orange, yellow and reddish yellow (i.e. red) colour flowers contain several pigments which appear to vary with source and soil nature. The active ingredients found in marigolds are plant sterols called calendulin, calendic acid, polysaccharides, polyunsaturated fatty acid, carotenoids, flavonoids, triterpenes saponins, like triterpenoid, Tocopherols, oleanolic acid glycosides.

The aqueous extract of flowers showed activity against gram positive bacteria. An infusion of plant is employed against rheumatism, cold and bronchitis. Root extract is employed as laxatives. Marigold leaves are utilised in kidney troubles and muscular pains. The infusion of florets is prescribed as a diuretic and carminative. The florets are utilised in the treatment of eye diseases and ulcers. The oil obtained from the fresh flowers incorporates a paralysing effect on the spinal marrow. It is used as antiseptic also. The flower heads are said to possess stimulant and anti-helminthic properties. Their juice contains iodine and is employed on cuts and wounds.

India is producing a big amount of marigold flowers. It is mainly used for floral offerings to Goddess and also for decorative purpose. After its use, the flowers were thrown away as garbage in ponds and rivers, which creates a lot of pollution. Our main aim of this chapter is to bring an awareness about the employability of marigold in many applications.

2. Uses of Marigold

The flowers of marigolds are very beautiful with a lot of benefits make a garden as more attractive. Marigold and its extracts are highly useful in therapeutic treatments like headaches, swelling, toothache, wounds and numerous skin problems. In addition, they have been used in cooking. Marigold was one of the earliest cultivated flower. The ancient Greeks used the petals of marigold for decorations and other purposes like make-up, colouring food (stews, soups, pudding, etc), dying fabrics and as part of medicines. The significant uses of marigold flowers are deliberated as follows:

2.1 As a garden plant

2.1.1 Nematode control

The roots and stems of marigolds emit a chemical that will suppress the population of root-knot nematodes, tiny soil borne worms that prey on the roots of decorative plants and vegetables. It appears that French marigolds, particularly the 'Tangerine' variety, are the best against the destructive pests.

2.1.2 Beneficial to insects

Marigolds itself attracts many insects that are harmful to other plants of the garden, so that the other plants get protected from the pests and do not require any pesticides.

2.1.3 Beautifies the garden

Marigolds are capable of growing in hot shades with many colours like orange, yellow, red, mahogany or with combinations. Flowers are of a single, double or multi-layered and sizes vary from 6 inches to 3 feet. So makes the garden more gorgeous and increases the beauty of the garden.

2.1.4 Marigold companion benefits on planting

When marigold is planted nearby cruciferous and tomato plants, these plants get protected from worms. It is because of the scent released from marigold, which confuses the pests. So it avoids usage of chemical pesticides. Marigold is additionally a decent companion when planted near bush beans, squash, cucumbers and brinjal.

2.2 As a medicinal plant

The very important uses of marigold with respect to antioxidant properties as medicine are as follows.

2.2.1 Homemade skin treatment

Ointments made up of marigold are used to heal sunburns, acne and ulcerations in addition to healing of wounds, dry skin and blisters.

2.2.2 Digestive-soothing tea

Tea powder made with marigold flowers lowers the symptoms caused by inflammatory bowel diseases. Marigold tea is useful in solving gastric and ulcer problems as well as in reducing stomach cramps. The florets are consumed in tea form to assist ease digestion internally and improve liver health.

2.2.3 Immunity booster

Marigold extracts are accustomed to manage symptoms of coughs, sore throats or fever. So it can also be used as immunity booster.

2.3 Treatment for fungal infections

2.3.1 Lowers inflammation and oxidative damage

Research shows that active chemicals of marigold make it a natural cytotoxic, hepato-protective, and spasmogenic herb, that has been used in both animal and human trials. Extracts obtained from the flower contains C-reactive protein and cytokine levels which protect cells from being damaged like ageing and cell deterioration.

Marigold controls oxidative damage from free radicals which will affect delicate tissues of the skin, GI tract and genitals caused by viruses or bacteria [1]. In addition, research also shows that marigold helps to reduce the growth of bacteria's in wounds and might even be ready to reduce symptoms related to chemotherapy and cancer treatments [2].

2.3.2 Reduces eye inflammation and conjunctivitis

Marigold extracts are used for treating conjunctivitis and other chronic ocular inflammatory conditions of animals. As these extracts have shown antibacterial, antiviral, antifungal and immuno-stimulating properties and which can be used on delicate tissues of the eyes against oxidative damage [3].

2.3.3 As a natural antiseptic

The extract of Marigold is employed to treat bacterial ear infections and reduce pain. It has been identified that marigold drops can lower inner-ear swelling and inflammation very easily in a short period and even without the use of antibiotics. Marigold extract may be used as antifungal for treating fungal infections of ear [4].

2.3.4 Heals skin wounds, burns and rashes

From the traditional days the marigold is employed for treating irritated skin to cut back itchiness, redness, sensitivity, dryness and swelling. It found that marigold has the power to push the expansion of healthy new tissues, increasing the rate of blood flow to the affected areas and boost collagen production, which firms and strengthen the skin, hydrate dry skin and speed up the method of skin repair following surgery or damage.

Marigold's ability to support the treatment of wounds is believed to be the secretion due to stimulation of somatic cell production, which contains glycoproteins and nucleoproteins. It is also associated with increased cell turnover and improved collagen metabolism stimulation.

In folklore medicine, marigold products were applied to varied ulceration (both internal and external) and on wounds to stop infections, and even today marigold is employed during post-surgery to push faster incision healing.

Marigold extract is combined with natural lubricating products and applied on dry and rash-prone skin that will help to enhance skin hydration and firmness. The extract of marigold is employed topically to reduce rashes on the sensitive skin, discoloration, unsightly varicose veins, dermatitis, eczema, bruises and scalp dandruff. It used to treat swollen bug bites, burns, and infected cuts and decreases other signs of poor blood flow and inflammation affecting the skin.

2.3.5 Eases cramps and spasms

Marigold's analgesic property is useful for relieving muscle spasms, stomach cramps and PMS/menstrual cramps. It reduces cramping by improving blood circulation to the painful area and lowering inflammatory responses [5].

2.3.6 Mosquito repellent

Due to their pungent odour, antioxidant content and volatile oils, marigolds are often accustomed naturally repel mosquitoes, pests and other insects. This is often one reason as marigold commonly planted in vegetable gardens. The flowers of Marigold are employed as extract form in candles, room/ bug sprays, and lots of skin lotions so as to forestall mosquito bites. Use of marigold products on our skin helps to repel bites. The flowers' aroma works underground to stay away nematodes (microscopic worms) [6].

2.4 Marigold as perfume

Many Tagetes sp. of marigold yield strong aromatic oil and referred as Tagetes oil. The tagetes oil is principally used for the compounding of high grade perfumes. The oil from French marigold L. is produced chiefly in France and finds a decent market within the perfumery trade.

In India French marigold L. appears to be a promising source of the oil on account of its fairly high yield with true floral character and favourable tagetone content. The flowers are regularly distilled in India for the assembly of attars by absorbing their steam distillate over sandalwood oil or liquid paraffin. It also acts as fly repellent and is credited with larvicidal properties.

2.5 Natural dye

India has a rich, diversified plant kingdom, which is a treasure house of varied natural products. Natural dye is one such product. The natural colourants mainly flavonoids and carotenoids present in the Marigold were extracted by different techniques and used as natural colourants on different fabrics. The use of natural colourants exists from ancient period.

3. Dye of marigold

Dyes are one of the foremost important yields of plants. In most of the countries, natural dyeing is practiced only on handicrafts and artificial dyes are being employed altogether for commercial dyeing processes. The problems related to synthetic dyes arise during their application, manufacture, and process of synthesis, intermediates formed and other raw materials involved.

Now a days with the global concern on the use of eco-friendly and biodegradable materials, around the world the solicitation of natural dyes in the textile industry is enhanced. The interest in the utilisation of eco-friendly and biodegradable materials create the employment of natural dyes. In contrast, natural dyes are environmental friendly, exhibit better biodegradability and customarily have the ensuing compatibility with the environment than synthetic dyes [7]. Natural Dyeing may be carried out by using eco-friendly bath methods like alkaline bath, acidic bath, or neutral bath. The method is economically viable because the raw materials are available at low cost, which in turn reduces the cost of production. There are several articles available on different methods of mordanting on different fabrics, for dyeing with different natural dyes.

Dyeing of cotton and silk with marigold brings a growing interest within the revival of natural dyes in textile colouration [8]. The main idea of extracting dyes from marigold plant sources is to avoid environmental pollution and its characteristics like antibacterial, antifungal, etc. The application of various mordants along with marigold gives innumerable shades like brown, yellow, orange and so on to the fabric.

Dyes derived from marigold sources have emerged as a significant alternative to synthetic dyes. The dyes produced were dyed on cotton fabric and tested for colour fastness to washing properties. Mordants were used to fix the colour on the fabrics. The dyes obtained from the marigold plant are replaceable to synthetic dyes.

Light fastness of the numerous natural dyes, particularly which are extracted from flower parts is found to be poor to medium [9]. In earlier days India was a significant exporter of herbal dyes to the world. Now days the demand for natural dyes has augmented, because of the ban on the assembly of a variety of synthetic dyes and intermediates within the developed countries, thanks to the pollution problem [10].

3.1 Process of dyeing

The procedure involved in dyeing of cotton with marigold extract comprises the following steps

- Pre-Treatment
- Extraction of colourant
- Mordanting (fixing dye with fibre)
- Dyeing

3.1.1 Pre-treatment

Before carrying out the dying process. Fabric undergoes scouring. Scouring is a process used to remove the dust and other colourants present on fabric. During scouring, the cotton fabrics were washed in a solution containing x g/L soda ash and a couple of g/L non-ionic detergent solution at 50° C for 25 min, keeping the fabric to required liquor ratio. The scoured material was thoroughly washed with H_2O and dried at moderate temperature. The scoured material must be soaked in clean water for 40 min before dyeing or mordanting.

3.1.2 Extraction of colourant

Flowers from the plant source were dried in trays, in thin layers, in an exceedingly current of warm air immediately after picking. Then crushed and dissolved in water and allowed to boil over a water bath for two hours for the quick extraction. All the colour was extracted from flowers was filtered for immediate use.

3.1.3 Mordanting

It is a method where mordant is used to fix the dye on fabric. Mordant binds dye with fabrics by forming a complex, which then confers to the fabric. Most of the natural dyes have no substantively on textile fabrics without the utilisation of a mordant. All most all the natural dyes need a mordanting chemical to make an affinity between the fabric and dye [11].

During mordanting, the weighed cotton samples were treated with a different metal salt. The different percentage of mordant was dissolved in water to create a required liquor ratio. The wetted sample is immersed into the mordant solution and so dropped at heating. The temperature of the dye bath was raised to 80°C over a period of half an hour and left at that temperature for 30 minutes. The mordanted material should be rinsed with water and dried in shadow. Mordanted cotton must be used immediately for dyeing because some mordants are very sensitive to light.

Generally mordants are metallic salts form a metal complexes with the fibres and dyes [12]. After completion of mordanting, the metal salts fixed to the fabrics, attract the dye pigment molecules. For example if aluminium sulphate mordant is used, it induced into the fabric and helps to fix the natural dyes through either coordination bonds or hydrogen bonds and by other inter molecular forces [13].

3.1.4 Dyeing

The cotton samples must be dyed with dye extract, keeping required M:L ratio. The marigold dye extract has prepared by adding × gm dye powder in y ml of water. Dyeing was carried out by standard dyeing method. After dyeing, the dyed material was washed with cold water and dried at low temperature. Then it has been dipped in brine for dye fixing.

4. Purpose of natural dye

Mainly the colours generated from natural resources like marigold are vibrant, biodegradable, and non-allergic. These features indicate that they are far compatible with the environment and to be used around humans. It is easy to extract the natural colour from plants, fruits or flowers. Many natural dyes even have antimicrobial properties, making them safer for teenagers specifically.

Natural dyes will not contain harmful chemicals nor carcinogenic components, common to artificial or synthetic dyes [14]. Usage of natural dyes will help to preserve the environment and lowering human dependence on harmful products. When toxic runoff and residuals from the textile manufacturing and dyeing process often find yourself in our delicate oceans, we must always do and make sure that we are using the nontoxic alternative, natural dyes.

Furthermore, the products employed in producing natural dyes, particularly plants, produce no waste, unlike the products employed in the synthetic dyeing process. Because plants bypass the complete production process which takes to form synthetic dyes. This can be one more reason why natural dyes are infinitely better for the environment. By using natural dyes instead of synthetic dyes, we are ready to be closely connected to nature and recognise the importance it plays altogether of our lives. The main advantage of naturally dyed fabric is that they are capable of absorbing higher UV rays. By wearing clothes dyed naturally, you are able to protect your skin more from the UV rays. The natural dyes can be used on fabrics for different purposes.

4.1 Intention of Marigold dye

4.1.1 To provide different colour effect on fabric

Marigold flower is grown altogether parts of India throughout the year and maybe an honest source of material for natural dyes. Three different shades of

Marigold flowers are available in India; those are light yellow, golden yellow and orange mix yellow. These were taken as raw materials for natural dyeing of cotton fabrics. Depending upon the thickness of the colour, the amount of flower is soaked in a specific amount of water to extract the required colour. The 30 g of extract powder is mixed with 100 ml of water for about 30 minutes for extraction. However, in the case of orange-yellow and light yellow, the extraction time was 50 minutes.

Cotton material is used to dye with the extracts obtained from three different shades of marigold after mordanting with eight different mordants in each case. All the specified cases of attractive shades may be produced by such dyeing. The colour fastness property to washing of most of the dyed samples was within the range of 2–3 percent in general. Colour fastness to light of the dyed samples varied with the change of mordant and therefore the substrate.

4.1.2 To enhance antibacterial/antimicrobial properties of fabric

Cotton fabric is employed in many applications in the textile industry due to its unique properties. However, the antibacterial properties of marigold extract will help within its applications in hygienic textiles and employed in medical fields. Flowers of marigolds are generally used to worship in temples and decorate vehicles [15]. The dyed SPF fabrics were then evaluated for colour values, fastness properties, anti-bacterial activities in addition to the durability of the identical. The studies give us clarity regarding the benefits of using such mordants in case of achieving antibacterial/antimicrobial functionality with addition of eco-friendliness [16]. Marigold dyeing by using most typically found alum mordant was also distributed for comparison of the aim.

5. Materials and experimental processes

5.1 Materials

Plant material: Marigold (*Tagetes erecta*).
The scientific classifications are as follows.
Kingdom – Plantae
Order – Asterales
Family – Asteraceae
Genus – Tagetes
Species – T erecta

5.1.1 Plant description

A stout, branching herb as shown in **Figure 2** is 60 cm tall, native of Mexico, extensively cultivated as a border - annual in gardens all over India. Roots usually taproot system, Stems usually erect; Leaves strong scented, pinnately dissected: segments 1–5 cm long, oblong or lanceolate, serrate; flower - heads solitary, yellow to orange, 5-9 cm across, florets bisexual, pistillate, functionally staminate, rays many, long-clawed, sometimes two-lipped or quilled, involucre campanulate; fruit achenes, 6–7 mm long, usually dry with relatively thick, tough pericarps, sometimes beaked (rostrate) and/or winged (late), often dispersed with aid from pappi; Seeds 1 per fruit, exalbuminous; embryos straight. The flower samples of four cultivars of *T. erecta* (marigold) i.e., marigold orange (MGO), marigold yellow (MGY), marigold reddish yellow or red (MGR) were collected (**Figure 3**).

Figure 2.
Marigold plant.

Figure 3.
Different colours of marigold.

5.2 Experimental process

5.2.1 Extraction of marigold powder

The petals were separated, sun-dried, powdered, and stored at −20°C for further studies (**Figure 4**).

5.2.2 Extraction of lutein ester

The fine powder of the petals of marigold orange, marigold yellow, marigold reddish yellow of about 30 gm was used to extract with various solvents. The extraction was carried out under dark conditions by covering the apparatus with black cloth together with the condenser. The extract was filtered and concentrated to dryness in an exceedingly rotary evaporator under reduced pressure and at 40° C, this stored in a container at −20° C (**Figure 5**).

5.2.3 Identification and extraction of fatty acids

The concentrated extract of different cultivars of marigold flower petals containing lutein esters was saponified in presence of 40% methanolic potassium hydroxide at 60°C for 1 h under dark condition. The unsaponifiable matter containing free lutein was extracted by three times using hexane. The water layer was acidified to

Figure 4.
Extraction process of marigold.

liberate the free fatty acids and extracted three times with petroleum ether. The ether fractions were collected and the solvent was removed under vacuum. The fatty acids were converted to methyl ester according to the standard method [13] and fatty acid composition was analysed by using a gas liquid chromatography.

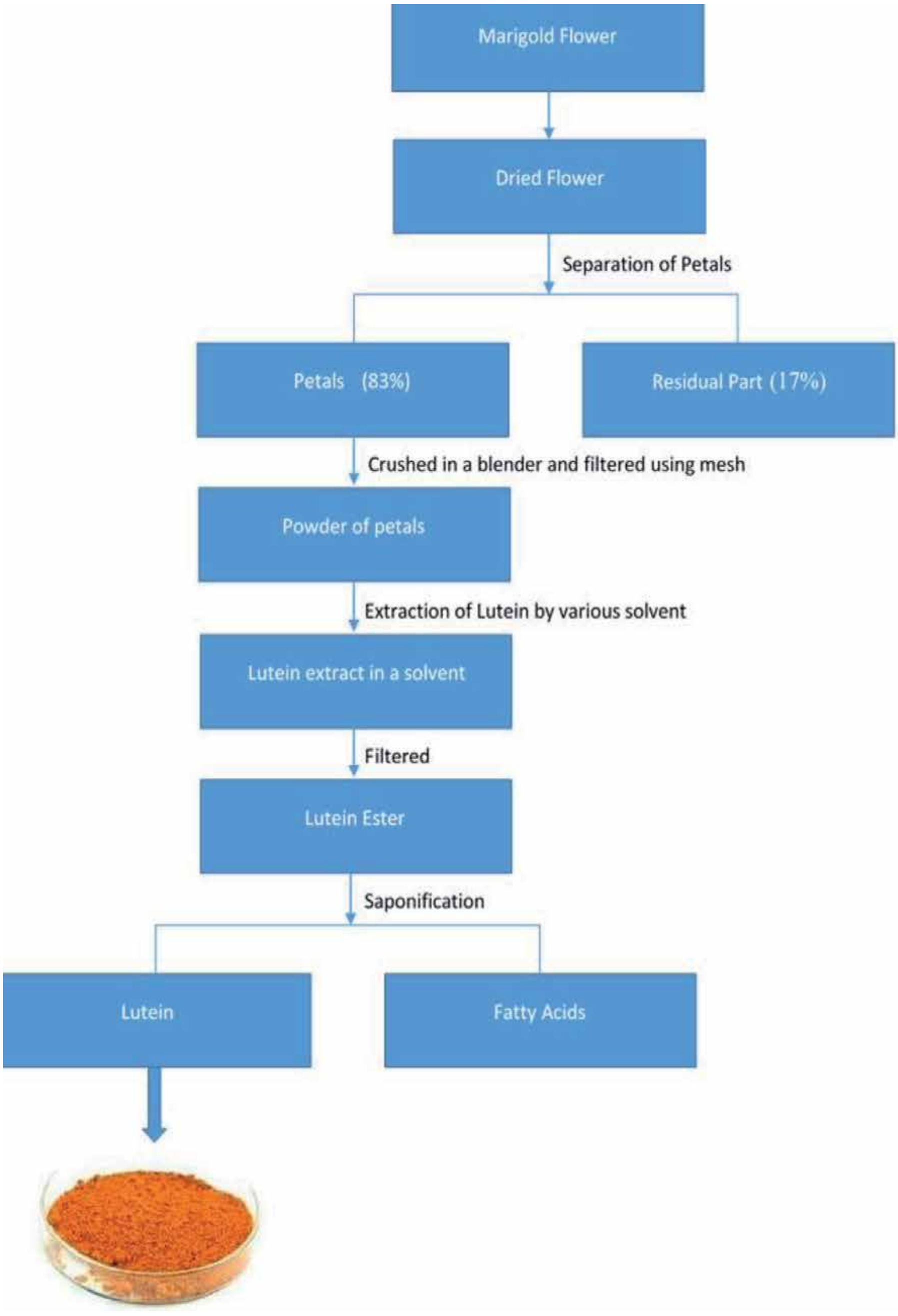

Figure 5.
Preparation and extraction of lutein.

6. Conclusion

The fabric dying process using marigold (*Tagetes erecta*) helps us to come to a general conclusion. Indian Marigold grows in all type of soil with different atmospheric condition and the required care to grow the marigold plant is very minimal. Marigold with rich quality of making garden beautiful have wide range of additional benefits. It has been used in the medicines, foods and in textile industry from ancient days. The marigold extracts are effective in protecting unsaturated fatty acids in the emulsion system. Marigold extract is used as a colouring agent.

Now days it is used in the fabrication of fabrics for colour dyeing and to provide antibacterial, antifungal and antimicrobial effects on fabric. Marigold dye arises as natural dye and free from harmful chemicals. Natural dye extraction is very simple and user friendly. Flower extract contains rich lutein esters. These extracts are used as antioxidant component in various food and pharmaceutical formulations.

Overall investigation has generated a lot of information on Indian marigold and its extract, which are highly beneficial in textile industry. This information can create commercially importance to produce viable products from Indian marigold for food, pharmaceutical industries and Textile industry.

Acknowledgements

Authors are thankful to Vemana Institute of Technology, Bangalore and Management of BVVs S R Vastrad Rural Polytechnic, Guledagudda for their support.

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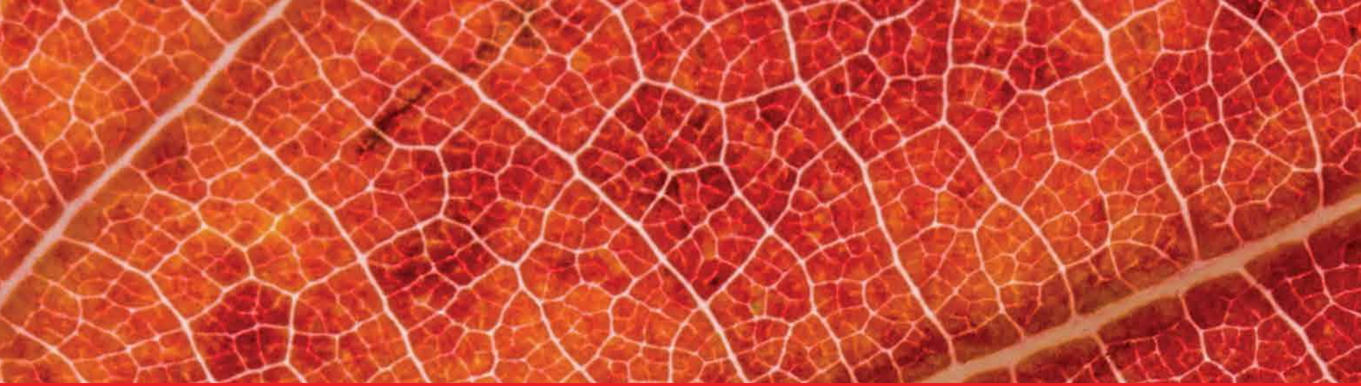

Edited by Shah Fahad, Shah Saud,
Yajun Chen, Chao Wu and Depeng Wang

Environmental insults such as extremes of temperature, extremes of water status, and deteriorating soil conditions pose major threats to agriculture and food security. Employing contemporary tools and techniques from all branches of science, attempts are being made worldwide to understand how plants respond to abiotic stresses with the aim to manipulate plant performance that is better suited to withstand these stresses. This book searches for possible answers to several basic questions related to plant responses towards abiotic stresses. Synthesizing developments in plant stress biology, the book offers strategies that can be used in breeding, including genomic, molecular, physiological, and biotechnological approaches that have the potential to develop resilient plants and improve crop productivity worldwide.

Published in London, UK

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ISBN 978-1-83881-063-4