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Tomato

From Cultivation to Processing Technology

*Edited by Pranas Viškelis,
Dalia Urbonavičienė and Jonas Viškelis*



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Meet the editors



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Preface

Tomatoes are one of the most valuable and popular vegetables worldwide. The fruit of each cultivar differs in size, shape, taste, and color, as well as the firmness of skin and flesh. Ideally, tomatoes should be fertile and disease resistant. With increased consumer demand for large salad-type tomato varieties, they also need to be robust to meet transportation conditions. More than 80% of tomatoes grown worldwide are processed into products such as tomato juice, paste, puree, ketchup, sauce, and salsa.

Tomatoes and tomato-based food products are rich in biologically active compounds such as polyphenols and carotenoids (mainly lycopene), which have numerous biological functions in the human body. Rising market demand has stimulated the development of diverse production methods for these compounds, and nowadays, lycopene is mainly produced through chemical synthesis. Nevertheless, bioactive compounds of natural origin enjoy both higher bioaccessibility and greater consumer trust. The industrial processing of tomatoes into tomato products generates large amounts of by-products (peel, pulp and seeds). These by-products are costly to dispose of and have a potentially negative impact on the environment, but they also represent a promising, low-cost source of carotenoids (primarily lycopene).

Among the interesting research topics covered in this book are the chemical composition, nutrition, production, and protection of tomato plants, and tomato processing applications, including sustainable technologies. This book will be of significant value to researchers, academics, and students in the field of agronomy, food, pharmacy, and other sectors.

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Section 1

Tomato Plant Nutrition and Production

Chapter 1

Foliar Application of Salicylic Acid on Growth and Yield Components of Tomato Plant Grown under Salt Stress

Salma Wasti, Salwa Mouelhi, Ferial Ben Aïch, Hajer Mimouni, Salima Chaabani and Hela Ben Ahmed

Abstract

Abiotic environmental stresses such as drought stress, mineral deficiency, heat stress, and salinity stress are major limiting factors of plant growth and productivity. Tomato (*Solanum lycopersicum* L.), one of the important and widespread crops in the world, is sensitive to moderate levels of salt in the soil. So many authors have reported large variation among tomato genotypes in their response to salinity. The present study was conducted to study the effect of different concentrations of salicylic acid on growth parameters, yield, and yield attributes of tomato under saline conditions. Tomato plants cv. Marmande were grown under normal or saline (100 mM NaCl) conditions. Different levels of salicylic acid: SA (0, 0.01, 0.1, and 1 mM) were applied as a foliar spray. The study was conducted at the vegetative and reproductive stage. Salt stress reduced significantly the whole plant growth at the two stages. Application of SA caused a significantly increase in biomass under non-saline conditions. However, in salt medium, treatment of leaves by SA induces a slight increase in biomass, leaf area and ameliorates the fruit diameter compared with plant grown only in the presence of salt. The beneficial effect of SA is more pronounced with the dose 0.01 mM.

Keywords: tomato, growth, foliar spray, fruit, salinity, salicylic acid

1. Introduction

During their development cycle, plants are exposed to several constraints under inappropriate environments without being able to escape them. Soil salinity is a major abiotic factor that reduced productivity of many crops.

About one third of the irrigated land in the world is affected by salinity to varying degrees [1]. According to FAO [2], more than 800 million hectares of land around the world are affected by salinity, accounting for more than 6% of the earth's surface. In Tunisia, saline soils cover about 23% of the total area, i.e., 8.7 million hectares of

arable land [2]. It caused various deleterious effects on morphological, physiological, biochemical, and nutritional attributes. During the onset and development of salt stress within the plant, all mechanism such as: photosynthesis and protein synthesis are affected. So, plants' first reaction was to reduce the extension of leaf area, followed by extension cessation with the increase of stress [3].

Tomatoes (*Solanum lycopersicum* L.) are today the most consumed vegetable in the world. They are an important greenhouse crop in semiarid coastal areas of Mediterranean countries. In these regions, soil and groundwater salinity are insidious problems that affect both tomato yield and quality [4]. It is known that dry biomass and fruit yield of tomato plants are strongly affected by soil salinity [5]. In a recent study, Ors and al. [6] reported that photosynthetic rate, plant dry weight, stomatal conductance, chlorophyll reading value decreased with salt in tomato seedlings. Plants exposed to NaCl stress were confronted with three fundamental problems, which are reduction of water potential, ion toxicity associated with the excessive accumulation of sodium (Na^+) and chloride (Cl^-) leading to essential cations potassium (K^+) and calcium (Ca^{2+}) deficiency, and production of ROS [7]. Salinity causes also unfavorable conditions that limit normal plant production. The increase of salinity most often causes a decrease in plants development and in general the average weight, the diameter of the stems, and size of the fruits were reduced significantly. Thus, driving with high salinity results in, therefore, a loss of production [8].

Furthermore, many research studies have focused on the physiological responses of plant subjected to salinity. The development of plants tolerant to environmental stress is seen as a promising approach, which can help satisfy the growing food demands in the world. Thus, overcoming NaCl stress is a major objective to ensure the stability of agricultural production. Salicylic acid (SA) is a signaling molecule that plays an important role in the induction of acquired systemic resistance (ASR) against pathogens; it was first demonstrated to play a crucial role in biotic stress such virus, fungi, [9]. Progressively, it was shown that SA induces tolerance to major abiotic stresses such as drought and salinity. Most papers, on this subject, have reported on the protective effect of exogenous salicylic acid against abiotic stress [9]. The exogenous application of SA affects various physiological, biochemical, and molecular processes in plants. Gharbi and al. [10] reported that SA (0.01 mM) enhanced shoot growth in *Solanum lycopersicum* cv Ailsa Craig and its wild salt-resistant relative *Solanum chilense*. Moreover, Mimouni and al. [11] found that the application of SA (0.01 mM) restored photosynthetic rates and photosynthetic pigment levels under salt (NaCl) exposure.

The purpose of this work was to study the effect of different concentrations of salicylic acid on growth parameters; yield and yield attributes of tomato under saline conditions. The study was conducted at two stages (vegetative and reproductive) and based on growth parameters (biomass production and leaf area).

2. Materials and methods

2.1 Plant material and growth conditions

Plant material studied is the cultivated tomato (*Solanum lycopersicum* L.) var Marmande. The tomato seeds were germinated in Petri dishes. Boxes containing 20 seeds are placed in an enclosure air-conditioned at a constant temperature (25°C) and under an illumination at low intensity ($10 \mu \text{mol m}^{-2} \text{s}^{-1}$). Eight days after, seedlings were transferred to nutrient solution composed by macroelements and microelements

as described by Wasti and al. [12] and placed in a *growth chamber* under controlled environmental conditions with relative humidity of 80%, temperature 25/18°C (day/night), artificial light 150 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and 16 h photoperiod. Two experiments were undertaken: one at the vegetative stage and the other at reproductive stage. The salicylic acid was applied as a foliar spray.

2.2 Experience 1

The plants were grown in pots, each pot containing four plants. The plants were grown for 11 days before the start of salt treatment. Each pot receives a basic nutrient solution. After an acclimation period of 11 days, the seedlings at three-leaf stage are divided into eight lots. Four control groups without NaCl continued to grow in the basic nutrient solution : Lot (1), the leaves are sprayed daily until the end of culture by distilled water, while the lots (2), (3), and (4) are sprayed with distilled water supplemented with SA (0.01, 0.1, 1 mM). The other four lots are transferred to nutrient solution enriched with NaCl (100 mM), and the leaves are sprayed daily until the end of the culture by distilled water added or not by salicylic acid (0.01, 0.1, 1 mM). The addition of salt is done gradually, with 25 mM every 24 hours, until a final concentration of 100 mM. The pH was adjusted to 5.9 with KOH (1N).

2.3 Experience 2

The second experiment was conducted under the same culture conditions as above, has tracked the growth and development of plants to produce fruit. The plants were divided into four groups. Two groups continued to grow on the nutrient solution, leaves of the first group were sprayed with distilled water while those of the second batch were sprayed with a solution of 0.01 mM SA. Plants of the third and fourth groups were transferred to a nutrient solution supplemented with 100 mM NaCl and leaves were sprayed with SA solution 0.01 mM. Spraying of SA continued until flowers were developed.

2.4 Growth parameters and ion analysis

* Dry mass (DM) was determined after desiccation at 80°C for 48h. * Sensitivity index (SI), i.e., the difference between dry matter production of treated plants and the control, expressed in percent of the latter, was calculated according to the following expression:

$$SI_{\text{treatment}} = \left(100 \times (DM_{\text{treatment}} - DM_{\text{control}}) / DM_{\text{control}} \right). \quad (1)$$

This parameter was more negative when the plant was sensitive to treatment

* *Leaf area* of the tagged leaf 5 was determined by using a leaf area meter AM 300.

3. Results

3.1 Plant growth

The tomato seedlings treated with 100 mM NaCl are less developed than the control plants. Indeed, salt stress reduced significantly the whole plant growth (42% compared

with control). Application of SA caused a significantly increase in biomass of whole plant under non-saline conditions. This increase is about 45, 30, and 32% (compared with control plants sprayed with distilled water) respectively for the concentrations 0.01, 0.1, and 1 mM. (Figure 1). This beneficial effect is more pronounced at the root system where there is a significant increase in biomass, about 66, 52, and 57% respectively, compared with control for the doses (0.01, 0.1, and 1 mM), whereas in aerial organs, stimulation is about 52, 33, and 32% for leaves and 26, 19, and 25% for stems compared with the control respectively for doses (0.01, 0.1, and 1 mM). (Figure 2).

Salt negatively affects the three organs. However, the roots were much less sensitive to NaCl than the aerial parts (leaves and stems). The decrease in dry matter was respectively 16, 40, and 50% (Figure 2). Foliar application of SA reduced the damaging effect of salinity on plant. The masses of dry matter increased compared with plants subjected only to NaCl. Indeed, in the presence of NaCl 100 mM, inhibition of growth of the whole plant was about 42% compared with the control plants, it was 40% when plants treated by salt were sprayed with Sa 1 mM and the inhibition was even more attenuated (29 and 27%) with the lower doses of SA (0.1 and 0.01 mM) (Figure 1). The root system of plants subjected to salt and sprayed by 0.01 mM SA was stimulated by 4% compared with the control (Figure 2).

3.2 Leaf area

The salt induced a decrease in leaf area by 50% in leaves of rank 5. Foliar application of SA induced an increase in leaf area of stressed plants. The stimulation was significantly with SA application at 0.01 and 0.1 mM (Table 1). While the foliar spray of SA did not affect the expansion of leaf plants grown without NaCl.

3.3 Na⁺ compartmentalization

Vacuolar compartmentalization of Na⁺ ions is one of the major strategies for salt stress tolerance. In tomato *var.* Marmande, when the content of Na⁺ ions increases,

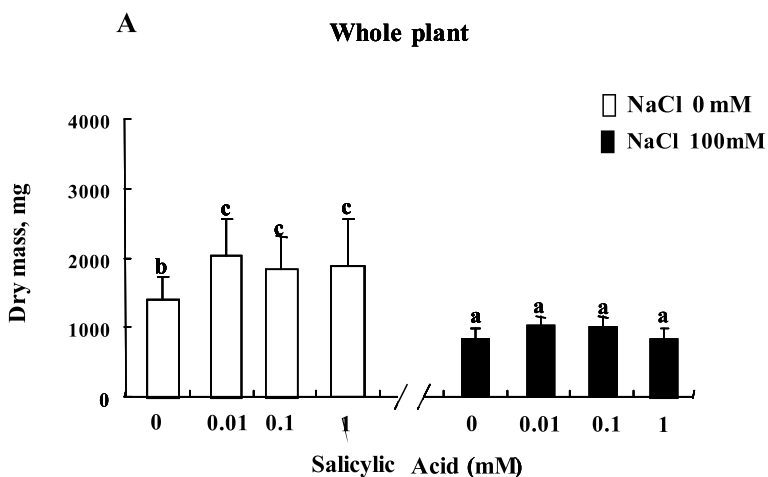


Figure 1. Dry mass of the whole plant of tomato *var.* Marmande submitted to 100 mM NaCl for 18 days and sprayed or not with salicylic acid (1; 0.1; 0.01 mM). Data are means of 16 replicates ± SE. Means with similar letters are not different at $P \leq 0.05$ according to Duncan's multiple range test at 95%.

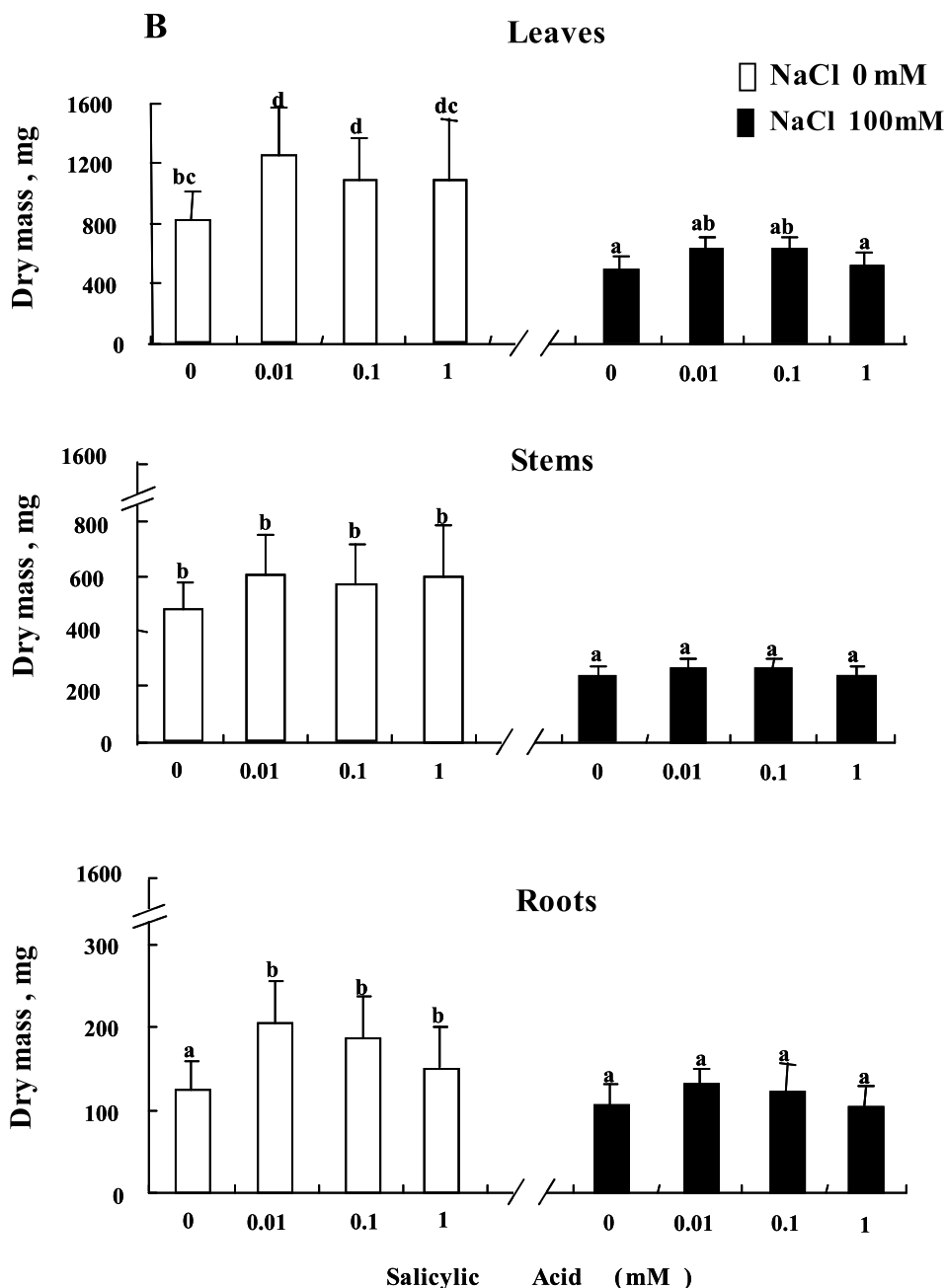


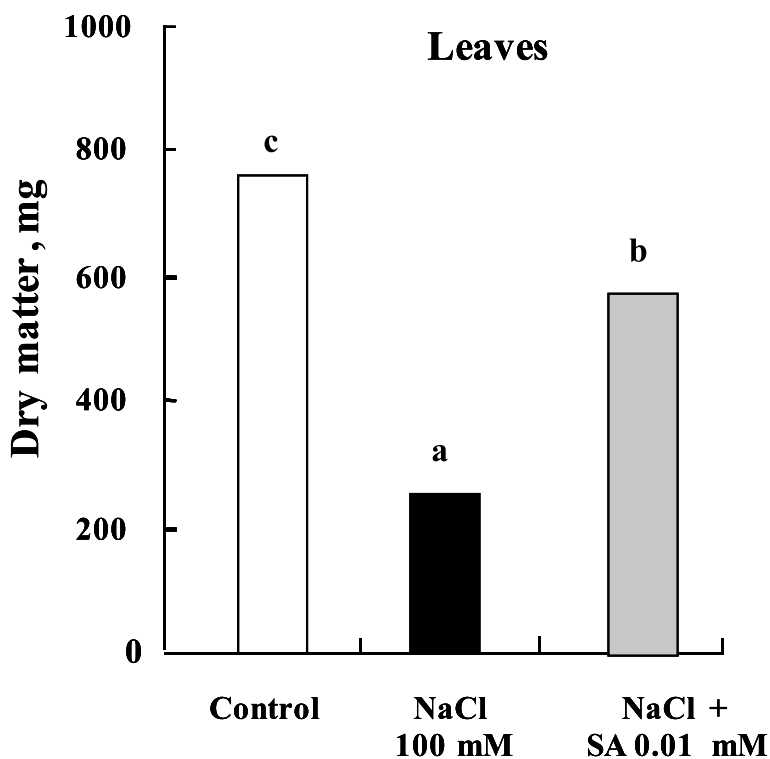
Figure 2. Dry mass of leaves, stems, and roots of tomato seedlings submitted to 100 mM NaCl for 18 days and sprayed or not with salicylic acid (1;0.1; 0.01 mM). Data are means of 16 replicates \pm SE. Means with similar letters are not different at $P \leq 0.05$ according to Duncan's multiple range test at 95%.

a drop in the water content of leaf tissue is observed (**Figure 3**); this decrease suggests that Na^+ is not properly compartmentalized in the leaf tissue vacuole; on the contrary, it is accumulated in the extracellular spaces. This accumulation is associated with a tissue dehydration. In the presence of NaCl, foliar spray of salicylic acid with

SA, mM	0	1	0.1	0.01
Leaf area	129.8a	115.0a	102.1a	128.8a
NaCl 100mM+ SA	0	1	0.1	0.01
Leaf area	67.6a	71.7a	79.7b	77.8b

Table 1.

Leaf area (cm^2) of order 5 of tomato seedlings submitted to 100 mM NaCl for 18 days and sprayed or not with salicylic acid (1; 0.1; 0.01 mM). Data are means of 16 replicates \pm SE.

**Figure 3.**

Dry mass of leaves of tomato seedlings cultivated to the reproductive growth stage in the absence (Control) or presence of NaCl 100 mM and sprayed or not with salicylic acid 0,01 mM. Data are means of 16 replicates per treatment. Means with similar letters are not different at $P < 0.05$ according to Duncan's multiple range test at 95%.

different concentrations (1, 0.1, and 0.01mM) improves vacuolar Na^+ compartmentalization, as shown in **Figure 3**, since leaf water contents are relatively stable, despite the accumulation of leaf with sodium. Maintaining leaf tissue hydration despite the accumulation of sodium suggests that the leaves have a light ability to compartmentalize Na^+ in the vacuoles.

3.4 Reproductive growth stage

Based on the results of the first experiment, the better amelioration of salt tolerance of tomato plants was obtained with SA 0.01 mM, for this we have used the dose (0.01 mM) in this part of our study. Salt stress at the reproductive stage caused

Parameters	Treatments			
	Control	SA	NaCl	NaCl+SA
Number of fruit	14b	10b	4a	6a
Number of stalk	24.5b	26.5b	16.5a	16.5a
Plant height	170b	168b	75.5a	76a
Plant diameter	32.65b	31.9b	10.46a	17.85a
Root length	31.5b	39.5b	26a	38.5a

Data are means of 16 replicates per treatment. Means with similar letters are not different at $P < 0.05$ according to Duncan's multiple range test at 95%.

Table 2.
 Yield parameters of tomato seedlings cultivated to the reproductive growth stage in the absence (Control) or presence of NaCl 100 mM and sprayed or not with salicylic acid 0.01 mM.

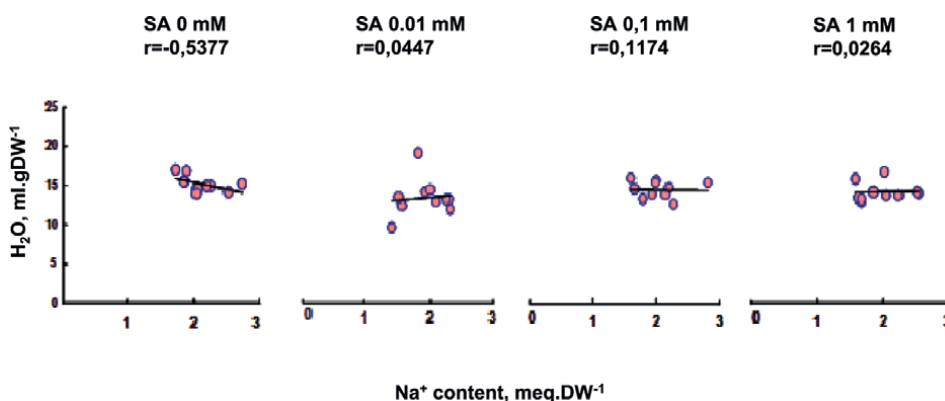


Figure 4.
 Relationship between sodium and water in the leaves of tomato seedlings submitted to 100 mM NaCl for 18 days and sprayed or not with salicylic acid (1; 0.1; 0.01 mM). Data are means of 16 replicates.

a reduction in plant size, number of stalk, and fruit diameter. In detail, the length of the plants decreased from 170 cm to 75 cm, so compared with control, reduction was about 56%. In control medium, plants sprayed or not by SA at the stage were at 25 foliar stages. In the presence of 100 mM NaCl, they only have 17 stages and thus have a developmental delay. In addition, there was a significant decrease in the number and the size of fruit; it is respectively about 70 and 67% compared with control (Table 2).

The salt has a depressive effect on weight of aerial organs in particular stems. The decrease was equal to 70%. The foliar spray of salicylic acid (0.01 mM) attenuated the effect of salt. The reduction was more than 30% (Figure 4). The fruit diameter was enhanced. The amelioration was about 16% compared with plants subjected only to NaCl.

4. Discussion

Saline soils and saline irrigations constitute a serious production problem for vegetable crops as saline conditions are known to suppress plant growth [13]. The present

study demonstrates salinity adversely affected the growth of tomato *cv* Marmande regardless of SA treatments. Earlier studies have shown that the concentration of 100 mM NaCl decreased total dry biomass and leaf area [11, 14]. Also, previous study reported a decrease in whole plant DW, shoot DW, root DW, and leaf area in tomato plant *cv*. Marmande under NaCl stress [11]. In fact, the reason of this reduction is due essentially to the nutritional imbalance and the specific ion toxicity [15]. On the other hand, it could be due to the decrease of the water content in relation to a decrease of external water potential [16]. However, foliar SA applications reduced the negative impact of salinity on growth of tomato plants. Application of SA caused a significantly increase in biomass under non-saline condition. Spraying leaves with SA at concentrations (0.01, 0.1, and 1 mM) in tomato seedlings grown on medium supplemented with 100 mM NaCl improves their tolerance to salinity, this increased tolerance is evidenced by the increase mass of dry matter and leaf area compared with plants that are subjected only to NaCl (**Figures 1 and 4**). Various studies on different plants, including quinoa [17], barley [18], and cowpea [19], showed that the use of SA ameliorates growth biomass under NaCl stress. Foliar applications of SA (0.01, 0.1, and 1 mM) in tomato seedlings grown on control medium induced a strong increase in biomass production at the whole plant, it is about 45, 30, and 32% respectively compared with the control for concentrations 0.01, 0.1, and 1 mM. These results are in agreement with those of El-Tayeb [8] and Arfan et al. [9], who reported that exogenous foliar application of SA ameliorated the adverse effects of salt stress on growth of barley and cowpea. Similarly the work of Noreen et al. [10] shows that exogenous application of SA stimulates foliar growth in sunflower plants grown in the absence or in the presence of salt. Similar results were obtained by Idrees et al. [11]. Also, Abdi et al. [12] showed that the salicylic acid causes a significant increase on the plant density and dry weight of root and shoot. Spraying maize plants “Single hybrid 10” with SA increased dry weight of stem, leaves, and whole plant [12].

To better assess the effect of salt and salicylic acid on growth of tomato seedlings *cv* Marmande, we have calculated a sensitivity index (SI) based on the dry matter production. **Table 3** shows that the presence of NaCl affects the three organs; however, it is the aerial organs, especially stems, that reflecting the greater depressive effect of salt. Foliar spraying of SA (0.01, 0.1, and 1 mM) reduces the effects caused by NaCl, this beneficial effect is more pronounced with the dose 0.01 mM, the improvement was about 15% compared with plants subjected only to NaCl. The protective effect of

Treatments	Whole plant	Roots	Stems	Leaves
NaCl	-42 a	-179 a	-50.6 a	-40.8 a
NaCl+SA 1mM	-40 a	-15.6 a	-51 a	-37.4 a
NaCl+SA 0.1mM	-29.3 b	-1,4 b	-45 a	-24.3 b
NaCl+SA 0.01mM	-27.6 b	+4.6 b	-43.7 a	-23 b
SA 1mM	+32.3 c	+57 c	+25.6 b	+32.6 c
SA 0.1mM	+30 c	+52.2 c	+19.3 b	+32.8 c
SA0.01mM	+45 c	+66.3 c	+26.4 b	+52.6 d

Table 3.

Index of salt sensitivity of roots, stems, leaves, and whole plant of Marmande tomatoes submitted to 100 mM NaCl for 18 days and sprayed or not with salicylic acid (1; 0.1; 0.01 mM).

exogenous salicylic on plants cultivated under abiotic stress have been also reported by Idrees et al. [21] on in lemongrass plants subjected to water stress and by Abdi et al. [22] on Marigold cultivated under salt stress.

Most commercial tomato cultivars are moderately sensitive to salinity at all stages of development, including seed germination, vegetative growth, and reproduction, and therefore yield is markedly reduced [23].

Our study reports that during the whole development cycle, tomato was sensitive to NaCl. Dry weight of the leaves decreased significantly (–70%). Salt affected negatively yield and yield attributes of tomato (plant size, number of stalk, number, and size of fruit). Decreased shoot and root weight, plant height, and leaf number were reported in soybean plant due to salt stress [24]. Also Lauchli and Grattan [25] reported that salinity adversely affected performances of grain crops and cowpea plants at flowering and seed filling stage. Kinsou et al. [26] also report that salinity reduced the number of fruits in tomato (*Lycopersicon esculentum* Mill.) var Akikon. This number has increased from approximately 7.67 in the control plants to 5 fruits at 30 mM NaCl. Salt stress reduces also the size of tomato fruits, the productivity and increases flowering time. Foliar spray of salicylic acid (0.01 mM) counteracted salt-stress-induced growth inhibition and improved yield attributes of tomato. The fruit diameter was enhanced, amelioration was about 16% compared with plants subjected only to NaCl. According to Shakirova et al. [27], the positive effect of salicylic acid on growth and yield can be due to its influence on other plant hormones. Salicylic acid altered the auxin, cytokinin, and ABA balances in wheat and increased the growth and yield under both normal and saline conditions. Stimulation of yield under foliar application of salicylic acid could be assigned to the well-known roles of these plant hormones on photosynthetic parameters and plant water relations. Some studies showed that SA increased membrane permeability facilitating absorption and utilization of nutrients [28]. This would contribute to ameliorating the growth of the stressed plants.

In conclusion, from the results of this study, it can be affirmed that exogenous application of SA (0.1 mM) as foliar spraying once at vegetative and second time at reproductive stage influences growth parameters; yield and yield attributes of tomato under saline conditions, which could be used as a useful strategy in order to enhance the tolerance of tomato plants to salinity and biological yield.

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Conflict of interests

The authors declare that the research was conducted in the absence of any commercial or relationships that could lead to a conflict of interest.

Disclosure

The authors alone are responsible for the content and writing of the paper.

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Chapter 2

Cultivation of Tomato under Dehydration and Salinity Stress: Unravelling the Physiology and Alternative Tolerance Options

Rowland Maganizo Kamanga and Patrick Alois Ndakidemi

Abstract

Tomato is an important fruit vegetable in the world, as a nutritional source and an income option for a majority of resource constrained households. However, tomato supply in developing countries is often fluctuating, with high scarcity in both supply and quality during rainy season. Unlike many crops, cultivation of tomato is a challenging task during rainy season, with high pest and disease infestation. Hence, dry season is the most favorable period for tomato cultivation. However, inadequate water supply poses a yet another significant hurdle, as the crop requires high soil moisture for optimum growth. According to a landmark study by FAO, Tomato has a yield response factor of 1.05, which signifies that a smaller decline in water uptake results into a proportionally larger decline in yield. Moreover, over the years, there have been increasing reports of soil salinization, which imposes similar effects to drought stress through osmotic effects of Na^+ in the soil solution and oxidative stress through excessive generation of reactive oxygen species. This chapter will dissect how tomato plants respond to these abiotic stress factors on physiological, anatomical, and molecular levels and suggest options to improve the crop's productivity under these constraining environments.

Keywords: drought, salinity, physiology, acclimation, osmotic tolerance

1. Introduction

Global changes in the climate scenario undeniably pose insurmountable challenge on global food supply system. Formerly agriculturally productive lands are insidiously becoming unarable. Yet, global population is steadily increasing, projected to reach an insane 9.8 billion by 2050 [1]. Therefore, finding ways to sustain agricultural productivity in light of the prevailing and worsening climate to support the growing population is one of the current and future's major global hurdles. Persistent droughts, extreme temperatures, soil salinization, and heat stresses have gradually become abiotic norms in the agricultural setting.

Tomato is among the most commercially important fruit vegetables globally [2, 3]. In one of the FAO's milestone publications on crop water relations, tomato was

established to have a yield response factor (Ky) of 1.05, indicating that a small decline in water uptake results into a proportionally larger yield decrease [4–6]. This substantiates the need for development of cultivars that are able to maintain yield or exhibit less yield decline under limited water conditions. Worldwide, agricultural productivity is confronted with accelerating environmental constraints such as drought and salinity. Coupled with the global changes in climate, water stress is progressively becoming a major environmental factor limiting plant growth, development, and yield [7]. Drought and salinity stress impose somewhat similar effects on growth of crop plants, as both result into reductions in soil water availability and plant water uptake capacity. When soil water potential and plant's turgor fall below a threshold, such that normal plant functioning is perturbed, the soil is said to be droughted [8] or in a state of water stress. Some authors refer to soil as droughted when plant's water deficiency results from evaporative demand of the atmosphere exceeding plant roots' capacity to extract soil water [9]. It is indicated that initial reductions in shoot growth under salinity stress are due to increases in plants' osmotic pressure due to heavy presence of salts around roots, resulting into hormonal signals that eventually reduce stomatal conductance and consequently growth [10]. These effects are similar to those generated by drought stress. Tomato (*Solanum lycopersicum* L.) is recognized as a crop of an immense economic importance globally [2]. What is more is that drought and soil salinity have considerable impacts on its production [11, 12]. Present understanding proves that water stress perturbs various physiological and biochemical processes [7, 13–16] eliciting expression of various stress-related genes [12, 17, 18]. Therefore, in order to achieve the required knowledge for attainment of water stress tolerance, it remains imperative to couple physiological analysis's descriptive power with biochemical, morphological, and transcriptomic analysis [19] in carefully screened and selected varieties with proven differential tolerance under drought stress.

2. Drought stress: an overview

Plant water deficit develops as its demand exceeds the supply of water. The supply is determined by the amount of water held in the soil to the depth of the crop root system. The demand for water is set by plant transpiration rate or crop evapotranspiration, which includes both plant transpiration and soil evaporation. Evapotranspiration is driven by the crop environment as well as major crop attributes such as plant architecture, leaf area, and plant development. Drought stress is often measured by the Palmer Index (the Palmer drought severity index, PDSI), a regional drought index commonly used for monitoring drought events and studying areal extent and severity of drought episodes [20]. The index uses precipitation and temperature data to study moisture supply and demand using a simple water balance model. Water moves into the plant within a physical system also known as the soil-plant-atmosphere continuum (SPAC). Here, water is driven through the plant from the soil to the atmosphere by the difference in water potential between the atmosphere (very low potential) and the soil (relatively high potential when wet).

Plants often receive excessive radiation, out of which only a small fraction is used for photosynthesis (photosynthetically active radiation), while the rest is dissipated as heat and transpiration [21], this led to the term “transpirational cooling.” Transpiration functions to cool leaves relative to ambient temperatures when the environmental energy load on the plant is high, without which plant leaves could heat up to lethal temperatures [22]. When a leaf transpires, leaf water potential becomes

more negative (lowers), creating a water potential gradient (pull) that drives water movement into the plant (assuming more water is available in the soil). As the soil gets drier, it is necessary that leaf water potential be reduced further in order to create the required pull to drive water into the plant leaf.

This brings a concept of osmotic adjustment (OA), defined as the net accumulation of solutes after the plant has been exposed to a predetermined rate of water deficit [23]. OA has been suggested as a prime drought stress adaptive engine in support of plant production. Osmotic adjustment (OA) and cellular compatible solute accumulation are widely recognized to have a role in plant adaptation to dehydration mainly through turgor maintenance and the protection of specific cellular functions by defined solutes. A typical leaf cell comprises a battery organic and inorganic osmolytes (osmotically active solutes), such as soluble sugars, proline, and glycine betaine, which determine the leaf osmotic potential. Relatively, osmotic potential is lower than leaf water potential, whose difference is what constitutes turgor potential, a critical determinant of cellular growth and function, devoid of which collapses the cells and wilts the leaves. A lower turgor is typified with stomata closure (as an attempt to reduce transpiration), this reduction reduces intercellular CO₂ concentration (C_i), consequently downregulating CO₂ fixation and photosynthetic assimilation [7, 24] and an increase in leaf temperature [18]. Rise in temperature may get excessive, causing heat damage to the leaf especially under hotter environments. Therefore, turgor maintenance and transpiration are two critical aspects for plants growing under dehydrated conditions. Turgor maintenance can be maintained by sustaining water uptake to keep leaf water potential higher or through accumulation of osmolytes (osmotic adjustment).

At a whole plant level, transpiration rate can be controlled by limiting total leaf area. For example, two plants growing in a pot of similar volume, a large plant will require irrigation more frequently than a smaller one. Reduction of plant size and growth rate has therefore been a key revolutionary feature for plants' adaptation to drier environments [25]. As such, it has been observed that as water deficit becomes severe, older leaves desiccate and shed off first as an attempt to reduce leaf area and slow down on water requirement, while younger leaves maintain stomatal opening and carbon assimilation. At the crop level, the relationship between plant size and the demand for water can be extrapolated by measurement of leaf area index (LAI), which expresses total area of live leaves per unit ground surface. When LAI is high, crop evapotranspiration (ET) also increases, at least until LAI reaches a maximum threshold beyond which ET does not increase. As the crop matures and leaves senesce, LAI is reduced and so does evapotranspiration. In response to desiccation, growth regulating hormone abscisic acid (ABA) is produced in the shoot, inducing a cascade of responses such as arrested growth, stomatal closure, and reproductive failure. ABA is also produced in the root in direct response to the drying soil and its hardness as it dries. Root ABA is translocated to the shoots via the transpiration stream, eliciting stomatal closure or arrested growth before any water deficit develops in the shoot. This "hormonal or chemical root signal" may therefore serve as an "early warning system" to the plant. This results into the ABA-dependent pathway of signal transduction under drought stress. In this pathway, ABA induces novel protein synthesis, which regulates expression of numerous "ABA responsive" genes. Alternatively, ABA may also regulate stress responsive genes without novel protein synthesis. These gene products are either functional (e.g., water-channel proteins or key enzymes) or regulatory (e.g., protein kinases), and they are involved in mediating various cellular responses. Presently, thousands of "drought stress responsive genes" have been identified that are either upregulated or downregulated under dehydration.

2.1 Responses of tomato plants to drought stress

Physiologically, photosynthesis is one of the highly regulated, sensitive, and primary traits affected by drought [26, 27]. Hence, ability to maintain photosynthetic capacity under water stress deserves solemn consideration when screening for drought stress. Ueda et al. [28] observed that water and salinity stresses downregulate photosynthesis through stomatal and non-stomatal limitations. Yuan et al. [7] observed that under different water stress conditions, reasons for decline in photosynthetic rates are different; with stomatal limitations being more apparent under mild stress while non-stomatal limitations were more prevalent under moderate and severe water stress. This may suggest that severing water stress affects photosynthesis, principally via photosystem damage, inhibition of RuBISCO enzyme and other enzyme activities [29], and these non-stomatal effects may be even more apparent in sensitive cultivars. Furthermore, water stress affects photosynthesis through stomatal closure triggered by root to shoot signaling after sensing lower plant water potential. Thus, cultivars that present higher stomatal conductance under water stress conditions indicate a higher adaptability to water stress [30]. A consequence of inhibited photosynthesis is downregulation of plant growth; however, this cause-effect relationship remains difficult to entangle [31]. Under water stress, accumulation of soluble sugars and other osmolytes has been implicated in osmotic adjustment in tomatoes. Several studies have thus observed and correlated an increase in sugars and proline accumulation with drought tolerance [32, 33]. As a consequence of photosynthetic downregulation, drought stress often results into accumulation of reactive oxygen species (ROS), which damage photosynthetic machinery and cell membranes consequently resulting into cell death [13, 34]. Malondialdehyde is a widely used marker for lipid peroxidation and shows greater accumulation under abiotic stresses [7, 35]. Cell membrane stability and electrolyte leakage have also emerged as important tools in assessing membrane damage elicited by abiotic stress [36, 37].

3. Salinity stress: an overview and effects

The global consequences of the rapidly changing climate scenario imply that the environment for crop growth and development is gradually becoming unbearably altered. Soil salinization for one has victimized agriculture since time immemorial and has remained an important factor constraining worldwide crop productivity [38]. As a result, remarkable strides have been made in unmasking plants' responses and tolerance mechanisms to salinity stress. Deposition of salts in agricultural fields is principally through rain and wind and in rare cases through weathering of rocks [39]. It is estimated that over 800 million hectares of land are saline representing more than 6% of world's land area [31]. Spatially, soil salinity is most widespread in arid and semiarid regions in addition to sub-humid and humid climatic conditions. In most cases, these regions experience lower precipitation, yet suffer from higher evapotranspiration rates. This imbalance results into capillary transport of salts from the water table to the ground surface [40]. There are various types of salts that accumulate in the soil and water to agriculturally lethal levels. However, sodium chlorides (NaCl) are considered the most soluble and abundantly released salts, hence have been the subject of considerable research attention insofar as soil salinity is concerned. Soil salinity increases electrical conductivity of soil, hence soils are

characterized as saline when its E_{Ce} is at least 4 dS/m at 25°C [41], approximately 40 mM NaCl, with about 15% exchangeable sodium [42].

Plants' responses to soil salinity are governed by complex interactions of morphological, physiological, and biochemical processes, thereby affecting plants from seed germination, vegetative growth, reproductive development [42], and uptake of water and soil nutrients [43]. The complexity of salinity stress renders it particularly difficult to manage as it is associated with interlinked yet dissimilar effects that require different tolerance strategies. The first observable primary effect of salinity on plant growth is the reduction of water uptake capacity of plants. Usually, this effect is as a result of salts outside the roots, which increase the osmotic pressure of water making it harder for plants to take up water [31]. This osmotic component of salinity is rapid, progressing a few hours after encountering soluble salts and is characterized by decreases in new shoot growth, through reductions in leaf expansion rates, slow new leaf emergence, and lateral buds development. As a consequence of the resultant decline in soil water potential and subsequent cell dehydration, osmotic stress induces stomatal closure and a decline in photosynthetic activity that eventually chucks growth [28, 44]. In addition, soil salinity may result into ion toxicity and nutritional imbalances. Na⁺ and K⁺ compete for binding sites, due to their similarity in physicochemical properties [45], such that excess availability of Na⁺ in the growth media results into replacement of K⁺ by Na⁺ in some key biochemical reactions [42], which may become inhibitory to some enzymes [46]. It is well understood that most enzymes rely on K⁺ as a cofactor and can thus not be substituted by Na⁺. Thereby, maintaining an optimal Na⁺/K⁺ ratio has emerged a crucial aspect of salinity tolerance [47]. The ion-specific phase is relatively slow and begins when salts accumulate to lethal concentrations particularly in older leaves that have ceased expanding and eventually die. According to Munns and Tester [31], ionic stress becomes a major concern in crop plants with uncontrollable accumulation of ions in the shoots coupled with an inability to tolerate the accumulated ions. Therefore, maintenance of a lower Na⁺ accumulation in relation to essential ions such as K⁺, Mg²⁺, and Ca²⁺ is a desirable trait under salinity stress. Toxic accumulation of Na⁺ and Cl⁻ salts in the cytosol and osmotic-effect-induced reductions in water uptake result into metabolic imbalances, which in turn cause oxidative stress [48]. Meanwhile, it is widely accepted that accumulation of reaction oxygen species (ROS) accounts for a major part of damage caused to macromolecules and cellular structure by most abiotic stresses [49] suggesting that generation of ROS might be the prime cause of lethality in stressed organisms. Under optimal conditions, plants' cellular homeostasis is dependent on a delicate balance of multiple interlinked pathways. However, water stress disrupts that balance, uncoupling the pathways resulting into transferring of high energy state electrons to oxygen, which generates reactive oxygen species [50, 51]. These include hydrogen peroxide (H₂O₂), superoxide radicles (O₂^{•-}), singlet oxygen (¹O₂), and hydroxyl radicles (OH[•]). When their generation exceeds their scavenging, they are potentially toxic and capable of causing oxidative stress to proteins, DNA, and lipids [13, 52, 53]. Therefore, tolerance to salinity stress requires a combination of multiple strategies and mechanisms that confront osmotic stress, specific ion toxicity and scavenge reactive oxygen species.

3.1 Tolerance mechanisms to salinity stress

As a result of the widespread nature of salinity stress, plants have developed multiple mechanisms and strategies to confront salinity stress. Tolerance to salinity

stress falls within three main categories; osmotic tolerance, ion exclusion, and tissue tolerance (**Figure 1**). A yet fourth tolerance strategy pertains to tolerance to oxidative stress elicited by excessive generation of ROS. Osmotic stress inhibits ability of plants to take up water due to excessive presence of salts around the roots. Thereby first mechanism, termed osmotic tolerance, is targeted at sustaining water uptake in plants and ensuring a well-hydrated leaf status of plants to maintain key metabolic activities such as photosynthesis. This is regulated by long distance signals that reduce shoot growth [54] way before Na^+ accumulates to the shoots. ROS and Ca^{2+} waves are speculated to be involved in the long-distance signaling under osmotic tolerance [55]. One important mechanism through which plants confront osmotic stress is through accumulation of solutes to balance extra osmotic pressure generated in the soil solution to maintain turgor [18]. This can be achieved by excluding saline ions from accumulating in the shoots and principally relying on accumulation of organic osmolytes such as sugars, proline, glycine betaine, etc. However, controversy sparks from this strategy as it comes with a larger trade-off in the form of energy cost [56]. That notwithstanding, this strategy is employed particularly among glycophytes through selective uptake of ions by roots, excluding uptake of Na^+ and Cl^- , preferentially loading K^+ in the xylem vessels, and controlling Na^+ loading and unloading of Na^+ from the xylem in the upper part of the roots, stems, and petiole [10]. Cognizant of the energy cost of synthesizing organic solutes for osmotic adjustment, some plants, mostly halophytes, rely on accumulation of Na^+ as a cheap osmoticum. This way, the plants transport Na^+ to the shoots to levels bearable and sequester them into vacuoles so as not to interfere with key cytosolic metabolic activities [57]. Works leading to the discovery of tissue tolerance as a strategy in plants were inspired by an earlier finding that in vitro, halophytic enzymes were not any more tolerant to high salt than those of glycophytic plants [58, 59]. Besides, several species have reported higher tissue Na^+ concentrations in leaves that are still functioning [60, 61]. When Na^+/Cl^- accumulates in the vacuole, K^+ and organic solutes must accumulate in the cytoplasm to balance

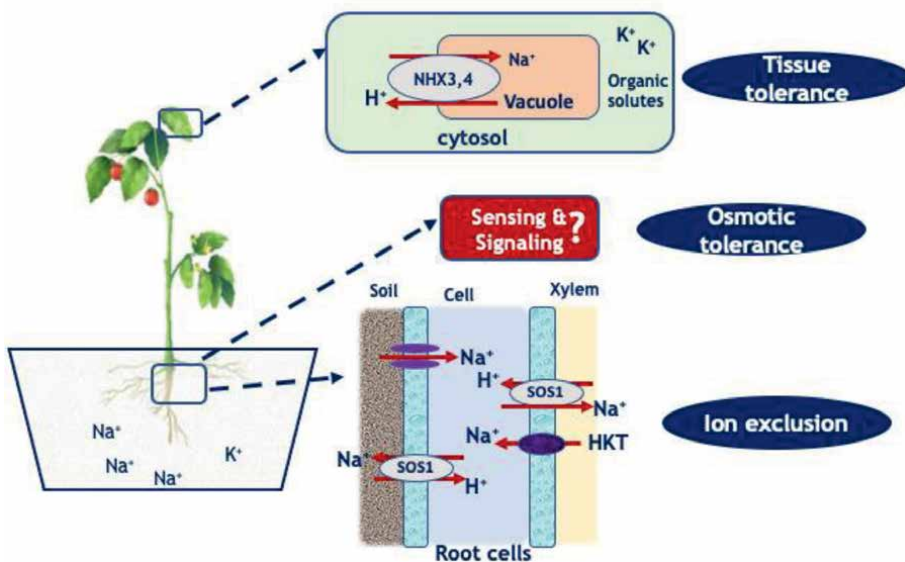


Figure 1.
A summary of mechanisms of salinity tolerance in tomato plants.

the osmotic pressure of ions in the vacuole. Common organic osmolytes in tomatoes are proline [62] and soluble sugars [24, 63].

All these compounds are found under both drought and salt stress and accumulate in higher levels in plants adapted to such environments. Under salt stress, their accumulation reflects more of an osmotic response than salt-specific (ionic) response. This strategy is termed tissue tolerance and in tomatoes, it is aided by Na^+/H^+ antiporters NHX, isoforms *SINHX3* and *SINHX4* [64]. It is important to note that plants transpire 30–70% more water than is used for cell expansion [10]. Salts carried in the transpiration stream are deposited in leaves as the water evaporates, gradually building up to toxic levels. In older leaves, salt toxicity becomes much higher than younger leaves since they are no longer expanding and cannot dilute incoming salts. Eventually, the salt concentration becomes high enough to kill the cells. Hence, some plants rely on Na^+ exclusion, to reduce the rate at which salt accumulates in transpiring organs. This can be achieved through (1) root cells and can selectively avoid uptake of Na^+ (2) preferentially loading of K^+ into the xylem at the expense of Na^+ and (3) unloading of Na^+ from the xylem, this is aided in tomato by high-affinity K^+ transporters (*SlHKT1;2*). Being a glycophytic plant, tomato is less efficient in sequestering Na^+ in the vacuoles but relies predominantly on exclusion of Na^+ from the leaves.

3.2 Screening techniques for drought and salinity tolerance

In view of the devastating effects of drought and salinity coupled with sensitivity of this agronomically important crop, it is substantiated to develop cultivars that are able to maintain yield or exhibit less yield decline under these environments. Such breeding goals can be aided with proper screening and selection for water stress-tolerant cultivars. Various techniques and parameters have been derived from screening for drought tolerance [65, 66]. A classical approach to investigating plants' responses to abiotic stresses is to use two genotypes with contrasting tolerance reputations. Some have argued that this approach is narrow, hence have advocated for the broadening of these types of analyses by using several genotypes before speculating about a species' performance [67]. Furthermore, when selecting a few contrasting genotypes, it is necessary to take into account the potential variability of the trait under study within the population, especially crops and plants with determinate and indeterminate growth habits such as tomato. In such cases, derivation of salt tolerance indices obtained as relative decreases in plant biomass by comparing plant biomass of stressed and control plants [68] is imperative. Stress susceptibility index (SSI) serves as a reliable measure of sensitivity to stress as it considers the intensity of stress and performance ratio between stress and their respective controls [69]. That notwithstanding, screening techniques need to be supplemented with other techniques to increase their reliability. Cluster analysis is one dependable tool that allows self-grouping of cultivars into groups of similar characteristics and has been widely used as a screening tool in tomato [24, 65, 66]. For reference to a variety of screening methods and their merits, refer to [70].

4. Salinity and water stress: where is the synergy?

Firstly, it is important to understand that plants' responses to salinity stress occur in two distinct temporal phases [71]: a rapid response to the increase in external

osmotic pressure, and a slower response due to the accumulation of Na^+ in leaves. Hence, in order to correctly dissect the physiological mechanisms associated with salinity tolerance of plants, it is necessary to first identify whether their growth is being limited by the osmotic effect of the salt in the soil or the toxic effect of the salt within the plant. According to [31], in the first few hours occurring immediately after plant roots are exposed to a saline media, plant's shoot growth is considerably reduced, largely as a result of osmotic effect of salts "outside" the plant roots. Evidently, plants experience a lower rate of leaf expansion, slower emergency of newer leaves, and slower development of lateral buds consequently forming fewer branches and lateral shoots. It is important to note that shoot growth is far much sensitive to the osmotic effects than root growth. This phenomenon is also common in drying soils under drought conditions. It has been suggested that reduction in leaf area development relative to root growth would decrease the water use by the plant, thus allowing it to conserve soil moisture and prevent an escalation in the salt concentration in the soil [31]. Relatively, it is the osmotic stress component of salinity stress that exhibits both an immediate and greater effect on growth than the ionic stress. The latter is manifested much later and lesser. Ionic effects of salinity stress are only higher either at very high salinity levels or in extremely sensitive species such as rice whose ability to control Na^+ transport is limited.

Now, the question may remain as to whether these osmotic responses are salt and species-specific. Experiments in maize [72, 73], rice [74] as well as wheat and barley [75] have all recorded rapid and transient reductions in leaf expansion rates after a sudden increase in salinity. Likewise, similar changes were reported when plants are exposed to KCl, mannitol, or polyethylene glycol (PEG) [76]. These results are indicative that the responses are neither salt- nor species-specific. This first phase growth reduction is quickly apparent and is due to the salt outside the roots. It is essentially a water stress or osmotic phase, for which there is surprisingly little genotypic variation. The growth reduction is presumably regulated by hormonal signals coming from the roots. It is from this point of view that salinity stress synonymizes drought stress, hence the usage of the term "physiological drought" by some authors [62, 77]. **Figure 2** shows this synergy, drought-specific responses involve synthesis of ABA and induction of ABA responsive genes involved in synthesis of water channels, enzymes, and protein kinases.

4.1 Comparative osmotic responses to salt and drought stress

Plants growing under salt stress are faced with three prime costs; (1) the cost of excluding Na^+ from uptake by roots, (2) the cost of compartmentalizing/sequestering Na^+ in the vacuole, and (3) that of excreting the salt through salt glands. In tomatoes, the latter cost is of less importance as tomatoes do not have the salt glands. Under drought stress, the prime cost is to synthesize organic osmolytes, a far much higher cost. While it remains unclear whether plants growing under saline media produce lesser organic solutes compared with those growing under non-saline media, a comparison of four tomato genotypes growing under PEG and NaCl at an isotonic solution, much greater accumulation of soluble sugars was observed under PEG than NaCl [78]. Correspondingly, the tomato plants growing under NaCl grew much better than under the isotonic PEG solution. This result suggests the higher cost of synthesizing organic osmolytes in tomatoes on growth, hence it may follow that plants growing under saline conditions grow faster than under non-saline media. However, this conclusion may not be overarching, as equivocal results have been obtained in other species, notably [10].

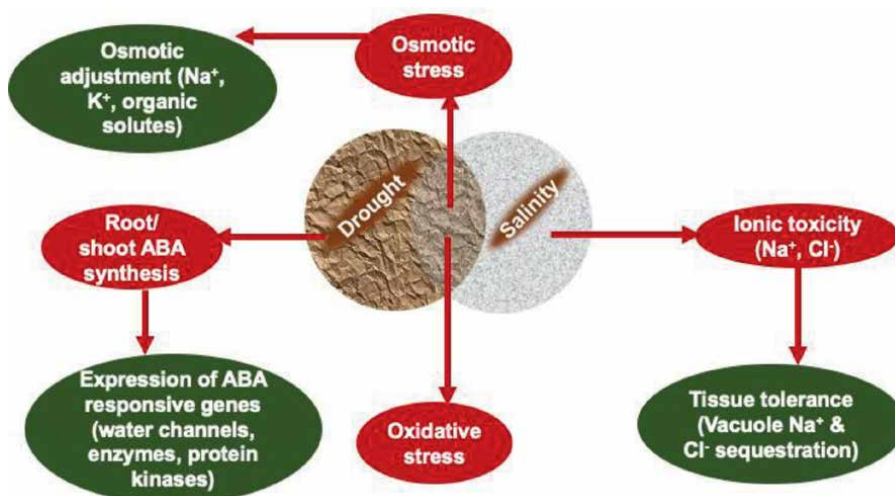


Figure 2.
The synergy of drought and salinity stresses. Osmotic stress and oxidative stress are common links of both salinity and drought stress, whereas salt specific response is ionic toxicity (Na^+ and Cl^-) and drought-specific responses include synthesis of ABA in either shoots or roots that trigger expression of ABA responsive genes.

5. Learning from tomato wild relatives

Despite that tomato remains sensitive to drought and salt stress, the genus *Solanum* has extensive wealth of genetic variation existing in wild relatives. Tomato, for example, has a number of wild relatives with remarkable reputation for tolerance to drought and salt stress, such as *Solanum pennellii*, *S. habrochaites*, *S. pimpinellifolium*, *S. hirsutum*, *Lycopersicon chillense*, and *L. peruvianum*. As such, they represent a valuable system in which to study local adaptation to drought and salt stress. A number of comparative studies have been conducted to evaluate physiological and molecular responses of cultivated tomato (*S. lycopersicum*) in comparison with wild relatives. For example, Egea et al. [17] reported substantial physiological differences, with the wild relative *Sp* leaves showing greater ability to avoid water loss and oxidative damage under drought stress. Similar results were also found in another wild relative *S. habrochaites* under root-chilling-induced water stress, in which *Sh* exhibited higher shoot turgor through enhanced stomata closure relative to cultivated tomato, which failed to close stomata and consequently wilted [79]. QTL mapping revealed a single QTL coincidental with the gene or genes contributing to shoot turgor maintenance under root chilling residing on chromosome 9 region that have been associated with abiotic stress tolerance in cultivated tomato. Under salinity stress, another study showed that *Sp* was able to reduce water loss by regulating transpiration through reduced stomatal density and aperture [18]. Furthermore, *Sp* leaves had larger and more turgid cells occupied by a giant vacuole, which was associated with higher water and Na^+ accumulation. On Na^+ homeostasis, the wild relative had higher expression of *SpHKT1;2* and *SpSOS1*, which played an important role in Na^+ translocation from root to shoot, and therefore, in the determination of the included behavior in the wild species, which was in concordance with the higher transcript levels of Na^+ vacuolar transporters *SpNHX3* and *SpNHX4* in *Sp* leaves. An association study in 94 genotypes of *S. pimpinellifolium* to identify variations linked to salt tolerance traits

(physiological and yield traits under salt stress) in four candidate genes identified five SNP/Indels in DREB1A and VP1.1 genes that explained substantially, phenotypic variation for various salt tolerance traits [80].

6. Improving salt and drought tolerance in tomato

Salinity stress affects many aspects of physiology and biochemistry of plants and, subsequently, yield. Growing knowledge and advances in molecular techniques provide room and opportunity for quicker enhancement of salt tolerance in tomatoes. Even though genetic transformation could become a powerful tool in plant breeding, it is necessary to integrate the growing knowledge from plant physiology with molecular breeding techniques. Notwithstanding the many relatively salt tolerant wild relatives of the cultivated tomato, it has proved difficult to enrich elite lines with genes from wild species that confer tolerance because of the large number of genes involved, most of them with small effect in comparison to the environment [81]. Critical in breeding for salt and drought tolerance is the need for the new cultivars to be both tolerant and maintain attributes of higher yield and quality shown by modern cultivars. Hypothetically, susceptible but productive cultivars should be converted to tolerant cultivars, while maintaining all the very valuable characters current cultivars possess, making the introduction of genes conveying salt tolerance to elite cultivars by transformation an attractive option. However, the problem of drought and salt tolerance is complex and multigenic, requiring a battery of strategies. Instead, scientists have resorted to a range of cultural techniques, each contributing to a small extent to allow plants to withstand better the deleterious effects of salt.

For many years to recent, it was believed that salt tolerance was solely a factor of expression of genetic information counteracting effects of stress [82]. However, present understanding tells that plants can improve their physiological ability and adapt to severe stresses when pretreated (PT) with mild stress, a phenomenon termed acclimation [61, 83–85] as shown in **Figure 3**. During the plant response and acclimation to abiotic stress, important changes in biochemistry and physiology take place and many genes are activated, leading to accumulation of numerous proteins involved in abiotic stress tolerance. Benefits of acclimation to salinity stress have been linked to improved growth via effective vacuolar Na^+ sequestration [61], improved survival rates [84], and reduced shoot Na^+ accumulation [62, 85, 86]. The successful adaptation of cell lines to salinity suggests that a genetic potential for salt tolerance is present in cells of plants from which the lines were derived and that exposure of the cells to salt triggers the expression of this information.

In tomato, success stories of acclimation have been reported through seedling pretreatment with NaCl [62, 87], pre-exposure to salicylic acid [88, 89], and seed priming with NaCl [82]. Another study by Gémes et al. [90] showed that pretreatment of tomato plants with salicylic acid attenuated oxidative stress by reducing H_2O_2 generation under salt stress, suggesting a cross talk between salicylic acid and salt-stress-induced ROS. H_2O_2 is considered functional link of cross-tolerance to various stressors, as also reported in rice under saline-alkaline stress [86]. Szepesi [91] found that salicylic acid pretreatment improved the acclimation of tomato plants to tolerance levels comparable to that of tomato's wild relative *L. pennellii*, a wild relative with a high reputation for stress tolerance. Humic acids pretreatment of tomato seedlings has also been explored and showed that seedlings primed by humic acids minimized the salinity stress by changing ion balance, promoting plasma membrane proton pumps activity and enhancing photosynthesis rate and plant growth [92].

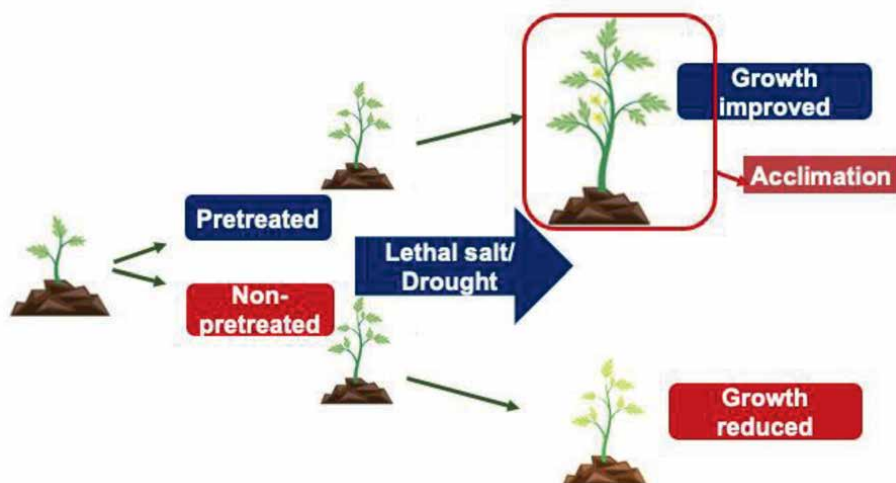


Figure 3. A schematic of acclimation in tomato plants, wherein seedlings' previous exposure to a mild level of stress (salt or drought) primes tomato plants to exhibit faster and stronger responses to subsequent lethal stresses.

Plant adaptation to abiotic stress has also been observed under drought stress. For example, a study by [93] showed that multiple exposures to drought stress trained transcriptional responses in *Arabidopsis*. In the study, it was shown that during recurring dehydration stresses, *Arabidopsis* plants displayed transcriptional stress memory demonstrated by an increase in the rate of transcription and elevated transcript levels of a subset of the stress-response genes (trainable genes). Four distinct types of dehydration stress memory genes in *Arabidopsis thaliana* have been further identified [94]. These observations of altered plants' subsequent responses following pre-exposure to various abiotic stresses by improving resistance to future exposures, have led to the concept of "stress memory" implying that during subsequent exposures, plants provide responses that are different from those during their first encounter with the stress. While these phenomena have not been reported in tomato, yet they might represent a general feature of plant stress-response systems and could lead to novel approaches for increasing the flexibility of a plant's ability to respond to the environment. In tomato, it has been shown that a moderate water deficit applied 10 days after anthesis induced acclimation to a subsequent more severe drought stress [95]. Similar results have also been reported in wheat [96]. It has been reported that plants exposed to one stress may show tolerance to other stresses, displaying a concept of cross-tolerance [86, 97, 98]. It is hypothesized that drought pretreatment could increase the tolerance to the osmotic effect, the main effect induced by salinity when moderate salt levels are used. This has been observed in tomato plants previously exposed to a drought stress pretreatment, which subsequently grew better than non-pretreated plants after 21 days of salt treatment [99]. Similar findings were reported by [100], who found improved salt tolerance of tomato plants following seedling pretreatment with PEG, a chemical drought (osmotic) stress simulator. This illustrates a concept of induced cross-tolerance (**Figure 4**), in which prior exposure to one stress induces tolerance to another stress, as opposed to inherent cross-tolerance that manifests itself as genetic correlation of gene expression under different stresses (**Figure 4**).

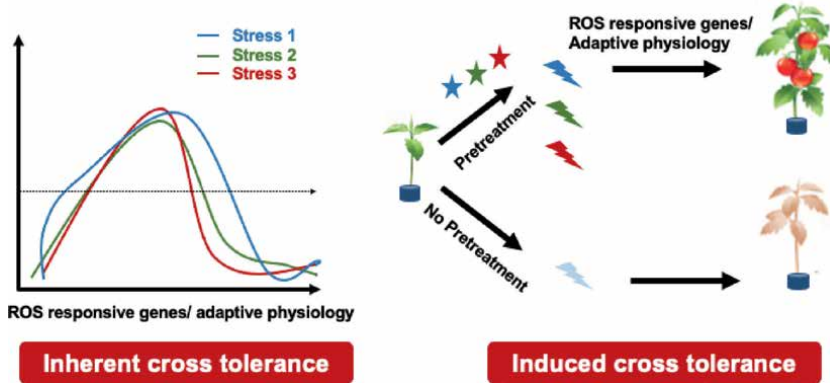


Figure 4. A schematic of cross-tolerance. Plants may exhibit inherent cross-tolerance, manifesting itself as genetic correlation of gene expression under different stresses. Cross-tolerance may also be induced by previous exposure to one stress that may develop tolerance to another stress compared with plants without prior exposure to any stress.

Plant adaptation to stresses is a complex process, involving numerous physiological and biochemical changes. The key components in a typical stress-response relationship involve stress stimulus, perception of stress by signals, expression of stress-induced genes, and resultant changes at morphological, biochemical, and physiological levels [98]. The signaling and response pathways have been reported to overlap during exposure to different abiotic stresses, including reactive oxygen species (ROS), hormones, protein kinase cascades, and calcium gradients as common elements [101]. In a case of cross-tolerance, it has been suggested that specific proteins are induced by one kind of stress and are involved in the protection against other kinds of stress [97, 98], although a common mechanism has not been found.

Aside from the use of pretreatments, another route for enhancing salt and drought stress tolerance in tomato would be to graft cultivars on to rootstocks able to reduce the effect of external salt or drought on the shoot. This strategy could also provide the opportunity to growers of combining good shoot characters with good root characters. In tomato, grafting has previously been used to limit soil-borne and vascular diseases such as *Fusarium*. Over the years, application of grafting technique has been widened across various uses such as improving yield, fruit quality, low and high temperature as well as Fe chlorosis as outlined in [81]. In *Citrus* spp., for example, the positive effect of rootstock is related to the ability of the rootstock to exclude chloride [102, 103]. However, grafting has rarely been used to increase the productivity of vegetables growing under adverse conditions. In tomato, a commercial tomato cultivar Jaguar as scion has been grafted onto roots derived from the same genotype (J/J) or other cultivars' root stocks that increased fruit yield by more than 60% under salinity stress by regulating the transport of Na^+ and Cl^- throughout the plant growth cycle, even after 90 days of salt treatment [104]. Similar results have also been reported by [105], further revealing that rootstock effect on the tomato salinity response depends on the shoot genotype. Furthermore, [106] also found higher improvements in vegetative growth as well as yield in a commercial tomato cultivar grafted on tomato wild relatives coupled with changes in morphological, physiological, and molecular attributes. The results suggest that the saline ion accumulation in leaves is predominantly controlled by the genotype of

Pretreatment	Abiotic stress	Crop species	Target stress factor	Studies
NaCl	Salinity	<i>O. sativa</i> L.	Osmotic, ionic	[85, 86, 107–109]
		<i>S. lycopersicum</i> L.	Osmotic, ionic	[62, 82, 87]
		<i>Z. mays</i> L.	Osmotic, ionic	[83]
		<i>P. sativum</i> L.	Osmotic, ionic	[61]
		<i>G. max</i> L. merr	Osmotic, ionic	[84]
		<i>V. radiata</i> L.	Osmotic, oxidative	[110]
Salicylic acid	Salinity	<i>S. lycopersicum</i> L.	Ionic	[88, 89]
	Alkaline	<i>S. lycopersicum</i> L.	Ionic, oxidative	[111]
Silicon	Alkaline	<i>S. lycopersicum</i> L.	Ionic, oxidative	[111]
ABA	Salinity	<i>O. sativa</i> L.	Osmotic, ionic	[112, 113]
	Alkali	<i>O. sativa</i> L.	Osmotic, ionic, oxidative	[114, 115]
Gibberellins	Salinity	<i>P. vulgaris</i> L.	Oxidative	[116]
Cytokinin	Saline-alkaline	<i>O. sativa</i> L.	Osmotic	[117]
	Salinity	<i>Lolium perenne</i>	Oxidative, ionic	[118]
	Salinity	<i>V. faba</i>	Oxidative, ionic, osmotic	[119]
NaHCO ₃	Saline-alkaline	<i>S. cereale</i> L.	Osmotic, oxidative	[120]
Drought	Drought	<i>S. lycopersicum</i> L.	Osmotic	[95]
	Drought	<i>T. aestivum</i>	Osmotic	[96]
	Salinity	<i>S. lycopersicum</i> L.	Osmotic	[99, 100]
Repeated drought	Drought	<i>A. Thaliana</i>	Oxidative	[93, 94]
H ₂ O ₂	Salinity	<i>T. aestivum</i> L.	Oxidative	[121]
	Salinity	<i>H. vulgare</i> L.	Oxidative	[122]
	Salinity	<i>Z. mays</i> L.	Oxidative, osmotic	[123]
	Saline-alkaline	<i>O. sativa</i> L.	Oxidative	[86]
Grafting	Salinity	<i>S. lycopersicum</i> L.	Ionic	[104–106]

Table 1.

List of some cultural techniques that have been used to enhance drought and salt tolerance in tomato and other crops.

the rootstock, providing an alternative way of enhancing salt tolerance in tomato – quicker and least costly.

An observation has been made that acclimation ability to abiotic stress in tomatoes is dependent on degree of tolerance of the cultivar such that more salt-sensitive cultivars benefit more from the acclimation process than tolerant cultivars [62, 87].

7. Concluding remarks

Despite being a crop of considerable agronomic importance, tomato remains a sensitive crop to droughts and salinity stress. Cultivation under these environments is an extremely challenging task; hence, it is imperative to develop tomato cultivars resistant to these adverse conditions. This, however, requires an understanding of their physiological and molecular mechanisms underpinning tolerance. This chapter has dissected in detail the key physiological and molecular changes that take place under both drought and salinity. These two stresses, albeit being distinct, pose considerable similarities and affect tomato growth in significantly comparable manners. The chapter also drew learning points from tomato's wild relatives that present the required variation for development of tolerant cultivated varieties. However, development of tolerant cultivars is often a long and costly endeavor and subject to country-specific regulatory frameworks. Moreover, considering the multigenic nature of drought and salt tolerance trait, the chapter suggests exploration of some quicker options that promote adaptation to adverse environments. In tomato, options such as acclimation, cross-tolerance, and grafting have proved effective in developing tolerance to abiotic and in some cases, biotic stress conditions (**Table 1**). These may provide some required short-term yield gains when cultivating tomato under adverse environmental conditions.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 3

Greenhouse Tomato Production for Sustainable Food and Nutrition Security in the Tropics

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Abstract

Greenhouse vegetable cultivation offers one of the optimistic approaches to ensuring sustainable food and nutrition security in the tropics. Although greenhouse vegetable production is known to be costly, this system of production is gaining popularity and contributes to sustainable tomato production with improved fruit quality and productivity, which results in higher economic returns. Among vegetable crops, tomato is the most cultivated under this system. A study was conducted to identify suitable soilless media for regenerating tomato cuttings from axillary stem of tomato plants and to assess the agronomic performance of the regenerated cuttings under greenhouse condition. The tomato cuttings were raised using 100% rice husk biochar, 100% rice husk, 100% cocopeat, 50% biochar +50% cocopeat, 50% cocopeat +50% rice husk. Two tomato hybrid varieties (Lebombo and Anna) were used. Cuttings from axillary stems were compared with those raised from seed. A 2 × 2 factorial experiment was arranged in a Completely Randomized Design (CRD) with four replications. From the study, 100% rice husk biochar was found to induce root development in stem cuttings of tomato. However, no significant differences in yield and fruit quality were found between plants raised from seed and those from stem cuttings.

Keywords: greenhouse, tomato production, food and nutrition security, tropics

1. Introduction

Tomato (*Solanum lycopersicum*) is a flowering plant belonging to the Solanaceae family, also known as Nightshade. It is one of the most popular vegetable crops grown in the world due to its fruit quality—taste, color, flavor and nutritional content [1]. Tomato fruits can be consumed in different forms; either fresh, partially cooked or processed. Tomatoes provide carotenoids, flavonoids, phyosterols, vitamins, and minerals which are essential in human nutrition. Carotenoids are the most abundant in tomatoes with the most common one being lycopene, followed

by beta-carotene, gamma-carotene, lutein, phytoene, and a few other minor carotenoids [2, 3] which have anti-cancer properties [4, 5]. It is also a great source of carbohydrates, fiber and a small amount of vitamin A, vitamin B complex (thiamin, riboflavin, and niacin) and vitamin C [6] and is also rich in iron, copper, phosphorus, manganese and potassium [7].

According to the statistical agency of the Food and Agriculture Organization of the United Nations (FAOSTAT) (2020), the world's total tomato production is estimated at 186,821 million tonnes with a cultivated area of about 5,051,983 hectares. In comparison, there has been a 3.35% increase in production from 180,766 million tonnes in 2019 to 186,821 million tonnes produced in 2020. China is the leading producer of tomatoes in the world accounting for about 34.67%. Egypt ranked fifth in global tomato production contributing 3.6% while leading the tomato production in Africa estimated at 6731.22 million tonnes cultivated on an area of 170.862 hectares. In addition to Egypt, other North African countries with both tropical and temperate conditions including Algeria, Tunisia and Morocco accounted for about 2.39% of the world's tomato production. Among the West African countries, the leading producers, Nigeria and Cameroun produced 3693.72 million and 1.246.65 million, respectively, while Kenya produced 1056.18 million to lead tomato production in East Africa [8]. In Ghana, according to the Ministry of Food and Agriculture (MoFA), tomato production is estimated at 420,000 tonnes in 2019 cultivated on 47,000 hectares [9, 10].

The rapid increase in tomato consumption in the tropics is one of the factors influencing emerging production practices and strategies to meet local and export demands. Thus, many tropical countries have expanded their tomato acreage to meet local needs and, in some cases, to generate foreign exchange due to the increased importance of tomatoes in food and nutrition security. Several different production systems have been used successfully in different parts of the world to produce tomatoes. For instance, in the tropics, particularly in Africa, the open field cultivation system is mostly adopted whereas, in the developed countries, there is a massive shift to controlled environment systems [11]. Tomato cultivars with a determinate or semi-determinate growth habit are typically grown in open fields which are usually for fresh consumption. This system is also distinguished by the use of either direct sowing or transplanting where a nursery is established. Currently, transplanting is commonly practiced since it ensures good stand establishment, uniformity, reduced weed competition, and improved survival rate and yield compared to direct sowing [12]. Nonetheless, open-field tomato seedlings tend to be weaker and have a lower rate of transplant survival, resulting in low yields [13]. Other constraints such as biotic (high incidence of pests and diseases) and abiotic stresses (such as drought and high temperature) pose serious threats to open-field tomato production [14]. Root-knot nematodes (including *Meloidogyne incognita*, *M. javanica* and *M. arenaria*) are soil-borne pathogens that cause yield losses of about 30% in tomatoes in the tropics [5]. Thus, they cause stunted growth making the tomato plants more susceptible to soil-borne fungal (such as *Fusarium wilt* caused by *Fusarium oxysporum*) and bacterial diseases (such as bacterial wilt caused by *Ralstonia solanacearum*) [5]. Several studies on grafting techniques to combat these soil-borne root-knot nematodes and fungal diseases have resulted in the identification of potential rootstocks such as *Solanum torvum*, *Solanum macrocarpon*, and *Solanum aethiopicum* [15] that confer tolerance to these soil-borne problems. However, due to the high cost of producing grafted seedlings in large quantities, grafting is not widely used in large-scale production in the tropics [16]. Furthermore, open-field tomato cultivation exposes the plants to a

variety of stinging and sucking insects, such as whitefly, thrips, and aphids, which cause moderate to severe physical damage as well as contribute to the transmission of viruses [5]. High temperatures observed in open-field tomato production in the tropics cause heat stress [17]. Tomato is an extremely sensitive crop to heat stress, which can lead to total yield loss [18]. A slight increase in night temperature especially can decrease pollen viability and female fertility thereby impairing fruit set and consequently yield reduction [19].

Increased tomato consumption [20] combined with unfavorable climatic conditions necessitates the development of urgent strategies to boost production while improving fruit quality in the tropics. Open field tomato production is hampered by climate change-related factors such as high temperatures, drought and high incidence of pests and diseases. In recent years, greenhouse tomato farming has proven to be the most efficient method of producing high-quality fresh tomatoes for both domestic and international markets [1]. In addition, it provides the opportunity for year-round production. Indeterminate tomato cultivars are usually used in this system, allowing the harvesting period to be extended, thereby, increasing the tomato productivity and revenue as well as improving the livelihood of farmers. This chapter discusses greenhouse structures and systems, agronomic practices, postharvest handling, prospects and challenges of greenhouse tomato production in the tropics and the use of axillary stem cuttings as an alternative method of producing true-to-type tomato seedlings for cultivation.

2. Greenhouse structures

Greenhouse farming systems have been adopted in some African countries, especially in Northern Africa (Algeria, Egypt, Morocco, and Tunisia), Eastern Africa (Kenya, Ethiopia, Uganda, and Rwanda), Western Africa (Ghana) and South Africa. In Northern Africa, the greenhouse system is mainly used for vegetable production while that of Eastern Africa (for e.g., Kenya), is for flower production. Furthermore, in Rwanda, South Africa and Ghana greenhouse system is mainly used for tomato production [21]. In all these countries, the greenhouse specifications are dependent on the availability of construction inputs, local climatic conditions and socio-economic status [11]. Generally, the initial investment cost of greenhouse construction is very high. Galvanized metals including steel or aluminum are the preferred construction material as they are durable and require less amount of material for construction thereby increasing light transmission (**Figure 1**). Wood such as bamboo is an alternative material (**Figure 2**). Though it is less expensive, more wooden materials are required to ensure a solid and firm structure. This, however, reduces light transmission. Also, the cost of maintenance in using bamboo is relatively higher compared to those constructed from metals [21].

High sidewalls in greenhouse construction are critical for maximizing the effectiveness of natural ventilation in greenhouses with roof venting. The direct/diffuse ratio in incident light, as well as the diffusion properties of covering materials [22, 23], greenhouse design, time of day, season, and location, all influence light transmission and spatial uniformity of light intensity inside the greenhouse [11]. To promote plant growth and development, an ideal greenhouse ensures that light is evenly distributed. Again, to ensure optimal light transmission in the greenhouse, the type of covering material should be considered. These include; (1) a non-waterproof net which provides partial shade and protection against insect permeability; (2) a plastic film



Figure 1.
Greenhouse of West Africa Center for Crop Improvement (WACCI), University of Ghana built from galvanized metals including steel or aluminum.



Figure 2.
Greenhouse of Institute of Applied Science and Technology (IAST), University of Ghana built from bamboo.

for protection against insects and rains and (3) a glass which is more durable and effective than plastic films. Glass is mostly used for high-tech greenhouses [21]. In most greenhouses in Africa, side nets are fixed to provide natural ventilation (**Figure 3**). Circulation fans (chimney) (**Figure 4**), misting/fogging and hosing (**Figure 5**) can also be used to regulate/manage the climatic conditions in the greenhouse. In addition, shade screens/nets are also used to reduce the intensity of solar radiation in the greenhouse (**Figure 5**) [21].

2.1 Greenhouse agronomic practices

Good greenhouse crop management practices serve as a gateway for ensuring sustainable production, increasing yield and high fruit quality, concomitant with increased income generation. Before plant establishment; raising vigorous and healthy seedlings, greenhouse fumigation media selection and sterilization, fertigation and irrigation, etc. need to be considered. In addition, other recommended



Figure 3.
Fixing of side nets (indicated with the arrow) to provide natural ventilation.



Figure 4.
Circulation fans (chimney) are fixed on greenhouses of IAST to regulate the climatic conditions in the greenhouse.



Figure 5.
Misting/fogging and hosing (blue arrow) are used to regulate the climatic conditions as well a shade net (red arrow) is used to reduce the intensity of solar radiations in the greenhouse.

greenhouse cultural practices such as plant spacing, pruning, topping, training/trellising and hormone application and pollination should be performed.

2.2 Tomato varieties and propagation

The cultivation of tomatoes in the tropics is solely by using seeds; either open-pollinated (OPV) or hybrids. Hybrid seeds of tomatoes are the most suitable planting materials because of their vigor and high yielding potential [24]. Since greenhouse cultivation is done in a limited area, indeterminate hybrid tomato varieties are cultivated [11]. For instance, in Ghana, hybrid tomatoes such as Cobra, Anna F1, Lebombo, Kwando, Jaguar, Gamharr, Jarrah, Eva, Ranja, and Sodaja are being introduced by seed companies for greenhouse cultivation. Several greenhouse screenings and evaluations of exotic tomato lines are being carried out to identify adaptable high yielding types with excellent fruit quality. However, cultivating these hybrid tomatoes in the tropics could be very expensive and as such, vegetative propagation of tomatoes could be a viable option for producing true-to-type tomato hybrid planting materials [25] to ensure sustainable production.

A study was conducted to identify a suitable soilless medium for regenerating tomato seedlings from axillary stem cuttings and to assess the agronomic performance of the regenerated seedlings under greenhouse condition. Cuttings (12–15 cm long) from mature tomato plants were taken and raised using 100% rice husk biochar, 100% rice husk, 100% cocopeat, 50% biochar + 50% cocopeat, 50% cocopeat + 50% rice husk. A 2 × 2 factorial experiment arranged in a Completely Randomized Design (CRD) with four (4) replications was used. Treatments consisted of two factors; two tomato hybrid varieties (Lebombo and Anna) and planting materials (cuttings and seeds). Seedlings were also raised using 100% rice husk biochar. Seedlings and rooted cuttings were sown and transplanted 28 days respectively into pots (22 × 25 cm) half filled with 100% cocopeat. The study identified rice husk biochar (**Table 1**) as a suitable medium for generating vigorous and healthy tomato stem cuttings obtained from pruned axillary shoots of tomato varieties, Lebombo and Anna F1 (**Figure 6**). Further evaluation using tomato plants generated from seeds and stem cuttings indicated that there were no significant differences in yield (**Table 2**) and fruit quality (**Table 3**). Hence, vegetative propagation via axillary stem cuttings could be used as an alternative method of raising tomato seedlings in the tropics. Seed companies and tomato nursery production operators can collaborate to leverage this method to supply tomato seedlings at affordable rates to ensure sustainable greenhouse tomato production in the tropics.

2.3 Substrate and sterilization

Plant roots are contained within a porous rooting medium called a 'substrate' or 'growing medium.' A suitable growing medium is required to provide root anchorage and a favorable environment for healthy root development, [26]. Growing media for greenhouse cultivation in the tropics comes in two basic types: soil- and organic-based. Field soil is the main component of the soil-based media and is the most simple and cheapest. However, it is associated with a high risk of soil-borne diseases such as bacterial wilt [21]. On the other hand, organic materials such as composted waste, peat, coconut peat/coir, sawdust, wood and bark are used to prepare the organic-based media [27]. Peat moss, vermiculite, and perlite which are premixed blends of organic and inorganic materials are commercially available. These products, however, are costly and difficult to obtain locally in the tropics, especially in Africa. Agricultural

Substrate	Root length (cm)	Survival (%)	Root volume (cm ³)	Shoot dry weight (g)	Root dry weight (g)	Total dry weight (g)
Rice husk biochar/Lebombo	16.6 b	95.8 de	1.71 b	1.74 bc	0.26 ab	1.44 b
Cocopeat/Lebombo	10.4 a	29.2 a	1.89 b	1.41 b	0.14 a	1.55 b
Biochar + Cocopeat/Lebombo	10.1 a	40.6 ab	1.66 b	0.96 a	0.15 a	1.11 a
Cocopeat + Rice husk/Lebombo	13.0 ab	45.8 ab	1.55 b	1.35 ab	0.20 a	1.52 b
Rice husk biochar/Anna	17.4 b	100.0 e	1.89 b	2.13 c	0.38 b	2.54 c
Cocopeat/Anna	10.4 a	50.0 abc	1.71 b	1.40 b	0.17 a	1.56 b
Rice husk biochar + Cocopeat/Anna	10.7 a	83.3 cde	0.97 a	1.37 ab	0.14 a	1.51 b
Cocopeat + Rice husk/Anna	10.6 a	72.9 bcd	1.58 b	1.45 b	0.20 a	1.62 b

Table 1. Mean Root length, Survival plants per replication, Root volume, shoot dry weight, root dry weight and Total dry weight. Means followed by the same letters within a column are not significantly different according to Fisher's Protected LSD at 5%.

and municipal wastes, which are locally available, affordable, and environmentally sustainable, should be investigated as alternatives to commercial products in the tropics. A good soil-free substrate should have excellent chemical, biological and physical characteristics with low nutrient content, low pH, a unique combination of high-water retention capacity, high air space, lightweight, pest, and disease-free [28]. Cocopeat, a waste product obtained from the mesocarp of coconut (*Cocos nucifera*) fruit is most widely used in Africa and Asian countries such as the Philippines, Indonesia, India and Sri Lanka, where lots of coconuts are produced [28]. It can be combined with rice husk biochar and oyster shells. Although cocopeat is a better substitute for peat moss, high levels of natural soluble salts, sodium, and chloride are present and could cause osmotic stress to plants. As a result, to make these materials suitable for crop production, they are buffered or flushed out to remove excessive salts [29]. Sterilization of growing media is required before use, especially the locally prepared ones to prevent the introduction of pathogens and weeds in the greenhouse. Heat sterilization is the most common method (Figure 7). Although the most popular and cheapest method is solar sterilization, other improvised systems have been developed. Regardless of the system, it is critical to ensure that the entire media is exposed to uniform and adequate heat for efficient and effective sterilization [27].

2.4 Plant spacing and density

Due to the high cost of greenhouse infrastructure, increasing plant density is one strategy for maximizing the limited space [30]. However, it is also important to



Figure 6. Lebombo (A) and Anna (B) tomato seedlings raised from stem cuttings.

Treatments	Days to 50% flowering	Days to 50% fruiting	Total number of fruits	Fruits per plant	Fruit weight per Plant (g)	Yield (kg/ha)	Shelf life (days)
Variety							
Anna	25	32 a	24 b	5 b	96.5	6431.0	5
Lebombo	27	34 b	21 a	4 a	97.6	6506.0	5
$P \leq 0.05$	0.143	<0.001	0.043	0.043	0.895	0.895	0.199
Propagule							
Seeds	32 b	37 b	23	5	97.6	6503.0	5
Cuttings	21 a	28 a	22	4	96.5	6434.0	5
$p \leq 0.05$	<0.001	<0.001	0.689	0.689	0.902	0.902	0.019
Variety * Propagule	NS	0.021	NS	NS	NS	NS	NS

Table 2. Days to 50% flowering and fruiting, the total number of fruits, number of fruits per plant, fruit weight per plant, yield and shelf life of tomato plants. Means followed by the same letters within a column are not significantly different according to Fisher's Protected LSD at 5%.

plant in rows at a recommended spacing (**Figure 8**) to achieve an optimum yield. The required spacing between tomato plants will ensure an even distribution of resources such as water, nutrients, light, and air [31]. For example, there is more competition for light due to the overlapping and shading of leaves when plants are closely spaced [32]. The amount of light intercepted by the basal leaves could be drastically reduced, lowering the plants' photosynthetic efficiency. Consequently, the plants may be

Treatments	Fruit girth (mm)	Fruit length (mm)	Brix (%)	Firmness (kg/lb)	Pericarp thickness (mm)	Juice volume (cm ³)	pH	Titratable acidity
Variety								
Anna	34.66 a	44.16	6.64	7.06	4.43 a	26.8	4.12	0.56 a
Lebombo	38.05 b	46.24	6.45	6.66	5.11 b	27.4	4.12	0.73 b
$p \leq 0.05$	<0.001	0.036	0.567	0.438	0.050	0.874	0.947	0.028
Propagule								
Seeds	36.20	44.50	6.47	6.73	4.49	27.8	4.13	0.58
Cuttings	36.51	45.90	6.62	6.99	5.05	26.4	4.10	0.71
$p \leq 0.05$	0.587	0.137	0.653	0.622	0.100	0.684	0.217	0.083
Variety * Propagule								
Anna * seeds	34.59	43.63	7.02 b	7.51 b	3.75 a	26.4	4.12	0.71 b
Anna * cuttings	34.73	44.69	6.27 ab	6.61 ab	5.11 b	27.3	4.11	0.41 a
Lebombo * seeds	37.82	45.37	5.93 a	5.95 a	5.23 b	29.2	4.09	0.71 b
Lebombo * cuttings	38.28	47.10	6.98 b	7.36 ab	4.99 b	25.6	4.14	0.75 b
$p \leq 0.05$	0.776	0.706	0.017	0.042	0.026	0.510	0.112	0.022

Table 3. Fruit girth, Fruit length, Brix, Firmness, Pericarp thickness, Juice volume, pH and Titratable acidity of tomato fruits. Means followed by the same letters within a column are not significantly different according to Fisher's Protected LSD at 5%.



Figure 7.
Dry heat from a flame used for the sterilization of growing media.



Figure 8.
Tomato plants planted in rows at a recommended spacing.

forced to trade off their energy for stem elongation and reduced assimilate transport to developing fruits [31], thereby, causing yield reduction and poor fruit quality [33]. There have been reports of great increases in tomato yield and yield components when recommended plant spacing was used [33–35]. A recent study by Nkansah et al. [36] suggested plant spacing of 0.2×1.3 m for greenhouse tomato production.

2.5 Irrigation and fertigation

Adequate water supply to plants is essential for various metabolic and physiological processes such as photosynthesis, nutrient transport, and cell expansion and development [27]. In the tropics, water for greenhouse production can be obtained from rivers, ponds or reservoirs, rain, groundwater (boreholes), and municipal sources (tap water). Unfortunately, water quantity, quality and seasonal availability are not guaranteed in most tropical environments. A good water should be free from pests (such as pathogenic bacteria, fungi, weeds and pesticide contamination) and high concentrations of dissolved salts and toxic ions (heavy metals) [27]. As a result, a thorough biological and chemical analysis of water for greenhouse tomato production



Figure 9.
Water tanks are elevated above the level of the field to allow for the natural flow of water and nutrients.



Figure 10.
Water and nutrients are applied using a computerized system with sensors and a pre-programmed fertigation regime.

is required as this can affect plant health, growth and development. The chemical property, for instance, is useful for the formulation of nutrient solutions.

In the tropics, the manual irrigation system is the cheapest but does not give precision in terms of the quantity of water and nutrients applied. Gravitational fertigation in combination with drip irrigation is the commonly adopted method. The water tank is elevated (**Figure 9**) to allow water and nutrients to flow naturally [37]. Water and nutrients can be reused by using a recirculation system [11]. Water recirculation, on the other hand, increases the risk of spreading soil-borne diseases, necessitating the use of a disinfection unit (UV or heat treatment) [38] which can be costly. Another means of supplying water and nutrients is using a computerized system with sensors and a pre-programmed fertigation regime (**Figure 10**). This system, however, is reliant on a constant supply of electricity, which is a major challenge in the tropics [21].

2.6 Pruning, topping and training/trellising

Tomato cultivars are divided into two categories based on their growth habits: determinate and indeterminate. Determinate tomatoes grow in a bush-like manner,



Figure 11.
Pruning of tomato vines by removing the stem suckers.

reaching a fixed mature size characterized by synchronized flower formation and fruit production. On the other hand, indeterminate tomatoes grow in a vine-like manner, continuing to grow throughout the growing season and thus, having continuous flower and fruit formation [39]. The indeterminate tomato cultivars are used in greenhouse tomato cultivation [11]. Tomato vines are pruned by removing the stem suckers (**Figure 11**). These are stem branches or side shoots that emerge from the leaf axils which are the junctions between the main stem and the true leaf. If not pruned, these suckers will grow into full shoots with leaves, flowers, and fruits, and even regenerate new suckers. When suckers are young and small, they can be pinched or cut using pruners such as knives, scissors and secateurs. In any of these pruning approaches, it is better to ensure decontamination either by using an alcohol-based sanitizer or washing with soap to prevent the spread of pathogens [40]. Pruning can be done on weekly basis to improve or ensure efficient air circulation/aeration [41]. In addition, pruning helps to prevent the diversion of assimilates from the developing fruits thereby, improving tomato fruit quality [40, 42].

Another important greenhouse technique is topping (**Figure 12**), which involves cutting or pinching off the terminal bud to break the apical dominance [43]. This technique is critical because tomato cultivars for greenhouse cultivation are indeterminate types characterized by indefinite growth. Topping has been shown to improve fruit quality and yield by causing assimilates to be redistributed to developing fruits [44, 45]. In the Solanaceae family, topping improved yield and yield components in eggplant [46], pepper [47] and tomato [36]. According to Nkansah et al. [36], tomato yields were increased by topping at truss 2.

The main stem of tomato plants is positioned upright immediately after transplanting to keep the leaves and fruits from touching the ground [48], facilitate pollination, maximize light interception of the younger leaves, and increase labor efficiency in pruning and harvesting [11]. This method known as stem training/trellising (**Figure 13**) is necessary for indeterminate tomato cultivars. It entails securing the main stem with a twine/rope suspended from a horizontal wire about 2.5–3.2 m above the ground [11, 49]. Non-slip loops or clips are used to secure the twine's tip



Figure 12.
Topping tomato plants by cutting or pinching off the terminal bud.



Figure 13.
Trellising or training of tomato plants by securing the main stem with a twine/rope suspended above the ground.

to the stem's base. The twine is then neatly wound in two or three spirals around the stem for each truss without damaging the stem [11].

2.7 Hormone application and pollination

Heat stress is a major problem hampering tomato production in the tropics [50]. Poor fruit set occurs in greenhouse systems where the microenvironment is not fully controlled or automated. Tomato is an extremely sensitive crop to heat stress, which can lead to total yield loss. The optimal day and night temperatures for tomato production are 21–29.5°C and 18.5–21°C, respectively. However, a slight increase in night temperature especially can decrease pollen viability and female fertility thereby impairing fruit set and consequently yield reduction [19]. Pollination and fertilization must both be completed before the fruit set can occur (**Figure 14**) [51]. Under heat stress, however, these processes are disrupted, resulting in flower abortion and flower drop [50]. Unfortunately, the molecular mechanisms underlying tomato fruit set are unknown, despite the fact that exogenous application of auxin and gibberellin to the



Figure 14.
Pollination and fertilization of tomato flowers before fruit set.

tomato stigma improved tomato fruit set. Bypassing pollination and fertilization, auxin or gibberellin can stimulate tomato fruit development (cell division and expansion) [51]. As a result, using these hormones can help increase greenhouse tomato production by increasing fruit set and yield [52]. The coordinated mechanism of auxin, gibberellin, and cytokinin has been investigated for the development of parthenocarpic tomato fruits [53], which improves fruit quality. Although this may be labor intensive, the high returns from increased productivity and improved fruit quality can compensate for this.

2.8 Greenhouse pest and disease management

One of the reasons for the rise in greenhouse tomato production in the tropics is the benefit of reducing pest and disease outbreaks, which can affect plant growth and development, resulting in lower yields and poor fruit quality. To control pest or disease outbreaks, an integrated pest management approach including cultural, biological and chemical measures (**Figure 15**) is used. Because prevention is the best approach, ensuring good environmental practices is an important first step [54]. Regular cleaning and washing of the greenhouse and its equipment with disinfectant (such as bleach) and fumigation prior to the start of the production cycle are examples of best practices. Another strategy is to keep a close eye on the crops in the greenhouse in case of a pest or disease outbreak [55]. Pheromone traps



Figure 15.
Chemical application for the management of pest and disease in greenhouse vegetable production.



Figure 16.
Pheromone traps (A) and sticky cards (B) are used to trap, detect, and determine pest population thresholds in greenhouses.

and sticky cards (**Figure 16**), for example, are used to trap, detect, and determine pest population thresholds of pests such as leaf miners, whiteflies aphids and thrips [8, 55]. A comprehensive pest management guide for tomato production is available [8]. Pruning, trellising, and proper plant density and spacing ensure good aeration. Avoidance of wet floors by preventing irrigation water spillage helps to reduce the creation of a microclimate that promotes disease outbreaks [55].

2.9 Harvesting and postharvest handling

Harvesting of greenhouse tomatoes is usually done at the breaker of color or when the fruit is orange-red, by handpicking. Thus, greenhouse tomatoes are typically

harvested riper than fresh market field-grown fruit, making them more susceptible to mechanical injuries due to their softer nature and shorter shelf life than mature-green fruit. Greenhouse-grown fruit harvesting is done twice or three times per week as it reaches the appropriate stage of fruit development [11]. Prior to temporary storage, tomato fruits are sorted and graded. Grading allows a grower to serve different qualities at different prices to different markets, such as a supermarket and a wet market. As such, good packaging is required to reduce losses during transportation [21]. Harvested tomato fruits are chilling sensitive. Breaker fruits can be stored at 10–12.5°C for a week while orange-red at 7–10°C for 3–5 days [11]. Even though greenhouse tomatoes are more expensive than field-grown fruits, they are primarily produced for local consumption in the tropics. On the other hand, Northern African countries (such as Egypt and Morocco) and South Africa, produce greenhouse tomatoes for export to Europe [21].

3. Prospects and challenges of greenhouse tomato production in the tropics

3.1 Prospects

In the tropics, greenhouse tomato production has the potential to create attractive jobs for youth and women in particular [56]. Greenhouse training programs have been introduced in West Africa, particularly in Ghana, to target entrepreneurs and young graduates to learn how to grow vegetables in greenhouses [57].

The increased demand for greenhouse tomatoes, owing to their superior fruit quality, benefits growers by earning appreciable income to improve their livelihoods [58]. People in urban and peri-urban cities have gradually accepted and are willing to pay more for greenhouse tomatoes, despite the fact they are more expensive than those grown in the field [59].

Greenhouse tomato production supplements local tomato production, which is primarily a field-grown system that is affected by biotic and abiotic factors. Thus, the introduction of greenhouses in the tropics has helped to ensure year-round tomato production and supply of high-quality fruits, ensuring sustainable food and nutrition security [60]. Also, there will be a constant supply of tomatoes to the processing industries for various industrial activities.

In addition, the greenhouse tomato production system contributes to the economic maximization of limited land and other resources [61]. This system, for example, ensures efficient water and nutrient supply to the plants while reducing losses such as leaching, which is common in field-grown systems. Also, unproductive lands, roof-tops and concreted areas can be utilized for greenhouse tomato cultivation [62].

Another advantage of greenhouse tomato production is the complete control over indiscriminate agrochemical (pesticides, fungicides and weedicides) application. Strict adherence to greenhouse agronomic practices and integrated pest management systems eliminates traces of these agrochemicals on tomato fruits, which are harmful to human health [58]. This could promote the use of traceability systems to encourage the export of greenhouse tomato fruits in order to generate foreign exchange to boost tropical economies [63].

The introduction of greenhouses has opened up new areas in the tropics for academic and research work. To improve greenhouse tomato cultivation in the tropics, researchers should look into areas such as greenhouse agronomic practices, breeding

for tropics-adapted greenhouse tomatoes, commercial adoption of grafting techniques for soil-based greenhouse cultivation, development of tropical soilless media and nutrient solutions, assessment and availability of raw materials for greenhouse constructions and so on.

3.2 Challenges

The initial cost of constructing a greenhouse is high which deters average income entrepreneurs to venture into greenhouse tomato production [64]. In addition to this, accessibility to credit facilities is difficult [65]. Lack of greenhouse technical know-how has also hindered the adoption of greenhouse tomato production in most tropical countries. In some areas, there are no greenhouse training centers for hands-on training to fully equip trainees in greenhouse design, construction, repair and maintenance and cultivation [66].

The unavailability of adaptable greenhouse tomato cultivation possess a major challenge. There is a high influx of imported tomato hybrids into various countries, however, some of these tomato hybrids are not adequately evaluated or screened to identify the promising candidates for further evaluations and official release. In addition, the available tomato hybrids are generally expensive for the local growers and may have fruit quality characteristics which are not preferred by the local market [45].

There is also a lack of greenhouse cultivation inputs and important resources. For instance, poor water quality and quantity prevent seasonal and year-round greenhouse tomato cultivation. Also, the unavailability of quality soilless substrates is a major challenge [58].

4. Conclusions

In conclusion, greenhouse tomato production is a promising technology that can ensure sustainable food and nutrition security in Africa. The selection of the proper greenhouse structure and system as well as the adoption of the appropriate agronomic practices and postharvest handling techniques would ensure enhanced tomato production under greenhouse condition in the tropics. Our research findings point to tomato cuttings as a viable source for raising planting material for tomato cultivation in the developing countries. The yields and fruit quality obtained from the use of seedlings versus stem cuttings were comparable.

It is therefore essential to encourage scientific research about greenhouse production in Africa to foster its adoption. Greenhouse tomato production has the potential of creating jobs and increasing income generation thereby improving the livelihood of the people in the greenhouse tomato value chain.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 4

Principles for the Production of Tomatoes in the Greenhouse

Olatunji Olabisi and Akeem Nofiu

Abstract

Greenhouse technology is the technique of regulating the environmental factors for the benefit of the plant (tomato) under protective cultivation. Production of tomatoes in the greenhouse involves two stages: nursery and greenhouse. In the nursery, the plants are seeded in small cavities of the nursery tray and arranged in the nursery chamber or a small-sized tunnel where they are given maximum care. At 3–4 weeks after seeding, when they must have developed four true leaves and a well-developed root system, the seedlings are transplanted into the bigger tunnel. The transplants are given water through drip irrigation. The nutrients are supplied through fertigation in the required quantity and concentration. Pest control is done by integrated pest management system (a combination of physical, biological, and sometimes chemical control).

Keywords: tomato, greenhouse, fertigation, integrated pest management, environmental factors

1. Introduction

Tomato is widely cultivated for its fleshy fruits that have special nutritive value. It is the world's second-largest vegetable crop following potato, and it is the most canned vegetable. Tomato is one of the most important vegetable crops produced by farmers in Nigeria with a demand gap of 2.3 million tons [1, 2]. Tomatoes can be eaten raw or processed. It could be processed into paste, tomato ketchup, soup, juice, diced, sauce, puree, etc. It is rich in nutrients, dietary fiber, and antioxidants such as lycopene and beta-carotene that prevent cells from cancer. It has high levels of vitamin A and C and some minerals such as iron and phosphorus [3].

Tomato production in Nigeria requires serious attention as the demand for domestic and industrial use has brought about peak rates in recent times. Tomato being one of the essential staple foods rich in minerals, carbohydrates, and vitamins is an important vegetable with premium and high processing values as well as a venture with production capacity to generate employment. In an attempt to achieve food-secured status as a nation, it is therefore pertinent to improve the production of tomatoes in Nigeria. However, generally, agriculture in Sub-Saharan Africa is rainfall-dependent, which is one of the factors debilitating the production output of agricultural produce. This dependence on climate/natural environment predisposes the crop plants to lots of dangers, such as pest and disease infestation and environmental stress due to various weather extremes, resulting in poor-quality fruits and ultimately low yield.

Kaduna and Kano states, which produce 43% of national production, have a yield of 7–10 tons and sometimes 15 tons per ha [2]. So now it is imperative to emphasize the adoption and use of a protected farming system, such as screen houses and greenhouses for the production of especially high-valued or premium vegetables or crops like tomatoes. This chapter, therefore, elucidates on basic principles of greenhouse tomato production.

Greenhouse production is more expensive than producing the same crop in the open field [4]. The most important factors determining costs are depreciation of the structure and equipment, labor, energy, and variable costs such as planting material, substrate, and fertilizer.

As the term implies, principle refers to a basic idea or rule that explains or controls how something happens or works.

1.1 Advantages of greenhouse tomato production

- i. The controlled environment allows for growing exotic fruits such as beefsteak for export.
- ii. It offers all-year-round production and quality produce.
- iii. Production in the greenhouse is more efficient than in the open field, which results in higher yield.
- iv. It is an intensive system that maximizes limited space and water.
- v. Efficient utilization of agrochemicals to control pests and diseases.

1.2 Disadvantages of greenhouse tomato production

- i. It is capital intensive
- ii. The energy requirement is high
- iii. Requires technical skills

2. Greenhouse structures

Tomatoes can be grown in every type of greenhouse, provided it is sufficiently high to manage and train the plants vertically. Generally, greenhouses can be classified into three based on structure: wooden (which could also be bamboo) framed, pipe framed, and truss framed. The cover could be glass, plastic film, or rigid panel. The cover must have high light transmission, and importantly photosynthetically active radiation (PAR) that falls within the range of 400–700 nanometers. In the central- and north-European countries, the Venlo-type glasshouses are mostly used. They typically have 3.2, 4, 6.4, 8, 9.6, 12, 12.8, and 16 m standard spans and 5–7 m gutter height to allow high wire planting systems [5–7]. There are variations in the dimensions, structures, and coverings used in the construction of greenhouses from one country to the other. For instance, most of the greenhouse facilities used in China are unheated [8].

3. Substrates and substrate systems

There is relatively little commercial tomato production done directly in the soil, except for organic growers. In large greenhouse complexes in developed countries, 95% of greenhouse tomatoes are grown on inert artificial substrates, a system usually referred to as soilless culture. The term “hydroponic” can refer to soilless culture or to systems such as the nutrient film technique (NFT), in which no solid substrate is used and water flows almost constantly down troughs holding plant roots [9–11].

There are many types of growing systems for greenhouse tomatoes, which include NFT (nutrient film technique), PVC pipes, sand, ground culture (in the soil), troughs, rock wool slabs, and various types of aggregate media. The various aggregate media include peat moss and peat–lite mixes, perlite, rock wool aggregate, glass wool, pine bark, and so on. In a trial of growing media at the University of Arizona [8], there were no significant differences in the yield of greenhouse tomatoes between five different media (coconut coir, perlite, peat–vermiculite mixes, coir/perlite, and rock wool).

4. Selection of variety

The first step in carrying out a successful crop production is the choice of good variety. Growing a variety that is not the best choice, or using seeds that are not of the best quality, reduces your potential for success at the outset. It is smart to start with the greatest potential rather than limiting yourself by using inferior seeds, even if it saves some money. Numerous tomato varieties are being pushed into the market, but only a few are suitable for greenhouse production. For greenhouse tomato growers, indeterminate tomato varieties are recommended. In indeterminate tomatoes, the growth of the stem is continuous and this allows for continual fruit production.

The selection of the best indeterminate seed to buy should be based on the following criteria:

- i. size of fruit desired.
- ii. disease resistance.
- iii. Lack of physiological problems, that is, cracking, cat-facing, blossom-end rot.
- iv. uniformity of fruit size.
- v. market demand.

5. Nursery

The success of a production cycle starts with acquiring healthy seedlings. Good healthy seedlings can be purchased from a commercial nursery. Also, the farmer can grow his seedling. Most greenhouse operators grow their seedlings. This is very desirable because it reduces the possibility of importing new diseases and insects [12]. Notwithstanding, in many other countries, seedlings are being raised successfully by special nursery farms that sell economical and high-quality seedlings to local growers

with the aid of modern technology. Transplant raising is a crucial stage in greenhouse vegetable production. The performance of a crop depends largely on the attention paid to the care given to it when it was in the nursery.

Quality seedlings are plants free from pests and diseases, quickly grown with no suppression of yield due to poor quality roots. Transplant production takes about 3 weeks, depending on temperature and light conditions. Tomato seed germinates best at 25°C, while seedling growth is optimal at 18°C night-time minimum and 27°C daily maximum. Germination rates are at least 95% and so only one seed needs to be planted per cavity. The ideal transplant size is when the seedling has four true leaves (Figure 1). A good seedling is as wide as it is tall and has not started flowering.

5.1 Step-wise procedure for raising a nursery

The following are the tools and materials needed for a successful nursery production: nursery tables, nursing trays, knapsack sprayer, substrate (peat moss, cocopeat, sawdust, or sterilized soil), indeterminate tomato seeds, and clean water.



Figure 1.
A typical tomato seedling ready for transplanting.

5.1.1 Procedure

1. The clean trays are arranged on the nursery table.
2. The substrate is collected in a clean bowl. Moisten the substrate by spraying with water while turning.
3. The growing cavities of the trays are filled with substrates.
4. Make a depression in the cavities which are already filled with substrates.
5. Place the seeds in the depression made at one per cavity.
6. Spray with water.
7. Cover the seeds with the substrate.
8. Spray with water again and place in a dark room.
9. At the sight of the emergence of the first plumule, remove all the trays from the dark room and place them in the nursery.
10. Spray with water two or three times daily depending on the weather. Just ensure it does not go dry.
11. At about 1 week after germination, you may supplement with fertigation depending on the type of substrate used.
12. A typical fertigation program for the nursery involves dissolving 10 g water-soluble poly feed fertilizer with micronutrients in 15 l water. The fertigation water is alternated with clean water every 2 days.
13. The seedlings are ready for transplanting when they have four true leaves and a well-developed root system. Typically, this is at 2–3 weeks for tomatoes and 3–5 weeks for peppers after seeding.

6. Transplanting

Transplanting of tomato seedlings into the greenhouse is often carried out when they have reached the height of 7.5 cm to 10 cm [13]. The media or substrate of any type chosen requires to be thoroughly wet with water or diluted nutrient solution several hours prior to planting. The plants should be checked for any individual that fails to establish after planting. They will have to be changed. To keep the substrate moist, plants will require irrigation with diluted fertilizer solution as often as necessary. A good general rule of thumb is to maintain moisture at a level where only a few drops of water are needed to compact the soil into a clump [14].

6.1 Plant population

It is important to use the proper planting density when growing greenhouse tomatoes. Using a higher planting density will cause the yield per plant to decrease. This is basically due to plants shading each other. The costs and the amount of labor required also increase with more plants. Likewise, crowding plants tends to encourage disease proliferation because sprays cannot easily penetrate the thick foliage and foliage does not dry as readily. Plants should be arranged in double rows, about 4 feet apart in the center. Within a row, plants will average 14–16 inches between stems [15].

6.2 Step-wise procedure for establishing plants in the greenhouse

Tools and materials needed are grow bags, plastic mulch, twine, soil, sterilizing pan, cooling pan, binding wire, spade, manure, wheelbarrow, firewood, lighter, NPK 15:15:15 fertilizer, iron rod, tape rule, hammer, and drip irrigation kit.

6.2.1 Procedure

1. Layout: four iron rods are used to mark a rectangle inside the tunnel. The beginning is marked at 1 m away from the front and back nets each and 0.5 m away from the right and left nets each. Along the width of the rectangle, the iron rod is used to mark the beds (0.75 m) and the furrows (0.5 m). This is done at the front and back of the tunnel. A twine is tied to the rod at the front and back, respectively, to make a straight line.
2. The beds are made along the line of the twine. As such an 8 × 24 m tunnel will contain six beds and each bed will contain two rows of tomatoes.
3. The iron rods are removed and drilled closer to each other to divide the width of the bed into three. This is done for each of the beds.
4. The plastic mulch is laid on the bed. The edges of the mulch are buried with soil.
5. Sterilization: soil sterilization is essential due to the prevalence of bacteria wilt and nematodes in the soil. The manure is first turned into the frying pan and continuously stirred. It is allowed to fry but not burnt after which the soil is turned in. The soil is mixed with the manure 2:1 ratio. The mix is continually turned now and then for about 30–60 mins depending on the intensity of the fire, and the soil will be taken out when 100°C is attained with the aid of a thermometer.
6. Thereafter, the soil is transferred into the cooling pan where it is allowed to cool before sharing into the grow bags at 20 kg each. The bagged soils are arranged on the beds in the tunnel at 80 bags per bed.
7. Base application of NPK 15:15:15 is applied and mixed thoroughly with the soil.
8. The drip lines are installed, and the soil is continually watered for about 2 weeks to mineralize the fertilizer before transplanting.

9. Transplanting should be done in the evening when the weather is cool.
10. The soil is watered and holes are drilled at the spot where the water drips.
11. The nursery is watered before transplanting to ease removal from the cavities.
12. At transplanting, the plantlets are carefully placed in the drilled holes and covered with the soil to the plant collar. The soil is pressed lightly to hold the plant root in place.
13. Water is supplied for about 5 mins. Care should be taken to ensure all the emitters are dripping and the plantlets are all receiving water.
14. At 2–3 weeks after transplanting, the binding wire is tied to the rod at the front and the corresponding one at the back along with the beg.

7. Growth and development

Growth and development continue in the greenhouse after transplanting (**Figure 2**). The management techniques include: Irrigation, fertigation, desuckering, staking and trellising, application of fruit-setting solution, defoliation, cleaning of filters, and flushing of driplines.

7.1 Irrigation

Large amounts of high-quality water are needed for plant transpiration, which serves both to cool the leaves and to trigger the transport of nutrients from roots to leaves and fruits. For instance, the amount of water needed by the greenhouse in the Netherlands is about $0.9 \text{ m}^3/\text{m}^2/\text{year}$ [16]. Therefore, before building a greenhouse, it is essential to ascertain that there is adequate, quality water available all year round. EC should be $<0.5 \text{ dS/m}$, pH from 5.4 to 6.3 and alkalinity $<2 \text{ meq/l}$ [9].

In the greenhouse, water supply is by drip irrigation (surface or sub-surface). This allows for efficient uptake of water and nutrients when mixed with fertilizer [17]. The water and nutrients are delivered to the active root zone thereby reducing nutrient loss by leaching or soil fixation. Also, the vegetative part of the plant does not come in contact with water, which reduces the growth of infection.

The frequency of irrigation varies with substrate rooting volume and its water-holding capacity. The water requirements of plants also depend on the growth stage of the plant and the season. The quantity of water required could vary from 1 to $14 \text{ l/m}^2/\text{day}$ ($0.4\text{--}5.6 \text{ l/plant/day}$) [4, 18, 19]. Generally, water consumption increases with the growth of the plant. The water is either gravity-fed or pumped with a mini pumping machine.

7.2 Fertilizer application

Nutrient supply to the greenhouse plants is done by nutrification or fertigation. Nutrification is an acronym for the two words “nutrient” and “irrigation” just as fertigation is a blend of “fertilizer” and “irrigation,” hence the application of water-soluble fertilizer with the irrigation water. This allows for precision and frequency in nutrient



Figure 2.
Tomato plants grown directly on the soil at 2 weeks after transplanting.

supply, especially when the water is delivered with drip lines. Also, the nutrient is delivered to the plant even when the plant is inaccessible. The fertilizer to be applied at a particular time depends on the developmental stage of the plant and the soil test result [20], which consequently inform the design of the fertigation program. For instance, more nitrogen is supplied at the vegetative stage of the plant, while potassium is supplied during flowering and fruiting. A typical fertigation program supplies 500 g poly-feed with micronutrients (e.g., Haifa Bonus) per 1000 L water for the first 2–3 weeks after transplanting. Potassium-nitrate (e.g., Maxi K) is supplied at 2 kg/1000 L water at pH 5.6–6.5 and E.C 1.2–1.6 from week 4 after transplanting onwards, and 2 kg calcium-nitrate (e.g., Haifa CalNit) at about 4 weeks after transplanting onwards. Pavani et al. [21] recommend supplying WSF 19% each of NPK at 3.75 G/M two times in a week from 21 days after transplanting, 3 g/l micro nutrient 2–3 times from 60 days after transplanting once in 30 days, and calcium nitrate 2–3 times once in 15 days.

The fertilizer is dissolved in a bucket of water before being added to the 1000 L tank full of water or supplied through a venturi system. In a venturi set-up, the fertilizer is mixed in a separate, smaller tank and a venturi injector is used to connect the fertilizer tank to the pure water pipe that goes into the greenhouse. The venturi injector operates on the principle that pressure drops accelerate the change of velocity of the water as it passes through the constriction [22].

7.2.1 Fertilizer compatibility

Two or more soluble fertilizers can be mixed in the same water and supplied to the plant provided they are compatible (**Figure 3**). For example, calcium fertilizer reacts with phosphate fertilizer to form a precipitate which blocks the emitters of the drip lines. This prevents the plants from getting water. Also, there should be no physical segregation of the components. As such it is always advisable to have two tanks in each tunnel: one aptly labeled for fertilizers that contain calcium or magnesium, and the other for those fertilizers that contain phosphorus or sulfur [24].

Where two tanks are not available, there should be a standing rule that fertilizers should not be mixed except the agronomist is present. Water-soluble fertilizers are mixed with the irrigation water depending on the fertigation program adopted.

7.3 Pruning

In greenhouse tomato production, the quality of the fruit is as important as the quantity of the yield gotten. That is, the greenhouse market prefers big, clean, and sweet fruits with higher Brix. As such, instead of allowing the growth of several branches that produces more flowers and consequently more fruits, the plant is pruned early giving fewer but bigger fruits. The tomato plants are pruned to a single stem for best production by removing all lateral shoots commonly referred to as “suckers.” Suckers are the buds that emanate from the node. Usually, one sucker will form at the inner angle of the point where the leaf petiole attaches to the main stem. If the suckers are not removed, they will grow into new stems, and produce more flowers and consequently fruits. The fruits, though plenty, will be small in size and poor in quality which is not desirable for the greenhouse market. Preferably, desuckering (which is the process of removing the suckers) is carried out to maintain one main stem. The fruits, though fewer in number, will be larger, of higher quality, and command premium prices in the market. The practice of desuckering is usually done once per week, continuously, throughout the life cycle of the plant. In the process of

Fertilizer	Abbr.	Ur	AN	AS	MAP	MKP	PN	PN+Mg	PN +P	SOP	CN	CaCl	Mg+N
Urea	(Ur)												
Ammonium nitrate	(AN)	C											
Ammonium sulfate	(AS)	C	C										
Mono-ammonium phosphate	(MAP)	C	C	C									
Mono-potassium phosphate	(MKP)	C	C	C	C								
Multi-K (potassium nitrate)	(PN)	C	C	L	C	C							
Multi-KMg (PN+Mg)		C	C	L	L	L	C						
Multi-NPK (PN +P)		C	C	C	C	C	C	X					
Potassium sulfate	(SOP)	C	C	C	C	C	C	C	C				
Calcium nitrate	(CN)	C	C	L	X	X	C	C	X	L			
Calcium Chloride (CaCl)		C	C	L	X	X	C	C	X	L	C		
Magnisal (magnesium nitrate)	(Mg+N)	C	C	C	X	X	C	C	X	C	C	C	
Magnesium sulfate (Epsom salt)	(MgS)	C	C	C	X	X	L	C	X	C	L	L	C

C – Compatible	L – Limited compatibility	X – Incompatible
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Figure 3. Water-soluble fertilizer compatibility chart [23].

removing the suckers, one or two top-most suckers at the shoot tip are left temporarily. One of them will be retained to continue the plant growth if the terminal of the main stem breaks.

7.4 Cluster thinning and fruit pruning

The purpose of fruit pruning is to increase fruit size and fruit quality and to balance fruit load. It also helps to maintain uniformity in fruit size. Distorted or undersized fruits at the end of each cluster are removed early because they are not desirable for the market and will reduce the size of the other good fruits in the cluster. Sometimes the clusters are generally pruned to the four proximal fruits. The decision to prune the clusters depends on the cultivar, that is, what is the expected fruit size and the number of fruits usually formed on a cluster of the variety. Also growing conditions and the market size preference are other factors that determine if to prune the cluster or not.

7.5 Staking and trellising

Staking is done 2–3 weeks after transplanting. For a distance of 2.5 m between the top and bottom binding wires, the twines should be cut into lengths of 3 m each. The twine is tied to the top and bottom binding wires and wound around the stem to keep the plant standing. The twine is made taut by tying it into a loop in the middle. As the plant grows taller, the loop is adjusted and wound around the newly grown shoot.

7.6 Application of fruit-setting solution

Pollination of the female flower part must occur before the fruit will set. Whatever prevents effective pollination reduces the number of fruit set per plant. Poor pollination could result in deformed fruit, smaller fruit, and fruit that is rough along the tops. Several factors, such as extreme temperatures, high humidity, drought, toxicities, nutrient deficiencies, and lack of pollen transfer, can cause poor pollination [25]. Pollination in greenhouse tomatoes is enhanced by the use of a fruit-setting solution. This operation starts when the plant starts flowering. The solution is diluted with water at 2 ml/L inside a spray bottle and sprayed directly into the flowers one by one. This is done twice weekly.

7.7 Defoliation

Defoliation is the removal of old and lower leaves. Usually, the old lower leaves are unproductive. Removing them reduces the number of sinks and allows more nutrients to be channeled to the fruits making them bigger and more qualitative. After every harvest, all the leaves below the last fruit at the lower part of the plant are cut. The lower leaves are detached up to the first fruit ground-up. A fungicide spray (copper or mancozeb preferably) should always follow every defoliation.

7.8 Cleaning of irrigation filter

The filter attached to the tank helps to sieve dirt from the irrigation water before it gets to the drip line. It is important to clean the filter frequently so that dirt will not accumulate in the drip lines.

7.9 Flushing

Occasionally, the emitters on the drip lines get blocked due to the dirt that passes through the filter. So, the end cap of the drip lines is removed and water is released to flush out the dirt every 2 weeks.

8. Environmental control

Computers are used to control environmental factors, such as temperature, relative humidity, light intensity, and CO₂ concentrations due to their capacity for automation and ease of use. They provide records of the history of the crop and its environment over time and alert operators to malfunctions in the greenhouse (greenhouse tomato production). Computers can control many mechanical devices within a greenhouse (vents, heaters, fans, evaporative pads, CO₂ burners, irrigation valves, fertilizer injectors, shade cloths, and energy-saving curtains) based on preset criteria, such as temperature, irradiance, humidity, wind, and CO₂ levels. Also, they can collect data from different sensors and process it. This capacity of the computer is called artificial intelligence. The computer uses the result to regulate the inner temperature or humidity of the greenhouse. The use of a computer to control the environmental factors makes it easier to balance plant growth [26–28]. Control of irrigation and fertilizer application regimes based on environmental conditions can also be computerized.

8.1 Relative humidity

In the greenhouse, humidity is a result of a precarious balance among the following: crop transpiration, soil evaporation, condensation on the greenhouse cover, and vapor escape due to ventilation. Vapor pressure deficit (VPD) affects transpiration and relative humidity. It changes as the ambient temperature changes. That is when there is low humidity and high temperature, the VPD increases resulting in increased stomatal resistance and consequently transpiration. Likewise, low VPD causes a reduction in plant transpiration that eventually results into dehydration, wilting, and necrosis [29, 30].

When the relative humidity is low, water is supplied by irrigation. However, high humidity encourages the proliferation of diseases. Generally, high relative humidity supports growth and enhances fruit setting, but if not managed, can cause water to condense on the leaf surface and lead to disease development [31].

There are limitations to the effectiveness of computers in controlling relative humidity. For example, humidity levels changes as vents are opened and closed to control temperature [32]. If the humidity goes higher than recommended and the temperatures remain at the normal level, the heating and ventilation systems should be adjusted to maintain acceptable levels of humidity and temperature. In glasshouses that have vents, the heating system should be turned on and the vents opened. In houses with fans, the fans should be turned on for a few minutes, and then the heater turned on to maintain air temperature. Venting for humidity control is most effective when the outside air is significantly cooler and drier than that inside the greenhouse. As the cool, dry air heats up in the tunnel, it absorbs the atmospheric moisture, which results in lower humidity. When the outside air is humid, venting can still be used to effectively control the relative humidity so far, the outside air is cooler than the inner air. However, practically, the cost of ventilation is justified only when the air outside is cooler and drier than the air inside.

8.2 Temperature control

The ambient temperature in the greenhouse is the primary environmental factor that affects plant vegetative growth, flower cluster development, fruit setting, fruit development, fruit ripening, and fruit quality. The average temperatures both day and night influence the growth of the crop. Higher temperature encourages faster growth [12]. Cuong and Munehiro [33] established that higher cumulative temperatures flowers bloom faster. Although maximum growth is known to occur at a day and night temperature of approximately 25°C, maximum fruit production is achieved with a night temperature of 18°C and a day temperature of 20°C (see **Table 1**). Hence, the recommended temperatures in **Table 1** are a compromise and are developed to sustain high fruit productivity while maintaining a modest crop growth all through the season. The use of a shade net is advised (where sophisticated means are not affordable) to reduce the direct impact of sunlight and heat in hot areas.

8.2.1 Maintaining optimal temperatures

Optimal day and night temperatures for different crop developmental stages are important. As temperatures increase within the range of 10–20°C, there is a direct linear relationship between increased growth and development. If daytime temperatures are warm, night-time temperatures can be allowed to fall to conserve energy as long as the mean temperature remains in the optimal range.

8.3 Light

Light is a prerequisite for plant growth. Photosynthesis, which produces plant matter, can only take in the presence of light. The chlorophyll present in the green parts of the plant, especially leaves, uses light energy to fix atmospheric carbon dioxide with water to produce carbohydrates. Generally, the rate of photosynthesis is related to light intensity [35]. The value of light in tomato production is seen when it is not adequate. At low light intensities, flower bud development is inhibited and flowers fail to set into fruits. This is because the plant is unable to produce adequate sugar and carbohydrates needed for bud, flower, and fruit formation from the low levels of radiant energy. Not only do the poor light conditions limit photosynthetic productivity but the limited carbohydrates produced during the day are expended by the respiring plant so that it can survive through the long nights [36]. Generally,

	Germination	Plant raising	Transplanting	harvesting	Full harvest
Temperature (°)					
Day	25	19–21	24	19	20–22
Night	25	19–21	24	19	17–19
EC (dS/m)	0.0–0.1	2.5–3.0	2.5–3.0	2.7–3.5	2.7–4.0
pH	5.8	5.8	5.8	5.8	5.8
Volume of feed (l/day)	—	0.2–0.3	0.2–0.3	0.5–1.5	0.5–2.5

Table 1.
Growing recommendations for tomato cropping [34].

increased natural light intensity benefits the tomato plants, especially when adequate water, nutrients, and carbon dioxide are made available to the plant and the air temperature is prevented from becoming too high.

8.4 Carbon dioxide

As ventilation is not needed during cold weather, a carbon dioxide concentration of 1000 ppm is recommended during the day. During summer, however, when ventilation is essential, supplementing with 400 ppm carbon dioxide is economically useful in other countries [37]. Regions with a moderate (sea) climate are more likely to benefit from carbon dioxide applied in the summer, while the procedure is uneconomical in regions that have continental climates [38].

8.5 Air movement

Horizontal air movement is beneficial for several reasons. Approximately 1 m/s airspeed, which causes leaves to move slightly, is beneficial [39]. It helps to minimize the air temperature gradients across the greenhouse by blowing the moisture under the foliage and distributing it to the other parts of the greenhouse. The motion also brings down the carbon dioxide from the top of the greenhouse into the leaf canopy where it is utilized for photosynthesis and may assist in pollination [40]. Air movement improves the uniformity of the greenhouse environment and this enhances crop productivity and energy conservation.

9. Pest and disease management

Pest and disease incidence is generally low in a greenhouse farm. The ones to watch out for are bacteria wilt, nematode, *Tuta absoluta*, thrips, mites, blossom end rot (BER), and early and late blight. Control is by integrated pest management.

The soil is sterilized to reduce the spread of soil-borne diseases (bacteria wilt and nematode), while it is eliminated in soil-less culture. The most damaging tomato disease is bacteria wilt (*Rastolnia solanacearum*). When tomatoes are grown in the soil, a combination of chemical soil treatment, soil solarization, and use of tolerant varieties are used to manage the bacteria wilt [41].

Insects such as *Tuta absoluta*, locusts, and crickets are absent in glasshouses and screened out in tunnels that are covered on the sides with a net. Sticky papers are also hung in the tunnel to trap insects. IPM program is desired for pest control.

BER is a physiological disorder that results from inadequate calcium at the blossom-end of the tomato fruit. Calcium as an immobile element in the phloem needs to be managed before the deficiency symptom becomes evident [42].

10. Harvest and storage of tomato fruits

Generally, the harvesting starts about 75 days after transplanting. There are three stages of fruit ripening (Table 2). The market patronizing greenhouse tomatoes prefers fruits harvested at the breaker stage or pink. The fruits are carefully plucked from the plant and placed in the basket. The baskets are taken to the sorting room where they are graded according to their colors. The pink fruits are labeled Grade A, while

Stage	Description
Breaker	Red stains appear on fruit skin
Pink	Tomato turns pink, not yet ready for consumption
Red	The tomato is red and completely ripe for consumption

Table 2.
Stages of fruit ripening [43].

the red ones are categorized as Grade B. Cracked tomatoes are removed and labeled Grade C after which they are all taken to the cold room where they are stored.

11. Conclusions

Tomato is a perishable vegetable fruit, which makes it difficult to preserve. Also, it is difficult to grow tomatoes in the rainy season due to the proliferation of diseases. Hence, the reason for the high market demand. Through the provision of ideal climatic conditions needed for optimal growth and possible output of any tomato variety planted, greenhouses offer a dependable alternative to growing high-quality tomatoes both in and out of season. The chapter makes it easier for a tomato farmer, an individual, or an entrepreneur who is interested in starting or expanding a tomato production firm to understand the fundamentals of greenhouse tomato production.

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Section 2

Tomato Plant Protection



Leaf Curl Disease a Significant Constraint in the Production of Tomato: Impact, Challenges, and Management

Indhravathi Chintapalli and Usha Rayalcheruvu

Abstract

Insect-borne plant viruses cause huge yield loss in the world's most important crops. Understanding viral transmission mechanisms involves defining plant virus receptors inside their insect vectors. Tomato leaf curl virus (ToLCV) is the most devastating virus for worldwide tomato production. Understanding the biology of ToLCV and devising management techniques are critical in combating this global threat. Researchers are looking into using advanced technologies to detect plant viruses quickly and handle them properly for long-term agriculture. This review's main goal is to highlight management solutions for effectively combating ToLCV outbreaks and worldwide spread. Resistance genes for plant viruses in agriculture have been identified using morphological, biochemical, and molecular markers from the ancient to the present era. Such techniques are extremely basic. Traditional virus identification methodologies should be integrated with current and advanced tools for efficient virus improvement in crops. This review's main goal is to highlight management solutions for effectively combating ToLCV outbreaks and worldwide spread. For this aim, we focus on the impact of ToLCV on the world's agriculture and the significance of recent advances in our comprehension of its interactions with its host and vector. Another important topic is the role of mutations and recombination in shaping the ToLCV genome's evolution and regional distribution.

Keywords: plant viruses, crop, yield, significant impact, challenges, molecular techniques

1. Introduction

In both tropics and subtropics of the world, tomato cultivation [*Solanum lycopersicon* L.] is significant and widespread. Tomato production has received greater attention recently because it is not only regarded as a dietary supply of the vitamins C, potassium, folate, and K, but also as a source of revenue and a significant factor in ensuring food security. China, India, the United States, Italy, Turkey, and Egypt are the world's top tomato-producing nations. The total area under

tomato cultivation worldwide is 4.582 Mha, with a yield of 150.51 mt. India is expected to have produced 21 million metric tons of tomatoes for the fiscal year 2021. India, which is second on the list of countries producing tomatoes during the measured period, accounts for 10.51 percent of the world's total tomato production cultivated 781,000 hectares or more. Production: 243,367 hg/ha. It is the second most significant vegetable. The major states in India are Andhra Pradesh, Karnataka, Orissa, Maharashtra, West Bengal, Bihar, Gujarat, Chhattisgarh, Tamil Nadu, and Jharkhand. The highest tomato producer in India is Andhra Pradesh, which produces 5962.21 thousand tons of tomatoes annually (from FY 2015 to FY 2020). In comparison to the prior fiscal year, the cultivated area increased. Tomato production has received greater attention recently, and tomatoes are often regarded as protective foods due to their high lycopene content, which aids in the prevention of various cancers. However, there are numerous obstacles to the production of tomatoes.

Plant viruses are considered to be predominantly damaging to their cultivated crop hosts' lives. In the majority of instances analyzed, virus-cultivated agricultural plant interactions have a detrimental impact on host morphology and physiology, resulting in disease [1, 2]. Viral diseases impact a lot of vegetable crops. The world's food supply is seriously threatened by crop diseases brought on by pathogenic microbes. Viruses, viroids, phytoplasma, bacteria, fungi, and nematodes are some of the pathogens that cause infectious plant diseases. Viral diseases pose a serious threat to sustainable and productive agriculture globally, causing annual economic losses. Plant pathogen infections are one of the main factors limiting crop productivity globally, and any destructive issues are caused by the wide range of viral isolates with highly variable degrees of virulence. They are immovable and often pass from one plant to another via a live thing called a vector or carrier. Since they have piercing-sucking mouthparts that enable them to reach and feed on the contents of plant cells, aphids, whiteflies, thrips, and leafhoppers are the most frequent carriers of plant viruses. Viruses can also be spread by other insects, mites, nematodes, fungi, contaminated seeds, pollen, vegetative propagation material, plant-to-plant contact, and other pests [3]. Emerging diseases, which are characterized by a rapid rise in disease incidence, geographic scope, and/or pathogenicity, have the most impact. Although the source of plant viruses is unknown, various suggestions have been put forth that suggest a possible insect vector as a possible explanation for the similarities between some plant and animal viruses. Plant viruses are challenging to control because they are widespread throughout the world and are effectively transmitted to their host plants by vectors. Although the length and specificity of the interactions between viruses and vectors vary, some recurring motifs in vector transmission have emerged: Plant viruses bind to specific sites in or on vectors and are retained there until they are transmitted to their plant hosts; viruses bind to specific sites in or on vectors and are retained there until transmission to their plant hosts; and viruses determine the virion's structural proteins, which are essential for transmission, as well as additional nonstructural helper proteins in some cases [4]. Entry, encapsulation, translation, replication, cell-to-cell movement, encapsidation, vascular transport, and plant-to-plant transmission—which can be horizontal through vectors or mechanical wounds, or vertical through seeds and pollen—are the basic steps in the successful infection of a plant by a virus. Viruses must combat host defenses such as RNA silencing and protein-mediated general and targeted immunity [5]. The majority of plant pathogenic viruses have an essential component to their infection cycle: acquisition and dissemination by an insect vector. Sap-sucking insects spread the virus in two ways: persistent transmission and nonpersistent transmission, which refer to how long it

takes an insect to acquire and transmit the virus, and circulative transmission; in some cases, it then involves virus replication in the cells of the insect host. Plant viruses can interact with their insect host in a variety of ways. Replicating viruses can also cause the insect host to mount general and targeted defenses. A recurring character is a need for specific molecular interactions between the virus and host, frequently via proteins, for the virus to interact with its insect host or carrier. By preventing virus absorption and transmission, plant protection strategies can be supported by knowledge of the interactions between plant viruses and the insects that serve as their hosts. Here, we offer a perspective centered on identifying existing and novel strategies with research directions to facilitate control of plant viruses by better understanding and focusing on virus-insect molecular interactions with these interactions in insect vectors of plant viruses, and we consider technical advancements for their control that may be more broadly applicable to plant virus vectors [6].

The increase of publications published on the topic during the past 15 years demonstrates the resurgence of interest in plant virus evolution. In the past 5 years, several new viruses have been described, some of which have novel genetic characteristics that have prompted the suggestion of the formation of new genera and the revision of the virus taxonomy status. There is a need for work aimed at understanding the processes involved in plant viral evolution, because contemporary plant virus evolution research has been regarded from a molecular, rather than populational, approach. Plant viruses create a significant amount of genetic variation that is present both within and between species using a variety of ways. Plant RNA viruses and pararetroviruses most likely have replication processes that are very error-prone, leading to a lot of mutations and a quasispecies nature. Although the origin of the diversity in the plant DNA viruses is not entirely apparent, it does exist. Recombination and reassortment are commonly used by plant viruses as evolutionary forces, as are occasionally other methods including gene duplication and hyperinflation [7].

Even though there is no proof of variation in the mutation rate, the amount of variety detected in different species of plant viruses is extremely varied. Plant viruses are thought to result in significant annual losses across the globe. Recent climate change events may have made this problem worse, and climate change will likely have an impact on how diseases spread in the future, which may affect how plant viruses spread. Increases in temperature, atmospheric carbon dioxide concentration, water availability, and the frequency of extreme weather events will all have a direct and indirect impact on plant viruses by affecting their hosts and vectors. Climate change may have an impact on plant viruses' virulence and pathogenicity, which will increase the frequency and scope of disease outbreaks. The natural defensive process of plants, known as autophagy, has become crucial in the interactions between plants and pathogens. In plants, it serves as an antiviral defense mechanism [8]. The virus alteration demonstrates how plant viruses can control, subvert, or even employ the autophagy system for pathogenicity. However, accumulating evidence from virus modification shows that: (1) high mutation rates are not necessarily adaptive, as a significant portion of the mutations is deleterious or lethal; (2) despite having a high potential for genetic variation, populations of plant viruses are not highly variable, and genetic stability is the norm rather than the exception; and (3) the degree of genetic variation constriction in virus-encoded proteins is comparable to that in their eukaryotic hosts and vectors [9].

Although it is difficult to trace the evolutionary history of viruses and practically impossible to regulate virus disease over the long term, their propensity for fast adaptation makes them a great model system for research on the broad mechanisms

underlying molecular evolution. More exemplary research was done in the second half of the twentieth century, demonstrating the infectiousness of RNA alone, (ii) the resolution of RNA-protein interactions in the structure determined by X-ray fiber diffraction, (iii) the existence of a distinct region on the virus for the start of encapsidation, (iv) the definition of the virus sequence and open reading frames (ORFs); (v) open reading frames and the definition of the viral sequence (ORFs); and (vi) cDNA clone that is biologically active [10]. This substantially contributed to our comprehension of reproduction and transmission, resulting in a new understanding of viruses among scientists in a subsequent generation. In addition to improving our knowledge of the local ecology and fitness of mechanically transmitted viruses, this upcoming research must expand our understanding of virus structure and transporters of small molecules. The process of developing effective host-virus interactions, including how different species move through a vector in different ways. The top nine virus list is shown in **Table 1**, in descending order [19].

1.1 Viruses, crops affected, and damage caused

Hence, there is an urgent need to improve its productivity with the help of modern technological implementation to shield the tomato plants against various biotic and abiotic factors.

One of the main biotic limitations is virus-associated. One of the most significant factors restricting its cultivation and productivity is tomato leaf curl disease (ToLCD). It frequently suffers from a range of infections, which causes significant yield losses. Infections caused by fungi, bacteria, and phytoplasma are only a few of the many viral diseases that affect it. The most significant and damaging viral pathogen in many regions of the world is the tomato leaf curl virus (ToLCV), a geminivirus, which is responsible for all documented viral diseases in tomatoes [20–27]. Based on the genome organization, host range, phylogenetic relationships, and insect vectors, geminiviruses have been classified into nine genera: Becurtovirus (two species), Begomovirus (>320 species), Curtovirus (three species), Mastrevirus (>30 species), Eragrovirus (one species), Topocovirus (one species), and Turncurtovirus (one species). Begomovirus is the largest genus in the Geminiviridae family, and it contains multiple notable species, including ToLCV, which infects tomato cultures in Asia and Australia. ToLCV is the name given to a group of vector-transmitted geminivirus genus [28]. Geminiviruses are made up of one or two circular ssDNA genomic components of 2500–3000 nucleotides encapsulated in paired icosahedra or geminate particles (called geminiviruses), as we know them now. Inside the host cell, their ssDNA genome is converted into a dsDNA intermediate and rapidly replicated (**Figure 1**). The introduction of high-yielding tomato varieties has been accompanied by ToLCV infection [29–31]. In India, in Andhra Pradesh, the disease is widespread in tomatoes during the summer season in southern parts and autumn in northern parts and causes yield losses ranging from 27 to 100% [32–34].

1.2 Genome organization

Plant viruses belonging to the Geminiviridae family have circular genomes made of single-stranded (ss) DNA, which encodes their genetic material. The genomes of all geminiviruses are identical, as is DNA-A, the section of bipartite begomoviruses that encodes the proteins necessary for replication, control of gene expression, getting past host defenses, encapsidation, and insect transmission. Two proteins that enable

S. No	Virus	Plant	Host range	Yield influence	Economic loss world wide	References
1.	Tobacco mosaic virus	Tobacco, pepper, cucumber, and ornamental crops	Tobacco, vegetables (especially in the Solanaceae family), and many ornamental species	Plant height, leaf length and width, and fresh and cured leaf yield are all factors to consider.	90%	[11]
2.	Tomato spotted wilt virus	Begonia, cowpea, impatiens, peanut, pepper, potato, squash, and tomato.	Monocots and dicots	Fruits' fresh weight, width, length, and marketability were all documented.	100%	[12]
3.	Tomato leaf curl virus	Vegetable crops, ornamental, and fruit crops	Solanaceae plants	Plant leaf number and area, biomass, height, root length, and stem diameter and yield are all factors to consider.	100%	[13]
4.	Cucumber mosaic virus	Carrot, celery, cucurbits, legumes, lettuce, onion, pepper, spinach, tomato, and rarely potato	Vegetable, woody and non-woody, and ornamentals plants	The US Department of Agriculture's Plant Yield and Nutritional Outlook Survey (PYTH) for 2014/15 shows an 89.89% drop in yield per plant compared to last year. The study also shows a decrease in plant height, root length and fresh plant weight.	60%	[14]
5.	Cauliflower mosaic virus	Brassicaceae (or mustard) family	Brassicaceae crops and solanaceous plants	Low seed yields. Lower temperature, plant development, especially in early infections, and the production of flowers can be blocked.	60%	[15]
6.	Cassava mosaic virus	Euphorbiaceae (for example, wild poinsettia and garden spurge)	Cassava (Manihotesculenta) and castor bean (Ricinuscommunis)	Height, stem diameter, leaf square, and yield of the plant	77.5% to 97.3%	[4]
7.	Plum pox virus	Peaches, apricots, plums, nectarine, almonds, and sweet and tart cherries.	Stone fruits	Fruit size and weight, pH, titratable acidity, total soluble solids, flesh hardness, and fruit skin color are all factors to consider.	80–100%	[16]
8.	Brome mosaic virus	Soybean and barley	Dicotyledonous and monocotyledonous plants	Plant height, stand establishment, grain production, and many yield components are all factors to consider.	7–10%	[17]

S. No	Virus	Plant	Host range	Yield influence	Economic loss world wide	References
9	Potato virus	Potato (<i>Solanum tuberosum</i>), tobacco (<i>Nicotianatabacum</i>), tomato (<i>Solanum lycopersicum</i>), and pepper (<i>Capsicum</i> spp.)	Solanaceae	Total yield, commercial and noncommercial yield, number of stems, number of tubers, and tuber weight	10–40%	[18]

The top nine list includes, in rank order, (1) tobacco mosaic virus, (2) tomato spotted wilt virus, (3) tomato leaf curl virus, (4) cucumber mosaic virus, (5) cauliflower mosaic virus, (6) African cassava mosaic virus, (7) plum pox virus, (8) Brome mosaic virus, and (9) potato virus X.

Table 1.

The top 10 viruses list in rank order.

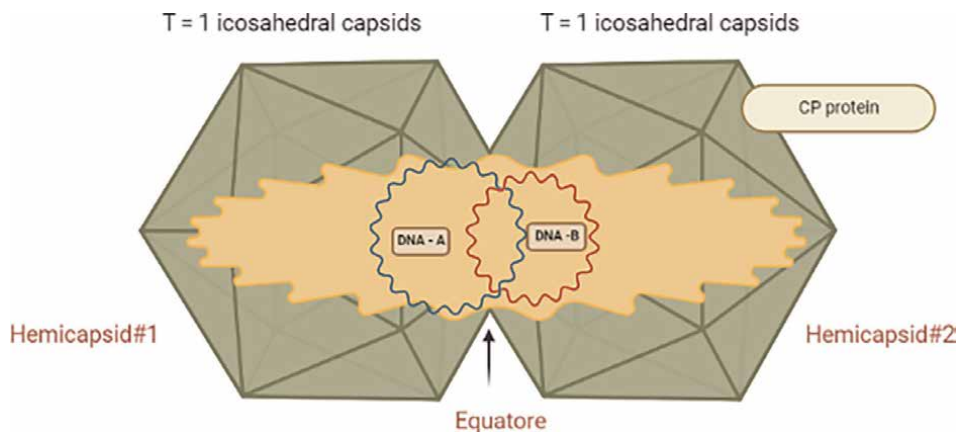


Figure 1.
Å structure of a plant geminivirus—Nanoviruses have T = 1 icosahedral capsids of ~18 nm in diameter.

intracellular and intercellular movement in host plants are encoded by the second component, DNA-B. The unknown is the origin of the DNA-B component. This work aimed to investigate the relationship between the bipartite begomovirus DNA-A and DNA-B components to unravel their evolutionary histories and gain insight into the potential origins of the DNA-B component [35].

One of the genomes recognized are begomovirus species, and they have all Old World origins (OW). Monopartite viruses have only one molecule of nucleic acid [36]. The majority of dsDNA viruses exist in monopartite forms. It has been demonstrated that monopartite begomoviruses interact with betasatellites, which are ssDNA satellites. In contrast to monopartite begomoviruses, which are phloem confined and only cause stunting and leaf curl symptoms, several bipartite begomoviruses infect both phloem and other tissues, causing leaf curling, crumpling, and mosaic/mottling symptoms, and are sap transmissible [37–40].

1.2.1 Gene functions

The six ORFs that make up DNA-genome A's are used to encode the signals for the six most significant viral proteins, which range in size from 11 to 40 kDa. Both viruses detect ORF. Monopartite begomoviruses have complementary-sense ORFs (C1–C4) and virion-sense ORFs (V1 and V2), respectively. Together with the V2 protein, the virion-sense coat protein (CP) promotes viral mobility in plants and is responsible for insect transmission. The sole viral protein necessary for viral DNA replication is the geminiviral replication-associated protein (Rep). The ToLCV intergenic region has a 120-bp segment that Rep particularly binds to. Two significant movement proteins, BV1 and BC1, are encoded by DNA-B and are in charge of long-distance mobility between cells. Globally, both economically significant and weed crops suffer enormous economic losses due to begomoviruses and their satellites. Monopartite begomoviruses typically develop a virus/satellite complex with a betasatellite and cause severe leaf curl in tomatoes. Betasatellites linked with geminivirus play a variety of roles in pathogenesis [41]. In India, the papaya leaf curl virus (PaLCuV) associated with aster betasatellite yellow vein disease was found. In Pakistan, PeLCV infection of *Pedilanthus tithymaloides* plants was connected to the betasatellite for tobacco leaf curl [42, 43]. Recently, the alphasatellite, a 1.4-kb additional satellite DNA, has been

connected to the tomato leaf curl virus (TYLCV). Although they multiply autonomously inside their hosts, they depend on the helper virus for encapsulation, movement inside the plant, and vector transmission. They can multiply independently within their hosts, but they, like betasatellites, depend on the helper virus for encapsulation, mobility inside the plant, and vector transmission. The earliest satellites discovered in relationship with the ToLCV were deltasatellites, an Australian example (ToLCV). Previously known as ToLCV-Sat, is currently called tomato leaf curl deltasatellite (ToLCD). The ToLCD (682 nt long) and the helper virus have no significant sequence similarities other than the origin of replication [44].

1.2.2 Geminiviruses replication

Geminiviruses employ their insect vectors to transfer their encapsidated DNA to plants. Due to the lack of a model system for eukaryotic replication, details of geminivirus replication remain unknown. Other research indicates that the replication initiator protein (Rep) is the viral protein most crucial to viral DNA replication. Yellow mungbean mosaic mutations in CR, Rep, AC5, and other replication-related components and viral proteins, as well as a lack of host factors, inhibited the replication of the India virus. When injected into protoplasts, DNA-A can replicate itself and contain the genes required for viral replication [45]. In mature plant cells, geminiviruses promote the expansion of the DNA replication apparatus to create a favorable environment for replication. On the other hand, a lot is still unknown about the process of C4-induced cell division. The physiological advantage of promoting cell division might produce a situation where DNA viruses can multiply easily. There is proof that ToLCV can be replicated within the vector, that is, whiteflies. A fivefold increase in viral accumulation was observed in the early stages of the insect's life cycle, followed by a decline in the later stages, according to the first study to demonstrate this phenomenon, which was published in 2015.

V1, V2, and C3 displayed increased expression after nonviruliferous flies fed on TYLCV-infected tomato plants for 1 to 3 days. The midgut epithelial cells were also found to have complementary viral DNA, a sign of viruses that replicate [5, 46]. This suggests that these cells served as viral replication hubs. Another study found that insects that consumed diseased tomato plants accumulated 10 times more viral gene transcripts. In the later stages of the investigation, the reduction in virus titers was also attributed to autophagy [47]. Contrarily, experimental evidence supports the notion that TYLCV cannot replicate within its vector. The quantity of viral transcripts increases only during the acquisition phase and thereafter remains constant, according to other research, indicating that replication is not occurring [48, 49].

Many DNA viruses depend on their hosts' transcription and replication systems. Geminiviruses only employ a small number of proteins, and they rely on host enzymes to carry out their functions. These genes are transcribed by begomoviruses using their bidirectional promoters. It produces several overlapping RNA species, some of which are polycistronic. The TATA box initiates transcription, and the RNA produced by the virus is polyadenylated, showing that the host transcription machinery aids in the geminivirus' transcription. One complementary-sense transcript (BC1) is produced for DNA-B.

1.3 Tomato leaf curl virus

The Food and Agriculture Organization of the United Nations estimates that the tomato is the most widely grown tomato crop in the world, producing about 180

million tons annually [50]. Growing for the processing industry accounts for one-fourth of the 160 million tons. The annual processing capacity of factories operated by significant food corporations is about 39 million tons of tomatoes. In all tropical locations of the world, ToLCV is one of the most virulent viruses that can cause catastrophic illnesses in vegetable crops [51]. Since ToLCV was originally identified in solanaceous crops, numerous instances of harm to other crops have surfaced all across the world (**Table 2**). It has a devastating impact on the development and production of several plant groups with significant agricultural economic importance. In 1948, Vasudeva and Samraj reported the first ToLCV case in India. ToLCNDV has expanded to other vegetable and fiber crops, according to several studies from the last 10 years [52]. Tomato leaf curl New Delhi virus with mosaic and leaf curl disease has just been discovered in chrysanthemum. This virus is attributed to diseases in a wide variety of plant species, including fruit crops and ornamental plants [53]. These begomoviruses may act as reservoirs for crops that are crucial to the economy [2, 11, 50]. Because it causes one of the most prevalent and economically significant tomato diseases in the world, ToLCNDV significantly hinders tomato output in general [54]. As it has spread across the Indian subcontinent, ToLCNDV's host range has significantly increased. India and Pakistan have reported cases of ToLCV linked to cotton leaf curl disease between 2013 and 2015 [55, 56]. The insects harm the plant directly by sucking phloem sap (**Figure 2**), which stunts growth, causes early wilting, premature defoliation, and ultimately results in yield loss. They also damage the plant indirectly by excreting honeydew, which promotes the growth of fungi on the surfaces of leaves and fruit. When a peach plant was infected with the leaf curl virus, the leaves had very little chlorophyll and performed little or no photosynthesis. Previous research has suggested that ToLCV causes increased reactive oxygen species (ROS) production in tomato leaves infected with it [57].

1.4 Occurrence and yield loss

According to geographic distribution, the ToLCD is expanding quickly. It affects tomatoes and significantly reduces agricultural yields in the Southeast United States and around the world [58]. If infected, susceptible tomato types could lose up to 100% of their production (142,251). The tobacco leaf curl virus on tomato first appeared naturally in India in 1942 according to Pruthi and Samuel, who were followed by Vasudeva and Samraj in 1948. Later, Andhra Pradesh, Hyderabad [59, 60], and Tamil Nadu [61] first reported the detailed characterization of ToLCV from Karnataka by Govindu and his coworkers in 1964. The prevalence of ToLCD in South India rapidly increases from 27 to 90 percent in susceptible cultivars, leading to yield losses of up to 90 percent [62]. The virus's extreme invasiveness and a dearth of efficient control methods allowed it to spread globally and cause a serious pandemic. Nine different ToLCV isolates have been found in India [11]. In the United States, the TYLCV-like ToLCV has been identified [63, 64]. The tomato leaf curl viral illness, which has a significantly high disease incidence in both the Rabi and Summer seasons, 96.80 percent and 98.43 percent, respectively, according to Khandare et al. [65], produces severe leaf curl disease. However, the first reports of tomato yellow leaf curl disease and the connection between radish leaf curl virus (RaLCV) isolates and a tobacco disease were both made in 2012 by Singh and his colleagues [66]. The majority of Indian isolates of ToLCVs are caused by a monopartite tomato leaf curl Joydebpur virus (ToLCJoV) that causes severe leaf curl. Numerous plants, including natural fibers and chillies, have been discovered to be infected by monopartite ToLCJoVs [67].

S. No.	Name of country	Name of the crop
1	Algeria	Tomato leaf curl New Delhi virus infecting cucurbit
2	Antigua and Barbuda	Tomato yellow leaf curl virus (TYLCV)
3	Argentina	Tomato rugose yellow leaf curl virus (TRYLCV)
4	Australia	Tomato leaf curl virus (TLCV)
5	Australia	Tomato yellow leaf curl virus (TYLCV)
6	France	Tomato leaf curl New Delhi virus infected in Cucurbita pepo (field pumpkin)
7	Azerbaijan	Tomato yellow leaf curl virus
8	Bangladesh	Tomato leaf curl New Delhi virus
9	Barbados	Tomato yellow leaf curl virus
10	Belize	Tomato mosaic leaf curl virus in pepper, red kidney bean, squash, string bean, and tomato
11	Benin	Tomato yellow leaf curl virus
12	Bolivia	Whitefly (tomato leaf curl virus vector) was detected
13	Brazil	Tomato mottle leaf curl virus
14	Burkina Faso	Tomato leaf curl disease
15	Burma	Tomato yellow leaf curl virus
16	Burundi	Tomato yellow leaf curl virus (TYLCV) and tomato leaf curl virus (ToLCV)
17	Cambodia	Tomato yellow leaf curl Kanchanaburi virus
18	Cameroon	Tomato leaf curl Cameroon virus (ToLCCMV)
19	Guatemala	Tomato yellow leaf curl virus infecting tomato, tomatillo, and peppers
20	Chad	Cotton leaf curl Gezira virus (CLCuGV)
21	Chile	Tomato yellow leaf curl disease (TYLCD)
22	China	Tomato yellow leaf curl virus
23	Colombia	Begomovirus identified
24	Comoros Archipelago	Tobacco leaf curl Zimbabwe virus
25	Costa Rica	Tomato yellow leaf curl virus in Tomato
26	Cote d'Ivoire	Okra leaf curl disease (OLCD) Cotton leaf curl Gezira virus (CLCuGeV)
27	Cuba	Tomato yellow leaf curl virus infecting pepper plants
28	Cyprus	Tomato yellow leaf curl virus (TYLCV)
29	Dominican	Tomato yellow leaf curl virus
30	Ecuador	Cabbage leaf curl virus infecting common bean, cowpea, pigeon pea, and Mucuna pruriens
31	Egypt	Squash leaf curl virus to Squash (Cucurbita pepo)
32	El Salvador	Tomato dwarf leaf curl virus Tomato saver leaf curl virus

S. No.	Name of country	Name of the crop
33	Estonia	Tomato leaf curl New Delhi virus (Begomovirus, ToLCNDV—EPPA Alert List)
34	Ethiopia	Tomato yellow leaf curl virus
35	France	Tomato leaf curl New Delhi virus
36	Georgia	Cabbage leaf curl virus
37	Ghana	Tomato leaf curl Ghana virus
38	Greece	Tomato leaf curl New Delhi virus
39	Grenada	Tomato yellow leaf curl virus
40	Guatemala	Tomato yellow leaf curl virus infecting tomato, tomatillo, and peppers
41	Haiti	TYLCV-Is was unintentionally identified
42	Hawaii	Tomato yellow leaf curl virus
43	India	Tomato leaf curl virus
44	Indonesia	Pepper yellow leaf curl Indonesia virus
45	Iran	Alfalfa leaf curl virus from alfalfa
46	Iraq	Tomato yellow leaf curl virus (TYLCV)
47	Israel	Tomato yellow leaf curl virus
48	Italy	Tomato yellow leaf curl virus
49	Jamaica	Tomato dwarf leaf curl virus
50	Japan	Tobacco leaf curl Japan virus
51	Jordan	Alfalfa leaf curl virus affecting alfalfa (<i>Medicago sativa</i>) in Jordan, Lebanon, Syria, and Tunisia
52	Yugoslavia	Pelargonium leaf curl virus
53	Korea	Papaya leaf curl virus
54	Kuwait	Tomato yellow leaf curl virus
55	Laos	Tomato yellow leaf curl Kanchanaburi virus infecting eggplant
56	Lebanon	First report of squash leaf curl virus in cucurbits
57	Libya	Tomato yellow leaf curl virus
58	Malaysia	Pepper vein yellows virus and pepper yellow leaf curl virus infecting chilli pepper (<i>Capsicum annum</i>)
59	Mali	Tomato leaf curl Mali virus and tomato yellow leaf crumple virus
60	Mauritius	Tomato yellow leaf curl virus in tomato
61	Mauritania	Tomato yellow leaf curl virus
62	México	Tomato yellow leaf curl virus
63	Mongolia	Tomato yellow leaf curl virus
64	Morocco	Tomato yellow leaf curl virus
65	Mozambique	Tomato curly stunt virus, a new begomovirus of tomato within the tomato yellow leaf curl virus
66	Namibia	Tomato leaf curl Kunene virus

S. No.	Name of country	Name of the crop
67	Nepal	Tomato leaf curl betasatellite infecting <i>Carica papaya</i>
68	Netherlands	Tomato yellow leaf curl virus in tomato
69	Nicaragua	Tomato severe leaf curl virus from Nicaragua
70	Nigeria	Tomato leaf curl disease
71	Oman	Tomato leaf curl Albatinah virus
72	Pakistan	Tomato leaf curl Palampur virus on Bitter Gourd
73	Panama	Tomato leaf curl Sinaloa virus infecting tomato crops
74	Philippines	Whitefly-transmissible geminiviruses-bgomovirus DNA fragments were detected
75	Piedmont-Sardinia	Tomato yellow leaf curl Sardinia virus
76	Portugal	Tomato yellow leaf curl virus
77	Saudi-Arabia	Tomato yellow leaf curl virus (TYLCV)
78	Senegal	Tomato yellow leaf curl
79	Seychelles	Tomato leaf curl virus
80	Somalia	Tomato leaf curl
81	South Africa	Tomato yellow leaf curl virus
82	Sudan	Tomato leaf curl Sudan virus
83	Spain	Tomato yellow leaf curl virus
84	Sri Lanka	Tomato yellow leaf curl virus
85	Syria	Alfalfa leaf curl virus affecting alfalfa (<i>Medicago sativa</i>)
86	Tanzania	Tomato yellow leaf curl Tanzania virus
87	Uganda	Sweet potato leaf curl Uganda virus (SPLCUV)
88	Saudi Arabia	Tomato yellow leaf curl virus [TYLCV]
89	Venezuela	Tomato yellow leaf curl virus
90	Vietnam	Corchorus yellow vein Vietnam virus (CoYVV)
91	Yemen	Tomato leaf curl Sudan virus
92	Zambia	Tobacco with leaf curling symptoms
93	Zimbabwe	Tobacco leaf curl Zimbabwe virus

Table 2.

There were reports of huge crop devastation all across the world due to ToLCV.

Tomato leaf curl Palampur virus (ToLCPaV) strains/variants' importance is an increasing hazard to cucurbit output in India. Because of the recombination breakpoint of the viral genome, these valuable crops are now being destroyed and impacted by ToLCV. ToLCV has emerged as a major limiting factor and problem for farmers and scientists alike, with a special focus on management initiatives for preventing the spread of ToLCD. Because of the economic importance of LCV, efforts have been made to understand LCV pathophysiology and generate tolerant plants using breeding and transgenic techniques [61].

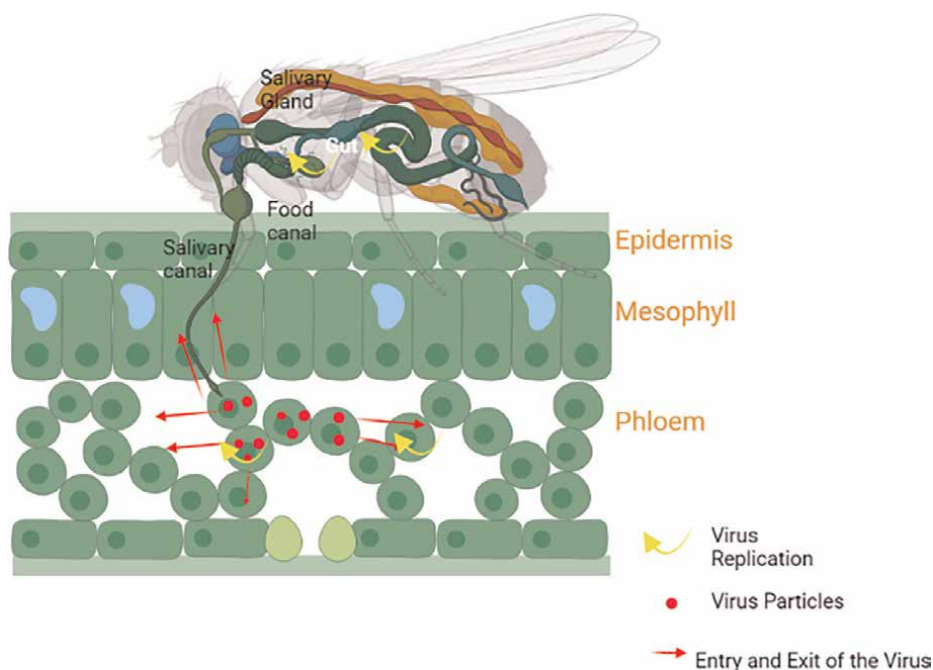


Figure 2. Schematic representation of *Bemisia tabaci* [insect vector]-mediated transmission of plant viruses in leaf—Spreads begomoviruses by sucking the sap from the phloem of leaf curl virus-infected plants (red geminate particles). As a result of the infection, the plants show characteristic begomovirus symptoms such as vein yellowing, foliar yellow mosaics, and leaf curling.

1.5 Geographical distribution

Cucumber mosaic virus (CMV), tomato spotted wilt virus (TSWV), tomato aspermy virus (TAV), tobacco mosaic virus (TMV), tomato bushy stunt virus (TBSV), potato Y virus (PVY), and ToLCV are among the viruses that infect tomatoes [68–70]. Additionally, mixed infections frequently contain these viruses [71]. In the southern region of India, a monopartite ToLCV is the most prevalent of the various ToLCVs [72]. There are several species of begomoviruses, viz. bipartite tomato leaf curl Palampur virus (ToLCPaV) and tomato leaf curl New Delhi virus (ToLCNDV), monopartite tomato leaf curl Kerala virus (ToLCKeV), tomato leaf curl Patna virus (ToLCPaV), tomato leaf curl Ranchi virus (ToLCRnV), tomato leaf curl Rajasthan virus (ToLCRaV), tomato leaf curl Pune virus (ToLCPV), tomato leaf curl Bangalore virus (ToLCBaV), tomato leaf curl Lucknow virus (ToLCLuV), tomato leaf curl Karnataka virus (ToLCKaV), tomato leaf curl Gujarat virus (ToLCGuV), and tomato leaf curl Joydebpur virus (ToLCJoV). The begomovirus, ToLCGuV, that exists in both mono and bipartite forms has been reported from Varanasi [73]. Both of these categories are common in India [74, 75]. ToLCNDV is a rare Old World bipartite begomovirus. It is ubiquitous on the Indian subcontinent but found elsewhere in the Far East, Middle East, North Africa, and Europe [76, 77]. According to Sahu et al., the occurrence of Guar leaf curl alphasatellite (GLCuA) could be attributed to whitefly migration from Pakistan [78, 79]. ToLCV has been linked to infections in a variety of hosts between 2000 and 2010 [80]. Only the DNA-A component appears to be present in the monopartite genomes of the tomato leaf curl Bangalore virus from Bangalore

[81, 82] and tomato leaf curl Karnataka virus from Karnataka [83]. The PaLCuV has been found in many different crops across the globe [84]. Numerous begomoviruses may pick papaya plants to survive in a variety of uncertain environments. The presence of chili leaf curl virus and its related tomato leaf curl betasatellite in the *Cucurbita maxima* host reveals the virus's potential harm to this crop [85]. There is a chance that this virus complex could speed up the spread to other crops. There is a risk that the virus could spread to other countries via air, sea, and possibly through borders, particularly among countries that share borders. It is critical to avoid any chance of the virus spreading to new hosts in other nations by enacting effective quarantine legislation.

1.6 Virus-vector interaction

There are often two components to a vector-transmitted pathogen: host-pathogen interaction and vector-virus contact (**Figure 3**). In the past, research on plant-virus interactions has focused on viral mobility, replication, symptom development, and the plant's reaction to infection. Vector-virus interaction, on the other hand, has been an insufficient investigation.

One of the most invasive creatures is the Bemisia tabaci [Hemiptera: Aleyrodidae], often known as the whitefly as a vector for ToLCV. It has been ranked among the top 100 worst invasive alien species in the world. It is a cryptic species that includes at least 39 Hemiptera species and is found naturally throughout the world's tropical and subtropical regions [86]. In the last 20 years, *B. tabaci* has spread around the globe, likely as a result of the transportation of agricultural products [87]. It has become one of the most destructive agricultural pests. The bug damages the plant directly by sucking phloem sap, which results in stunted development, early wilting, premature defoliation, and ultimately a loss of production, as well as indirectly by excreting

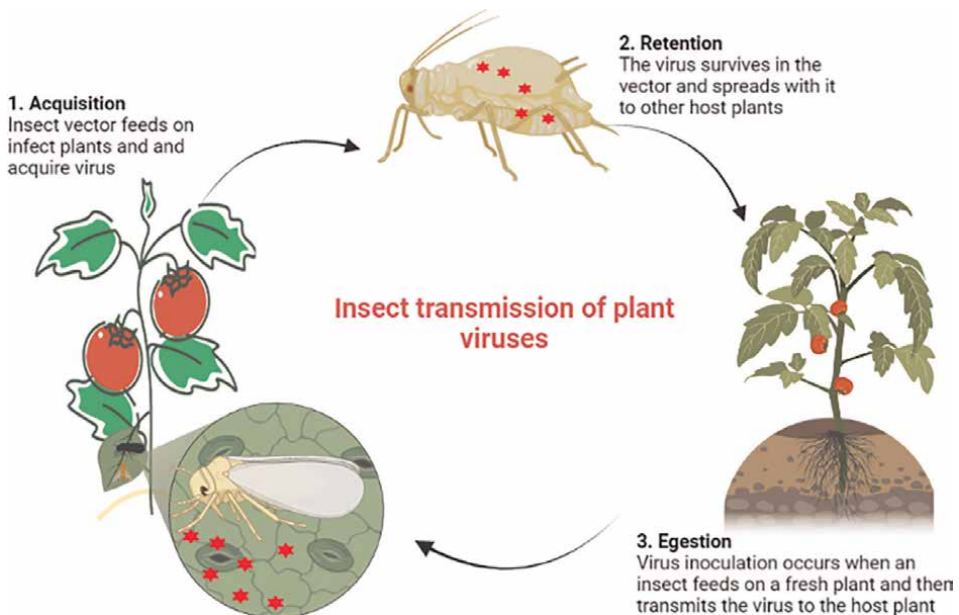


Figure 3.

The infection cycle starts when the vector comes into contact with the virus in the plant and acquires it. The virus must then survive long enough in or on the vector to be transmitted to a new host and released into the plant cell.

honeydew, which encourages fungal growth on the surfaces of leaves and fruits [88]. Over 600 plant species are targeted by Bemisia tabaci, and viruliferous whiteflies creating a feeding site directly contributes to leaf curl virus transmission to plant hosts [89]. Alfalfa leaf curl virus (ALCV), which is spread by Aphis craccivora [90], is one of two Capulavirus species that are spread by aphids. Aphid transmission was only found in 2015, even though the Aphididae family contains most species described as plant virus vectors [91, 92]. An unclassified geminivirus from the genus Capulavirus can spread ALCV [93]. Being polyphagous explains B. tabaci wide host range and ability to spread a lot of viruses to more than 300 plant species from 63 families [94]. Forty-nine begomovirus species have been related to ToLCD, while 17 have been linked to TYLCD [95, 96]. The two primary tomato geminiviruses that infect tomatoes and significantly reduce output are ToLCV and TYLCV [97].

The key features of ToLCV acquisition, retention, and egestion by the vector have been the focus of investigations into how viruses interact with their host's machinery fluids. The maxillary stylet is made up of three stylets: a mandibular stylet and the salivary canal. Individual whiteflies may acquire varying amounts of viral particles in their exoskeletons even when fed on the same tissue for the same significant period. In insects, the precibarium and cibarium taste organs control whether virus particles move through circulatory or noncirculatory channels. Circulating persistent viruses circulate and stay in the body of the insect for the majority of its life, whereas noncirculative viruses never breach the gut barrier [77]. Any alteration to the protein that supports virus retention and transmission (CP) interferes with both processes [98]. ToLCV uses endosymbionts from whiteflies that release proteins to stop damage in the open circulatory system. ToLCV's CP interacts with GroEL, a molecular chaperone, and is shielded from deterioration. It has been demonstrated that Hsp70 interacts with CP and blocks transmission. The continuous cycle between the host and vector due to their circulative pathway affects prospective agricultural output all over the world. ToLCV particles enter the salivary glands of viruliferous flies while they feed on phloem sap and are then discharged into plants [99].

1.7 Plant-virus interaction

Geminiviruses are intracellular parasites that must successfully influence plant cell activities to multiply, block antiviral defenses, and spread throughout the plant [44, 100]. They inject viral DNA into the host cell during infection, disrupting the host gene silencing system. During viral infection, the production of ROS in the host cells is raised to prevent systemic virus migration up to specific cells [63]. To counteract the self-damage produced by ROS, the host creates glutathione peroxidases (GPXs), which lower ROS levels in the cells. The competence of a virus to co-opt and alter processes in a particular host plant will influence how the virus-plant relationship turns out. To hijack the molecular machinery of the host cell, geminiviruses produce a small number (between 4 and 8) of tiny, rapid evolving, multifunctional proteins, encoded by bidirectional and partially overlapping ORFs [101]. ToLCV is a whitefly-transmitted vector-borne disease (**Figure 4**), and this is the first time ToLCNDV has been identified as a seed-transmissible virus in zucchini squash plants in Italy. The leaf curl virus was found in early seedlings sprouted organically from fallen fruits [18]. After being injected into the host, virus particles multiply and travel to other areas of the body, causing symptoms [102]. Geminiviruses manipulate E2F transcription factor activity to produce an S-phase environment [103]. Beet curly top virus (BCTV) infection causes cell expansion (hypertrophy) and division (hyperplasia) [104].

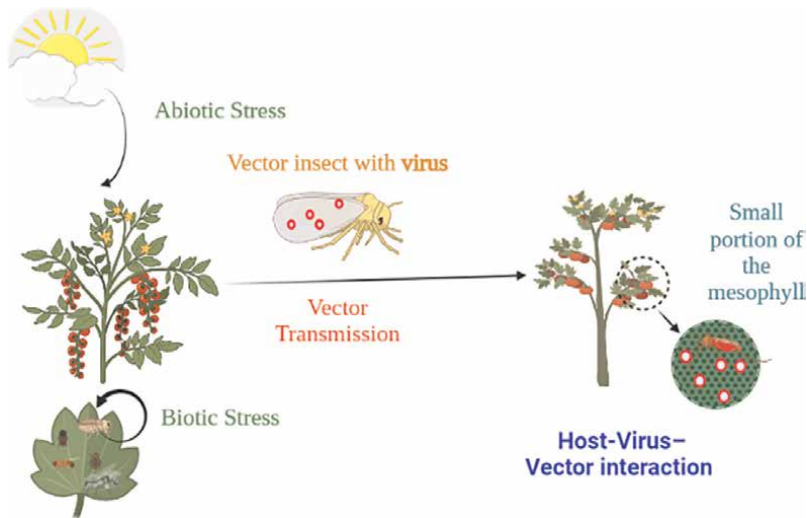


Figure 4. *Plant-pathogen interactions-global environmental change, study the environmental parameters (biotic and abiotic) influencing the biology of plant viruses and their transmission by vectors.*

DNA virus replication in differentiated plant cells requires the induction of cell division. To achieve efficient replication, DNA viruses encode the C4 protein, which encourages cell proliferation. The viral protein may interact with yeast and alter its growth and development, since the expression of BCTV C4 caused a 100-fold decrease in transformation efficiency [105, 106]. The glycogen synthesis kinase-3 (GSK3) homolog in *N. benthamiana*, NbSK, is hijacked by the C4 protein of the tomato leaf curl Yunnan virus (TLCYNV). According to Mei and his coworkers in 2018 [107], SK is a target of geminivirus C4, and activation of cell division is necessary for DNA virus replication in differentiated plant cells. According to Chandan et al., infected tomato plants exhibited increased expression of LeCTR1, one of the ethylene signaling pathway's negative regulators. LeCTR1 gene silencing caused by the tobacco rattle virus (TRV) increased ToLCJov infection tolerance [101].

1.8 ToLCV recombination, mutation, and evolution

Begomovirus disease complexes are quickly developing to increase their host range and overcome resistance sources through recombination, component capture, and mutations.

1.8.1 Mutation

Genetic variation in the tomato yellow leaf curl Sardinia virus [TYLCSV] is a result of interspecific recombination as well as mutation, natural selection, and genetic drift [108]. RNA viruses have a high mutation rate as a result of error-prone replication mediated by viral RNA-dependent RNA polymerases (RDRP). Recombination that occurs during mixed infections and mutations is what drives viral evolution. DNA viruses like TYLCV, whose replication is facilitated by plant DNA polymerases, should have modest mutation rates as a result of proofreading activity [109]. However,

studies disagree with these assumptions, and considerable variability has been reported. Serious outbreaks have been triggered by recombinant TYLCVs with resistance-breaking capabilities [110, 111].

1.8.2 Recombination and evolution

Mixed infections and high viral replication levels are two conditions that may promote recombination [103]. As recombination hotspots, the IR, V1, V2, and C1 regions of the genome have been identified [112–118]. Studies from the Punjab regions of Pakistan and India recently suggest that CLCuMuV has reemerged in the Indian subcontinent, which is consistent with observations from earlier studies. Its dominance and frequent emergence in North Indian regions have been demonstrated by recent studies [55]. Researchers examined and studied recombination events in the viruses to see if they had any consequence on viral transmission due to the high diversity of begomoviruses in North India. Geminiviruses have evolved and appeared as a result of recombination. The Cotton Leaf Curl Multan betasatellite [CLCuMBC1]'s satellite conserved region (SCR) and A-rich portions were particularly prone to recombination [67]. Additionally, recombination was found in the Rep and A-rich regions of the GLCuA, supporting earlier findings that suggested these sites were recombination hotspots [11]. As shown by DNA-A components of ToLCNDV isolates [119], recombinations involving genomic sequences from other begomoviruses or sequences of unknown origin are frequent in this genus.

Recombination happened in 458 occurrences among begomoviruses related to cotton leaf curl disease during 459 mixed infections [120]. If these viruses travel to other locations, 460, where they are common, new viruses may emerge [4]. The Recombination Detection Program version 4 [RDP4] was used to identify possible recombination events in sequences [121]. SeqMan and GenBankBLASTn were used to put together sequence reads (**Table 3**). MEGA7 was used to perform pairwise nucleotide sequence analysis and build phylogenetic trees [139]. This sequence analysis is useful to determine the threshold level at 91 percent sequence identified demarcation for begomovirus classification that has been set [140].

2. Plant viruses management and detection

Human activity is creating conditions that encourage the spread of begomoviruses. To avoid ToLCV outbreaks and agricultural losses, administrative, legal, and technological procedures should be implemented. Insect vector biological management may also be explored to minimize insect vector infestation and disease dissemination. We discuss recent developments in the identification, characterization, and detection of plant viruses and virus-like compounds using nano-coupled molecular method approaches in this review article [141]. The objective of this essay was to accurately identify the most significant plant viruses as reported by Molecular Techniques contributors. We are well aware that between disciplines and regions, importance and priority might differ locally (**Table 4**). In the past, biological assays were a crucial tool for determining a plant's health condition as well as for the identification and characterization of a specific virus. The labor- and time-intensive biological indexing has few practical uses nowadays, though it is still important for plant virus research. Instead, molecular diagnostics is the primary method used for the precise and sensitive detection of the majority of viruses and virus-like diseases [151]. Some recent developments

Genus	Species	Host	Genome	Genbank Accession Number	Reference
Alfavirus	Alfalfa mosaic virus	Potato (<i>Solanum Tuberosum</i>), pea (<i>Pisum Sativum</i>), tobacco (<i>Nicotiana Tabacum</i>), tomato (<i>Lycopersicon Esculentum</i>), and bluebeard (<i>Caryopteris Incana</i>)	(+)Ssma-9.1 Kb	LC485016 To LC485018	[12]
	Alphaendomavirus BPEV (bell pepper alphaendomavirus)	Capsicum annuum	Dsrna-17.6 Kb	MH094752.1	[74]
Betanecrovirus	Tobacco necrosis virus D (TNV-D)	Herbaceous Species	Linear, Ssrna(+)-4 kb	FN543421	[61]
Begomovirus	Cabbage leaf curl virus	Dicotyledonous plants—tomatoes, beans, squash, cassava, and cotton	(+)Ssma-2.8 Kbp	MK087038-DNA-A MK087039-DNA-B	[12]
	Cleome leaf crumple virus				
	Euphorbia mosaic virus				
	Sri Lankan cassava mosaic virus				
	Tomato yellow leaf curl virus				
South African cassava mosaic virus					
Bromovirus	Brome mosaic virus	Cowpea	Ssrna (+) 4.7 Kb	LG146066	[20]
	Cassia yellow blotch virus	Cassia			
	Cowpea chlorotic mottle virus	Cowpea			
Spring beauty latent virus					
Carmovirus	Cardamine chlorotic fleck virus	Cowpea	Ssrna - 4.8 Kbp.	NC_001600	[122]
	Turnip crinkle virus	Brassicaceae family			
Carlavirus	Potato latent virus	Allium species Onion, leek, garlic, shallot, and Allium scorodoprasum	Single-Stranded Positive-Sense RNA-8.7 Kb	AF271218	[123]
Caulimovirus	Cauliflower mosaic virus	Vegetables, ornamentals, and weeds	Ds DNA	M90541	[15]

Genus	Species	Host	Genome	Genbank Accession Number	Reference
Cheravirus	Apple latent spherical virus	Caryophyllaceae, Chenopodiaceae, Cryptomeria, Fabaceae, Cucurbitaceae, Gentianaceae, Pinus, Rosaceae, Rutaceae, Solanaceae, and Arabidopsis	Ssma (+)	NC_003788	[124]
Cilevirus	Citrus leprosis virus C	Citrus, Chenopodium quinoa, C. Amaranticolor, and Gomphrena globosa	Linear Ssma (+)	MT554553	[125]
	Solanum Violaefolium ringspot virus	Solanaceae family			
Comovirus	Turnip ringspot virus	Legumes (bean, cowpea, pea, soybean, and clover)	Bipartite Linear Ssma (+)	GQ222382	[126]
Cucumovirus	Cucumber mosaic virus	Legumes and solanaceous	Tripartite Linear Ssma (+) Genome	HV194209	[127]
Curtovirus	Beet curly top virus	Dicotyledonous plants	Monopartite, Circular, Ssdna Genome	MW234427	[31]
	Beet severe curly top virus	Beetroot		NC_043649	
	Spinach curly top virus	Spinach			
Dichorhavirus	Clerodendrum chlorotic spot virus, coffee ringspot virus	Orchid, citrus, coffee, clerodendrum, and hibiscus	Negative-Stranded RNA Linear-6.4–6.7 Kb	KF812525 and KF812526	[128]
Elaviroid	Elvd (eggplant latent viroid)	Eggplant (Solanum melongena), tomato, cucumber, chrysanthemum, and citron	Viroid	NC_039241	[129]
Fabavirus	BBWV (broad bean wilt virus)	Fabaceae, Solanaceae, Cucurbitaceae, and Chenopodiaceae, including Vigna unguiculata, Vicia faba, Pisum sativum, Nicotiana occidentalis, Nicotiana benthamiana, Solanum lycopersicum, Cucurbita moschata, Cucumis sativus, Chenopodium amaranticolor, and C. quinoa	(+)Ssma	KT923141	[130, 131]
Illavirus	TSV (tobacco streak virus)	Woody plants—cowpea	(+)Ssma	MF784816	[125]

Genus	Species	Host	Genome	Genbank Accession Number	Reference
Ipomovirus	Tmmov (tomato mild mottle virus)	Non-gramineous or gramineous host plants	(+)Ssma	NC_038920	[1]
Macluravirus	Arlv (artichoke latent virus)	Monocots and dicots	(+)Ssma	NC_026759	[49]
Mastrevirus	Cpcdv (chickpea chlorotic dwarf virus)	Cereals and some vegetable crops Solanaceae and Fabaceae Monocot plant species	Ssdna	LN865163	[128]
Nanovirus	Faba bean necrotic yellow virus	Only the monocot species	The Double-Stranded DNA Viruses The Single-Stranded DNA Viruses The Double-Stranded RNA Viruses Single-Stranded-Sense Strand (+ Form) RNA Viruses Double-Stranded DNA With RNA Intermediate- 1 kb	MN716783	[132]
Nepovirus	Arabis mosaic virus Cherry leaf roll virus Tobacco ringspot virus Tomato spotted wilt virus	Woody And Herbaceous Plants Plums, cherries, peaches Tobacco Tomato	Bipartite Linear Ssrna (+) Genome— 1.8 Kbp	GQ369530	[133]
Orthotospovirus	TSWV (tomato spotted wilt orthotospovirus), IYSV (iris yellow spot orthotospovirus),	Weeds, woody plants such as kiwifruit, mulberry, and macadamia nut		OK469365	[134, 135]
Potterovirus	Beet mild yellowing virus Beet western yellows virus Turnip yellows virus	Solanaceous hosts and weed Taproots plant White, fleshy taproot	Positive-Sense Single-Stranded RNA —6.2 Kb	NC_003491	[136]
Potexvirus	Plantago asiatica mosaic virus	Mono- and dicotyledonous plant species	Monopartite Ssrna Genome Of 5.9– 7.0 Kb	LC592412	[14]

Genus	Species	Host	Genome	Genbank Accession Number	Reference
Potyvirus	Lettuce mosaic virus	N. Benthamiana is one of the most permissive hosts	Positive-Sense, Single-Stranded RNA—9000–12,000 Nucleotide	MZ318158	[21]
	Plum pox virus	Each, almond, and cherry, and ornamentals			
	Tobacco etch virus	Tobacco			
	Turnip mosaic virus	Turnip			
	Watermelon mosaic virus	Watermelon			
Sobemovirus	Turnip rosette virus	Dicotyledonous and monocotyledonous plants	Single-Stranded RNA—4149 Nt.	AY177608	[137]
Tobamovirus	Oilseed rape mosaic virus	Tobacco, potato, tomato, and squash	Single Linear Positive-Sense Single-Stranded RNA—6.3–66 Kb	EF432728	[138]
	Tobacco mosaic virus	Tobacco			
	Turnip vein-clearing virus	Turnip			
Tobravirus	Pepper ringspot virus	Herbaceous and few woody plant species	Bipartite, Positive-Sense, Single-Stranded RNA Around 26.84. 5 kb-8600–11,300 Nucleotides	NC_003669	[135]
	Tobacco rattle virus	Tobacco			
Tospovirus	Iris yellow spot virus	Ornamental, vegetable, fruit, and other annual and perennial plants	Tripartite Negative And Ambisense RNA—8.9-Kb	MF359021	[123]
Tymovirus	Turnip yellow mosaic virus	Monocotyledonous plant	Single Molecule Of Positive-Sense Single-Stranded RNA Of 6.0–7.5 Kb In Length.	AH002387 J02418 K00602 V01419	[126]
	Umbrovirus	TBTV (tobacco bushy top virus) Tmov (Tobacco mottle virus)	(+) Ssma-Linear, Positive-Sense, Single-Stranded RNA, 4200–6900 Nucleotides	TBTV-YBSh, MW579556; TBTV-YKMPL, MW579557	[10]

Table 3. Some plant viruses are shown in the table, along with their Genbank accession numbers.

Viruses	Material DNA/ RNA	Method of detection	Advantages	Limitations	References
Asparagus viruses	DNA	cDNA macroarray	Detect multiple viruses	High cost and the large number of probe	[27, 142]
Potato viruses	RNA	Generic methods	Potential to test for a large number of targets in a single assay	Cannot use casts or instance of with parameterized types	[18]
Viruses with non-polyadenylated genomes	RNA	rRNA-selective depression method	To detect a broad range of viruses	High-throughput and cost-effective method	[143]
Tomato yellow leaf curl virus (TYLCV)	DNA	LAMP-coupled CRISPR-Cas12	Rapid and sensitive detection	May be not yet	[144]
Any viruses	Both DNA and RNA	Oxford Nanopore Technologies (ONT) sequencing	Nucleotide sequences in real time without the need for prior amplification.	It tends to be error-prone	[145]
Citrus tristeza virus	RNA	High-throughput sequencing (HTS)	More comprehensive and standard operating procedures	Short-read technology is that short read must be mapped to genome that is not always available	[146]
Potyvirus	DNA/ RNA	'Direct RT-RPA	Target virus directly from plant leaf extracts. The minimal sample preparation requirements and the possibility of storing RPA reagents without cold chain storage	Detection with CDV RNA extracted from strains	[147]
Latent viruses	RNA	RT-qPCR assays	Amplifying the target from positive controls without showing any detectable amplification in negative and nontarget controls and revealed high sensitivity by reliably detecting	It requires separate priming reactions for each target. It is also wasteful if only limited amounts of RNA are available	[148]
Grapevine viruses	RNA	A multiplex RT-PCR	Simultaneous detection of nine viruses at once	(1) The self-inhibition among different sets of primers; (2) low amplification	[149]

Viruses	Material DNA/ RNA	Method of detection	Advantages	Limitations	References
				efficiency; and (3) no identical efficiency on different templates	
Both DNA/ RNA virus	DNA/ RNA	Dot- immunobinding assay	Economical to screen more than 1000 samples against 15 known plant viruses in a very short time	The biotinylation process may alter the structure and properties of the proteins of interest	[150]
Cucurbit- Infesting Viruses	DNA/ RNA	Fluorescent microsphere- based assay	Simplicity, speediness, and sensitiveness	Not yet	[16]

Table 4.
 For the identification of plant viruses and virus-like pathogens, nanotechnology integrated molecular method techniques.

in point-of-care (POC) nucleic acid extraction technology are summarized in this study. Emerging bacterial, viral, fungal, and other pathogen-caused human and plant diseases present a persistent threat to global health and food security [152]. Although there are numerous pipelines for finding plant viruses, they all have a similar structure. In POC diagnostics, plant samples are examined right away at the sampling site for disease screening. Rapid point-of-care (POC) molecular diagnostics of plant diseases is becoming increasingly important for disease control and agricultural protection. The identification of the disease-causing pathogens and their pathogenesis is revealed at the genomic level by nucleic acid-based molecular diagnostics. One of the most important and efficient steps in creating control strategies for plant viral infections is still the development of reliable and early detection technologies [117].

2.1 Conventional measures before, during, and after vegetation

The virus itself is frequently not the main problem, but rather the vector that it travels on. To reduce the population of *B. tabaci*, insecticides including imidacloprid, acetamiprid, dinotefuran, and thiamethoxam are frequently applied. Whitefly infestation in nurseries may be avoided by using netting. Eretmoceris eremicus and other biocontrol agents could be very effective control agents. One of the main challenges in managing pathogens is keeping a pathogen alive in many hosts. Alternate hosts serve as a repository for inoculum both during the growing season and during periods when there is no crop. Recent research indicates that the effectiveness of using chitosan as a biocontrol agent against ToLCV has increased when combined with pseudomonas. Because begomoviruses are so common, breeders should take them into account when making selections for resistance.

2.2 Biotechnological approaches for viral disease diagnosis

The family Geminiviridae (genus Begomovirus) has more than 100 different viruses. Because of improvements in cloning and low-cost sequencing, the number of

accessible genome sequences has significantly increased. Tomatoes have a small DNA genome that is simple to clone. Numerous tomato viruses can be identified, as well as new or emerging viruses and viroids, using general virus detection technologies like enzyme-linked immunosorbent assay (ELISA) or polymerase chain reaction (PCR). For the accurate identification of well-known and novel viruses and viroids, a bioinformatics pipeline based on the alignment and assembly of sRNA or DNA sequences is necessary. Key viral genes called ToLCNDV miRs affect the fundamental processes involved in virus emergence. RNAi-based viral gene silencing and sense/antisense technology have been used to create transgenic resistance. The use of next-generation sequencing [NGS] technology to quickly, precisely, and affordably detect miRs has become commonplace. The generation of markers for marker-assisted selection (MAS) of resistant genotypes may now be done swiftly because to the development of NGS technology and high-throughput genotyping platforms [153]. The use of transgenic methods to control viral infections is extremely successful. The public hostility to genetically modified organisms (GMOs) in developing countries like India has inhibited their implementation. Infection is reduced by 75% in transgenic tomato plants that produce dsRNA-containing sequences from the IR, V1, and V2 regions of TYLCV-Oman. The identification of VSR may lead to the development of disease resistance strategies and other biotechnological applications. RNA silencing sometimes referred to as RNA interference or RNAi shields plants from viroids and invading viruses [111]. The AC4/C4 protein is a pathogenicity protein that impacts plant development and can be used as a useful tool for research into cell cycle regulation, hormone signaling, cell differentiation, and plant development. It has been demonstrated that transgenic lines of AC4 viruses led to abnormal phenotypes and developmental patterns in several different host plants. Disease-resistant tomato varieties can assist with gene-pivoting and resistance-breeding [154].

Elite tomato breeding lines were chosen using a mix of phenotypic and molecular screening techniques for ToLCD, late blight, and RKN. To develop fresh market tomato lines that are begomovirus-resistant, AVRDC, The World Vegetable Center, initiated a campaign. A lipid transfer protein, nucleotide-binding site, and leucine-rich repeat (NBS-LRR) proteins, posttranscriptional gene silencing machinery and other defense genes are expressed specifically in the host in response to ToLCNDV infection. To build resistance against various ToLCV strains, six resistance loci have been employed in tomato plants. Of the six resistance/tolerance loci, Ty-1, Ty-2, and Ty-3 are the ones that are used most frequently. These results suggest that these Ty loci increase host defense via a different mechanism from the R gene-mediated hypersensitive response (HR). Increased expression of genes associated with the lignin and SA biosynthesis pathways has been related to improved plant virus defense, according to reports. A global miR profiling study has shown that a large number of miRs were differently changed in ToLCV-ND-infected Pusa Ruby tomato leaf tissue. The study also identified miRs that demonstrated differential expression between the sensitive and tolerant cultivars. ToLCD resistance is conferred by the expression of artificial microRNA targeting the ATP-binding region of AC1 in transgenic tomatoes without affecting tomato output. The antisera's usefulness in begomovirus identification in field samples and reservoir hosts is demonstrated by polyclonal antibodies generated using purified intact virus and rCP of tomato leaf curl Bangalore virus [155]. From tomato and *N. benthamiana* leaves that had been treated with the virus, ToLCBV was successfully purified. Different Indian isolates of the begomovirus in plant and weed species might be recognized using monoclonal antibodies. ToLCBVC viral infections were caused by biologist Devaraja in tomato samples as well as other

crop and weed plant types. Total viral resistance is provided by transgenic plants that produce Cas9 and dual gRNAs that target different regions of the cotton leaf curl Multan virus (CLCuMuV) single-stranded DNA genome, offering a special method for developing geminivirus resistance. Clustered, regularly interspaced short palindromic repeats/CRISPR-associated proteins are the foundation of the genome-editing technique CRISPR/Cas. It originated from the bacterial and archaeal adaptive immune systems that resist viruses [156, 157].

2.3 Nanotechnology-based nucleic acid/viral particle detection

High-throughput sequencing (HTS) has enabled virologists to identify an unprecedented number of viruses, advancing our understanding of the variety of viruses in nature and, in particular, revealing the virome of many crops. The gaps in our knowledge of virus biology have, however, frequently become wider as a result of these new virus discoveries. Enzyme-linked immunosorbent assays (ELISA) and direct tissue blot immunoassays are two immunological methods now utilized to detect infections in plants (DTBIA). For the identification and detection of pathogens, DNA-based techniques such as polymerase chain reaction (PCR), real-time PCR (RT-PCR), and dot blot hybridization have also been proposed [158].

The sensitivity and specificity of virus nucleic acid sequence identity are predicted to be improved by using nanotechnology-based techniques, which are thought to be more effective, safer, and target-specific. Compounds made of nanoparticles (NPs) can simulate ligand and receptor binding to particular target-specific plant diseases, such as the interaction between an antigen and an antibody. The gold standard for plant disease diagnostics uses molecular assays based on nucleic acids and antibodies. Being one of the most fascinating and dynamic fields of research, nanotechnology has a significant impact on a wide range of fields, including science, engineering, medicine, and agriculture. Nanomaterials, whose diameters typically vary from 1 to 100 nm, can offer enhanced surface-to-volume ratios as well as special chemical, optical, and electrical properties, making them excellent candidates for the analysis of plant diseases. For the quick detection of a variety of human and plant illnesses, lateral flow assays (LFA) and electrochemical sensors have been used as some nano-based approaches (**Table 5**). Fast identification of plant diseases is now possible because of portable imaging equipment (such as cellphones) backed by nanostructures. Due to the extraordinary biosecurity of designed molecular recognitions at the nanoscale, which has seen exceptional development in the past decade, nanoscale materials are promising possibilities for plant disease detection. Overall, thanks to recent advancements in rapid plant DNA extraction technology made possible by microneedles, tiny DNA sequencing chips, and smartphone-based volatile organic (VOC) sensors, many traditional laboratory tests, like nucleic acid amplification, sequencing, and volatile organic compound (VOC) analysis, may now be performed directly in the crop field in a much faster and more affordable manner [163].

2.3.1 Challenges in nanotechnology

The environmental impact and toxicity of engineered nanomaterials, the quickness of data sharing and disease forecasting, and long-term sensor stability in extreme conditions like extreme cold or heat, prolonged sun exposure, and heavy wear are the three main challenges that currently exist for plant diagnostic tools. The first issue is that safety issues must be resolved before any nanosensors can be commercialized and

Virus	Plant	Nano-based device	Advantages	Limitations	References
Orchid viruses	Cattleya, Cymbidium, Dendrobium, Odontoglossum hybrids, and Phalaenopsis	Gold nanorods (AuNRs) Fiber optic particle plasmon resonance (FOPPR)	Faster analysis, better reproducibility, and lower detection limit than ELISA	Conventional UV-vis spectroscopy is constrained by weak resolution and a low signal-to-noise ratio, which may not be enough to detect proteins at low quantities. The LSPR optical fiber biosensor, which is based on wavelengths, might experience the same restriction.	[159]
Citrus tristeza virus (CTV)	Citrus trees	Gold nanoparticles (AuNPs) to develop a specific and sensitive fluorescence resonance energy transfer (FRET)-based nanobiosensor for detecting	Rapid, sensitive, specific, and efficient in detecting viruses	Mass transfer limitation. Process at low frequencies	[6]
Cauliflower mosaic virus 35 s (CaMV35S)	Brassicaceae and Solanaceae species	Forster resonance energy transfer	Applied to identify the real sample. Most utility and reliable for quantification of GMOs in food.	Low signal-to-noise ratio	[83, 160]
Citrus tristeza virus	Rutaceae	Amplification (RPA) detection on gold nanoparticle-modified electrodes	In-field diagnostic application and effective replacement to polymerase chain reaction (PCR)	Heat sources are needed for getting detectable signals, which represents an important practical limitation	[161]
Citrus tristeza virus	Citrus	Solid-phase isothermal recombinase polymerase	Sensitive, specific, rapid, and efficient in detecting viruses	Not yet	[162]

Table 5. Only a few methods use nanotechnology-based electrochemical nucleic acid sensing in disease diagnosis.

used in the field because some nanoparticles, like QDs, may be hazardous. More thorough toxicity testing and regulation are required for nanosensors that will be used on living plants or consumable agriculture and food items, because dangerous

nanomaterial residues may infiltrate the food chain and be consumed by end users. Regarding the second issue, the new generation of nanosensors is anticipated to be more wirelessly connected and capable of providing measurement in close to real time as the primary requirement for disease diagnosis is consistently the timely report and forecast of infection events on-site. Finally, before any sensors can be deployed to the actual yield, more resilient and robust sensors that can endure a wide range of environmental factors (such as temperature, humidity, air pollution, etc.) in the agricultural yield are predicted [164].

2.4 Need to resolve

2.4.1 Several outstanding questions and future directions are highlighted

- Luan et al. show that TYLCV infection can trigger whitefly immune responses, potentially leading to viral particle destruction within the body of viruliferous whiteflies. These findings suggest that TYLCV infection causes physiological changes and immunological responses in whiteflies, which is harmful. However, the precise mechanisms driving the whitefly's immunological responses to begomoviruses remain unknown [49].
- Ashish Prasad et al. [102].
 - What role in the pathophysiology of TYLCV do endogenous noncoding RNAs, such as sRNAs and lncRNAs, play?
 - Is there another way to safeguard against the virus? It is imperative to hunt for new sources of resistance given the virus's quick global spread.
 - Investigations focusing on this area may help to fix the problem, because there is still debate on whether TYLCV can reproduce inside the whitefly.
 - The effect of suppressor TYLCV proteins in dampening the host RNA-silencing machinery has been investigated extensively. It is yet unclear if they can inhibit proteins involved in other processes, such as the ubiquitin-proteasome system or autophagy.
- Zaidi et al. [77].
 - By pyramiding various poisons, can we develop broadspectrum resistance against chewing and sap-sucking insects?
 - Tma12 kills *B. tabaci* in two ways.
 - Will phloem-specific promoters improve the performance of dsRNA and Tam12?
 - Will the poisons cause *B. tabaci* to become resistant?
 - Can the simultaneous expression of many toxins and/or dsRNAs prevent resistance breaking?

- Will these strategies work against other begomoviruses including those that cause cassava mosaic viruses (CMVs) and tomato yellow leaf curl virus (TYLCV)?
- Adapted from a drawing of *B. tabaci* sucking phloem sap.
- Rolling circle replication (RCR)-dependent geminivirus replication requires the viral replication initiator protein (Rep) and the conserved common region (CR), but the precise mechanisms of geminivirus replication are still unknown due to the lack of a eukaryotic model system [97].
- One of the viral symptom determinants that have the potential to lead to abnormal cell division is the C4 protein expressed by the geminivirus [107]. On the other hand, it is unclear what chemical process C4 uses to promote cell division.
- GAs are a defensive measure, geminiviruses encode viral proteins that lower viral DNA methylation and transcriptional gene repression (TGS). However, it is still uncertain how viral proteins contribute to TGS suppression at the molecular level.
- According to DNA-A components of ToLCNDV isolates, recombinations in this genus frequently involve genomic sequences from other begomoviruses or sequences of unknown origin. The taxonomic status of these recombinants is determined by their relatedness to the original virus(es) and putatively altered biological characteristics [77].
- The dsDNA intermediates used by geminiviruses to replicate their genome in the nucleus may be a target for methylation-mediated TGS [98]. Cytosine DNA methylation is an efficient defense strategy against geminiviruses in plants, because methylation of the viral genome results in transcriptional gene repression (TGS). Geminiviruses produce viral proteins that, as a defense mechanism, reduce viral DNA methylation and TGS. On the other hand, it is yet unclear how viral proteins contribute to TGS suppression at the molecular level.
- The insect vector Bemisia tabaci does not appear to be the site of tomato yellow leaf curl virus replication [81]. Due to the characteristic of an extended host range, controlling and reducing losses brought on by mixed begomoviral infection is challenging. To lessen agricultural losses brought on by begomoviruses, a long-term disease management strategy must be devised while the effects of begomovirus infection on commercially farmed crops like papaya must be continuously monitored [73].

3. Concluding remarks

One of the most widely investigated plants viral diseases is the ToLCV. On the Indian subcontinent, ToLCV is the most hazardous bipartite begomovirus, and it has quickly spread to other regions of the world. The implementation of innovative management techniques is dependent on the availability of information regarding

ToLCV-associated viruses and their epidemics, which is lacking. ToLCNDV has an incredibly diverse population, with mutations that have different host ranges and some that are better adapted to infecting particular host plants. The invention of novel techniques to defend plants from infection will be facilitated by an understanding of the fundamental mechanisms behind such host adaptation. GM techniques based on gene silencing have presented exceptionally significant options for plant viral resistance tactics, and their long-term promise should not be overlooked. Conventional control procedures alone are insufficient for ToLCV control. However, combining many of these approaches following suggestions based on an understanding of the disease's epidemiology may make managing ToLCNDV outbreaks easier. As a result, it is suggested that integrated management techniques integrating numerous control practices be used. However, more research about the epidemiology and ecology of this multifaceted disease is needed to develop efficient management strategies. Because this virus does seem to have a quicker response time than available controls, we should be able to predict the nature and diversity of ToLCV outbreaks in a more dynamic environment, which will have drastic effects on virus vectors. The discovery of more potent and long-lasting strategies to prevent epidemics also depends on a detailed awareness of ToLCV polymorphism and the factors that influence the growth of its inhabitants.

Conflicts of interest


The authors declare no conflicts of interest.

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Chapter 6

Host-Pathogen and Pest Interactions: Virus, Nematode, Viroid, Bacteria, and Pests in Tomato Cultivation

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Abstract

Several pathogens and pests damage tomato plants, and only one and/or more pathogens and pests can coexist in the same plant at the same time. As several numerous pathogens are found in the same plant, the damage to the tomato plants is higher. Pathogens such as nematodes, viruses, viroids, bacteria, and insects adversely affect the growth and development of tomato plants. They may infect roots or upper part of the plant and can cause not only slow down the growth of plants, but also crop losses and their death. Damaging of plant caused by pathogens and pests reduces the market value of plant products. Those pathogens and pests are also called biotic stress agents. The damage, mode of infection, and the mechanism of infection in each tomato plant and pathogens might be different. This situation is crucially important to understand plant pathogen relationship in detail in terms of controlling pests and pathogen. The effect of each pest/pathogen on tomato plants during the cultivation, the type of damage, and new developments and perspectives on morphological and molecular aspects in tomato-pathogen interactions will be discussed in this chapter.

Keywords: nematode, viroid, bacteria, virus, insects, pathogens, resistance, pest, biotic stress

1. Introduction

Tomato (*Solanum lycopersicum* L.), member of the family Solanaceae, is a cultivated plant with a very large cultivation area in the world. According to 2021 FAO data, the amount of tomatoes produced in the worldwide is 187 million tonnes. The highest production amount is in China, followed by Turkey in the third place [1]. Tomato (*S. lycopersicum* L., family Solanaceae) is one of the most produced crops worldwide, and Turkey is placed in top five countries in terms of the production of Solanaceae family [2, 3]. At least 12% of the world's agricultural products are lost every year due to plant

diseases caused by some pathogenic microorganisms and 20% due to some insect pests. Disease factors, pest organisms, and weeds in agricultural products can cause significant economic losses and damage. If the necessary controls against these factors are not made, crop losses can reach from 35% to 100%. The 60–75% of the diseases observed in plants are caused by fungal and bacterial diseases, 10–15% by viral disease (virus and viroids), and 10% by other pathogens and some environmental stress factors [4].

Viruses are commonly encountered in the living ecosystem. Since it does not have a complete cellular structure, it interacts with prokaryotic and eukaryotic organisms and maintains its own existence [5, 6]. In recent years, plant viruses and their mechanisms of action have been widely studied due to the loss of agricultural products and their effects on fruit-vegetable quality. Plant viruses have either single-stranded RNA (ssRNA) or double-stranded RNA or DNA nucleotides [7].

Nematodes are one of the most abundant multicellular organisms on the earth. They may live as plant and animal parasites and/or free living. Parasitic nematodes may infect humans, plants, and animals [8]. Among nematodes, about 4100 nematode species have been identified as plant-parasitic nematodes [8]. They cause significant crop losses on tomato plants.

Bacterial, viroid diseases, and insect pests give also significant crop losses affecting tomato production in many regions in the world.

In this chapter, the effect of each pest/pathogen (virus, nematode, viroid, bacteria, and pests) on tomato plants during the cultivation, the type of damage, and new developments and perspectives on morphological and molecular aspects in tomato-pathogen interactions are given.

2. Viruses disease

Tomato viruses are transmitted by vector insects, plant material, and seeds [9]. Transmission of tomato viruses is important to determine the plant material used in the diagnosis, to choose the method of diagnosis, to prevent the spread of the virus, and to develop a method of struggle against the virus. In this part, we examine under two subtitles that some viral diseases, the main host of which is tomato, are transmitted only by plant materials including seeds and are transmitted by vector insects and/or plant material together. In addition, in this section, the general information and classification of viruses, their genetic characteristics, symptoms and damage in tomato plants, and preventing against the viruses have been briefly explained.

2.1 Tomato viruses transmitted by plant parts including seeds

2.1.1 Tomato brown rugose fruit virus

Tomato brown rugose fruit virus (ToBRFV) was first reported in tomato in Jordan [10]. ToBRFV belongs the family *Virgaviridae* and genus *Tobamovirus*, has rod-shaped particles with encapsidating a positive-sense single-stranded RNA (ssRNA) [11, 12]. ToBRFV is basically transmitted by mechanical ways as plant-plant contact, workers, tools, equipment, and irrigation water. The virus is also effectively transmitted by seeds [10]. In addition, bumblebees transmit the virus on tomatoes [13]. The virus has severe symptoms as mosaic blotch, narrowing on leaves and brown rugose, yellowing spots on fruits. Moreover, the virus reduces the quality of the fruit and causes the

fruit to be unmarketable [14]. ToBRFV is detected by enzyme-linked immunosorbent analysis (ELISA), polymerase chain reaction (PCR)-based analysis by specific primers, and genome sequencing, NGS (next-generation sequencing) [10, 14–16].

2.1.2 *Pepino mosaic virus*

Pepino mosaic virus (PepMV) was originally identified in pepino (*Solanum muricatum*) in Peru, in 1974 [17]. Following pepino, the virus was firstly detected in tomato, in Netherlands [18]. PepMV belongs to the family Flexiviridae and genus *Potexvirus*, has a positive-sense ssRNA genome with non-enveloped, flexible, rod-shaped particles [17]. Although PepMV isolates show a high genomic similarity, they differ from the original source isolate that causes disease in tomato [9]. Observing leaf symptoms are yellow and mosaic spots, scorching, and deformations [9]. The common transmission way of PepMV is mechanical basis such as plant sap, contaminated tools, and surfaces [9]. The virus has been also transmitted by recirculating hydroponic system, bumblebees, and the root-infecting fungus *Olpidium virulentus* between tomato plants [19–21]. In addition, conventional polymerase chain reaction (PCR), quantitative PCR (qPCR) methods as TaqMan assays and restriction fragment length polymorphism (RFLP) are also have been used for detection of virus and identification of different genotypes [19, 22].

2.1.3 *Tobacco mosaic virus*

Tobacco mosaic virus (TMV) was the first virus detected [23], belongs the family Virgaviridae and genus *Tobamovirus* [24, 25]. TMV has rod-shaped and encapsulating particles with a single-stranded RNA (ssRNA) [26–28]. The first viral protein structure sequenced belongs to TMV [29, 30]. TMV is transmitted by mechanically including workers, tools, and propagating materials [31]. Because the virus has oldest genomic information, it has widespread host plants including tomato [32]. TMV has characteristic symptoms on the leaves such as light and dark green spots and malformation. Moreover, TMV infections have also caused necrotic rings, browning, and number and size reducing on fruits [33]. In addition to the serological analysis method for TMV, numerous molecular detection methods and diagnostic studies have been carried out [34]. In general, virus-free seeds, plantlets, and hygienic measures have to be used to prevent from virus like other tobamoviruses.

2.1.4 *Tomato mosaic virus*

Tomato mosaic virus (ToMV) belongs the family Virgaviridae and genus *Tobamovirus* [12, 35]. The particles of virus are rod-shaped and encapsulating with a genome single-stranded RNA (ssRNA) [26]. ToMV has high rate of infectivity, effective seed transmission, and mechanic transmission easily by working hands, tools, soil, and plants parts [12, 36]. Like as other tobamoviruses, ToMV causes malformation, spotting and clearing on tomato leaves, and malformation on fruit and reducing the yields [36]. As with other tobamoviruses, virus-free seeds, plantlets, and hygiene measures should generally be used to prevent the virus.

2.1.5 *Tomato mottle mosaic virus*

Tomato mottle mosaic virus (ToMMV) was firstly identified in Mexico in 2013, belongs the family Virgaviridae and genus *Tobamovirus*, has four open reading frames

(ORFs) including the movement protein (MP) and coat protein (CP) in genome [37]. As other tobamoviruses, ToMMV is inclined to mechanical transmission including contacts, hands, tools, the greenhouse structure, and bumblebees. Moreover, seed transmission is also possible with infected seeds [12]. ToMMV causes the mosaic symptoms, chlorosis, and leaf deformation on tomato plants [38]. The virus can be detected by using polymerase chain reaction (PCR) basis methods [39]. Management of the ToMMV is possible by using virus-free seeds and plantlets and using hygienic measures [40].

3. Plant-parasitic nematodes

Plant-parasitic nematodes are significant pests and cause crop losses, with an estimated yearly loss of USD 173 billion [41]. It is likely that 10% of world crop production is lost as a result of plant-parasitic nematode damage [42]. Most of the plant-parasitic nematodes feed on roots and decrease the uptake of water and nutrients [43]. Stylets of the plant-parasitic nematodes are important apparatus used to puncture plant cells and uptaking nutrient contents. The main signs shown by plants affected by nematodes are stunted development, wilting, and susceptibility to contamination by other plant pathogens [44]. Although there are many plant-parasitic nematodes, the most vital plant-parasitic nematodes in the USA are *Heterodera glycines*, *Meloidogyne fallax*, *Meloidogyne chitwoodi*, *Globodera pallida*, *Ditylenchus dipsaci*, *Litylenchus crenatae*, *Globodera rostochiensis*, *Meloidogyne enterolobii*, *Pratylenchus fallax* and *Bursaphelenchus xylophilus* [45]. Similarly, *Meloidogyne* spp., *Aphelenchoides besseyi*, *Nacobbus aberrans*, *Pratylenchus* spp., *B. xylophilus*, *Heterodera* and *Globodera* spp, *Xiphinema index*, *Radopholus similis*, *D. dipsaci*, and *Rotylenchulus reniformis* are most important nematodes in terms of plant pathology [46]. Root-knot nematodes: The nematodes belonging to the *Meloidogyne* genus termed root-knot nematodes are polyphagous plant pathogens [47]. They may be found worldwide and parasitize the species of higher plants [47]. Root-knot nematodes, *Meloidogyne* genus, which are obligate plant parasites, are economically important and damage plants. They are found in many parts of the world and have the ability to parasitize any high plants [47]. They disrupt plant physiology and decrease crop quality and yield [9, 48]. Root-knot nematodes have 106 species [47]. *M. hapla*, *M. incognita*, *M. arenaria*, and *M. javanica* are major species; however, *M. fallax*, *M. minor*, *M. chitwoodi*, *M. exigua*, *M. paranaensi*, and *M. enterolobii* (= *M. mayaguensis*) are minor root-knot nematode species [41].

The genus of *Meloidogyne* compromises more than 100 species in the world [46]. Root-knot nematodes are named because of their characteristic features, as they typically cause root galls. While young plants may not survive high infection by a nematode, mature plants often show low yield and growth retardation. Among the root-knot nematodes, *M. graminicola* may cause damage to cereals in South Africa, the USA, Australia, and Mexico [44]. *M. arenaria*, *M. incognita*, and *M. javanica* are good hosts of some cereal cultivars such as rye, barley, oat, and wheat under greenhouse conditions [49]. *M. hapla* is distributed in temperate regions, and yield losses caused by some root-knot nematode species are valued at approximately \$10 billion [50]. Root-knot nematodes cause damage and induce a unique feeding site structure termed giant cells within the plant roots. Cell wall molecular architecture of nematode feeding site is changed [51]. *M. javanica*, *M. arenaria*, *M. graminicola*, *M. incognita*, and *M. hapla* are some of the most damaging species; some species cause more damage to their host than other species. For instance, *M. graminicola* is one of the main



Figure 1. The root-knot nematode, *M. incognita*, induced root galls in tomato plants (left) and control-uninfected healthy tomato plant roots (right). The nematode cause galls in tomato roots (right).

problems in rice fields that develop special hook-like knots on the roots of rice plant roots [52]. Root-knot nematodes induce feeding cells and become sedentary within approximately 48 hours after nematode infection [53]. The second stage juveniles of root-knot nematodes can infect the plant roots. More than one species of root-knot nematodes in the same plant tissues can be found. The nematode causes galls in the root system (Figure 1), disrupts the vascular tissues, and restricts the exchange of water and nutrients. Growth slows down, wilting, stunting, and yellowing of leaves are seen. During a severe infection, the plant may completely dry out. The secondary damage of root-knot nematodes is that soil-borne pathogens may enter nematode-induced wounds in plants [54].

4. Tomato pests and their control

4.1 *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)

Tuta absoluta is the main pest in open field and greenhouse tomato cultivation. Adult butterflies are active at night. They lay their eggs, usually under the leaves, in the lower part of the sepals of buds and immature fruits. Its larvae damage all parts of the tomato plant except the root and in each period. The larva feeds by opening

galleries between the two epidermes on the leaves of the tomato. The plant may dry out completely due to the galleries opened in the green part of the plant. The pest enters under the sepals of immature tomato fruits. The damaged fruit loses its market value, and rots occur when secondary microorganisms settle in the galleries opened in the fruit [55]. As a biotechnical method, pheromone + water trap or pheromone + light + water trap can be used in greenhouse tomato cultivation for mass trapping against tomato moth [55].

4.2 *Bemisia tabaci* (Genn.) and *Trialeurodes vaporariorum* (Westw.) (Hemiptera: Aleyrodidae)

The damage of these pests is important in tomatoes, cucumbers, peppers, beans, and eggplant [56]. Whitefly adults use the underside of leaves for feeding, laying eggs and resting. Larvae and adults feed by sucking plant sap. As a result of suction, yellowing occurs in the form of spots on the leaf. In addition, the pest secretes a sweet substance during feeding, with the development of fumagine fungi on this substance, a black layer forms on the leaves, and these parts cannot assimilate. For this reason, the plant weakens, plant growth is adversely affected, yield and quality decrease. Whiteflies give an average of 9–10 offspring per year, depending on the temperature, and a female lays an average of 200-300 eggs. Whitefly adults also play an important role in the transmission of some viral diseases. Especially *Tomato yellow leaf curl virus* (TYLCV) is carried by Tobacco whitefly [55].

4.3 *Liriomyza trifolii* (Burgess), *L. bryoniae* (Kalt.), *L. huidobrensis* (Blanchard) (Dip.: Agromyzidae)

Especially tomato, cucumber, and beans are among the important hosts of leaf fly, which is a polyphagous pest. Adults and larvae of the pest cause damage to the plant. Adults lay their eggs between the two epidermes of the leaf [55]. Larvae emerging from the egg feed on the parenchyma tissue between the two epidermes in the leaf, and as a result, galleries are formed. In the following periods, these areas turn yellow, dry, and fall off. It indirectly causes loss of product and value by delaying development in young seedlings and plants [55]. A female can lay about 400 eggs in her lifetime at 30°C. It can give about 10 offspring under greenhouse conditions. In order to obtain healthy plants in the cultural struggle, precautions should be taken against pests, especially during the seedling period, For this purpose, ventilation openings must be covered with gauze. Weeds around and inside the greenhouse must be destroyed. Contaminated plant residues must be destroyed. The soil must be kept moist and the pupae must rot from moisture by mulching, and larvae should be prevented from becoming pupae by passing into the soil. Entry-exit and ventilation openings in greenhouses should be covered with gauze or fine-hole wire to prevent the entry of adults. Yellow sticky traps are used in biotechnical control since planting seedlings. One of the most important parasitoids is *Diglyphus isaea* Walker (Hym.: Eulophidae). In case of 10 larvae per leaf in tomato, chemical control is decided [55].

4.4 Aphids [*Myzus persicae* (Sulz.), *Aphis gossypii* Glov., *A. fabae* Scop., *Macrosiphum euphorbiae* (Thomas) (Hem.: Aphididae)]

Aphids are particularly damaging to tomatoes, peppers, eggplants, cucumbers, and zucchini. Aphids cause damage by sucking plant sap. Due to the suction, the

leaves take a shrivelled, curled appearance. As a result of this suction, the plant weakens, development stops, the yield and quality of the product deteriorate. The sweet substances they secrete cover the plant surface by causing fumagine, and damage occurs as a result of the plant's obstruction to assimilation and respiration. It is also the vector of viral diseases. It is known that only *M. persicae* is the vector of 50 different viruses [55]. Contaminated plants and weeds should be cleaned from inside the greenhouse. Among the predators, especially the species belonging to the Coccinellidae, Chrysopidae, and Syrphidae families and the parasitoids *Aphidius* species are very important in terms of biological control. For chemical control against Aphids in tomato, it is decided to apply if 20 individuals are seen per leaf [55].

4.5 *Tetranychus urticae* Koch. (Acarina: Tetranychidae)

As a polyphagous pest, *T. urticae* is particularly damaging to tomatoes, beans, cucumbers, eggplant, peppers, and zucchini [83]. The females lay their eggs on the underside of the leaves, between the webs they weave along the leaf veins. The larva that emerges from the egg becomes adult by passing the protonymph and deutonymph stages. Larvae change three shirts until they reach adulthood [55]. A female can lay 100–200 eggs. Depending on the climatic conditions and the host, it can produce 10–12 offspring per year in greenhouses [56]. As a cultural precaution in the fight against spider mites, plant residues contaminated with the pest should be removed from the environment. Soil cultivation should be done, and weeds should be combated. In its biological control, especially Phytoseids, Coccinellids, and predatory thrips are the first preferred natural enemies. If five nymphs + adults per leaf are determined in chemical control against spider mites in tomato, the application is decided [55].

4.6 Thrips [*Thrips tabaci* Lind., *Frankliniella occidentalis* Pergande. (Thys.: Thripidae)]

Thrips particularly give damage to tomatoes, cucumbers, peppers, eggplants, and beans. Adults and larvae injure the epidermis layer of leaves, stems, and fruits of plants and feed by absorbing the sap. The cells in the area where the thripsin is fed die and white silvery spots appear. As a result, the assimilation capacity of the leaves decreases and the leaf edges curl. As a result of feeding on fruit or capsules, silvery spots appear, and deformities occur. *T. tabaci* lay 70–100 eggs during their lifetime. It completes one offspring in an average of 14–30 days. It gives 3–10 offspring per year. *F. occidentalis* lays 150–300 eggs during its lifetime. It gives a maximum of 15 offspring per year. As a cultural precaution, plant residues contaminated with pest should be destroyed. Of the natural enemies, especially *Orius* spp., it is important for biological control. In the chemical control of thrips, if 20 nymphs per leaf or three nymphs + adults (adults-larvae) are determined per flower, the application is decided [55].

5. Tomato bacterial diseases

5.1 Bacterial canker *Clavibacter michiganensis* subsp. *michiganensis* (Smith)

Clavibacter michiganensis subsp. *michiganensis* (CMM) is a xylem-inhabiting bacteria [57]. Optimal growth conditions are at 24–38°C and 7 and 8 P. But it found to grow



Figure 2. Vascular color change of tomato plant by *Clavibacter michiganensis* subsp. *michiganensis* (CMM). The bacteria inhabit in the xylem. The color of the plant vascular tissues is cream-yellow to brown.

in plant xylem at pH 5 [57, 58]. The disease is seed-borne, and bacteria may survive in or on the seed coat. Contaminated soil equipment and other materials serve as inoculum sources for short periods. Infected plant materials and soils with infected plant debris are important inoculum sources by providing long life periods of bacteria. After the plant is infected, bacteria invade xylem vessels, and it moves systemically throughout a plant. Disease causes weak and stunted plants. Infected seedlings may be quickly collapsed. Bacterial canker caused vascular (systemic) and parenchymal (superficial) symptoms. The early symptoms are wilting, curling browning, and wilting of the leaves, especially along one side of the plant. Wilting of the lower leaves can be seen toward the flowering stage. The wilting may progress upward of the plant. The wilted parts can dry out in a short time. As a result of the superficial infections, necrotic or slightly raised spots may appear on the surfaces of leaves, on the stems, and on petioles. In infected plant, cream-yellow to brown coloring of the vascular tissues can be seen (Figure 2).

5.2 Bacterial pith necrosis

Bacterial pith necrosis disease is caused by several pathogenic bacteria, *Pseudomonas corrugata* (Scarlett et al.) Roberts and Scarlett, *P. cichorii* (Swingle) Stapp, *P. mediterranea* Catara et al., *P. viridiflava* (Burkholder) Dowson, *P. fluorescens*, *Pseudomonas marginalis* Brown (Stewens), *Dickeya chrysanthemi*, *Pectobacterium carotovorum* subsp. *carotovorum* [59–61]. The disease affects tomato plants (*S. lycopersicum*), especially in greenhouse production. The disease was first described in Britain in 1970 by Scarlett et al. [62]. Disease-causing agents are generally opportunistic bacteria to cause disease when the plant is under stressful conditions. High humidity, high N fertilizer, and low night temperatures encourage rapid plant growth, and the formation of the juicy structure is a disease favorable condition [63]. The major entry place for bacteria is the wounds caused after secondary sprout removal, which is a common practice in staked tomato fields. Disease agents generally survived in seeds, soil, and infected plant debris for 6–8 months [64]. The disease may occur in



Figure 3. Bacterial pith necrosis: general wilting and stem necrosis by tomato pith necrosis and stem necrosis and vascular coloring of tomato plants caused by tomato pith necrosis. The brown discoloration is seen.

the field and covered greenhouse crops, especially during winter in greenhouse crops. The symptoms are similar to the infections caused by the pathogens *P. viridiflava*, *P. corrugata*, *P. mediterranea*, *P. carotovorum*, or *Pectobacterium atrosepticum* [65–67]. Typical symptoms of pith necrosis on tomato plants consisted of general plant wilting, yellowing, and brown to black spots or lesions developing on the stem, petiole, and fruit stalk (**Figure 3**). Internally, pith tissues developed water-soaking, brown discoloration, hollowing, and soft rotting. In some cases, browning also occurs in the vascular tissues (**Figure 3**).

5.3 Bacterial speck disease *Pseudomonas syringae* pv. *tomato* (Okabe) Young, Dye, Wilkie

Bacterial speck of tomato is a serious problem in many greenhouse and field production areas. Disease can occur at every growing stage of tomato, but it causes severe infections at cool, moist conditions. The optimal growth temperature of the bacteria is 24–30°C. Disease development stops in hot weather conditions. The disease is ubiquitous [68], Bacteria can survive epiphytically on weed hosts [69]. Bacteria can maintain the viability for 1–2 years as saprophytically on diseased plant residues in the soil [70].

The disease is seed-borne. Infection may begin with soil with contaminated seeds or plant debris. Secondary contamination occurs from wounds or natural openings. Water droplets play an important role in the spread of the disease. During the seedling period, brown-black spots sometimes surrounded by chlorotic margin are seen on the leaves and stems of the seedlings, and sometimes these spots spread and cause drying of the seedling. The spots on the leaves are small, round, dark in color, and unlimited. A yellow halo is usually seen around these spots, which are 1–3 mm in diameter. The spots coalesce over time and form large necrotic areas that lead to deformation and drying of the leaf. Superficial large brown spots are seen on the main stem and branches, leaves, and flower stalks (**Figure 4**) [71].



Figure 4. The symptoms of bacterial speck disease *P. syringae* pv. Tomato (*Pst*). Large spots on tomato stems (left), flower spots (middle), spots on fruit stalks and fruits (right) by *Pst*.

5.4 Bacterial spot of tomato *Xanthomonas vesicatoria* Vauterin et al., *Xanthomonas euvesicatoria* (Jones et al.); *Xanthomonas perforans* (Jones et al.).

Bacterial spot of tomato is a worldwide disease. *X. vesicatoria* Vauterin et al., *X. euvesicatoria* X. *perforans* have been identified to cause bacterial spot disease on tomato. The disease was firstly discovered in South Africa in 1914 [72]. High relative humidity and overhead irrigation are optimal conditions for disease development. The optimum growth temperature of these bacteria is 29°C. 20–35°C temperature conditions promote disease development, while night temperatures lower than 16°C suppress disease development. Infected seeds may serve as a major inoculum source. The agent can survive on or in the seed for a year or more. *Xanthomonads* may also survive epiphytically in the tomato phyllosphere. Under favorable conditions, epiphytic populations can cause severe infections or outbreaks, especially in transplants [73]. Tomato bacterial spot caused necrotic lesions on the leaves, stems, petals, and flowers, and fruit [74]. Circular water-soaked lesions appear on seedlings (Figure 5). They later dry and turn dark brown to black [75]. Sometimes, halos are present around the spots. Primary lesions coalesce, resulting in extensive necrosis and a blighted appearance (Figure 5).

5.5 Bacterial wilt of tomato *Ralstonia solanacearum*

Bacterial wilt (BW) is the most important disease affecting tomato production in many regions [76]. It causes severe wilting of economically important crops such as tomato, potato, eggplant, chili, and non-solanaceous crops such as banana and groundnut. *R. solanacearum* is an aerobic obligate organism. It was classified as four races and five biovars. Race 1 has a very wide host range mainly flowering crops. Race 2 attacks bananas, race 3 has worldwide effects on tomatoes, potatoes, and other *Solanaceae* plants, and race 4 infects ginger [77]. *R. solanacearum* can survive on weeds and alternative non-host plants epiphytically. Infected soil and crop residues may serve as important inoculum sources [78]. The pathogen is carried in tomato seeds [79].

Initial symptom of bacteria in tomato is wilting of upper leaves (Figure 6). Complete wilting of the plants is observed in a short time. Brown discoloration of the infected vascular tissues and visible white or yellowish bacterial ooze can be seen [80].



Figure 5.
The symptoms of bacterial spot of tomato *Xanthomonas* spp. Water-soaked lesions of the disease on seedlings (left), leaf spots of *X. euvesicatoria* in greenhouse grown tomatoes (right). N. YILDIZ.



Figure 6.
The symptoms of BW of tomato *R. solanacearum*. Wilting caused by *R. solanacearum* is seen on the leaves of tomato plant.

6. Tomato viroids

Some viroids are pathogenic, some can continue to multiply asymptotically in susceptible plant species. Viroids are classified in two families, Avsunviroidae

and Pospiviroidae. It has been reported that there are eight species in the family Pospiviroidae, which cause symptoms to occur intensely in tomatoes, especially in the Solanaceae family [81].

Common symptoms of viroid infection depending on viroid species and variant (species and strain), variety, temperature, and light conditions include chlorosis, tanning, leaf deformation, reduced plant growth, severe yield loss, and non-marketable fruit symptoms in tomato plants [82].

6.1 Potato spindle tuber viroid

The genus *Pospiviroid* of the family Pospiviroidae; *Potato gothic virus*, *Potato spindle tuber pospiviroid* (PSTVd), *Potato spindle tuber virus*, *Tomato bunchy top viroid* has been named under different names. The PSTVd factor is included in the EPPO A2 list. PSTVd was the first to be identified as a new viroid and is quite different from bacteria and viruses [83]. PSTVd is located in the family Pospiviroidae of the *Pospiviroid* genus [84]. While the main host is potato (*Solanum tuberosum* and other *Solanum* spp.), tomato (*S. lycopersicum*), pepper (*Capsicum* spp.), and other vegetables and ornamental plants and weeds from the Solanaceae family also constitute the host series. Infections in ornamental plants and weeds are generally asymptomatic. It has been determined that many species in the Solanaceae family and a few species in other families can be transmitted experimentally [85].

The type and severity of PSTVd symptoms vary depending on the viroid strain, host species and variety, and environmental conditions. PSTVd infections can be asymptomatic or produce symptoms ranging from mild to severe. PSTVd may cause more severe symptoms at higher temperatures [86]. In tomato, early in infection, infected plants show slow growth and chlorosis in the upper part of the plant, while in advanced stages the growth reduction may become more severe and leaves may turn red and/or purple and become more fragile (**Figure 7**). At this stage, flowering and fruiting may stop. In advanced stages, plants may die or partially recover.

6.2 *Citrus exocortis* viroid (*Citrus exocortis pospiviroid*) (Indian tomato bunchy top viroid)

The disease agent was observed for the first time with the symptom of bark scaling on the three-leaf rootstock of citrus fruits, and it was revealed that it was transmitted



Figure 7. *Potato spindle tuber pospiviroid* (PSTVd) induced plant symptoms on tomato plants. PSTVd symptoms of tomato plant (*Money maker* cv. (left) and *H5656* cv. *Standard* cultivar (right)). Control plant represents the uninfected plants.



Figure 8.
The symptoms of CEVd (*Citrus exocortis pospiviroid*) (Indian tomato bunchy top viroid). CEVd symptoms of *Gynura aurantiaca* indicator plant and tomato plant (H5656 cv. Standart cultivar). Control plant represents the uninfected plants.



Figure 9.
The symptoms of PSTVd (A) and CEVd (B) in *S. lycopersicum* L. (Hünkar cv.) and *C. annuum* L. (Sunam F1 cv.) plants.

by the bud [87]. In 1972, this factor was determined to be a viroid [88]. The agent is classified as a *Citrus exocortis viroid* (CEVd) species in the *Pospiviroid* genus of the family Pospiviroidae. CEVd is one of the best characterized viroids today. Exocortis disease is called citrus dwarfing viroid disease in our country. CEVd can cause scaling in the bark tissue of citrus trees, peeling and general stunting of the plant [89, 90]. Decreased growth, stunting may occur, chlorosis in leaves may become more severe, turning into reddening, bruising, and/or necrosis (Figures 8 and 9).

6.3 *Columnnea latent viroid*

Columnnea latent viroid (CLVd) agent was first detected in the *Columnnea erythrophaea* plant in the US state of Maryland in 1989, and it was stated that the agent was present asymptotically in this plant [91] but it was later determined that it

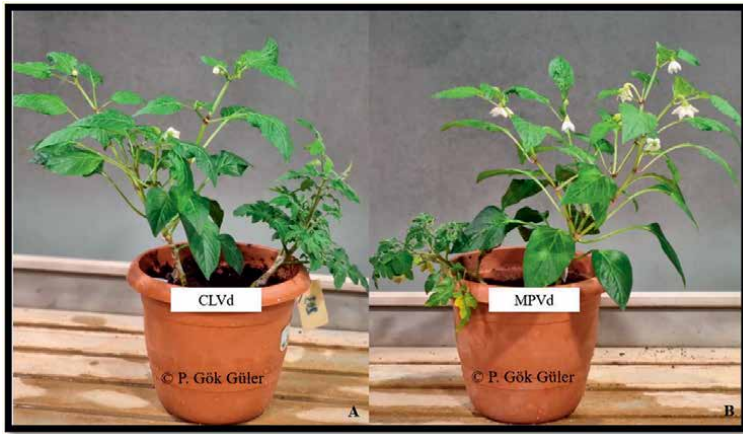


Figure 10. The symptoms of CLVd in pepper plants. CLVd (A) and Mexican papita viroid (MPVd) (B) symptoms of *S. lycopersicum* L. (Hünkar cv.) and *C. annuum* L. (Sunam F1 cv.) plant.

produced PSTVd-like symptoms in potatoes and tomatoes [92]. The agent is *Brunfelsia* spp., *Columnnea* spp., *Gloxina* spp. and *Nematanthus* species are generally asymptomatic (latent) in ornamental plants [93, 94]. Both PSTVd and MPVd were found naturally in wild *Solanum* species [95]. In tomatoes, CLVd can cause general stunting, deterioration of leaf structure, formation of thin-stemmed plants, tanning of leaves, chlorosis and leaf epinasticity, as well as necrosis of leaves, stems, and petioles (**Figure 10A**).

6.4 Mexican papita viroid

The MPVd agent was first identified in 1996 in the plant *Solanum cardiophyllum*, a wild solanum species in Mexico [95]. The symptom caused by MPVd in plants is observed as a general stunting and the formation of chlorotic and purplish spots on the leaves (**Figure 10B**). Depending on the severity of the infection, the fruit size decreases and/or no fruit is formed. There are uncertainties about how the agent is transported. The sequence of MPVd was determined to be very similar to that of TPMVd (93%) and PSTVd [95].

6.5 Tomato apical stunt

Tomato apical stunt (TASVd) causes severe symptoms in tomato plants shortening of the internodes, leaf deformation and yellowing, shrinkage, and less coloration of fruits (**Figure 11A**). TASVd has been reported in the Ivory Coast, Tunisia [96], Senegal [97]. TASVd has also been detected asymptotically in some ornamental plants (e.g., *Brugmansia*, *Cestrum*, *Solanum jasminoides*, *S. rantonetii*, *Streptosolen jamesonii*). TASVd is transported by seed, by plant sap during mechanical processes (during pruning, etc.). While it is not carried by pests such as *M. persicae* and *B. tabaci*, it is carried with pollen with the help of bumblebees during pollination. There is insufficient data on the geographical distribution, host range and epidemiology of TASVd, and control of viroids is difficult in practice [98].

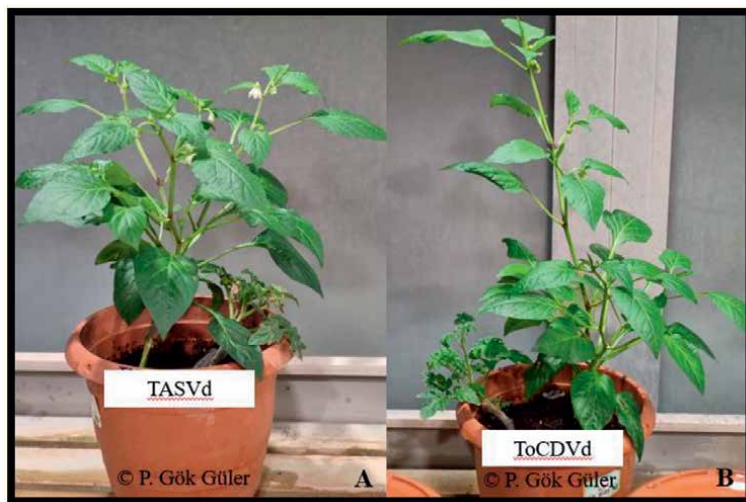


Figure 11.
The symptoms of *TASVd* in plants. *TASVd* (A) and *ToCDVd* (B) symptoms of *S. lycopersicum* L. (*Hünkar* cv.) and *C. annuum* L. (*Sunam F1* cv.) plant.

6.6 Tomato chlorotic dwarf viroid

Tomato chlorotic dwarf viroid (TCDVd) agent was first detected in 1996 in a tomato greenhouse in Manitoba, Canada [99]. As the hosts of the agent; *Brugmansia* spp. and hybrids, *Petunia* spp., *Solanum melongena*, *Verbena* spp., and *Vinca minor* plants have been reported. The agent has been found in Arizona and Hawaii [100, 101], India [102], Slovenia [103]. It has caused disease in tomatoes grown in greenhouses in [104]. General stunting, curling of leaves, chlorosis that may turn bronze or purple in later periods (**Figure 11B**), necrosis in petioles and veins, leaf epinasticity, apical bunching, small It causes losses in total yield with the appearance of cracked fruit formations [105].

6.7 *Tomato planta macho viroid*

Tomato planta macho viroid (TPMVd) agent was first detected in the tomato state of Morelos, Mexico, in 1982 [106]. Seven species in the Solanaceae family have been reported as natural hosts of TPMVd to date. Since the fruits of the infected plants are in the size of balls and they are completely unmarketable, great commercial losses have been experienced. Although this factor was initially thought to be a viral disease, it was later determined to have a viroid etiology [107–109]. In infected tomato plants, the first symptoms begin 10–15 days after the infection as growth cessation. Chlorosis, epinasty, wrinkling, wrinkling are seen on the leaves and the leaves become brittle. Later, the leaves shrink and turn yellow and stand upright. Although excessive and early fruit formation is seen, the fruits remain small. No seeds are formed in the fruit or fruits with very few seeds are formed. In general, severe stunting is observed in the plant and the fruits may lose their market value. The main symptom occurring within the cells is necrosis caused by the collapse of the phloem [106]. TPMVd affects plant growth (**Figure 12**). It has been reported that the agent can be transmitted mechanically and by the vector *M. persicae*, but there is no conclusive evidence of seed transmission [110].



Figure 12. The effect of TPMVd on plants. TPMVd (A (60 days), B (21 days)) symptoms of *S. lycopersicum* L. (Hünkar cv.) and *C. annuum* L. (Sunam F1 cv.) plant.

7. Plant resistance to pathogens

Many devastating diseases widely distribute throughout the world in tomato-growing areas and tomato hosts more than 200 species of pests and pathogens [111]. Bacterial canker caused by seed-borne organism *Clavibacter michiganensis* subsp. *michiganensis* (CMM) is a destructive disease in both field and protected cultivation of tomato crops. *S. hirsutum*, *S. peruvianum*, *S. pimpinellifolium*, and *S. chilense* are the wild relatives to improve resistance source of *S. lycopersicum* [112–115]. Inheritance of the resistance was controlled by four-gene model [116]. Inheritance of the CMM resistance in wild relatives has been explained by at least four genes [117] and quantitative trait loci (QTL) associated with resistance in interspecific cross [118]. Two major loci Rcm 2.0 and Rcm 5.1 introgressed from LA407 (*S. hirsutum*) have been identified on second and fifth chromosome and explained epistatically 68% of the variation [119].

Whitefly transmitted tomato yellow leaf curl virus (TYLCV Genus *Begomovirus*, Family Geminiviridae) has been threatened to tomato production throughout the temperate regions of the world since 1930s [120]. TYLCV and/or TYLCV-like viruses have many strains and genomic recombinants causing similar symptoms [121]. TYLCV-resistant tomato breeding program was initiated in Israel where first symptoms were observed in the world [122]. TY-20 has been improved as the first hybrid variety resistant to TYLCV from *S. peruvianum* (line M-60) and *S. lycopersicum* (line 10) [123]. Cucumber mosaic virus (CMV) has been divided into subgroups (I and II) and generates stunting, filiform leaves, and necrosis. A single dominant resistance gene *Com* derived from chromosome 12 of *S. chilense* accession (LA458) contributes complete or partial resistance to cultivars [124]. Potato virus Y (PVY) and tobacco etch virus (TEV) are two of main viruses belonging potyviridae transmitted by many species of aphids infect to tomato plants. The recessive gene *pot-1* sourced from PI 247087 contributes resistance by single recessive genes both TEV and PVY [125, 126]. ToMV and TMV are named synonymously vice versa. Three dominant resistance genes *Tm-1*, *Tm2*, and *Tm22* are used to improve resistant varieties derived from PI 235673 (*S. lycopersicum*) [127], PI 126926 (*S. peruvianum*) [128], and PI 128650 (*S. peruvianum*) [129], respectively. *S. peruvianum* is the wild relative used as genetic resource for resistance to *Meloidogyne* spp. Resistance is conferred by a single eight

Mi-1 to Mi-8 dominant gene located on chromosome 6 and 12, controls *M. incognita*, *M. arenaria*, and *M. javanica* [130]. Resistance sources to *Meloidogyne* spp. are PI128657 (Mi or Mi-1), PI270435-2R2 (Mi-2) PI126443-1MH (Mi-3), LA1708-1 (Mi-4) PI126443-1MH (Mi-5), PI270435-3MH (Mi-6 and Mi-7) PI270435-2R2 (Mi-8). Mi-3, Mi-7 and Mi-8 genes confer resistance to virulent strain *M. incognita* 557R. Nematode resistance is heat-sensitive in tomato. Mi-4, Mi-5, and Mi-6 genes contribute resistance over 30°C. LA2884 (*S. chilense*) line has heat stable resistance [131]. Potato spindle tuber viroid (PSTVd), tomato chlorotic dwarf viroid, citrus exocortis viroid, Columnea latent viroid, TASVd, tomato planta macho viroid (including Mexican papita viroid), and pepper chat fruit viroid have been identified as causal agents of pospiviroids in tomato. There is no commercial variety resisting to pospiviroids [132]. Potato spindle tuber viroid (PSTVd) causes yield loss, plant stunting, leaf chlorosis, smaller fruits. It is one of the most prevalent viroid species attacked to tomato plants. Four accessions belonging *S. chilense* and *S. habrochaites* have been reported less than 50% of PSTVd infection [133]. *S. pimpinellifolium* (LA0373, LA0411) and *S. chmielewskii* (LA1028) plants reported highly tolerant to PSTVd [134].

8. Conclusions

Plant-pathogens and pests are significantly important and cause an immense amount of crop losses worldwide. Plant-parasitic nematodes, insects, bacteria, viroid, and viruses damage crops at a high rate. Some groups of those diseases and pests parasitize the specific host plant, while others are polyphagous. Identification of plant-parasitic nematodes, insects, bacteria, viroid, and viruses and determination of the parasitism mode of action are important in terms of controlling pests and disease. Plant pathogens and pests show very different symptoms in plants, for example, root knot nematodes cause galls, bacteria cause color changes in plant stems and roots, viruses and viroids cause color changes and deformities in plants. The species of some insects that cause not only their own damage, but also secondary damages due to the fact that some of them carry viruses (for instance *M. persicae* is the vector of numerous viruses). Therefore, in order to grow disease-free plants, it has to be protected of healthy plants from plant-pathogens and pests. In controlling diseases and pests, it is important to have a deep understanding of the host-parasite interactions using cutting-edge technology and techniques. It is also crucially significant for future studies to fully understand host parasite interactions at morphological, molecular, and genetics level.

Conflict of interest

The authors declare no conflict of interest.

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† Authors contributed equally to this work. Plant parasitic nematodes (by R Bozbuga and M Imren), insects (by PA Kara), viroid diseases (by PG Guler), bacterial diseases (by HN Yildiz), plant resistance to pathogens (by BB Arpaci), virus diseases (by SY Ates) in tomato plants are written in this book chapter.

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Viral Diseases of Tomato – Origins, Impact, and Future Prospects with a Focus on Tomato Spotted Wilt Virus and Tomato Yellow Leaf Curl Virus

Stephen F. Hanson

Abstract

Tomatoes are affected by a number of viruses, with tomato spotted wilt virus (TSWV) and tomato yellow leaf curl virus (TYLCV) being two of the most damaging. TSWV and TYLCV have severely impacted tomato production worldwide for the past several decades at levels that led to both of these viruses being included in the list of top ten most important plant viruses. While they were first described in the early 1900s, both of these viruses emerged in the 1980s to become the severe and persistent problems they are today. The emergence of both viruses was facilitated in part by the emergence and expansion of more efficient insect vectors. Natural sources of resistance, especially from wild relatives of tomato, have provided some measure of control for both viruses to date. This chapter summarizes the origins, emergence, and impacts of these viruses, along with current approaches and future prospects for control, including both natural and engineered resistance.

Keywords: tomato spotted wilt virus, TSWV, tomato yellow leaf curl virus, TYLCV, RNAi, SIGS, spray-induced gene silencing, RNA interference

1. Introduction

Tomato (*Solanum lycopersicum*) is a member of the Solanaceae family of plants that also includes potato, chili and bell peppers, and eggplant. Tomato is a ubiquitous crop produced worldwide for a variety of uses ranging from high value fresh fruit to use in a variety of processed products including ketchup, pastes, soups and stews, and canned pasta sauces. Tomatoes are grown under a variety of conditions including open fields, plastic or green houses, screenhouses, and indoor growth rooms.

Tomatoes are one of the most important vegetable crops in the world, valued for both their flavor and nutritional qualities including being rich in vitamins A and C as well as minerals like calcium, potassium, and phosphorus. According to FAO statistics, tomatoes are the most widely produced vegetable, with production levels

of ~170 million tons annually and accounting for ~16% of all vegetable production worldwide [1] coming from ~5 million cultivated hectares. Tomato production has been steadily increasing over recent decades, with China, the US, and India being the largest producers.

Tomato was likely domesticated by indigenous peoples in Mexico and became an important vegetable crop in Central America prior to the arrival of Europeans. Tomatoes were first introduced to Europe by conquistadors returning from the Americas then spread across Europe and the Spanish empire. Tomatoes spread quickly around the globe and even reached China during the 16th century [2].

Tomatoes are affected by many diseases, like all domesticated crops that have been extensively bred and grown in high-density monoculture. Diseases affecting tomato include those caused by bacteria, fungi, viruses, and nematodes. Viruses cause some of the most consistent and severe losses of tomato crops (reviewed in [3–5]). This chapter will focus on two viruses that have caused serious problems in tomato production for several decades, tomato spotted wilt virus (TSWV) and tomato yellow leaf curl (TYLCV). Both of these viruses were included in the top ten most damaging plant viruses, with TSWV and TYLCV occupying the second and third spots on the list, respectively [6].

TSWV and TYLCV provide interesting contrasts on a number of levels including genome structure (RNA for TSWV and DNA for TYLCV), the origin of TSWV appearing to have been disseminated around the globe along with tomatoes and/or peppers, while TYLCV has emerged more recently and its spread has been partly facilitated by humans; TSWV has an extremely broad host range that includes plants and animals, while the host range of TYLCV is much more limited. These two viruses also share some common themes including the role of insect transmission in their emergence as leading pathogens, the strong potential for natural resistance to play a role in controlling damage, and the potential for biotech/genetic engineering solutions to reduce damage caused by these viruses. This chapter will examine some of the commonalities and differences between TSWV and TYLCV as well as current and potential future prospects for control of these highly damaging pathogens.

2. Tomato spotted wilt virus background

TSWV causes severe losses in tomato and many other crops worldwide. Symptoms of TSWV in tomato include spotting, often ring spots, and uneven ripening that renders the fruit unmarketable, along with bronzing and wilting of vegetative tissue (**Figure 1**). The first known report of spotted wilt disease on tomatoes was in 1915 in Australia [7]. This spotted wilt disease was shown to be thrips transmitted in 1927 [8] and attributed to a virus in 1930 [9]. TSWV was subsequently reported in various regions around the globe, including Hawaii and Europe, where it appeared sporadically for several decades until emerging as a more regular and profound problem in the 1980s. Since that time, TSWV has become one of the most damaging plant viruses in the world, being cited for regularly causing over \$1 billion in annual crop losses worldwide since the mid-1990s [10] and being recognized as the second most damaging plant virus in the world [6].

TSWV is a member of the *Tospovirus* genus within the family *Bunyaviridae*. TSWV virions are pleomorphic pseudo-spherical, with a diameter ranging from ~70 to 120 nm, and are enveloped in a host-derived membrane [11]. The RNA genome segments inside the envelope are encapsidated in N protein [12]. The virions also



Figure 1. *Tomato spotted wilt on tomato and chili pepper fruit. Typical symptoms of TSWV, including uneven ripening and spotting of fruit on tomato (left) and chili pepper (right).*

contain the L protein, which is the viral RNA-dependent polymerase [13]. TSWV is mechanically transmissible to most plant species it infects, and plants can be infected with either virions or membrane-free ribonucleoprotein complexes that contain the N protein-encapsidated genome segments [14].

Tospoviruses have a tripartite negative sense (or ambi-sense) genome (**Figure 2**). The three genomic RNAs are designated by size as large (L), medium (M), and small (S) RNAs. The L RNA have an entirely negative sense, while the M and S RNAs have ambi-sense and encode genes in both the viral genome sense and viral complement senses [15]. The TSWV genome codes for five proteins overall [16]. The L protein is coded on the viral or negative sense on the L RNA and is the viral RNA-dependent polymerase [13, 17]. The M RNA has ambi-sense and codes for the NSm protein in the genome sense and the polyprotein that is processed into the two structural glycoproteins in the genome complement sense. The non-structural protein NSm has been shown to promote cell to cell and long-distance movement during infection [16, 18]. The glycoproteins were formerly referred to as G1 and G2 but are now denoted as Gn and Gc, indicating their N- or C-terminal location in the precursor polyprotein. The glycoproteins decorate the surface of the virions and are required for thrips transmission [19, 20]. The ambi-sense S RNA codes for the nonstructural protein NSs in the genome sense and the N protein in the genome complement sense. The NSs protein promotes thrips transmission and also functions as a suppressor of RNA silencing

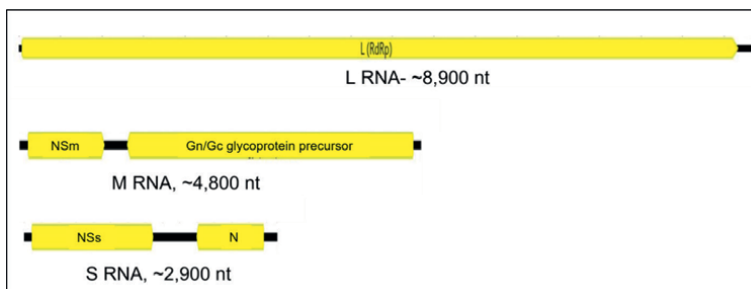


Figure 2. *Genome structure of TSWV. Cartoon representation of the tripartite TSWV genome showing the L, M, and S RNAs approximately to scale. Yellow boxes show positions of open reading frames in the genomic (L, NSm, NSs) and genome complement (glycoprotein precursor and N) senses.*

[21, 22], while the N protein is the nucleocapsid that encapsidates viral RNA to form RNPs [23]. The N protein is also required for local spread, suggesting that RNPs may be the functional viral unit involved in local spread [24].

Reverse genetic systems have been a valuable tool that enabled in vitro infection from cloned cDNA and DNA copies of plant virus genomes, mutational analysis of virus genes, evaluation of chimeric viruses, and more. Unfortunately, reverse genetics systems have been unavailable or difficult to develop for viruses with negative or ambi-sense genomes, including TSWV. The recently reported rescue of TSWV from cloned cDNAs is an exciting step forward that will enable reverse genetic analysis of TSWV to TSWV researchers [25].

TSWV has an extremely broad host range and is a rare case of a virus that infects hosts in two different kingdoms as it replicates in both plants and its thrips vector [26]. This observation led to the speculation that TSWV may be a thrips-infecting virus that evolved to also infect plants, which may partially explain its severity as a plant virus. For plants, the host range of TSWV includes over 1000 different plant species in 82 botanical families encompassing both monocotyledonous and dicotyledonous plants [27]. This extremely broad host range likely contributes to TSWV disease persistence since there is a high likelihood that alternate hosts will be present even when susceptible crops are not being grown.

TSWV is transmitted by at least 10 different species of thrips with *Frankliniella occidentalis*, commonly known as the western flower thrips, being the most efficient vector species [28, 29]. Transmission is circulative and propagative [30, 31]. While adult thrips can acquire TSWV, they are unable to transmit it, and transmission only occurs when thrips acquire TSWV as first- or second-stage larva [29, 32, 33]. While adult thrips can acquire TSWV, they are unable to transmit; thus, the acquisition of TSWV by adult thrips is a dead end for TSWV. Thrips larvae can acquire TSWV with acquisition access periods as short as 15 min although transmission efficiency increases with longer acquisition access periods, and an acquisition period of 4 days was reported to result in 74% of emerging adult thrips being competent for TSWV transmission [34]. Thrips that acquire TSWV remain infected and able to transmit TSWV for life due to the circulative propagative nature of transmission.

TSWV is thought to be acquired by thrips via an animal virus-like receptor-mediated interaction that is rare among plant viruses. The demonstration that a truncated soluble form of the TSWV glycoprotein Gn interferes with thrips transmission of TSWV, presumably by blocking TSWV receptors in the thrips midgut, suggests that the glycoproteins are the viral proteins that mediate virion acquisition [35]. Identification of thrips receptors for TSWV has been an area of interest since it may lead to strategies for blocking thrips transmission of TSWV. While early reports of thrips proteins that interact with TSWV [36] generated some excitement, these initial leads appear to have been dead ends (S. Hanson, unpublished). More recent work has identified different thrips proteins that interact with TSWV virions or glycoproteins and are therefore promising candidates for receptors that mediate TSWV acquisition in thrips [37].

TSWV was described as occurring in many different parts of the world going back to the mid-1900s. This worldwide distribution as a minor pathogen before emergence as one of the most damaging agricultural viruses suggests that TSWV may have spread around the world with host plants like tomato and pepper as they were brought back from meso-America and subsequently spread around the globe. Molecular phylogeny studies that have shown that TSWV often exists as a stable populations in geographically isolated regions and may have spread around the world

with tomatoes and/or peppers when these plants were introduced to Europe and beyond by Spanish explorers returning from the Americas [38]. The emergence of TSWV as a more widespread and damaging disease started in the 1980s, likely due to the spread of the more efficient western flower thrips vector into areas that were already infested with TSWV.

3. TYLCV background

Serious outbreaks of tomato yellow leaf curl disease were reported in the late 1920s in the Jordan Valley [39]. Typical symptoms of TYLCD include mosaic chlorosis and stunting of affected plants (**Figure 3**). Since then, numerous outbreaks of TYLCD happened around the Mediterranean in the 1960s. From there, it spread throughout the Middle East to Central Asia, Africa, and the Americas. TYLCV is now considered to be ubiquitous across tropical, subtropical, and temperate regions [40]. During the 1980s, outbreaks of TYLCV became more common and widespread, with some being noted as causing up to 100% loss in affected areas of Italy and the Dominican Republic [41, 42]. All of this led to TYLCV being recognized as one of the most severe viral pathogens of tomato worldwide [43, 44] and to TYLCV being ranked the third most important plant virus in the world [6].

TYLCV is a member of the geminiviridae family, characterized as having single stranded genomes that replicate via a rolling circle type of mechanism and unique twinned icosahedral capsids (reviewed in [45]). There are nine recognized genera within the geminiviridae, and TYLCV is part of the begomovirus genus, which is characterized as being transmitted by whiteflies and infecting dicot plants [46]. The large number of individual viruses within the begomovirus genus has led to several revisions for how groupings are determined and individual viruses are named within this group [47, 48]. The begomovirus genus contains numerous distinct tomato-infecting members, with the TYLCV subgroup being recognized as one of the most damaging to agriculture [47]. With so many closely related members, the TYLCV subgroup is often treated as a complex of closely related strains that are individually identified by including the location where the strain was recognized in the name, such as for tomato yellow leaf curl sardinia virus denoted as TYLCSV (recent listing in table 1 of [47]). In addition to the large number of strains identified to date, mixed infections that produce recombinant/chimeric variants are believed to happen frequently [49].



Figure 3. TYLCV symptoms. Typical symptoms of TYLCV on tomato, including stunted plants (left) and mosaic chlorosis (right).

TYLCV was the first member of the begomovirus genus with a monopartite genome, with most begomoviruses having bipartite genomes (**Figure 4**). The genome of TYLCV is ~2.7 Kb and codes for genes in both the viral and complementary senses [50]. The relatively small and simple nature of geminivirus genomes has facilitated extensive reverse genetic analysis via infectious DNA clones that have been obtained for many geminiviruses including TYLCV.

The viral sense codes for two open reading frames (ORFs), with V1 encoding the capsid protein and V2 coding for a multifunctional protein that functions to both facilitate movement and suppress RNA silencing [51, 52]. The genome complementary sense strand encodes four overlapping ORFs that have broad functions in viral replication, transcription, and host interactions. The C1 ORF encodes the replication-associated protein that contains ATPase and DNA nicking domains [53]. The C1 protein promotes rolling circle replication directly by initiating and terminating rolling circle replication via DNA nicking and ligase activities and indirectly by recruiting host factors involved in viral DNA replication. The C2 ORF codes for a transcriptional activator protein (TrAP) that regulates early and late gene expression. The C3 ORF codes for a replication enhancer protein (Ren). The C4 ORF is involved in symptom development and movement [54]. Like all geminiviruses, TYLCV contains a large intergenic region that facilitates bidirectional transcription and contains the origin of replication, including a requisite stem-loop sequence, where rolling circle replication begins and ends.

TYLCV, like all begomoviruses, is transmitted by whiteflies (*Bemisia tabaci*) in a circulative manner (reviewed in [55]). Acquisition and inoculation can both

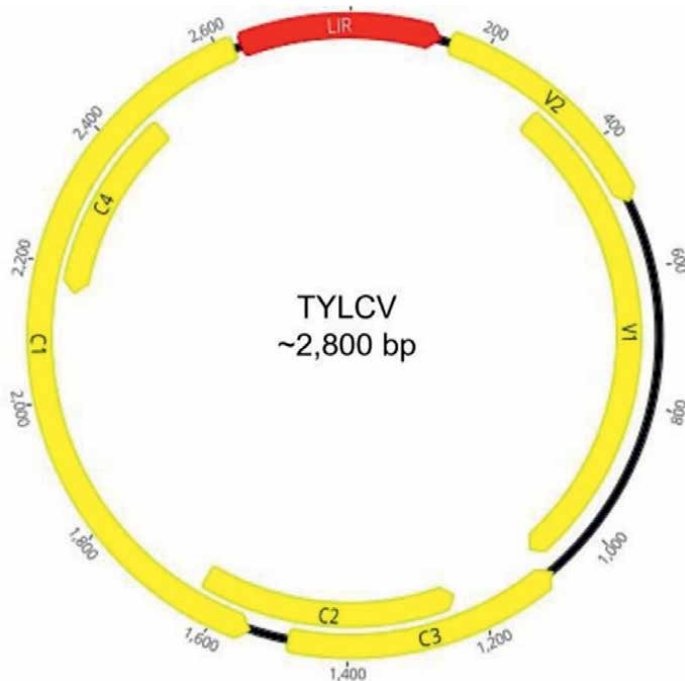


Figure 4. TYLCV genome. Cartoon representation of the circular ssDNA genome of TYLCV. Open reading frames are shown in yellow. The large intergenic region containing the origin of replication and bidirectional promoters is shown in red.

be as quick as 15 minutes. While several *B. tabaci* biotypes are able to transmit geminiviruses, the emergence and spread of the B biotype that is highly efficient at transmitting geminiviruses played a key role in increasing the spread and severity of geminivirus diseases, including TYLCV, that started in the 1980s.

4. Management of TSWV and TYLCV

In spite of many differences in virus biology, the factors that lead to the emergence of these viruses and measures for control share a lot of commonalities. A great deal of work has gone into reducing losses caused by TSWV and TYLCV over the past few decades, with some promising advances, although much remains to be done as both viruses still cause extensive losses in tomatoes at present. Standard IPM-based practices, especially those that limit insect vectors, are widely used for controlling both TSWV and TYLCV [56–58]. Although these IPM-based approaches can produce modest reductions in disease, they are not able to prevent all diseases. Breeding for disease resistance has shown some success for both TSWV and TYLCV, and thus, resistance breeding programs are likely to continue as a focus into the future. While not broadly adopted at present, genetic engineering (GE) has shown great potential for controlling both TSWV and TYLCV. The high cost of developing GE lines, extensive regulatory requirements, and concerns about consumer acceptance of GE crops have severely limited the adoption of these approaches for control of diseases in agricultural crops (reviewed in [59]). Thus, GE-based approaches hold great promise for controlling diseases if GE crops become more widely accepted for use in agriculture.

Since the emergence and expansion of more efficient vector species was a major driver in increasing damage caused by these viruses, a number of approaches, especially integrated disease management approaches, have focused on reducing populations of insect vectors or managing production aspects like time of planting to reduce exposure of plants to viruliferous insect populations [58, 60]. Strategies based on insect vector control remain challenging for several reasons, including the lack of effective insecticides, the rapid evolution of insecticide resistance, the fact that both thrips and whiteflies are successful on a number of alternate hosts, and very quick transmission when viruliferous insects enter agricultural fields.

5. Genetic resistance

Natural resistance has been a highly successful and long-relied-upon strategy for controlling many plant pathogens. Often, wild relatives are found to contain sources of resistance that can be introgressed back into domesticated lines where resistance has been lost.

Several resistance genes have been described for TSWV. These include the single dominant R genes like the Tsw gene from *Capsicum chinense* and the Sw-5 from *Lycopersicon peruvianum* that have provided commercially useful resistance to TSWV in tomato [61–63]. Both of these genes confer a typical hypersensitive response (HR)-based resistance that usually prevents systemic infection by stopping pathogens at the site of inoculation [64]. Molecular studies on TSWV strains with re-assorted genomes were used to determine that the NSm gene is the avirulence determinant recognized by the Sw-5 gene [65].

Natural resistance genes have also been described for several geminiviruses, with many of the resistance genes coming from non-domesticated relatives (reviewed in [66]). This is especially true for TYLCV [66]. The tomato relative *Solanum chilense* is noted as the most common source of TYLCV resistance genes identified to date [66]. At least twelve different sources of resistance to TYLCV were described as of 2020 (summarized in [67]). The Ty-2 gene appears to be a canonical R gene with typical nucleotide binding (NB) and leucine-rich repeat (LRR) regions [68], while others are clearly not classical R genes, but are rather genes involved in RNA metabolism, basic metabolism, cell status sensing, or signaling. The Ty-1 and Ty-3 resistance genes appear to be alleles of a gene [69] that encodes for RNA-dependent polymerase and cause increased cytosine methylation in replicated genomes [70]. Members of the WRKY group III transcription factors have been shown to play a role in TYLCV defense signaling [71]. Still other genes involve in hexose transport or other metabolic processes [72].

Unfortunately, single dominant R genes tend to have limited durability and are often overcome as pathogens evolve to escape the resistance. This is the case for many of the resistance genes described above. Resistance breaking strains of TSWV that overcome the Sw-5 genes have emerged several times independently in different areas including Europe, the US, and Australia [73–75]. Multiple independent cases of resistance breaking TSWV variants have also been reported for the Tsw genes [61, 76]. Resistance breaking has also been observed for several of the described TYLCV genes. Ty-2 mediated resistance was reported to be overcome by TYLCV-Sardinia [77] and an isolate of the mild strain of TYLCV [78]. The Ty-1 gene has been shown to be overcome occasionally under high disease pressure [79].

The generation of resistance breaking strains does not mean that R genes are not useful for control of TSWV and TYLCV. On the contrary, genetic resistance has proven to be one of the most effective tools for limiting TSWV and TYLCV losses to date. And the generation of resistance breaking strains is both typical and expected for any single dominant R gene against any evolving pathogen. For R genes to provide long-term utility, they need to be cycled through, with tomorrow's R genes being discovered while today's are in use. Fortunately, wild relatives of tomato appear to be a robust source for the discovery of new R genes that may be able to supply novel sources of genetic resistance to these viruses well into the future. This is evidenced by one recent study that has evaluated ~700 accessions derived from 13 wild tomato species, where ~140 of the lines were symptom free after inoculation with TYLCV [66]. Based on this, it is likely that wild species will continue to be a robust source of natural resistance genes that will help in reducing TSWV- and TYLCV-caused losses for the foreseeable future.

It should also be noted that while R genes are the most common form of resistance gene found historically, single dominant R genes are not the only type of genetic resistance to pathogens. There are several examples of multigenic resistance and tolerance that provide long-term stable reductions in pathogen losses. One current example is a multigenic field resistance that appears to be providing long-term durable control of TSWV in peanuts [80]. Sequence-level population analysis of multiple TSWV genes did not detect any resistance-related selection in TSWV populations, indicating that this multigenic resistance is likely to be durable. While this resistance appears to be based on high-level tolerance, it provides commercially useful control of TSWV in peanuts, a crop that suffered serious losses from TSWV prior to deployment of this

resistance. Future work using marker-assisted breeding and similar approaches may be useful for developing tomato lines with similar multigenic resistance to TSWV and TYLCV in the future.

6. Engineered resistance

Genetic engineering is an approach that has proven useful for developing resistance to many plant pathogens including many plant viruses. This is true for TSWV and TYLCV, where numerous approaches for creating engineered resistance have been reported over the past several decades. While several approaches have been described, gene silencing/RNAi approaches (reviewed in [81]) are the most common. Despite promising research results, genetically engineered virus resistance has not been widely adopted due to several barriers, including the high costs for the development of commercial lines approved for human consumption and public resistance to GMO crops (reviewed in [59]).

The first description of engineered resistance to TSWV was described in 1991 [82]. Since that time, several additional examples of engineered resistance to TSWV have been reported, including the use of chimeric RNAi-inducing genes that confer broad spectrum tospovirus resistance [83, 84]. Despite these promising results, engineered resistance to TSWV has yet to be deployed in commercial crops.

Numerous examples of engineered resistance have also been described for geminiviruses in general and TYLCV in particular (reviewed in [85]). Similar to TSWV, the first reports of engineered geminivirus resistance also date back to the 1990s, with many of these attempts using virus-derived resistance targeting the viral genes involved in replication, movement, or encapsidation [86–88]. Examples also include numerous descriptions of anti-sense RNA- and RNAi-based resistance. There are also some interesting examples of non-pathogen-derived resistance, including the use of peptide aptamers that interfere with the function of geminivirus replication-associated proteins that were found to confer high-level tolerance to several diverse begomoviruses, including TYLCV and tomato mottle virus [89]. Still other approaches have targeted host functions like those involved in modulating host defenses [90, 91]. Approaches that modulate host resistance responses have also shown promising results.

Geminiviruses are one rare example where engineered resistance has been approved and deployed in crops produced for human consumption [92]. In this case, common beans engineered to express an RNAi construct targeting the Rep gene of bean golden mosaic virus (BGMV) proved to be highly resistant to begomoviruses affecting bean production in Brazil [93]. The lack of natural resistance sources for BGMV, in spite of decades of screening, made engineered resistance an attractive alternative for BGMV. Extensive multi-year field testing showed that this gene effectively protected common beans from BGMV-caused losses, which had previously reduced yields by 40–100% [94]. So far, this resistance is only approved for use in Brazil. The effectiveness of this approach for controlling BGMV-caused losses, and similar levels of conservation among the Rep genes of TYLCV isolates, suggests that this approach has strong potential for controlling TYLCV-caused losses. While genetic engineering holds great promise for controlling TYLCV, the substantial barriers associated with development costs, regulatory approval, and consumer acceptance must still be overcome before engineered resistance approaches can be broadly utilized.

The time, cost, and consumer acceptance barriers to deploying genetically engineered resistance in crop plants intended for human consumption have spurred innovation aimed at producing similar resistance mechanisms without using transgenic plants. Promising approaches in this area include the use of exogenous double-stranded RNAs that are sprayed on plants to induce an RNAi response in a process referred to as spray-induced gene silencing (SIGS; [95]). SIGS has shown promise against several viral pathogens including TSWV [96]. Another similar approach uses endophytic bacteria engineered to express dsRNAs that can induce an RNAi response in plants. This bacterial-mediated RNAi, sometimes referred to as transkingdom RNAi, has shown promise in reducing infection by fungal and viral plant pathogens [97]. It will be interesting to see if SIGS or transkingdom RNAi evolve into useful technologies that provide control of plant pathogens while successfully skirting the barriers that have prevented more widespread adoption of genetically engineered approaches for control of plant pathogens like TSWV and TYLCV.

7. Summary

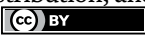
Tomatoes are the most widely produced vegetable on earth, and viruses have been a persistent problem in tomato production for as long as tomato has been cultivated as a crop. TSWV and TYLCV have been serious yield-limiting constraints on tomato production for the past several decades. Tried and true practices like traditional resistance breeding and integrated disease management have allowed continued production of tomatoes in spite of the severe losses these viruses can cause. It is likely that both of these viruses will be better controlled in the future based on the rich body of knowledge developed to date for these viruses. In particular, the abundance of natural resistance sources that are known to be present in wild relatives will continue to be a valuable source of natural resistance genes. Biotech is also likely to play a bigger role in the future on several levels. Marker-assisted breeding and other related approaches will speed introgression of natural resistance resources into commercial cultivars. And if (or when) the cost and societal acceptance barriers are reduced, approaches like engineered resistance and technologies like SIGS are certain to reduce virus caused losses.

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Chapter 8

Management of Branched Broomrape in Field Processing Tomato Crop

Francesco Lops, Laura Frabboni, Antonia Carlucci, Annalisa Tarantino, Maria Luisa Raimondo and Grazia Disciglio

Abstract

In recent years, there has been a considerable increase in land area used for tomato (*Lycopersicon esculentum* Mill.) in many countries around the world. The essential role is played by Italy at a worldwide level as the country with the third biggest production of tomatoes for processing. *Phelipanche ramosa* (L) Pomel, commonly known as branched broomrape, is a root holoparasitic weed for many crops, particularly for the processing tomato. Due to its physical and metabolic overlap with the crop, its underground parasitism, and hardly destructible seed bank, the control of this parasite in the field is difficult. Results of research studies, many of them on environmental-friendly methods such as preventive, agronomic, and biological carried out in southern Italy, are discussed and summarized. The results can constitute a relevant basis for further experimental studies.

Keywords: orobanche, *Phelipanche ramosa*, control methods, processing tomato crop, cultural practices

1. Introduction

Tomato (*Lycopersicon esculentum* Mill.) is the vegetable crop with the highest demand and the greatest economic value in the world. Tomato trade and production have particular importance in tropical, subtropical, and mild regions of the world, for both fresh and processing markets [1]. In recent years, there has been a considerable increase in the world land area used for tomato production. The essential role is played by Italy at a worldwide level as the country with the third biggest production of tomatoes for processing after the United States and China. The 2021 tomato processing campaign in Italy closed with a production of just over 6 million tons of processed product, up 17% compared with 2020. Italy's production is 13% of the world's and 53% of Europe [2].

In Italy, as in other areas of the world [3, 4], and especially in the Mediterranean basin, the tomato crop and other species (broccoli, fennel, parsley, celery, and chamomile) are undergoing increased attack of a holoparasitic plant with obligate

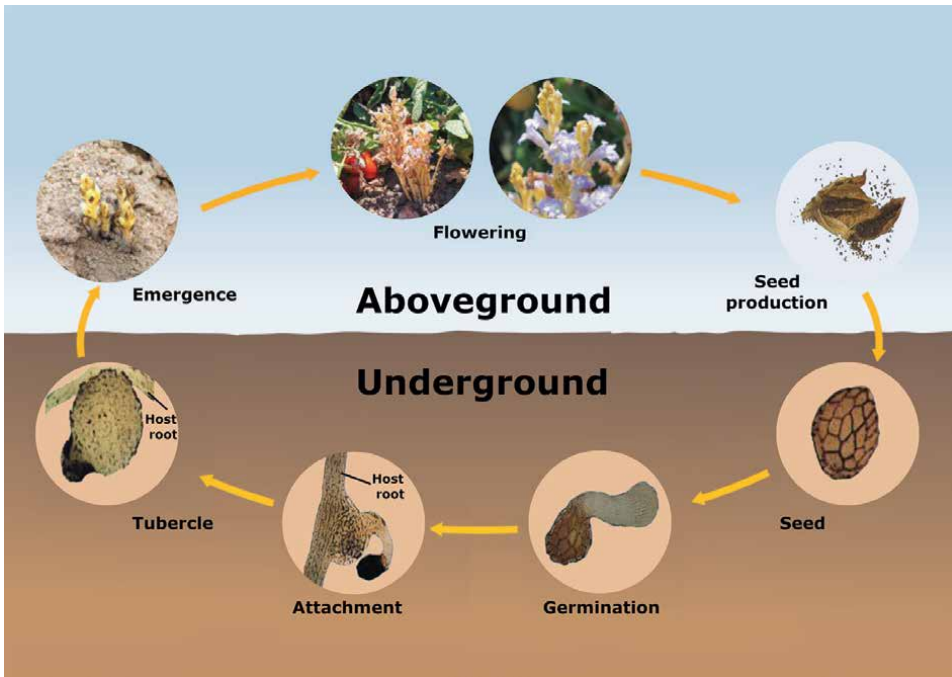


Figure 1. A summarized life cycle of a branched broomrape (from Osipitan et al., 2021) [6].

root belonging to the *Orobanchaceae* family, the *Phelipanche ramosa* (L.) Pomel (syn. *Orobanche ramosa* L.), commonly known as the branched broomrape. Tomato is highly vulnerable also to similar species, as the *Phelipanche aegyptiaca* Pomel (syn. *O. aegyptiaca*) and *O. cernua* Loefl., which are known to cause damage and yield reductions in this crop [5]. The broomrape seeds only germinate in response to specific chemicals (strigolactones) released by the host plant, and the plant spends most of its life cycle underground (**Figure 1**) [7, 8].

Following germination, the seedlings attach to the host roots by the production haustoria that penetrate the host tissues until they reach the vascular system for uptake of water and nutrients, assimilate, and grow at the expense of the host plant's resources [5]. *P. ramosa* attacks tomato roots early in the growing season, within 14–28 days after transplanting (DAT), depending on the temperature conditions, and the shoot usually emerges within 35–56 DAT [9]. Once connected to a host plant, broomrape grows rapidly, forming a tubercle (a storage organ for nutrients and water extracted from the host) underground. Multiple shoots (up to about 20) develop from the tubercle and emerge above the soil surface, and then grow to stalks from 15 cm to 30 cm in height (**Figures 2 and 3**). Flowering begins within 3–7 days after a broomrape shoot emerges above the soil surface. A mature broomrape plant can release more than 500,000 seeds (from 0.2 to 0.4 mm), which can remain dormant and viable for many years (> 20) in soil [5]. The number of emerged shoots per surface unit, and/or number and dry weight of parasitic plants per host plant, can be used as indicator to monitor *Phelipanche infestation* [10].

The air and soil temperature are the main factors that influence the dynamic of host/parasite interaction and development. Moreover, the optimum temperature for maximum germination of *Orobanche* seeds decreases as the level of their water stress increased [11].



Figure 2.
Branched P. ramosa plant (F. Lops).



Figure 3.
P. ramosa infestation in tomato (F. Lops).

The presence of the parasite causes a significant reduction in the photosynthetic capacity of tomatoes, as shown by the higher SPAD chlorophyll indices detected on the leaves of infested tomato crop compared with the non-infested one (**Figure 4**).

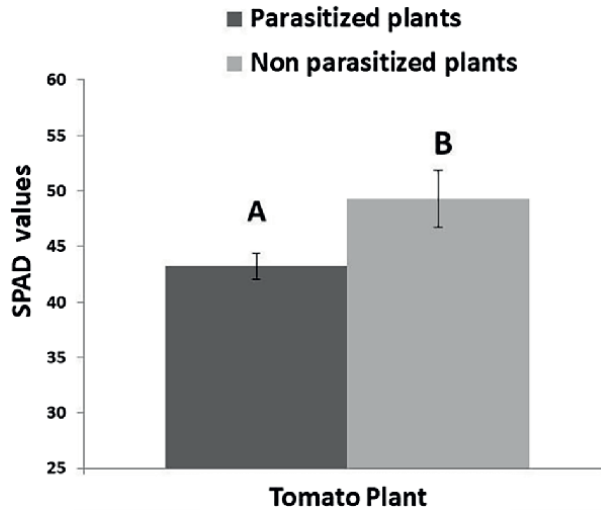


Figure 4. Average SPAD values \pm SD of parasitized and non-parasitized tomato plants, measured at 53 days after transplanting. Different letters indicate significant differences at $P < 0.05$ according to Tukey's test [12].

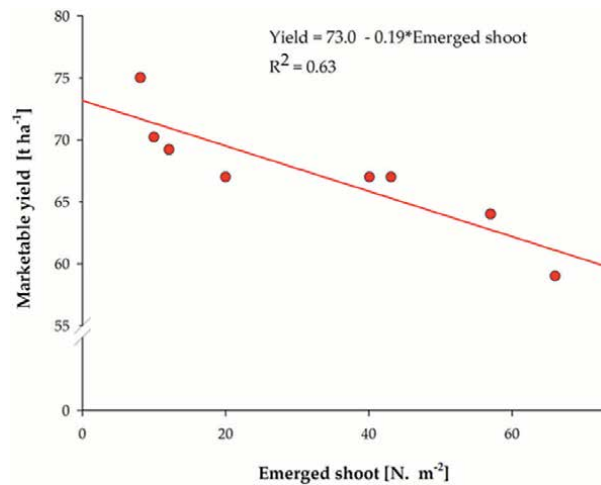


Figure 5. Relationship between tomato marketable yield and number of emerged branch shoots of *P. ramosa* detected at the end of tomato cycle (harvesting time) [14].

This generates a loss of biomass of their aerial organs [13] and a significant decrease in crop yield (**Figure 5**), mesocarp thickness, fruit color, compactness, content of soluble solids, of ashes, and of ascorbic acid [15].

2. Management of *P. ramosa* in the field

Effective control of *P. ramosa* is difficult because, as already mentioned, most of its life cycle occurs below the soil surface. Thus, the effective management of this parasitic weed will require a long-term and an integrated approach. Measures to

successfully contain the problems due to *P. ramosa* need to be targeted at: i) reduction of the existing *P. ramosa* seed bank in the soil; ii) prevention of further seed production; and iii) prevention of seed dissemination. These objectives are mutually dependent. Practices to control this parasite include several methods (preventive, chemical, agronomic, and biological), which help to avoid germination, infection, or strong reproduction of the weed [16, 17].

2.1 Prevention methods

Preventing the movement of parasitic weed from infested into un-infested areas or its spread in recently infested fields is a crucial component of control. Principal measures are to remove the *Orobanch*e prior to flower opening; the quarantine for a period of at least 2 years, and in subsequent years only rotational crops may be cultivated (e.g., in California, these crops are those approved by the local agricultural commissioner); clean and disinfect all equipment used in a field with broomrape infestation [6, 17]. As for seed eradication on farm equipment, quaternary ammonium compounds have been found effective in *Phelipanche* and *Orobanch*e spp. [18].

2.2 Chemical methods

Herbicides that currently are in use for parasitic weed broomrape control in various crops are sulfonyleurea and imidazolinones. Sulfonyleurea herbicides are absorbed through the host plant foliage and roots with rapid acropetal and basipetal translocation. Imidazolinone herbicides are absorbed and translocated through the host to the meristematic tissues. The most successful method to the parasite control in processing tomato is to apply sulfonyleurea herbicides, on foliage and by injection through the drip irrigation system in preplanting, or post-emergence, or post-planting [19]. Soil herbigation (saturating the soil with sulfonyleureas) effectively controls pre-attached stages of broomrapes [20], but this is hardly compatible with other agricultural cropping practices, as detrimental for many crop seedlings for several weeks or months. Applying sulfosulfuron to the soil three times, at 200, 400, and 600 growing degree days, followed by two applications of imazapic to the tomato foliage late in the season, effective Egyptian broomrape control has been achieved [21, 22]. In the conditions of southern Italy, the best parasite control and tomato yield performances were obtained with sulfonyleureas (rimsulfuron and chlorsulfuron) applied through drip irrigation in pretransplant at 25.0 and 5.0 g a.i. ha⁻¹, and in post-transplant at 75.0 and 15.0 g a.i. ha⁻¹, respectively [23].

2.3 Agronomic methods

In order to integrate the use of chemical methods, there has been an increased effort to research suitable methods (fertilization, soil solarization, long-term rotation, soil management, sowing, or transplanting date) for the control of this parasitic weed, even because there is an increasing market for organically grown tomatoes, where the use of chemical pesticides is not an option [24].

2.3.1 Fertilization

Broomrape infestations occur mainly in soils poor nitrogen (0.2 and 1.8 ‰) and organic matter (1–2%) such as many soils of southern Italy [25], where the Italian

research studies related in this chapter were carried out. Also, phosphate in deficient soil showed a suppressive effect of *P. ramosa* parasitism [26]. Therefore, soil fertility management can contribute to the management of this parasite. Phosphorous and nitrogen have been described to downregulate strigolactones exudation in some crop species [27–29].

Direct contact with fertilizer, such as urea and ammonium, may be toxic to broomrape, inhibiting seed germination and seedling growth [30]. Urea fertilizer, due to hydrolysis in soil, produces ammonium ion, which probably exerts the toxic effect on the parasite [31].

Nitrogen fertilizer ($80 \text{ kg ha}^{-1} \text{ N}$) or sulfur ($8 \text{ t ha}^{-1} \text{ S}$) applied prior to the tomato seedling transplant showed a suppressive effect on the seed germination of *Phelipanche* [32]. Also, the mixtures of chicken manure and sulfur significantly reduced the dry weight of *Orobanche* and increased eggplant and potato yield compared with the control [33].

Organic compounds are widely used in cropping systems to increase soil organic matter, structural stability, water holding and cation exchange capacities, and as a source of nutrients [34].

Recently, in the olive production and/or processing areas, as those of southern Italy, the use of oil mill wastewater (OMW) has been proposed as a suitable method for the containment of *P. ramosa*. In this regard, several trials dealing with the OMW distributed on the heavy infested soils at the dose of $80 \text{ m}^3 \text{ ha}^{-1}$, 40 days prior to tomato seedling transplant (**Figure 6**), and incorporated into the soil later, revealed a significant reduction (between 34 and 76%) of emerged *P. ramosa* plants with respect to the untreated control (**Figure 7**), limiting the additional seed production of this parasite [35]. This could be due to the organic and mineral compounds, as nitrogen, phosphorus, and potassium contained in the OMW, which could improve the nutrient status of the tomato plants in addition to the effects of phenols present in the OMW that could produce a reduction of *P. ramosa* seed germination [36–38]. Therefore, the tomato marketable yield showed a significantly higher value in the OMW treatment than the untreated control. No significant differences for the fruit qualitative characteristics were observed [35].



Figure 6. Mechanical distribution of OMW on the soil (F. Lops).

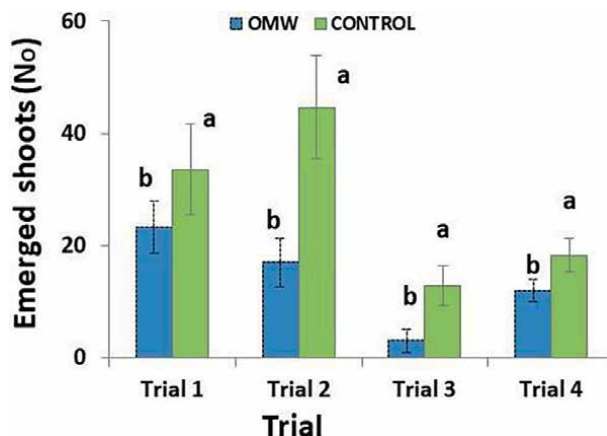


Figure 7. Average number per m^{-2} of *P. ramosa* for OMW and control at the time of the tomato harvest in the different trials. Different letters indicate significant differences at $P < 0.05$ according to Tukey's test [35].

Furthermore, in recent years, the use of organic fertilizers or “plant biostimulant” compounds has encountered increasing interest in agriculture because they play roles in various soil and plant functions [39]. Some of these compounds of natural origin, such as natural amino acids, were also suggested for use in *P. ramosa* management strategies being able to inhibit seed germination [40, 41]. Experimental results in Italy indicated that using the commercial product “Radicon®” (a suspension-solution containing humic substances), at the time of transplanting (immersing the root of the seedlings in a 1.5% solution), and incorporating it into the soil in the first 3 irrigation interventions, produced a reductions of 68.1% of emerged shoots in comparison with the untreated control. These substances introduced into the soil rhizosphere can cause severe physiological disorders of the germinating *P. ramosa* seeds, thus reducing the number of developing tubercles of the parasite [42].

2.3.2 Soil solarization

Solarization is used in many warm climate countries, as pre-tomato planting treatment. Its consists of heating the soil through sun energy achieving temperatures above 45°C , by covering a wet soil with transparent polyethylene sheets for a period of 4–8 weeks during the warmest season [43]. This method for the high cost per surface unit is not readily applicable at large scale [44]. Solarization may be more effective if combined with added nitrogen fertilizers as chicken manure [45].

2.3.3 Rotation

Decreasing the frequency of tomato cultivation prevents *P. ramosa* seed bank increases, maintaining the seed bank dormant and reducing the rate of seed bank replenishing. However, it is a long-term strategy due to the long viability of seed bank [16], which requires at least a nine-course rotation in order to prevent broomrape seed bank increases [46]. Its efficacy for broomrape cultural control can be increased including trap and/or catch crops as components in the rotation [16].

The trap crops are species (e.g., *Medicago sativa*, *Vigna unguiculata*, *Pisum sativum*, and *Linum usitatissimum*) whose root exudates induce broomrape seed germination,

but these species do not allow attachment or support broomrape seedling growth and survival [47].

Catch crops are host plants that support normal parasitism, but they are harvested as green vegetables after the parasite seeds germinated and before the flowering and seed dispersal stages of the parasite itself. For instance, *Brassica campestris* when managed properly as a catch crop can result in up to a 30% reduction in the size of broomrape seed bank [48].

2.3.4 Soil management

The soil tillage management must aim at reducing the seed bank, while minimizing the production of new seeds. In this regard, inversion plowing results in burial of a large proportion of seed in the tillage layer, carrying them at a depth from which they cannot germinate, although they remain viable in the deep soil for a long period of time [49, 50]. Deep plowing has been suggested to bring seeds of parasitic weeds to a depth with less oxygen availability and therefore a reduction in its germination capacity [51, 52]. Eizenberg et al., 2007 observed that the deep plowing ≥ 12 cm strongly reduced broomrape infection severity in terms of number of parasites, total parasitic biomass, delayed broomrape emergence and prevention of flower initiation, and seed set. Results of another study [53], carried out in two heavily infested fields in southern Italy, showed significant lower parasite attachments on tomato roots, the lower dry weight of emerged and underground-branched shoots per host plant in 50 cm deep plowing compared with 30-cm-deep plowing (**Table 1**).

2.3.5 Sowing or transplanting date

The air and soil temperature are the main factors influencing the dynamic of host/parasite interaction and development. Temperature is strongly connected with the climatic conditions, which are themselves related to the periods for crops seedling into the field. Delayed sowing is consistently reported to reduce infection of winter crops such as oilseed rape [30]. Also, in spring-summer crops such as sunflower, modified planting dates provided the indirect effect of temperature on *Orobanche* parasitism [54]. In this regard, a study by Kebreab et al., 1999 [55] reports that at supra-optimal temperatures for germination of *O. crenata* seeds (i.e., above 25°C), they will not

Field trials	Plowing depth (cm)	Total attachments (no)	Shoot (DW) (g)	Tubercles (g)
Field trial A	30	9.7 ± 2.4 a	56.9 ± 12.9 a	106.1 ± 11.8 a
	50	5.1 ± 1.5 b	29.9 ± 6.7 b	56.1 ± 8.2 b
Field trial B	30	12.8 ± 2.8 a	73.0 ± 16.4 a	140.7 ± 15.6 a
	50	7.9 ± 1.6 b	46.2 ± 10.4 b	87.4 ± 9.7 b
Average plowing depth	30	11.2 ± 2.6 a	64.9 ± 14.6 a	123.4 ± 9.0 b
	50	6.5 ± 1.5 b	38.0 ± 8.4 b	71.7 ± 8.9 b

Table 1.

Mean value ± SD of total attachments, dry weight of emerged shoots, and tubercles per tomato plant of 30-cm-deep plowing compared with 50 cm one. Different letters in each column of each field and plowing treatment are differing significantly at $P \leq 0.05$, according to Tukey's test.

germinate. In a research carried out in southern Italy [14], a delay in seedling transplanting date from April to the hottest May reduced the *P. ramosa* infestation by 77%. Indeed, the daily maximum temperature was almost always below 25°C from April to mid-May, the period corresponding to the first stage of the tomato cycle for the early crop (transplant in April), while it increased to the threshold values always higher than 25°C starting from mid-May. This technique would give the host plant a time advantage over the *P. ramosa* and thereby make the tomato crop more competitive against this parasitic weed.

2.4 Biological methods

2.4.1 Bioherbicide

Biological agents such as pathogens *Fusarium* spp. (e.g., *Fusarium oxysporum* and *Fusarium arthrosporioides*) or *Ulocladium botrytis*, incorporated into the soil by drip irrigation in field, are able to infect the pre-attached broomrape stages, and efficacy in reducing number and weight of emerging broomrapes [56, 57]. Due to the parasitic plant life cycle, multiple applications of *Fusarium* at the soil level would be necessary [58]. Conidial suspension of two *F. oxysporum* isolated reduced *O. crenata* and *P. ramosa* germination *in vitro* by 76–80%, in root chambers by 46–50%, and in polyethylene by 40–55% [59]. Fungi can be applied in the field together with solid growth media (such as wheat, corn, or rice grains) or in granules containing the biocontrol agent nutrients [60]. Compost activated by *Fusarium* was efficient in reducing the infection, by minimizing the number of parasitic spikes on the host tomato plant. This might be due to the additive effects on the seed germination of the parasite of the organic compound along with the soilborne fungi [61, 62]. Both granular soil applications and conidial suspensions of *Fusarium sp.* caused extensive mortality of *P. ramosa* in pot experiments. On the contrary, in field experiments, results were inconsistent as reduction *P. ramosa* shoot number and biomass [63, 64]. The main obstacle to the use and development of biocontrol agents is the poor field efficacy of the known pathogens. Soil-active biocontrol agents for *Phelipanche* must be able to contend with soil microorganisms without negatively affecting the host crop [65].

2.4.2 Resistant varieties

Cultivation of resistant varieties is another sustainable method to control *Phelipanche* [66, 67]. In addition, it is a useful component of an integrated approach, because easy to combine with other measures such as soil fertility amendments, land preparation, or soil tillage. Several mechanisms underlying the resistance of plants to the *P. ramosa* parasite have been described [68]. These include low stimulation of broomrape seed germination, pre-haustorial resistance, phytoalexin induction, high levels of peroxidase activities, lignification of host endodermis and xylem vessels, cell wall deposition, development of an encapsulation layer in the cortical parenchyma, induction of pathogenesis-related proteins, and sealing of host xylem vessels by deposition of mucilage [69]. Considered that this parasite requires stimulants exuded by the host roots, in order to germinate and reach the host root, varieties that exude stimulants at low levels or secrete inhibitors, they could be suitable for reducing parasite infection [70, 71]. An example of tomato cultivars resistant to *P. ramosa* infestation was reported by Qasem and Kasraw, 1995 [72]. The low germination stimulant phenotype of tomato has been reported in mutants owing to reduced exudation of strigolactones [73]. A successful screening program in a heavily

broomrape-infested field, to locate a resistant tomato line from a fast neutron-mutagenized M2 tomato population, was reported in Israel [74]. However, at present there are no commercial varieties for the broomrape control in tomato [6]. Research is needed in this regard to select from the wide range of varieties resistant to this parasite.

2.5 Integrated method

The single control practices described above are often only partially effective and sometimes inconsistent. Therefore, the most feasible way of coping with the weedy root parasites is *via* the integration different preventive measures and control instruments on a long-term basis into the given farming system [75]. The real challenge is to integrate practices that obtain optimum efficiency in terms of reduction of existing seed banks, prevention of seed production, and avoidance of seed dissemination with affordable costs. A computer simulation on integrated approach with a selection of appropriate cultural methods such as hand weeding, trap/catch cropping, delayed planting, resistant cultivars, and solarization demonstrates the importance of preventing new seeds entering the soil seed bank [76]. Resistant crop varieties and delayed transplant, for instance, are generally considered the useful components of an integrated approach that are usually easy to combine with other measures such as rotation, soil fertility amendments, and land preparation or soil tillage, and suitable to promote tomato plant growth and to reduce the *P. ramosa* infestation. Advantages of these sustainable approaches are no chemical applications that are known to cause damage to the environment.

3. Conclusion


The spread of branched broomrape is of great concern in tomato and other susceptible crop production systems in many countries around the world. This review summarizes the main control measures for the weedy root parasites *Phelipanche* and *Orobancha* in processing tomato, namely prevention, chemical, agronomic, and biological control. Some of these methods are commercially widely used by farmers (herbicidal control), some are in the final stages of development toward commercialization (resistant varieties), and some still require further development and improvement before commercial implementation (bioherbicide control). As for chemical control of broomrape, it should take the environment into consideration by encouraging reductions of herbicides, by carefully calibration of doses and timing of treatments depending on the underground phenology of broomrape determined by local conditions. One of the most promising directions is the precision agriculture approach of site-specific weed management. In this approach, herbicide is applied only in the infested area according to the spatial variation of parasite infestation in the field. Furthermore, it is desirable to improve the environmentally friendly, sustainable, and practical parasite control methods and use them in an integrated way. Therefore, future efforts must aim at improving these parasite control methods in accordance with new cultivation technologies suitable for the development of the processing tomato.

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Section 3

Tomato Processing
Applications

Tomatoes By-products Extracts Mediated Green Synthesis of Silver Nanoparticles and Their Application as Antimicrobial Agent

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Abstract

Silver nanoparticles (AgNPs) biosynthesized using by-products of tomatoes extracts as reducing and capping agents show multiple possibilities for solving various biological problems. The aim of this study was to expand the boundaries on AgNPs using novel low toxicity and production cost phytochemical method for the biosynthesis of nanoparticles from tomatoes aqueous extracts. Biosynthesized AgNPs were characterized by various methods (SEM, EDS). Determined antioxidative and antimicrobial activity of plant extracts was compared with the activity of the AgNPs. TEM results show mainly spherical-shaped AgNPs, size distribution of which depends on the plant leaf extract type; the smaller AgNPs were obtained with tomatoes extract (6–45 nm AgNPs). Besides, AgNPs show strong antimicrobial activity against broad spectrum of Gram-negative and Gram-positive bacteria strains and fungi.

Keywords: silver nanoparticles, green synthesis, tomatoes by-products, antimicrobial activity

1. Introduction

There are various bacterial, fungal, viral, and other microscopic life forms in our environment. Microorganisms make up 80–90% of the earth's total biomass, and even under “clean” conditions, several thousand fungal spores can be inhaled per day. Many microorganisms are harmless or even beneficial, but others can be extremely dangerous or even deadly. The current way of life creates favorable conditions for the spread of infections (food from distant lands, work in air-conditioned rooms, frequent trips to foreign countries, visits to hospitals, etc.). The human body is not sterile, and it is colonized by many microorganisms that are part of the normal microflora and live like harmless commandants.

Microorganisms living under normal conditions on the skin, nasopharynx, and intestine play an important protective role, as they prevent the growth of pathogenic

microorganisms in these places. As the bodies condition changes (weakened immunity, disease, or trauma), the so-called non-pathogenic bacteria can become pathogenic and cause infections. Wounds are susceptible to contamination by microorganisms both externally and from internal sources in the body, such as the nasopharynx, skin, and gastrointestinal tract. Infection is the result of a constantly changing interaction between microorganisms, the human being as their host, and the environment around them. Exposure of the Gram-positive and Gram-negative bacteria strains to the host's defense capacity interferes with wound healing and potentially dangerous changes in the body due to infection.

According to research, more than 23,000 people die each year in Europe from invasive (or systemic) infections caused by *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*). It has also been observed that these infections are increasing rapidly due to the progressive, excessive use of antimicrobials, which allows pathogenic microorganisms to evolve and acquire multiple antibiotic resistance. Therefore, scientists around the world are constantly looking for new ways and materials to combat the colonization of pathogenic microorganisms [1]. Thus, the problems associated with unwanted bacterial adhesion to the surfaces of medical equipment, as well as the colonization of surgical equipment, implants, and other health-related products, pose a significant risk to public health. The formation of biofilms also directly affects many industrial processes: food processing and storage, water treatment processes, maritime transport and management. Various antimicrobial agents are commonly used against biofilms and their infections, but microbiological control of the process is hampered by the ability of pathogenic microorganisms to attenuate or acquire full resistance to antimicrobial compounds, including antibiotics. Despite ongoing efforts by scientists to avoid bio-contamination and additional control measures implemented by industry, there is still no effective solution to protect the surface of equipment from colonization by pathogenic microorganisms. For these reasons, the need for antimicrobials is greater than ever before.

2. Antimicrobial agents

The increasing level of pollution by microorganisms and infections creates the need for new antimicrobial agents. Therefore, the research on the development and application of polymer composites with antimicrobial activity is of great interest.

Plant-mediated synthesis imparts several advantages to metal nanoparticles (MNPs) technology for the development of alternative products against infectious diseases. Indeed, most of green MNPs from plant-derived materials are highly effective and nonspecific antimicrobial agents, showing remarkable activities against the growth of a broad spectrum of bacterial and fungal species, in both planktonic and biofilm forms, including nosocomial and multidrug-resistant strains [2, 3].

Materials with antimicrobial activity are abundant. One of the largest groups is natural or synthetic antibiotics, which inhibits the appropriate stage of synthesis of the microorganism's cellular proteins. However, excessive use of antibiotics has led to the emergence of strains of bacteria that are resistant to most antibiotics, posing a significant risk to public health. As a result, other substances with antimicrobial activity are increasingly being used. Their nature can be very different. These are, in particular, substances of natural origin: vegetable (various essential oils, medicinal plant extracts, etc.) and animal (e.g., lysozyme, lactoferrin), microbes (nisin, naticin, etc.), as well as inorganic and organic synthetic and hybrid derivatives of a

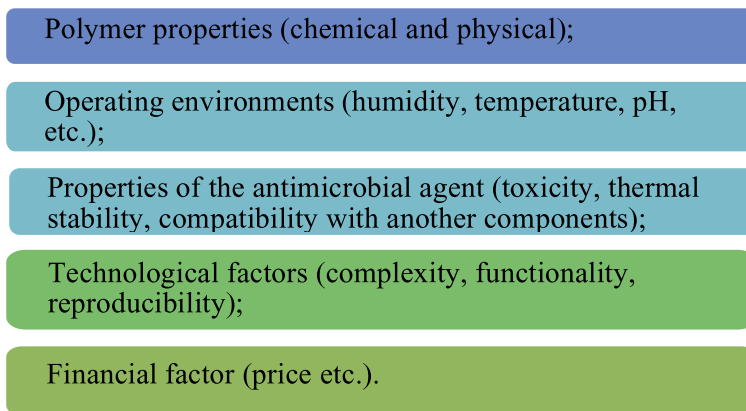


Figure 1.
The choice of antimicrobial modification method.

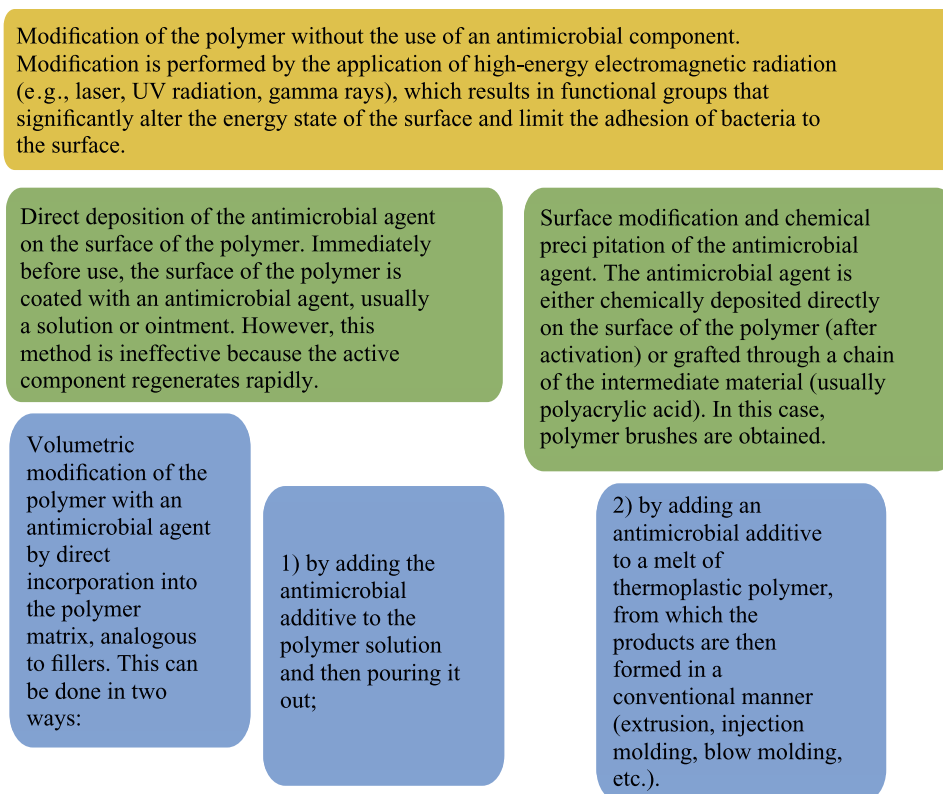


Figure 2.
The main methods of antimicrobial modification of polymers [4].

nature. Polymeric materials that are resistant to the colonization of microorganisms and the spread and multiplication of pathogenic microorganisms are also one of the groups of antimicrobials. They usually consist of a polymeric matrix and an embedded antimicrobial component.

The choice of antimicrobial modification methods depends on many factors (**Figure 1**).

The main methods of antimicrobial modification of polymers are as presented in **Figure 2**.

3. Antimicrobial activity of metal nanoparticles

One of the most abundant groups of substances with antimicrobial activity suitable for polymer modification is inorganic compounds and metal nanoparticles. This group consists of metals (Ag, Au, Cu, etc.), metal oxides (ZnO, TiO₂, etc.) [5], nonmetallic oxides (SiO₂). In most cases, the size of antimicrobial nanomaterials ranges from 1 to 100 nm. They can be of organic or inorganic origin, but inorganic substances are most commonly used. Nanoparticles are the most widely used because they have broad-spectrum antibacterial properties against both Gram-negative and Gram-positive bacterial strains [6]. The main reason why nanoparticles are an alternative to antibiotics is their ability to inhibit multiresistant microorganisms in some cases. Nanoparticles have a large surface area that increases interaction with microorganisms, resulting in strong antimicrobial activity. Nanoparticles with a smaller size and a higher surface area to weight ratio are more efficient at breaking biofilms. The particle shape also has a significant effect on the degradation efficiency of biofilms (e.g., rod-shaped particles are much more efficient than spherical forms). There are various methods for the synthesis of nanoparticles, which can be divided into two main classes: (1) bottom-up, and (2) from top to bottom [7].

In general, the chemical, physical, mechanical, and antimicrobial properties of nanoparticles depend on their chosen precursor. Nanoparticle microorganisms act in different ways, and the mechanism of their action depends on the origin of the nanoparticle [8]. Nanoparticles have antibacterial (inhibits DNA replication, enzyme functions, etc.), antiviral (blocks the attachment of viruses to the cell wall), antifungal (breaks down the cell membrane), and other effects.

Nanotechnology is the science, engineering, and technology that studies matter at the atomic, molecular, or supramolecular levels to yield nanometric materials and nanosystems with improved properties such as high surface-to-volume ratio and high dispersion in solution. With size typically ranging between 1 and 100 nm, these nanomaterials and nanosystems can be synthesized by chemical, physical, and/or biological methods [9]. In comparison with chemical and physical methods that involve costly and toxic chemicals, the biological synthesis pathway based on the usage of biological sources (plants, bacteria, fungi, and algae) is hoisted as a real rescue route. In spite of that, the biological methods do not envisage the use of toxic catalysts and reagents, dealing exceptionally with the intracellularly or extracellularly produced metabolites within fermentation routes, and this method requires big input of costly materials, well-developed protocols and guidelines, and microbiological hands-on experience to ensure cell culture and nanoparticles purification under highly aseptic conditions.

In contrast, the use of plant-derived extracts, juice, hydrolysates, etc., for the biosynthesis of metal nanoparticles (MNPs) seems to be an environmentally friendly, cost-effective, robust, sustainable alternative with moderate reaction conditions [10]. The plant-mediated synthesis of nanoparticles is also biocompatible, clinically adaptable, and easily up-scalable for industrial production [11]. Plants could represent

continuous source of natural antioxidants and antimicrobials (polyphenols, flavonoids, tannins, terpenoids, alkaloids, essential oils, etc.) suitable for green synthesis of nanoparticles with desirable properties. Under proper extraction conditions dealing with nontoxic organic solvents, diverse spectrum of non-deleterious reducing agents could be acquired [12].

Recent evidence in the field of nanotechnology revealed that the morphological parameters of nanoparticles (e.g., size and shape) can be modulated by varying the concentrations of bioactive compounds and reaction conditions (e.g., temperature and pH). Due to multiple therapeutic and biological activities such as antioxidative, antimicrobial, anti-inflammatory, anticancer, eugenol as a representative of phenylpropanoids received tremendous interest among researchers. The crude extracts recovered from such herbal plants as Lamiaceae, Lauraceae, Myrtaceae, and Myricaceae, the major compound of which was eugenol, have been investigated in terms of reducing ability for nanoparticles synthesis. However, less explored are other sources of this unique molecule, especially by-products that also could provide adequate quantities of eugenol. Considering the evidence on the presence of eugenol, a principal component of lignin in cereals and their by-products (bran), and already established protocol for lignocellulose biomass hydrolysis, it is speculated that the process of biorefining could represent a sustainable and reliable way of bran utilization for the production of eugenol-based nanoparticles, thereby contributing to waste reduction. Additionally, using different sources of metals (salts or oxide) in combination with plants, the biological reduction method allows the synthesis of a large number of green MNPs, including silver (Ag), gold (Au), zinc oxide (ZnO), platinum (Pt), palladium (Pd), copper (Cu), iron oxide (Fe_2O_3 and Fe_3O_4), nickel oxide (NiO), magnesium oxide (MgO), titanium dioxide (TiO_2), and indium oxide (In_2O_3).

Considering the above, exploration of the plant systems as potential bio-factories for MNPs has gained considerable attention, especially for researchers working in the field of phytonanotechnology, pharmaceutical, and clinical microbiology as well as medicine [7]. Indeed, due to the surging popularity of green methods, more than 2000 research papers and reviews related to antibacterial, antifungal, and antibiofilm properties of MNPs have been published. Noteworthy, most of the reviews and research articles published so far focused mainly on predicting the antimicrobial mechanisms of MNPs and parameters that may influence their antibacterial, antifungal, and activities such as.

The type (and origin) of plants used as bioreactor sources for biosynthesis;

The reduction process of the metal salts (mainly silver, zinc and gold) used during the bio-fabrication of nanoparticles;

The particulate characteristics of MNPs (size, zeta potential and shape) as well as the characterization techniques allowing their determination;

The general protocols.

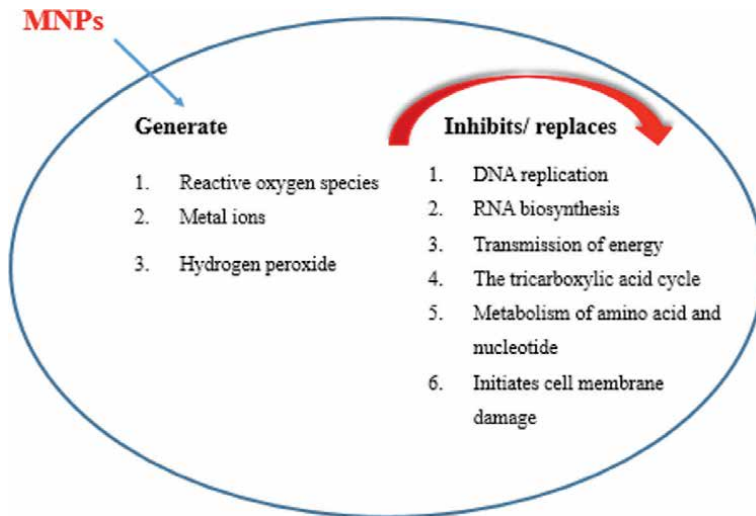


Figure 3.
Mechanisms of antimicrobial action of nanoparticles on bacterial cells.

Unfortunately, it appears from these reviews that the methods used for assessing the antibacterial, antifungal, and antibiofilm efficiency of MNPs are only partially elaborated in terms of standardization process; therefore, it is hard to correlate or compare data from different studies to pinpoint the high values of antimicrobial nanoparticles. Moreover, such methodologies and models are usually hard to extrapolate to real products.

Metal nanoparticles can affect the bacterium even in several ways, causing extremely strong antimicrobial activity (**Figure 3**). The most common and widely used silver nanoparticles, elemental silver has been widely used as an antimicrobial agent since ancient times. To improve their antibacterial activity and reduce their toxicity, silver ions can be transformed into metallic silver nanoparticles through biological and biomimetic methods of synthesis. Green AgNPs have demonstrated the ability to reduce microbial infections in the skin and burn wounds and prevent bacterial colonization on the surface of various medical devices such as catheters and prostheses. Acting as capping agents, different multifunctional phytochemicals contribute efficiently to these antimicrobial activities [8]. Moreover, AgNPs can express synergism with standard antibiotics such as gentamycin and streptomycin [13]. Hence, these combinations can effectively be used against antibiotic-resistant pathogens. Additionally, antifungal activities of AgNPs have extensively been studied and demonstrated in the literature [14]. In the frame of the fight against antibiotic resistance, green synthesized AgNPs may be used as vehicles to transport oligonucleotide-based antimicrobial. Their synthesis can be performed by physical, chemical, or biological methods. Particle size, morphology, and antimicrobial activity differ according to the chosen method [15].

Numerous studies have shown that silver nanoparticles, in both colloidal and ionic forms, have a broader spectrum of antibacterial activity than most other nanoparticles. Due to their unique optical, electrical, and chemical properties, silver nanostructures are widely used in a variety of industries. However, they are most commonly used in health care and medicine due to their strong antimicrobial activity against many pathogenic microorganisms—Gram-positive, Gram-negative, and antibiotic-resistant bacterial species, fungi, and viruses.

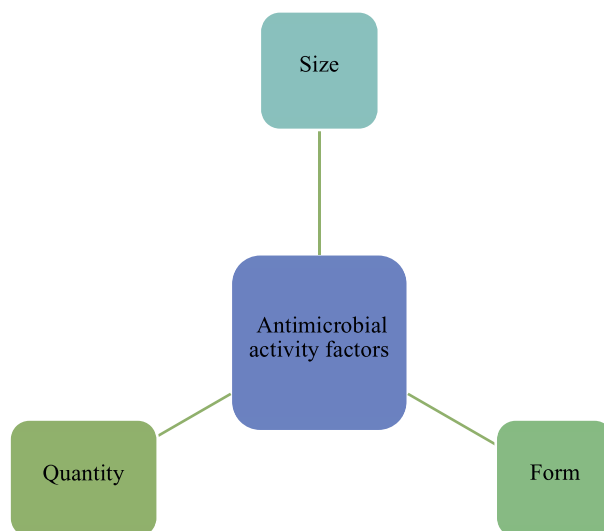


Figure 4.
Factors determining the antimicrobial activity of silver nanoparticles.

Rapid wound healing is due to decreased matrix metalloproteinase and increased neutrophil apoptosis in the wound induced by silver compounds. Matrix metalloproteinase is thought to be able to initiate inflammation and thus slow wound healing, so its regulation is very important [16]. Silver nanoparticles are also used in bone cement in various disinfectants [13] as antimicrobial agents. Beside this, their effect depends on several factors presented in the figure (**Figure 4**).

Antimicrobial activity of silver ions is obtained by reacting with the main components of the bacterium:

- cell wall and plasma membrane,
- DNA and proteins.

Due to the small size and very large specific surface area, silver nanoparticles adhere firmly to the surface of the bacterium. Silver ions, by interacting with the bacterial cell membrane and the sulfur compounds present in its proteins, impair its functionality. Further, silver nanoparticles penetrate the cell and damage DNA. Silver ions react with phosphorus compounds present in DNA, disrupting the process of DNA replication, which inhibits bacterial proliferation. They also degrade bacterial proteins, especially enzymes that catalyze metabolic reactions and other vital cellular processes. In addition, nanoparticles lead to the formation of reactive oxygen species, which are active and unstable molecules that can damage cellular DNA, protein structures, and cell membranes [17].

The antimicrobial activity of silver ions in Gram-positive and Gram-negative bacterial cultures may be different due to differences in bacterial cell structure. The cell wall of Gram-positive and Gram-negative bacteria has a complex, semi-rigid structure. The structure of the wall is very important because it determines the ability of the bacteria to cause disease and resistance to certain antibiotics. The wall thickness of the bacterial cells is unequal. The cell wall of Gram-positive prokaryotes is composed of a network of macromolecules called peptidoglycan or murein,

polysaccharides, lipids, and proteins. The wall thickness is much higher (20–80 nm) than that of Gram-negative bacteria. Their prokaryotic cell wall is composed of several layers: The inner dense electron layer (2–3 nm) is composed of peptidoglycan, two dense electron bands separated by an electron-conducting cavity, a space separated by the periplasmic cavity of the cytoplasmic membrane. The cell wall of Gram-positive microorganisms adheres closely to the cytoplasmic membrane [18]. These differences between bacterial species lead to unequal interactions between antimicrobial compounds. It is clear that metal nanoparticles are promising as antimicrobial agents and therapeutic agents due to their biological, physical, and chemical properties. They can solve many problems in the field of nanomedicine. However, there is a lack of knowledge about the long-term effects of nanoparticles on human health and the environment. Nanoparticles are stable and can accumulate in the environment; they have a tendency to agglomerate and can therefore change their dimensions. Toxicity studies of nanoparticles have shown that metal nanoparticles can act at the organ, tissue, cell, muscle, and protein levels. Nanoparticles are extremely small in size and can easily spread through air or water and adversely affect the skin, lungs, and brain (especially nanoparticles with dimensions 10 nm).

Therefore, the search for other substances with antimicrobial activity, such as the use of plant-derived substances to obtain antimicrobial compounds, is intensifying [19].

4. Morphology and antimicrobial activity of tomatoes by-products and green AgNPs

The aim was to compare the morphological differences of tomato pulp by variety. From **Figure 5**, the micrographs presented by SEM can show that the tomato particles are irregular in shape, with an uneven, layered surface, and that the particles appear to be composed of discrete slender shapes without any visible particles on the surface. The average particle diameter is very uneven as it was not fractionated, but the particle size could be harmonized by choosing milling techniques and conditions. Also, the particle size may vary depending on the desired properties.

The aim is to obtain stable and externally resistant colloidal solutions of silver nanoparticles and to investigate the antimicrobial efficacy of synthesized nanoparticles. Silver nanoparticles (AgNPs) were obtained by crude metal synthesis by reducing and stabilizing silver nitrate in extracts from bioactive compounds.

The morphology of lyophilized AgNPs of biologically active tomato pulp used in the work was investigated by SEM methods. From **Figure 6**, the microstructures of tomato by-products AgNPs can be concluded from the irregularly shaped particles but do not form agglomerations, which will have a positive effect on antimicrobial

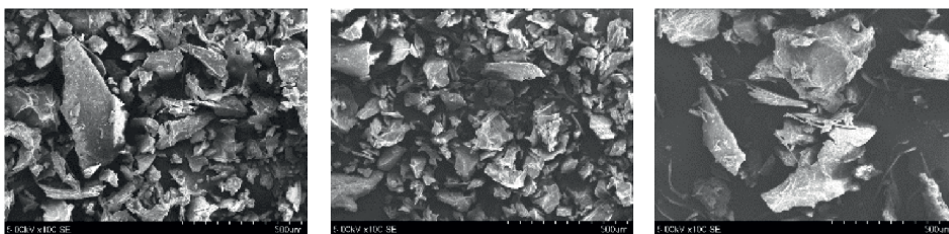


Figure 5.
SEM images of tomato by-products particles

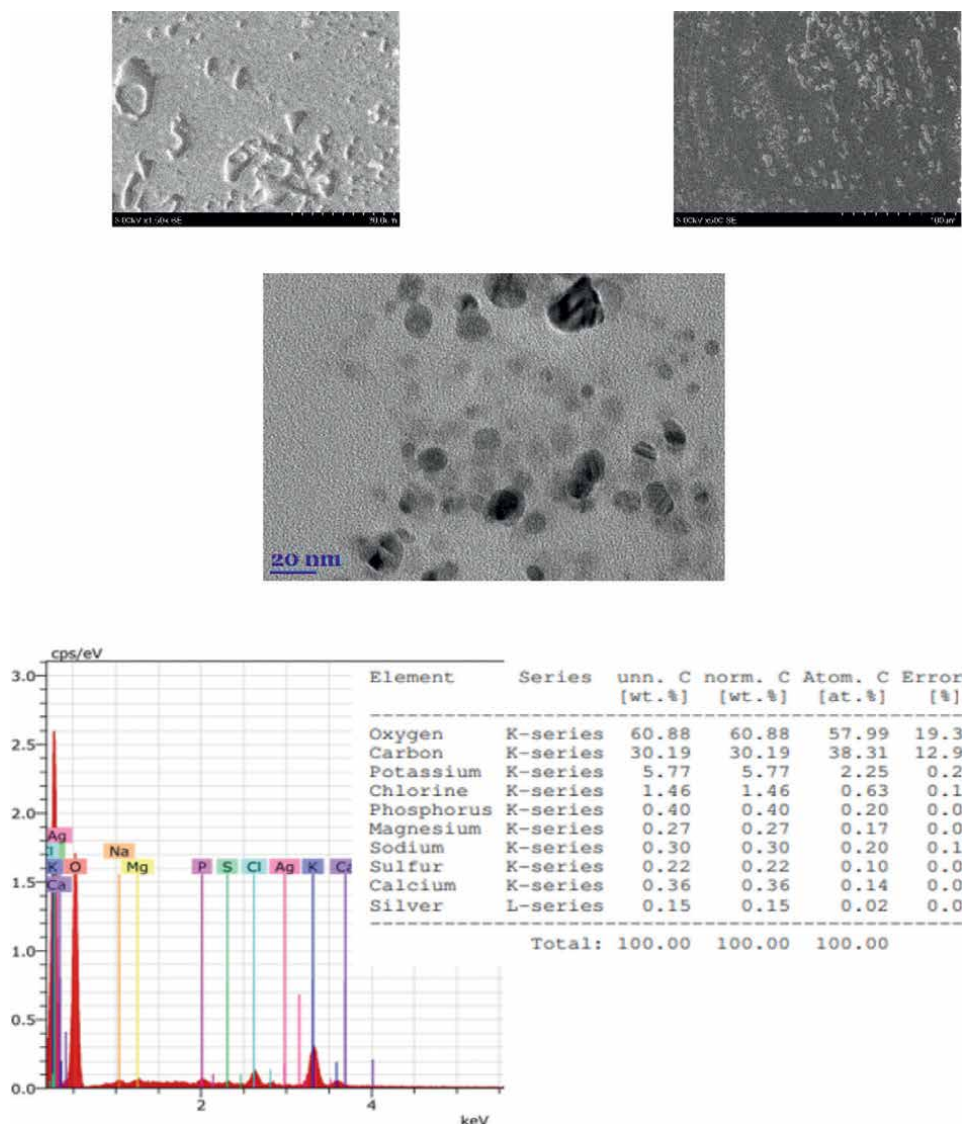


Figure 6. Tomatoes “Vilina” by-products TEM micrographs, surface EDS spectra, and elemental analysis

activity. From the photos provided by TEM, we can see a clearer morphology of the particles. The particles are spherical and do not form agglomerates (Figure 6).

In this case, individual agglomerations can already be observed. Scanning of metal particles at selected locations where AgNPs are suspected shows peaks in the 3.0 keV region of the EDS spectra that can be attributed to silver binding energy, and this can be detected at first and third samples. In second, sample AgNPs could not be found, but the biomatrices had antimicrobial activity. Therefore, we can say that the particles formed. With the help of TEM microscopy, we can see that the particles obtained are particles with a clear spherical shape, but in individual cases we can observe the formation of agglomerates (Figure 7).

The TEM images show an uneven surface with AgNPs. A high silver content in the biomatrix was identified (Figure 8). The particles remain irregular in shape and do not

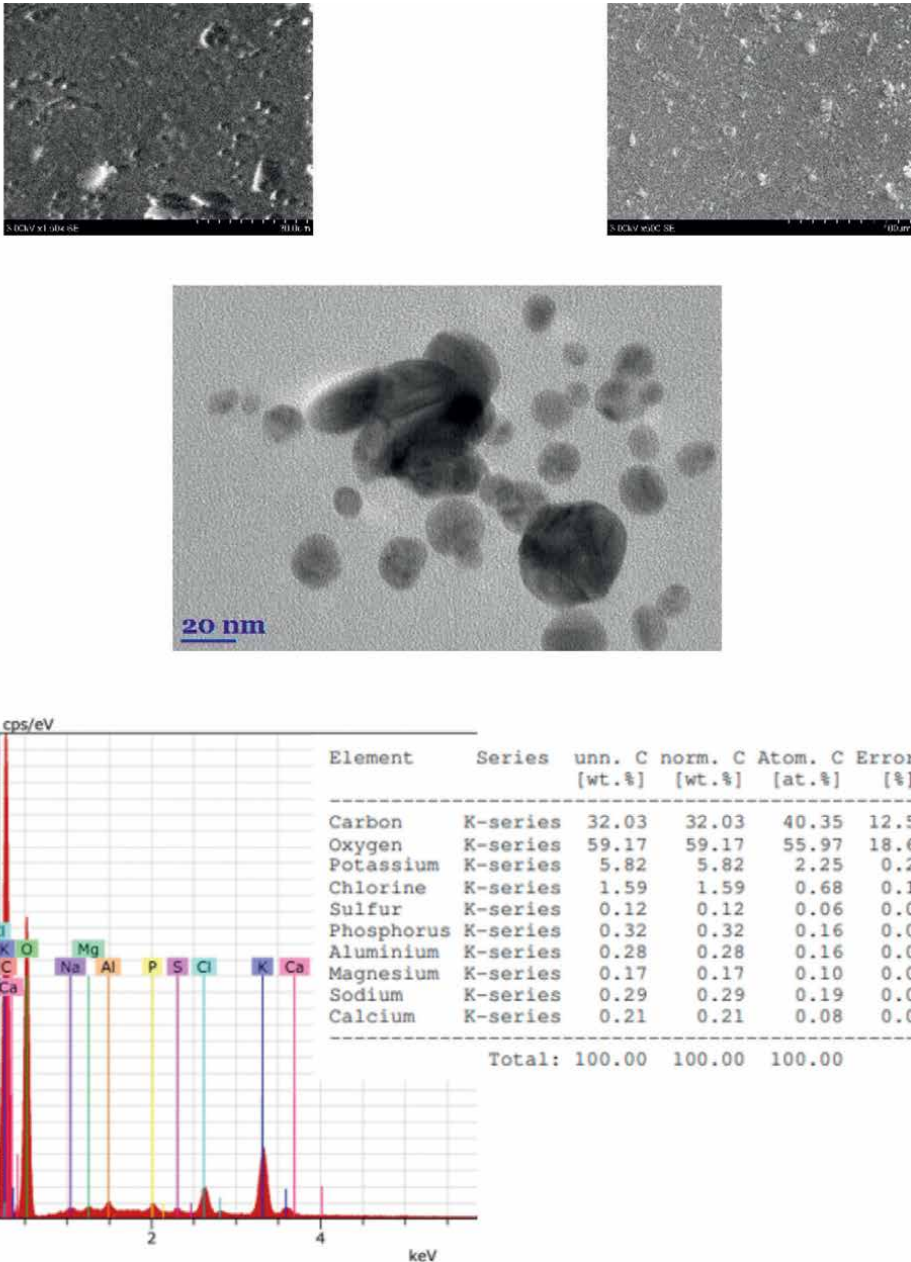


Figure 7. Tomatoes “Laukiai” by-products TEM micrographs, surface EDS spectra, and elemental analysis

tend to form large aggregates, which is likely to have a positive effect on antimicrobial activity. From the presented photos, we can see the particle shape, size distribution, and agglomeration tendency of AgNPs. In this case, the largest particles are obtained. Also in their form the resulting spheres. The particles obtained have a relatively high polydispersity, which is likely to have a positive effect on antimicrobial activity.

The antibacterial activity of organic colloidal solutions of AgNPs was tested for both Gram-negative and Gram-positive bacterial strains and fungi. From the results

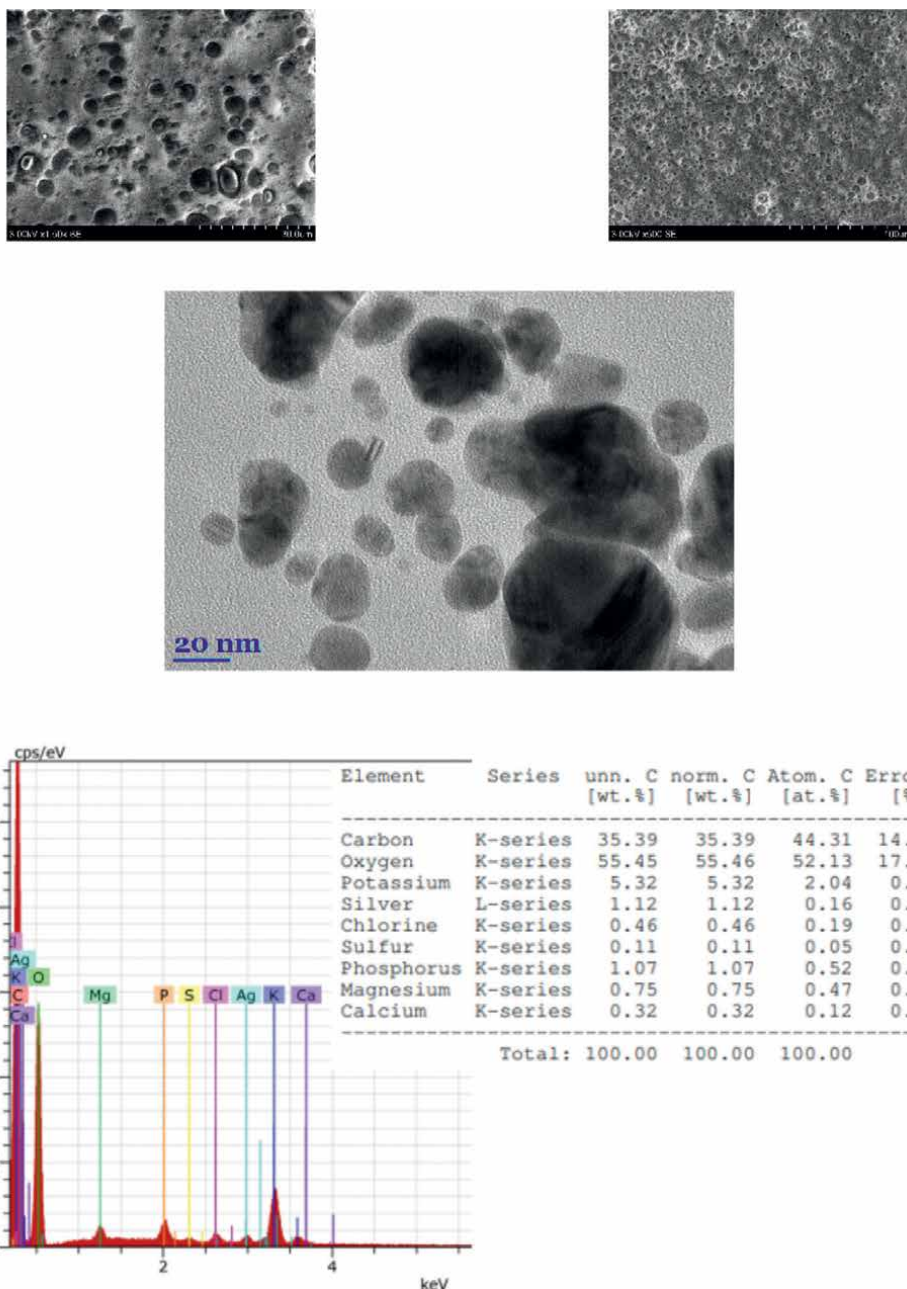


Figure 8. Tomatoes by-products mix TEM micrographs, surface EDS spectra, and elemental analysis

presented in **Table 1**, it can be concluded that silver nanoparticles in organic media actively interact with the bacterial membrane and disrupt their functions.

The results of the antifungal efficacy studies of AgNPs are presented in **Table 1**. Two different fungal cultures were selected: *Candida albicans* (*C. albicans*) and *Rhodotorula glutinis* (*R. glutinis*). In humans, *C. albicans* can cause external infections and life-threatening systemic infections, and *R. glutinis* is an opportunistic pathogen that can cause infection in

Reference (standard) cultures of microorganisms	Samples					
	1	2	3	4	5	6
<i>Staphylococcus aureus</i>	0.0 ± 0.0	10.8 ± 0.1	0.0 ± 0.0	10.10 ± 0.1	0.0 ± 0.0	12.2 ± 0.1
<i>Staphylococcus epidermidis</i>	0.0 ± 0.0	9.7 ± 0.3	0.0 ± 0.0	10.1 ± 0.3	0.0 ± 0.0	11.5 ± 0.2
<i>β-streptococcus</i>	0.0 ± 0.0	10.1 ± 0.1	0.0 ± 0.0	11.5 ± 0.1	0.0 ± 0.0	13.5 ± 0.1
<i>Escherichia coli</i>	0.0 ± 0.0	5.8 ± 0.3	0.0 ± 0.0	5.6 ± 0.2	0.0 ± 0.0	6.2 ± 0.2
<i>Klebsiella pneumoniae</i>	0.0 ± 0.0	5.3 ± 0.1	0.0 ± 0.0	4.9 ± 0.2	0.0 ± 0.0	5.5 ± 0.4
<i>Pseudomonas aeruginosa</i>	0.0 ± 0.0	4.2 ± 0.1	0.0 ± 0.0	3.5 ± 0.3	0.0 ± 0.0	4.1 ± 0.2
<i>Proteus vulgaris</i>	0.0 ± 0.0	6.8 ± 0.2	0.0 ± 0.0	6.5 ± 0.1	0.0 ± 0.0	7.1 ± 0.4
<i>Bacillus cereus</i>	0.0 ± 0.0	8.7 ± 0.2	0.0 ± 0.0	9.7 ± 0.1	0.0 ± 0.0	6.9 ± 0.2
<i>Enterococcus faecalis</i>	0.0 ± 0.0	7.6 ± 0.3	0.0 ± 0.0	8.2 ± 0.5	0.0 ± 0.0	5.4 ± 0.1
<i>Candida albicans</i>	0.0 ± 0.0	5.3 ± 0.2	0.0 ± 0.0	7.4 ± 0.4	0.0 ± 0.0	6.4 ± 0.3
<i>Rhodotorula glutinis</i>	0.0 ± 0.0	4.4 ± 0.1	0.0 ± 0.0	6.3 ± 0.2	0.0 ± 0.0	5.7 ± 0.2

Table 1.
Antimicrobial activity of the greenly synthesized AgNPs.

a weakened immune system. From the results presented in the table, it can be concluded that AgNPs obtained using different Russian tomatoes with different syntheses inhibited the growth of *C. albicans* and *R. glutinis* colonies. Meanwhile, extracts without particles did not show this effect.

It is clear that metal nanoparticles are promising as antimicrobial agents and therapeutic agents due to their biological, physical, and chemical properties. They can solve many problems in the field of nanomedicine. However, there is a lack of knowledge about the long-term effects of nanoparticles on human health and the environment. Nanoparticles are stable and can accumulate in the environment, and they have a tendency to agglomerate and can therefore change their dimensions. Toxicity studies of nanoparticles have shown that metal nanoparticles can act on the organ, tissue, cell, muscle, and protein levels. Nanoparticles are extremely small in size and can easily spread through air or water and adversely affect the skin, lungs, and brain (especially nanoparticles with dimensions ≤ 10 nm). Therefore, the search for other substances with antimicrobial activity, such as the use of plant-derived substances to obtain antimicrobial compounds, is intensifying.

5. Conclusions

Green nanoparticles obtained by green synthesis methods, which have a wide range of antibacterial properties against both Gram-negative and Gram-positive bacterial strains and fungi, expand their applications in orthopedics, biomedicine, and medicine, as well as in other industries. Recently, the range of substances resistant to microbial colonization and multiplication of pathogenic microorganisms are increasing due to the increasing use of extracts of medicinal plants and plant by-products, which are strong antioxidants with anticancer, antibacterial, anti-inflammatory, antiallergic, antiviral, hepatoprotective effects. One of the most important antioxidants accumulated in plants is phenolic compounds, the

mechanism of action of which is related to their ability to neutralize free radicals, protect against diseases caused by oxidative stresses, and reduce various forms of reactive oxygen species. It can be assumed that the modification of green nanoparticles with multifunctional hybrid particles can increase and expand their scope. Such antimicrobial and functional biomatrices are obtained using secondary by-products and Ag.

Stable colloidal solutions of AgNPs with high antibacterial activity in organic media have been obtained, which completely inhibit various bacterial cultures.

Acknowledgements

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Conflict of interest

The authors declare no conflict of interest.

Appendices and nomenclature


silver nanoparticles	AgNPs
<i>Bacillus cereus</i>	<i>B. cerues</i>
<i>Candida albicans</i>	<i>C. albicans</i>
<i>E. coli</i>	<i>E. coli</i>
<i>E. faecalis</i>	<i>Enterococcus faecalis</i>
<i>Klebsiella pneumonia</i>	<i>Klebsiella pneumoniae</i>
<i>Pseudomonas aeruginosa</i>	<i>P. aeruginosa</i>
<i>Proteus vulgaris</i>	<i>P. vulgaris</i>
<i>R. glutinis</i>	<i>Rhodotorula glutinis</i>
<i>S. aureus</i>	<i>S. aureus</i>
<i>S. epidermidis</i>	<i>Staphylococcus epidermidis</i>
β . <i>Streptococcus</i>	<i>beta - streptococcus</i>
SEM	Scanning electron microscope
TEM	Transmission electron microscopy
MNPs	Metal nanoparticles

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Perspective Chapter: Accelerating Demand-Led Tomato Breeding for Emerging Markets in Africa

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Abstract

Tomato production in Africa has increased due to increased population, rising consumer demands for nutritious and healthy food and potential use of improved technologies. Demand-led' plant breeding puts producers and consumers at the heart of research and development involving stakeholders even before the research starts. These 'stakeholders' are not only farmers but key actors along the tomato value chain. They influence how the tomato is traded as: fresh food and processing product. This chapter focuses on different approaches to fast-track tomato breeding so as to contribute to the transformation of African agriculture by enabling small scale farmers to compete in local and regional markets, by increasing the availability and adoption of high performing tomato varieties that meet market demands. It further outlines development of varieties that meet farmer needs, consumer preferences, and market demand in Africa. These new varieties are designed to meet client needs by connecting plant breeders with crop value chains, seed distribution organizations, and encouraging enterprise and entrepreneurship in transforming agriculture in Africa. Lastly, it outlines the prospects and challenges associated with demand-led breeding of tomato and offers suggestions to increase food security in Africa.

Keywords: demand-led, tomato, breeding, emerging markets, consumers, producers

1. Introduction

The production and utilization of tomato has increased over the years in Africa [1]. However, demand for the crop exceeds supply owing to its economic importance and increasing popularity as a result of processing, value addition and consumption [2]. According to ref. [3], open field cultivation dominates production of tomato in Africa hence exposes the crop to biotic and abiotic stresses, resulting in yield reduction and poor fruit quality [4, 5]. Increasing population, reduced natural resources as well as extreme climate change has further worsened the glitches of food security. The focus

of most plant breeding programmes is to develop and make available to end-users, crop varieties that meet their needs and capable of solving their everyday problems. Therefore, to facilitate the adoption and utilization of the end product, there is the need to take into consideration the expectations or demands of the prevailing market. Plant breeding approaches that consider active participation of farmers in the identification of their challenges, mitigation approaches and preferences in new varieties is crucial in the adoption of the resultant varieties [6].

Conventional methods of crop improvement require longer period, are time-consuming, costly, and restrict supply of genetic diversity. These challenges are exacerbated by the growth of human population (7.8 billion) in 2020, projected to nearly 10 billion by 2050 [7]. The need for strategies to increase the genetic advance in foods crops including tomatoes [8] for achieving food and nutrition security especially in Africa cannot be overemphasized. Speed breeding (SB) provides an opportunity to fast-track the breeding cycle, a key component of breeder's equation [9]. In diverse and marginal environments like Africa [10], appropriate plant breeding strategies should be developed to enhance the adoption and utilization of new varieties by farmers, consumers and industry.

The demand-led breeding approach will not only increase development of new tomato varieties but meet the needs of changing market preferences. Demand-led breeding combines the best practices in market led new variety design with innovative plant breeding methods and integrates both of these with the best practices in business as a new way of breeding crops to deliver benefits. The approach puts stakeholders especially producers and consumers at the heart of research and development by involving stakeholders even before the research starts. These 'stakeholders' are not only farmers but include other actors along the tomato value chain. They influence how the tomato is traded either as fresh food or processed product. According to ref. [11], demand-led breeding takes an integrated approach to new variety development and requires a comprehensive analysis that takes into consideration the target clients, their needs and how these may change.

This chapter focuses on different approaches to fast-track demand-led tomato breeding so as to contribute to the transformation of African agriculture, by empowering small-scale farmers to better compete in local and regional markets. It stresses on demand-led breeding and strategies to hasten breeding of tomato varieties that will be appropriate for consumers as well as other relevant stakeholders. It further discusses seed system in the sub-Saharan Africa and projects seed security as paramount in food security. It outlines the development of varieties that meet farmer needs, consumer preferences, and market demand in Africa. Finally, it outlines the challenges and prospects associated with demand-led breeding of tomato and offers suggestions to enhance food security in Africa.

2. Tomato breeding objectives in Africa

Tomato production in Africa has increased [1], but demand for the vegetable crop continues to surpass supply due to its economic relevance and growing popularity as a result of processing, value addition, and consumption [2, 5]. In Africa, less than 5% of areas dedicated to vegetable crop production including tomato are subjected to protected or controlled environments such as greenhouses [12]. Open field cultivation is paramount in tomato production in Africa [3, 13], exposing the crop to numerous biotic and abiotic stresses, causing reduction in yield and fruit quality [4, 5, 14].

Approaches to improve tomato resilience as well as productivity include breeding for resistance/tolerance to biotic and abiotic stresses and improvement of fruit quality to meet consumer/industry preferences.

2.1 Breeding for resistance/tolerance to biotic stresses

In Africa, one of the limiting factors impeding tomato production is biotic stress. This is induced by the living components of the environment [15] such as weeds, insect pests and diseases [2, 16–18]. In an attempt to address this situation, the indiscriminate use of chemicals such as pesticides which is harmful to human health [19] and the environment [18, 20] has made the demand-led breeding approach environmentally safe. Plant breeding is one of the most cost-effective and environmentally friendly methods of controlling biotic stresses in tomato production. In Africa, a number of breeding programmes have been undertaken to enable breeders develop tomato cultivars that are resistant to diseases and insect-pests [21]. In the tropics, major diseases affecting tomato production include bacterial wilt caused by *Ralstonia solanacearum*, late blight caused by *Phytophthora infestans*, *Stemphyllium spp* and Fusarium wilt caused by *Fusarium oxysporum* [22, 23]. Major pests of tomato include nematodes, thrips, aphid, cotton bollworm and mites [24]. Outbreaks of the tomato leaf miner (*Tuta absoluta*) have caused substantial damages in some West African countries [25, 26].

The pests and pathogens causing the above-mentioned diseases are genetically diverse with vast potential to generate new forms and hence difficult to control [16]. In view of this, conventional breeding together with marker-assisted selection (MAS) [21, 23], Quantitative Trait Loci analysis, and Hybridization [27] have been adopted for improving tomato resistance to biotic stresses. For instance, in recent decades extensive breeding programmes via the use of a series of in-region trials and collection of germplasm have been used to screen and develop varieties that are commercially acceptable and resistant to diseases [24]. Currently, the Crops Research Institute under the Council for Scientific and Industrial Research (CSIR), Ghana and West African Centre for Crop Improvement (WACCI), University of Ghana have released tomato varieties that have shown reasonable tolerance to late blight, Fusarium wilt and nematodes [28, 29].

2.2 Breeding for tolerance to abiotic stress

Similarly, abiotic factors tend to cause a myriad of considerable qualitative and quantitative crop losses to tomato production, especially in open field production. Due to threats posed as a result of climate change, tomato-producing environments are bedevilled with abiotic stresses such as heat, drought, water logging and salinity [24, 30]. Water deficit [31] and heat stress [32] are the most predominant abiotic factors that threaten tomato production in sub-Saharan Africa. Abiotic stresses can cause up to 70% yield losses [33]. Though considerable efforts have been made to ameliorate the continental menace through the use of agronomic approaches such as increased irrigation and temperature regulation, some of these efforts have proven futile over recent decades. An alternative promising approach to address these stresses is the development of tolerant tomato cultivars/lines through breeding [30]. One of the major constraints affecting tomato production is heat stress [34]. This is due to the increasing day and night temperatures as a result of climate change [35]. Breeding for heat tolerance has become one of the primary objectives of breeding programmes in

Africa [5]. In Ghana, Nkansah, King 5, DV2962 have been identified as heat-tolerant tomato cultivars [21, 32]. Currently, the CSIR-Crops Research Institute is developing heat tolerant tomato varieties with funding from the Korea government through Korea Africa Food and Agriculture Co-operation Initiative (KAFACI).

Most farmers in Africa grow tomatoes under open field conditions and as such rely solely on rainfall. Under such conditions, drought stress which is one of major constraints in open field tomato production [36] occurs due to erratic rainfall pattern. This consequently affects plant growth and development, resulting from reduced nutrient uptake [37] leading to increase in flower abscission, low percentage of fruit set, reduction in yield as well as fruit quality [38, 39]. There is therefore the need to breed and develop improved drought-tolerant varieties [38]. However, developing tomato cultivars tolerant to drought stress has been a neglected objective in many tomatoes breeding programmes [40], since the breeding objectives tend to focus much more on biotic stresses, prolonging shelf life, and determination of genetic variability among continental accessions. Although advances in molecular research and plant breeding have resulted in the introduction of drought-tolerant tomato cultivars in most developed countries, breeding efforts in sub-Saharan Africa (SSA) have focused on yield as the primary selection criteria, with little attention for drought tolerance [5, 40]. Nonetheless, a few screening trials have been conducted in countries such as Kenya to evaluate the susceptibility and tolerance of tropical cultivars derived from the AVDRC-The World Vegetable Center and the National Gene Bank of Kenya to drought stress [31].

2.3 Breeding to improve tomato fruit quality to meet consumer/industry preferences

Breeding for fruit quality is one of the major objectives of the tomato breeding programmes in Africa [41]. Due to the economic and nutritional importance of this perishable crop, breeders over the decades have put in great efforts to prolong its shelf life and organoleptic quality. Extensive studies have been reported on fruit quality traits such as the size, shape, total soluble solids, pH, colour, firmness, ripening, nutritional content, and flavour [42–44]. Therefore, tomato breeding initiatives have focused on boosting fruit quality and understanding its genetic and molecular diversity [44]. Recently, fruit colour is becoming increasingly important in the fresh market due to the awareness of the health benefits of carotenoids in the tomato fruit. Regarding processing of the tomato fruit in the industries, content of total soluble solids has also received lot of attention [44]. For instance, technologies such as pure line selection, hybridization, irradiation-induced mutation, and the crossing of local cultivars with exotic ones are ongoing breeding schemes in African countries such as Ghana to improve the fruit quality and shelf life of tomato [5, 14]. In addition, [45] reported studies on the utilization of single nucleotide polymorphism (SNPs) to evaluate the shelf life and fruit quality of F1 tomato progenies.

3. Demand-led tomato breeding

3.1 Overview of demand-led breeding

To facilitate the adoption and utilization of the end product, there is the need to take into consideration the expectations or demands of the prevailing market.

According to [6, 46, 47] plant breeding approaches that consider active participation of farmers in the identification of their challenges, mitigation approaches and preferences in new varieties is crucial in the adoption of the resultant varieties. The concept of demand-led breeding encompasses the approach whereby the situation in the prevailing market for new crop varieties considers the type of traits to incorporate into new varieties that will meet the expectations and satisfy the consumer or end-user needs. Whereas, participatory plant breeding or variety selection considers farmers involvement at different stages of the breeding programme, demand-led breeding encompasses various considerations from different actors such as processors, aggregators, marketers and consumers [48].

3.2 Demand-led principles and approaches for tomato breeding

Unlike participatory breeding that is more localized with limited scope, demand-led breeding involves more global focus. It takes into consideration a broader range of tools such as market research, value addition and modern product promotion strategies. It focuses more on the demands of the market rather than adoption for cultivation thereby producing a product that would be in high demand once released for cultivation. Demand-led approaches focus on the use of market information and intelligence to develop indices that are used to rank traits based on the monetary value and preferences from all potential end-users of the final product [49]. According to [50] demand-led variety design is based on six core principles; client needs and preferences, value chain analysis, market research, market trends and drivers, public and private sector linkages and multidisciplinary teams.

Demand-led or client-oriented breeding should consider client needs and preferences. This is crucial in considering the breeding objectives whether for industrial processing or home consumption. In Ghana, tomato production is either for processing into paste or direct consumption by consumers who purchase from the open market [51]. A demand-led programme should consider value chain analysis and innovation systems that involve all the actors in the value chain of the crop. For instance, in the value chain, common actors include farmers, aggregators or middlemen, transporters, traders, processors and consumers. Another consideration for demand-led breeding is market research. This allows the breeder to define the standard and priority for the traits or client preferences and validate the key assumptions at every stage of the breeding process. As a result, the breeder is kept abreast with the demands of the market in order to provide a product which will be readily adopted by the producers, marketers and consumers alike.

Demand-led breeding is also based on market trends and drivers which normally influence farmers' choices of crop varieties to adopt for cultivation. Prevailing circumstances and future occurrences such as climate change, national policies regarding certain commodities can all influence the kind of varieties that would be needed for cultivation [50]. For instance, a government initiative on tomato production or establishment of tomato processing factory may change the focus of variety design towards home consumption to varieties with good processing attributes. Another key principle guiding demand-led breeding is integration or linkage between private and public sector. It focuses on fostering cordial relationships between breeders and other actors in the value chain such as seed producers and distributors as well as other actors in the value chain. All these actors are involved in the identification and priority setting of client needs that result from the market research. Through this approach, breeders know what to breed for, the farmers also know what to cultivate

to meet the market requirements. Farmers are also linked to ready market. This approach promotes synergies between the various actors and culminates in benefits that far exceed what can be achieved with the different actors acting independently [51]. Demand-led breeding relies on multidisciplinary teams to achieve its objective. Demand-led breeding follows an innovative approach that utilizes a broad range of expertise and competencies of different actors with specific roles towards the design and development of the proposed variety. It is expected that the different experts will contribute to the development of the ideal product profile which possesses all the desired attributes and is responsive to the needs of the target group irrespective of the gender [52].

3.3 New variety design and product profile for tomato breeding: Ghana as a case study

To facilitate large scale adoption and commercialization of a new crop variety, such variety must meet the needs and expectations of the intended end-users [53]. Therefore, product profiles are developed for such a desired variety. A product profile encompasses a number of traits in a new variety that farmers would prefer compared to the variety they are already cultivating [54]. Product profiles are developed based on the array of clients that are targeted by the breeding programme following market research or broadscale stakeholder consultation. Several factors may influence the choice of crops to cultivate or the variety of a particular crop a farmer may cultivate and these have implications on the overall product design. A study by ref. [55, 56] revealed that the number of attributes or traits preferred by tomato farmers in the Wenchi municipal was positively influenced by the gender, education level, access to credit, household size, level of education, contact with extension staff, membership of farmer-based organization, farm size and off-farm income. This implies that different varieties are likely to be adopted by farmers in the different categories. As a result, these factors need to be considered by breeders and breeding programmes in designing and developing product profiles of new tomato varieties to meet the needs of the different clientele. For this reason, a particular breeding programme can have several product profiles that will define the type of varieties that would be developed [48].

Another survey carried out in seven regions of Ghana (Bono, Ahafo, Bono East, Ashanti, Greater Accra, Eastern and Upper East regions) involving 12 tomato growing communities found that tomato farmers in these areas prefer tomato varieties with large fruit size, high rounded shaped and red in colour [51]. A similar study by ref. [55, 56], indicated that majority of the farmers interviewed prefer firmness and extended shelf-life in their new tomato varieties. Though past plant breeding efforts in tomato have focused on morphological and molecular diversity studies, screening against biotic and abiotic stresses [5], breeding objectives must target other traits that may be of benefits to a wide array of end-users. Current efforts have targeted breeding for extended shelf-life through incorporation of genes from wild relatives [21, 45, 55, 56]. Development of an early maturing tomato varieties was achieved through hybridization of cherry tomato and Pectomech, a popular commercial variety [27].

In order to meet the current market demands and changing climate, there is the need to design and develop new tomato varieties that meet the requirements of different clients. As must-have traits, all new tomato varieties must be resistant to common pests and diseases such as whiteflies (*Bemisia tabaci*), tomato leaf miner (*Tuta absoluta*), bacterial wilt, Fusarium wilt as well as resilient to the prevailing

environmental conditions such as heat and drought. For home consumption, the new tomato varieties must be rounded in shape with large fruit sizes and red in colour. In order to meet the industrial market, there is the need to develop new varieties with high pulp content and/or brix for good paste production. To facilitate rapid adoption by farmers, the new varieties need to be resistant to most biotic stresses that prevail in most of the growing ecologies.

4. Fast track/speed breeding for demand-led tomato varieties

Speed Breeding involves manipulation of light, temperature, plant population and application of single seed descent method to identify major traits [57, 58]. Various methods which have been used to improve the cycle of turnover and are extensively classified as speed breeding (SB). SB is exploited in several tomato breeding programmes involving population generation, pyramiding traits, phenotyping, assessment of agronomic traits, genomic selection and genomic editing [58, 59]. Majority of the SB approaches target improvement in the tomatoes for fruit quality, fruit yield, and tolerant to stresses. Tomato is a model crop of the Solanaceae family that supports SB due to its short maturity period, diploid genome, convenience of *Agrobacterium*-mediated transformation thus supporting mutation breeding, CRISPR-Cas9 application [60, 61]. SB strategies used in developing demand-led tomato varieties such as *Flavr Savr* in America as the first engineered tomato by biotechnology method [62]. SB strategies such as marker-assisted selection, participatory plant breeding, mutation, and clustered regularly interspaced short palindromic repeat (CRISPR-/Cas9) system could be used by Africans in developing demand-led tomatoes. There is little information as to a tomato variety developed in Africa via the SB strategies. This implies that African tomato breeders must take advantage of current breeding tools.

4.1 Marker assisted breeding

Marker-assisted selection (MAS) and marker assisted breeding (MAB) progresses the effectiveness of crop improvement via accurate transfer of genomic sections of significance and hasten the recovery of the recurrent parent genome. The application of MAS and MAB support genomic selection which rely on molecular markers in assisting crop breeding. MAS in tomato improvement is traced around the 1930s [63]. MAS have been used to improve the traits that are related to disease, morphological, and physiological in most crops [59]. Specifically, in tomatoes, integration of SB into MAS results into transfer of beneficial alleles. For instance, SB and genomic led to purify tomato hybrids [64]; identify heat tolerant tomatoes [20, 65, 66].

The achievement of MAS is highly dependent on several critical issues including the number of target genes to be transferred, the distance between the target gene and the flanking markers, number of genotypes selected in each breeding generation, the nature of germplasm and the technical options available at the marker level. The power and efficacy of genotyping are anticipated to develop with the advent of markers like single nucleotide polymorphisms (SNP).

4.2 Participatory plant breeding

When farmers and other actors are involved in a breeding programme to either to participate or collaborate with scientists in every stage of the breeding programme is

termed as a participatory plant breeding (PPB) [67]. PPB is recommended as a one of the breeding approaches that enhance crop improvement, empower and promote farmers right, and increases the acceptance rate when new varieties and or technologies are introduced to farmers [68–70].

Tomato is an essential crop to Africans, hence there is continual increase in demand. Therefore, PPB serves to intensify farmers access and openness to breeding of new tomatoes in Africa. Thus, PPB will aid in rapid improvement and delivery of farmers and customers preferred tomato variety [71]. In Africa, the smallholder farmers serve as the foundation for the food system [72] PPB is observed to be useful to the smallholder farmers, especially in Africa. Application of PPB strategies in Tanzania assisted in evaluating and releasing a tomato variety resistant to late blight resistant [73] PPB has the potential to improve farmers preferred tomato but it has not been fully utilized. Hence, the various actors in the tomato breeding must take full advantage of PPB.

4.3 Mutation

Mutation is among the efficient approaches for enhancing crop traits without changing the well-optimized genomic background of the crop. Mutation is used to study variation and traits of interest for improving fruit quality, male fertility and disease resistance [74, 75].

For instance, tomato variety M82 through mutation developed mutant line with a variant of *eIF4E 1* showed resistance to the potyvirus strains [76]. Tomato fruits are known to be influenced by *rin* (ripening inhibitor) and PL gene that relates to fruit softening. Mutating of *rin* and silencing of PL gene results in delaying ripening. However, *rin* reduced nutritional components, flavour and colour of the fruits but PL gene had recorded otherwise [77, 78]. Tomato mutant *iaa9-3* line is capable of developing parthenocarpic variety that would be seedless and of high quality [79]. Mutation resulted in generating heat-tolerant tomatoes which exhibited high pollen fertility and fruit set [80, 81]. These are evidence which support the contribution of mutation breeding to the tomato industry around the globe. Tomato breeding in Africa has not witnessed the anticipated progress hence breeders should be encouraged to use mutation.

4.4 Clustered regularly interspaced short palindromic repeat (CRISPR-/Cas9) system

Gene editing technologies (zinc finger nuclease—ZFN; transcription activator-like effector nucleases—TALENs; and clustered regularly interspaced short palindromic repeat—CRISPR/Cas) applications have witnessed some successes in some crops within the Solanaceae family [82]. These technologies aim at modifying genome by generating novel desirable alleles that will foster the improvement and subsequent release of a new varieties and/or augmenting the genetic pool of desired alleles. The CRISPR/cas9 is the desired technology due to its high specificity and low cost [83]. Gene editing in tomatoes has been successful due the availability of its genome sequence (<https://solgenomics.net/organism>) and its annotation [84]. Thus, resulting in several research relating to improvement to stresses (biotic & abiotic), fruit quality (nutritional value, shelf life, & colour), and plant architecture [85].

CRISPR-Cas9 was first used in tomatoes in generating the first needle-leaf mutant in 2014 by knocking out *Argonaute 7* [86]. Most products produced by

gene editing have no commercial value [87, 88] but tomato is an exception [61]. Application of gene editing in tomato by Mlo1 mutant showed resistance to powdery mildew. Then again, through selfing at T₀, a mutant of mlo1 T-DNA was achieved [89]. Tomato is more sensitive to fusarium wilt disease by knocking out Solyc08g075770 by CRISPR-Cas9 [90]. Similarly, tomato bsr1 mutant via CRISPR reduced the production of hydrogen peroxide (H₂O₂) which improved heat tolerance in tomato [91]. Tomato plant's response to drought has been improved through gene editing technologies by manipulating CBF1 (C-repeat binding factor 1) and MAPK3 [92, 93]. CRISPR-Cas9 application have resulted in obtaining herbicide-resistant tomatoes plants. A study by ref. [94] resulted in over 70% edited tomatoes plants exhibiting resistant to the pesticide chlorsulfuron. Similarly, CRISPR-Cas9 mutated carotenoid dioxygenase 8 (*CCD8*) and more Axillary Growth1 (*MAX1*) involved in the promotion of strigolactone synthesis, a key component required for the germination of *Phelipanche aegyptiaca* seeds thereby resulting in producing *Podalirius aegyptiaca*-resistant tomato plants [95, 96]. Fruit set in tomatoes is influenced by pollination and fertilization, a CRISPR/Cas9 via mutation developed parthenocarpic tomato which is attractive to farmers as it reduces labour cost of fruit setting [97].

There is numerous evidence that support that, CRISPR-/Cas9 system has the potential to facilitate SB in tomatoes. However, there is little evidence that show it application in Africa. Other researchers confirm that, agricultural production is low in Africa compared to other continents [98]. A study by ref. [14] recommended that Africa should embrace technologies such as CRISPER to develop novel crops such as tomato genotypes to sustain its production. It is time that Africa embrace modern breeding technologies that support SB for tomato industry to be sustained due to increase demand.

5. Tomato seed system in sub-Saharan Africa

Achieving food security is dependent on seed security as well as timely availability of quality seed in adequate quantity at the right price and time. This is very fundamental in increasing production and productivity. Population increase, depleted natural resources and extreme climate variability has worsened the problems of food security to help mitigate this problem, a functional seed system is needed. Seed systems include interrelated institutions that develop new cultivar, produce, test, certify and market the seed. An effective seed system has the potential to increase productivity in a marginal way as good quality seed alone has the potential of increasing yield of crop by up to 20–30% [99]. Tomato is a food security and high value crop that improves the livelihood and income among smallholder farmers in the sub-Saharan Africa. Farmer's access to high quality tomato seeds is the surest way to ensure a resilient tomato industry in Africa.

5.1 Types of tomato seed system

In Africa, farmers have different ways of obtaining their quantity and quality of seed which they need for production. Tomato seed system varies greatly depending on the locality, market availability and farmers knowledge on seed system and supply and can be basically grouped into formal, informal or the combination of the formal and the informal.

5.1.1 Formal seed system

Formal seed system in Africa is usually government supported with the active involvement of the public institutions (**Figure 1**). It is a holistic approach involving evaluations of genetic resources, breeding and the development of new materials, certification and distribution of the planting materials to farmers. The tomato seed system is not formalized in most parts of Africa but a form of semi-formal seed system where seed companies are involved in the distribution of imported tomato seed to farmers. In sub-Saharan Africa tomato seed is still imported from outside the continent, while local companies continue to produce seed of open-pollinated varieties. Countries like South Africa and Tanzania have a formal tomato seed system where both the government and the private sector (Seed companies) are involved in

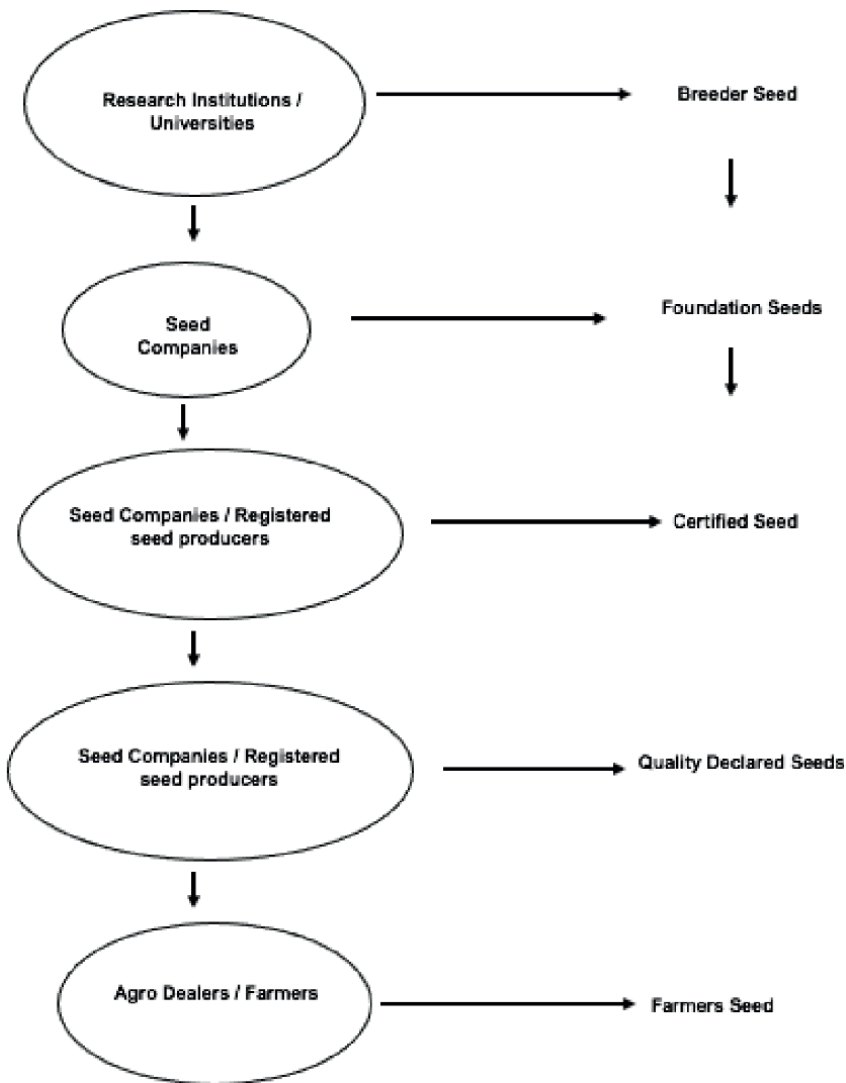


Figure 1. Schematic distribution of the formal seed system.

the development and distribution of tomato varieties [100]. The first private seed company in Tanzania (Alpha seed) was established almost three decades ago. It was into the sale of open-pollinated tomato varieties developed by the World Vegetable Centre [100].

Currently, Tanzania can boast of about 25 vegetable seed companies involved in tomato seed production and is expected to grow from 25 million USD in 2018 to 65 million USD by 2023 [101]. In most African countries for example., Ghana and Nigeria, seed companies are involved in on-farm trials of breeding and participatory cultivar selection, seed-related research, seed multiplication, seed conditioning and quality assurance, repackaging of the seeds, storage and distribution to final end users in this case farmers [102]. Currently, in Ghana, a formal tomato seed system is about springing up due to the release of the first official tomato varieties by CSIR-Crops Research Institute, Kumasi, Ghana and West Africa Centre for Crop Improvement (WACCI) at the University of Ghana.

5.1.2 Informal tomato seed systems

The informal tomato seed system, otherwise termed as “local or farmers saved-seed system” account for about 80% of the seed stock [103] supply to the farmers. It is an unorganized system that includes identification, seed saving, seed exchange, production and distribution by the farmer according to his/her knowledge of the plant and it is highly localized (**Figure 2**). The informal tomato seed system in Africa apart from being farmer or community-based practices; also includes different local level seed production initiatives organized by either farmer group, non-governmental organization or both, working outside the formal regime of the organized seed sector. Other characteristics of the informal tomato seed sector within the sub-region includes the non-law regulatory system, farmer to farmer seed exchange and this deals with individual community with small seed quantities usually demanded by farmers [104].

In Africa, countries like Tanzania, Ethiopia, Mali, Burkina Faso, Nigeria, Kenya, and South Africa although have some kind of formal seed system for some vegetables but the tomato seed system is largely informal [24]. Farmers within these countries purchase the imported hybrid or open pollinated tomato seed from agro-dealers and seed companies to produce their own tomato seed. These seeds once they are produced are easily marketed and exchanged from farmer to farmer by irregular means for many seed generations. On the other hand, farmers after acquiring some seed production skills from the extension officers often develop their own seeds from hybrid tomatoes i.e., they advance it to the F₂ themselves for local marketing. This is usually as a result of high cost of the F₁ hybrids [104].

5.2 Tomato seed value chain

A seed value chain is a series of actors or stakeholders. The value chain involves input suppliers, producers and processors, to exporters and buyers engaged in the activities required to bring an agricultural product from production to end consumer through various actors as shown in **Figure 3**. A value chain, therefore, incorporates productive transformation and value addition at each stage of any value chain. At each stage in the tomato value chain, the product changes hands through chain actors, transportation costs are incurred, and generally, some form of value is added. Tomato value chain results from diverse activities including input

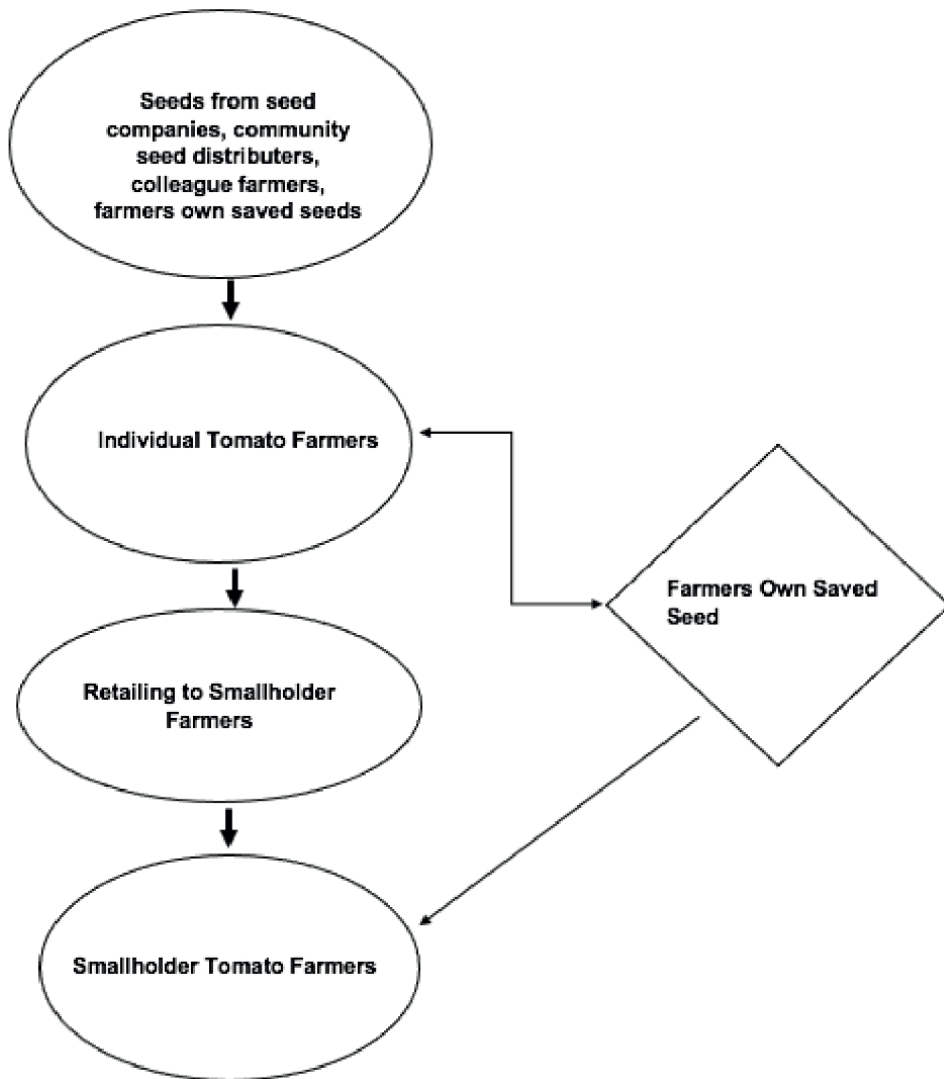


Figure 2.
Schematic distribution of the informal tomato seed system.

supply, production, transportation, marketing, processing, distributions, retailing, and consumption.

5.2.1 Input suppliers

Input suppliers are the producers of agricultural inputs such as seeds, pesticides, fertilizers, mulching sheets, etc. needed for the production of tomatoes. Through company owned, and other company dealers they sell their products to the farmers. Moreover, they also provide technical guidance on inputs usage and timely supply of inputs to the tomato farmers. They do maintain good relationships with the farmers and act as one of the informal sources of finance. Regarding the delivery of inputs like

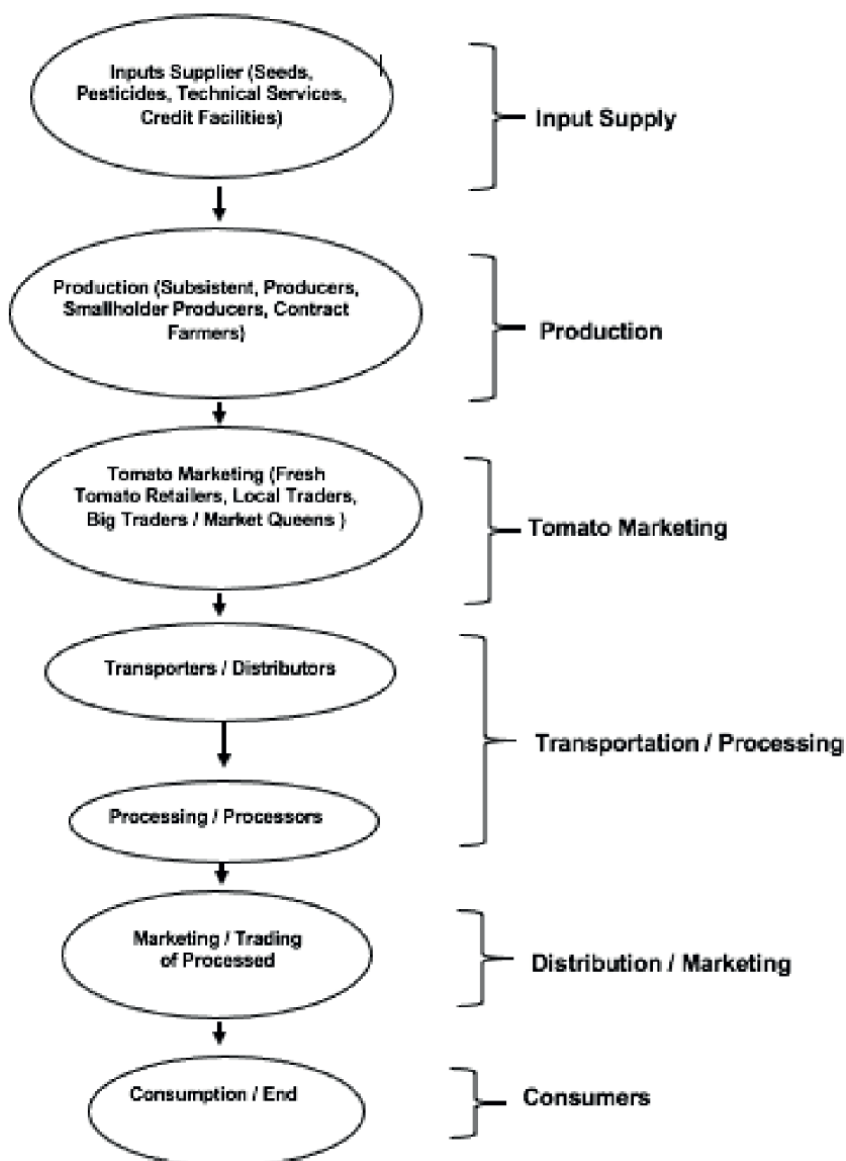


Figure 3.
Linkages and flow of tomato value chain in Africa.

improved seed, herbicides and pesticides, and credit among others, public and private extension services provide extension services to the farmers.

5.2.2 Producers

They are the initial link in tomato value chain. Producers decide what to produce, how much to produce when to grow and sell. Three types of the production system can be observed viz., subsistence production, small-scale commercial production, and largescale commercial production. Subsistence production is carried out for

household consumption and produced in small quantities. The produce from the first category of farmers generally does not enter the market or enters in a very limited quantity especially in the local marketplace. Small- and large-scale commercial farmers sell most of their products to various market intermediaries. The producers generally deal with traders and wholesalers. In most cases, farmers depend on village level traders for price information.

5.2.3 Marketing

The main aggregators usually buy the initial tomato from the main farmers to a special location within the village where traders buy them and transport to desired markets. Such collection and transporting activities are carried out either by the local trader, or an outside trader regularly visiting the location.

5.2.4 Wholesalers

They usually depend on the various intermediate sized loads and put the tomatoes into large uniform units. These activities all contribute to price determination. Wholesalers are market participants who buy large quantities of tomato and resell to other traders. Wholesalers often buy the tomatoes at the farm gate and other road site.

5.2.5 Processors

Processors are the secondary processing industries. The tomato processed products manufactured by the sample processors include tomato paste, sauce and ketchup. They usually collect fresh tomatoes from wholesalers and other sellers in major tomato production areas during peak season and glut in the market at cheaper prices.

5.2.6 Distributors

The distributors normally buy processed tomato products from processors and supply to small grocery stores and supermarkets. They generally sell products of different companies in different formats of retailers.

5.2.7 Retailers

They are the middlemen that include the supermarket and another large-scale retailer who divide large shipments of produce and sell it to consumers in small units. The basic function they provide is bulk breaking. Retailers are the sellers of tomatoes to the ultimate consumers through multiple channels such as small grocery stores, exclusive fruits and vegetable shops, supermarkets and exporters. They normally buy from wholesalers and sell both fresh tomatoes and other tomato processed products in smaller quantities with a higher profit margin. The retailers are the final connection that deliver tomato to consumers. They are many as compared to others and their function is selling tomato to consumers in small volumes after receiving large volumes from producers.

5.2.8 Consumers

It is the last link in the tomato market value chain. The consumers always make production meaningful and they usually set the pace for production. From the

consumers' perspective, the shorter the value chain the more likely the retail price going to be cheaper and affordable. The consumers are mostly classified into individual/household consumers and larger consumers like the restaurants, hotels and local food joints [105].

5.3 Constraints in tomato seed production

In Africa, the production and supply of most agricultural commodities is seasonal and tomato is not an exception [106]. This has contributed to the price adjustment of tomatoes based on the trend of supply [107] which follows the normal curve of demand and supply, as the demand increase the supply decreases and the reverse is true. In Ghana, Nigeria and most African countries where tomatoes production is considered as the game changer in both nutritional and in the economic sense, but yet cannot meet their production demand has for the past decades seen fluctuation in the rural wholesale price by a marginable percentage [108, 109].

This trend in price fluctuation of tomatoes in Africa has not only affected the quantity and quality of the tomatoes [110] but also affected the tomato seed production. The following are some of the challenges faced by tomatoes producers in the sub-region;

- Inadequate government support
- Lack of quality germplasm for fresh and processing markets
- Insufficient numbers of trained tomato breeders and seed producers
- Absence of seed laws and enabling environment
- Underdeveloped private sector and seed system
- Biotic and abiotic challenges to productivity: tomato yellow leaf curl, viruses, nematodes, heat and drought stress among others

6. Challenges and prospects of demand-led tomato breeding

The demand-led breeding approach will not only increase development of new tomato varieties but rather meet the needs of changing market preferences. Below are few highlighted prospects and challenges of demand-led tomato breeding methods in Africa.

6.1 Challenges

The major challenge for demand-led tomato breeding is lack of adequate funding covering aspects of research, training of researchers and technical officers, establishment of tomato breeding infrastructures, etc. [111]. Researchers, governments and private investors should partner to strategize and examine the potential benefits, implementation and how to sustain demand-led tomato breeding.

In addition, there is inadequate genetic resources to meet demand-led tomato breeding programmes in Africa [112]. To improve future tomato breeding

programmes, countries in Africa should prioritize the collection and conservation of local landraces which possess useful agronomic genes to sustain future breeding programmes [113]. Overreliance of improved exotic tomato lines/cultivar in most African countries, however, makes demand-led tomato breeding programmes unsustainable. This is because, the imported tomato variety may not meet the actual needs of the actors in the tomato value chain.

Last but not least, well- resourced laboratories to explore modern techniques in crop improvement are lacking in Africa. Most African countries are lagging behind regarding the utilization of basic to advanced biotechnology techniques such as marker assisted selection, genetic engineering and genome editing to facilitate plant breeding programmes [14]. Demand-led tomato breeding programmes would require most of these above-mentioned techniques to reduce the time for variety development.

6.2 Prospects

Tomato breeding based on demand has the potential to gather and understand information about tomato value chain actors' preferences for single or multiple traits. In order to accomplish this, more comprehensive quantitative research methodologies will be required to identify well-informed preferred traits by tomato value chain actors. To develop excellent demand-driven tomato breeding, scouting for traits insights along the value chain is a key [49]. Furthermore, using market intelligence, a comprehensive quantitative breeding index to rank the preferred traits for demand-led tomato breeding schemes can be developed. The “monetary values, preferences from all potential breeding clients, from the farm to the consumer's table” [49] may be the basis for trait ranking. As a result, this analysis will aid in determining trait improvement priorities and maximizing not only genetic gains, but also actual variety adoption, while ensuring that released varieties have traits that are preferred by all key value chain actors and stakeholders. This strategy will hasten the adoption of new tomato varieties.

Demand-driven tomato breeding can facilitate and harness the adoption of a customer- and data-driven approach that adds value to released tomato variety, while meeting the actual needs of a diverse range of customers and breeding clients [114]. Thus, involving various actors along the value chain especially in the early breeding process will promote early adoption, acceptance and consumption of newly released tomato varieties. As such, this breeding method will contribute enormously in achieving broader societal goals including increased food and nutrition securities concomitant with improved health, poverty reduction, climate resilience and environmental conservation.

Again, demand-led tomato breeding can help increase the economic value for targeted tomato traits. Since, traits of significant importance to various actors in the tomato value chain are known and ranked, economic value for specific or highly ranked traits could be improved. For instance, a change in dietary preferences and requirements has caused a shift for tomato fruits with improved quality such as fruits with high lycopene content [115] and sugar level [116]. A situation where consumers' preferred traits are successfully introgressed into a newly released tomato varieties, they will be willing to pay premium prices for these tomato fruits. Thus, the market value can be increased and then improve the livelihoods of various actors in the tomato value chain.

7. Conclusion

Tomato breeding programmes have focused on breeding for resistance to biotic and abiotic constraints which cause severe yield reduction. In addition to yield, tomato varieties are also bred for quality traits such as colour, firmness, flavour and extended shelf-life to meet consumer or industry preferences. Demand-led breeding which targets consumer and market preferences is based on six core principles which need to be considered in designing new tomato varieties. Attributes desired by Ghanaian and tomato producers from other African countries considered in new variety designs include pests and diseases resistance, tolerance to environmental conditions as well as for quality traits and attributes such as shape, colour, and high pulp content.

Various fast-track breeding approaches are employed for rapid progress in tomato breeding. Speed breeding techniques reduce the long breeding cycle compared to conventional tomato breeding. Examples of such approaches include marker assisted breeding (MAB), participatory plant breeding (PPB), mutation breeding and clustered regularly interspaced short palindromic repeat (CRISPR-/Cas9) system which have been used to accelerate the breeding process in tomato. Two key seed systems are common in the tomato seed value chain. These are the formal system which is regulated, and the informal system which mainly operates in the rural areas. The tomato seed system is faced with challenges such as: inadequate government support, absence of seed laws and enabling environment, under-developed private sector among others. Challenges facing demand-led tomato breeding include lack of involvement of all stakeholders in the tomato value chain. Inadequate research infrastructure, few trained personnel and inadequate genetic resources. These have limited to scope of most tomato breeding programmes to meet consumer needs.

Finally, demand-led tomato breeding has the potential to gather relevant information on the preferences of tomato value chain actors to inform breeders on what product profile to develop. Comprehensive quantitative research methodologies will help identify the requisite traits that would be preferred by current and future markets. Demand-driven tomato breeding can help breeders to design products that have the potential of satisfy consumer needs and facilitate rapid adoption by farmers and other end-users. Adequate funding, governmental support and active collaboration between researchers, private investors and farmers, and education on the potential benefits demand-led breeding are needed to implement and sustain demand-led tomato breeding.

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Recovery and Valorization of Tomato By-products in R&D EU-Funded Projects

Marcello Casa and Michele Miccio

Abstract

In the last years, the European Commission has been funding numerous projects regarding the valorization of food wastes. Tomato by-products received great attention especially in Spain, Italy, Greece, and Portugal due to high volumes and high concentration of valuable compounds. Among 40 funded projects about the management of tomato wastes in general, 14 projects are strictly connected to the valorization and exploitation of the tomato residues/by-products after processing and are of great interest for their scientific, technical, and economical outcomes. They received an overall budget of around 37 M€ over 35 years, involving 20 European and 4 non-European countries, with project coordinators located in Germany, the Netherlands, and Italy in most of the cases. This chapter delivers general information about these projects, assessing and reporting scientific and technical results. Moreover, the interconnection is highlighted among them by focusing on the contribution they gave to the European know-how, the management of the by-products and the progress they reached in waste minimization and valorization. Finally, the industrial and environmental outcomes of these projects have been reported by highlighting issues and problems that are still to be overcome.

Keywords: tomato by-products, waste valorization, European Union, funded projects

1. Introduction

In the last years, the European Commission has been funding projects regarding the valorization of food wastes. Tomato by-products received great attention especially in Spain, Italy, Greece, and Portugal due to high volumes and high concentration of valuable compounds. Among 40 funded projects about the management of tomato wastes in general, 14 projects are strictly connected to the valorization and exploitation of the tomato residues/by-products after processing and are of great interest for their scientific, technical, and economical outcomes. They received an overall budget of around 37 M€ over 35 years, involving 20 European and 4 non-European countries, with project coordinators located in Germany, the Netherlands, and Italy in most of the cases. This chapter delivers general information about these projects, assessing and reporting scientific and technical results. Moreover, the interconnection is highlighted among them by focusing on the contribution they gave to the European expertise, the

management of the by-products and the progress they reached in waste minimization and valorization. Finally, the industrial and environmental outcomes of these projects have been reported by highlighting issues and problems that are still to be overcome.

2. Funded projects

The Community Research and Development Information Service (CORDIS) [1], namely the European Commission's primary source of results from the projects funded by the EU's framework programs for research and innovation, was used to gather all information such as project factsheets, participants, reports, deliverables, and links to open-access publications about tomato by-products valorization. In the first instance, from research in this database, it came up that on 352 funded projects including the keyword "TOMATO" only 10% take into consideration wastes or by-products produced by harvesting, transformation, and use of this vegetable. In particular, the research on CORDIS with "TOMATO" and "WASTE" as keywords gives forty projects as a result. Other searches with other keywords were conducted with less significant results: for example, "TOMATO" and "VALORIZATION" give 9 projects as a result, or "TOMATO" and "RESIDUE" return 23 projects as a result. As it is possible to see from **Figure 1** the number of funded projects in this field of application had a strong increase in the last 5 years, probably due to the growing interest, shown by academia and industries, in waste reduction, valorization of materials so far considered as undesirable by-products, and exploitation of the high-value compounds contained in these waste streams.

Then, these forty projects were deeply studied, and it was possible to divide them into eight categories regarding the topic:

- Production of bioplastic from tomato residues
- Extraction of high-value compounds from residues
- Production of food additives from residues
- Production of biogas from residues

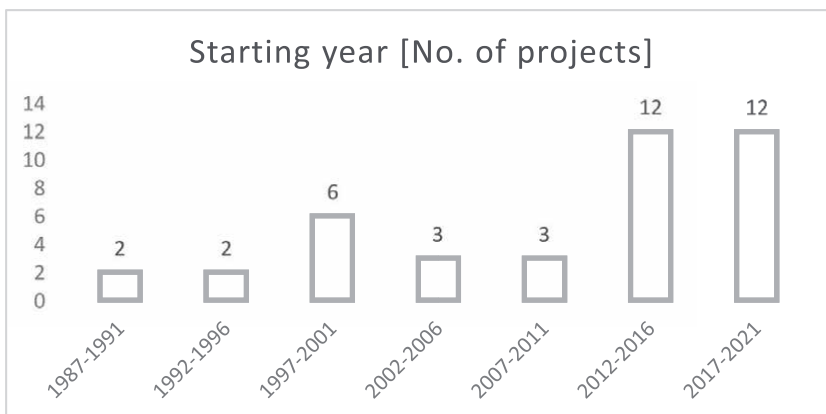


Figure 1. Distribution during last years of funded research projects on tomato waste.

- Biorefining of residues
- Harvesting optimization
- Shelf life of processed tomato
- Other (not included in the previous categories)

Figure 2 reports a bar chart of the number of projects per field of application. Among these, only fourteen projects are strictly connected to the valorization and exploitation of the tomato residues/by-products after transformation processes. In **Table 1**, the main information is reported about these projects of interest, sorted by topic.

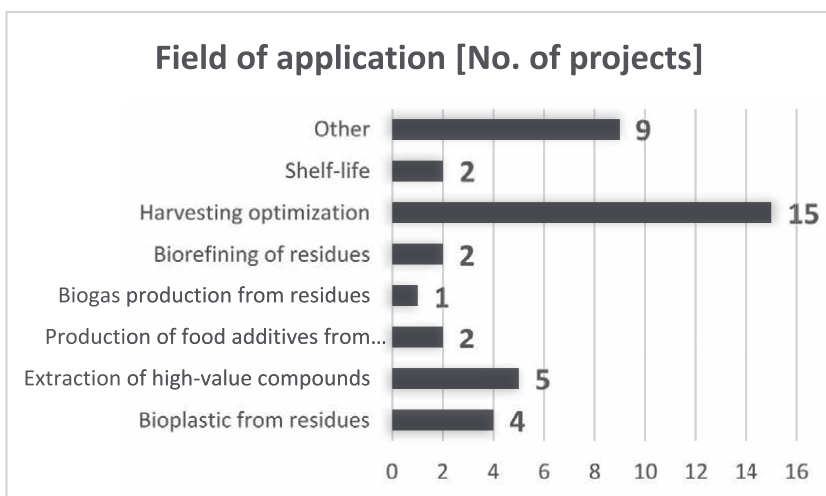


Figure 2.
 Number of projects per field of application.

Acronym	Start	Duration [months]	Budget [M€]	Coordinator	Partners	Status
Bioplastic production						
<i>BIOCOPAC</i>	2011	33	1	SSICA (Italy)	10	✓
<i>BIOPROTO</i>	2014	24	0.2	IIT (Italy)	—	✓
<i>ECOFUNCO</i>	2019	33	5.6	CNISTM (Italy)	17	Ongoing
<i>TOMAPAINTE</i>	2020	24	3	TOMAPAINTE SRL	—	Ongoing
Extraction of high-value compounds						
<i>QLK1-CT-2000-2041,137</i>	2000	12	0.03	Conservas Vegetales De Extremadura (Spain)	1	✓
<i>QLK1-CT-2000-2040,942</i>	2000	12	0.03	Hac Le Poole (Netherlands)	1	✓
<i>TOM</i>	2003	24	0.9	Catchmabs (Netherlands)	8	✓

Acronym	Start	Duration [months]	Budget [M€]	Coordinator	Partners	Status
BIOACTIVE-NET	2006	24	0.6	Hochschule Bremerhaven (Germany)	7	✓
LYCOSOL	2019	6	0.07	Biocapsol (Turkey)	—	✓
Production of food additives						
QLK1-CT-2001-2042,093	2001	12	0.03	ChiPro (Germany)	1	✓
PRO-ENRICH	2018	36	3.3	Teknologisk Institut (Denmark)	15	✓
Biogas production						
AVI*940005	1995	30	0.1	Universität Stuttgart (Germany)	2	✓
Biorefining						
REFRESH	2015	48	9.4	Wageningen University (Netherland)	26	✓
AGRIMAX	2016	48	15.5	Iris Technology (Spain)	29	✓

Table 1. Main information about funded European projects on valorization and exploitation of tomato wastes.

The information reported in the previous table was analyzed and summarized in the next chart to synthetically show the distribution of budget and participants among the considered application categories (**Figure 3**).

The overall budget is around 40 M€ involving 20 European and 4 non-European countries, with project coordinators located in Germany, the Netherlands, and Italy in most cases. It is worth notice that the field of biorefining, the one in which this thesis is involved, even if it is not the one with the highest number of the funded project, exhibits the highest budget and is the one with more partners involved. It is so probably because, even if the application of the biorefinery concept to tomato residual

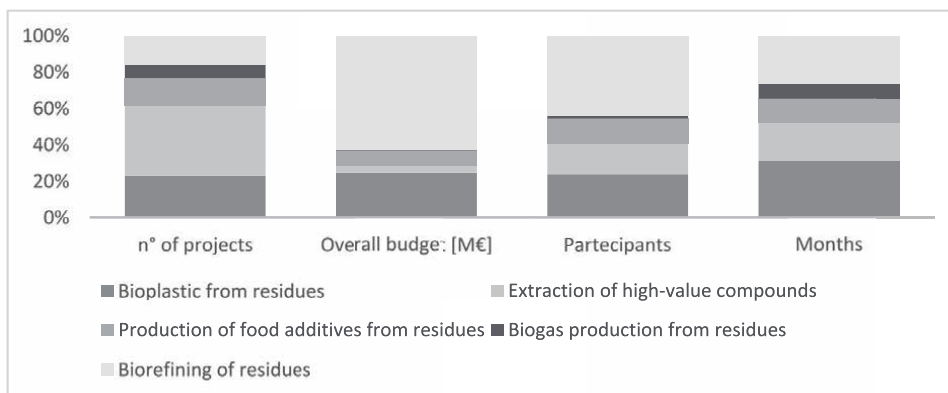


Figure 3. Distribution of budget and participants among the considered application categories.

by-products is quite new, the European Commission believes that research in this field could strongly increase the EU technological level. In the next paragraph, the outcome of these projects will be reported and briefly discussed.

2.1 Early projects

Projects funded before 2001 lack results reports, for different reporting policies of the European Commission. Anyway, the project QLK1-CT-2000-2041,137 had likely as an outcome a patent EP1676888B1 entitled Method of obtaining lycopene from tomato skins and seeds [2], assigned to *Conservas Vegetales de Extremadura SA*, which was the coordinator of the project. The patent refers to a process for obtaining lycopene from tomato skins and seeds. The carotenoid is obtained after a series of steps of dehydration, seed separation, pelletization, extraction, distillation, and crystallization. The extraction solvent is hexane and the purity of the lycopene obtained is between 65% and 85%, depending on the raw material.

2.2 TOM

The title of the project was “Development of new food additives extracted from the solid residue of the tomato processing industry for the application in functional foods.” Partners of the TOM project had developed and optimized an extraction process whereby lycopene is extracted in tomato seed oil from tomato plant processing residue. This can then be used in functional food products and cosmetics. The carried-out process involves the use of supercritical carbon dioxide (CO₂) [3]. The yield in tomato seed oil is 3–6%. The lycopene yield depends on raw material and ranges between 15 and 180 ppm, which is very low considering the extraction yield nowadays.

2.3 Bioactive-net

The title of the project was “Cultivation and processing of tomato, olive, and grape are the main agricultural businesses in the South European countries. Production of tomato paste, olive oil, and grape” and the main objectives of the project were:

- Create a broad information platform for dissemination of research results and state of art regarding the extraction of bioactive compounds from tomato, olive, and grape processing residues as well as their application facilities in the food and cosmetic industry
- Implement dissemination workshops in the South European countries aimed at transferring expertise and evaluating economic feasibilities of the extraction
- Strengthen the European market on natural ingredients

Remarkable was the study on the best available technologies (BATs) to separate vitamins, antioxidants, essential oils, and other valuable compounds from the processing residues. In *Guida pratica sui COMPOSTI BIOATTIVI ottenibili dai SOTTOPRODOTTI della TRASFORMAZIONE DEL POMODORO* they reported the main technologies available for: residues drying, lycopene extraction, and lycopene purification. Moreover, an economic assessment that compares solvent and supercritical extraction for this compound was reported [4]. The report clearly shows from

an economic and technological point of view that supercritical CO₂ is rarely favorable, while solvent extraction is profitable only when a high amount of tomato by-products is processed.

2.4 Lycosol

The title of this 2019 project is “Feasibility Analysis on the Extraction of Lycopene from Tomato Peel through Organic Synthesis.” LycoSOL project proposes an environmentally friendly solution based on natural ingredients. The method involves extracting and processing healthy ingredients from the waste from food processing. The project aims to develop the process of extraction and encapsulation from plant waste, targeting production from tomato peels. No results reports or scientific papers have been already disseminated.

2.5 Pro-enrich

The title of this 2018 project is “Development of novel functional proteins and bioactive ingredients from rapeseed, olive, tomato and citrus fruit side streams for applications in food, cosmetics, pet food.” Pro-Enrich was aimed at optimizing existing biomass fractionation technologies and validating novel extraction approaches beyond the current state of the art with reference to the Technology Readiness Level (TRL) assessment system (i.e., from TRL2 through to TRL 4/5) to isolate and purify proteins, polyphenols, and dietary fibers and pigments. The products being targeted are food ingredients, pet food, cosmetics, and adhesives. These were to be developed through an iterative process of feedstock mapping, laboratory process development, functionality/performance testing of samples by upscaling to pilot plant and industry level. Rapeseed, tomato peels and citrus waste were studied in the project. First, a review paper on waste composition and edible protein extraction for the selected feedstock was published [5]; then, a first pilot plant for protein production from rapeseed was started [6]; finally, the following bioactive ingredients were successfully extracted, and are waiting for Scale-up to demonstration scale:

1. Hesperidin (flavonoid/antioxidant) from citrus peels after juice production. It has market and applications in:
Pharma: In diosmin/hesperidin products for its venotonic activity, protection, anti-cellulite, and more.
Cosmetics: In products for alleviation of eye wrinkles.
Feed: As antioxidant supplementation for pets and horses.
2. Lycopene (carotenoid/antioxidant) from tomato peels after canned food production. It has market and applications in:
Pharma: for prostate health.
Food: As a coloring agent in food and drink products.
Nutraceuticals: In dietary supplements for heart and brain health, sunburn protection, and more.
Cosmetics: In anti-aging products and for healthy skin appearance.
3. Rapeseed protein (isolate >90% wt. protein and concentrate >50% wt. protein) from rapeseed press cake after rapeseed oil and biodiesel production. It has market and applications as a replacement for:

Food: animal-based protein.
Pet food: animal-based protein.
Adhesives: petrochemically derived phenolics up to 40%.

2.6 BIOCOPAC

The title of the project is “Development of bio-based coating from tomato processing wastes intended for metal packaging.” BIOCOPAC initiative looked at tomato by-products to satisfy some of these needs. The goal was to develop a natural lacquer liner for tins that are made from the cutin raw material contained in discarded tomato skins. The coating was aimed to be applied to internal and external surfaces of food tins to ensure consumer health and safety. The next step was to develop the bio-resin and the lacquer. Scientists developed two different formulas to produce the lacquer, one specifically designed for tinplate and a generic one for all types of metal can. BIOCOPAC produced canned goods using these lacquers, demonstrating that the lacquer performs as well as current products. An interesting outcome of the project is a Life Cycle Assessment (LCA) conducted using the SimaPro software, version 7.1. The analyses compared the LCA of a conventional epoxy-based lacquer to a bio-lacquer, tomato cutin based, obtained from tomato processing waste. The results showed clear environmental benefits of the “Bio-lacquer.” The benefit of the cutin lacquer lies in the saving of natural resources and the recovery of part of the skins. This can lead to lower consumption of fossil fuels and lower CO₂ emissions.

BIOCOPAC project merged with the BIOCOPAC+ project, funded under LIFE+ Environment Policy and Governance project application (Grant Agreement No. LIFE13 ENV/IT/000590). The project was started on the 1st of June 2014 and lasted for 36 months. The project was industry-driven and focused on demonstration activities aimed to prove the technical feasibility and effectiveness of the cutin extraction and production systems currently developed at a laboratory scale. Its outcomes were a prototype pilot plant for cutin extraction, installed at *Azienda Agricola Virginio CHIESA* (IT) and a cutin-based lacquer production site in SALCHI (IT) plant [7].

2.7 BIOPROTO

The project title is “Bioplastic production from tomato peel residues.” The team investigated the possibility of creating a bioplastic film from discarded tomato skins. The idea proved feasible, yielding scalable and biodegradable options for food packaging. Results yielded a new set of films and coatings taken from the lipid portion of plant cuticles, reported in **Figure 4**. The outcome also represented a potentially scalable and cheap process for the manufacture of bioplastics intended for use in food packaging. BIOPROTO’s new plastic was biodegradable, with minimal environmental impact [8].



Figure 4.
Photographs of bioplastic made by tomato cuticle during the BIOPROTO project.

2.8 ECOFUNCO

The tile project is “ECO sustainable multi-FUNctional biobased COatings with enhanced performance and end-of-life options.” The overall objective of project ECOFUNCO [9] was to select, extract and functionalize molecules (proteins, polysaccharides, cutin) from highly available, low valorized biomass such as tomato, legumes, sunflower, etc. for the development of new bio-based coating materials to be applied on two different substrates (i.e., cellulosic and plastic-based), with improved performances compared to currently available products and at the same time with the more sustainable end of life options. The products to be developed in the project were in particular:

- Antimicrobial-antioxidant coatings based on chitin nanofibrils, and/or chitosan, for cellulose tissues (personal care), paper and cardboard (packaging for fresh products like pasta, tableware), woven and nonwoven (sanitary), plastic substrates (bio-polyesters) for active packaging
- Cutin-based formulations for water-repellent coatings (paper cups, service paper, etc.), water vapor barrier (packaging), and protective properties (non-food packaging)
- Protein-based barrier adhesive for multilayer food packaging (bio polyesters based), with sustainable end-of-life options (composting, recyclability).

The ECOFUNCO project final event has been taken on June 17–18, 2022 in the form of “1st Conference on Green Chemistry and Sustainable Coatings.”

The event confirmed that the ECOFUNCO project developed sustainable bio-based and compostable coatings to be applied on bioplastics and cellulose substrates to reach the same properties as fossil-based packaging materials. Also, the use of nanofibrils to add antimicrobial properties to tissues has been demonstrated.

The ECOFUNCO coordination and management demonstrated a great mind opening and a forward-looking sensitivity as they dedicated a session of the final conference to other related EU-funded projects, that is, FISH4FISH, PRESERVE, RECOVER, PROLIFIC, and Agrimax, in order to foster the cooperation between European projects.

2.9 Refresh

The title of the project is “Resource Efficient Food and dRink for the Entire Supply cHain.” The overall aim of the REFRESH project was to significantly contribute toward the objective of reducing food waste across the EU by 30% by 2025 and maximizing the value from unavoidable food waste and packaging materials. The project aims to gather information about the main and most present food waste in the European countries, find the known way to exploit these by-products, and create a simplified tool to help the decision-maker to valorize at best these side streams, both in terms of economic feasibility and environmental impact. Tomato by-products are one of the considered waste streams. The project outcomes are 6 scientific publications regarding food waste, from their management to their reduction, a website and a software tool [10]. One of the main outcomes of the REFRESH project is a deliverable with the TOP20 waste streams in Europe, carefully reporting

3.1 Top 20 food waste streams appropriate for valorisation

The priority waste streams identified (organised alphabetically by food product) along with their current management are shown in the table below.

Table 1: Top 20 food waste streams appropriate for valorisation

Food product	Waste stream	Current management	Reason for selection
Spirits	Organic wastes, mash from grain, fruit or potato	Animal feed, composting	High volumes, regionally important for United Kingdom, France, Germany, Poland & Italy.
Sugar	Sugar beet pulp	Marketed in fresh / ensiled form as pressed pulp or blended with molasses to give molassed sugar beet feed (MSBF)	High volumes, regionally important for France, Germany, United Kingdom & Poland.
Tomatoes	Pomace (skin, pulp & seeds)	Animal feed	High volumes, regionally important for Spain, Italy, Greece & Portugal, rich source of lycopene.
Vegetable oil	Crude & extracted press	Production of fuels, industrial uses (kernel oil, wood,	High volumes of meal produced across Europe (~30

Figure 5.
 Tomato pomace is one of the TOP20 food wastes in Europe.

their current management and the reason for selection. Tomato by-products are in the list (see **Figure 5**).

Another main outcome is FORKLIFT, a spreadsheet learning tool that applies a partial lifecycle greenhouse gas impact and costing calculation approach for six key examples of unpreventable food processing co-products, by-products, or wastes (collectively referred to as *side flows*):

1. Apple pomace
2. Pigs blood
3. Brewers spent grains
4. Tomato pomace
5. Whey permeate
6. Oilseed press cake

FORKLIFT allows users to interpret the results of the effects of intervention while making it possible to compare the results with alternative products available on the market [11]. For tomato pomace conventional solutions for its exploitation were selected and modeled in the FORKLIFT® tool, allowing for evaluation via LCA and LCC, cost and CO₂ emission for different scenarios of valorization, and to easily compare them as a support to decision making. **Figure 6** shows the interface of the tool. In the analysis of tomato pomace, the following valorization routes were considered:

- Lycopene production
- Preparation of fodder
- Anaerobic digestion
- Land spread

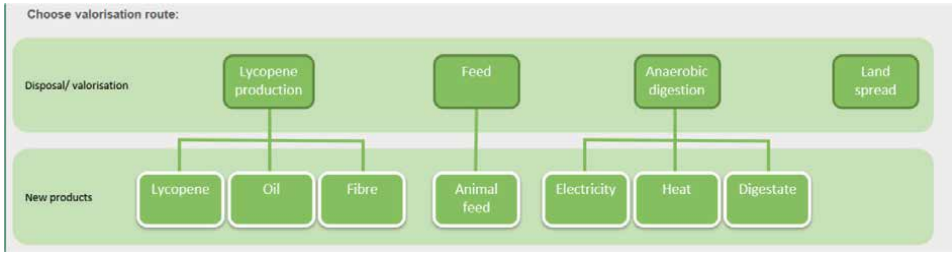


Figure 6. Valorization routes available on the FORKLIFT spreadsheet.

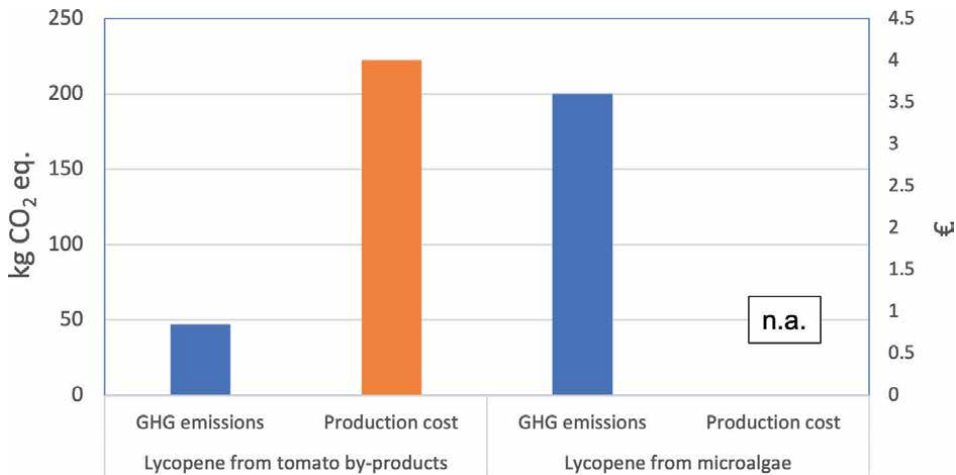


Figure 7. FORKLIFT output for lycopene production.

For example, with this spreadsheet is possible to compare lycopene production cost and emission with carotenoid production from microalgae (Figure 7).

2.10 AGRIMAX

The project title is “Agri and food waste valorization co-ops based on flexible multi-feedstocks biorefinery processing technologies for new high added value applications.” The goal of the project was to extract the significant amounts of valuable compounds contained in food industry wastes, AgriMax [12] combined affordable and flexible processing technologies for the valorization of side streams from the horticultural culture and food processing industry to be used in a cooperative approach by local stakeholders. The project merged previous knowledge and outcome of other European projects, such as cutin extraction and exploitation studied in the BIOCOPAC project. LCA and LCC studied the best approach to minimize the environmental impact of the new value chains. Moreover, a pilot multi-feedstock bio-refinery process was set up at two demonstration sites in Spain (Pilot Plant at Indulleida S.A.) and Italy Pilot Plant (at Chiesa Virginio EC). Currently, the Italian pilot plant is valorizing the tomato by-products, producing cutin bioplastic, a small amount of lycopene and compost. The pilot plant flow-sheet is reported in Figure 8.

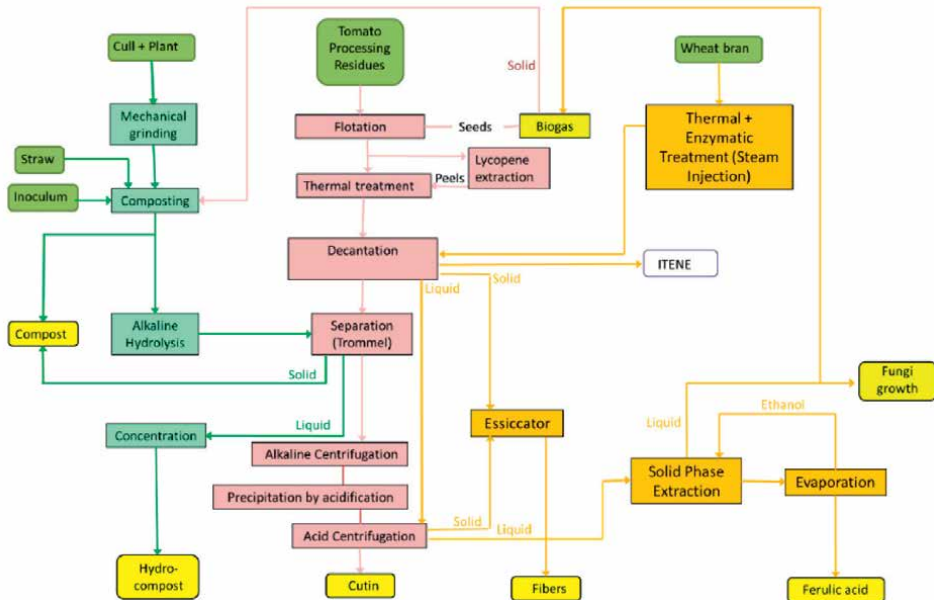


Figure 8. Flowsheet of Italian pilot plant located in the factory of Azienda Agricola Virginio Chiesa, Canneto Sull'Oglio (MN), Italy.

3. Conclusion

In conclusion, 11% of funded European projects having tomato as a topic are dealing with tomato wastes and by-products. Forty projects were found when searching CORDIS with “tomato” and “waste” as keywords; 14 regard by-products valorization, categorizable in the following topics: production of bioplastic or biofilm, extraction of high-value compounds, preparation of food additives or fodder, biogas production via fermentation and biorefining of tomato by-products. The overall budget, that European Commission furnished to the participants, has been around 40 M€ in about 35 years. These projects involved 130 participants coming from all over the world. Extraction of compounds is the topic of most projects, but the highest budget has been awarded to biorefining. This is also the main focus of the research activities first explored and then directly pursued by the authors [13, 14] of this chapter. Projects on extraction technology development had as an outcome the optimization of commercial techniques, leading to patents; moreover, some studies showed that supercritical CO₂ is never economically feasible for lycopene extraction. PRO-ENRICH is the only project about food additives that were recently found, to start a pilot plant for protein production from different waste streams, including tomato pomace. In the last years, bioplastic production from tomato by-products received great attention and funding, leading a pilot plant in Italy to produce metal packaging cover with a biofilm obtained from tomato peels. Recent projects (AGRIMAX and REFRESH) aim to best exploit food waste, making recourse to a biorefining approach. Main problems remain in the tomato by-products valorization: the high economic or environmental cost of lycopene extraction, as also underlined [15] by the authors of this chapter; the absence of a ‘green’ alternative for cutin extraction, and the difficulty in finding a biomass

similar to tomato pomace in order to overcome the seasonality issue. Moreover, a lack of data, studies, and projects on energy recovery from tomato by-products was evidenced by the present survey.

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*Edited by Pranas Viškelis,
Dalia Urbonavičienė and Jonas Viškelis*

The tomato is a valuable vegetable, popular all over the world. This book covers interesting research topics including tomato plant nutrition, production and chemical composition, tomato plant protection, and sustainable tomato processing technologies. This book will be of value to researchers, academics, and students in the field of agronomy, food, pharmacy, and other sectors.

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