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Cotton

Edited by Ibrokhim Y. Abdurakhmonov

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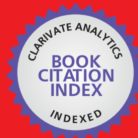

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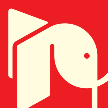

Meet the editor

Ibrokhim Y. Abdurakhmonov received his BS in Biotechnology from the National University, Uzbekistan in 1997, an MS in Plant Breeding from Texas A&M University in 2001, and a Ph.D. in Molecular Genetics and DSc from the Academy of Sciences of Uzbekistan in 2002 and 2009, respectively. He became a full professor at the same university in 2011. He founded the Center of Genomics and Bioinformatics of Uzbekistan in 2012. He received the 2010 TWAS prize and ICAC Cotton Researcher of the Year 2013 for his outstanding contribution to cotton genomics and biotechnology. Dr. Abddurakhmonov was elected as a fellow of The World Academy of Sciences (TWAS) in 2014 and a member of the Academy of Sciences of Uzbekistan in 2017. He was appointed Minister of Innovative Development of Uzbekistan in 2017. He was honored as the 2022 Ambassador of Silk Road Friendship (Individual) by the China International Culture Exchange Center (CICEC) and *Global People Magazine*.

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Preface

Cotton cultivars (*Gossypium* spp.) are the single most important natural fiber crop in the world. The oil and proteins produced from cottonseed contribute to the world's food security as human diet and animal feed products with important economic value. Cotton was first cultivated as a fabric in prehistoric times. Very old pieces of cotton cloth have been found in Peru (dating back to 6000 BCE) as well as Mohenjo-Daro and other sites of the Bronze Age Indus Valley civilization (dating back to 5500 BCE).

There are five allotetraploid species and forty-five diploid species, representing the primary, secondary, and tertiary genetic sources (gene pool) used for the genetics and breeding of cotton cultivars. Cotton research has advanced globally over the past half-century. Researchers have addressed the key challenges and limitations of cotton farming worldwide by initiating largely coordinated multi-institutional research projects on cotton. These efforts have greatly accelerated cotton research worldwide and helped to improve cotton production and farming.

A century of cotton research activity analysis shows that investigations on this crop have rapidly increased. Based on our analysis of PubMed indexed scientific publications, there were six major bumps around 1966, 1973–1975, 2001, 2007, 2013, and 2016 (see introductory chapter for details) with increased publication activities. Although the number of cotton science publications has plateaued in the last five years, their focus has narrowed to biotic/abiotic and fiber or plant developmental studies, utilizing more genomic tools than ever. Therefore, there is a need for a timely review of the current state of advancements in cotton research.

This book provides a comprehensive overview of the latest advances in cotton science. It is a useful resource for university students, life science researchers, and other interested readers.

I greatly acknowledge the assistance of Dr. Kater Hake at Cotton Incorporated Inc., Dr. Venkatesh N. Kulkarni at Nath Bio-Gene Ltd., and Prof. Govind Sharma at Alabama A&M University for their help in reviewing some of the book chapters and for their valuable opinions in improving the book's content. I also thank all the authors for their efforts and invaluable contributions to this volume. Finally, I extend my thanks to the staff at IntechOpen, especially the Author Service Manager Ms. Jasna Bozic.

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Dedication

This book volume and my introductory chapter are dedicated to the memory of the distinguished cotton geneticist, mentor, and friend Dr. Sukumar Saha who sadly passed away on September 3, 2021. Dr. Saha will always be remembered for his invaluable contribution to the cotton research program of Uzbekistan and for his pioneering scientific research in cotton genomics, cytogenetics, and breeding.

Section 1

Introduction

Chapter 1

Introductory Chapter: Global Cotton Research Development Trends for the Past Five Years – Key Directions

Ibrokhim Y. Abdurakhmonov

1. Introduction

Cotton, represented by more than 50 *Gossypium* species, is the world's most important natural textile fiber crop. The cottonseed is an important source of feed, foodstuff, and oil. Because of the importance of this cash crop worldwide, it is very important to timely review the current state of advancement in cotton research for determining the development trends. For this purpose, I previously compiled two book volumes in the InTechOpen open-access publication platform on cotton research in 2014 and 2016 [1, 2]. Later, in 2018, Mehboob-Ur-Rahman and Zafar [3], and in 2020, Ansari [4] had compiled two other dedicated book volumes on the advancement of cotton research. All of these volumes highlighted the latest advancement in cotton investigations and covered periodical success in cotton science and farming. However, in recent years, we have witnessed that cotton research progressively has advanced over a decade to address the key challenges and limitations of cotton farming worldwide. Researchers have initiated largely coordinated multi-institutional research projects on cotton. These efforts have greatly accelerated cotton research globally to improve cotton production and farming [5].

2. Novel focuses

In recent years, cotton researchers have focused on cotton genetics/genomics and physiology, cotton germplasm collections and its biodiversity, traditional and molecular breeding for cultivar development, tagging important genes, whole-genome sequencing, genome-wide marker-trait association, transcriptome and proteome analyses useful for new-generation superior biotechnology crops, transgenic and new-generation gene-editing technologies, including CRISPR/Cas9. Cotton crop physiology in a complex view from seed germination to maturation stages under different temperatures, water, light, and nutrient applications [5] has been the key propriety and under the targeted focus of the world research community.

Efforts on novel and emerging innovations and game-changing technologies are ongoing to address current cotton farming limitations and for the effective management of sustainable cotton agriculture. These include but are not limited to virtual

breeding, bioinformatics, smart communication and mobile phone technologies, artificial intelligence, and augmented reality-based applications helping cotton breeding and farming in the era of climate change and technological advances [5].

Eminent cotton scientists of the world have reported that "cotton farming and management practices, the utilization of new generation of chemical and biological fertilizers and their assessment tools, including modern conservation tillage, winter cover cropping, site-specific nutrient applications, and remote sensing technologies, as well as integrated pest and disease management programs" [2, 5] and cotton seed science development, will be the key research directions toward 2030.

3. Past 5-year development trends

Cotton-research-related publications over more than one century (1908–2022), retrieved from the *PubMed* database [6] using the keyword search of "*Gossypium*," revealed over 9060 scientific publications as of March 2022 (**Figure 1**). We observed a publication increase in early 1966 that had doubled in number compared with earlier years. The next twofold increase was observed around 1973–1975, and publication activity remained in the same range of about 100 publications per year until 2000, after which a sharp 1.6-fold increase (167 publications) was observed in 2001. The next bumps were in 2007 (227 publications/year), 2013 (385 publications/year), and 2016 (420 publications/year), remaining at the plateau till today.

The literature analysis and research publication on the *Gossypium* science for the past 5 years clearly showed the advancement trends of cotton research worldwide for the current time. The number of publications per year for the past 5-year period was at the plateau and was within the range of 420 publications/year, and overall, we found over 2009 available articles in *PubMed*, retrieved using the "*Gossypium*" keyword, out of which 1948 were directly related to the cotton study. Interestingly, analysis based on PubMed indexed journals showed that, over the past 5 years, at least, 283 internationally known peer-reviewed plant science journals including but are not limited to Front Plant Sci (125 publications), PLoS One (103 publications), Sci Rep (103 publications), BMC Plant Biol (92 publications), Int J Mol Sci (81 publications), and BMC Genomics

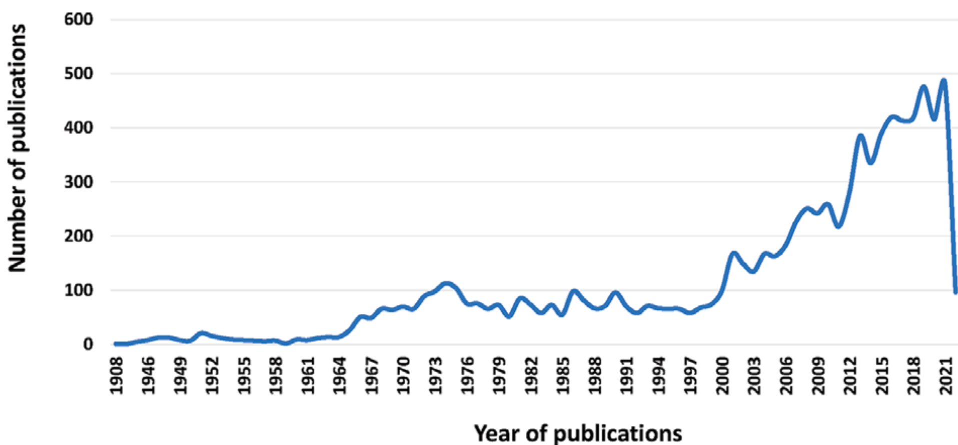

Figure 1.
PubMed [6] indexed scientific publications, retrieved using the "Gossypium" keyword in March 2022.

(72 publications) have published cotton articles. Among all *PubMed-i*ndexed publications, the majority of works (1872 publications) were research articles, while 55 articles were literature review works. The remaining 21 articles were Brief Communications (3 publications), Database (3 publications), Protocols (3 publications), Reports (3 publications), Research highlights (2 publications), Scientific opinions (2 publications) as well as Disease notes, Methods, News & Views, Brief Reports, and Short communications (1 publication of each).

These publications have also used or investigated a diverse type of cotton species including but are not limited to *G. hirsutum* (796 publications), *Gossypium barbadense* (219 publications), *G. arboreum* (226 publications), G. *raimondii* (191 publications), *Gossypium herbaceum* (17 publications), *G. davidsonii* (7 publications), *G. longicalyx* (5 publications), and *Glossoloma anomalum* (1 publication). The main AD-genome allotetraploid (*G. hirsutum* and *G. barbadense*) and A- and D-genome diploid (*G. herbaceum*, *G. arboretum,* and *G. raimondii*) cotton species were the subjects to study a diverse set of traits and/or plant ontogenesis or resistance-related processes, while some of the diploid species including *G. davidsonii, G. longicalyx, and G. anomalum* have been mainly used to study the specific traits such as cytoplasmic male sterility, pant development, salt stress tolerance, nematode resistance, and fiber development.

In this context, in-depth analysis and review of the past 5-year publications have revealed the main directions of cotton research that the world research community or leading researchers have focused on lately (**Figure 2**). In particular, researchers have more focused on studying biotic stresses [e.g., 7–10], plant development [e.g., 11–13], abiotic stresses [e.g., 14, 15], fiber development [e.g., 16, 17], and genomics [e.g., 18–21] compared with other directions. Studies on cotton agronomy [22, 23], genetics and breeding [24, 25], evolution [26, 27], yield improvement [28, 29], processing of cotton product [30, 31], biochemical components [32, 33], and mutagenesis [34, 35] were also carried out.

The past 5-year published literature analysis demonstrated that cotton plant protection, in particular, resistance to biotic pressure (670 publications) and abiotic

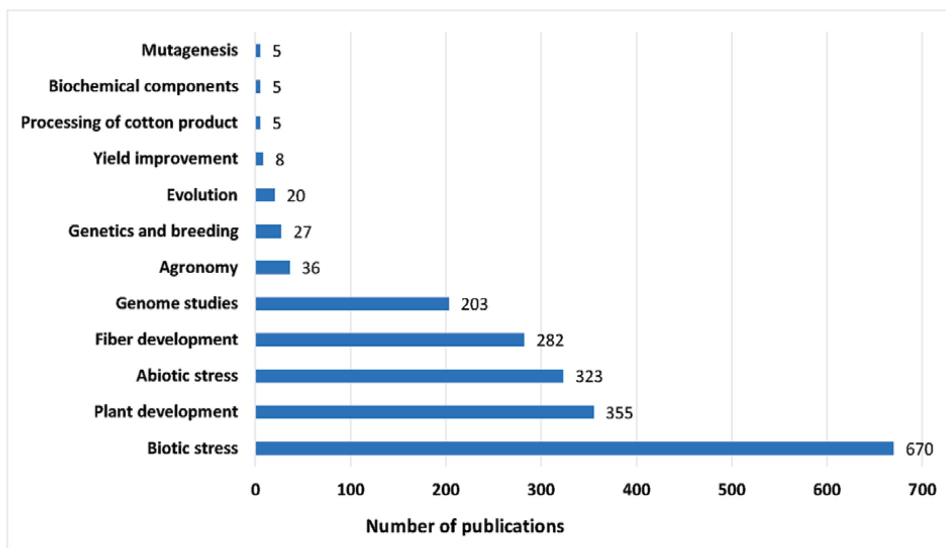

Figure 2.
Research directions of the past 5-year scientific publications.

stresses (323 publications) have been the two major research trends of cotton research for the past 5-year period. Researchers have also focused on plant development (355 publications), fiber development (282 publications), and genome (203 publications) studies. Each main direction, in turn, has addressed the diverse research problems in cotton based on national or global level issues. Here, interestingly, we observed the leading number of biotic stress-related publications with more focus on insect resistance (171 publications) or pest control (166 publications), following the *Verticillium* wilt disease (152 publications) topics. Similarly, publications on abiotic stress-related topics have mainly addressed salinity tolerance, drought tolerance, cold tolerance, heat and drought tolerance, soil mineral deficiency, and its impact on many other traits and crop productivity. Salt (94 publications) and drought (72 publications) or their combinations (43 publications) were the main targets of researchers. These directions could also be the result of the need for cotton farming for the improvement of cotton cultivar development under harsh environmental conditions and/or increased disease/pest pressure over the past years. Moreover, these key research directions are most likely to be in our current and future cotton research program agenda under the rapidly growing challenges and limitations of the global climate change scenario worldwide.

This *Cotton* book volume has compiled scientific chapters from the international research community that covers the current status and latest advances in cotton science. Chapters have presented a wide range of novel discussions on cotton genetics, biochemistry and physiology of important trait(s), bioinformatics and genomics resources to understand the cotton plant, genomics and transcriptomic approaches to the identification and characterization of important genes, novel transgenic tools for acceleration of cotton breeding against climate issues and biotic/abiotic stress pressures, biological control and machinery tools for cotton cultivar protection, and cottonseed meal production as well as sustainable and effective farming in the era of climate change and technological advance. These peer-reviewed chapters have also presented novel views and discussions on the abovementioned research trends observed in the past 5-year publications, which will be an excellent addendum for readers.

4. Conclusion

Thus, this Cotton book chapters from international cotton research communities have timely provided an overview of the current state of advancement in cotton research. I am confident that these peer-reviewed chapter discussions should help to determine the current and future development trends in cotton science, cultivar development, and sustainable cotton agriculture in the world.

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References

[1] Abdurakhmonov IY. World Cotton Germplasm Resources. London: IntechOpen; 2014. p. 322. DOI: 10.5772/56978

[2] Abdurakhmonov IY. Cotton Research. London: IntechOpen; 2016. p. 310. DOI: 10.5772/61914

[3] Mehboob-Ur-Rahman M, Zafar Y. Past, Present and Future Trends in Cotton Breeding. London: IntechOpen; 2018. p. 184. DOI: 10.5772/intechopen.69672

[4] Ansari M. Advances in Cotton Research. London: IntechOpen; 2020. p. 84. DOI: 10.5772/intechopen.77828

[5] Cotton vision 2030. ICAC. Recorder. 2020;**38**(4):18-20 ISSN 1022-6303

[6] PubMed Database [Internet]. 2022. Available from: https://pubmed.ncbi.nlm.nih.gov/. [Accessed: January 3, 2022].

[7] Cox KL, Meng F, Wilkins KE, Li F, Wang P, Booher NJ, et al. TAL effector-driven induction of a SWEET gene confers susceptibility to bacterial blight of cotton. Nature Communications. 2017;**8**:15588. DOI: 10.1038/ncomms15588

[8] Zubair M, Zaidi SS, Shakir S, Farooq M, Amin I, Scheffler JA, et al. Multiple begomoviruses found associated with cotton leaf curl disease in Pakistan in early 1990 are back in cultivated cotton. Scientific Reports. 2017;**7**(1):680. DOI: 10.1038/s41598-017-00727-2

[9] Liu XD, Xu TT, Lei HX. Refuges and host shift pathways of host-specialized aphids *Aphis gossypii*. Scientific Reports. 2017;**7**(1):2008. DOI: 10.1038/s41598-017-02248-4

[10] Wang G, Xu J, Li L, Guo Z, Si Q, Zhu G, et al. GbCYP86A1-1 from *Gossypium barbadense* positively regulates defense against *Verticillium dahliae* by cell wall modification and activation of immune pathways. Plant Biotechnology Journal. 2020;**18**(1):222-238. DOI: 10.1111/pbi.13190

[11] McGarry RC, Ayre BG. Cotton architecture: Examining the roles of *SINGLE FLOWER TRUSS* and *SELF-PRUNING* in regulating growth habits of a woody perennial crop. Current Opinion in Plant Biology. 2021;**59**:101968. DOI: 10.1016/j.pbi.2020.10.001

[12] Zhi X, Han Y, Li Y, Wang G, Feng L, Yang B, et al. Root growth and spatial distribution characteristics for seedlings raised in substrate and transplanted cotton. PLoS One. 2017;**12**(12):e0190032. DOI: 10.1371/journal.pone.0190032

[13] Ayubov MS, Mirzakhmedov MH, Sripathi VR, Buriev ZT, Ubaydullaeva KA, Usmonov DE, et al. Role of MicroRNAs and small RNAs in regulation of developmental processes and agronomic traits in *Gossypium* species. Genomics. 2019;**111**(5):1018-1025. DOI: 10.1016/j.ygeno.2018.07.012

[14] Mahmood T, Khalid S, Abdullah M, Ahmed Z, Shah MKN, Ghafoor A, et al. Insights into drought stress signaling in plants and the molecular genetic basis of cotton drought tolerance. Cell. 2019;**9**(1):105. DOI: 10.3390/cells9010105

[15] Wei Y, Xu Y, Lu P, Wang X, Li Z, Cai X, et al. Salt stress responsiveness of a wild cotton species (*Gossypium klotzschianum*) based on transcriptomic analysis. PLoS One. 2017;**12**(5):e0178313. DOI: 10.1371/journal.pone.0178313

[16] Hernandez-Gomez MC, Runavot JL, Meulewaeter F, Knox JP. Developmental features of cotton fibre middle lamellae in relation to cell adhesion and cell detachment in cultivars with distinct fibre qualities. BMC Plant Biology. 2017;**17**(1):69. DOI: 10.1186/s12870-017-1017-3

[17] Martínez-Sanz M, Pettolino F, Flanagan B, Gidley MJ, Gilbert EP. Structure of cellulose microfibrils in mature cotton fibres. Carbohydrate Polymers. 2017;**175**:450-463. DOI: 10.1016/j.carbpol.2017.07.090

[18] Gao W, Long L, Tian X, Xu F, Liu J, Singh PK, et al. Genome editing in cotton with the CRISPR/Cas9 system. Frontiers in Plant Science. 2017;**8**:1364. DOI: 10.3389/fpls.2017.01364

[19] Wang M, Tu L, Yuan D, Zhu D, Shen C, Li J, et al. Reference genome sequences of two cultivated allotetraploid cottons, *Gossypium hirsutum* and *Gossypium barbadense*. Nature Genetics. 2019;**51**(2):224-229. DOI: 10.1038/s41588-018-0282-x

[20] Grover CE, Arick MA, Conover JL, Thrash A, Hu G, Sanders WS, et al. Comparative genomics of an unusual biogeographic disjunction in the cotton tribe (*Gossypieae*) yields insights into genome downsizing. Genome Biology and Evolution. 2017;**9**(12):3328-3344. DOI: 10.1093/gbe/evx248

[21] Zaidi SS, Mansoor S, Paterson A. The rise of cotton genomics. Trends in Plant Science. 2018;**23**(11):953-955. DOI: 10.1016/j.tplants.2018.08.009

[22] Song X, Li Y, Yue X, Hussain Q, Zhang J, Liu Q, et al. Effect of cotton straw-derived materials on native soil organic carbon. Science in the Total Environment. 2019;**663**:38-44. DOI: 10.1016/j.scitotenv.2019.01.311

[23] Bansal S, Yin X, Schneider L, Sykes V, Jagadamma S, Lee J. Carbon footprint and net carbon gain of major long-term cropping systems under no-tillage. Journal of Environmental Management. 2022;**307**:114505. DOI: 10.1016/j.jenvman.2022.114505

[24] Wang L, Wang X, Maimaitiaili B, Kafle A, Khan KS, Feng G. Breeding practice improves the mycorrhizal responsiveness of cotton (*Gossypium* spp. L.). Frontiers in Plant Science. 2021;**12**:780454. DOI: 10.3389/fpls.2021.780454

[25] Billings GT, Jones MA, Rustgi S, Hulse-Kemp AM, Campbell BT. Population structure and genetic diversity of the PEE DEE cotton breeding program. G3 (Bethesda). 2021;**11**(7):jkab145. DOI: 10.1093/g3journal/jkab145

[26] Bao Y, Hu G, Grover CE, Conover J, Yuan D, Wendel JF. Unraveling cis and trans-regulatory evolution during cotton domestication. Nature Communications. 2019;**10**(1):5399. DOI: 10.1038/s41467-019-13386-w

[27] Zhang TT, Liu H, Gao QY, Yang T, Liu JN, Ma XF, et al. Gene transfer and nucleotide sequence evolution by *Gossypium* cytoplasmic genomes indicate novel evolutionary characteristics. Plant Cell Reports. 2020;**39**(6):765-777. DOI: 10.1007/s00299-020-02529-9

[28] Li C, Zhao T, Yu H, Li C, Deng X, Dong Y, et al. Genetic basis of heterosis for yield and yield components explored by QTL mapping across four genetic populations in upland cotton. BMC Genomics. 2018;**19**(1):910. DOI: 10.1186/s12864-018-5289-2

[29] Ma L, Wang Y, Ijaz B, Hua J. Cumulative and different genetic effects contributed to yield heterosis using

maternal and paternal backcross populations in upland cotton. Scientific Reports. 2019;**9**(1):3984. DOI: 10.1038/s41598-019-40611-9

[30] Wang C, Zhang S, Wu S, Cao Z, Zhang Y, Li H, et al. Effect of oxidation processing on the preparation of post-hydrothermolysis acid from cotton stalk. Bioresource Technology. 2018;**263**:289-296. DOI: 10.1016/j.biortech.2018.05.008

[31] Xu SS, Qiu SW, Yuan ZY, Ren TZ, Bandosz TJ. Nitrogen-containing activated carbon of improved electrochemical performance derived from cotton stalks using indirect chemical activation. Journal of Colloid and Interface Science. 2019;**540**:285-294. DOI: 10.1016/j.jcis.2019.01.031

[32] Resco de Dios V, Anderegg WRL, Li X, Tissue DT, Bahn M, Landais D, et al. Circadian regulation does not optimize stomatal behaviour. Plants (Basel). 2020;**9**(9):1091. DOI: 10.3390/plants9091091

[33] Xu J, Chen L, Sun H, Wusiman N, Sun W, Li B, et al. Crosstalk between cytokinin and ethylene signaling pathways regulates leaf abscission in cotton in response to chemical defoliants. Journal of Experimental Botany. 2019;**70**(5):1525-1538. DOI: 10.1093/jxb/erz036

[34] Chen X, Lu X, Shu N, Wang S, Wang J, Wang D, et al. Targeted mutagenesis in cotton (*Gossypium hirsutum* L.) using the CRISPR/Cas9 system. Scientific Reports. 2017;**7**:44304. DOI: 10.1038/srep44304

[35] Abid MA, Wang P, Zhu T, Liang C, Meng Z, Malik W, et al. Construction of *Gossypium barbadense* mutant library provides genetic resources for cotton germplasm improvement. International Journal of Molecular Sciences. 2020;**21**(18):6505. DOI: 10.3390/ijms21186505

Section 2

Cotton Genetics, Genomics, Breeding and Farming

Chapter 2

Studies on Colored Cotton: Biochemical and Genetic Aspects

Sathees Nagarajan, Yazhni Purushothaman, Monika Selvavinayagam, Pandidurai Govindharaj and Aasif Musthafa

Abstract

Cotton (*Gossypium hirsutum* L.) is a commercially important fiber crop used as the primary raw material in the textile industry and is cultivated throughout the world. Normally cotton fiber is white color and various dyes are used to color the fiber. In textile industry, the process of artificial dying is a major source of pollution to the environment and the cost of dying is also higher. Apart from the white fiber, several cotton species have colored fiber which can be used to reduce the dying process and its ill effects to the environment. The cotton fiber color inheritance pattern is an urgent problem. The physical and chemical properties of colored cotton are determined by its chemical composition. The naturally colored cotton contain some important properties such as, greater hygiene, hypoallergenic properties, lower flammability and higher ultraviolet protection value compared to traditional white cotton. The natural colored cotton loss their market value due to the poor fiber quality. Understanding of the colored cotton pigment composition, biochemical and genetic prospects of colored cotton will be useful for the development of high quality of colored cotton.

Keywords: colored cotton, fiber quality, colored pigment, biochemical and genetic property

1. Introduction

In world, cotton is an important cash crop and it is a most traded commodity [1]. China, India, United States, Pakistan, and Brazil are the largest cotton producers [2]. With 312 lakh bales, India has the world's largest cotton area of around 12.7 million hectares and is now the world's second largest cotton producer (each of 170 kg) [3]. The cotton is a dicotyledon comes under the malvaceae family and *Gossypium* genus. Globally, *Gossypium* genus is spread in 5 continents. It contains 50 species in the world which are woody and herbaceous form [4]. The 50 species contain 45 diploid (2 n = 2x = 26) and five allotetraploid (2 n = 4x = 52) species [4]. Among these two diploid species (*G. arboreum* L. and *Gossypium herbaceum* L.) and two tetraploid species (*G. hirsutum* L. and *Gossypium barbadense* L.) comes under cultivated species. The 95 percentage of world cotton production was fulfilled by two tetraploid cotton cultivars such as *G. hirsutum* L. (upland cotton) and *G. barbadense* L. (Sea Island or

Egyptian cotton) because it contains good fiber yield and broad adaptation to several environments [5]. In global fiber market, the polyester and other synthetic fibers have the robust competition during 1990 s which increases the competition in beginning of 2000 s [1]. Most of textile products are manufactured by cotton fiber and lint [6]. In *Gossypium* genus, the formation of fibers is abnormal. The surface of the ovule has the outward elongated cells growing. Additionally, as the fiber matures, the protoplast dies, and the cell wall collapses inward to form a convoluted ribbon [5]. One of the most significant raw materials for the textile industry is cotton (*Gossypium hirsutum* L.). Nearly all cotton fiber used for textiles is white, dyes are necessary throughout the fiber processing process to color the cloth. The massive usage of dyes has resulted in pollution, which has had a significant impact on human health [7]. Cotton that is naturally colored is made up of pigmented fibers with color embedded in the lumen [8]. The colorful strain of *G. hirsutum* is crossed with a white linted strain to create hybrids that outperform the color parents in terms of fiber length, strength, and color fastness. Natural colored cotton will be the next big thing in the market as the world shifts toward pollution-free organic fabrics and products. This is due to the fact that the production of naturally colored cotton avoids the most polluting activity of textile product manufacturing (dyeing) [9]. In ecology textiles, naturally colored cotton is an important raw material which eliminates the dying during the processing. It would significantly decrease the processing cost, environmental pollution and chemical residue [8, 10]. Furthermore, when compared to standard white cotton, naturally colored cotton may have a reduced flammability and a greater UV protection rating [11]. One of the most efficient solutions is to breed cotton varieties that naturally contain colored cotton fibers (CCFs), which are environmentally safe. Cotton plants with colorful fibers have been cultivated for a long time [12]. However, for the following two reasons, their development has been slower than that of white fiber cotton. To begin with, colored fiber cotton yields far less than white fiber cotton [13]. Various dye products that have been employed in the textile industry since the industrial revolution are available, as well as the negative consequences of their use has long been forgotten [14]. Cotton fiber is the most important fabric material on the planet, with almost all the industrially used cotton coming from white cotton fiber (WCF). However, with rising environmental concerns and improved human life quality, interest in naturally colored cotton (NCC, *G. hirsutum*) fiber has steadily increased over the previous decade. Natural colors are used in NCC fibers. The use of NCC fiber in fabrics would cut textile processing and the generation of harmful chemical wastes significantly [15]. Nonetheless, poor fiber quality and drab colors have hampered NCC fiber adoption on a big scale [16]. The genetics and plant breeding of cotton goal is improving fiber quality while increasing the cotton yield [17]. Fiber quality is a complex trait which includes fiber length, strength, and fineness. The following traits determine the cotton yield such as number of bolls per plant, number of plants per unit area, lint percentage, and single boll weight [18]. The fiber quality and yield have the negative correlation, so the synchronous improvement of that traits through conventional breeding techniques is difficult. So, understanding of the colored cotton biochemical and molecular aspects will be used to improve that cotton.

2. Genes regulating pigmentation and its inheritance pattern

Knowledge on the genes responsible for pigmentation can help the breeders to develop colored cotton and to overcome the barriers in developing it. The natural colored

lint of cotton shows great variability for lint color of which brown and green are the most stable and predominant types. The pigments are accumulated in the lumen of the lint [19] only when exposed to sunlight [20]. The developed color fades out when exposed to sunlight for a longer time and moisture content also affects the developed color [15]. The poor fiber quality *viz.,* fiber length, fiber percent and resistance is attributed to the pleiotropic effects of genes controlling fiber quality [21]. Presence of modifying genes also affects the color and quality of pigments. By expression analysis, *GhF3'5'H* and *GhCHS3* genes were found to be higher in colored cottons than in white cotton [22].

Green color results due to the deposition of caffeic acid in the suberin layer. Brown pigmentation results due to the pro anthocyanidin (PA) (condensed tannin) accumulation in cell vacuoles [23]. The prime sequences in PA biosynthesis were highly conserved in both white (*G. arboreum*) and brown (*G. raimondii* or *G. stocksii*) fibers [24]. Earlier it was reported that both the green and brown pigments are governed by single gene with incomplete dominance [25]. Later, in 1944, conventional approaches revealed the existence of six loci (L_{c1}- L_{c6}) for brown pigment, out of which L_{c1} was located on chromosome 7, L_{c2} on chromosome 6 and one locus (L_g) for green pigment. Further, colored cotton is dominant over white fiber cotton [26]. But in texas green and brown lint, they are controlled by single incompletely dominant gene [25]. Generally, these genes are pleiotropic in nature [10].

Genetically, green color genes are dominant over both brown and white colors in cotton. From the findings of several researchers, it is evident that, the brown color is controlled monogenically with incomplete dominance [27]. Also, the brown lint and brown fuzz color was found to be correlated and controlled by single gene with incomplete dominance [28, 29]. Further investigation advocated that the *GhTT2-AO7* gene of *LC 1* controls the brown color fiber trait [30]. Additionally, in brown color cottons, the flavanoid genes (*GhCH1, GhF3H, GhDFR, GhANS & GhANR*) were involved in proanthocyanidin flavanoid production which is responsible for the brown pigmentation [22]. The gene *Gh3GT* coding flavonoid 3- glucosyltransferase leads to green color even in brown fiber cotton [31]. Flavonoid biosynthesis is regulated by several transcription factors such as R2R3-MYB type factors, basic helix-loop- helix and WD40 repeats [32]. The genes *GhMYB10* and *GhMYB36,* homologous to genes which encode the R2R3-MYB type transcription factors were noticed in cotton and they enhance the PA synthesis [33]. *GhTT2-A07* and *GhTT2-3A* also involve in the production of brown pigment. *GhTT2-3A* and *GhbHLH130D* drive the structural genes *GhANR* and *GhLAR* to accumulate PA in fiber [30]. Also, the genes *GhTTG1* and *GhTT3* genes play an inevitable role in PA synthesis and fiber development [34]. The genes which are responsible for anthocyanin pigment were also found to act in the regulation of PA synthesis [35].

Caffeic acid biosynthesis in green fiber takes place via phenyl propanoid pathway. The expression of the gene *GhPAL* was found higher at the initiation stage of secondary cell wall thickening. The genes *Gh4CL1- GhCL4* convert the caffeic acid to its ester form. Out of these four above mentioned genes, *Gh4CL2* showed higher expression in green fiber, which was confirmed by its expression level and enzymatic activity studies [36].

3. Biosynthetic pathway/molecular basis of pigment synthesis and deposition

In brown fiber cotton, the amount of oxidized pro anthocyanidin increases with the maturation of bolls and its structure was observed to be modified by a galloyl

group [37]. MALDI- TOF MS proteomic analysis [38] in brown fiber revealed that out of 21 proteins responsible for pigmentation, 15 were the members of flavonoid biosynthesis process. PAs are polymers of polyhydroxy flavan-3-ol units and addition of leucoanthocyanidin (flavan-3, 4-diol) molecules. Digital gene expression (DGE) analysis showed that 34 PA synthase genes are involved out of which only 24 were upregulated [39]. These upregulated genes coding for the enzymes involved in the synthesis of PA including, 3- phenylalanine ammonia lyases (PAL), cinnamic acid-4-hydroxylase (C4H), 1, 4- coumarate CoA ligase (4CL), chalcone synthase (CHS), chalcone isomerase (CHI), flavone 3- hydroxylase (F3H), flavonoid 3′ hydroxylase (F3'H) and flavonoid 3′ 5′- hydroxylase (F3'5'H), dihydroflavonol 4- reductase (DFR), anthocyanidin synthase (ANS) and anthocyanidin reductase (ANR) [39]. Out of these, CHI plays a major role in the coloration of brown fiber [31]. Individual biochemical pathways were identified in brown fiber cotton some involving the major role of ANR [23] and some with major role of leucoanthocyanin reductase (LAR) [39]. Also, the activity of PAL was found to be higher in brown fiber than white ones [40]. The accumulation of PA in the brown fiber was found to be in peak at 30 DAS and decreased due to their oxidation toward maturation [37]. The biosynthetic pathway involved in the synthesis of pro anthocyanins is illustrated in **Figure 1.**

Green color in fibers is the result of Caffeic acid (CA) accumulation. Nearly 70% ω- hydroxydocosanoic acid and 25% decosanedoic acid, which are the components of caffeic acid, were isolated from green cotton whereas only 0.5% was reported in white fiber. UV and Nuclear magnetic resonance (H-NMR) spectroscopic studies revealed that wax portion of green fiber is mostly composed of glycerol, CA and its esterified form. Isolated fatty acids from green fiber showed the presence of 22-O-caffeol-22-hydroxydocosanoic acid and 22-O-caffeol-22- hydroxydocosanonin. They were responsible for green and yellow pigmentation and increasing the concentration of

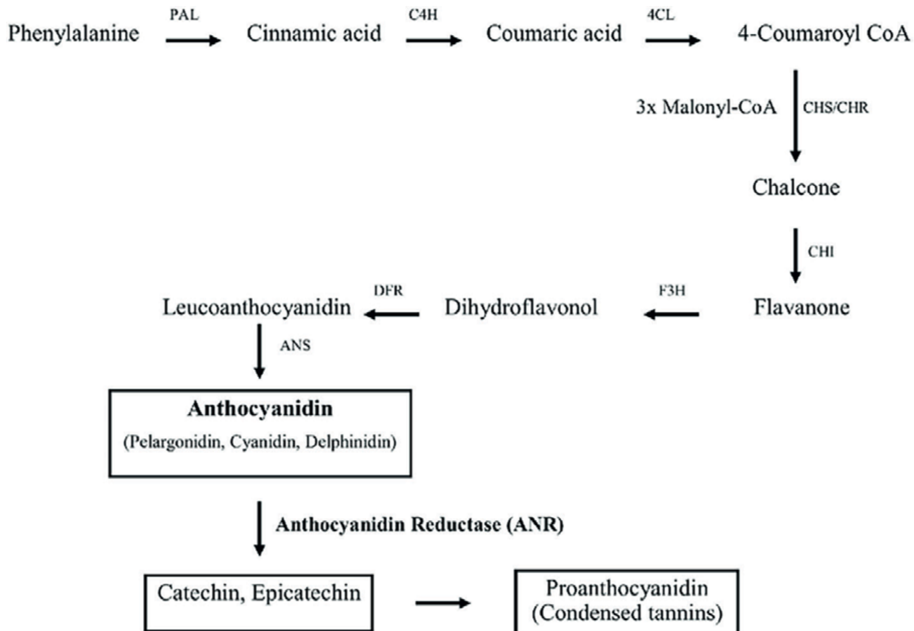

Figure 1.
Biosynthesis of pro anthocyanins [41].

the latter, leads to deep green color [23]. Another important point to be noted was the suberization of seed coat in the plant, producing green fiber, while it was absent in white and brown cotton [42].

4. Characterization of colored cotton pigments

Comparing to white cotton, naturally colored cotton has more flavonoids, which would reach 1 mg/g at maturity. This forms the major portion of pigments synthesized in colored cotton. pH values also tend to vary in the colored and white cotton, which was 5.60 in white fiber and 5.63 in colored cotton at 30 days post anthesis (DPA). But in brown cotton, it rose to 6.07 at 35 DPA and may reach 6.38. Generally, a drop in pH, favors cell elongation and secondary cell wall thickening during fiber cell development and this rise in pH may lead to poor development of fiber [13]. Cellulose content of colored cotton differs from that of white cotton after 20 DPA and this may be due to the fact that flavonoid synthesis in CCF may make use of the available simple carbohydrates. This in turn may affect the quality and quantity of fiber [43]. Pigment development in green cotton takes more time than that of brown cotton. The fibers of naturally colored cotton give low lint yield, produce short, weak and coarse fiber. Also, the distribution of pigment may not be uniform [10]. The brown pigmented cotton fiber was found to be superior to green. In addition, the green pigment deposits in fiber during 15 to 20 days post anthesis period. While testing the amount of Nitrogen in the colored cotton, it was higher in colored cotton. Potassium level was lower in the colored cotton particularly in green. Correlation studies indicated negative relationship between the pigment with fiber quality parameters. The pleiotropic nature of the color genes inhibits the fiber development and this becomes the reason in the difficulty of developing colored cotton with good fiber quality [13]. Thus, cotton with high cellulose level, low N and P and high K levels with acceptable level of pigment is desirable.

Suberin lamella was present in the cell wall of green cotton fiber cells [44]. Presence of glycerol also has been found in the green cotton fibers. The presence of some yellow green pigments due to the presence of caffeic acid derivatives [23].

5. Evaluation of quality of colored cotton

Colored cotton fibers are currently available and can be combined with conventional white cottons. They are shorter, weaker, and finer than regular upland cotton fibers. Due to smaller bolls and low ginning outturn, color-linted cultivars are often low yielders with low productivity per unit area. Other issues with these cottons include high whiteness per cent, higher wax content, isolation distance requirements, the availability of only a few hues, and unpredictability and non-uniformity of fiber color across seasons and locales. To make these environmentally friendly color cottons commercially viable, researchers must focus their efforts on improving the genetics of agronomic features, fiber quality, and color uniformity [45]. The correct assessment of basic fiber properties and quality classification is a major issue for dealers, spinners, and farmers who are working to improve cotton production characteristics [46]. The degree of reflectance (Rd) and yellowness (+b) as specified by official criteria and measured by the high volume instrument determine the color grade (**Figure 2**). The equipment specification for Rd. and + b is specified in the **Table 1**. The brightness

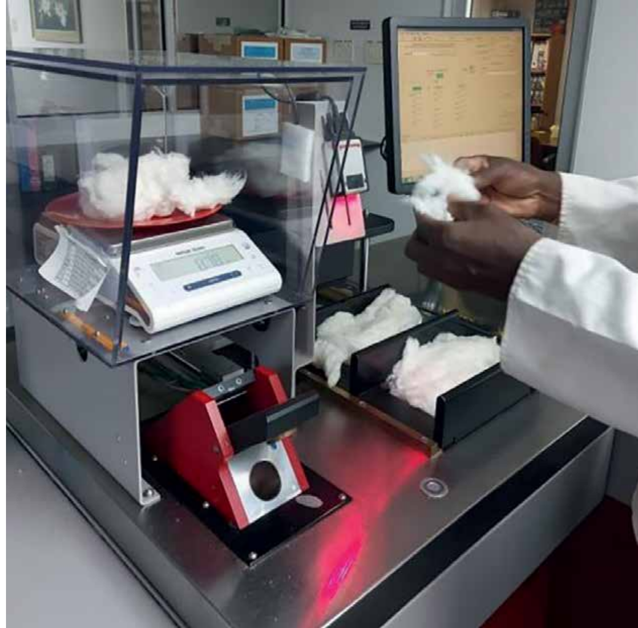

Figure 2.
The cotton color grading instrument [46].

Fiber propperty	Equipment specifications	
	Precision specifications	Calibration tolerances
Color (Rd) (units)	0.700	0.400
Color (+b) (units)	0.300	0.400

Table 1.
Color cotton equipment specifications [47].

or dullness of a sample is determined by its reflectance, while the degree of pigmentation is determined by its yellowness. Finding the intersection of the Rd. and + b values on the color chart for American Upland cotton yields a three-digit color code [47]. The color of cotton fibers can be affected by rainfall, freezes, insects, fungi, and staining through contact with soil, grass, or cotton-plant leaf. Color can also be affected by excessive moisture and temperature levels during storage, both before and after ginning. Color deterioration because of environmental conditions affects the fibers' ability to absorb and hold dyes and finishes and is likely to reduce processing efficiency [48].

Several tools and programs are in place to manage quality. These include laboratory conditioning, sample conditioning, equipment performance specifications, instrument calibration, in-house monitoring, and USDA's Quality Management Program.

5.1 Laboratory conditioning

The measurement of cotton fiber characteristics is influenced by atmospheric conditions. As a result, the classing laboratory's temperature and humidity must be

strictly controlled. The temperature is kept at 70 degrees Fahrenheit plus or minus 1 degree Fahrenheit (about 21 degrees Celsius plus or minus 1/2 degree Celsius), and the relative humidity is kept at 65 percent plus or minus 2 percent.

5.2 Sample conditioning

The moisture content of the samples is conditioned to match the permitted atmospheric conditions. Moisture level in conditioned samples will range from 6.75 to 8.25 percent (on a dry-weight basis). The moisture content of the conditioned samples is examined at random to ensure that the correct moisture content has been achieved. Samples can be passively or actively conditioned. The samples are put in single layers in trays for passive conditioning.

6. Breeding methods for the development of Colored cotton

Natural colored cotton cultivation dates back to 2300 BC [49]. Anciently, colored cottons were domesticated from *G. hirsutum* and *G. barbadense*. Subsequently, Due to the varied dyeing advantages in white cotton, the colored cotton was underrated and lost its preference among people and industrialist during the latter half of 19th century. But with the increasing environmental concern, demand for natural colored cotton gained its momentum during the past decade [16]. Generally the fiber color was negatively correlated with fiber yield, fiber quality [50] and limited color choice [51]. Correspondingly, the studies on genetics, inheritance and correlation of cotton fiber color and fiber yield, quality, pigmentation were stenuously carried out [29, 52]. These studies have led to the development of next generation colored cotton to overcome the short-comings encountered previously.

Primarily, colored cottons were identified as mutants of white cotton predominant from *G. hirsutum* and *G. barbadense* [53]. So far varieties of colored cotton were developed mostly by selection and recurrent crossing approaches from the germplasm [54]. Hybrids were also developed from crossing suitable germplasm with white cotton varieties to enhance the yield and fiber quality. Cocanada 1 & 2 and Red northers were the brown linted tree cotton varieties selected from *G. arboretum*. Vaidhehi 95 (MSH 53) is a introgressed cultivar developed from *G. hirsutum* which is also a brown linted variety produced by Central Intsitute for Cotton Research, Nagpur, India (CICR). CICR also produced some brown linted cotton varieties *viz.,* CNA 405, CNA 406 and CNA 407 but no green linted cotton varities were developed so far. In order to develop more vibrant, diverse fiber color, high yielding, quality colored cotton varieties needs the combined usage of conventional and biotechnological method of plant breeding. Also integrating the results of omics studies on pigmentation of colored cotton would help in a big leap in developing colored cotton varieties [16].

7. Conclusion

To recapitulate, environmentalists are urging scientists and farmers to improve and grow NCC due to their concern about the effects of dyes and the advantages of naturally colored cotton over white cotton. Color development methods in green and brown cotton have been disclosed by the studies on the biochemical and molecular mechanisms of fiber color development in cotton and flavonoid biosynthesis genes.

GhC4H, *GhCHS*, *GhCHI*, *GhF3'H*, *GhDFR*, and *GhANR* are the genes responsible for the structural flavonoid biosynthesis pathway that play a major role in color formation in brown fiber cotton. Cloning and genome-wide association studies of the fiber color genes help us comprehend the complicated biological mechanism of color development in cotton fiber which would have been hampered if the cotton genome sequences were not developed. Changing the cellulose and flavonoid biosynthesis pathways to improve fiber quality will be a step toward manufacturing cotton fiber with a wide range of hues and high quality for the textile industry. The recent improvements in several NCC research areas offer opportunities to overcome barriers to commercial NCC breeding, while further studies including multi-omics techniques are needed. The ability to reduce the fiber-quality and yield gap between NCC and white-fiber cotton will determine its growth in the textile market.

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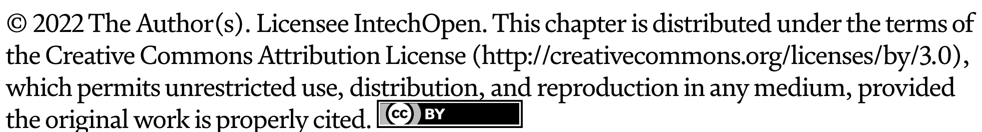
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References

[1] Barros MA, Silva CR, Lima LM, Farias FJ, Ramos GA, Santos RC. A review on evolution of cotton in Brazil: GM, white, and Colored cultivars. Journal of Natural Fibers. 2020;**18**:1-3

[2] Statista. Cotton production by country worldwide in 2017/2018 (in 1, 000 metric tons). The Statistics Portal. 2018. Accessed September 12. https://www.statista.com/statistics/263055/cotton-production-worldwide-by-top-countries/

[3] Meshram, JH, Mahajan SS, Nagrale D, Gokte-Narkhedkar N, Kumbhalkar H. Understanding Root Biology for Enhancing Cotton Production. In Plant Roots; IntechOpen: London, UK, 2021; p. 13

[4] Fryxell PA. A revised taxonomic interpretation of *Gossypium* L. (Malvaceae). Rheedea. 1992;**2**(2): 108-165

[5] Lee JA, Fang DD. Cotton as a world crop: Origin, history, and current status. Cotton. 2015;**57**:1-23

[6] Buainain AM, Alves E, Silveira JM, Navarro Z. O mundo rural no Brasil do século 21: a formação de um novo padrão agrário e agrícola. Brasília, DF: Embrapa, 2014;**4**:1125-1156

[7] Weisburger JH. Comments on the history and importance of aromatic and heterocyclic amines in public health. Mutation Research, Fundamental and Molecular Mechanisms of Mutagenesis. 2002;**506**:9-20

[8] Kimmel LB, Day MP. New life for an old Fiber: Attributes and advantages of naturally Colored cotton. Aatcc Review. 2001;**1**(10):32-35

[9] Rathinamoorthy R, Parthiban M. Colored cotton: Novel eco-friendly textile material for the future. In: Handbook of Ecomaterials. Springer, New York; 2017. pp. 1-21

[10] Murthy MS. Never say dye: The story of coloured cotton. Resonance. 2001;**6**(12):29-35

[11] Crews PC, Hustvedt G. The ultraviolet protection factor of naturally-pigmented cotton. The Journal of Cotton Science. 2005;**9**:47-55

[12] Yatsu LY, Espelie KE, Kolattukudy PE. Ultrastructural and chemical evidence that the cell wall of green cotton fiber is suberized. Plant Physiology. 1983;**73**(2):521-524

[13] Dutt Y, Wang XD, Zhu YG, Li YY. Breeding for high yield and fibre quality in coloured cotton. Plant Breeding. 2004;**123**(2):145-151

[14] Von Tunzelmann GN. Technology and Industrial Progress: The Foundations of Economic Growth. Edward Elgar Publishing limited, UK; 1995

[15] Gong W, Du X, Jia Y, Pan Z. Color cotton and its utilization in China. In: Cotton Fiber: Physics, Chemistry and Biology. Springer, USA; 2018. pp. 117-132

[16] Sun J, Sun Y, Zhu QH. Breeding next-generation naturally colored cotton. Trends in Plant Science. 2021;**26**(6):539-542

[17] Naoumkina M, Thyssen GN, Fang DD, Jenkins JN, McCarty JC, Florane CB. Genetic and transcriptomic dissection of the fiber length trait from a cotton (*Gossypium* hirsutum L.)

MAGIC population. BMC Genomics. 2019;**20**(1):1-4

[18] Zhang D, Chen L, Zang C, Chen Y, Lin H. Antibacterial cotton fabric grafted with silver nanoparticles and its excellent laundering durability. Carbohydrate Polymers. 2013;**92**(2):2088-2094

[19] Gong W, He S, Tian J, Sun J, Pan Z, Jia Y, et al. Comparison of the transcriptome between two cotton lines of different fiber color and quality. PLoS One. 2014;**9**(11):e112966

[20] Rauf S, Shehzad M, Al-Khayri JM, Imran HM, Noorka IR. Cotton (*Gossypium* hirsutum L.) breeding strategies. In: Advances in Plant Breeding Strategies: Industrial and Food Crops. Springer, USA; 2019. pp. 29-59

[21] Carvalho LP, Farias FJ, Lima MM, Rodrigues JI. Inheritance of different fiber colors in cotton (*Gossypium* barbadense L.). Crop Breeding and Applied Biotechnology. 2014;**14**(4):256-260

[22] Xiao YH, Zhang ZS, Yin MH, Luo M, Li XB, Hou L, et al. Cotton flavonoid structural genes related to the pigmentation in brown fibers. Biochemical and Biophysical Research Communications. 2007;**358**(1):73-78

[23] Feng H, Yang Y, Sun S, Li Y, Zhang L, Tian J, et al. Molecular analysis of caffeoyl residues related to pigmentation in green cotton fibers. Journal of Experimental Botany. 2017;**68**(16):4559-4569

[24] Sun Y, Zhang D, Zheng H, Wu Y, Mei J, Ke L, et al. Biochemical and expression analyses revealed the involvement of Proanthocyanidins and/or their derivatives in fiber pigmentation of *Gossypium* stocksii. International Journal of Molecular Sciences. 2022;**23**(2):1008

[25] Richmond TR. Inheritance of green and brown lint in upland cotton. Journal of the American Society of Agronomy. 1943;**35**(11):967-975

[26] Fletcher E. Mendelian heredity in cotton. The Journal of Agricultural Science. 1907;**2**:281-282

[27] Li Z, Su Q, Xu M, You J, Khan AQ, Li J, et al. Phenylpropanoid metabolism and pigmentation show divergent patterns between brown color and green color cottons as revealed by metabolic and gene expression analyses. Journal of Cotton Research. 2020;**3**(1):1-1

[28] Shi YZ, Du XM, Liu GQ, Qiang AD, Zhou ZL, Pan ZE, et al. Genetic analysis of naturally colored lint and fuzz of cotton. Cotton Science. 2002;**14**(4):242-248

[29] Sun DL, Sun JL, Du XM. Genetic study on the color of fiber and linter in brown cotton. Journal of Anhui Agricultural Sciences. 2008;**36**:6254-6255

[30] Yan Q, Wang Y, Li Q, Zhang Z, Ding H, Zhang Y, et al. Up-regulation of Gh TT 2-3A in cotton fibres during secondary wall thickening results in brown fibres with improved quality. Plant Biotechnology Journal. 2018;**16**(10):1735-1747

[31] Liu Q, Luo L, Zheng L. Lignins: Biosynthesis and biological functions in plants. International Journal of Molecular Sciences. 2018;**19**(2):335

[32] Li T, Fan H, Li Z, Wei J, Lin Y, Cai Y. The accumulation of pigment in fiber related to proanthocyanidins synthesis for brown cotton. Acta Physiologiae Plantarum. 2012;**34**(2):813-818

[33] Lu N, Roldan M, Dixon RA. Characterization of two TT2-type

MYB transcription factors regulating proanthocyanidin biosynthesis in tetraploid cotton, *Gossypium* hirsutum. Planta. 2017;**246**(2):323-335

[34] Humphries JA, Walker AR, Timmis JN, Orford SJ. Two WD-repeat genes from cotton are functional homologues of the Arabidopsis thaliana TRANSPARENT TESTA GLABRA1 (TTG1) gene. Plant Molecular Biology. 2005;**57**(1):67-81

[35] Aleksandra M, Ksenia S, Elena K. The genes determining synthesis of pigments in cotton. Biological Communications. 2019;**64**(2):133-145

[36] Fan L, Shi WJ, Hu WR, Hao XY, Wang DM, Yuan H, et al. Molecular and biochemical evidence for phenylpropanoid synthesis and presence of wall-linked phenolics in cotton fibers. Journal of Integrative Plant Biology. 2009;**51**(7):626-637

[37] Feng H, Li Y, Wang S, Zhang L, Liu Y, Xue F, et al. Molecular analysis of proanthocyanidins related to pigmentation in brown cotton fibre (*Gossypium* hirsutum L.). Journal of Experimental Botany. 2014;**65**(20):5759-5769

[38] Li YJ, Zhang XY, Wang FX, Yang CL, Liu F, Xia GX, et al. A comparative proteomic analysis provides insights into pigment biosynthesis in brown color fiber. Journal of Proteomics. 2013;**78**:374-388

[39] Xiao YH, Yan Q, Ding H, Luo M, Hou L, Zhang M, et al. Transcriptome and biochemical analyses revealed a detailed proanthocyanidin biosynthesis pathway in brown cotton fiber. PLoS One. 2014;**9**(1):e86344

[40] Hua S, Yuan S, Shamsi IH, Zhao X, Zhang X, Liu Y, et al. A comparison of three isolines of cotton differing in fiber color for yield, quality, and photosynthesis. Crop Science. 2009;**49**(3):983-989

[41] Campbell BT, Dever JK, Hugie KL, Kelly CM. Cotton fiber improvement through breeding and biotechnology. In: Cotton Fiber: Physics, Chemistry and Biology. Springer, USA; 2018. pp. 193-215

[42] Schmutz A, Jenny T, Amrhein N, Ryser U. Caffeic acid and glycerol are constituents of the suberin layers in green cotton fibres. Planta. 1993;**189**(3):453-460

[43] Hua S, Wang X, Yuan S, Shao M, Zhao X, Zhu S, et al. Characterization of pigmentation and cellulose synthesis in colored cotton fibers. Crop Science. 2007;**47**(4):1540-1546

[44] Ryser U, Meier H, Holloway PJ. Identification and localization of suberin in the cell walls of green cotton fibres (*Gossypium* hirsutum L., var. green lint). Protoplasma. 1983;**117**(3):196-205

[45] Basavaradder AB, Maralappanavar MS. Evaluation of eco-friendly naturally coloured *Gossypium* hirsutum L. cotton genotypes. International Journal of Plant Sciences. 2014;**9**(2):414-419

[46] Frydrych I. Cotton quality evaluation: New possibilities. In: 62nd ICAC Plenary Meeting, Gdańsk. ICAC. Poland. 2003

[47] Nath J, Patil PG, Shukla SK, Arude VG. Design of pocket colorimeter for cotton. Agricultural Engineering Today. 2002;**26**(3 and 4):79-88

[48] Naeem-Ullah U, Ramzan M, Bokhari SH, Saleem A, Qayyum MA, Iqbal N, et al. Insect pests of cotton crop

and management under climate change scenarios. In: Environment, Climate, Plant and Vegetation Growth. Springer, Nature Switzerland; 2020. pp. 367-396

[49] Vreeland J. The revival of colored cotton. Scientific American. 1999;**280**(4):112-118

[50] Feng H, Guo L, Wang G, Sun J, Pan Z, He S, et al. The negative correlation between fiber color and quality traits revealed by QTL analysis. PLoS One. 2015;**10**(6):e0129490

[51] Blas-Sevillano RH, Veramendi T, La Torre B, Velezmoro-Sánchez CE, Oliva AI, Mena-Martínez ME, et al. Physicochemical characterization of several types of naturally colored cotton fibers from Peru. Carbohydrate Polymers. 2018;**197**:246-252

[52] Ruhsora R, Muhabbat R. The nature of inheritance of the colored cotton fiber trait. Nature. 2021;**7**(5):229-232

[53] Kohel RJ. Genetic analysis of fiber color variants in cotton 1. Crop Science. 1985;**25**(5):793-797

[54] Khan NU, Hassan G, Kumbhar MB, Marwat KB, Khan MA, Parveen A, et al. Combining ability analysis to identify suitable parents for heterosis in seed cotton yield, its components and lint% in upland cotton. Industrial Crops and Products. 2009;**29**(1):108-115

Chapter 3

Bioinformatics Tools and Genomic Resources Available in Understanding the Structure and Function of *Gossypium*

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Abstract

Gossypium spp. (Cotton) is the world's most valuable natural fiber crop. *Gossypium* species' variety makes them a good model for studying polyploid evolution and domestication. The past decade has seen a dramatic shift in the field of functional genomics from a theoretical idea to a well-established scientific discipline. Cotton functional genomics has the potential to expand our understanding of fundamental plant biology, allowing us to more effectively use genetic resources to enhance cotton fiber quality and yield, among with using genetic data to enhance germplasm. This chapter provides complete review of the latest techniques and resources for developing elite cotton genotypes and determining structure that have become accessible for developments in cotton functional genomics. Bioinformatics resources, including databases, software solutions and analytical tools, must be functionally understood in order to do this. Aside from GenBank and cotton specific databases like CottonGen, a wide range of tools for accessing and analyzing genetic and genomic information are also addressed. This chapter has addressed many forms of genetic and genomic data now accessible to the cotton community; fundamental bioinformatics sources related to cotton species; and with these techniques cotton researchers and scientists may use information to better understand cotton's functions and structures.

Keywords: *Gossypium*, genomics, bioinformatics, structure, gene sequencing

1. Introduction

Providing fiber for one of the biggest and most significant sectors (textiles), cotton is the world's major natural fiber crop. It has a global economic effect of around $500 billion every year. Cotton genetic resources, which comprise germplasm from more than 50 distinct cotton species, have allowed researchers to investigate the transformation of fibers of lint and the impact of polyploidy in enhancing lint output. It allow researchers to gain a better understanding of how domesticated cotton evolved from its wild counterpart. Bio-based alternatives to petroleum-based chemicals, such as assessing the degree of genetic diversity and exploiting it to increase

cotton yield and lint quality, are also being carefully researched [1]. A staple of the world economy, cotton (*Gossypium hirsutum*) is appreciated for its valuable renewable fiber resource and is a cornerstone of the global economy. Various biological investigations, including polyploidization [2], single-celled biological processes and genome evolution, may be carried out using this plant as a model [3]. Polyploidy and genomic size disparities with the *Gossypium* genus, as well as cotton's evolution, may be better understood through decoding cotton's genome [4].

A summary of the technological advancements achieved during the previous two decades have presented in this article. For example, progress has been made in understanding differences in a variety of physiological, biochemical, morphological and genetically relevant features, all of which have been explored in this volume. Many cutting-edge genomic methods were used to study the cotton genome of the genus *Gossypium*, as was done with other significant crop species. This work significantly contributed to laying the groundwork for maintaining lint production around the globe [5]. There are still a lot of things to look into [6]. Cotton's genetic foundation has become more limited as a consequence of extensive domestication [7]. In previous generations of cotton production, conventional genetic resources were used; nevertheless, cotton productivity has been declining over the last several years [8]. As it is imperative for producing fresh sources in order to confront the difficulties of the new millennium.

Genomic methods have been used extensively for enhancing fiber characteristics, producing cotton cultivars that are resistant to insect pests and diseases, and developing cotton varieties that are resistant to abiotic pressures. In the minds of many, genetic alteration has led to an improvement in genomes [9]. The scope of this investigation of cotton's genetic resources, both traditional and modern, as well as its potential for future use, is broad. Improved usage of existing genetic resources has the potential to alleviate the concerns to cotton production that are now being faced.

2. Genomics and genetic diversity of *Gossypium* spp

Members of the Malvaceae family, *Gossypium* spp., belong to the genus *Gossypium*. 50 species (45 diploids 2n =2 × =26; and 5 tetraploids 2n = 4 × =52) are found in the *Gossypium* genus [10]. The tetraploid species were formed after interbreeding of the A and "D" genome species around 4–11 million years ago (MYA), and they diverged about 1–2 lakh years ago from their common ancestor. As two sub-genomes contains a single copy of practically all genes, the tetraploid cotton contains more than two copies of each genes [11]. Furthermore, these genes were organized in the same manner as the diploid progenitors [12]. There are two tetraploid species of cotton that are among the most frequently farmed in the world: *Gossypium* barbadense and *G. hirsutum*.

A through G and K in the alphabet represent the eight genome groups, which are based on chromosomal pairing affinities [13]. (AD)1 through (AD)5 are the five tetraploid species, based on their genomic constitutions. On the basis of phylogenetic analyses, *Gossypium* species were categorized into two lineages: the 13 D-genome species lineage and the 30 ~ 32 A-, B-, E-, F-, C-, G- and K-genome species lineage. Based on phylogenetic analyses, the polyploid species have been divided into one lineage, with the 5 AD-genome lineage being the most notable (**Figure 1**). Allotetraploids (*G. hirsutum* and *G. barbadense*) and diploids (*Gossypium herbaceum* and *G. arboreum*) are two of the four *Gossypium* species grown in agriculture. It is estimated that *G. hirsutum*, or "Upland cotton," is responsible for 90% of the world's cotton production. Cotton varieties like *G. herbaceum* (Levant Cotton), and *G. arboretum* (Tree Cotton),

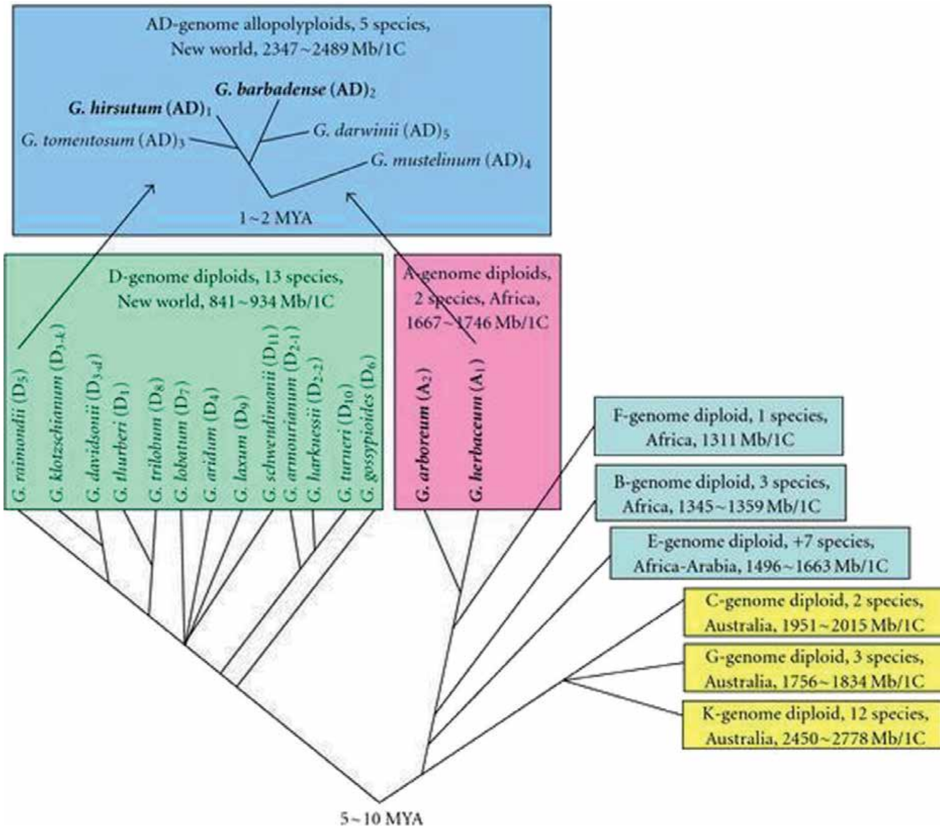

Figure 1.
Phylogeny and evolution of Gossypium *spp. [14].*

account for about 2% of the world's production of cotton, while *G. barbadense*, (Egyptian Cotton), American Pima Cotton, Sea Island Cotton or Extra Long Staple Cotton provides 8% of the world's production of cotton [13].

3. Genome sequencing of *Gossypium* spp

The advances made possible by genome sequencing show that functional genomics research is working to become more efficient. Insect and herbicide-resistant cotton cultivars have advanced at breakneck pace during the previous two decades [15]. When it comes to genetic modification of cotton for plant morphology and blooming as well as for fiber quality as well as yield and resistance to biological and environmental stresses, however, process was slow. The advancement of a cotton genome research collaboration depending on Arabidopsis and rice has been made possible thanks to the efficient deployment considering the availability of well-known whole-genome sequences. While setting up an approach for cotton genome sequencing, the Consortium of cotton genome [6], decided to focus on simpler diploid genomes which is applied to tetraploid cotton. Among the D-genome species like *G. raimondii* has been prioritized for full sequencing in order to meet the personal milestone of cotton genome sequence completion. Both Paterson [16] and Wang [17] authored the

review genome sequence of *G. raimondii* in 2012, which was an obvious first step in categorizing the bigger "A" diploid as well as "AD" tetraploid cotton genomes. They were not only ones to write the review genome sequence in 2012. Cotton genome sequencing in 2012 began with this as an initial financial funding source.

It wasn't long until the same study team published their results on the 1694-MB genome for *G. arboreum*, which is assumed to be a cotton's donor species. Group of A chromosome in tetraploids [18]. There are two known cotton progenitors, *G. raimondii* and *G. arboreum* both of which have their genomes sequenced, however it is still uncertain which species was responsible for the growth of the tetraploid cotton species around lakhs of years ago [19]. Furthermore, when compared to diploid cotton species, *G. hirsutum* showed significant alterations in economic characteristics and structures of plants. This indicates that throughout development, both natural and artificial selection took place. As a result, the allotetraploid cotton species must be sequenced in order to learn more about the plant's evolutionary history and fiber biology. Li [20] and Zhang [21] sequenced the allotetraploid *G. hirsutums* genomes using genes from the A and D progenitor species as a preparatory step. Also of note is Sea Island cotton, which is renowned for its great durability and excellent fiber. Its use in textile manufacturing sounds perfect for the production of high-quality goods. Both Liu et al. [22] and Yuan et al. [23] have sequenced the genome of the genus *G. barbadense* and found that it spans 2470 Mb of the genome [23].

As a result, experts feel that because of the way they were constructed, a number of the recently disclosed genome sequences reference for tetraploid and diploid species of cotton are flawed. *G. raimondii* [16, 17] as well as *G. hirsutum* [19, 20] sequenced and assembled review genomes differed in chromosomal lengths as well as the number of annotated genes between the 2 categories. At least on a large scale, it is plausible that such differences are the consequence of assembly errors. As a result of this, there has been a huge amount of genetic study done on diverse cotton species. For the time being, we must put in more effort to gather these genome assemblies for a more skeptical eye in order to do thorough comparisons, assessments, and repairs of their misassembles, among many other things.

When a reference genome is available, it is possible to investigate the link between sequence alterations and other properties by re-sequencing the genome. In order to find genomic regions that are indicators of choice in cotton, recent comprehensive genome studies on 34 [24] as well as 318 [25], along with 147 [26], and 352 [27] cotton accessions constitute considerable collection. Cotton molecular breeding has benefited tremendously from the discoveries made in these experiments, which have yielded valuable new genetic resources. It is possible to transfer favorable genes associated with high yield, wide adaptability, with high fiber grade across many gene pools under the guidance of sequencing information, in order to considerably enhance cotton output.

4. Genome database/bioinformatic tools available for *Gossypium* spp

Identifying the genetic features that are important for the biological behavior of cotton is just the first step in the process of genome sequencing and resequencing. Cotton genomics have disclosed various DNA's physiologically active states in the same manner as studies of epigenetic alterations, fine map platforms, SNP array platforms, high density genetic, and transcript abundance across different species and tissues have done so for other model crop plants. All other model crop plants have done the same thing. Cotton plantation industry genetic research and breeding was

hindered by limited ultra-precision genetic mapping before the publishing of the complete genome sequences for four *Gossypium* species in 2013. The cotton plantation industry's access to fairly large cotton-genome linkage maps may enable gene mapping, high-throughput markers, cotton cloning and gene isolation [28, 29]. In the previous 10 years, approx. 1075 QTLs in 58 *G. hirsutum* studies and 1059 QTLs in interspecific *G. hirsutum* and 9 *G. barbadense* populations were submitted as yield, fiber quality, seed quality, and biotic and abiotic challenge tolerance. In the case of marker-aided selection, the newly identified QTLs provide only coarse resolution due to their location in vast genomic domains that may comprise several genes. When selecting a marker, it is crucial to have a large number to choose from, so that the genes presence in the target locus may be cloned more effectively. The glandless gene [30], leaf shape [31], and quality of fiber related QTLs [32, 33], have all been mapped in cotton along with Many genes and quantitative trait loci (QTLs).

Single nucleotide polymorphism variations (SNP) have been discovered at the 2.5-Gb of whole genome level for allotetraploid cotton genome in recent years thanks to better in silico techniques and next-generation sequencing (NGS). SNP63K is formed in cotton, that includes tests for 45,104 and 17,954 possible intraspecific as well as interspecific SNP markers [34]. SNP63K was created by Ashrafi et al. [34]. The SNP63K cotton array is a foundational high-throughput genotyping technique as well as a platform for genetic research for commercially and agronomically relevant methods. CNVs, which stand for a larger proportion of the genome than SNPs, may be beneficial in discovering phenotypic changes that are not recorded by SNPs, since they stand for more of the genome. Many studies have shown that plant genomes are full with copy number variants (CNVs), which may affect gene regulation, dosage and gene structure [35]. The vast majority of genes impacted by CNVs are linked to important traits. In a recent study, researchers found that cotton contains 989 CNV-infected genes that influence plant type, cell wall structure, and translational control [26].

A decade ago, transcriptome analysis identified as the most essential tool for determining how sequencing data might be used to get insights into the activities of individual genes. Whole-genome transcriptome profiling may be achieved with RNA-Seq, it allows high-throughput sequencing tools to sequence transcripts directly. The freshly published transcriptome assembly for the *G. hirsutum* TM-1 inbred line, as well as assembly of all publically accessible to expressed sequence tags (ESTs), were utilized as a reference for SNP detection in cotton [34]. The utilization of diploid and tetraploid genome sequences, as well as next-generation sequencing (NGS) technologies, was also described in RNA-Seq analyses for large-scale gene expression in the cotton plant. Many activities in plants have been studied using transcriptome analysis, along with the study of leaf sense [36], fiber growth [37], biotic stress [38], along abiotic stress [39]. However, there are certain obstacles with the RNA-Seq approach, such as library creation and the development of efficient techniques for storing and processing vast volumes of information [40]. As soon as these limitations to widespread use of RNASeq are removed, it has envisaged that this approach has taken over as the primary tool for evaluation of transcriptome [41].

Many characteristics of living creatures are influenced by processes known as epigenetic modifications in addition to genetic variations. Gene expression is influenced by these alterations, which alter when, how many, and how much they are expressed. Among the several epigenetic signaling approaches available, DNA methylation [42] has been shown a crucial role in agricultural plant growth and morphological variety [43]. DNA methylation changes the cotton which is connected with seasonal fluctuations in fiber production [44] and different tissue [45]. CHH methylation mediated

with RNA-directed DNA methylation (RdDM) has been associated with ovules gene activation, while CHH methylation mediated by chromomethylase2 (CMT2) has been connected to gene repression in fiber development [46].

It has been shown that between wild and domesticated cotton varieties, 519 cotton genes have been epigenetically changed, some of which have been linked to domesticated and agronomic properties [47], and others of which have not. As a result of this research, we have a better understanding of how epigenetic regulation affects many aspects of cotton's development and its polyploid evolution. In terms of bringing this technique to reality, we need to know how the methylome has evolved and been domesticated.

4.1 Functional genomics databases for cotton

To do an examination of genome, data in the map location, protein expression and mRNA, allelic variation genome sequence, and metabolism must all be accessible at the same time. With the increase of omics sets of data, it is important than ever to get a database of functional genomics that helps users to easily access and display genetic data. "CottonGen (https://www.cottongen.org) [48], Cotton Genome Resource Database (CGRD; http://cgrd.hzau.edu.cn/index.php) [49], Database for Co-expression Networks with Function Modules (ccNET; http://structuralbiology.cau.edu.cn/*Gossypium*/) [50], Join Genome Institute (JGI; http://jgi.doe.gov) [51], Cotton Genome Database (CottonDB; http://www.cottondborg) [52], Evolution of Cotton (https://learn.genetics.utah.edu/content/cotton/evolution/) [53], Platform of Functional Genomics Analysis in *Gossypium raimondii* (GraP; http://structuralbiology.cau.edu.cn/GraP/about.html) [54], Cotton Functional Genomic Database (CottonFGD; https://cottonfgd.org) [55], Cotton Genome Project (CGP; http://cgp.genomics.org.cn/page/species/index.jsp) [56], https://www.cottongen.org/data/markers [57], https://bacpacresources.org/ [58], https://scienceweb.clemson.edu/cugbf/clemson-genomics-and-bioinformatics-courses/ [59]." As a result, Cotton FGD provides accessibility to most of the sequenced genomes of *Gossypium*, and also other plant genomes, and also from transcriptome data along with re-sequencing data. The ccNET database contains 1155 and 1884 functional modules from the diploid *G. arboreum* as well as *G. hirsutum*, respectively, in respect of cotton species' founder patterns and structural modules.

5. Advances in cotton genomics research

It has been shown that genome research may be used to maintain and improve agricultural plant genetics as a consequence, attempts in cotton genetic studies, notably the creation of genetic tools, as well as the establishment of breeding stock for genetic and genomics research, have been made. Genomic markers like simple sequence repeats or microsatellites, random amplification of polymorphic DNA, restriction fragment length polymorphism, amplified fragment length polymorphism, resistance gene analogues, sequence-related amplified polymorphism are some of the tools available. Cotton genome sequencing is taking place at the same time as genetic mapping as well as genome-wide Bacterial artificial chromosome (BAC) libraries, plant-transformation-competent binary bacterial artificial chromosome (BIBAC)-based integrated physical map is being created. Study on cotton's genome lags behind that of soybean, rice and maize mostly due to the lack of funding provided for the species in contrast to these other important crops. The following section provides an overview of recent significant advancements in research of cotton genomics [14].

5.1 DNA markers and molecular linkage maps

RFLPs were first DNA markers to be utilized in cotton genomic research, and they were also found in the most of plant species at the moment of their discovery, indicating that they were widely distributed. The development of the first *Gossypium* species genetic linkage map [60], which was formed from the F2 population of interspecific *G. barbadense*, *G. hirsutum* and founded in RFLPs, should come as no surprise to those familiar with the genus. On the map, 705 locations were included, which was organized with 41 linkage groups along with a total area of 4675 square kilometers. Rong et al. [61] designed it to be more comprehensive than the previous *Gossypium* genus map, which contained 2584 loci spaced at 1.74-cM intervals which includes all 13 homeologous chromosomes of cotton, making it most comprehensive genetic map for the species to date. Crosses among the D-genome diploid species *G. trilobum* x *G. raimondii* [61] and a diploid species *G. arboreum* x *G. herbaceum* [62] revealed a large quantity of DNA probes from the map, In addition, there are hybrids between *G. arboreum* and *G. herbaceum*, which are a A-genome diploid species. In-depth research on the relationship among the tetraploid AD subgenomes and the diploid A and D genome maps, as well as the cross-species transfer of these insights, produced important results.

RFLPs are time-consuming and need large quantity of DNA, labor-intensive blot hybridization, autoradiography processes, all of which are now being superseded by DNA marker systems based on polymerase chain reaction (PCR). The development of a broad range of markers for diverse applications has resulted from the utilization of PCR-based DNA markers in genetic investigations of cotton. Multiple techniques, including as AFLP, RAPD, SRAP and RGA, provide an ideal chance for scanning a large number of DNA loci in a short period of time, focusing on DNA elements that are quickly developing and hence more likely to include loci that vary between genotypes [63]. Using a population obtained from an interspecific cross between Texas Marker-1 (TM-1) and 3–79, Kohel [63], collected 355 DNA markers into 50 linkage groups, which covered a total of 4766 cM, to construct a genetic map of the species. This map was initially published by Brown and Brubaker [64], which was based on an interspecific *Gymnodinium nelsonii* x *G. australe* population that was geneticall linked by AFLP. For the *Gossypium* G-genome, this was the first AFLP genetic linkage map. In a *G. australe* hexaploid bridging family, it was observed that AFLPs could be used to detect chromosomal-specific molecular markers that were unique to the G-genome, and that the frequency of chromosome transmission of *G. australe* could be monitored using AFLPs.

A novel class of cottons genetic markers has been developed that is more easy to use and greatly polymorphic. as a result, the introduction of SSR or microsatellite markers in the cotton industry. Upland cotton has a low level of intraspecific polymorphism, which is especially helpful to the crop's cultivation because of the crop's minimum intraspecific polymorphism. As a result of the presence of flanking primer sequences, SSRs are easily transferred between laboratories and are highly transferable from one population to another, SSRs are the PCR-based markers that are commonly co-dominant, extensively distributed all along the genome, and readily transferable across populations [65]. SSRs have been created in cotton, according to http://www.cottonmarker.org [60], for a total of about 5484 SSRs [66].

5.2 Gene and QTL mapping

Even while maps of molecular linkage made significant advances in the knowledge of the development and organization of cotton genomes, a main motive of molecular

linkage map building was to locate the genes that impact qualitative as well as quantitative features. If DNA markers are linked to genes that impart critical agronomic characteristics that are costly or time-consuming to analyze, it is less expensive and more reliable to select for acceptable progenies in breeding programs.

5.3 Mapping qualitative traits

Whether it's a qualitative or plain evaluation, Mendelian hereditary features are qualities that are passed down from one generation to the next that differ in type rather than degree. All of these characteristics are generally managed with a single gene, further the phenotypic diversity in the offspring of the segregating parent may be divided into several groups. It has been discovered that the qualitative characteristics of *G. arboreum* and *G. herbaceum* are present in both the diploid (*G. arboreum*) and tetraploid species (mostly *G. hirsutum* and *G. barbadense*) species [1]. Pollen color, leaf shape, lint color, leaf color, pubescence, bract morphology, and other traits are examples of such characteristics. Many qualitative characteristics in crop production, for example, are the result of morphological mutants that have arisen as a result of natural variation among species with interspecific hybrids, or morphological mutants that have arisen as a result of irradiation, spontaneous mutation. As a result, only a few attempts have been undertaken to map qualitative features onto the molecular genetic map as a result of this predicament. A recent publication [67] presented an overview of the qualitative qualities which are mapped with molecular markers. As a consequence, many of these features were included in the map as a kit for linking the different linkage groups to the chromosomes allocated with the classical map, which was the main goal. Genes for leaf shape and development, genes for fiber production (including fiber strength), genes for disease and insect pest resistance (including insect pest resistance), and genes for fertility restoration (including fertility restoration genes) are among those associated with cotton quality and productivity [67].

5.4 Mapping quantitative traits

Qualities with quantitative approach are characteristics of persons which fluctuate in degree rather than kind, as opposed to other traits. They're usually assumed to be the result of interactions between several loci, and they show continuous variation in a segregating population, as well as being quickly altered by environmental change. In recent years, there has been an explosion of activity in the discovery and detection of quantitative trait loci (QTLs), Since the previous decade, there has been a growth in the number of DNA markers that can be used in cotton genetic mapping. Among the quantitative trait loci (QTLs) that have been found in cotton are those that affect plant architecture, disease resistance, insect resistance, and blooming date, to name a few [14].

5.5 BAC and BIBAC resources

Significant-insert BAC and BIBAC libraries are necessary and sought for advanced genetics and genomics research, according to a large number of publications [68–70]. Due to the simplicity of increased purification of DNA cloned insert, low levels of chimerism, and high levels of stability in the host cell, bacteria and bacterial-infected cells (BACs) have swiftly established themselves as a significant component of genome research [71, 72]. Gene and QTL mapping [73], whole-genome or chromosome physical mapping [74, 75], large genome sequencing [76, 77],

isolation and characterization of structural and regulatory genes [78, 79] and cytologically based gene discovery are only some of the applications that this technique has been used for in genomics. For a range of taxa, including plants, animals, insects, and bacteria, artificial chloroplast (BAC) libraries have been produced. The public may access these libraries via following websites: (i) https://bacpacresources.org/ [58], and (ii) https://scienceweb.clemson.edu/cugbf/clemson-genomics-and-bioinformatics-courses/ [59]. *G. hirsutum*, an upland cotton variety, has had BAC and BIBAC libraries produced to help in the study of the cotton genome. A number of *G. hirsutum* genotypes have been screened, and libraries of BAC and BIBAC are developed for making cotton genome research more efficient. Further on May 1, 2007, the construction of minimum six binary data libraries, as well as their availability to the general public, was accomplished. Using five different genotypes of upland cotton, containing Auburn 623, Tamcot HQ95, 0-613-2R and TM-1, Maxxa, each of these libraries was constructed. The construction was carried out in 4 different BAC vectors and 1 Agrobacterium-mediated along with plant-transformation competent BIBAC vector, each of which contained three restriction enzymes, and each of which was carried out in a BAC vector containing three restriction enzymes. When all libraries are merged, the average insert size in each library varies from 93 to 175 kb, with genome coverage ranging from 2.3 to 8.3x genome equivalents, resulting in a total of >21x haploid cotton genomes in the polyploid cotton species. Other *Gossypium* species that have been studied include G. raimindii, *G. barbadense* (Pima S6), G. longicalyx, and *G. arboreum* (AKA8401), and among others. All the libraries of BAC and BIBAC are necessary additions to the field, providing crucial resources for advanced genetics and genomics research on cotton.

5.6 Microarray

Gene identification, mutational tests, gene expression profiling, gene expression mapping (eQTL mapping), high-throughput genetic mapping, and comparative genome analysis, among other applications, have all benefited from the widespread use of microarrays in genomics research in recent years. For the process of array printing to take place, long (70-mer) gene-specific oligonucleotides are printed as array elements on chemically-coated glass slides, followed by the hybridization of the slide with one or more fluorescently-labeled cDNA or mRNA targets obtained by extracting specific tissues, organs, or cells from the mRNA source. As a consequence, researchers may save time and money by observing the expression and activity of all the genes represented on the microarray in a single hybridization experiment. To further the progress of research in cotton genomics, microarrays created from cotton ESTs have been built in various labs across the world to help in the discovery of new cotton ESTs. In order to produce the first batch of cotton microarrays [80], 70-mers oligos were used to generate the first batch of unigene ESTs of *G. arboretum*. NR fiber ESTs are represented by 12,227 elements in each microarray, each of which corresponds to 12,227 NR fiber ESTs. Each element is replicated twice in each microarray. Arpat et colleagues [80] found a statistically significant difference in gene expression between 10-dpa fibers during the manufacturing stage or elongation of primary cell wall and 24-dpa fibers during the stage of secondary cell wall disposal using microarrays (**Figure 2**). According to the findings, fiber gene expression changes from primary cell wall biogenesis or elongation to secondary cell wall biogenesis, with 2553 fiber genes possibly down-regulated and 81 greatly up-regulated in this phase.

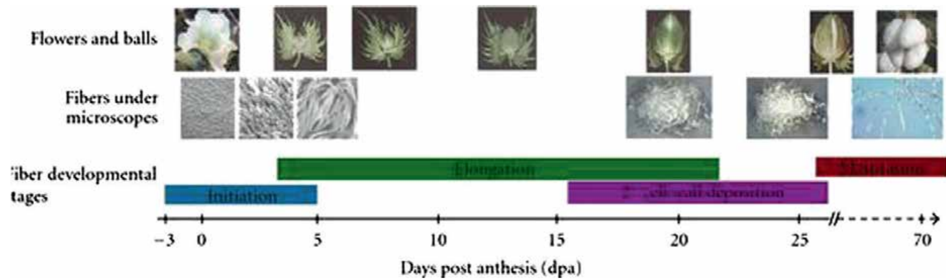

Figure 2.
Cotton fiber development and corresponding morphogenesis stages. The initiation stage is characterized by the enlargement and protrusion of epidermal cells from the ovular surface; during the elongation stage the cells expend in polar directions with a rate of 2 mm/day; during the secondary cell wall deposition stage celluloses are synthesized rapidly until the fibers contain 90% of cellulose; and at the maturation stages minerals accumulate in the fibers and the fibers dehydrate [14].

According to the findings of this research, the expression of fiber genes seems to be stage-specific or cell-expansion-dependent rather than continuous. As a result of our research, we discovered that most of the genes that were upregulated in secondary cell wall synthesis when compared with primary cell wall biogenesis belonged to three main functional categories: energy and metabolism; cellular organization and biogenesis; and cytoskeleton (cytoskeleton was the most frequently observed). The fact that such a large amount of cellulose synthesis and cell wall biogenesis is taking place at this moment makes it feasible to suppose that it is taking place in large quantities. Recent additions to the fiber gene microarrays include almost 10,000 gene elements acquired from ESTs of the tetraploid farmed cotton, *G. hirsutum*, as well as ovary ESTs of the tetraploid farmed cotton. It was necessary to employ *G. hirsutum* fiber and ovary ESTs in order to generate the fiber gene microarrays, which were later upgraded to include over 10,000 gene elements that were derived from *G. hirsutum* fiber and ovarian ESTs [14].

6. Application of bioinformatics-genomic tools

Undoubtedly, one of the most important the employment of genomic technology is one of the aims of genome research. That have been developed so as to promote or assist in the continued growth of agricultural genetics in the future. It is now possible to answer a myriad of vital scientific questions in the area of cotton because of advancements in genetic resources and technology. It is possible to employ genomic resources and techniques to encourage or support cotton genetic improvement in a number of ways, depending on the situation. According to current and future projections, marker-assisted selection (MAS) has been one of the most significant and beneficial applications in the field of computer science in the present and near future. The MAS technology has the potential to bring various benefits to a breeding program in a variety of circumstances. For example, using DNA linked to a gene of interest in the first generation of a mating cycle may be utilized to boost the efficiency of selection in the subsequent generations.

When screening for phenotypes in situations where selection is costly or difficult to perform, such as when dealing with a large number of recessive genes, seasonal or geographical issues, or late expression of the characteristic, the adoption of this approach offers substantial benefits [81]. Because the majority of research in cotton genome over the last decade has been devoted for the growth of resources and

genomics techniques, for the improvement of cotton genetics as the ultimate end goal of the research, cotton breeding programs have only recently begun to use MAS.

6.1 Fiber quality

Glossoloma anomalum introgression line 7235 was used by Zhang [82] with excellent fiber quality attributes to uncover molecular markers related with fiber strength QTLs. The results showed that molecular markers associated with fiber strength QTLs were found in the introgression line 7235. QTLFS1, a big quantitative trait locus (QTL), was identified in the Hainan and Nanjing field sites in China, as well as in the College Station field site, Texas. QTLFS1 was discovered in the Nanjing and Hainan field sites in China, as well as in the College Station field site, Texas, USA. This QTL is shown to be joined with eight markers and to be responsible for more than 30% of the phenotypic variation in the study population. QTLFS1 is originally thinked to be located on chromosome 10, further study revealed it was actually positioned on LGD03 [81]. As established by Guo et al. [83], an unique SCAR4311920 marker was employed to undertake large-scale screening for the absence or presence of this important fiber strength QTL in breeding populations using a genetic marker [83–85]. It is possible that this QTL, as well as the DNA markers that are closely associated to it, has been crucial in the commercial cultivars with superior fiber length attributes.

The researchers detected stable fiber length QTL, qFLD2-1, in the population of Xiangzamian 2 by evaluating it in four distinct settings at the same time, as reported in Wang et al. [86]. Because of its high degree of stability, it is conceivable that this QTL has been important for use in MAS algorithms due to its high degree of stability. By applying an in-depth RFLP map to 15 parameters that reflect fiber length in 3662 BC3F2 plants from 24 independently derived BC3 families using *Gossypium barbadense* as the donor parent, Chee and coauthor [87] dissected the molecular basis of genetic variation in *G. barbadense*-derived BC3 families that governs 15 parameters that reflect fiber length. The finding of many QTLs that are identical to each characteristic shows that, to obtained the largest genetic gain, breeding works that target each trait are necessary to target each trait individually. Lacape et al., [88] done a quantitative trait locus investigation of 11 fiber characteristics in BC1, BC2, and BC2S1 backcross generations created from a cross between *G. hirsutum* "Guazuncho 2" and *G. barbadense* "VH8," which resulted in the BC1 and BC2S1 backcross generations. They founded 15, 12, 21, and 16 quantitative trait loci for strength, length, color and fineness, in atleast one populations, with the number of QTLs varied from population to population.

The data indicated that the vast majority of QTLs had advantageous alleles coming from the *G. barbadense* parent, and that QTLs colocalization for diverse traits was much prevalent to isolated placement of QTLs for unique features. By considering these QTL-rich chromosomal sites, scientists were able to identify 19 spots on 15 different chromosomes that may be used as prospective target regions in the marker-assisted with introgression approach. *G. barbadense* quantitative trait loci linked to genetic markers may allow breeders to more effectively transmit and keep favorable characteristics gained from foreign sources throughout cultivar development as a result of the sources of DNA markers related to QTLs.

6.2 Cytoplasmic male sterility

The D8 restorer (D8R), which is formed for use with the D2 cytoplasmic male sterile alloplasm, and the D2 restorer (D2R), which is formed for use with the D2

cytoplasmic male sterile alloplasm, both work to restore cytoplasmic male sterility by the D8 alloplasm (CMS-D8) to fertility in cotton (CMS-D2). Following these findings, Zhang and Stewart [89] examined that the two restorer loci are not only nonallelic, as well as they are also genetically closely connected, with an approx. Genetic distance between them of 0.93 cM on average. Restoration of the D2 restorer gene has been renamed Rf1, and restoration of the Rf2 restorer gene has been assigned to the restoration of the D8 restorer gene It is possible that a molecular marker that is closely related to the restorer genes of cytoplasmic male sterility are identified and utilized to help hybrid cotton parental lines creation.

According to the findings of Guo et al. [90], one of the DNA markers utilized in the investigation, dubbed OPV-15(300), was shown to be significantly related to the fertility-restoring gene Rf1. They uncovered three RAPD markers which are linked to the restorer gene and, more crucially, they turned the three RAPD markers into markers of genome specific sequence tagged site (STS). It was identified by Liu et al. [22] on the chromosome 4 long arms, which was previously unknown, that the Rf1 locus was located. It was observed that the Rf1 gene is significantly related with two RAPD and 3 SSR markers, for a total of 4 markers. Because they are specific to restorers, MAS should find these markers to be beneficial in the creation of restorer parental lines. Later, Yin [91] developed a genetic map of Rf1 in high resolution that had 13 markers that were separated by a genetic distance of 0.9 cM. This map was utilized to determine the location of Rf1 mutations. Using the Rf1 locus physical map, the researchers determined that the gene's likely location was atleast of two Bacterial Artificial Chromosome clones with an interval of generally 100 kb among them, which were identified as 081-05 K and 052-01 N, respectively, with an interval of generally 100 kb. The method of extracting the Rf1 gene from cotton is now in the process of being completed.

6.3 Resistance to diseases and insect pests

An important consideration in breeding programs of cotton is resistance of diseases. For this purpose, the researchers identified and described the family of NBS-LRR expressing genes in the Auburn 634 Upland cotton cv. in order to allow investigation, modification and cloning of genes imparting resistance to diverse diseases including fungus, viruses, bacteria and nematodes. It was discovered that only a less percentage of AD-genome chromosomes of cotton include members of the RGA gene family, and that members of one subfamily tend to cluster together on the genetic map of cotton, with many RGAs found in subgenome. Than in subgenome D. Wright et al. [92] discovered two RGAs that comapped with previously identified QTLs for cotton bacterial blight resistance. Cotton RGAs from the NBS-LRR gene family have been crucial in the manipulation, characterization and cloning of resistant genes to a variety of pests and pathogens, accounting for approx. 80% of the genes (>40 genes) that have been cloned to date and confirmed resistance to fungus, viruses, bacteria and nematodes.

Meloidogyne incognita, an RKN, has the potential to significantly reduce cotton yields. CIR316, a SSR marker on linkage group A03, was found by Wang et al. [93] using the *G. hirsutum* "AaclaNemX." resistant cultivar. This marker was closely attached to a critical gene resistant RKN (rkn1). A bulked segregant analysis in combination with AFLP is also used in a parallel study to find additional rkn1-associated molecular markers [94]. When an AFLP marker called GHACC1 that was previously linked to rkn1 was converted to a CAPS marker, it resulted in the creation of the CAPS

markers. MAS patients might benefit from the use of these two markers. Researchers from Shen et al. [95] found that RFLP markers which are on chromosomes 7 as well as 11 are related to RKN resistance in the source of Auburn 634,which is another source of resistant germplasm than the AcalaNemX source [96].

On chromosomes 7 and 11, an SSR marker-based search for a minor and major dominant quantitative trait locus further verified this relationship. It was shown that when two SSR markers were combined, they accounted for 31% of the galling index. Short arm chromosome 14 mapping is handled by BNL 3661, while long arm chromosome 11 mapping is handled by BNL 1231. It is fair to believe that minimum two genes are included in RKN resistance, given the link between RKN resistance and two different chromosomes.

Blight produced with the bacteria Xanthomonascampestris is other commercially essential disease of cotton (Xcm). There have been two studies that looked at the genetic genes location that provide bacterial resistance that cause blight disease, Wright et al. and Rungi et al., respectively [92, 97]. RFLP markers linked to specific locations on the chromosome were used in both experiments to look for genes that give resistance to the virus. Maps show an association between the B12 resistance gene marker on chromosome 14 and the resistance locus that was initially discovered in African cotton varieties. As an additional step, AFLP and SSR markers were used to discover novel markers that may be used to introduce the Xcm resistance gene into *G. barbadense* through MAS.

7. Conclusion and future prospectives

A vast amount of genetic information about the cotton plant and its products has been made available despite the fact that cotton genomics research has lagged behind that of rice, maize, wheat, and soybean. Numerous genes and quantitative trait loci (QTLs) joined with quality of cotton fiber, production of fiber, biotic and abiotic stresses are seen and mapped using these resources and methodologies. At Texas Tech University, the laboratory of T. A. Wilkins includes cotton fiber microarrays that may be used for research and development purposes. In the four arrays one is printed on a single slide have seen in the picture above. Biology of cotton, as well as plant biology in general, is explored. These tools and methodologies, however, need a lot more effort to be properly utilized in improvement of cotton genetic and biology study, as well as made more accessible for usage in applications. Cotton genomes research should be emphasized, including but not limited to the following: Based on whole-genome BAC/BIBAC sequencing, we are developing physical maps for cottons. Till date it has not been an accurate and trustworthy based on BAC/BIBAC whole-genome, which is for cotton's physical/genetic map. The maps should contain minimum two species of *Gossypium*. Both Upland and *Gossypium* Raimondi cottons have 90% of world cotton output. Genome of *Gossypium*'s is the smallest among its species, which means that it has the largest density of genes among *Gossypium*'s. Mostly current genetics and research for genomics initiatives may benefit from the usage of whole-genome integrated physical or genetic maps, which are shown to be strong platforms and freeways in model and other species, such as the fruit fly, the human genome and the mouse genome [74, 75]. Additional advantages of developing integrated physical maps include a more speedy and effective integration of all current mapped genes, genetic maps, and QTLs, along with genetic resources, which resulted in enhanced research efficiency and cheaper costs.

QTLs are being finely mapped. Even though many genes and quantitative trait loci (QTLs) related to cotton fiber output and fiber quality, as well as stressors from both the natural and man-made environments have been genetically mapped, a couple of issues must be addressed: first, virtually all QTLs are discovered using F2, BC1, and early generations in only one setting, if not a few. Because quantitative elements are very subject to environmental change, findings obtained by using early generations in just one or a few conditions that differ it from one research to the next. Furthermore, DNA markers and most QTLs genetic distances are just too great for MAS applications to be successful. This is the second challenge. For mapping QTLs, huge and advanced population, such as RILs or DHs, in varied settings, and nearly connected DNA markers, comprehensive physical maps are required. Accurate mapping of QTLs and formation of DNA markers which are well-equiped for MAS (i.e., tightly connected and user-friendly) are necessary for the ultimate isolation of QTL genes for map-based cloning. Genes which are isolated as best candidates for generating MAS markers as gene and markers have no recombination between them which is making them perfect choices. More than one key for genomes of cotton are being sequenced. The most effective method for identifying and decoding all cotton genes is whole genome sequencing, despite its high cost with current sequencing technology. It also generates the most sought-after and highly detailed map of the cotton genome, both physically and genetically integrated. Although the genome sizes of *Gossypium* species vary widely used studies of comparative genomics which show the gene content and order of genes for these species are very consistent [60, 61]. *Gossypium raimondii* has small genome for all *Gossypium* species, despite the fact that it is not cultivated in culture. This makes it an excellent candidate for genome sequencing. The sequence data from *G. raimondii* is transfers to the most important farmed cotton, If a physical map for this larger genome is available, end sequences of BAC for the integrated physical map may be used as anchors for *G. hirsutum*.

Cells at the stage of secondary cell wall development, including those produced from nonfiber and nonovary tissues and fibers. To be sure, Cotton ESTs are now more plentiful than ever before, but the distribution of these ESTs across different tissue types is still rather uneven, as seen above. After 20 dpa, when secondary cell wall deposition has occurred, there are relatively few ESTs from nonfiber/nonovary tissues as well as fibers. This is especially true during the 15–45-dpa stage. It is clear that even while the expressed genes first set do not contribute directly to fiber output with quality, the second set of expressed genes has a major influence on fiber yield and quality. A large influence on fiber output and quality may be found in the first set of expressed genes, despite the fact that they do not directly contribute to fiber strength. Researchers are working to profile and identify genes related with certain biological processes with an emphasis on genes involved in fiber production. There have been several advances in molecular biology that have been made possible by the creation and widespread availability of microarrays based on cDNA or unigene EST. Cotton research has not made much progress in any of these areas, unfortunately. The capacity of cotton breeders to improve cotton genetics have considerably enhanced by incorporating and defining genes used in the process of fiber creation, development as well as growth of plant, and responses of cotton plants to biotic and abiotic challenges.

Cotton breeders benefit greatly from the capacity to translate changes in gene activity or expression in different tissues and developmental stages into changes in fiber quality and yield. However, it is not clear what the upregulation or downregulation of fiber gene activity or active expression in developmental stages and organs means to cotton's final fiber yield or in order to discover genes included in fiber

introduction [98, 99], expansion [80, 98] along with secondary cell wall deposition [80], cotton genotypes are employed. Are longer fibers inferred by the presence of a gene that is actively expressed during the elongation stage of the fiber? There needs to be more study done on using the data of gene expression for cotton germplasm analyses and development programs.

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Conflict of interest

The authors declare no conflict of interest.

Acronyms and abbreviations

Cotton FGD	Cotton functional genomic database
Cotton DB	Cotton genome database
CGP	Cotton genome project
CGRD	Cotton genome resource database
JGI	Joint genome institute
CMT2	chromomethylase2
CNVs	Copy number variants
CMS-D8	Cytoplasmic male sterility by the D8 alloplasm
D2R	D2 restorer
D8R	D8 restorer
ESTs	Expressed sequence tags
MAS	marker-assisted selection
NGS	Next-generation sequencing
PCR	Polymerase chain reaction
QTL	Quantitative trait loci
RdDM	RNA-directed DNA methylation
SNP	Single nucleotide polymorphism
TM-1	Texas Marker-1

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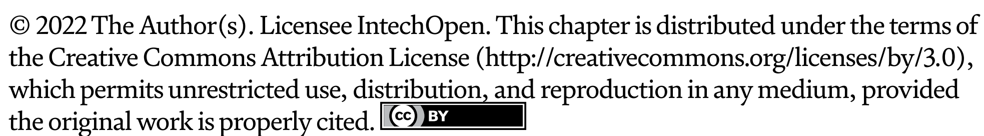

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References

[1] Shaheen T, Tabbasam N, Iqbal MA, Ashraf A, Zafar Y, Peterson AH. Cotton genetic resources. A review. Agronomy for Sustainable Development. 2012;**32**:419-432. DOI: 10.1007/s13593-011-0051-z

[2] Qin YM, Zhu YX. How cotton fibers elongate: A tale of linear cell growth mode. Current Opinion in Plant Biology. 2011;**14**:106-111. DOI: 10.1016/j.pbi.2010.09.010

[3] Shan CM, Shangguan XX, Zhao B, Zhang XF, Chao LM, Yang CQ, et al. Control of cotton fibre elongation by a homeodomain transcription factor GhHOX3. Nature Communications. 2014;**5**:5519. DOI: 10.1038/ncomms6519

[4] Chen ZJ, Scheffler BE, Dennis E, Triplett BA, Zhang T, Guo W, et al. Toward sequencing cotton (Gossypium) genomes. Plant Physiology. 2007;**145**:1303-1310. DOI: 10.1104/pp.107.107672

[5] Rahman M, Zafar Y, Paterson AH. Gossypium DNA markers types, number and uses. In: Paterson AH, editor. Genomics of Cotton. Berlin: Springer; 2009. pp. 101-139. DOI: 10.1007/978-0-387-70810-2_5

[6] Chen H, Qian N, Guo WZ, Song QP, Li BC, Deng FJ, et al. Using three overlapped RILs to dissect genetically clustered QTL for fiber strength on chro.24 in Upland cotton. Theoretical and Applied Genetics. 2009;**119**:605-612. DOI: 10.1007/s00122-009-1070-x

[7] Rahman M, Yasmin T, Tabassum N, Ullah I, Asif M, Zafar Y. Studying the extent of genetic diversity among Gossypium arboreum L. genotypes/cultivars using DNA fingerprinting. Genet. Resour. Crop Evoluation. 2008;**55**:331-339. DOI: 10.1007/s10722-007-9238-1

[8] Helms AB. Yield study report. In: Duggar P, Richder D, editors. Proceedings of Beltwide Cotton Production Conference. San Antonio TX: National Cotton Council; 2000. pp. 4-9

[9] Abelson PH. A third technological revolution. Science. 1998;**279**(5359):2019. DOI: 10.1126/science.279.5359.2019a

[10] Fryxell PA, Craven LA, Stewart JM. A revision of Gossypium sect. grandicalyx (malvaceae), including the description of six new species. Systematic Botany. 1992;**17**:91-114. DOI: 10.2307/2419068

[11] Rong J, Abbey C, Bowers JE, Brubaker CL, Chang C, Chee PW, et al. A 3347-locus genetic recombination map of sequence-tagged sites reveals features of genome organization, transmission and evolution of cotton (Gossypium). Genetics. 2004;**166**:389-417. DOI: 10.1534/genetics.166.1.389

[12] Brubaker CL, Paterson AH, Wendel JF. Comparative genetic mapping of allotetraploid cotton and its diploid progenitors. Genome. 1999;**42**:184-203. DOI: 10.1139/g98-118

[13] Endrizzi JE, Turcotte EL, Kohel RJ. Qualitative genetics, cytology, and cytogenetics. In: Kohel RJ, Lewis CF, editors. Cotton. Madison, Wisconsin: American Society of Agronomy; 1984. pp. 81-129. DOI: 10.2134/agronmonogr24.c4

[14] Zhang HB, Li Y, Wang B, Chee PW. Recent advances in cotton genomics. International Journal of Plant Genomics. 2008;**742304**:14. DOI: 10.1155/2008/742304

[15] Yu LH, Wu SJ, Peng YS, Liu RN, Chen X, Zhao P, et al. Arabidopsis EDT1/HDG11 improves drought and

salt tolerance in cotton and poplar and increases cotton yield in the field. Plant Biotechnology Journal. 2016;**14**:72-84. DOI: 10.1111/pbi.12358

[16] Paterson AH, Wendel JF, Gundlach H, Guo H, Jenkins J, Jin D, et al. Repeated polyploidization of Gossypium genomes and the evolution of spinnable cotton fibres. Nature. 2012;**492**:423-427. DOI: 10.1038/nature11798

[17] Wang K, Wang Z, Li F, Ye W, Wang J, Song G, et al. The draft genome of a diploid cotton Gossypium raimondii. Nature Genetics. 2012;**44**:1098-1103. DOI: 10.1038/ng.2371

[18] Li F, Fan G, Wang K, Sun F, Yuan Y, Song G, et al. Genome sequence of the cultivated cotton Gossypium arboreum. Nature Genetics. 2014;**46**:567-572. DOI: 10.1038/ng.2987

[19] Wendel JF. New world tetraploid cottons contain old world cytoplasm. Proceedings of the National Academy of Sciences. 1989;**86**:4132-4136. DOI: 10.1073/pnas.86.11.4132

[20] Li F, Fan G, Lu C, Xiao G, Zou C, Kohel RJ, et al. Genome sequence of cultivated Upland cotton (Gossypium hirsutum TM-1) provides insights into genome evolution. Nature Biotechnology. 2015;**33**:524-530. DOI: 10.1038/nbt.3208

[21] Zhang T, Hu Y, Jiang W, Fang L, Guan X, Chen J, et al. Sequencing of allotetraploid cotton (Gossypium hirsutum L. acc. TM-1) provides a resource for fiber improvement. Nature Biotechnology. 2015;**33**:531-537. DOI: 10.1038/nbt.3207

[22] Liu L, Guo W, Zhu X, Zhang T. Inheritance and fine mapping of fertility restoration for cytoplasmic male sterility in Gossypium hirsutum L. Theoretical and Applied Genetics. 2003;**106**(3):461-469. DOI: 10.1007/s00122-002-1084-0

[23] Yuan D, Tang Z, Wang M, Gao W, Tu L, Jin X, et al. The genome sequence of Sea-Island cotton (Gossypium barbadense) provides insights into the allopolyploidization and development of superior spinnable fibres. Scientific Reports. 2015;**5**(1):1-16. DOI: 10.1038/srep17662

[24] Page JT, Liechty ZS, Alexander RH, Clemons K, Hulse-Kemp AM, Ashrafi H, et al. DNA sequence evolution and rare homoeologous conversion in tetraploid cotton. PLoS Genetics. 2016;**12**:e1006012. DOI: 10.1371/journal.pgen.1006012

[25] Fang L, Wang Q, Hu Y, Jia Y, Chen J, Liu B, et al. Genomic analyses in cotton identify signatures of selection and loci associated with fiber quality and yield traits. Nature Genetics. 2017;**49**: 1089-1098. DOI: 10.1038/ng.3887

[26] Fang L, Gong H, Hu Y, Liu C, Zhou B, Huang T, et al. Genomic insights into divergence and dual domestication of cultivated allotetraploid cottons. Genome Biology. 2017;**18**:33. DOI: 10.1186/s13059-017-1167-5

[27] Wang M, Tu L, Lin M, Lin Z, Wang P, Yang Q, et al. Asymmetric sub genome selection and cis-regulatory divergence during cotton domestication. Nature Genetics. 2017;**49**:579-587. DOI: 10.1038/ng.3807

[28] Li X, Jin X, Wang H, Zhang X, Lin Z. Structure, evolution, and comparative genomics of tetraploid cotton based on a high-density genetic linkage map. DNA Research. 2016;**23**:283-293. DOI: 10.1093/dnares/dsw016

[29] Wang S, Chen J, Zhang W, Hu Y, Chang L, Fang L, et al. Sequence-based ultra-dense genetic and physical maps reveal structural variations of allopolyploid cotton genomes. Genome Biology. 2015;**16**:108. DOI: 10.1186/s13059-015-0678-1

[30] Cheng H, Lu C, Yu JZ, Zou C, Zhang Y, Wang Q, et al. Fine mapping and candidate gene analysis of the dominant glandless gene Gl2 e in cotton (Gossypium spp.). Theoretical and Applied Genetics. 2016;**129**:1347-1355. DOI: 10.1007/s00122-016-2707-1

[31] Andres RJ, Bowman DT, Kaur B, Kuraparthy V. Mapping and genomic targeting of the major leaf shape gene (L) in Upland cotton (Gossypium hirsutum L.). Theoretical and Applied Genetics. 2014;**127**:167-177. DOI: 10.1007/s00122-013-2208-4

[32] Fang X, Liu X, Wang X, Wang W, Liu D, Zhang J, et al. Fine-mapping qFS07. 1 controlling fiber strength in upland cotton (Gossypium hirsutum L.). Theoretical and Applied Genetics. 2017;**130**:795-806. DOI: 10.1007/s00122-017-2852-1

[33] Xu P, Gao J, Cao Z, Chee PW, Guo Q, Xu Z, et al. Fine mapping and candidate gene analysis of qFL-chr1, a fiber length QTL in cotton. Theoretical and Applied Genetics. 2017;**130**:1309-1319. DOI: 10.1007/s00122-017-2890-8

[34] Ashrafi H, Hulse-Kemp AM, Wang F, Yang SS, Guan X, Jones DC, et al. A long-read transcriptome assembly of cotton (L.) and intraspecific single nucleotide polymorphism discovery. The Plant Genome. 2015;**8**:1-14. DOI: 10.3835/plantgenome2014.10.0068

[35] Mills RE, Walter K, Stewart C, Handsaker RE, Chen K, Alkan C, et al. 1000 genomes project. Mapping copy number variation by population scale genome sequencing. Nature. 2011;**470**:59-65. DOI: 10.1038/nature09708

[36] Lin M, Pang C, Fan S, Song M, Wei H, Yu S. Global analysis of the Gossypium hirsutum L. Transcriptome during leaf senescence by RNASeq. BMC Plant Biology. 2015;**15**:43. DOI: 10.1186/s12870-015-0433-5

[37] Islam MS, Thyssen GN, Jenkins JN, Zeng L, Delhom CD, McCarty JC, et al. A MAGIC population-based genome-wide association study reveals functional association of GhRBB1_A07 gene with superior fiber quality in cotton. BMC Genomics. 2016;**17**:903. DOI: 10.1186/s12864-016-3249-2

[38] Artico S, Ribeiro-Alves M, Oliveira-Neto OB, de Macedo LL, Silveira S, Grossi-de-Sa MF, et al. Transcriptome analysis of Gossypiumhirsutum flower buds infested by cotton boll weevil (Anthonomusgrandis) larvae. BMC Genomics. 2014;**15**:854. DOI: 10.1186/1471-2164-15-854

[39] Bowman MJ, Park W, Bauer PJ, Udall JA, Page JT, Raney J, et al. RNA-Seqtranscriptome profiling of upland cotton (Gossypiumhirsutum L.) root tissue under water-deficit stress. PLoS ONE. 2013;**8**(12):e82634. DOI: 10.1371/journal.pone.0082634

[40] Wang Z, Gerstein M, Snyder M. RNA-Seq: A revolutionary tool for transcriptomics. Nature Reviews. Genetics. 2009;**10**:57-63. DOI: 10.1038/nrg2484

[41] Zhao S, Fung-Leung WP, Bittner A, Ngo K, Liu X. Comparison of RNA-Seq and microarray in transcriptome profiling of activated T cells. PLoS One. 2014;**9**:e78644. DOI: 10.1038/nrg2484

[42] Phillips T. The role of methylation in gene expression. Nature Education. 2008;**1**:116

[43] Cubas P, Vincent C, Coen E. An epigenetic mutation responsible for natural variation in floral symmetry. Nature. 1999;**401**:157-161. DOI: 10.1038/43657

[44] Jin X, Pang Y, Jia F, Xiao G, Li Q, Zhu Y. A potential role for CHH DNA methylation in cotton fiber growth patterns. PLoS One. 2013;**8**:e60547. DOI: 10.1371/journal.pone.0060547

[45] Osabe K, Clement JD, Bedon F, Pettolino FA, Ziolkowski L, Llewellyn DJ, et al. Genetic and DNA methylation changes in cotton (Gossypium) genotypes and tissues. PLoS One. 2014;**9**:e86049. DOI: 10.1371/journal.pone.0086049

[46] Song Q, Guan X, Chen ZJ. Dynamic roles for small RNAs and DNA methylation during ovule and fiber development in allotetraploid cotton. PLoS Genetics. 2015;**11**:e1005724. DOI: 10.1371/journal.pgen.1005724

[47] Song Q, Zhang T, Stelly DM, Chen ZJ. Epigenomic and functional analyses reveal roles of epialleles in the loss of photoperiod sensitivity during domestication of allotetraploid cottons. Genome Biology. 2017;**18**(1):99. DOI: 10.1186/s13059-017-1229-8

[48] CottonGen. Available from: https://www.cottongen.org [Accessed: December 4, 2021]

[49] Cotton Genome Resource Database. CGRD. Available from: http://cgrd.hzau.edu.cn/index.php [Accessed: December 4, 2021]

[50] Database for Co-expression Networks with Function Modules. Available from: http://structuralbiology.cau.edu.cn/Gossypium/ [Accessed: December 4, 2021]

[51] Join Genome Institute. Available from: http://jgi.doe.gov [Accessed: December 4, 2021]

[52] Cotton Genome Database. Available from: http://www.cottondborg [Accessed: December 4, 2021]

[53] Evolution of Cotton. Available from: https://learn.genetics.utah.edu/content/cotton/evolution/ [Accessed: December 4, 2021]

[54] Platform of Functional Genomics Analysis in Gossypium raimondii. Available from: http://structuralbiology.cau.edu.cn/GraP/about.html [Accessed: December 4, 2021]

[55] Cotton Functional Genomic Database. Available from: https://cottonfgd.org [Accessed: December 4, 2021]

[56] Cotton Genome Project. Available from: http://cgp.genomics.org.cn/page/species/index.jsp [Accessed: December 4, 2021]

[57] Abd El-Moghny AM, Santosh HB, Raghavendra KP, Sheeba JA, Singh SB, Kranthi KR. Microsatellite marker based genetic diversity analysis among cotton (Gossypium hirsutum) accessions differing for their response to drought stress. Journal of Plant Biochemistry and Biotechnology. 2017;**26**(3):366-370

[58] Zeng C, Kouprina N, Zhu B, Cairo A, Hoek M, Cross G, et al. Large-insert BAC/YAC libraries for selective re-isolation of genomic regions by homologous recombination in yeast. Genomics. 2001;**77**(1-2):27-34. DOI: 10.1006/geno.2001.6616

[59] Decker CJ, Steiner HR, Hoon-Hanks LL, Morrison JH, Haist KC, Stabell AC, et al. dsRNA-Seq: Identification of viral infection by purifying and sequencing dsRNA. Viruses. 2019;**11**(10):943

[60] Reinisch AJ, Dong JM, Brubaker CL, Stelly DM, Wendel JF, Paterson AH. A detailed RFLPmap of cotton, Gossypium hirsutum x Gossypium barbadense: Chromosome organization and evolution in a disomic polyploid genome. Genetics.

1994;**138**(3):829-847. DOI: 10.1093/genetics/138.3.829

[61] Rong J, Abbey C, Bowers JE, Brubaker CL, Chang C, Chee PW, et al. A 3347-locus genetic recombination map of sequence-tagged sites reveals types of genome organization, transmission and evolution of cotton (Gossypium). Genetics. 2004;**166**:389-417. DOI: 10.1534/genetics.166.1.389

[62] Desai A, Chee PW, Rong J, May OL, Paterson AH. Chromosome structural changes in diploid and tetraploida genomes of Gossypium. Genome. 2006;**49**(4):336-345. DOI: 10.1139/g05-116

[63] Kohel RJ, Yu J, Park YH, Lazo GR. Molecular mapping and characterization of traits controlling fiber quality in cotton. Euphytica. 2001;**121**(2):163-172. DOI: 10.1023/A:1012263413418

[64] Brubaker CL, Brown AHD. The use of multiple alien chromosome addition aneuploids facilitates genetic linkage mapping of the Gossypium G genome. Genome. 2003;**46**:774-791. DOI: 10.1139/g03-063

[65] SaghaiMaroof MA, Biyashev RM, Yang GP, Zhang Q, Allard RW. Extraordinarily polymorphic microsatellite DNA in barley: Species diversity, chromosomal locations, and population dynamics. Proceedings of the National Academy of Sciences of the United States of America. 1994;**91**(12):5466-5470. DOI: 10.1073/pnas.91.12.5466

[66] Blenda A, Scheffler J, Scheffler B, Palmer M, Lacape JM, Yu JZ, et al. CMD: A cotton microsatellite database resource for gossypium genomics. BMC Genomics. 2006;**7**:132. DOI: 10.1186/1471-2164-7-132

[67] Ulloa M, Brubaker C, Chee P. Cotton. In: Kole C, editor. Technical Crops. Genome Mapping and Molecular Breeding in Plants. Vol. 6. Berlin, Heidelberg: Springer; 2000. DOI: 10.1007/978-3-540-34538-1_1

[68] Lichtenzveig J, Scheuring C, Dodge J, Abbo S, Zhang H-B. Construction of BAC and BIBAC libraries and their applications for generation of SSR markers for genome analysis of chickpea, Cicer arietinum L. Theoretical and Applied Genetics. 2005;**110**(3):492-510

[69] He L, Du C, Li Y, Scheuring C, Zhang HB. Large insert bacterial clone libraries and their applications. In: Liu Z, editor. Aquaculture Genome Technologies. Ames, Iowa, USA: Blackwell; 2007. pp. 215-244

[70] Ren C, Xu ZY, Sun S, Lee MK, Wu C, Scheuring C, Zhang HB. Genomic DNA libraries and physical mapping. In: Meksem K, Kahl G, editors. The Handbook of Plant Genome Mapping: Genetic and Physical Mapping. Wiley-VCH Verlag GmbH; Weinheim: Germany; 2005. p.173-213. DOI: 10.1002/3527603514.ch8

[71] Ioannou PA, Amemiya CT, Garnes J, Kroisel PM, Shizuya H, Chen C, et al. A new bacteriophage P1-derived vector for the propagation of large human DNA fragments. Nature Genetics. 1994;**6**(1):84-89. DOI: 10.1038/ng0194-84

[72] Shizuya H, Birren B, Kim UJ, Mancino V, Slepak T, Tachiiri Y, et al. Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in Escherichia coli using an F-factor-based vector. Proceedings of the National Academy of Sciences of the United States of America. 1992;**89**(18):8794-8797. DOI: 10.1073/pnas.89.18.8794

[73] Zhang H-B. Map-based cloning of genes and QTLs. In: Kole C, Abbott A,

editors. Plant Molecular Mapping and Breeding. New York, NY, USA: Springer; 2007

[74] Wu C, Sun S, Lee MK, Xu ZY, Ren C, Zhang HB. Whole genome physical mapping: An overview on methods for DNA fingerprinting. In: Meksem K, Kahl G, editors. The Handbook of Plant Genome Mapping: Genetic and Physical Mapping. Wiley-VCH Verlag GmbH. Weinheim, Germany; 2007. p. 257-283. DOI:10.1002/3527603514.ch11

[75] Zhang HB, Wing RA. Physical mapping of the rice genome with BACs. Plant Molecular Biology. 1997;**35**(1-2):115-127

[76] International Rice Genome Sequencing Project. The map-based sequence of the rice genome. Nature. 2005;**436**(7052):793-800. DOI: 10.1038/nature03895

[77] Tyler BM, Tripathy S, Zhang X, Dehal P, Jiang RH, Aerts A, et al. Phytophthora genome sequences uncover evolutionary origins and mechanisms of pathogenesis. Science. 2006;**313**(5791):1261-1266. DOI: 10.1126/science.1128796

[78] Chen M, SanMiguel P, de Oliveira AC, Woo SS, Zhang H, Wing RA, et al. Microcolinearity in sh2-homologous regions of the maize, rice, and sorghum genomes. Proceedings of the National Academy of Sciences of the United States of America. 1997;**94**(7):3431-3435. DOI: 10.1073/pnas.94.7.3431

[79] Patocchi A, Vinatzer BA, Gianfranceschi L, Tartarini S, Zhang HB, Sansavini S, et al. Construction of a 550 kb BAC contig spanning the genomic region containing the apple scab resistance gene Vf. Molecular & General Genetics. 1999;**262**(4-5):884-891. DOI: 10.1007/s004380051154

[80] Arpat AB, Waugh M, Sullivan JP, Gonzales M, Frisch D, Main D, et al. Functional genomics of cell elongation in developing cotton fibers. Plant Molecular Biology. 2004;**54**(6):911-929. DOI: 10.1007/s11103-004-0392-y

[81] Dreher K, Morris M, Khairallah M, Ribaut JM, Pandey S, Srinivasan G. Is marker-assisted selection cost-effective compared to conventional plant breeding methods? The case of quality protein maize. In: Evenson RE, Santaniello V, Zilberman D, editors. Economic and Social Issues in Agricultural Biotechnology. Wallingford, UK: CABI Publishing; 2002. pp. 203-236

[82] Zhang T, Yuan Y, Yu J, Guo W, Kohel RJ. Molecular tagging of a major QTL for fiber strength in Upland cotton and its marker-assisted selection. Theoretical and Applied Genetics. 2003;**106**(2):262-268. DOI: 10.1007/s00122-002-1101-3

[83] Guo W, Zhang T, Shen X, Yu JZ, Kohel RJ. Development of SCAR marker linked to a major QTL for high fiber strength and its usage in molecular-marker assisted selection in upland cotton. Crop Science. 2003;**43**(6):2252-2256. DOI: 10.2135/cropsci2003.2252

[84] Shen X, Guo W, Zhu X, Yuan Y, Yu J, Kohel R, et al. Molecular mapping of QTLs for fiber qualities in three diverse lines in Upland cotton using SSR markers. Molecular Breeding: New Strategies in Plant Improvement. 2005;**15**(2):169-181

[85] He DH, Lin ZX, Zhang XL, Nie YC, Guo XP, Feng CD, et al. Mapping QTLs of traits contributing to yield and analysis of genetic effects in tetraploid cotton. Euphytica. 2005;**144**:141-149. DOI: 10.1007/s10681-005-5297-6

[86] Wang BH, Wu YT, Huang NT, Zhu XF, Guo WZ, Zhang TZ. QTL mapping

for plant architecture traits in upland cotton using RILs and SSR markers. Yi ChuanXueBao. 2006;**33**(2):161-170. DOI: 10.1016/S0379-4172(06)60035-8

[87] Chee PW, Draye X, Jiang CX, Decanini L, Delmonte TA, Bredhauer R, et al. Molecular dissection of phenotypic variation between Gossypium hirsutum and Gossypium barbadense (cotton) by a backcross-self approach: III. Fiber length. Theoretical and Applied Genetics. 2005;**111**(4):772-781. DOI: 10.1007/s00122-005-2062-0

[88] Lacape JM, Llewellyn D, Jacobs J, Arioli T, Becker D, Calhoun S, et al. Meta-analysis of cotton fiber quality QTLs across diverse environments in a Gossypiumhirsutum x G. barbadense RIL population. BMC Plant Biology. 2010;**10**:132. DOI: 10.1186/1471-2229-10-132

[89] Zhang JF, Stewart MDJ. Inheritance and genetic relationships of the D8 and D2-2 restorer genes for cotton cytoplasmicmale sterility. Crop Science. 2001;**41**(2):289-294. DOI: 10.2135/cropsci2001.412289x

[90] Guo W, Zhang T, Pan J, Kohel RJ. Identification of RAPD marker linked with fertility-restoring gene of cytoplasmic male sterile lines in upland cotton. Chinese Science Bulletin. 1998;**43**(1):52-54

[91] Yin J, Guo W, Yang L, Liu L, Zhang T. Physical mapping of the Rf1 fertility-restoring gene to a 100 kb region in cotton. Theoretical and Applied Genetics. 2006;**112**(7):1318-1325. DOI: 10.1007/s00122-006-0234-1

[92] Wright RJ, Thaxton PM, El-Zik KM, Paterson AH. D-subgenome bias of Xcm resistance genes in tetraploid Gossypium (cotton) suggests that polyploid formation has created novel avenues for evolution. Genetics. 1998;**149**(4):1987-1996. DOI: 10.1093/genetics/149.4.1987

[93] Wang C, Ulloa M, Roberts PA. Identification and mapping of microsatellite markers linked to a root-knot nematode resistance gene (rkn1) in AcalaNemX cotton (Gossypiumhirsutum L.). Theoretical and Applied Genetics. 2006;**112**(4):770-777. DOI: 10.1007/s00122-005-0183-0

[94] Wang C, Roberts PA. Development of AFLP and derived CAPS markers for root-knot nematode resistance in cotton. Euphytica. 2006;**152**(2):185-196. DOI: 10.1007/s10681-006-9197-1

[95] Shen X, Van Becelaere G, Kumar P, Davis RF, May OL, Chee P. QTL mapping for resistance to root-knot nematodes in the M-120 RNR Upland cotton line (Gossypium hirsutum L.) of the Auburn 623 RNR source. Theoretical and Applied Genetics. 2006;**113**(8):1539-1549. DOI: 10.1007/s00122-006-0401-4

[96] Shen X, Zhang T, Guo W, Zhu X, Zhang X. Mapping fiber and yield QTLs with main, epistatic, and QTL x environment interaction effects in recombinant inbred lines of Upland cotton. Crop Science. 2006;**46**(1):61-66. DOI: 10.2135/cropsci2005.0056

[97] Rungis D, Llewellyn ES. Dennis, Lyon BR. Investigation of the chromosomal location of the bacterial blight resistance gene present in an Australian cotton (Gossypiumhirsutum L.) cultivar. Crop & Pasture Science. 2002;**53**(5):551-560. DOI: 10.1071/AR01121

[98] Shi YH, Zhu SW, Mao XZ, Feng JX, Qin YM, Zhang L, et al. Transcriptome profiling, molecular biological, and physiological studies reveal a major role for ethylene in cotton fiber cell elongation. The Plant Cell.

2006;**18**(3):651-664. DOI: 10.1105/tpc.105.040303

[99] Wu Y, Machado AC, White RG, Llewellyn DJ, Dennis ES. Expression profiling identifies genes expressed early during lint fibre initiation in cotton. Plant & Cell Physiology. 2006;**47**(1): 107-127. DOI: 10.1093/pcp/pci228

Chapter 4

Transcriptome Analysis Using RNA Sequencing for Finding Genes Related to Fiber in Cotton: A Review

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Abstract

The cotton crop is economically important and primarily grown for its fiber. Although the genus *Gossypium* consists of over 50 species, only four domesticated species produce spinnable fiber. However, the genes determine the molecular phenotype of fiber, and variation in their expression primarily contributes to associated phenotypic changes. Transcriptome analyses can elucidate the similarity or variation in gene expression (GE) among organisms at a given time or a circumstance. Even though several algorithms are available for analyzing such high-throughput data generated from RNA Sequencing (RNA-Seq), a reliable pipeline that includes a combination of tools such as an aligner for read mapping, an assembler for quantitating full-length transcripts, a differential gene expression (DGE) package for identifying differences in the transcripts across the samples, a gene ontology tool for assigning function, and enrichment and pathway mapping tools for finding interrelationships between genes based on their associated functions are needed. Therefore, this chapter first introduces the cotton crop, fiber phenotype, transcriptome, then discusses the basic RNA-Seq pipeline and later emphasizes various transcriptome analyses studies focused on genes associated with fiber quality and its attributes.

Keywords: *Gossypium*, species, gene, expression, sequencing, fiber, transcriptome, and RNA-Seq

1. Introduction

Cotton is a globally and economically important fiber, oil, and protein source. It belongs to the family Malvaceae and genus *Gossypium* with more than 50 species, but only four domesticated species produce spinnable fiber. Of these, *G. hirsutum* and *G. barbadense* are allopolyploids that originated in the United States. The remaining two species are diploids (*G. herbaceum* and *G. arboreum*) from Africa and/or Asia [1]. Genus *Gossypium* consists of one tetraploid (AD) and eight diploid genome groups (A – G and K). It is believed that the allotetraploid, upland cotton, *G. hirsutum*

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($A_tA_tD_tD_t$; ~2.5 Gb) is most likely evolved from the diploid A-genome ancestor, *G. arboreum* (A_2A_2; ~1.8 Gb) or *G. herbaceum* (A_1A_1; ~1.6 Gb), and the diploid D-genome progenitor, *G. raimondii* (D_5D_5; ~900 Mb) [1–3]. According to the United States Department of Agriculture (USDA) and Foreign Agricultural Service (FAS), the acreage, yield, and production projections for the year 2021–2022 across the world and the United States are ~32 and ~4 million hectares, ~810 and ~950 kilograms per hectare, and ~120 and ~18 million 480-lb bales, respectively [4]. However, the United Nations (UN) projected that the global population would surpass the mark of 10 billion by 2050 [5]. Therefore, to meet the clothing needs of a constantly expanding population, the yield or production must be increased by developing or employing better cotton crop improvement strategies.

The percent composition of cotton fibers includes cellulose (94%), waxes (0.6%), pectin (0.9%), proteins (1.3%), minerals (1.2%), organic acids (0.8%), sugars (0.3%), and miscellaneous (0.9%) substances [6–8]. Cellulose is a homopolymer containing repeated units of β-(1→4)-D-anhydroglycopyranose. Polymerization and crystallinity of these units impart strength to the fiber [6–8]. Cotton fibers are chemically composed of five layers: (i) cuticle, (ii) primary wall, (iii) winding (transition) layer, (iv) secondary wall, and (v) lumen. The outermost layer of the cotton fiber is the cuticle, and it is composed of waxes (cutin and suberin), pectins, proteins, sugars, ash, and other substances. The primary wall and winding layer comprise amorphous cellulose, hemicelluloses, esterified and non-esterified pectins, proteins, and metal ions. The secondary cell wall (SCW) is made of crystallinity cellulose. Finally, the innermost layer is the lumen, and it comprises proteins, malic, citric, and other organic acids [6–8]. Mature cotton fibers are cellulose-rich with a thicker secondary wall and smaller lumen, while immature fibers are low in cellulose content with a thin wall and a large lumen. Based on the length, the mature cotton fibers can be classified as lint (long) and fuzz (short) fiber [9]. The unicellular cotton fibers emerge from the ovule surface immediately after flowering (days post-anthesis, DPA). The temporal progression of fiber development occurs in approximately 50 days, and it can be divided into four overlapping phases based on morphological features: (i) initiation (0–4 DPA), (ii) elongation (3–21 DPA), (iii) SCW formation and thickening (15–40 DPA), and (iv) maturation (38–52 DPA) [10]. However, genotype and environmental interactions affect the duration of phases and the rate of progression. Moreover, an array of transcripts expressed at these phases vary substantially. Thus, enabling us to study these differences in gene expression (GE).

The term transcriptome is referred to as a repertoire of RNA types found in a cell or a tissue or an organism at a given period or a collection point or a location or a specific treatment or a developmental phase or an environmental condition or a physiological state [11, 12]. The transcriptome is dynamic, and it tends to respond to subtle changes in environment or experimental condition or treatment [11, 12]. The earlier GE or transcript analysis methods such as *in situ* hybridization (ISH), northern blot, Quantitative reverse transcription PCR (RT-qPCR), microarrays, serial gene expression analysis (SAGE), and expressed sequence tags (EST) analysis primarily focused on a single gene or a group of genes, while RNA-Seq or whole transcriptome sequencing (WTS) technique aimed at studying a wide range of transcripts [11, 13]. WTS is a contemporary and more promising strategy that has been widely used in studying the similarity or variability of GE, depending on the objective of the study. Although microarrays is an inexpensive and user-friendly method with a decent throughput, the fundamental limitation is the requirement of prior knowledge for immobilizing a probe set on the chip. RNA-Seq has largely replaced microarrays as the

predominant method for quantifying GE due to its significant advantages in providing a more comprehensive overview of the transcriptome [14, 15]. RNA-Seq can be used to study differential expressed genes (DEGs), a dynamic range of transcripts including, novel transcripts, alternative splice variants (ASVs), novel isoforms, and gene fusions, non-coding RNA (ncRNA), and single nucleotide variants (SNVs) from a complex landscape of transcripts collected from a sample [11, 15].

RNA-Seq is also the method of choice because of its throughput, ease, and portability in using hypothesis-free experimental designs. RNA-Seq has been widely used in profiling the transcriptomes of diverse crop species, including cotton [3] and its fiber [16]. In cotton, RNA-Seq based differential gene expression (DGE) for fiber-related genes among diverse species [17–19], genotypes [20, 21], and in various biotic [22, 23] and abiotic [24, 25] stresses have been reported. The inherent variation in the biochemical composition of the cotton fiber, and the temporal and spatial GE in fiber development phases, offers broad scope for analyzing the fiber transcriptome using RNA-Seq. Combining biochemical, molecular, and microscopic approaches to analyze cotton fiber showed that the significantly enriched genes were associated with the cytoskeleton, cell wall, cellulose biosynthesis, carbohydrate, and energy metabolism during fiber development [26, 27]. Since the fiber transcriptome has been reported [28], various transcriptome-based studies reported in cotton have shown the differential expression (DE) of several genes among different phases of fiber development [26]. Further, RNA-Seq enabled researchers to investigate a comprehensive set of genes involved in fiber yield and quality [20, 29, 30]. This chapter reviews the basic RNA-Seq analysis pipeline and tools currently being used in Section 2 below. Also, a few RNA-Seq studies primarily aimed at fiber quality attributes such as fiber length, strength, development, initiation, elongation, and color are discussed in Section 3. However, fiber single-cell transcriptome analysis is beyond the scope of this chapter.

2. RNA-seq analysis pipeline

RNA-Seq is a complex subject with several aspects to be considered. A few key factors include experimental design, sequencing, and data analysis. This section provides an overview of the experimental design and primarily focuses on the basic pipeline of RNA-Seq analysis.

2.1 Experimental design

The time, effort, and cost of RNA-Seq analysis primarily rely on the experimental design [31]. A single sample on a short-read (Illumina) and long-read (PacBio SMRT/Oxford Nanopore) sequencing including RNA-Seq are offered as low as $100 [32]. However, the sequencing costs vary with the experimental design (control vs. treatment; normal vs. condition; early vs. late collection time point; and the number of samples vs. the number of replicates), throughput (30 vs.100 million reads), NGS platform (Illumina vs. PacBio), sequencing time (today vs. a year later), and setup (academic vs. commercial). In addition, there are several factors one needs to consider before RNA-Sequencing that include the size of the genome (e.g., ~2.5 Gb), the number of genes (e.g., ~50,000), gene density (e.g., 20 genes/1 Mb), the targeted coverage (1X vs. 10X), sequencing chemistry (short-read vs. long-read), the read type (single-end vs. paired-end), the library type (stranded vs. non-stranded), the number of reads (e.g., >30 million) per sample, and the number of samples per lane (1 vs. 12) [11].

It is ideal for including six biological/technical replicates per sample in any experiment [33]. However, in most cases, a minimum of three replicates are used to draw statistically significant conclusions [11].

Different variations in RNA-Seq methodology are available, and they are primarily based on coding (e.g., mRNA-Seq) and non-coding regions (e.g., small RNA-Seq). Besides total RNA-Seq, targeted RNA-Seq, digital gene expression (DGE)-Seq, and single-cell RNA-Seq (scRNA-Seq) are widely used [11], but only mRNA-Seq is highlighted in this chapter as it represents the coding portion of the genome. A typical short-read-based RNA-Seq experiment generally starts by selecting a population of cells or tissue, then extracting total RNA, enrichment of mRNA, fragmenting mRNA, converting fragments into cDNAs, adding adapters to cDNAs, creating a library, sequencing (Illumina), and data analysis [11]. The detailed RNA-Seq methodology has been reviewed earlier [15, 34–36], and it is beyond the scope of this chapter.

2.2 Read processing and quality check

After collecting the raw read data from an NGS platform, we must consider several metrics. The most important statistic is Phred quality (numeric) scores calculated at each position or base within the read. Phred score indicates the probability of base calling at each position is likely accurate. In addition, the Phred score is the mean value calculated at each position for all reads in that sample. For instance, in a read library of 100 bases, the Phred score is the mean value calculated across each sample within the reads between positions 0 and 100. Therefore, a higher Phred score value is expected at each position in a read and across all reads in a sample as an indicator of quality data. The second quality metric to consider is the adapter content, the percent of sequences containing the adapter. It is often detected *in silico* by checking for the adapter contamination at each read position and across all reads in a sample. Sometimes, adapter contamination is seen towards the end of the read. Especially when the sequencing fragment (cDNA) is too short, the DNA polymerase continues to sequence until the end of the fragment and into the opposite adapter. Another quality metric to consider is GC content, in which we expect to see a single peak with a smooth progression without multiple peaks. The presence of multiple peaks in GC content represents the possible contamination during the library preparation.

RNA-Seq is primarily performed in core laboratories or offered as a charge per service. However, it is obvious to find traces of ribosomal RNA from humans or other species, if contaminated during the sample handling and processing. Before analysis, we can eliminate such contamination issues by comparing query sequences against the reference databases such as DeconSeq [37] and PrinSeq [38]. Some efficient algorithms like FASTQC [39] will take a random subset of the reads from each sample and map them to various possible contaminant datasets to determine the quality. A clean RNA-Seq data should have the vast majority of reads (80–90%) from the organism being sequenced, and the remaining reads (10–20%) can be from related species owing to their homology. Also, the genomic origin of the reads is determined by their exonic proportion. In mRNA-Sequencing, ~80% of the reads must have exonic regions, and the remaining (~20%) can be intronic or intergenic regions. Before performing the alignment, raw reads collected are preprocessed for filtering low-quality reads and removing non-biological sequences such as adapter sequences, barcodes, and indexes using trimming tools such as Cutadapt [40], TrimGalore [41], Scythe [42], Trimmomatic [43], HTStream [44], and BBduk [45]. The popular tools

for quality checking of raw reads before and after trimming include iSeqQC [46], MultiQC [47], NGS QC [48], FASTQC [39], and FASTX-Toolkit [49].

2.3 Read alignment

The alignment approaches available for both short and long reads for alignment and quantification are: (i) traditional reference-based splice-aware alignment, and (ii) pseudo alignment. The reads are mapped directly to the reference genome in reference-based alignment without ignoring the splice junctions across the exons. For instance, pre-mRNA contains exons and introns, but the processed mRNA joins the spliced-out exons. So, it is inevitable to find some short reads that intersect these exon-exon junctions that do not map directly back to the reference genome. The selected aligner must be aware of such spliced products and junctions. It is important to realize that if our goal is to discover novel isoforms, we must consider exon-exon junctions. These methods typically run-on clusters because they require a large amount of memory or central processing unit (CPU) time. There are several read-alignment tools available for both short and long reads. The popular reference-based read alignment tools are PuffAligner [50], STAR [51], HISAT2 [52], HTSeq [53], TopHat2 [54], and Bowtie2 [55].

The other relatively advanced approach in RNA-Seq is pseudo alignment. In which, the mapper joins reads together based on their compatibility with the transcripts and not based on precise alignment, as its primary goal is to quantify the transcript expression. The main idea of pseudo alignment is to ignore the location of mapped reads and to consider only the aligned reads. Computationally, pseudo alignment is more efficient, faster, less memory intensive, and a better prediction tool than reference-based alignment. However, a transcriptome or a transcript repertoire related to an organism of our interest is used as a reference in pseudo alignment. A splice-aware aligner is not required here, as reads are mapped to a reference transcriptome but not the genome. The popular pseudo alignment tools such as Salmon [56], Kallisto [57], and Sailfish [58] are currently being used. A few tools available for checking the alignment quality are QC3 [59], QoRTs [60], Qualimap [61], RseQC [62], and RNA-SeQC [63]. Further, %GC, base quality, and mapping efficiency of aligned reads are assessed along with distribution of read count, insert size, and depth to detect sample bias resulting from library preparation.

2.4 Transcript abundance estimation and quantification

In a standard RNA-Seq pipeline, read quantification is performed after filtering low-quality reads, aligning them to an annotated genome or the transcriptome to identify their genomic origin. The standard alignment and counting methods mainly relied on base-to-base alignment or by mapping to an annotated or unannotated genome. The major limitation of standard tools is that genes often have multi-mapped reads. As a result, the algorithms such as STAR [51], HTSeq [53], and Tophat2 [54] underestimate gene expression (GE), thus resulting in false negatives. In contrast, the Cufflinks [64] overestimates GE and results in many false positives (FPs). Most transcriptome-based tools avoid base-to-base alignment of the reads, thus reducing the computational time and costs. Also, transcriptome-based tools provide quantification estimates much faster and more accurately at the transcript level. Less memory intensive tools such as Salmon [56], Kallisto [57], and Sailfish [58] are used for transcript quantification and abundance estimation with minor differences. For instance,

Salmon first quantifies pseudo-counts, then quasi-mapping, and finally estimates transcript abundance. The pseudo-counts obtained can be used to find the differential gene or isoform-level expression. A few tools available for transcript abundance estimation and quantification are StringTie [65], STAR [51], tximport [66], DESeq2 [67], and Cufflinks [64].

The most straightforward approach for quantifying GE by RNA-Seq is to count the reads that align with each gene. The gene-level quantification approaches such as HTSeq commonly utilize annotated information, where gene models correspond to the structure of transcripts. Raw read counts are usually affected by transcript length and the total number of reads. For instance, the longer transcripts have higher read counts at the same expression level. Thus, the raw read counts are normalized to compare expression levels between samples. The reads per kilobase (kb) of the exon per million mapped reads (RPKM) are used to normalize the single-end read data for sequencing depth and gene length differences. While fragments per kb of transcript per million reads mapped (FPKM) is used to normalize the paired-end read data for differences in sequencing depth and gene length. In contrast to RPKM and FPKM, transcripts per million reads (TPM) is used to normalize the differences in gene length first and library size later [68]. Therefore, correcting gene length within the same gene across samples is avoided. However, it is required to precisely rank the GE levels within the sample to accurately report longer genes with relatively more reads at the same expression level.

2.5 Finding differentially expressed genes

Normalized read count data is taken in differential expression (DE) analysis, and statistical analysis is performed to identify quantitative changes in GE levels between experimental groups. For instance, statistical testing determines whether the observed differences in read counts are significant compared to natural random variation. Often the selection of the analysis tool depends on the experimental design and availability. We can use DE analysis tools for pair-wise or multiple comparisons between or among the samples. When the same GE contribution is observed in several samples, their average value is taken as the eventual GE level. Testing for DE across thousands of genes requires correction for multiple comparisons. The two common ways in statistics for correction are Bonferroni correction and false discovery rate (FDR). FDR is the most widely adopted approach in RNA-Seq as it operates on the whole population and aims to keep the false positive rate below the acceptable threshold ($<5\%$). The upregulated or downregulated DEGs are typically represented using volcano plots or MA plots. Top-ranked significant (p-value < 0.05; Log2 Fold Change, Log2FC > 1.00; and FDR < 0.05) DEGs are usually shown as heatmaps. The average linkage method is used to compute the hierarchical clustering, whereas the euclidean algorithm computes the closeness or distance between rows and columns. Dimensionality reduction on expression data is obtained to eliminate outliers and batch effects. Principal component analysis (PCA) is most commonly used as it reduces the complexity of expression data by showing relationships among samples or replicates as clusters in two-dimensional space.

Even though different tools such as Glimma [69], Ballgown [70], EBSeq [71], limma [72], voom [73], DESeq2 [67], edgeR [74], and baySeq [75] are available for DE analysis, DESeq2 and edgeR were most widely used. DESeq2 normalizes the gene read counts by library size and composition to avoid sampling bias and batch effects. In addition, it models gene read counts with the negative binomial distribution

and uses hierarchical modeling to stabilize the gene variance. Further, it uses the Benjamini-Hochberg (BH) statistic to calculate the false discovery rate. Although DESeq2 and edgeR rely on the negative binomial distribution assumption, they differ in the test statistic. For example, DESeq2 depends on the Wald test, and edgeR relies on the quasi-likelihood F-test. Also, the distribution assumption of Bayesian approaches, baySeq, and EBSeq is the negative binomial model. While limma, voom, limma+voom tools use a normal linear model. The test statistic used for these tools is empirical Bayes moderated t-statistic. In comparison, Ballgown uses a nested linear model and parametric F-test. In addition, quality control on expression data is determined using tools such as iSeqQC [46], DEGreport [76], NOISeq [77], and EDASeq [78] for detecting the sample heterogeneity, outliers, and cross-sample contamination. These tools mostly rely on statistical approaches such as correlation analysis and dimensionality reduction.

2.6 Functional annotation

After finding DEGs or gene clusters, assigning a function is commonly employed. These genes or gene sets are screened to see whether they are enriched in a particular pathway, localized to a specific cell location, or have a specific function. Based on these features, the DEGs are classified into Biological Process (BP), Cellular Component (CC), and Molecular Function (MF) [79]. In gene ontology (GO) analysis, initially, an individual or a set of DEGs are assigned with functions or GO terms, and then the enrichment analysis is performed on gene sets. Finally, filters lowly expressed genes to reduce the number of hypotheses to be tested. For instance, given a set of DEGs that are upregulated among samples under a particular condition, an enrichment analysis will find GO terms that are overrepresented or underrepresented using functional annotations for that gene set. For example, we can use the goana and camera functions in the limma Bioconductor package to find the most enriched GO terms on the gene sets and enrichment analysis. A few routinely used functional annotation and enrichment tools include: Panther [80], FoldGO [81], DAVID [82], ReviGO [83], and AmiGO [84].

2.7 Enrichment and pathway analysis

The three methods used to assess the gene sets or pathways are enrichment-based, pathway topology-based, and combined. The enrichment-based approaches, overrepresentation (ORA) and functional class scoring (FCS) analysis/gene set enrichment analysis (GSEA) are widely used. At the same time, pathway topology tools help us understand GE as a set in a coordinated network [85]. While the combined approach utilizes the features of both enrichment-based and pathway topology approaches.

A typical ORA pipeline includes DE analysis to find the number of DEGs and their reference genes associated with each pathway. ORA is simple and robust in identifying a few significant genes or gene-sets, i.e., it relies on a portion of the data. As the background assumption is based on low-input, independent genes or gene sets in a pathway are treated as separate entities, and the interaction among the genes or gene clusters are ignored, it may result in many false positives. The GSEA is more accurate than ORA as the entire list of genes is considered. A typical GSEA first enriches significant genes and gene sets based on their P-values, rank order, and weighted scoring, and then identifies independent pathways. However, GSEA also ignores

the interaction between the gene sets or pathways. A few widely used pathway tools are Cytoscape [86], BioCyc [87], and EcoCyc [88]. Pathway topology is developed to mimic the biological perspective as, in reality, genes work in a coordinated or regulated environment in the form of networks or pathways. The idea is to perturb a pathway and thus leverage the topology to study the effect on a single gene or gene set. Pathway topology analysis predicts the gene function, gene position, fold change, and interactions among genes. It relies on more data and is computationally intensive, and it is currently limited to signaling pathways alone. A few integrated tools such as iDEP [89], ingenuity pathway analysis [90], and ipathway guide [91] are gaining more attention recently with the availability of cloud-based and data science tools in RNA-Seq analysis. We can visualize the up-regulated and down-regulated genes on a Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway using the Pathview package [92] and KEGG mapper [93]. Protein-protein interactions (PPIs) and their enrichment among up-regulated or down-regulated genes can be retrieved using the STRING database [94].

Most transcriptome analysis studies include a combination of tools discussed above based on the objective or biological question under investigation. In general, the upstream analysis includes read processing and alignment, while the downstream analysis includes quantification, annotation, enrichment, and pathway assignment. A few recent RNA-Seq studies in identifying DEGs associated with fiber quality and its characteristics are discussed in the next section.

3. Transcriptome analysis in cotton for finding fiber related genes and pathways

3.1 Transcriptome analysis for fiber quality

Several attributes that determine fiber quality include fiber length (mm), strength (cN/tex), development: initiation and elongation (%), uniformity (%), and micronaire (μg/inch). Fiber strength and length are important in deciding the spinning and yarn quality [95]. In addition, the micronaire value reflects the fiber fineness and maturity, which influences its processing and dyeing [96]. We reviewed a few recent RNA-Seq studies in identifying DEGs associated with fiber quality attributes below (**Table 1**).

In an integrated study, a high-density mapping has been used to identify 36 stable and 18 novel quantitative trait locus (QTLs) associated with fiber quality in the CCR170 RIL hybrid generated from sGK156 and 901-001 varieties of *G. hirsutum*. Their RNA-Seq analysis included these two parental types and two RILs (MBZ70-053 and MBZ70-236) to identify 24,941 unique and 473 DEGs associated with pectin and phenylpropanoid biosynthesis and plant hormone signaling. Their bioinformatics analysis included Trimmomatic for trimming reads, HISAT2 for read alignment, StringTie for quantification of genes, DESeq2 for differential gene expression, BLASTX program and GO tools for gene set enrichment, and KAAS for pathway analysis [99]. In a different transcriptome-based study, a high yielding cotton cultivar Jimian 5 and a high fiber quality *G. thurberi* introgression line, DH962 have been used to identify 780 DEGs linked with fiber quality at 10 DPA [97]. Also, their study integrated DEGs from transcriptome data and QTLs from phenotypic data to identify 31 genes associated with nine QTLs. Further, their study included Bowtie and TopHat tools for aligning clean reads to the reference genome, Cufflinks for transcript

S. No	Significant DEGs (~5) identified	Associated function/pathway	Citation
1	ABC transporter G family member 10 (Gh_A01G0397); Protein DETOXIFICATION 19 (Gh_A01G0453); RING-H2 finger protein (Gh_A09G2087); Probable ubiquitin-conjugating enzyme 24 (Gh_A10G0253); and Aquaporin TIP1-1 (Gh_D04G1049)	Secondary metabolites biosynthesis	[97]
2	Heat shock protein 90-6 (Gh_A07G1723); Nuclear pore complex protein 133 (Gh_A07G1727); Transcriptional regulator STERILE APETALA (Gh_A07G1730); SNF1-related protein kinase regulatory subunit beta-2 (Gh_A07G1735); and Fasciclin-like arabinogalactan protein 2 (Gh_A07G1742)	Primary and secondary cell wall (SCW) synthesis	[98]
3	Aldehydedehydrogenases (Gh_A06G1256); xyloglucan endotransglycosylase/hydrolase 8 (Gh_A06G1277); glycosylphosphatidylinositol (GPI)-anchored protein (Gh_A06G1301); and Aminomethyltransferase (Gh_A06G1313)	Cell wall and cellulose biosynthesis	[30]

Table 1.
Transcriptome analysis for fiber quality.

prediction, DESeq for DEGs, OmicShare tools for functional annotation, and Cotton Functional Genomics Database for finding pathways associated with the significantly enriched DEGs [97].

In a different study, RNA-Seq analysis has been performed on data obtained from different TM-1 tissues from NCBI to identify genes controlling fiber quality. Results showed that 91 genes had been expressed at different fiber developmental stages (5, 10, 20, and 25 DPA). Functional annotation of these genes using GO analysis revealed that most of the genes have been involved in binding and enzymatic activity [98]. Further, their study revealed 11 candidate genes for fiber quality linked with the genomic locations of chromosomes, A07 and A13, by combining the genome-wide association data (GWAS) with publicly available RNA-Seq datasets [98]. A transcriptome study has been conducted between two recombinant inbred lines, L1 and L2, with a varied fiber quality, to underline the differences in gene expression (GE) during fiber development stages and identify the genes responsible for the fiber quality in upland cotton (*G. hirsutum*) [100]. Their RNA-Seq analysis utilized Trimmomatic, Bowtie, TopHat2, Cufflinks, and DESeq2 tools to find over 1000 DEGs between L1 and L2 at 15, 20, 25, and 30 DPA. Among these, 363 DEGs colocalized within the fiber strength QTL. Further, DEGs have been annotated, and pathways were assigned by STEM, BLASTX, KOBAS, WGCNA, and Cytoscape tools. In addition, their co-expression network analysis revealed five modules closely associated with fiber-development stages. The significantly enriched genes belonged to leucine-rich repeat (LRR) receptor-like protein kinase, Rho GTPase-activating protein, bHLH transcription factor (TF), TPX2 protein, and actin-1 protein classes [100]. In a separate study, by integrating fine-mapping data with RNA-Seq, four DEGs linked with a QTL responsible for fiber quality traits have been identified in *G. hirsutum* RIL118 and Yumian 1 lines [30]. Their analysis included Bowtie and TopHat for mapping reads to the reference genome, HTSeq for transcript abundance estimation, DEGSeq for finding DEGs, KEGG, and KOBAS for gene enrichment analysis and finding KEGG pathways [30].

3.2 Transcriptome analysis for fiber length

The cotton fiber length and other attributes such as strength, elongation, and evenness determine the spinning or yarn quality. However, the cotton fiber length varies from one variety to the other and generally ranges between 0.9 and 1.6 inches. Longer fibers are preferred over shorter ones in the textile industry due to their uniformity, fineness, and strength. However, the fiber length is determined by the underlying molecular mechanisms, including gene expression and regulation. Recent RNA-Seq studies related to fiber length attribute are presented below (**Table 2**).

Integrated transcriptome and genotyping study aimed at deciphering molecular mechanisms associated with cotton fiber length identified 2662 significant DEGs that belonged to energy metabolism during fiber initiation and auxin signaling pathway during fiber elongation by utilizing ovule and fiber samples collected at −3, 0, 5, and 10 DPA from two contrasting RILs (MBZ70-053 and MBZ70-236) of *G. hirsutum* hybrid, CCRI70 [104]. Their pipeline included Trimmomatic for trimming adapters from raw reads, HISAT2 for aligning clean reads to the reference genome, StringTie2 for quantifying genes, DESeq2 for finding DEGs, BLASTX and GO tools for functional annotation, and KAAS, WGCNA, and Cytoscape for gene set enrichment and pathway analysis [104]. A study conducted in Upland cotton investigated the role of class II KNOX protein (GhKNL1) in fiber development, which primarily acts as a transcription repressor in regulating SCW formation. The comparative transcriptome profiling of two transgenic cotton varieties (silenced and dominant repression for GhKNL1 gene) and a genetic standard (TM-1). The GhKNL1 silenced variety showed improved fiber length and thickened SCW, whereas the dominant repression for GhKNL1s showed shortened fiber length and thinner SCW. Furthermore, it has been reported that GhKNL1 could bind to promoters to facilitate cellulose synthesis and SCW development, thus affecting the cotton fiber length [101].

In a study conducted to evaluate Germin-like proteins (GLPs) in regulating cotton fiber development, the RNA-Seq analysis between the wild type and RNAi line for GbGLP1 (YZ-1) gene with an overexpression promoter revealed that higher expression levels of GhGLP1 lead to shortened fibers. Their RNA-Seq analysis identified

S. No	Significant DEGs (~5) identified	Associated function/pathway	Citation
1	Homeobox protein knotted-1-like (Gh_D12G244300); Cellulose synthase A catalytic subunit 4 (Gh_A07G229300 and Gh_A08G054700); Transcription factor MYB46 (Gh_A13G243300); and Cellulose synthase A catalytic subunit 8 (Gh_D05G155000)	Modulating cellulose synthesis	[101]
2	4-coumarate—CoA ligase-like 7 (Gh_D03G1318); DNA ligase 1 (Gh_D03G1330); Protein UPSTREAM OF FLC (Gh_D03G1331); E3 ubiquitin-protein ligase 1 (Gh_D03G1332); and Protein CHUP1 (Gh_D03G1337)	Sucrose synthesis	[102]
3	1-aminocyclopropane-1-carboxylate oxidase-like (Gh_A08G1512); 3-ketoacyl-CoA synthase 19 (Gh_A06G1558); gibberellin-regulated family protein 2 (Gh_A06G0045); Ethylene responsive element binding factor 3 (Gh_A04G0106); and zinc finger transcription factor constans 21 (Gh_D06G0023)	Secondary metabolites biosynthesis	[103]

Table 2.
Transcriptome analysis for fiber length.

566 DEGs in the RNAi lines, while most of them belonged to genes and TFs involved in SCW biosynthesis. Also, comparative transcriptome screening of thirty long and short fiber varieties of cotton revealed that the GhGLP1 promotes fiber elongation by delaying the SCW thickening. Moreover, the YZ-1 knockdown line for the GhGLP1 gene resulted in improved fiber length and retarded SCW thickening, thus suggesting its negative role in fiber elongation [105]. A separate study combined GWAS and linkage mapping identified a sucrose synthesis-like gene linked with a significant QTL on chromosome D03 that affects fiber length in Upland cotton [102]. Their RNA-Seq and qRT-PCR results consistently showed elevated expression levels of eleven candidate genes related to fiber length in *G. hirsutum*, including the sucrose synthesis gene at -5 DPA to 20 DPA with increments of five. In addition, their study included Bowtie2 for mapping clean reads to the reference genome and cufflinks for obtaining GE levels for analyzing the publicly available data [102].

Comparative transcriptome analysis between *G. hirsutum* CSB25 line developed for fiber elongation and genetic standard, TM-1 has been performed to evaluate fiber traits. Their data analysis included Tophat2 for aligning clean reads to the reference genome, HTSeq to estimate GE, EdgeR for finding DEGs, GAGE and REVIGO for GO enrichment, and KEGG tools for finding gene sets and pathways. They identified 1872 DEGs in their study, and most of them belonged to cytoskeleton and cell wall metabolism. In addition, their investigation revealed that most of the genes were enriched in plant hormone signaling, phenylpropanoid, amino acid, sucrose, and starch biosynthesis [103].

3.3 Transcriptome analysis for fiber strength

There is a huge demand for stronger cotton fiber in the global textile industry. Individual fiber strength determines the yarn strength. However, fiber strength is often measured as bundle fiber strength (BFS), i.e., grams per tex (g/tex), where 'g' is the breaking force, and tex (g/km) is the fineness. BFS is usually not an accurate measure for yarn strength because of the variability in fiber properties and interaction between the fibers. The fiber strength of Upland cotton has been improved considerably through molecular breeding approaches. However, efforts on gene expression and regulation of fiber strength during its development are limited, and a few such studies are discussed here (**Table 3**).

Using comparative fiber transcriptome analysis between *G. mustelinum* introgression line (IL9) and its recurrent parent (PD94042), over 250 significantly enriched DEGs associated with the fiber strength QTL have been identified at 17 and 21 DPA. Among which, 52 DEGs have been identified as candidate genes and two DEGs associated fiber strength QTL regions. Their GO enrichment and KEGG analysis showed that most of these DEGs belonged to the biosynthesis of secondary metabolites and metabolic pathways [106]. An RNA-Seq analysis study aimed to understand the molecular mechanism underlying the fiber development and quality included a CSL line (SL7) and *G. hirsutum* line (L22) to identify 70 significantly enriched DEGs associated with plant hormone transduction pathways. Their findings indicated that the introgressed chromosomal segment of SL7 plays a crucial role in expressing a transcription factor that contributes to the fiber strength [109]. A study that screened publicly available transcriptomic data for bHLH transcription factors found that GhbHLH18 is coexpressed with most lignin biosynthesis genes [107]. Furthermore, they suggested that GhbHLH18 is preferentially expressed during the early fiber elongation and is negatively regulates fiber strength and length by binding to the E-box of its promoter and enhancing peroxidase-mediated (GhPER8) lignin biosynthesis [107].

S. No	Significant DEGs (~5) identified	Associated function/pathway	Citation
1	Pentatricopeptide repeat-containing protein (CotAD_13630 and CotAD_22605); methyl-sterol monooxygenase 1-1-like protein (CotAD_19764); homeobox-leucine zipper protein HAT22-like (CotAD_36040); and Phospholipase (CotAD_36045)	Secondary metabolites biosynthesis	[106]
2	Peroxidase (GhPER8); serine/threonine-protein kinase-like protein (GhCCR2); Cinnamyl alcohol dehydrogenase 4 (GhCAD4); Hydroxcinnamoyl-CoA quinate (ChHCT6); and Catechol-O-MethylTransferase family (GhCOMT4)	Lignin biosynthesis	[107]
3	Expansin A10 (Gh_D13G0786); Pectin methylesterase 3 (Gh_A05G1180); Sucrose synthase 5 (Gh_A07G0665); COBRA-like protein 2 precursor (Gh_D12G0298); and Chitinase/ Chitinase-like (Gh_D06G0479)	Cell wall modification	[108]

Table 3.
Transcriptome analysis for fiber strength genes.

Transcriptome analysis in chromosome segment substitution lines (CSSLs) revealed 71 significant DEGs associated with fiber strength among four lines, CCRI45, MBI7561, MBI7747, and MBI7285, collected at 15, 20, 25, and 28 DPA [110]. They suggested the possible roles of these genes in cell wall biogenesis, SCW deposition, and cotton fiber strength. Their analysis further identified 16 DEGs consistently found in the introgressed segments from the *G. barbadense* chromosomes across all possible comparisons. Their data analysis included NGS QC Toolkit for trimming and filtering raw reads, TopHat for aligning clean reads to the reference genome, HTSeq for quantifying expression levels, GFOLD for DEGs, and BLAST2GO for functional annotation and protein class assignment [110]. Comparative transcriptome analysis of two contrasting near-isogenic lines (NILs) of *G. hirsutum*, MD90ne, and MD52ne for fiber strength at 15 and 20 DPA revealed over 1000 significant DEGs [108]. In addition, the fiber elongation and cell wall integrity genes have been enriched in ethylene and receptor-like kinases (RLKs) signaling pathways. In data processing, they utilized Sickle, GSNAP, Bedtools, EdgeR, and AgriGO tools for trimming, mapping, annotation, differential gene expression, and enrichment analysis, respectively [108]. Further, they compared the RNA-Seq data with previously published microarray data [111].

3.4 Transcriptome analysis for fiber development

Cotton fibers are natural, unicellular outgrowths that emerge from the epidermis of the ovules. The differentiation and developmental phases (initiation, elongation, SCW synthesis, and maturation) of the cotton fiber determine the other attributes such as fiber length and strength. The variation in GE at different developmental stages of cotton fiber can be assessed using RNA-Seq. A few RNA-Seq studies related to fiber development are discussed below (**Table 4**).

Comparative transcriptome analyses of three fiber developmental stages with non-fiber tissues (leaf, root, stigma, and anther) identified 1205, 1135, and 937 significantly upregulated, and 124, 179, and 213 downregulated DEGs at 7, 14, and 26 DPA, respectively in *G. hirsutum* during fiber development. Moreover, the identified DEGs have been enriched in functional and metabolic pathways, including signal transduction, catalytic activity, and carbohydrate metabolism [26]. Their pipeline included cutadapt for collecting quality reads, TopHat2 for aligning clean reads to the

S. No	Significant DEGs (~5) identified	Associated function/pathway	Citation
1	Tubulin beta-2 chain 6 (CotAD_51888); Tubulin alpha-3 chain 1 (CotAD_75071); Actin-7 (CotAD_57082); Kinesin-related protein 11 (CotAD_24245); and Clathrin light chain 1 (CotAD_46336)	Fatty acid metabolism	[26]
2	Zinc finger protein 5 (Gh_A03G1255); Histone deacetylase 1 (Gh_D05G0849); UBX domain-containing protein 1 (Gh_D02G2408); Zinc finger protein 10 (Gh_D01G1033); and Protein indeterminate-domain 5 (Gh_A01G2114)	Transcriptional and apoptosis regulation	[112]
3	WRKY transcription factors: WRKY11 (Gh_A07G0017); WRKY33 (Gh_D03G1371); WRKY40 (Gh_A06G1923); WRKY41 (Gh_D08G1232); and Abscisic acid 8-hydroxylase 1 (Gh_A08G1344).	Metabolic pathways	[113]

Table 4.
Transcriptome analysis for fiber development.

reference genome, StringTie for assembling mapped reads into transcripts, HTSeq for quantification of GE, DeSeq for finding DEGs, and GOseq R and KOBAS for finding protein classes and pathways [26]. A study aimed to understand the genes and complex networks associated with cotton fiber development and its domestication utilized *G. hirsutum* and screened transcriptomes collected at 5, 10, 15, and 20 DPA to reveal convergence and divergence in duplicated and homoeologous coexpression networks. Their analysis included GSNAP for mapping, PolyCat for homoeolog-specific expression, HTseq for read count data, DEseq2 for finding DEGs, and weighted gene coexpression network analysis (WGCNA) for coexpression data to corroborate the idea of widespread gene usage in cotton fibers, subgenome-specific expression bias, and similarities and differences in coexpression modules within the subgenomes of a polyploid [114].

In a study aimed at screening publicly available datasets for myeloblastosis (MYB) like TFs and finding their role in fiber development, a research group has identified 36 R2R3-MYBs highly expressed at 20 DPA in Upland cotton suggested them as potential SCW synthesis regulators [115]. Comparative transcriptome analysis of *G. arboreum*, *G. hirsutum*, and transgenic line revealed that the GhTCP4 TF plays an essential role in activating SCW genes by interacting with cis-elements in the promoter region. In contrast, GhHOX3 regulates TCP gene expression, thus promoting fiber cell elongation [116]. A comprehensive genome-wide analysis of *G. arboreum*, *G. raimondii*, and *G. hirsutum* from publicly available data revealed 196, 195, and 386 C2H2-like zinc finger genes, respectively. Also, the phylogenetic analysis of C2H2-like zinc finger proteins identified seven subgroups with similar exon-intron and protein motif compositions. Further, the differential expression (DE) pattern of 16 C2H2-like zinc finger genes identified in RNA-Seq data has been validated with RT-qPCR analysis in Ligon-lintless-1 (Li1) mutant and TM-1 at 0, 5, 8, and 10 DPA and suggested the role of these transcription factors in biochemical and physiological functions during cotton fiber development [112]. In another study, comparative transcriptome analysis of phytochrome A1 gene (PHYA1) RNAi line and its parent Coker 312 using RNA-Seq to study the GE profiles of 10 DPA fibers identified 142 DEGs that play an essential role in fiber development. Their pipeline included Trimmomatic for trimming low-quality reads, ArrayStar for aligning clean reads to the reference genome, DeSeq2 for finding DEGs, Blast2GO and InterProScan for functional annotation, and KEGG database for functional protein classes and pathways [113].

3.5 Transcriptome analysis for fiber initiation and elongation

Cotton fibers are single and elongated cells derived from epidermis of seed as external outgrowths. Therefore, cotton fibers are ideal for studying cell developmental stages such as differentiation and elongation. Further, the initiation step is critical in the fiber development process because it is the stage where the cell fate is determined or committed to developing into a fiber. Therefore, fiber initiation and elongation are ideal stages for undertaking RNA-Seq analysis to understand early fiber development, and a few such studies are discussed here (**Table 5**).

A recent study combined the Laser-capture microdissection (LCM) technology with RNA-Seq to understand the cotton cell types during the fiber developmental shifts. LCM can differentiate the epidermal cells from the fiber, while RNA-Seq can identify the subtle differences between these cell types [29]. Their results suggested that the fiber cell initiation in cotton can be triggered by phytohormones and MYB-like transcription factors, cell cycle arrest, ribosome biosynthesis, and homoeolog expression bias of cell cycle and ribosome biosynthetic genes [29]. A recent omics-based study conducted in the ovules collected immediately after anthesis in upland cotton showed DE of several MYB-like TFs and early fiber development genes associated with biosynthesis and signaling of phytohormones, indole-3-acetic acid, cytokinins, gibberellic acid, jasmonic acid, and brassinosteroids [27]. Another RNA-Seq study has been conducted to understand effect of temperature on fuzz fiber initiation in a thermo-sensitive variety of *G. barbadense*, L7009 subjected temperature stress at 4 DPA to identify 43,826 DEGs. Of these, 189, 9667, 240, and 901 DEGs belonged to plant hormone signal transduction, fiber development, fuzz fiber initiation, and transcription factors, respectively. Also, they reported that high temperatures could induce fiber development, fiber quality, and fuzz initiation. Further, the significantly enriched DEGs belonged to stress response, asparagine, and cell wall biosynthesis. However, the fuzz initiation can be inhibited by low-temperature treatment in L7009. Furthermore, they reported the 4 DPA stage as the most susceptible stage to temperature stress during the fuzz initiation [117].

A genome-wide transcriptome profiling of fiber-bearing ovules of *G. arboreum* at an increment of 0.5 from -0.5 DPA till 3.0 DPA has been investigated to understand the molecular basis for fiber initiation. A total of 12,049 DEGs and 1049 DE

S. No	Significant DEGs (~5) identified	Associated function/pathway	Citation
1	Myb-related proteins: Myb 16 (Gh_A01G187100); Myb 306 (Gh_D04G053900); Myb 330 (Gh_A13G137700); Myb 16 (Gh_A05G373500); and Transcription factor WER (Gh_A05G364100)	Cell cycle and translational regulation	[29]
2	Transcription factor LABRA 3, (GB_D11G0963); BURP domain protein RD22 (GB_D05G0504); Protodermal factor 1 (GB_A07G1636); Homeobox-leucine zipper protein PROTODERMAL FACTOR 2 (GB_D06G1803); and Zinc finger protein 8 (GB_D01G1500)	Asparagine and cell wall biosynthesis, and stress responses	[117]
3	Trehalose phosphatase/ synthetase 11 (Gh_D08G0936); Ethylene responsive element binding factor 1 (Gh_D11G0426); Methyl esterase 1 (Gh_D11G1910); Tubulin beta-1 chain 1 (Gh_D01G0939); and Tubulin alpha-2 chain 2 (Gh_D02G2420)	Carbohydrate metabolism, ethylene response, and microtubule synthesis	[118]

Table 5.
Transcriptome analysis for fiber Initiation and elongation.

transcription factors have been detected from the analyses. Most identified DEGs belonged to the ribosome and amino acid biosynthesis and carbon metabolism. A few significantly enriched DEGs belonged to fatty acid degradation and flavonoid biosynthesis. Further, during fiber initiation, the significantly induced DE transcription factors belonged to the trihelix family, referred to as GaGTs, and often found on 12 of 13 chromosomes in *G. arboreum* [119]. In a study aimed at screening the transcriptome profiles for finding variations in fiber initiation and elongation among diverse fiber types of *G. hirsutum*, for example, long-staple cotton (LSC), short-staple cotton (SSC), long fiber group (LFG), and short-fiber group (SFG) to identify twelve genes in fiber development; among these, glycosyl hydrolase, Pectin lyase-like superfamily protein (PER64), and Pectin lyase (PL) were down-regulated in fiber elongation [118]. Their pipeline included Trimmomatic for filtering low-quality reads, TopHat2 for aligning clean reads to the reference genome, StringTie for assembling mapped reads into transcripts, HTSeq for quantification of GE, DeSeq for finding DEGs, GO for functional annotation, and KEGG for finding protein classes and pathways [118].

3.6 Transcriptome analysis for fiber color

Most cotton (*G. hirsutum*) fibers produced worldwide are white, despite the lint and fiber of tetraploid cotton (*G. barbadense*), exhibiting various colors including red, blue, green, and several shades of brown. The fiber color trait in cotton is genetically inherited, resulting from pigments blended with cellulose. Generally, the yields of colored cotton are typically lower, and the fiber is shorter and weaker but softer when compared with white cotton. However, the fiber qualities such as fiber length, strength, and color have been improved in hybrids between *G. barbadense* and *G. hirsutum*. More recently, colored cotton fiber has gained importance due to its unique and desirable characteristics and emerged as an eco-friendly dye-free textile material. A few recent RNA-Seq studies focussed on fiber color-related genes are presented below (**Table 6**). Using multi-omic approaches (metabolome and RNA-Seq analysis), the biochemical and regulatory roles of genes involved in light-induced green color formation in cotton have been reported [120]. Their study

S. No	Significant DEGs (~5) identified	Associated function/pathway	Citation
1	Cryptochrome 1 (Gh_A05G1941); Asparagine synthetase 1 (Gh_A07G0951); E3 ubiquitin-protein ligase 1 (Gh_A10G0098); Glutamate dehydrogenase 1 (Gh_A11G2354); and Mannose-6-phosphate isomerase 2 (Gh_A12G0488)	Phenylpropanoid pathway	[120]
2	Phenylalanine ammonia-lyase 6 (Gh_D10G2528); Cytochrome P450 84A (Gh_D11G1805); Aldehyde dehydrogenase family 2 member 4 (Gh_D07G0047); Flavonoid 3',5'-methyltransferase (Gh_D04G1818); and Shikimate O- hydroxycinnamoyltransferase 1 (Gh_A05G1005)	Phenylpropanoid pathway	[121]
3	Shikimate O-hydroxycinnamoyltransferase (Gorai.009G122600); Cinnamoyl-CoA reductase 2 (Gorai.003G055600); Flavonoid 3-O-glucosyltransferase (Gorai.012G009500); Chalcone synthase 1 (Gorai.005G035100); and Dihydroflavonol-4-reductase (Gorai.009G200600)	Flavonoid and phenylpropanoid pathways	[122]

Table 6.
Transcriptome analysis for fiber color.

compared early initiation (15 DPA) and late accumulated (45 DPA) metabolites under different lighting conditions and identified 236 differential metabolites. Among which, 20% of metabolites belonged to the phenylpropanoid pathway. Their RNA-Seq analysis included gene set enrichment and KEGG pathway analysis to identify genes and regulatory networks linked with light-induced fiber color formation. These networks are highly correlated with the corresponding phenylpropanoid metabolites [120].

Another study compared transcriptomes and metabolomes of Green Colored Fiber (GCF) accession and its near-isogenic line, White Colored Fiber (WCF) at 12, 18, and 24 DPA, to identify 2047 non-redundant metabolites enriched in eighty pathways, including biosynthesis of phenylpropanoid, wax, cutin, and suberin [121]. Their metabolome analysis identified higher levels of metabolites (sinapaldehyde) linked with the phenylpropanoid pathway in the GCF line compared with the WCF phenotype. Moreover, the metabolites identified in their study overlapped with the transcriptome analysis showing significant up-regulation of the genes responsible for the biosynthesis of select metabolites. The WGCNA analysis on DEGs identified between GCF and WCF has shown 16 gene modules co-expressed with fiber color at selected time points. At a visually different fiber color stage between GCF and WCF, the blue module at 24 DPA was of prime importance due to the upregulation of 56 hub and two homoeologous Gh4CL4 genes that have a potential role in green pigment biosynthesis [121]. A study aimed at understanding the gene expression and regulation of the pigment biosynthesis generated RNAi lines for the chalcone flavanone isomerase gene in the brown-colored fiber (BCF) line [122]. In addition, they compared the transcriptome profiles of BCF with its transgenic fiber phenotypes, white and green, to identify 13 significantly enriched DEGs in flavonoid and phenylpropanoid pathways [122].

4. Conclusions

In conclusion, comprehensive phenotyping, genotyping, and transcriptome approaches coupled with integrated bioinformatics pipelines have considerably improved our understanding of genes associated with fiber quality and yield traits. However, besides the genes related to the fiber quality characteristics discussed in this chapter, fiber uniformity, fineness, and micronaire attributes must also be considered in cotton germplasm improvement programs. Further, functional annotation, enrichment, and gene network analyses tools will continue to evolve with better features to visualize subtle changes in gene expression associated with biological pathways. Furthermore, to better understand complex traits (e.g., fiber quality and yield) and polyploid plant genomes (e.g., Upland cotton), more advanced computational pipelines need to be developed to integrate multi-omic and multi-dimensional phenotypic data. Moreover, fiber cell is a single elongated structure that serves as an ideal model for single-cell genomics. Thereby, dissecting the complexity associated with the initial input mRNA quantity in single-cell RNA-Seq will aid in screening thousands of samples per sequencing run. Therefore, the bulk RNA-Seq data generated by such voluminous efforts demands lightweight data science tools that utilize less memory footprint.

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Conflict of interest

The authors declare no conflict of interest.

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References

[1] Wendel JF, Grover CE. Taxonomy and evolution of the cotton genus, *Gossypium*. Cotton. 2015;**57**:25-44

[2] Hendrix B, Stewart JM. Estimation of the nuclear DNA content of *Gossypium* species. Annals of Botany. 2005;**95**(5):789-797

[3] Sripathi VR, Buyyarapu R, Kumpatla SP, Williams AJ, Nyaku ST, Tilahun Y, et al. Bioinformatics tools and genomic resources available in understanding the structure and function of *Gossypium*. Bioinformatics-Updated Features and Applications. 2016. p. 231

[4] FAS-USDA. 2022. Cotton: World Markets and Trade: February 2022 report. Available from: https://apps.fas.usda.gov/psdonline/circulars/cotton.pdf

[5] United Nations. World Population Prospects: The 2017 Revision, Key Findings and Advance Tables. Department of Economics and Social Affairs PD, editor. New York: United Nations; 2017. p. 46

[6] Liu Y. Chemical composition and characterization of cotton fibers. In: Cotton Fiber: Physics, Chemistry and Biology. Cham: Springer; 2018. pp. 75-94

[7] McGrath JE, Hickner MA, Höfer R. Polymers for a sustainable environment and green energy. Polymer Science. 2013;**10**:849

[8] Dochia M, Sirghie C, Kozłowski RM, Roskwitalski Z. Cotton fibres. In: Handbook of Natural Fibres. Cambridge, England: Woodhead Publishing; 2012. pp. 11-23

[9] Hu H, Wang M, Ding Y, Zhu S, Zhao G, Tu L, et al. Transcriptomic repertoires depict the initiation of lint and fuzz fibres in cotton (*Gossypium hirsutum* L.). Plant Biotechnology Journal. 2018;**16**(5):1002-1012

[10] Salih H, Leng X, He SP, Jia YH, Gong WF, Du XM. Characterization of the early fiber development gene, Ligon-lintless 1 (Li1), using microarray. Plant Gene. 2016;**6**:59-66

[11] Sripathi VR, Anche VC, Gossett ZB, Walker LT. Recent applications of RNA-sequencing in food and agriculture. Applications of RNA-Seq in Biology and Medicine. 2021. p. 97

[12] Lowe R, Shirley N, Bleackley M, Dolan S, Shafee T. Transcriptomics technologies. PLoS Computational Biology. 2017;**13**(5):e1005457

[13] Morozova O, Hirst M, Marra MA. Applications of new sequencing technologies for transcriptome analysis. Annual Review of Genomics and Human Genetics. 2009;**10**:135-151

[14] Wolf JB. Principles of transcriptome analysis and gene expression quantification: an RNA-seq tutorial. Molecular Ecology Resources. 2013;**13**(4): 559-572

[15] Wang Z, Gerstein M, Snyder M. RNA-Seq: a revolutionary tool for transcriptomics. Nature Reviews. Genetics. 2009;**10**(1):57-63

[16] Yoo MJ, Wendel JF. Comparative evolutionary and developmental dynamics of the cotton (*Gossypium* hirsutum) fiber transcriptome. PLoS Genetics. 2014;**10**(1):e1004073

[17] Chen ZJ, Sreedasyam A, Ando A, Song Q, De Santiago LM, Hulse-Kemp AM, et al. Genomic diversifications of five

Gossypium allopolyploid species and their impact on cotton improvement. Nature Genetics. 2020;**52**(5):525-533

[18] Parekh MJ, Kumar S, Fougat RS, Zala HN, Pandit RJ. Transcriptomic profiling of developing fiber in levant cotton (*Gossypium* herbaceum L.). Functional and Integrative Genomics. 2018;**18**(2):211-223

[19] Lin M, Pang C, Fan S, Song M, Wei H, Yu S. Global analysis of the *Gossypium hirsutum* L. Transcriptome during leaf senescence by RNA-Seq. BMC Plant Biology. 2015;**15**(1):1-8

[20] Ma Q, Wu M, Pei W, Wang X, Zhai H, Wang W, et al. RNA-Seq-mediated transcriptome analysis of a fiberless mutant cotton and its possible origin based on SNP markers. PLoS One. 2016;**11**(3):e0151994

[21] Peng Z, He S, Gong W, Sun J, Pan Z, Xu F, et al. Comprehensive analysis of differentially expressed genes and transcriptional regulation induced by salt stress in two contrasting cotton genotypes. BMC Genomics. 2014;**15**(1):1-28

[22] Naqvi RZ, Zaidi SS, Akhtar KP, Strickler S, Woldemariam M, Mishra B, et al. Transcriptomics reveals multiple resistance mechanisms against cotton leaf curl disease in a naturally immune cotton species. *Gossypium* arboreum. Scientific reports. 2017;**7**(1):1-5

[23] Xu L, Zhu L, Tu L, Liu L, Yuan D, Jin L, et al. Lignin metabolism has a central role in the resistance of cotton to the wilt fungus Verticillium dahliae as revealed by RNA-Seq-dependent transcriptional analysis and histochemistry. Journal of Experimental Botany. 2011;**62**(15):5607-5621

[24] Zhang F, Zhu G, Du L, Shang X, Cheng C, Yang B, et al. Genetic regulation of salt stress tolerance revealed by RNA-Seq in cotton diploid wild species, *Gossypium* davidsonii. Scientific Reports. 2016;**6**(1):1-5

[25] Bowman MJ, Park W, Bauer PJ, Udall JA, Page JT, Raney J, et al. RNA-Seq transcriptome profiling of upland cotton (*Gossypium hirsutum* L.) root tissue under water-deficit stress. PLoS One. 2013;**8**(12):e82634

[26] Yang J, Gao L, Liu X, Zhang X, Wang X, Wang Z. Comparative transcriptome analysis of fiber and nonfiber tissues to identify the genes preferentially expressed in fiber development in *Gossypium* hirsutum. Scientific Reports. 2021;**11**(1):1-8

[27] Wang L, Wang G, Long L, Altunok S, Feng Z, Wang D, et al. Understanding the role of phytohormones in cotton fiber development through omic approaches; recent advances and future directions. International Journal of Biological Macromolecules. 2020;**15**(163):1301-1313

[28] Wilkins TA, Arpat AB. The cotton fiber transcriptome. Physiologia Plantarum. 2005;**124**(3):295-300

[29] Ando A, Kirkbride RC, Jones DC, Grimwood J, Chen ZJ. LCM and RNA-Seq analyses revealed roles of cell cycle and translational regulation and homoeolog expression bias in cotton fiber cell initiation. BMC Genomics. 2021;**22**(1):1-6

[30] Liu D, Zhang J, Liu X, Wang W, Liu D, Teng Z, et al. Fine mapping and RNA-Seq unravels candidate genes for a major QTL controlling multiple fiber quality traits at the T 1 region in upland cotton. BMC Genomics. 2016;**17**(1):1-3

[31] Conesa A, Madrigal P, Tarazona S, Gomez-Cabrero D, Cervera A, McPherson A, et al. A survey of best

practices for RNA-Seq data analysis. Genome Biology. 2016;**17**(1):1-9

[32] Naphade S, Bhatnagar R, Hanson-Smith V, Choi I, Zhang A. Systematic comparative analysis of strand-specific RNA-Seq library preparation methods for low input samples. Scientific Reports. 2022;**12**(1):1-10

[33] Schurch NJ, Schofield P, Gierliński M, Cole C, Sherstnev A, Singh V, et al. How many biological replicates are needed in an RNA-Seq experiment and which differential expression tool should you use? RNA. 2016;**22**(6):839-851

[34] Hrdlickova R, Toloue M, Tian B. RNA-Seq methods for transcriptome analysis. Wiley Interdisciplinary Reviews: RNA. 2017;**8**(1):e1364

[35] Jazayeri SM, Melgarejo Munoz LM, Romero HM. RNA-Seq: a glance at technologies and methodologies. Acta Biológica Colombiana. 2015;**20**(2):23-35

[36] Nagalakshmi U, Waern K, Snyder M. RNA-Seq: a method for comprehensive transcriptome analysis. Current Protocols in Molecular Biology. 2010;**89**(1):4-11

[37] Schmieder R, Edwards R. Fast identification and removal of sequence contamination from genomic and metagenomic datasets. PLoS One. 2011;**6**(3):e17288

[38] Schmieder R, Edwards R. Quality control and preprocessing of metagenomic datasets. Bioinformatics. 2011;**27**(6):863-864

[39] Andrews, S. FastQC: A Quality Control Tool for High Throughput Sequence Data. 2010. Available from: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/

[40] Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet. journal. 2011;**17**(1):10-12

[41] Krueger F. Trim galore. A wrapper tool around Cutadapt and FastQC to consistently apply quality and adapter trimming to FastQ files. Babraham Bioinformatics. 2015. Available from: https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/

[42] Buffalo V. Scythe—A Bayesian adapter trimmer. UC Davis Bioinforma. Core. 2011. Available from: https://github.com/ucdavis-bioinformatics/scythe

[43] Bolger AM, Lohse M, Usadel B. Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics. 2014;**30**(15):2114-2120

[44] David S. HTStream: A toolkit for high throughput sequencing analysis. In: Theses and Dissertations Collection, Digital Initiatives. University of Idaho Library; 2017. Available from: https://www.lib.uidaho.edu/digital/etd/items/streett_idaho_0089n_11229.html

[45] Bushnell B. BBDuk. Jt Genome Inst. 2020. Available from: https://jgi.doe.gov/data-and-tools/bbtools/bb-tools-userguide/bbduk-guide/ [Accessed: June 25, 2020]

[46] Kumar G, Ertel A, Feldman G, Kupper J, Fortina P. iSeqQC: A tool for expression-based quality control in RNA-sequencing. BMC Bioinformatics. 2020;**21**(1):1-10

[47] Ewels P, Magnusson M, Lundin S, Käller M. MultiQC: summarize analysis results for multiple tools and samples in a single report. Bioinformatics. 2016;**32**(19):3047-3048

[48] Patel RK, Jain M. NGS QC Toolkit: A toolkit for quality control of next generation sequencing data. PLoS One. 2012;**7**(2):e30619

[49] Gordon A, Hannon GJ. Fastx-toolkit. FASTQ/A short-reads preprocessing tools (unpublished). 2010. Available from: http://hannonlab.cshl.edu/fastx_toolkit.

[50] Almodaresi F, Zakeri M, Patro R. PuffAligner: A fast, efficient and accurate aligner based on the pufferfish index. Bioinformatics. 2021;**37**(22):4048-4055

[51] Dobin A, Gingeras TR. Mapping RNA-seq reads with STAR. Current Protocols in Bioinformatics. 2015;**51**(1):11-14

[52] Kim D, Langmead B, Salzberg SL. HISAT: a fast spliced aligner with low memory requirements. Nature Methods. 2015;**12**(4):357-360

[53] Anders S, Pyl PT, Huber W. HTSeq—A Python framework to work with high-throughput sequencing data. Bioinformatics. 2015;**31**(2):166-169

[54] Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Salzberg SL. TopHat2: Accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biology. 2013;**14**(4):1-3

[55] Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nature Methods. 2012;**9**(4):357-359

[56] Patro R, Duggal G, Love MI, Irizarry RA, Kingsford C. Salmon provides fast and bias-aware quantification of transcript expression. Nature Methods. 2017;**14**(4):417-419

[57] Bray NL, Pimentel H, Melsted P, Pachter L. Near-optimal probabilistic RNA-Seq quantification. Nature Biotechnology. 2016;**34**(5):525-527

[58] Patro R, Mount SM, Kingsford C. Sailfish enables alignment-free isoform quantification from RNA-Seq reads using lightweight algorithms. Nature Biotechnology. 2014;**32**(5):462-464

[59] Guo Y, Zhao S, Sheng Q, Ye F, Li J, Lehmann B, et al. Multi-perspective quality control of Illumina exome sequencing data using QC3. Genomics. 2014;**103**(5-6):323-328

[60] Hartley SW, Mullikin JC. QoRTs: A comprehensive toolset for quality control and data processing of RNA-Seq experiments. BMC Bioinformatics. 2015;**16**(1):1-7

[61] García-Alcalde F, Okonechnikov K, Carbonell J, Cruz LM, Götz S, Tarazona S, et al. Qualimap: evaluating next-generation sequencing alignment data. Bioinformatics. 2012;**28**(20):2678-2679

[62] Wang L, Wang S, Li W. RSeQC: quality control of RNA-Seq experiments. Bioinformatics. 2012;**28**(16):2184-2185

[63] DeLuca DS, Levin JZ, Sivachenko A, Fennell T, Nazaire MD, Williams C, et al. RNA-SeQC: RNA-Seq metrics for quality control and process optimization. Bioinformatics. 2012;**28**(11):1530-1532

[64] Trapnell C, Williams BA, Pertea G, Mortazavi A, Kwan G, Van Baren MJ, et al. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nature Biotechnology. 2010;**28**(5):511-515

[65] Shumate A, Wong B, Pertea G, Pertea M. Improved Transcriptome Assembly Using a Hybrid of Long and Short Reads with StringTie. bioRxiv. 2021

[66] Soneson C, Love MI, Robinson MD. Differential analyses for RNA-Seq: transcript-level estimates improve gene-level inferences. F1000Research. 2015;**4**: 1521

[67] Love MI, Huber W, Anders S. Moderated estimation of fold change and

dispersion for RNA-Seq data with DESeq2. Genome Biology. 2014;**15**(12):1-21

[68] Monga I, Kaur K, Dhanda SK. Revisiting hematopoiesis: applications of the bulk and single-cell transcriptomics dissecting transcriptional heterogeneity in hematopoietic stem cells. Briefings in Functional Genomics. 2022. Elac002

[69] Su S, Law CW, Ah-Cann C, Asselin-Labat ML, Blewitt ME, Ritchie ME. Glimma: Interactive graphics for gene expression analysis. Bioinformatics. 2017;**33**(13):2050-2052

[70] Pertea M, Kim D, Pertea GM, Leek JT, Salzberg SL. Transcript-level expression analysis of RNA-Seq experiments with HISAT, StringTie and Ballgown. Nature Protocols. 2016;**11**(9):1650-1667

[71] Leng N, Dawson J, Kendziorski C. EBSeq: An R package for differential expression analysis using RNA-Seq data. R Package Version. 2015;**1**(10):1-39

[72] Ritchie ME, Phipson B, Wu DI, Hu Y, Law CW, Shi W, et al. limma powers differential expression analyses for RNA-Sequencing and microarray studies. Nucleic Acids Research. 2015;**43**(7):e47

[73] Law CW, Chen Y, Shi W, Smyth GK. voom: Precision weights unlock linear model analysis tools for RNA-Seq read counts. Genome Biology. 2014;**15**(2):1-7

[74] Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics. 2010;**26**(1):139-140

[75] Hardcastle TJ, Kelly KA. baySeq: Empirical Bayesian methods for identifying differential expression in sequence count data. BMC Bioinformatics. 2010;**11**(1):1-4

[76] Pantano L, Hutchinson J, Barrera V, Piper M, Khetani R, Daily K. DEGreport: Report of DEG analysis 2017. R package version 1.16.0. Available from: https://bioconductor.org/packages/release/bioc/html/DEGreport.html.

[77] Tarazona S, Furió-Tarí P, Turrà D, Pietro AD, Nueda MJ, Ferrer A, et al. Data quality aware analysis of differential expression in RNA-Seq with NOISeq R/Bioc package. Nucleic Acids Research. 2015;**43**(21):e140

[78] Risso D, Schwartz K, Sherlock G, Dudoit S. GC-content normalization for RNA-Seq data. BMC Bioinformatics. 2011;**12**(1):1-7

[79] Gene Ontology Consortium. Gene ontology consortium: Going forward. Nucleic Acids Research. 2015;**43**(D1):D1049-D1056

[80] Mi H, Ebert D, Muruganujan A, Mills C, Albou LP, Mushayamaha T, et al. PANTHER version 16: A revised family classification, tree-based classification tool, enhancer regions and extensive API. Nucleic Acids Research. 2021;**49**(D1):D394-D403

[81] Wiebe DS, Mukhin AM, Omelyanchuk NA, Mironova VV. FoldGO for functional annotation of transcriptome data to identify fold-change-specific GO categories. In: Mathematical Modeling and High-Performance Computing in Bioinformatics, Biomedicine and Biotechnology (MM-HPC-BBB-2018). 2018. p. 73

[82] Jiao X, Sherman BT, Huang DW, Stephens R, Baseler MW, Lane HC, et al. DAVID-WS: A stateful web service to facilitate gene/protein list analysis. Bioinformatics. 2012;**28**(13):1805-1806

[83] Supek F, Bošnjak M, Škunca N, Šmuc T. REVIGO summarizes and

visualizes long lists of gene ontology terms. PLoS One. 2011;**6**(7):e21800

[84] Carbon S, Ireland A, Mungall CJ, Shu S, Marshall B, Lewis S. AmiGO Hub, Web Presence Working Group. AmiGO: online access to ontology and annotation data. Bioinformatics. 2009;**25**(2):288-289

[85] Fernandes M, Husi H. ORA, FCS, and PT strategies in functional enrichment analysis. In: Proteomics Data Analysis. New York, NY: Humana; 2021. pp. 163-178

[86] Smoot ME, Ono K, Ruscheinski J, Wang PL, Ideker T. Cytoscape 2.8: New features for data integration and network visualization. Bioinformatics. 2011;**27**(3):431-432

[87] Green ML, Karp PD. The outcomes of pathway database computations depend on pathway ontology. Nucleic Acids Research. 2006;**34**(13):3687-3697

[88] Karp PD. Pathway databases: A case study in computational symbolic theories. Science. 2001;**293**(5537):2040-2044

[89] Ge SX, Son EW, Yao R. iDEP: An integrated web application for differential expression and pathway analysis of RNA-Seq data. BMC Bioinformatics. 2018;**19**(1):1-24

[90] Krämer A, Green J, Pollard J, Tugendreich S. Causal analysis approaches in ingenuity pathway analysis. Bioinformatics. 2014;**30**(4):523-530

[91] Draghici S, Khatri P, Tarca AL, Amin K, Done A, Voichita C, et al. A systems biology approach for pathway level analysis. Genome Research. 2007;**17**(10):1537-1545

[92] Luo W, Pant G, Bhavnasi YK, Blanchard SG, Brouwer C. Pathview web: User friendly pathway visualization and data integration. Nucleic Acids Research. 2017;**45**(W1):W501-W508

[93] Kanehisa M, Sato Y. KEGG mapper for inferring cellular functions from protein sequences. Protein Science. 2020;**29**(1):28-35

[94] Szklarczyk D, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S, et al. The STRING database in 2021: Customizable protein–protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic Acids Research. 2021;**49**(D1):D605-D612

[95] Yang X, Wang Y, Zhang G, Wang X, Wu L, Ke H, et al. Detection and validation of one stable fiber strength QTL on c9 in tetraploid cotton. Molecular Genetics and Genomics. 2016;**291**(4):1625-1638

[96] Rodgers J, Zumba J, Fortier C. Measurement comparison of cotton fiber micronaire and its components by portable near infrared spectroscopy instruments. Textile Research Journal. 2017;**87**(1):57-69

[97] Wang H, Zhang R, Shen C, Li X, Zhu D, Lin Z. Transcriptome and QTL analyses reveal candidate genes for fiber quality in Upland cotton. The Crop Journal. 2020;**8**(1):98-106

[98] Dong C, Wang J, Yu Y, Ju L, Zhou X, Ma X, et al. Identifying functional genes influencing *Gossypium hirsutum* fiber quality. Frontiers in Plant Science. 2019;**9**:1968

[99] Jiang X, Gong J, Zhang J, Zhang Z, Shi Y, Li J, et al. Quantitative trait loci and transcriptome analysis reveal genetic basis of fiber quality traits in CCRI70 RIL population of *Gossypium* hirsutum. Frontiers in Plant Science. 2021;**12**:753755-753755

[100] Zou X, Liu A, Zhang Z, Ge Q, Fan S, Gong W, et al. Co-expression network analysis and hub gene selection for high-quality fiber in upland cotton (*Gossypium* hirsutum) using

RNA-Sequencing analysis. Genes. 2019;**10**(2):119

[101] Wang Y, Li Y, Gong SY, Qin LX, Nie XY, Liu D, et al. GhKNL1 controls fiber elongation and secondary cell wall synthesis by repressing its downstream genes in cotton (*Gossypium* hirsutum). Journal of Integrative Plant Biology. 2022;**64**(1):39-55

[102] Zhang C, Li L, Liu Q, Gu L, Huang J, Wei H, et al. Identification of loci and candidate genes responsible for fiber length in upland cotton (*Gossypium hirsutum* L.) via association mapping and linkage analyses. Frontiers in Plant Science. 2019;**10**:53

[103] Hsu CY, Arick MA, Miao Q, Saha S, Jenkins JN, Ayubov MS, et al. Transcriptome analysis of ten days post anthesis elongating fiber in the upland cotton (*Gossypium* hirsutum) chromosome substitution line CS-B25. American Journal of Plant Sciences. 2018;**9**(06):1334

[104] Jiang X, Fan L, Li P, Zou X, Zhang Z, Fan S, et al. Co-expression network and comparative transcriptome analysis for fiber initiation and elongation reveal genetic differences in two lines from upland cotton CCRI70 RIL population. PeerJ. 2021;**9**:e11812

[105] Sun M, Ye Z, Tan J, Chen S, Zhang X, Tu L. A cotton germin-like protein GbGLP2 controls fiber length via regulating genes involved in secondary cell wall synthesis. Molecular Breeding. 2020;**40**(10):1-4

[106] Chen Q, Wang W, Khanal S, Han J, Zhang M, Chen Y, et al. Transcriptome analysis reveals genes potentially related to high fiber strength in a *Gossypium hirsutum* line IL9 with *Gossypium mustelinum* introgression. Genome. 2021;**64**(11):985-995

[107] Gao Z, Sun W, Wang J, Zhao C, Zuo K. GhbHLH18 negatively regulates fiber strength and length by enhancing lignin biosynthesis in cotton fibers. Plant Science. 2019;**286**:7-16

[108] Islam MS, Fang DD, Thyssen GN, Delhom CD, Liu Y, Kim HJ. Comparative fiber property and transcriptome analyses reveal key genes potentially related to high fiber strength in cotton (*Gossypium hirsutum* L.) line MD52ne. BMC Plant Biology. 2016;**16**(1):1-9

[109] Song Z, Chen Y, Zhang C, Zhang J, Huo X, Gao Y, et al. RNA-Seq reveals hormone-regulated synthesis of non-cellulose polysaccharides associated with fiber strength in a single-chromosomal-fragment-substituted upland cotton line. The Crop Journal. 2020;**8**(2):273-286

[110] Lu Q, Shi Y, Xiao X, Li P, Gong J, Gong W, et al. Transcriptome analysis suggests that chromosome introgression fragments from sea island cotton (*Gossypium* barbadense) increase fiber strength in upland cotton (*Gossypium* hirsutum). G3: Genes, Genomes, Genetics. 2017;**7**(10):3469-3479

[111] Hinchliffe DJ, Meredith WR, Yeater KM, Kim HJ, Woodward AW, Chen ZJ, et al. Near-isogenic cotton germplasm lines that differ in fiber-bundle strength have temporal differences in fiber gene expression patterns as revealed by comparative high-throughput profiling. Theoretical and Applied Genetics. 2010;**120**(7):1347-1366

[112] Salih H, Odongo MR, Gong W, He S, Du X. Genome-wide analysis of cotton C2H2-zinc finger transcription factor family and their expression analysis during fiber development. BMC Plant Biology. 2019;**19**(1):1-7

[113] Miao Q, Deng P, Saha S, Jenkins JN, Hsu CY, Abdurakhmonov IY, et al.

Transcriptome analysis of ten-DPA fiber in an upland cotton (Gossypium hirsutum) line with improved fiber traits from phytochrome A1 RNAi plants. American Journal of Plant Sciences. 2017;**8**(10):2530-2553

[114] Gallagher JP, Grover CE, Hu G, Jareczek JJ, Wendel JF. Conservation and divergence in duplicated fiber coexpression networks accompanying domestication of the polyploid *Gossypium* hirsutum L. G3: Genes, Genomes, Genetics. 2020;**10**(8):2879-2892

[115] Huang J, Guo Y, Sun Q, Zeng W, Li J, Li X, et al. Genome-wide identification of R2R3-MYB transcription factors regulating secondary cell wall thickening in cotton fiber development. Plant and Cell Physiology. 2019;**60**(3):687-701

[116] Cao JF, Zhao B, Huang CC, Chen ZW, Zhao T, Liu HR, et al. The miR319-targeted GhTCP4 promotes the transition from cell elongation to wall thickening in cotton fiber. Molecular Plant. 2020;**13**(7):1063-1077

[117] Cheng G, Zhang L, Wei H, Wang H, Lu J, Yu S. Transcriptome analysis reveals a gene expression pattern associated with fuzz fiber initiation induced by high temperature in Gossypium barbadense. Genes. 2020;**11**(9):1066

[118] Qin Y, Sun H, Hao P, Wang H, Wang C, Ma L, et al. Transcriptome analysis reveals differences in the mechanisms of fiber initiation and elongation between long-and short-fiber cotton (*Gossypium* hirsutum L.) lines. BMC Genomics. 2019;**20**(1):1-6

[119] Mo H, Wang L, Ma S, Yu D, Lu L, Yang Z, et al. Transcriptome profiling of *Gossypium* arboreum during fiber initiation and the genome-wide identification of trihelix transcription factors. Gene. 2019;**709**:36-47

[120] Tang Z, Fan Y, Zhang L, Zheng C, Chen A, Sun Y, et al. Quantitative metabolome and transcriptome analysis reveals complex regulatory pathway underlying photoinduced fiber color formation in cotton. Gene. 2021;**767**:145180

[121] Sun S, Xiong XP, Zhu Q, Li YJ, Sun J. Transcriptome sequencing and metabolome analysis reveal genes involved in pigmentation of green-colored cotton fibers. International Journal of Molecular Sciences. 2019;**20**(19):4838

[122] Liu HF, Luo C, Song W, Shen H, Li G, He ZG, et al. Flavonoid biosynthesis controls fiber color in naturally colored cotton. PeerJ. 2018;**6**:e4537

Chapter 5

Transgenic Technology Can Accelerate Cotton Breeding: Transgenic *ScALDH21* Cotton Significantly Improve Drought Tolerance in Southern and Northern Xinjiang

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Abstract

Aldehyde dehydrogenases (ALDHs) contribute to cellular protection against oxidative stress. These enzymes are crucial to organisms' ability to cope with environmental stress. The ALDH21 gene was introduced into upland cotton (*Gossypium hirsutum* L.) from desiccant-tolerant *Syntrichia caninervis* moss, created stable genetic transgenic lines. As a result, drought tolerance is increased and yield penalty is reduced in those transgenic lines. The first study to demonstrate overexpression of *ALDH21* enhances drought tolerance in cotton under multi-location field experiments is presented here. Cotton genotypes containing *ScALDH21* exhibit significant morphological, physiological, and economic benefits. *ScALDH21* functions in the physiology of cotton plants to protect them by scavenging ROS and reducing osmotic stress. The yield of transgenic cotton in northern Xinjiang showed up to 10% improvement under full irrigation and up to 18% improvement in deficit irrigation conditions on fields with purple clay loam soils. Additionally, transgenic cotton can be grown in sandy loam soil in southern Xinjiang with an average yield increase of 40% on different irrigation levels in the desert-oasis ecotone. Using *ScALDH21* as a candidate gene for cotton improvement in arid and semi-arid regions was demonstrated. In addition, we assessed different irrigation protocols and optimized irrigation methods with minimal water requirements for *ScALDH21*-transgenic cotton that could be used in production agriculture.

Keywords: transgenic cotton, molecular breeding, *ScALDH21*, drought tolerance, yield improved

1. Introduction

Plants are restricted in their habitat range and productivity by adverse environmental conditions [1]. Drought is the foremost constraint on agricultural production. Cotton (Gossypium spp.) is a major source of textile fibers and oil around the world. More than 32 million ha of cotton are produced in 76 countries [2]. In terms of cotton production, China is ranked among the top two countries in the world [3]. However, cotton production in China, as well as other countries, has recently declined due to increasing drier environments [3, 4]. Chinese agriculture consumes 62% of the country's annual water consumption, and the country is in a moderate water shortage [4]. In agriculture, cotton is the crop with the highest water consumption. In China, cotton is grown mainly in the Xinjiang-Uygur Autonomous Region, an area characterized by very low air humidity and a severe water shortage.

Cotton is the most important crop in China, accounting for around 25% of global fiber production. There is more than one-third of all agricultural land in the Xinjiang-Uyghur Autonomous Region dedicated to cotton plantations [5]. This region has a warm climate with average temperatures of 11.4°C and 49 mm precipitation annually, low groundwater levels, sandy soils, and severe soil salinization [6–9]. In southern Xinjiang, cotton has low germination rates, low survival rates, and low yields [10].

Plants are able to generate significant amounts of reactive aldehydes when faced with a variety of abiotic stresses (such as salinity, desiccation, and cold) [11], which can impair plant growth and crop productivity. Cotton varieties that survive droughts and other adversities must be developed urgently to combat these conditions. In arid lands where freshwater scarcity is a severe constraint on agricultural production, it is necessary to develop more tolerant varieties of plants. It is often difficult to obtain drought-tolerant crops through traditional breeding programs because of the time and labor involved, in addition to the need for large-scale facilities, such as rainout shelters. Interestingly, biotechnological improvements have been attempted since the 1990s, which is inspiring. However, the majority of transgenic cotton is aimed at controlling insect pest damage by expressing a variety of insecticidal proteins from *Bacillus thuringiensis* (i.e., Bt cotton). In transgenic plants, a number of genes have been discovered and studied that have a high potential for improving drought resistance, and some of these genes have shown promise for crop improvement [12–16]. However, only a few of these genes have been successfully deployed in fields of agriculture [17–20].

Plants that are known as bryophytes (mosses, hornworts, liverworts, etc.) are among the oldest species in the world's flora; they are thought to be small, non-vascular, and green plants. Many bryophytes survive even with a total loss of water in their vegetative tissues [21, 22]. The study of drought-tolerant mosses is of particular interest because their genetic engineering properties can be used to increase drought tolerance in arid-zone crops. The desiccation-tolerant moss *Syntrichia caninervis* is distributed in the Gurbantunggut desert in western China and can survive almost complete water loss and recover within 30 seconds after rehydrating [23]. Thus, *S. caninervis* may be a natural gene base for desiccation tolerance (**Figure 1**).

Aldehyde dehydrogenase (ALDH) genes show promise as candidate genes to increase plant resistance, especially *ALDH21* gene from moss *S. caninervis,* which is not found in seed plants. In a desert-oasis ecotone, non-transgenic cotton has an advantage over plants that overexpress *ALDH21* from desiccant tolerant moss, even under different irrigation practices. In addition, we are seeking the best irrigation scheme to reduce the consumption of irrigating water and increase crop production

Figure 1.
The natural habitat of S. caninervis *distributed in the Gurbantunggut desert of western China. Different morphology of moss: dried state and recovered state after applied water.*

in desert areas. Zhu et al. reported that different cotton planting lands gave different yields for some cotton lines [17]. To evaluate the efficacy of transgenes in cotton, irrigation strategy has a crucial role to play, especially in climate-dependent arid regions.

2. Cotton drought tolerance breeding with transgene technology

2.1 The overexpression gene types in current drought-tolerant cotton

It is possible to improve cotton drought tolerance using transgenic technology. The drought tolerance of transgenic cotton has recently been enhanced by using several genes (**Table 1**). As an example, AtLOS5, encoding an aldehyde oxidase cofactor sulfurase; GhAnn1, an annexin gene; isopentenyl transferase (IPT), an enzyme responsible for cytokinin biosynthesis; and 14-3-3 genes involved in plasma membrane H^+-ATPase activity [17, 24–27]. Increased drought tolerance was also observed in transgenic cotton overexpressing the OsSIZ1 gene from *Oryza sativa*, which encodes a SUMO E3 protein [36]. Researchers have used several transcription factor genes as transgenes in cotton to increase drought tolerance, including AtEDT1/HDG11 (homeodomain-START transcription factor), GhABF2 (bZIP transcription factor), NAC (a transcription factor) in rice (O. sativa L.), and AtRAV (for ABA insensitive3/viviparous1) in cotton [28–32]. It has been demonstrated that the expression of the vacuolar proton-pumping pyrophosphatase gene (AVP1) from Arabidopsis in cotton results in an increase in fiber yield of 20% compared to non-transgenic cotton [33, 34]. Drought tolerance is further improved by co-overexpression of AVP1 and AtNHX1 in cotton [35]. Nonetheless, only AtEDT1/HDG11, transgenic IPT, and

Gene name	Gene types/function	Gene source	References
AtLOS5	Molybdenum cofactor sulfurase gene/ aldehyde oxidase activity	*Arabidopsis*	Yue et al. [24]
GhAnn1	Annexin gene	*G. hirsutum*	Zhang et al. [25]
IPT	Isopentenyl transferase gene/rate-limiting enzyme for cytokinin biosynthesis	*Arabidopsis*	Kuppu et al. [26]; Zhu et al. [17]
14-3-3	Regulate the activity of plasma membrane H^+-ATPase	*Arabidopsis*	Yan et al. [27]
GhABF2	bZIP transcription factor family gene	*Arabidopsis*	Liang et al. [28]
AtEDT1/ HDG11	Homeodomain-START transcription factor gene	*Arabidopsis*	Yu et al. [29]
AtRAV	Related to ABA insensitive3/viviparous1	*Arabidopsis*	Mittal et al. [30]
SNAC1	Transcription factor gene	*Oryza sativa* L.	Liu et al. [31]
AtSAP5	Zinc-finger protein gene	*Arabidopsis*	Hozain et al. [32]
AVP1	Vacuolar proton-pumping pyrophosphatase (H^+-PPase) gene	*Arabidopsis*	Pasapula et al. [33]; Zhang et al. [34]
AtNHX1	Vacuolar Na^+/H^+ antiporter gene	*Arabidopsis*	Shen et al. [35]
OsSIZ1	SUMO E3 protein gene/participates in a sumoylation reaction	*Oryza sativa*	Mishra et al. [36]
ScALDH21	Aldehyde dehydrogenases gene	*Syntrichia caninervis*	Yang et al. [37, 38]
AtHUB2	Histone H2B monoubiquitination E3 ligase gene	*Arabidopsis*	[39]
StDREB2	Dehydration-responsive element binding (DREB) transcription factors	*Solanum tuberosum* L.	[40]
ABF	bZIP AREB/ABF transcription factor orthologs	*Arabidopsis*; *G. hirsutum*	[41]
GHSP26	Heat-shock proteins gene	*Gossypium arboreum*	[42]

Table 1.
Overexpression of various genes in cotton that reported to enhance drought tolerance.

transgenic AtAVP1 cotton showed a simultaneous increase in drought tolerance as well as cotton or fiber yield.

2.2 Transgenic *ScALDH21* cotton significantly improve drought tolerance in southern and northern Xinjiang

A number of fiber quality parameters and yield were improved with cotton *ScALDH21*. Planting transgenic *ScALDH21* cotton lines on purple clay loam soils in northern Xinjiang, field experiments demonstrated an increase in yields of 10.0% under full irrigation and >18.0% under deficit irrigation conditions (**Figure 2**). Compared to the non-transgenic cotton variety "Xin Nong Mian 1," the transgenic cotton showed an average yield increase of at least 40% grown on sandy loam soil in southern Xinjiang. Compared to the recipient cultivar "Xin Nong Mian 1,"

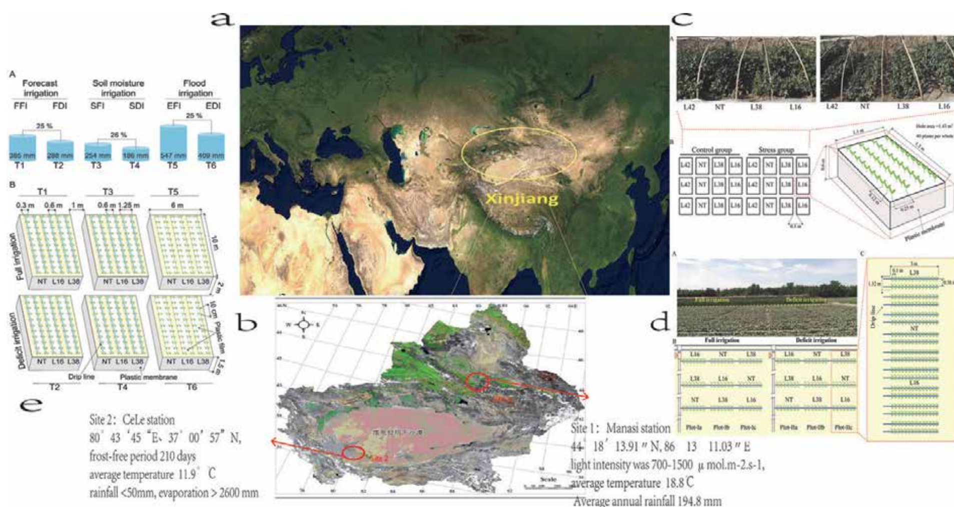

Figure 2.
The experiment sites location and experiment design. (a) Site of Xinjiang in the world map; (b) experiment site in Xinjiang map; (c) experiment design in the north of Xinjiang (44° 18′13.91″ N, 86° 13′11.03″ E, average temperature 18.8°C, average annual rainfall 194.8 mm); (d) experiment design in south of Xinjiang (80° 43′45″ E, 37° 00′57″ N, frost-free period 210 days, average temperature 11.9°C, rainfall < 50 mm, evaporation > 2600 mm).

ScALDH21-cotton had substantially improved performance under deficit irrigation, ensuring a more sustainable cotton production in the desert-oasis ecotone [37].

A variety of irrigation protocols were evaluated and optimized to use *ScALDH21*-cotton genotypes in production agriculture with minimum water requirements. The following paragraphs describe the characteristics of transgenic *ScALDH21* cotton.

2.2.1 The aldehyde dehydrogenase (ALDH) enzyme superfamily and its functions

As ROS are generated, oxidative stress is induced, lipid membranes are destroyed, and 200 types of aldehydes are accumulated, many of which are highly reactive and toxic. Aldehydes must be effectively removed and detoxified in arid environments to improve plant productivity. Plants have developed many enzymatic and non-enzymatic mechanisms to scavenge these toxic compounds [24]. Aldehyde dehydrogenase (ALDH) superfamily proteins may also play a role in scavenging ROS enzymatically [43]. Aldehyde dehydrogenases (ALDHs) have been found to play a central role in plants exposed to stressful conditions in the detoxification of aldehyde [44]. This superfamily of enzymes metabolizes endogenous and exogenous aldehydes to their carboxylic acids by using the coenzyme $NAD(P)^+$, producing NAD(P)H and thereby reducing oxidative/electrophilic stress [45]. ALDHs belong to a group of $NAD(P)^+$-dependent enzymes. Based on sequence similarity, the ALDH gene superfamily has been classified into 24 protein families by the ALDH Gene Nomenclature Committee (AGNC) [46]. There are 14 ALDH enzyme families in plants. Two of them (ALDH21 and ALDH23) are unique to bryophytes, and the rest (ALDH10, ALDH11, ALDH12, ALDH19, ALDH21, ALDH22, ALDH23, and ALDH24) are unique to higher plants [11, 47, 48]. ALDH21A1 plays a crucial role in the detoxification of aldehydes generated by desiccation stress, and it is proposed that ALDH21A1 expression is a unique stress resistance mechanism. Two classes of resistance pathways have been linked

to the ALDHs superfamily as abiotic or biotic resistance genes. An ALDH acts as an 'aldehyde scavenger' [49]. The increased activity of the Arabidopsis aldehyde dehydrogenase Ath-ALDH3 and soybean ALDH7 was reported to act as a detoxification mechanism that limits the accumulation of aldehyde and oxidative stress in Arabidopsis [1, 50]. In addition, metabolized ALDH products are directly involved in maintaining cellular osmotic homeostasis by catalyzing the synthesis of osmolytes [51, 52]. POD activity was the primary reason for the reduced peroxide levels in transgenic BADH tomatoes compared with SOD, APX, and CAT [52]. ALDH21 confers tolerance to osmotic and oxidative stress in cotton, according to our data. Under deficit stress, *ScALDH21*-cotton showed lower MDA production, increased POD activity, and higher proline and soluble sugar levels than non-transgenic cotton. This indicates that the *ScALDH21* gene may play a significant role in drought tolerance. Several ALDHs participate in drought-tolerant pathways in plants [53–55]. The transcriptional level is believed to be the mechanism by which ALDHs mediate environmental stress. An et al. [56] reported that the treatment of maize plants with NaCl and mannitol increased levels of *ZmALDH7B6* mRNA transcripts. Results of quantitative real-time PCR revealed that osmotic and H_2O_2 stress increased the expression of the *SiALDH7B1*, *SiALDH12A1,* and *SiALDH18B2* genes of *foxtail millet* (*Setaria italica*) [57]. ALDH overexpression has been shown to positively mitigate environmental stress. In contrast to non-transgenic plants, transgenic Arabidopsis *AtALDH2B8*, *AtALDH3I1*, *AtALDH7B4,* and *SpBADH* were able to survive on media containing high levels of H_2O_2. Moreover, ROS content in detached leaves of ALDH plants was significantly lower than that of WT [58, 59]. Plants overexpressing *ZmALDH22A1* show increased stress tolerance [60]. *SpALDH10* encodes the drought-inducible betaine aldehyde dehydrogenase (BADH) that catalyzes the oxidation of betaine aldehyde to the compatible solute glycine betaine, resulting in enhanced drought and salinity tolerance in potato plants [61]. ALDHs appear to play an important role in cell metabolism and stress physiology, according to these results. Recently, cotton transformed with *ALDH* genes has been reported to be tolerant to drought and salinity [62]. Transgenic cotton harboring the betA gene (part of the ALDH10 family) improved salt tolerance and cotton yield [62]. However, in only a few cases has a member of *ALDH* been reported as performing a specific function. *ALDH21A1* has previously been identified as a novel eukaryotic aldehyde dehydrogenase that is transcriptionally activated by abiotic stress [11]. Recombinant *Escherichia coli* expressing *ScALDH21* showed higher drought tolerance than control *E. coli* [23]. Compared with the control, tobacco overexpressing *ScALDH21* was more drought-tolerant [63]. Abiotic stress tolerance has been demonstrated in transgenic cotton using *ScALDH21* [37, 38, 64].

2.2.2 The genetic background of transgenic ScALDH21 cotton

2.2.2.1 The plant expression vector and the plant transformation

To make *ScALDH21* expression under CaMV 35S promoter, the open reading frame of *ScALDH21* cDNA (GQ245973) was amplified and cloned into the *Sal* I and *Kpn* I sites of the pCAMBIA2300. A recombinant vector containing selective neomycin phosphotransferase (NPTII) gene was transformed into *Agrobacterium tumefaciens* strain EHA105. Xin Nong Mian 1 (*Gossypium hirsutum*) has been transformed through Agrobacterium-mediated transformation. The Economic Crop Research Institute,

Xinjiang Academy of Agricultural Sciences, China, developed this cotton variety for specific arid zones in Xinjian. It displays good agronomic traits and economic characteristics.

2.2.2.2 PCR, RT-PCR detection, and Southern blot analysis

Using the cetyltrimethylammonium bromide method, genomic DNA was isolated from cotton seedlings at the five-leaf stage. PCR was used to detect the *ScALDH21* transgene in cotton plants using gene-specific primers [38]. The PCR amplification conditions were as follows—initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, primer annealing at 60°C for 30 s, and elongation at 72°C for 90 s, with a final elongation at 72°C for 5 min. Electrophoresis of 1% (w/v) agarose gels was used to visualize the PCR products. DNA extracted from cotton seedlings of the T5 generation was digested, run through gel electrophoresis, and transferred to a positively charged nylon membrane (Amersham, USA). Hybridization and chemiluminescence detection were performed according to the manufacturer's protocol (Roche, Germany) using digoxygenin dUTP-labeled probes of the *ScALDH21* gene product. Total RNA was extracted from young cotton leaves to analyze transgene expression. The genomic DNA was removed from the total RNA using DNase I (TaKaRa, Dalian, China). DNA was codified with the cDNA synthesis kit (TaKaRa, Dalian, China) according to the manufacturer's instructions. *ScALDH21* cDNA was used as a positive control for qPCR detection [38]. As an internal control, the *UBQ7* gene (GenBank accession no. DQ116441) was amplified with specific primers [24].

2.2.2.3 The transcriptome background of transgenic ScALDH21 cotton

We collected root samples from cotton seedlings after 1month of growth. Three biological replicates of each treatment were carried out. The total RNA was extracted using the RNAprep Pure Plant Kit (Tiangen, Beijing, China) following the manufacturer's instructions. In accordance with the manufacturer's instructions, sequencing libraries were prepared using the NEB Next Ultra RNA Library Prep Kit for Illumina (NEB, Beverly, CA, USA). Illumina HiSeq 4000 platform was used for sequencing with 150 bp paired-end reads. Based on the length of the gene and the number of reads mapped to the gene (Novogene company, China), the expected fragments per kilobase of genes per million mapped reads (FPKM) of each gene were calculated. Differentially expressed genes were defined using the DESeq R package with an adjusted P-value (q-value) of 0.05. We used the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg/) to test the statistical enrichment of DEGs in KEGG pathways. Five hundred and seventy-eight co-expressed genes were detected in the two *ScALDH21* transgenic lines, which were differentially expressed from NT and indicate that the target gene *ScALDH21* affected gene expression (**Figure 3a**). In **Figure 3b**, transcription expression patterns for those genes are shown. On the basis of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG), 578 genes were identified as overlapping across two transgenic lines compared to NT (**Figure 3c** and **d**). GO shows that ADP binding, O-methyltransferase activity, sulfotransferase activity, and transferase activity are significantly different from those of NT (**Figure 3c**). KEGG annotation shows no significant differences compared with that of NT (**Figure 3d**). Photosynthesis-antenna proteins, phenylpropanoid biosynthesis, and plant-pathogen interactions are the top

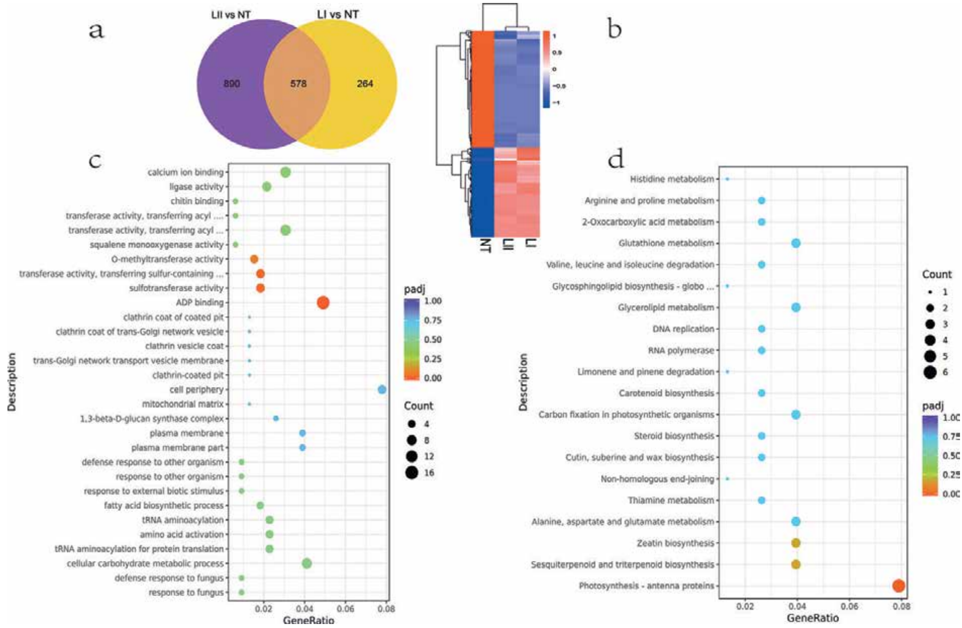

Figure 3.
The analysis of the difference between two ScALDH21 transgenic lines and non-transgenic cotton based on transcriptomic data. (a) Show the different expression gene numbers in two transgenic lines compared with that in NT in Venn graphs (log2 foldchange > 2, p_{adj} < 0.05). (b) Gene Ontology (GO) annotation of the 578 overlap genes in two transgenic lines after compared with NT separately. (c) Kyoto Encyclopedia of Genes and Genomes (KEGG) of annotation the 578 overlap genes in two transgenic lines after being compared with NT separately. NT, non-transgenic cotton; LI and LII are the two ScALDH21 transgenic lines.

three different pathways. These differences may have contributed to the different biofunctions and phenotypes.

2.2.3 The phenotype of ScALDH21-transgenic cotton

Following the identification of *ScALDH21* transgenic cotton, the growth performance of non-transgenic cotton (NT) and transgenic cotton (TC) under drought stress was examined and compared in northern Xinjiang from 2011 to 2014 and in southern Xinjiang from 2016 to 2018. Plants of all three independently transformed *ScALDH21* transgenic lines grew significantly taller than NT recipient plants in both full irrigation (26% higher) and deficit irrigation (23% higher). Transgenic and non-transgenic lines did not differ in leaf shape (length/width ratio) in either condition. However, the *ScALDH21* cotton had a greater leaf area compared to the NT plants in both full irrigation (79% increased) and deficit irrigation (51% increased). A significant difference was not observed between transgenic and NT plants in the full irrigated group. However, 24% more branches and 32% more bolls were observed in the deficit stress group. In general, the results showed that transgenic plants outperformed NT in morphological features like plant height, leaf area, leaf number, stem diameter, and root length. Similarly, morpho-physiological traits like fresh weight and dry weight of transgenic plants were greater than those of recipient plants. In transgenic plants, drought stress triggered lateral roots and increased leaf area significantly [38, 64]. Under drought stress conditions, *ScALDH21* overexpression appeared to

enhance plant growth in TC. Thus, overexpression of *ScALDH21* in cotton significantly increased the number of lateral roots, which consequently accelerated leaf growth following drought stress compared to NT. The height, leaf area, and leaf color of the transgenic *ScALDH21* cotton were all enhanced under normal and stress conditions in addition to the root system. The phenotypic results were consistent with the performance of other transgenic cotton [25, 31]. Under normal and drought conditions, overexpression of the rice NAC gene improves the root system [31]. In cotton, overexpressing a vacuolar pyrophosphatase gene increased root length and lateral root number, which improved the plant's water-absorbing abilities [33, 65]. After a 90-day water deficit, expression of the isopentenyl transferase gene IPT in cotton led to increased cotton height and roots. Overexpression of the Arabidopsis 14-3-3 protein gene GF14 in cotton results in a "stay-green" phenotype [27]. A potato sucrose synthase gene ectopically expressed in cotton accelerates leaf expansion and vegetative growth [66]. Additionally to demonstrating drought-tolerant phenotypic characteristics in *ScALDH21* transgenic cotton, our data also explain cotton's stress memory in terms of phenotype and physiology with two continuous water retention experiments that showed double water deficiency was worse than single water deficiency [38].

2.2.4 The physiological character of ScALDH21-transgenic cotton

Various biotic and abiotic stresses trigger ROS accumulation in plant cells, which leads to oxidative stress with lipid peroxidation, which also causes free radical reactions involving membrane polyunsaturated fatty acids [67]. ROS are produced when toxic aldehydes accumulate from lipid peroxidation [68]. Aldehyde-detoxifying enzymes ALDH3I1 and ALDH7B4 are both significant ROS scavengers and proteins that inhibit lipid peroxidation in Arabidopsis. In Arabidopsis, overexpression of these genes reduced lipid peroxidation under drought and salt stress [60].

It remains to be seen whether overexpression of *ScALDH21* improves overall plant health and performance under deficit irrigation. As a result of the toxicity of ROS produced by environmental stress, plants experience reduced growth, delayed development, and decreased yield. The *ScALDH21* cotton lines in this study responded to deficit irrigation with an increase in POD activity. This is consistent with previous reports [69], and therefore, it is reasonable to assume that they are better equipped to negate drought-induced ROS production. The transgenic cotton plants also showed decreased levels of MDA, an indicator of peroxidation [70] that indicates an improved ability to combat oxidative stress. Additionally, the *ScALDH21* cotton lines exhibit a more pronounced proline accumulation response to deficit irrigation, which is a well-distributed, multifunctional osmolyte that aids osmotic stress tolerance. *ScALDH21* overexpression reduced ROS-induced membrane peroxidation (lower MDA), increased ROS protection (elevated POD activity), and increased proline levels. It is likely that *ScALDH21* maintains a more intact cell system to counteract the negative effects of water deficiency. The level of proline and soluble sugars in transgenic cotton was increased by overexpression of *ScALDH21*. After one or two continuous water-withholding treatments, we measured the levels of free proline and soluble sugar in the leaves of seedlings and flowers [38]. It was found that proline accumulation increased after two continuous water withholdings, compared with other treatments. As a result of all drought treatments, NT and TC accumulate more soluble sugar, but TC accumulates more.

ScALDH21 cotton lines are able to maintain photosynthetic homeostasis and chlorophyll levels despite drought-induced oxidative stress. Southern Xinjiang

experienced an increase in SPAD values in either 2017 or 2018 [37]. Furthermore, the photosynthetic characteristics of plants under drought stress conditions were investigated at different stages of development (germination and flowering). As a result of full irrigation, the TC plants had a greater photosynthetic rate than the NT plants. The net photosynthetic rate of both TC and NT cotton was significantly reduced under drought stress, however, the TC cotton still maintained a significantly higher rate than NT cotton. The TC showed higher stomatal conductance and transpiration rates than the NT cotton [38]. Those can provide insight into the possible reason for the increase in biomass in *ScALDH21*-cotton plants.

2.2.5 The yield and fiber performance of ScALDH21-transgenic cotton

From 2013 to 2018, transgenic lines of cotton were grown in northern and southern Xinjiang to determine whether the *ScALDH21*-cotton was effective in the field.

TC lines in northern Xinjiang have been found to be better in growth and development than NT lines to some extent, after applying six different water retention treatments at different growth stages. Water deficit stress during the bud stage will cause the plant stalk length and boll number to decrease. Cotton yields were significantly decreased if twice deficit stresses were met during the bud or flower stage. Cotton growth and yield are critically dependent on water availability during the bud stage. Fiber parameters such as fiber strength, ginning out-turn of the fibers, fiber length, and length uniformity of the *ScALDH21*-cotton lines were not significantly different from NT plants. Comparatively to NT plants, TC lines produced stronger, more uniform, and longer fibers. TC had a micronaire value similar to or slightly lower than NT [38].

During harvesting season, boll weight, seed index, cotton yield, and fiber yield were measured in managed treatment plots under full and deficit irrigation conditions in northern Xinjiang in 2014 and 2016. Under drought stress, both *ScALDH21* transgenic cotton lines and NT lines had greatly reduced boll weight. Compared with NT plants, the seed index was higher in transgenic lines under both full and deficit irrigation conditions, and it reached up to 22% under stress. In both conditions, cotton yield per hectare and fiber yield did not differ significantly [64]. Under both full and deficit irrigation conditions in both managed treatment plots and production fields, the fiber length, uniformity, strength, elongation, and micronaire value of *ScALDH21* transgenic lines were improved or significantly improved compared with NT.

To determine the performance of the *ScALDH21* transgenic cotton under oasis field conditions in southern Xinjiang, three kinds of irrigation schedules were used (root zone model-simulated forecast irrigation (F), soil moisture sensor-based irrigation (S), and flood irrigation based on experience estimates (E) and two full (FI) and deficit (DI) irrigation conditions were employed [37]. Under all deficit irrigation conditions, the number of bolls and cotton yield of TC plants decreased compared to full irrigation, however, they were higher than those of NT plants. Over 3 years of experiments, TC plants showed a significant increase in cotton yields of up to 58.7% compared to NT plants [37]. Furthermore, in soil moisture sensor-based deficit irrigation (SDI) treatment, cotton yields are the lowest. In years, the cotton yield of *ScALDH21*-cotton lines grown under forecasted full irrigation (FFI) increased from 37–73% compared to NT plants. NT and Forecasted deficit irrigation (FDI), Soil moisture sensor-based full irrigation (SFI), SDI, Experience-based full irrigation (EFI), and Experience-based deficit irrigation (EDI) differed for all 3 years.

Furthermore, yield increases of transgenic lines were highest in SDI (from 67.5% to 92.3% in 3 years), compared to NT plants. Average data for 3 years in the SDI showed a large increase in cotton yield with a smaller deviation (83.5%).

In addition to treatments, cotton yields vary by year. As with the boll number per plant, the cotton yield per hectare, fiber yield per hectare, and cotton yield per plant were significantly higher in the transgenic lines than in the NT lines. The average seed yield for all treatments was 68% (variable from 14–128%) and 41% (variable from 10–102%) in 2017 and 2018, respectively [37]. Fiber elongation was increased in transgenic lines. Fiber strength also increased in transgenics after irrigation. There were no significant differences in fiber uniformity and micronaires between genotypes.

2.2.6 The irrigation strategy of ScALDH21-transgenic cotton

Cotton productivity and yield are largely influenced by a variety of factors, including genetics and irrigation methods. In this study, the TC lines performed better than the NT lines, despite soil, air humidity, and temperature affecting plant yield [71–73]. This study evaluated the drought tolerance ability of *ScALDH21*-cotton lines at field stations located in southern Xinjiang, China, a region classified as a desert-oasis ecotone with sandy loam soils, as well as at Manas Experimental Station, northern Xinjiang (**Figure 2**). The results indicated that our transgenic cotton had improved performance and could adapt to a wide range of cotton culture environments.

The lack of rainfall makes irrigation vital for agricultural production in arid and semi-arid lands. In arid zones, for example, normal irrigation above 600 mm during the vegetation period is sufficient for stable cotton harvesting [5]. Our desert oasis drought experiments in southern Xinjiang with sandy loam soils designed the 75% deficit irrigation and less than 600 mm different irrigation strategies to conserve more irrigation water and keep cotton yield constant. We used three irrigation schedules: DSSIS (Decision Support System for Irrigation Scheduling) forecasts (F), soil moisture sensors (S), and experience irrigation (E). Full irrigation (FI) and deficit irrigation (DI, 75% of full) were applied from 2016 to 2018 (**Figure 2**). Different irrigation protocols and water consumption affected the growth and yield of cotton, and the "Smart Irrigation" irrigation scheme based on the Root zone water quality model (RZWQM2) was found to be the best irrigation scheme for sustainable cotton production in an arid land. The results indicated that deficit irrigation schemes can be utilized in the desert-oasis ecotone, and in conjunction with the use of *ScALDH21*-cotton lines, the yields are sufficient for viable and sustainable agriculture.

Moreover, through mixed model analysis, we found that the cotton line always has a significant effect on plant phenotype, physiology, and yield components in southern Xinjiang, and cotton line and irrigation scheduling both have significant effects on cotton growth and development separately. In addition, irrigation scheduling and irrigation levels have a significant interaction effect. The relationship between yield and crop water use was calculated as overall water use efficiency (WUE). The EI schedule consumed more water (EFI, 547 mm, and EDI, 409 mm) than either the FI (FFI, 385 mm, and FDI, 288 mm) or SI (SFI, 254 mm, and SDI, 186 mm) schedules. Compared to NT plants, WUE was higher in *ScALDH21*-cotton lines each year. A high WUE was observed in the forecast, with drip irrigation leading to the highest WUE, and flood irrigation leading to the lowest WUE. In all treatments, the WUE value of *ScALDH21*-cotton lines increased by 59.6% compared to NT.

The individual irrigation level and timing significantly affected vegetative growth parameters, plant height, and leaf area, but the differences did not differ substantially between years despite differing precipitation levels. In each irrigation treatment, the TC, and especially the L16, grew significantly higher than the NT controls from 2016 to 2018. There was a dramatic reduction in the leaf area of NT under SDI in all years, but there was no difference in the leaf area of TC [37]. Therefore, the irrigation treatments can be ranked as forecast irrigation > flood irrigation > soil moisture irrigation based on their ability to maintain high instantaneous water use efficiency (IWUE).

We also used managed treatment plot experiments and field-scale in purple clay loam soil sites at Manas Experimental Station, northern Xinjiang. The two experiments differed in terms of growth space and water consumption. In the managed plot experiment, 50% less of full irrigation significantly reduced cotton vegetative growth and cotton yield (*50% loss of cottonseed and lint yield compared with full irrigation), whereas, in the field, 30% less of full irrigation did not affect cotton vegetative growth or yield. The reason for this can be explained by the amount of water used, which was 675 L of water m^{-2} with full irrigation and 472 L m^{-2} with deficient irrigation in the field, 566 L m^{-2} (control), and 283 L m^{-2} (stress) in the managed treatment areas.

The study also provides guidelines for optimal irrigation protocol and minimum water requirements for the use of *ScALDH21*-transgenic cotton lines in arid regions.

3. Conclusions and perspectives

ScALDH21-transgenic cotton exhibits improved plant growth and developmental phenotype through sustained net photosynthetic rate, greater tolerance to osmotic and oxidative stress, and improved cotton yield and fiber quality. Transgenic cotton can also be grown in sandy loam soils in southern Xinjiang and purple clay loam soils in northern Xinjiang that are more productive than non-transgenic recipients. This transgenic variation of *ScALDH21* is significantly better than recipient cultivars that can be commercially exploited when irrigation is scarce, enabling a more sustainable cotton production in the desert oasis ecotone. For *ScALDH21* transgenic cotton that can be used in agricultural production, we evaluated various irrigation protocols and optimized irrigation regimes with minimal water requirements. Using multiple growing seasons and multiple studies, ectopic expression of the moss *ScALDH21* gene in cotton improves drought tolerance and reduces yield penalties. *ScALDH21* overexpression enhances drought tolerance in cotton, suggesting that *ScALDH21* could be a candidate gene for improving cotton in arid-arid regions. To ensure the safety of transgenic lines, they must be tested with a GMO undergone a biosafety protocol. Currently, biosafety and risk assessment based on GMO requirements are being tested. To breed the next generation of crop varieties, updated germplasm, knowledge, and breeding techniques will be needed [74]. There are numerous reports of genes that confer stress tolerance, most of which are from Arabidopsis, very few of which have been successfully tested in crop plants due to potential side-effects of candidate genes on growth and morphology. It is therefore urgent to develop many drought-tolerant gene resources from drought-resistant plants. Reports indicate that introducing foreign genes from xerophytic plants or overexpressing certain cotton genes improve its performance under drought conditions [35, 36, 38]. Therefore, it is reasonable to assume that an in-depth comparative study of the expression and function of members of the same gene families in extreme xerophytes will eventually aid in breeding drought-tolerant crop plants.

It has been widely used in plant biotechnology to improve crop traits with CRISPR/Cas9 technology [75, 76] and it will be applied to crop breeding in the near future [77], especially for gene knock out in crops. To overcome complex and changing adversity, crop breeding must be multi-resistant because climate change leads to an increase in both biotic and abiotic stresses. The pursuit of homologous genes from extreme xerophytes plants, but with a low degree of identity to crop, will significantly increase drought tolerance. *ScALDH21* orthologs were not found in cotton genomes, but transgenic lines exhibited better growth and development, as well as greater photosynthesis ability in a water-scarce environment. Furthermore, this gene was also discovered to be salt-tolerant as well as resistant to *Verticillium*, which indicates that moss as the first landing plant may be an excellent resistant gene resource library, especially the extreme drought-tolerant moss.

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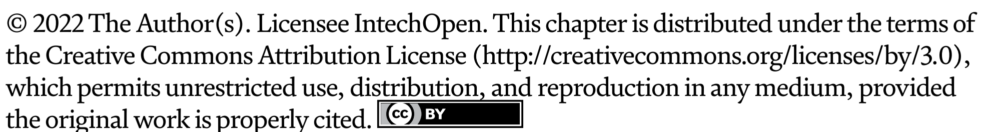
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References

[1] Sunkar R, Bartels D, Kirch HH. Overexpression of a stress-inducible aldehyde dehydrogenase gene from *Arabidopsis thaliana* in transgenic plants improves stress tolerance. The Plant Journal. 2003;**35**:452-464. DOI: 10.1046/j.1365-313X.2003.01819.x

[2] Burkitbayeva S, Qaim M, Swinnen J. A black (white) hole in the global spread of GM cotton. Trends in Biotechnology. 2016;**34**:260-263. DOI: 10.1016/j.tibtech.2015.12.017

[3] Liu BC, Mu JQ, Zhu JB, Liang ZQ, Zhang L. *Saussurea involucrata* Sidhn2 gene confers tolerance to drought stress in upland cotton. Pakistan Journal of Botany. 2017;**49**:465-473

[4] Guo RQ, Sun SC, Liu BA. Difference in leaf water use efficiency/photosynthetic nitrogen use efficiency of Bt-cotton and its conventional peer. Scientific Reports. 2016;**6**:33539

[5] Yang CJ, Luo Y, Sun L, Wu N. Effect of deficit irrigation on the growth, water use characteristics and yield of cotton in Arid Northwest China. Pedosphere. 2015;**25**:910-924

[6] Gui DW, Lei JQ, Zeng FJ. Farmland management effects on the quality of surface soil during oasification in the southern rim of the Tarim Basin in Xinjiang, China. Plant, Soil and Environment. 2010;**56**:348-356

[7] Shareef M, Gui DW, Zeng FJ, Waqas M, Zhang B, Iqbal H. Water productivity, growth, and physiological assessment of deficit irrigated cotton on hyperarid desert-oases in northwest China. Agricultural Water Management. 2018;**206**:1-10

[8] Yang YM, Yang YH, Han SM, Macadam I, Liu DL. Prediction of cotton yield and water demand under climate change and future adaptation measures. Agricultural Water Management. 2014;**144**:42-53. DOI: 10.1016/j.agwat.2014.06.001

[9] Kuang WN, Gao XP, Gui DW, Tenuta M, Flaten DN, Yin MY, et al. Effects of fertilizer and irrigation management on nitrous oxide emission from cotton fields in an extremely arid region of northwestern China. Field Crops Research. 2018;**229**:17-26

[10] Zhong RS, Tian FQ, Yang PN, Yi QX. Planting and irrigation methods for cotton in southern Xinjiang, China. Irrigation and Drainage. 2016;**65**:461-468

[11] Chen XB, Zeng Q, Wood AJ. The stress-responsive *Tortula ruralis* gene ALDH21A1 describes a novel eukaryotic aldehyde dehydrogenase protein family. Journal of Plant Physiology. 2002;**159**:677-684. DOI: 10.1078/0176-1617-0813

[12] Chaves MM, Maroco JP, Pereira JS. Understanding plant responses to drought - from genes to the whole plant. Functional Plant Biology. 2003;**30**:239-264. DOI: 10.1071/Fp02076

[13] Cooper M, Messina CD, Podlich D, Totir LR, Baumgarten A, Hausmann NJ, et al. Predicting the future of plant breeding: Complementing empirical evaluation with genetic prediction. Crop & Pasture Science. 2014;**65**:311-336. DOI: 10.1071/Cp14007

[14] James C. Global adoption, impact and future prospects of biotech/GM crops. Journal of Biotechnology.

2008;**136**:S213-S214. DOI: 10.1016/j.jbiotec.2008.07.452

[15] Ronald PC. Lab to farm: Applying research on plant genetics and genomics to crop improvement. PLoS Biology. 2014;**12**(6):e1001878

[16] Vinocur B, Altman A. Recent advances in engineering plant tolerance to abiotic stress: Achievements and limitations. Current Opinion in Biotechnology. 2005;**16**:123-132. DOI: 10.1016/j.copbio.2005.02.001

[17] Zhu XL, Sun L, Kuppu S, Hu RB, Mishra N, Smith J, et al. The yield difference between wildtype cotton and transgenic cotton that expresses IPT depends on when water-deficit stress is applied. Scientific Reports. 2018;**8**:2538

[18] Castiglioni P, Warner D, Bensen RJ, Anstrom DC, Harrison J, Stoecker M, et al. Bacterial RNA chaperones confer abiotic stress tolerance in plants and improved grain yield in maize under water-limited conditions. Plant Physiology. 2008;**147**:446-455

[19] Deikman J, Petracek M, Heard JE. Drought tolerance through biotechnology: Improving translation from the laboratory to farmers' fields. Current Opinion in Biotechnology. 2012;**23**:243-250. DOI: 10.1016/j.copbio.2011.11.003

[20] Xiao BZ, Chen X, Xiang CB, Tang N, Zhang QF, Xiong LZ. Evaluation of seven function-known candidate genes for their effects on improving drought resistance of transgenic rice under field conditions. Molecular Plant. 2009;**2**:73-83. DOI: 10.1093/mp/ssn068

[21] Proctor MCF, Oliver MJ, Wood AJ, Alpert P, Stark LR, Cleavitt NL, et al. Desiccation-tolerance in bryophytes: A review. Bryologist. 2007;**110**:595-621

[22] Wood AJ. Invited essay: New frontiers in bryology and lichenology—The nature and distribution of vegetative desiccation-tolerance in hornworts, liverworts and mosses. Bryologist. 2007;**110**:163-177. DOI: 10.1639/0007-2745(2007)110[163:Ienfib]2.0.Co;2

[23] Yang H, Zhang D, Wang J, Wood AJ, Zhang Y. Molecular cloning of a stress-responsive aldehyde dehydrogenase gene ScALDH21 from the desiccation-tolerant moss *Syntrichia caninervis* and its responses to different stresses. Molecular Biology Reports. 2012;**39**:2645-2652. DOI: 10.1007/s11033-011-1017-6

[24] Yue YS, Zhang MC, Zhang JC, Tian XL, Duan LS, Li ZH. Overexpression of the AtLOS5 gene increased abscisic acid level and drought tolerance in transgenic cotton. Journal of Experimental Botany. 2012;**63**:3741-3748. DOI: 10.1093/Jxb/Ers069

[25] Zhang F, Li SF, Yang SM, Wang LK, Guo WZ. Overexpression of a cotton annexin gene, GhAnn1, enhances drought and salt stress tolerance in transgenic cotton. Plant Molecular Biology. 2015;**87**:47-67

[26] Kuppu S, Mishra N, Hu R, Sun L, Zhu X, Shen G, et al. Water-deficit inducible expression of a cytokinin biosynthetic gene IPT improves drought tolerance in cotton. PLoS One. 2013;**8**:e64190. DOI: 10.1371/journal.pone.0064190

[27] Yan JQ, He CX, Wang J, Mao ZH, Holaday SA, Allen RD, et al. Overexpression of the Arabidopsis 14-3-3 protein GF14 lambda in cotton leads to a “Stay-Green” phenotype and improves stress tolerance under moderate drought conditions. Plant and Cell Physiology. 2004;**45**:1007-1014. DOI: 10.1093/Pcp/Pch115

[28] Liang CZ, Meng ZH, Meng ZG, Malik W, Yan R, Lwin KM, et al. GhABF2, a bZIP transcription factor, confers drought and salinity tolerance in cotton (*Gossypium hirsutum* L.). Scientific Reports. 2016;**6**:35040

[29] Yu LH, Wu SJ, Peng YS, Liu RN, Chen X, Zhao P, et al. Arabidopsis EDT1/HDG11 improves drought and salt tolerance in cotton and poplar and increases cotton yield in the field. Plant Biotechnology Journal. 2016;**14**: 72-84

[30] Mittal A, Jiang YW, Ritchie GL, Burke JJ, Rock CD. AtRAV1 and AtRAV2 overexpression in cotton increases fiber length differentially under drought stress and delays flowering. Plant Science. 2015;**241**:78-95

[31] Liu GZ, Li XL, Jin SX, Liu XY, Zhu LF, Nie YC, et al. Overexpression of rice NAC gene *SNAC1* improves drought and salt tolerance by enhancing root development and reducing transpiration rate in transgenic cotton. PLoS One. 2014;**9**(1):e86895. DOI: 10.1371/journal.pone.0086895

[32] Hozain M, Abdelmageed H, Lee J, Kang M, Fokar M, Allen RD, et al. Expression of AtSAP5 in cotton up-regulates putative stress-responsive genes and improves the tolerance to rapidly developing water deficit and moderate heat stress. Journal of Plant Physiology. 2012;**169**:1261-1270. DOI: 10.1016/j.jplph.2012.04.007

[33] Pasapula V, Shen GX, Kuppu S, Paez-Valencia J, Mendoza M, Hou P, et al. Expression of an Arabidopsis vacuolar H^+-pyrophosphatase gene (AVP1) in cotton improves drought- and salt tolerance and increases fibre yield in the field conditions. Plant Biotechnology Journal. 2011;**9**:88-99. DOI: 10.1111/j.1467-7652.2010.00535.x

[34] Zhang KW, Guo N, Lian LJ, Wang J, Lv SL, Zhang JR. Improved salt tolerance and seed cotton yield in cotton (*Gossypium hirsutum* L.) by transformation with betA gene for glycinebetaine synthesis. Euphytica. 2011;**181**:1-16. DOI: 10.1007/s10681-011-0354-9

[35] Shen GX, Wei J, Qiu XY, Hu RB, Kuppu S, Auld D, et al. Co-overexpression of AVP1 and AtNHX1 in cotton further improves drought and salt tolerance in transgenic cotton plants. Plant Molecular Biology Reporter. 2015;**33**:167-177

[36] Mishra N, Sun L, Zhu XL, Smith J, Srivastava AP, Yang XJ, et al. Overexpression of the rice SUMO E3 ligase gene OsSIZ1 in cotton enhances drought and heat tolerance, and substantially improves fiber yields in the field under reduced irrigation and rainfed conditions. Plant and Cell Physiology. 2017;**58**:735-746

[37] Yang HL, Bozorov TA, Chen XP, Zhang DW, Wang JC, Li XS, et al. Yield comparisons between cotton variety Xin Nong Mian 1 and its transgenic *ScALDH21* lines under different water deficiencies in a desert-oasis ecotone. Agronomy-Basel. 2021;**11**:1019. DOI: 10.1910.3390/agronomy11051019

[38] Yang H, Zhang D, Li X, Li H, Zhang D, Lan H, et al. Overexpression of *ScALDH21* gene in cotton improves drought tolerance and growth in greenhouse and field conditions. Molecular Breeding. 2016;**36**:34. DOI: 10.1007/s11032-015-0422-2

[39] Chen H, Feng H, Zhang XY, Zhang CJ, Wang T, Dong JL. An Arabidopsis E3 ligase HUB2 increases histone H2B monoubiquitination and enhances drought tolerance in transgenic cotton. Plant Biotechnology Journal. 2019;**17**:556-568. DOI: 10.1111/pbi.12998

[40] El-Esawi MA, Alayafi AA. Overexpression of *StDREB2* transcription factor enhances drought stress tolerance in Cotton (*Gossypium barbadense* L.). 2019;**10**(2). DOI: ARTN14210.3390/genes10020142

[41] Kerr TCC, Abdel-Mageed H, Aleman L, Lee J, Payton P, Cryer D, et al. Ectopic expression of two AREB/ABF orthologs increases drought tolerance in cotton (Gossypium hirsutum). Plant Cell and Environment. 2018;**41**(5):898-907

[42] Maqbool A, Abbas W, Rao AQ, Irfan M, Zahur M, Bakhsh A, et al. Gossypium arboreum GHSP26 enhances drought tolerance in Gossypium hirsutum. Biotechnology Progress. 2010;**26**(1):21-25

[43] Wenzel P, Muller J, Zurmeyer S, Schuhmacher S, Schulz E, Oelze M, et al. ALDH-2 deficiency increases cardiovascular oxidative stress—Evidence for indirect antioxidative properties. Biochemical and Biophysical Research Communications. 2008;**367**:137-143. DOI: 10.1016/j.bbrc.2007.12.089

[44] Gao C, Han B. Evolutionary and expression study of the aldehyde dehydrogenase (ALDH) gene superfamily in rice (*Oryza sativa*). Gene. 2009;**431**:86-94

[45] Kirch HH, Bartels D, Wei YL, Schnable PS, Wood AJ. The ALDH gene superfamily of Arabidopsis. Trends in Plant Science. 2004;**9**:371-377. DOI: 10.1016/j.tplants.2004.06.004

[46] Vasiliou V, Bairoch A, Tipton KF, Nebert DW. Eukaryotic aldehyde dehydrogenase (ALDH) genes: Human polymorphisms, and recommended nomenclature based on divergent evolution and chromosomal mapping. Pharmacogenetics. 1999;**9**:421-434. DOI: 10.1097/00008571-199910000-00004

[47] Brocker C, Vasiliou M, Carpenter S, Carpenter C, Zhang YC, Wang XP, et al. Aldehyde dehydrogenase (ALDH) superfamily in plants: Gene nomenclature and comparative genomics. Planta. 2013;**237**:189-210. DOI: 10.1007/s00425-012-1749-0

[48] Wood AJ, Duff RJ. The aldehyde dehydrogenase (ALDH) gene superfamily of the moss Physcomitrella patens and the algae *Chlamydomonas reinhardtii* and *Ostreococcus tauri*. Bryologist. 2009;**112**:1-11

[49] Singh S, Brocker C, Koppaka V, Chen Y, Jackson BC, Matsumoto A, et al. Aldehyde dehydrogenases in cellular responses to oxidative/electrophilic stress. Free Radical Biology and Medicine. 2013;**56**:89-101. DOI: 10.1016/j.freeradbiomed.2012.11.010

[50] Rodrigues SM, Andrade MO, Gomes APS, DaMatta FM, Baracat-Pereira MC, Fontes EPB. Arabidopsis and tobacco plants ectopically expressing the soybean antiquitin-like *ALDH7* gene display enhanced tolerance to drought, salinity, and oxidative stress. Journal of Experimental Botany. 2006;**57**(9):1909-1918

[51] Fu XZ, Khan EU, Hu SS, Fan QJ, Liu JH. Overexpression of the *betaine aldehyde dehydrogenase* gene from *Atriplex hortensis* enhances salt tolerance in the transgenic trifoliate orange (*Poncirus trifoliata* L. Raf.). Environmental and Experimental Botany. 2011;**74**:106-113. DOI: 10.1016/j.envexpbot.2011.05.006

[52] Li MF, Li ZM, Li SF, Guo SJ, Meng QW, Li G, et al. Genetic engineering of glycine betaine biosynthesis reduces heat-enhanced photoinhibition by enhancing antioxidative defense and alleviating lipid peroxidation in tomato. Plant Molecular Biology Reporter. 2014;**32**:42-51. DOI: 10.1007/s11105-013-0594-z

[53] Brocker C, Cantore M, Failli P, Vasiliou V. Aldehyde dehydrogenase 7A1 (ALDH7A1) attenuates reactive aldehyde and oxidative stress induced cytotoxicity. Chemico-Biological Interactions. 2011;**191**:269-277. DOI: 10.1016/j.cbi.2011.02.016

[54] Munoz-Clares RA, Riveros-Rosas H, Garza-Ramos G, Gonzalez-Segura L, Mujica-Jimenez C, Julian-Sanchez A. Exploring the evolutionary route of the acquisition of betaine aldehyde dehydrogenase activity by plant ALDH10 enzymes: Implications for the synthesis of the osmoprotectant glycine betaine. BMC Plant Biology. 2014;**14**. DOI: 10.1186/1471-2229-14-149

[55] Wei YL, Lin M, Oliver DJ, Schnable PS. The roles of aldehyde dehydrogenases (ALDHs) in the PDH bypass of Arabidopsis. BMC Biochemistry. 2009;**10**. DOI: 10.1186/1471-2091-10-7

[56] An X, Duan F-Y, Guo S, Chen F-J, Yuan L-X, Gu R-L. Transcriptional regulation of expression of the maize aldehyde dehydrogenase 7 gene (ZmALDH7B6) in response to abiotic stresses. Journal of Integrative Agriculture. 2014;**13**:1900-1908. DOI: 10.1016/s2095-3119(13)60518-3

[57] Chen Z, Chen M, Xu ZS, Li LC, Chen XP, Ma YZ. Characteristics and expression patterns of the aldehyde dehydrogenase (ALDH) gene superfamily of foxtail millet (*Setaria italica* L.). PLoS One. 2014;**9**. DOI: 10.1371/journal.pone.0101136

[58] Xu X, Guo R, Cheng C, Zhang H, Zhang Y, Wang X. Overexpression of ALDH2B8, an aldehyde dehydrogenase gene from grapevine, sustains Arabidopsis growth upon salt stress and protects plants against oxidative stress. Plant Cell, Tissue and Organ Culture (PCTOC). 2013;**114**:187-196. DOI: 10.1007/s11240-013-0314-2

[59] Yang C, Zhou Y, Fan J, Fu Y, Shen L, Yao Y, et al. SpBADH of the halophyte *Sesuvium portulacastrum* strongly confers drought tolerance through ROS scavenging in transgenic Arabidopsis. Plant Physiology and Biochemistry. 2015;**96**:377-387. DOI: 10.1016/j.plaphy.2015.08.010

[60] Kotchoni SO, Kuhns C, Ditzer A, Kirch HH, Bartels D. Over-expression of different aldehyde dehydrogenase genes in *Arabidopsis thaliana* confers tolerance to abiotic stress and protects plants against lipid peroxidation and oxidative stress. Plant, Cell & Environment. 2006;**29**:1033-1048. DOI: 10.1111/j.1365-3040.2005.01458.x

[61] Zhang N, Si HJ, Wen G, Du HH, Liu BL, Wang D. Enhanced drought and salinity tolerance in transgenic potato plants with a BADH gene from spinach. Plant Biotechnology Reports. 2011;**5**:71-77. DOI: 10.1007/s11816-010-0160-1

[62] Zhang KW, Wang J, Lian LJ, Fan WJ, Guo N, Lv SL. Increased chilling tolerance following transfer of a betA gene enhancing glycinebetaine synthesis in cotton (*Gossypium hirsutum* L.). Plant Molecular Biology Reporter. 2012;**30**:1158-1171. DOI: 10.1007/s11105-012-0433-7

[63] Yang H, Zhang D, Li H, Dong L, Lan H. Ectopic overexpression of the aldehyde dehydrogenase ALDH21 from *Syntrichia caninervis* in tobacco confers salt and drought stress tolerance. Plant Physiology and Biochemistry. 2015;**95**:83-91. DOI: 10.1016/j.plaphy.2015.07.001

[64] Yang HL, Zhang DW, Zhang DY, Bozorov TA, Abdullaev AA, Wood AJ, et al. Overexpression of ALDH21 from *Syntrichia caninervis* moss in upland

cotton enhances fiber quality, boll component traits, and physiological parameters during deficit irrigation. Crop Science. 2019;**59**:553-564

[65] Zhang H, Shen G, Kuppu S, Gaxiola R, Payton P. Creating drought- and salt-tolerant cotton by overexpressing a vacuolar pyrophosphatase gene. Plant Signaling & Behavior. 2011;**6**:861-863. DOI: 10.4161/psb.6.6.15223

[66] Xu SM, Brill E, Llewellyn DJ, Furbank RT, Ruan YL. Overexpression of a potato sucrose synthase gene in cotton accelerates leaf expansion, reduces seed abortion, and enhances fiber production. Molecular Plant. 2012;**5**:430-441. DOI: 10.1093/Mp/Ssr090

[67] Halliwell B, Gutteridge J. Protection against oxidants in biological systems: The superoxide theory of oxygen toxicity. In: Free Radicals in Biology and Medicine. Clarendon, Oxford; 1989. pp. 86-123

[68] Bolwell GP. Role of active oxygen species and NO in plant defence responses. Current Opinion in Plant Biology. 1999;**2**:287-294. DOI: 10.1016/S1369-5266(99)80051-X

[69] Luo XL, Wu JH, Li YB, Nan ZR, Guo X, Wang YX, et al. Synergistic effects of GhSOD1 and GhCAT1 overexpression in cotton chloroplasts on enhancing tolerance to methyl viologen and salt stresses. PLoS One. 2013;**8**. DOI: 10.1371/journal.pone.0054002

[70] Mittler R. Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science. 2002;**7**:405-410

[71] Bozorov TA, Usmanov RM, Yang HL, Hamdullaev SA, Musayev S, Shavkiev J, et al. Effect of water deficiency on relationships between metabolism, physiology, biomass, and yield of upland cotton (*Gossypium hirsutum* L.). Journal of Arid Land. 2018;**10**:441-456. DOI: 10.1007/s40333-018-0009-y

[72] Basal H, Dagdelen N, Unay A, Yilmaz E. Effects of deficit drip irrigation ratios on cotton (*Gossypium hirsutum* L.) yield and fibre quality. Journal of Agronomy and Crop Science. 2009;**195**:19-29. DOI: 10.1111/j.1439-037X.2008.00340.x

[73] Zonta JH, Brandao ZN, Rodrigues JID, Sofiatti V. Cotton response to water deficits at different growth stages. Revista Caatinga. 2017;**30**:980-990. DOI: 10.1590/1983-21252017v30n419rc

[74] Li CD. Breeding crops by design for future agriculture. Journal of Zhejiang University: Science B. 2020;**21**:423-425. DOI: 10.1631/jzus.B2010001

[75] Hua K, Zhang JS, Botella JR, Ma CL, Kong FJ, Liu BH, et al. Perspectives on the application of genome-editing technologies in crop breeding. Molecular Plant. 2019;**12**:1047-1059. DOI: 10.1016/j.molp.2019.06.009

[76] Zhu HC, Li C, Gao CX. Applications of CRISPR-Cas in agriculture and plant biotechnology. Nature Reviews Molecular Cell Biology. 2020;**21**:661-677. DOI: 10.1038/s41580-020-00288-9

[77] Miki D, Wang R, Li J, Kong DL, Zhang L, Zhu JK. Gene targeting facilitated by engineered sequence-specific nucleases: Potential applications for crop improvement. Plant and Cell Physiology. 2021;**62**:752-765. DOI: 10.1093/pcp/pcab034

Chapter 6

Cotton Breeding in the View of Abiotic and Biotic Stresses: Challenges and Perspectives

Venera Kamburova, Ilkhom Salakhutdinov and Ibrokhim Y. Abdurakhmonov

Abstract

Global climate change manifested in average annual temperature rise and imbalance of most natural factors, such as changes in annual mean rainfall, air humidity, average temperature of cold and warm months, soil quality, etc., lead to climatic zones displacement. All these have a significant impact on agricultural production in total, including cotton growing. Cotton is one of the most important technical crops in the world. However, it is very sensitive to environmental changes. The influence of abiotic stresses (high temperature, changes in the mean rainfall and soil salinity) causes a dramatic decrease yield of this crop. Moreover, temperature anomalies and climatic zones displacement cause a change in the area of pathogens and pests distribution, which also reduces the cotton yield. One of the possible ways to increase the cotton yield under the influence of abiotic and biotic stresses is the development of new resistant varieties, using both classical breeding methods and genetic engineering achievements.

Keywords: cotton, global climate change, abiotic and biotic stresses, cotton breeding, genetic engineering

1. Introduction

Cotton (*Gossypium* ssp. L.) is the major source of quality natural fiber and widely contributed to textile and seed oil industry [1]. Currently, the annual global cotton fiber production is about 25 million metric tons; the market value estimated is $ 12 billion [2].

Because cotton is a subtropical plant, it is well adapted to survive with dry and hot environment [3]. Despite this, cotton nevertheless reacts to an environmental change such as temperature and rainfall in instance. Long-term exposure of negative factors such as a drought, salinity, and temperature stress causes a significant decrease of yield and fiber quality [4, 5]. Such negative effect on cotton is due to the fact that drought, salinity, and temperature stress cause osmotic imbalance, membrane disorganization, growth decrease, inhibition of cell fission and reproduction; this

also leads to decline of photosynthesis level and hyperproduction of reactive oxygen species (ROS) [6, 7].

In addition to abiotic stresses, the cotton production is greatly influenced by biotic factors, such as pests and diseases that also cause a significant (up to 10–30%) reduction in yield [8]. At the same time, global climate changes responsible for temperature factors and climatic zone displacement also affect their development, geographical distribution, pathogenicity or injuriousness [4].

In this regard to these threats to cotton production, breeders are facing the important task of new cotton varieties resistant to abiotic and biotic stresses. However, this problem-solving by the classical genetics methods has become complicated due to the resistance traits generally having multigenic nature with a complex type of inheritance [9]. Additionally, the breeding oriented on resistance is further complicated by the "bottle-neck" effect such as narrow genetic basis typical for cultivated cotton [10]. Nevertheless, these disadvantages may be successfully overcome by the use of genetic engineering methods: transgenesis, RNA interference, and genome editing approaches.

In this chapter, we would like to analyze and summarize information about increase of cotton resistance to abiotic and biotic factors using genetic engineering approaches.

2. Increasing resistance to abiotic stresses

Abiotic stresses are a direct consequence of climate change. The world increase of temperature is primarily caused by carbon dioxide effect, i.e., its content in the atmosphere. The increase in the average annual temperature is the cause of increase of water evaporation from the soil, which directly leads to osmotic (by drought) and salt stress. One of the features of abiotic stresses is their simultaneous exposure. In other words, they have usually a similar effect on plants and defense mechanisms appearance in plants [11].

Abiotic stresses may affect cotton upon all development stages and lead to significant decrease in both yield and quality of cotton fiber [12, 13]. Thus, an increase of temperature at 2–3°C from the optimum can decrease biomass and yield, as well as increase fiber micronaire [13]. Drought and salinity also cause a decrease in the yield and quality of cotton fiber [6, 14].

In this regard, increase of a cotton resistance to abiotic stresses will reduce a negative effect and can raise the yield and quality of fiber. In this chapter, we consider the impact of abiotic stresses on the morphological and physiological parameters, as well as the mechanisms of resistance development and methods for increasing the adaptive potential of cotton to negative environmental factors.

2.1 Influence of abiotic stresses on morphophysiological parameters in cotton

Influence of abiotic stresses on cotton plants manifested in various forms of morphophysiological and biochemical changes, which reduce yield and fiber quality of cotton [6, 13, 14]. They negatively affect both morphological (seed germination, plant height and architecture, length and area of root system, leaf area, shoot and root biomass, boll development) and physiological parameters (chlorophyll content, photosynthetic efficiency, transpiration rate, stomatal conductance) [6, 13, 14].

In addition, prolonged exposure to abiotic stresses leads to a decrease in yield and fiber quality. Yield reduction manifested by both a decrease in the number and weight of bolls and fiber yield [6, 13, 14]. At the same time, this negative effect on yield is explained by a decrease in the activity of catabolic processes, including photosynthesis [6, 13, 14]. The fiber quality reduction manifested in a decrease in fiber length and an increase in micronaire. Such influence of abiotic factors on one of the most important agronomic traits of cotton is caused both by reduction of carbohydrate synthesis due to reduction of photosynthesis activity and by disruption of elongation process due to changes of membrane permeability and organization of microtubules and cytoskeleton [13, 14].

Disruption of photosynthesis under abiotic stresses is associated with an increase in ion permeability of chloroplast thylakoids and a decrease in chlorophyll levels, as well as inhibition of the activity of the key enzyme of carbohydrate synthesis 1,5-bisphosphate carboxylase [13–16].

2.2 Mechanisms of resistance to abiotic stresses

To reduce the negative impact of abiotic stresses in plants, including cotton, they have developed some adaptations on physiological and molecular level.

Physiological adaptations include accumulation of soluble substances in vacuoles to maintain cell turgor and decrease of stomatal conductance to reduce transpiration [11, 13, 14].

Molecular defense mechanisms against abiotic stresses include accumulation of osmolytes (proline, betaine, and soluble sugars), changes in activity of antioxidant system reducing level of ROS, regulation of cell ion balance and hormonal activity, as well as changes in activity of heat stress proteins [6, 7, 13, 14, 17]. Let us consider each mechanism separately.

Antioxidant system. One of the aftereffects of abiotic stresses on cotton is an increased level of ROS due to disruption of cell respiration and photosynthesis [6, 7, 13, 14, 17–20]. An increased ROS level leads to oxidative damage to proteins, DNA and lipids, destabilization of membranes, and increase of their permeability [19, 21]. Neutralization of ROS in plants is carried out by antioxidant system that includes nonenzymatic antioxidants (flavonoids, carotenoids, tocopherols, glutathione, etc.) and various antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, peroxidase, ascorbate peroxidase, and glutathione reductase) [21–24]. In most crops, including cotton, the increased activity level of antioxidant enzymes is associated with resistance to abiotic stresses: higher activity of antioxidant enzymes had been seen in more resistance varieties [17, 19, 25].

Ion balance regulation in cell. Ion imbalance and toxicity accompanied by Na^+ accumulation are the main consequences of salt and osmotic stress [6, 7, 11, 14, 18, 26]. To reduce ion toxicity and restore ion balance, plant cells use the Ca^{2+}-dependent salt supersensitive (SOS) regulatory pathway, which regulates ion homeostasis by modulating Na^+/H^+-antiporter activity during salt stress [7, 14, 26, 27]. The SOS pathway consists of plasma membrane Na^+/H^+-antiporter (SOS1), protein kinase (SOS2), and two calcium sensors—SOS3 and SCaBP8 (SOS3-like calcium-binding protein 8) [26, 28].

The excessive accumulation of Na^+ in the cytoplasm also results in the accumulation of Ca^{2+}, which interacts with SOS3/SCaBP8, activating the serine/threonine protein kinase SOS2. Then, SOS2 phosphorylates SOS1, which increases Na^+/H^+-antiporter activity, restoring the ion balance in the cell and enhancing salt tolerance [7, 20, 26]. SOS3/SCaBP8-SOS2 also regulates the activity of other transporters involved in ion

homeostasis: K^+- and Na^+-transporters, vacuolar Na^+/H^+-exchanger (NHX), vacuolar H^+-ATPases, and pyrophosphatases (PPase) [18, 20, 26].

Accumulation of osmolytes. Most abiotic stresses lead to water imbalance and, as a consequence, the induction of osmotic stress, which reduces cell turgor and the activity of many enzymatic systems [7, 11, 14, 18, 20]. To reduce the osmotic stress affects, plant cells accumulated the following osmoprotectors such as proline, betaine, soluble sugars, etc. [7, 11, 14, 18]. These agents protect membrane lipids and proteins from oxidative damage, increase the photosynthesis rate, and restore the osmotic potential of the cell [7, 20, 26, 29, 30].

Hormonal regulation. Abscisic acid (ABA), ethylene, salicylic acid (SA), jasmonic acid (JA), and brassinosteroids (BR) are the main plant stress hormones [7, 26, 31]. ABA is considered a major stress hormone whose activity increases during drought and salinity [7, 31]. ABA promotes the accumulation of K^+, Ca^{2+}, and osmolytes, reducing the inhibitory effect of abiotic stresses [7, 26, 31]. SA and BR are also involved in plant responses to abiotic stress [7, 26, 31]. An increase in BR under salt stress contributes to the maintenance of ion and osmotic homeostasis, increasing the stress tolerance of plants [7, 26, 31]. In addition, the BR signaling cascade intersects with the SOS pathway. BR leads to calcium accumulation in the cytosol, which activates the SOS pathway through SOS3/SCaBP8 [7, 26, 31]. The protective effect of SA and JA under stress is due to the activation of plant antioxidant system [7, 26, 31].

Heat shock proteins (HSPs). HSPs are molecular chaperones and play an important role in plant resistance to temperature stress [13, 32]. Depending on the molecular weight, the following HSP groups are distinguished: small HSP (sHSP), HSP60, HSP70, HSP90, and HSP100 [13, 32]. sHSP and HSP100 bind to proteins, prevent their denaturation and aggregation, promote their refolding with the participation of ATP-dependent chaperones (ClpB/DnaK) [13, 32]. HSP60 (mitochondrial chaperone or chaperonin 60) contributes to the maintenance of normal mitochondrial and chloroplast functioning under heat stress by keeping the native state of the inner mitochondrial membrane proteins and chloroplast thylakoids [13, 32]. HSP70 is involved in protein folding and prevention of protein aggregation [13, 32]. In addition, inhibition of HSP70 gene expression in cotton leads to oxidative stress by increasing H_2O_2 levels, which suggests the involvement of this chaperone in the regulation of several antioxidant enzymes activity [13, 32]. HSP90 together with HSP70 regulates protein folding by participating in signal transduction through signaling kinases and hormone receptors [13, 32].

Thus, plants have numerous mechanisms to promote abiotic stresses resistance. The genes mediating these defense mechanisms may be potential objects of interest for enhancing the adaptive potential of plants to environmental stress conditions.

2.3 Improving the adaptive potential of cotton to abiotic stresses

A significant decrease in the yield and fiber quality under the influence of abiotic stresses assigns a task for breeders to create cotton varieties resistant to these stresses. To solve this problem, it can use the methods of classical breeding, methods of molecular selection, and genetic engineering. Let us consider the application, advantages, and disadvantages of these methods.

Classical breeding. Inheritance of abiotic stress tolerance traits in cotton is multigenic with complex intergenic interaction including additive and nonadditive, dominant, and epistatic effects [9, 14]. The complex mechanism of trait inheritance

and "bottle-neck" effect make it difficult to use classical breeding methods to obtain cotton varieties resistant to abiotic stress. Moreover, these methods require a lot of time to develop new varieties.

Marker-associated selection (MAS). The use of molecular markers and Quantitative Trait Loci (QTL) mapping made it possible to overcome the disadvantages of classical breeding in developing cotton varieties resistant to abiotic stress [13, 14, 33–35]. Simple sequence repeat (SSR) and single-nucleotide polymorphisms (SNPs) are most commonly used to identify QTL [13, 14, 33–35]. Thus, using SSR markers, 11 QTLs localized on eight chromosomes (c9, c11, c15, c16, c21, c23, c24, c26) associated with salt tolerance traits were identified in the test population from *G. tomentosum* and *G. hirsutum* cross [36]. In the same population, QTLs associated with drought tolerance were also localized on chromosomes c5, c8, c9, and c16 as well as some QTL clusters for same trait on chromosomes c2, c3, c5, c6, c9, c14, c15, c16, and c21 [37]. Additionally, 165 QTLs have been identified in an introgressed population of *G. hirsutum* under abiotic stress conditions using 481 SNPs and 523 SSR markers covered of most cotton chromosomes. In total, 15 of them have been common QTLs of tolerance to abiotic stresses localized in 12 chromosomes: c1, c2, c5, c6, c8, c9, c10, c12, c20, c23, c25, and c26 [14].

Presently, various strategies, including genotyping by sequencing (GBS), SNP arrays, and genome-wide association study (GWAS), as well as populations of recombinant inbred lines (RIL) and backcross inbred lines (BIL), are used to improve the efficiency of QTL mapping [14]. Thus, 95 loci that associated with salt tolerance in *G. hirsutum* were found using GWAS in combination with SSR markers [38]. GWAS in combination with polymorphic SNPs of the CottonSNP63 K array applied to determine resistance of upland cotton has revealed a drought tolerance QTLs on chromosomes c8, c15, c21, c24, c25, and c26 and salt tolerance QTLs on chromosomes c1, c9, c11, c12, c13, c14, c18, c21, and c24 [39]. These data have confirmed using GWAS in combination with SNPs for MAGIC population of *G. hirsutum* including of 550 RILs. It has found that 11 QTLs associated both drought and salt tolerance [40].

In addition, the use of meta-analysis allows improving the accuracy of QTL mapping associated with abiotic stresses. For example, this approach has identified 23 stress tolerance QTL clusters on 15 different cotton chromosomes: c3, c4, c5, c6, c7, c11, c14, c15, c16, c19, c20, c23, c24, c25, and c26 [41].

Summarizing the above, the use of molecular markers and associative mapping data can significantly reduce the time to breed resistant cotton varieties.

Transgenic approaches. These approaches are widely used to increase cotton resistance to abiotic stresses. Thus, overexpression of *AVP1* and *OsSIZ1* genes in cotton enhances its resistance under both drought and heat shock stresses [42]. Overexpression of *HSP101* gene also increases resistance of cotton to temperature stress [13]. Further, transformation of cotton by *AsHSP70* gene from *Agave sisalana* resulted in improvement of a number of physiological parameters under heat stress [43].

Application of transgenic approaches also allows increasing cotton resistance to drought and salinity. Many transcription factors, regulating the activity of functional genes, can influence drought and salt tolerance in cotton [13]. Thus, overexpression of transcription factor *GhABF2* increases both drought and salt tolerance in cotton through regulation of ABA cascade genes [44]. Overexpression of other transcription factor genes (*AtRAV1/2*, *AtABI5,* and *SNAC1*) also increases cotton resistance to drought and salinity [13].

Increase in defense capacity of cotton due to increase level of osmoprotectants and activity of antioxidant enzymes and ion antiporters also enhance the adaptive resistance of the crop to abiotic stresses [13]. Overexpression in cotton of *AtEDT1/HDG11* gene from *A. thaliana* led to the increase of proline level and activity of antioxidant enzymes, increasing the resistance to salt and osmotic stress [45]. Moreover, transformation of cotton by the H^+-phosphatase gene (*TsVP*) from *Thellungiella halophile* allowed to reduce the negative effect of salt stress on photosynthetic activity [13]. Individual and coexpression of H^+-pyrophosphatase (*AVP1*) and vacuolar Na^+/H^+-antiporter (*AtNHX1*) genes from *A. thaliana* led to the increase of cotton salt tolerance due to more efficient regulation of ion balance [13].

Regulation of hormonal status by overexpression of their biosynthesis genes can also increase the adaptive potential of cotton resistance to salt and osmotic stress. Thus, overexpression of isopentenyltransferase (*IPT*) gene, one of cytokinin biosynthesis genes, increased cotton resistance to drought and salinity [13, 46]. Furthermore, cotton transformation with AtLOS5 gene (involved in ABA biosynthesis) from *A. thaliana* increased drought tolerance of the crop [13].

In this way, the application of transgenic methods makes it possible to effectively increase cotton resistance to abiotic stresses. However, those approaches are limited by the legislative regulation of GMO in many countries, according to this all transgenic crops obliged to undergo a full cycle of biosafety assessment [47].

Modern methods of genetic engineering. To overcome the biosafety constraints of transgenic cotton, researchers use modern genetic engineering methods including RNA interference (RNAi) and genome editing (GE) approaches.

RNAi is one of promising approaches both for studying of resistance genes and developing new cotton varieties resistant to abiotic stresses [10, 48]. For example, the use of VIGS-mediated RNAi revealed that R2R3-type *GbMYB5* transcription factor increases cotton resistance to abiotic stresses due to proline accumulation and increase antioxidant enzymes activity [10]. It has been also found that the expression levels of several miRNAs in leaves (miR156, miR157, miR162, miR172, miR397, miR398, miR399) and roots (miR172, miR397, miR398, miR399) change under salt and osmotic stress [49]. In addition, RNAi of phytochrome A1 gene increased the resistance of cotton to salt stress by activation of antioxidant enzymes [17].

Application of GE approaches to increase the adaptive potential of cotton in accordance to abiotic stresses is currently quite limited. However, there are successful applications of GE in cotton. For example, the target editing of *GhRDL1* and *GhPIN1–3* genes by the use of CRISPR/Cas9 system has allowed to obtain drought-resistant cotton lines [50].

Summarizing the above, it should be noted that presently, marker-associated selection and transgenic methods have the greatest importance in breeding of cotton resistant to abiotic stresses.

3. Improving resistance to biotic factors

Biotic factors (insect pests and pathogens) are among the most important factors that reduce cotton productivity [4, 8, 51]. For example, losses of cotton yield from pests may be up to 84% [51] and due to pathogens up to 30% [8]. As in the case of abiotic factors, global climate change leads to a shift of climatic zone, affecting the growth, development, and spread of insect pests and pathogens [4]. As results, this leads to the emergence of new pests and pathogens in these areas.

In this regard, improving plant resistance to biotic factors allows effectively control of pests and pathogens to reduce yield losses. In this part, we are looking at the characteristics of the main pests and pathogens, as well as a natural defense mechanisms and methods of improving cotton resistance to them.

3.1 Characteristics of major pests and pathogens of cotton

Insect pests. Cotton pest insects can be divided into two groups according to the mechanism of plant damage: chewing and piercing-sucking [52]. The first group includes insects that feed the plant biomass: cotton bollworm (*Helicoverpa armigera*), fall armyworm (*Spodoptera frugiperda*), pink moth (*Pectinophora gossypiella*), spotted bollworm (*Earias vittella*), and cotton leafworm (*Alabama argillacea*). The pests of this group of insects are larvae (caterpillars) that feed on immature bolls and leaves [8, 52].

The second group includes sap feeding insects that damage phloem: boll weevil (*Anthonomus grandis*), cotton aphid (*Aphis gossypii*), thrips (*Frankliniella spp*, *Thrips tabaci*, *Neohydatothrips variabilis*, and *Scirtothrips dorsalis*), cotton seed bug (*Oxycarenus hyalinipennis*), tarnished plant bug (*Lygus lineolaris*), cotton fleahopper (*Pseudatomoscelis seriatus*), and two-spotted spider mite (*Tetranychus urticae*) [8, 52]. The pests in this group are adults and/or nymphs [52].

In addition, soil nematodes can also cause a significant cotton yield reduction [8]. Nematodes parasitizing on cotton include the root-knot nematode (*Meloidogyne incognita*), reniform nematode (*Rotylenchulus reniformis*), and sting nematode (*Belonolaimus longicaudatus*) [8].

Phytopathogens. Cotton pathogens include viruses, bacteria, and fungi [8, 53]. Fungi of genera *Fusarium*, *Rhizoctonia*, *Pythium,* and *Thielaviopsis* affect cotton seedlings causing seedling root rot [53]. Blackspot causes with fungus of *Alternaria macrospora* Zimm, leading to leaves damage. *Ramularia areola* causes Ramularia blight of cotton [53]. Cotton boll rot is a complex disease caused by several fungal pathogens such as *Fusarium moniliforme*, *Calletotrichum gossypii*, *C. capsici*, *Aspergillus flavus*, *A. niger*, *Rhizopus nigricans*, *Nematospora nagpuri,* and *Botryodiplodia sp*. This disease affects the bolls, spreads to inner tissues, and leads to rotting of the seeds and fibers [53]. The most dangerous form of boll-rot is anthracnose, caused by *Calletotrichum gossypii* Southw. Anthracnose in cotton can occur in all growth stages of the plant, and it can affect all tissues, causing seedling or young plants to wilt and die, as well as a severe reduction in fiber and seed yields [53].

F. oxysporum f. sp. *vasinfectum* causes development of cotton Fusarium wilt at seedling stage with cotyledon lesions [53]. Verticillium wilt is caused by *Verticillium dahliae* Kleb, which affects cotton leaves in the budding or immature bolls stages [53].

The viral diseases include cotton leaf curl disease (CLCuD) and (CLCrD) [53]. CLCuD is caused by begomoviruses that lead to leaf injury (swollen veins, leaf curl, enation, and stunting). When affected in the early stages of development, there is a significant reduction in yield [53]. The cotton leaf curl virus (CLCrD) affects the leaves resulting in leaf discoloration and vein hypertrophy, leaf curl, shortening of internodes, and growth stunting. The infestation degree depends on the stage of plant development [53].

Bacterial blight of cotton is one of the most serious diseases causing significant yield losses [8, 53]. Disease results from infection by *Xanthomonas citri* pv. *malvacearum* [8, 53]. Affected plants show the following symptoms such as defoliation, swelling and darkening of stems, bolls detachment. By severely affecting, the fiber quality is decreased due to coloration and the plant death [53].

3.2 Mechanisms of resistance to biotic factors

The long coevolution of cotton and insect pests and pathogens has resulted in mechanisms to reduce the damage from biotic factors. Molecular mechanisms of pathogen resistance include the activation of resistance genes (R-genes) in response to exposure. R-gene activation triggers a large number of intracellular cascades leading to the synthesis of protective substances that reduce the damage by pathogens [54, 55]. Morphological and chemical defense mechanisms have been developed in cotton to reduce the pest influence degree [56, 57]. Let us in more detail consider mechanisms of resistance to insect pests and pathogens correspondingly.

Resistance to pests. Morphological defenses and chemicals (secondary metabolites) of cotton directly influence the insect (imago or larvae) affecting important parameters of their life cycle [56]. Trichomes are considered as the major morphological adaptation of cotton that increases its insect resistance. These provide protection by forming a physical barrier or excreting chemical repellents, toxins, or adhesive substance [56].

Terpenoids, flavonoids, tannins, and anthocyanins are among the secondary metabolites providing direct protection of cotton plants from insects [56]. Terpenoids are the most studied protectors of cotton. Terpenoids synthesized in cotton include gossypol, hemigossypol, hemigossypolone, and heliocides H1, H2, H3, and H4 contained in small subepidermal and intracellular pigment glands [56]. Cotton terpenoids have direct toxic effect on insect pests including *H. virescens*, *H. zea*, *H. armigera*, *P. gossypiella*, *Estigmene acrea*, *E. insulana,* and *E. vitella*. In addition, gossypol and gossypol-like compounds are toxic to the gall nematode *Meloidogyne incognita* [56].

It should also be noted that damage of cotton by pests and pathogens causes induction of terpenoid biosynthesis by activation of JA-, SA-, and ethylene-dependent signaling pathways [56, 58]. These pathways activation occurs due to elicitors, which, interacting with specific receptors, lead to an increase in intracellular Ca^{2+}. This in turn activates calcium-dependent proteins, including Ca^{2+}-dependent protein kinases (CDPKs) [58]. CDPK, by phosphorylating proteins and changing gene expression patterns, activates mitogen-activated protein kinases (MAPK), leading to JA and SA formation, on the one hand, and the ethylene pathway, on the other [58].

Resistance to pathogens. Plant resistance to pathogens (plant immunity) is controlled by resistance genes (R-genes) [54]. R-genes encode surface (receptor-like kinases—RLK) or intracellular receptors (nucleotide-binding proteins with leucine-rich repeats—NLR) activating a various mechanism under interaction with them [54, 59–61]. One consequence of receptor activation is an increase intracellular Ca^{2+} concentration, leading both to the activation of the Ca^{2+}-dependent signaling cascade and an increase in ROS levels [59–61]. At the same time, ROS play the function of an intracellular signaling molecule contributing to the development of systemic acquired resistance (SAR). It should also be noted that both Ca^{2+}- and ROS-dependent signaling cascades by activation of JA-, SA-, and ethylene-dependent signaling pathways lead to synthesis of phytoalexins (mainly gossypol and gossypol-like terpenes), which play an important role in cotton resistance to pathogens [59–61]. For example, induction of gossypol synthesis in cotton has been proved by infestation with *Verticillium dahlia*, *Fusarium oxysoporum* f.sp. *vasinfectum*, *Rhizoctonia solani*, *Rhizobium rhizogenes,* and *Xanthomonas spp* [56].

Thus, plants have a various mechanisms that provide resistance to pests and pathogens. Genes mediating these defense mechanisms may be potential genes for improving cotton resistance to biotic factors. In addition, the control of genes of the

causative agents themselves, playing an important role in their life activity, may also be of potential interest.

3.3 Improving cotton resistance to biotic factors

Biotic factors (pathogens and pests) are one of the main reasons for significant yield losses (up to 84% due to insects and up to 30% for pathogens) in agriculture [4, 8, 51]. At the same time, strategies to control infestations are an increase in the internal defense mechanisms of plants or introduction of pathogen-targeted constructs into the genome [62, 63]. Methods of classical breeding, molecular breeding, and genetic engineering are used to develop new varieties that are resistant to the impact of biotic factors. Let us consider the application, as well as advantages and disadvantages of each of these methods.

Classical breeding. Classical breeding methods increase the internal defense mechanisms of the plants and use the cotton germplasm reserves to produce new resistant varieties. For example, among all cultivated cotton species (*G. hirsutum* L., *G. barbadense* L., *G. arboretum* L., and *G. herbaceum* L.), only *G. barbadense* has sufficient resistance to *Verticillium dahlia*. However, transgenesis of the resistance into upland cotton by classical breeding methods has so far not been successful [59].

Such interspecific crossing for the purpose of transfer wilt resistance genes is complicated by different type of these traits inheritance in *G. hirsutum* and *G. barbadense L*. Studies of interspecific crossing show dominant or partially dominant inheritance of resistance traits, while by intraspecific crossing of *G. hirsutum*, the traits inheritance is more complex [59]. A number of studies report wilt resistance control by a single dominant gene, while others state that resistance is a quantitative trait [59, 64]. An additional difficulty is the fact that varieties with high wilt resistance have low fiber yield and quality, as well as crop yield [59]. In addition, it should be noted that classical breeding methods are time-consuming, which reduces the effectiveness of this approach in breeding pathogen-resistant cotton varieties.

Marker-associated selection (MAS). MAS and QTL mapping have been widely used in the development of cotton varieties resistant to Verticillium and Fusarium wilt. For example, more than 400 QTL of resistances to both kind of wilt have been identified, which are distributed over all 26 pairs of chromosomes [34, 59, 64]. These data were obtained both using mapping of chromosome-substituted and RIL populations with the help of various markers type and GWAS [59, 64–68].

Furthermore, a meta-analysis of the consensus map of Cotton Marker Database (CMD) based on *G. hirsutum* × *G. barbadense* cross, five mutagenesis “hot spots” of wilt resistance were identified on c16 and c23 chromosomes [69]. Same meta-analysis revealed that 74 QTLs of nematode resistance are localized on all chromosomes. Thus, 71 QTLs of them are associated with resistance to root-knot nematode, and three remains with resistance to reniform nematode. Especially, the greatest number of QTLs for this trait was identified on chromosomes c7 and c11. The mutagenesis hotspot of nematode resistance is also located on chromosome c7 [69, 70]. Additionally, this study shown that two QTLs of resistance to *Xanthomonas campestris* pv. *Malvacearum* are localized on chromosomes c5 and c14 [69].

The obtained data of a QTL mapping can be successfully used in further MAS and genomic breeding programs.

Transgenic approaches. These approaches are currently the most effective method for creating cotton varieties resistant to insect pests [8]. According to ISAAA, transgenic cotton occupies about 79% of the total cultivated area of this crop [71]. Despite

the existence of various strategies to develop insect-resistant transgenic crops (use of several genes with insecticidal properties such as inhibitors of insect's digestive proteases, α-amylase, lectin, etc.), most transgenic insect-resistant (IR) crops, including cotton, are based on insertion of *cry* genes encoding *Bacillus thuringiensis* (Bt) toxin in the host plant genome [8, 72]. Bt (or Cry) toxins have specific activity against insect from orders such as *Lepidoptera*, *Coleoptera*, *Hymenoptera,* and *Diptera*, as well as for nematodes [8, 72]. The cultivation of Bt-cotton has significantly reduced the use of insecticides in cotton-growing countries [72]. The use of transgenic cotton plants with Bt-gene sets further expands the potential of transgenic cotton and reduces the emergence of resistant insect populations [8, 72].

Thus, vegetative insecticidal proteins (*Vip*s) from *B. thuringiensis* with insecticidal activity against *Gossypium spp*. pests are promising for the creation of transgenic IR-cotton [8]. The proteins *Vip1* and *Vip2* are binary toxins, which are very toxic to some representatives of *Coleoptera* and *Hemiptera*. The action mechanism of *Vip3* is similar to that of Bt-toxins [8].

In order to create varieties resistant to fungal pathogens (*Rhizoctonia solani*, *Alternaria alternata*, *Alternaria macrospora,* and *Fusarium oxysporum*), transformation of cotton with genes encoding chitinases has been used [8]. Glucose oxidase genes were introduced into the cotton genome to improve resistance to *V. dahliae*, while the harpin encoding gene (*hpa1Xoo*) from *Xanthomonas oryzae* pv. *oryzae* is used to provide resistance to various pathogens [8]. Transformation of cotton with the antisense movement protein (*AV2*) and antisense coat protein (*ACP*) genes from CLCuV results in resistance to CLCuD [8].

In accordance with above, the application of transgenic technology is currently the most used and commercially successful for creating pest and pathogen resistant crops. However, the most serious disadvantage of this technology is the need for long-term biosafety assessment of transgenic cotton to minimize risks of human health and the environment [47].

RNA interference (RNAi). The host-induced gene silencing (HIGS) approach, in which a construct is introduced into the host genome that induces posttranslational suppression of gene expression in pathogen or pest through dsRNA upon infection, is most commonly used to achieve resistance to biotic factors with RNAi [8, 10, 59]. Thus, introduction of RNAi construct to hygrophobins1 (*VdH1*) gene of *V. dahliae* into cotton genome provides resistance to this pathogen [73]. A similar effect is achieved by HIGS to *V. dahliae VdRGS1* gene mediated by tobacco rattle virus [74].

The use of HIGS to the genes encoding proteins that play an important role in the life maintenance of insect allows the development of cotton IR lines. Thus, silencing of cytochrome P450 gene of insect monooxygenase (*CYP6AE14*) involved in gossypol detoxification leads a significant increase in cotton resistance to cotton bollworm (*Helicoverpa armigera*) [10, 75].

Another approach to improve cotton resistance to biotic stresses is virus-induced gene silencing (VIGS) of the host genome [10]. Thus, VIGS-mediated suppression of *GhNDR1*, *GhMKK2,* and *GbVE1* gene expression in cotton increased its resistance to *V. dahliae* [10].

Summarizing these, RNAi is a promising approach to develop cotton varieties resistant to biotic stresses. However, the application of this approach is limited by high probability of effect on nontarget organisms and complexity of cotton genome, due to tetraploidy [10].

Genome editing approaches. GE methods are also promising for developing pathogen and pest-resistant cotton varieties. For example, CRISPR/Cas9-mediated editing

of Gh14–3-3d gene, which is a negative regulator of disease resistance, has allowed obtaining cotton lines with high resistance to *V. dahliae* [50, 76]. However, despite the promise of GE methods, their application is limited by the complexity of the genome of cultivated tetraploid cotton species, needing to edit both homologs in A- and D-subgenomes.

Thus, summarizing the data above, transgenic methods are currently the most used and commercially successful strategy for developing of new insect pest and pathogen-resistant varieties.

4. Conclusion and future perspectives

Global climate change has a significant impact on cotton production through the complex impact of abiotic and biotic factors, reducing yields and fiber quality [4, 8, 13, 14, 51]. This poses a task to breeders of developing new cotton varieties that are resistant to abiotic and biotic stresses. To challenge it, breeders use both classical and molecular breeding methods and genetic engineering.

By developing cotton varieties resistant to abiotic stresses, molecular breeding methods are more often used, while genomic transgenomic methods improve resistance to insect pests and pathogens [8, 14]. However, the use of modern genetic engineering approaches, including cis- and intragenesis methods, is limited by the complexity of the genome of cultivated tetraploid cotton species. Therefore, the application of RNAi and GE methods to obtain cotton varieties resistant to abiotic and biotic stresses is currently insignificant [8, 14].

In addition, the insignificance of using molecular breeding methods to create pest and pathogen-resistant cotton varieties should be noted. This is due to the insignificant number of mapped insect and pathogen resistance loci in the cotton genome [59–70].

Fundamental understanding of molecular and genetic mechanisms underlying cotton resistance to abiotic and biotic stresses will allow application of cis- and intragenesis methods as well as RNAi and GE technologies in new resistant varieties development. Thus, the genes encoding DRE-binding protein 1 (*GhDBP1*), Na^+/H^+-antiporter (*SOS1*; *GhNHX1*), and H^+-pyrophosphatase (*AVP1*) are promising genes for improving drought and salt tolerance [14]. Overexpression of own heat shock genes can be used to improve resistance to heat stress [13]. RNAi and GE technologies can also be used to reduce the expression level of negative regulators of resistance to abiotic stresses.

Studying the mechanisms of interaction between the host plant and insect pests or pathogens, as well as the molecular and genetic basis of life support functions of causative agents, will allow more successful use of the HIGS, RNAi, and GE technologies to suppress key genes and cisgenesis technologies to enhance the host plant defense mechanisms. Genes encoding Vacuolar-type ATPase (*V-ATPaseE*), tubulin-folding cofactor D (*TBCD*), choline acetyltransferases, receptor for activated C kinases (*RACK*), and zinc finger transcription factor (*HUNCHBACK*) are promising for HIGS approach application. Overexpression of key genes of stress hormone biosynthesis (SA, JA, and ethylene) can be used to enhance the protective properties of cotton. In addition, pyramiding the genes for different resistance traits to develop varieties with combined resistance to stresses is promising.

Thus, modern molecular biology technologies have great potential to reduce the negative effect of global climate changes on cotton yield and fiber quality.

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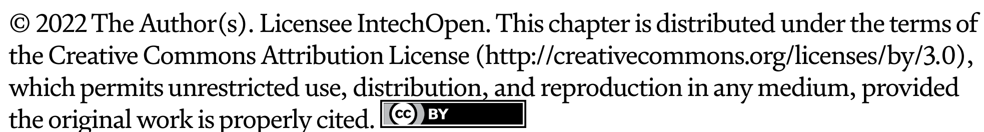

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References

[1] Munir H, Rasul F, Ahmad A, et al. Diverse uses of cotton: From products to byproducts. In: Ahmad S, Hasanuzzaman M, editors. Cotton Production and Uses. Singapore: Springer Nature Singapore Pte Ltd; 2020. pp. 629-641. DOI: 10.1007/978-981-15-1472-2_30

[2] Khan MA, Wahid A, Ahmad M, Tahir MT. World cotton production and consumption: An overview. In: Ahmad S, Hasanuzzaman M, editors. Cotton Production and Uses. Singapore: Springer Nature Singapore Pte Ltd; 2020. pp. 1-7. DOI: 10.1007/978-981-15-1472-2_1

[3] Arshad Awan Z, Khaliq T, Masood Akhtar M, et al. Building climate-resilient cotton production system for changing climate scenarios using the DSSAT model. Sustainability. 2021;**13**:10495. DOI: 10.3390/su131910495

[4] Bange M, Baker JT, Bauer PJ, et al. Climate Change and Cotton Production in Modern Farming Systems. Boston: CAB International; 2016. p. 61

[5] Jans Y, von Bloh W, Schaphoff S, Müller C. Global cotton production under climate change—Implications for yield and water consumption. Hydrology and Earth System Sciences. 2021;**25**:2027-2044. DOI: 10.5194/hess-25-2027-2021

[6] Sharif I, Aleem S, Farooq J, et al. Salinity stress in cotton: Effects, mechanism of tolerance and its management strategies. Physiology and Molecular Biology of Plants. 2019;**25**:807-820. DOI: 10.1007/s12298-019-00676-2

[7] van Zelm E, Zhang Y, Testerink C. Salt tolerance mechanisms of plants. Annual Review of Plant Biology. 2020;**71**:24.1-24.31. DOI: 10.1146/annurev-arplant-050718-100005

[8] Tarazi R, Jimenez JL, Vaslin MF. Biotechnological solutions for major cotton (*Gossypium hirsutum*) pathogens and pests. Biotech Res Innov. 2020;**3**:19-26. DOI: 10.1016/j.biori.2020.01.001

[9] Surabhi GK, Badajena B, Sahoo SK. Genome editing and abiotic stress tolerance in crop plants. In: Wani SH, editor. Recent Approaches in Omics for Plant Resilience to Climate Change. Switzerland: Springer Nature; 2019. pp. 35-56. DOI: 10.1007/978-3-030-21687-0_2

[10] Abdurakhmonov IY, Buriev ZT, Saha S, Jenkins JN, Abdukarimov A, Pepper AE. RNA interference for functional genomics and improvement of cotton (*Gossypium* spp.). Frontiers in Plant Science. 2016;**7**:202. DOI: 10.3389/fpls.2016.00202

[11] Rani S, Kumar P, Suneja P. Biotechnological interventions for inducing abiotic stress tolerance in crops. Plant Gene. 2021;**27**:100315. DOI: 10.1016/j.plgene.2021.100315

[12] Noreen S, Ahmad Sh, Fatima Z, et al. Abiotic stresses mediated changes in morphophysiology of cotton plant. In: Ahmad S, Hasanuzzaman M, editors. Cotton Production and Uses. Singapore: Springer Nature Singapore Pte Ltd; 2020. pp. 341-366. DOI: 10.1007/978-981-15-1472-2_18

[13] Majeed S, Rana IA, Mubarik MS, et al. Heat stress in cotton: A review on predicted and unpredicted growth-yield anomalies and mitigating breeding strategies. Agronomy. 2021;**11**:1825. DOI: 10.3390/agronomy11091825

[14] Abdelraheem A, Esmaeili N, O'Connell M, Zhang J. Progress and perspective on drought and salt stress tolerance in cotton. Industrial Crops and Products. 2019;**130**:118-129. DOI: 10.1016/j.indcrop.2018.12.070

[15] Karademir E, Karademir C, Sevilmis U, Basal H. Correlations between canopy temperature, chlorophyll content and yield in heat tolerant cotton (*Gossypium hirsutum* L.) genotypes. Fresenius Environmental Bulletin. 2018;**27**:5230-5237

[16] Chaudhary S, Devi P, Bhardwaj A, Jha UC, et al. Identification and characterization of contrasting genotypes/cultivars for developing heat tolerance in agricultural crops: Current status and prospects. Frontiers in Plant Science. 2020;**11**:587264. DOI: 10.3389/fpls.2020.587264

[17] Kamburova VS, Ubaydullaeva KA, Shermatov SE, Buriev ZT, et al. Influence of RNA interference of phytochrome A1 gene on activity of antioxidant system in cotton. Physiological and Molecular Plant Pathology. 2022;**117**:101751. DOI: 10.1016/j.pmpp.2021.101751

[18] Gong Z. Plant abiotic stress: New insights into the factors that activate and modulate plant responses. Journal of Integrative Plant Biology. 2021;**63**:429-430. DOI: 10.1111/jipb.13079

[19] Hasanuzzaman M, Bhuyan MHMB, Zulfiqar F, et al. Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. Antioxidants. 2020;**9**:681. DOI: 10.3390/antiox9080681

[20] Liang W, Ma X, Wan P, Liu L. Plant salt-tolerance mechanism: A review. Biochemical and Biophysical Research Communications. 2018;**495**:286-291. DOI: 10.1016/j.bbrc.2017.11.043

[21] Caverzan A, Casassola A, Brammer SP. Reactive oxygen species and antioxidant enzymes involved in plant tolerance to stress. In: Shanker A, Shanker C, editors. Abiotic and Biotic Stress in Plants—Recent Advances and Future Perspectives. Vol. p. Croatia: IntechOpen; 2016. pp. 463-480. DOI: 10.5772/61368

[22] Nouman W, Qureshi M.K., Shaheen M., Zubair M. Variation in plant bioactive compounds and antioxidant activities under salt stress. In: Vats Sh, editor. Biotic and Abiotic Stress Tolerance in Plants. Singapore: Springer Nature Singapore Pte Ltd; 2018. pp. 77-102. DOI: 10.1007/978-981-10-9029-5_4

[23] Saini P, Gani M, Kaur JJ, Godara LC, et al. Reactive oxygen species (ROS): A way to stress survival in plants. In: Zargar SM, Zargar MY, editors. Abiotic Stress-Mediated Sensing and Signaling in Plants: An Omics Perspective. Vol. p. Singapore: Springer Nature Singapore Pte Ltd; 2018. pp. 127-154. DOI: 10.1007/978-981-10-7479-0_4

[24] Sachdev S, Ansari SA, Ansari MI, Fujita M, Hasanuzzaman M. Abiotic stress and reactive oxygen species: Generation, signaling, and defense mechanisms. Antioxidants. 2021;**10**:277. DOI: 10.3390/antiox10020277

[25] Guo H, Hu Z, Zhang H, Min W, Hou Z. Comparative effects of salt and alkali stress on antioxidant system in cotton (*Gossypium hirsutum* L.) leaves. Open Chemistry. 2019;**17**:1352-1360. DOI: 10.1515/chem-2019-0147

[26] Zhao S, Zhang Q, Liu M, et al. Regulation of plant responses to salt stress. International Journal of Molecular Sciences. 2021;**22**:4609. DOI: 10.3390/ijms22094609

[27] Park HJ, Kim WY, Yun DJ. A new insight of salt stress signaling in plant.

Molecules and Cells. 2016;**39**(6):447-459. DOI: 10.14348/molcells.2016.0083

[28] Quan R, Lin H, Mendoza I, Zhang Y, et al. SCABP8/CBL10, a putative calcium sensor, interacts with the protein kinase SOS2 to protect *Arabidopsis* shoots from salt stress. The Plant Cell. 2007;**19**:1415-1431. DOI: 10.1105/tpc.106.042291

[29] Alhasnawi AN. Role of proline in plant stress tolerance: A mini review. Research on Crops. 2019;**20**:223-229. DOI: 10.31830/2348-7542.2019.032

[30] Peng J, Liu J, Zhang L, Luo J, Dong H, et al. Effects of soil salinity on sucrose metabolism in cotton leaves. PLoS One. 2016;**11**(5):e0156241. DOI: 10.1371/journal.pone.0156241

[31] Yu Z, Duan X, Luo L, Dai S, et al. How plant hormones mediate salt stress responses. Trends in Plant Science. 2020;**2020**(25):1117-1130. DOI: 10.1016/j.tplants.2020.06.008

[32] Ahmad F, Perveen A, Mohammad N, Ali MA. Heat stress in cotton: Responses and adaptive mechanisms. In: Ahmad S, Hasanuzzaman M, editors. Cotton Production and Uses. Singapore: Springer Nature Singapore Pte Ltd; 2020. pp. 393-428. DOI: 10.1007/978-981-15-1472-2_20

[33] Katageri IS, Gowda SA, Prashanth BN, Biradar M, et al. Prospects for molecular breeding in cotton, *Gossypium* spp. In: Abdurakhmonov IY, editor. Plant Breeding—Current and Future Views. Vol. p. London: IntechOpen; 2020. pp. 231-266. DOI: 10.5772/intechopen.94613

[34] Kushanov FN, Turaev OS, Ernazarova DK, Gapparov BM, et al. Genetic diversity, QTL mapping, and marker assisted selection technology in cotton (*Gossypium* spp.). Frontiers in Plant Science. 2021;**12**:779386. DOI: 10.3389/fpls.2021.779386

[35] Bolek Y, Hayat K, Adem B, Azhar MT. Insight in the utilization of marker assisted selection in cotton (a review). Molecular Plant Breeding. 2016;**7**(10): 1-17. DOI: 10.5376/mpb.2016.07.0010

[36] Oluoch G, Zheng J, Wang X, Khan MKR, et al. QTL mapping for salt tolerance at seedling stage in the interspecific cross of *Gossypium tomentosum* with *Gossypium hirsutum*. Euphytica. 2016;**209**:223-235. DOI: 10.1007/s10681-016-1674-6

[37] Zheng JY, Oluoch G, Riaz MK, Wang XX, et al. Mapping QTLs for drought tolerance in an F2:3 population from an inter-specific cross between *Gossypium tomentosum* and *Gossypium hirsutum*. Genetics and Molecular Research. 2016;**15**:gmr.15038477. DOI: 10.4238/gmr.15038477

[38] Du L, Wu S, Zhang F, Hou S, Guo W. Evaluation and exploration of favorable QTL alleles for salt stress related traits in cotton cultivars (*G. hirsutum* L.). PLoS One. 2016;**11**(3):e0151076. DOI: 10.1371/journal.pone.0151076

[39] Abdelraheem A. Joint genetic linkage mapping and genome-wide association study of drought and salinity tolerance and Verticillium wilt and thrips resistance in cotton [PhD dissertation]. Vol. 24. Las Cruces, NM, USA: New Mexico State University; 2017

[40] Abdelraheem A, Fang DD, Zhang JF. Quantitative trait locus mapping of drought and salt tolerance in an introgressed recombinant inbred line population of upland cotton under the greenhouse and *fi*eld conditions. Euphytica. 2018;**214**:8. DOI: 10.1007/s10681-017-2095-x

[41] Abdelraheem A, Liu F, Song M, Zhang JF. A meta-analysis of quantitative trait loci for abiotic and biotic stress resistance in tetraploid cotton. Molecular Genetics and Genomics. 2017;**292**(6):1221-1235. DOI: 10.1007/s00438-017-1342-0

[42] Esmaeili N, Cai Y, Tang F, Zhu X, et al. Towards doubling fibre yield for cotton in the semiarid agricultural area by increasing tolerance to drought, heat and salinity simultaneously. Plant Biotechnology Journal. 2021;**19**:462. DOI: 10.1111/pbi.13476

[43] Batcho AA, Sarwar MB, Rashid B, Hassan S, Husnain T. Heat shock protein gene identified from *Agave sisalana* (As HSP70) confers heat stress tolerance in transgenic cotton (*Gossypium hirsutum*). Theoretical and Experimental Plant Physiology. 2021;**33**:141-156. DOI: 10.1007/s40626-021-00200-6

[44] Liang C, Meng Z, Meng Z, Malik W. GhABF2, a bZIP transcription factor, confers drought and salinity tolerance in cotton (*Gossypium hirsutum* L.). Scientific Reports. 2016;**6**:35040. DOI: 10.1038/srep35040

[45] Yu LH, Wu SJ, Peng YS, Liu RN, et al. Arabidopsis *EDT1*/*HDG11* improves drought and salt tolerance in cotton and poplar and increases cotton yield in the field. Plant Biotechnology Journal. 2016;**14**:72-84. DOI: 10.1111/pbi.12358

[46] Zhu X, Sun L, Kuppu S, Hu R, et al. The yield difference between wild-type cotton and transgenic cotton that expresses IPT depends on when water-deficit stress is applied. Scientific Reports. 2018;**8**:2538. DOI: 10.1038/s41598-018-20944-7

[47] Hilbeck A, Meyer H, Wynne B, et al. GMO regulations and their interpretation: How EFSA's guidance on risk assessments of GMOs is bound to fail. Environmental Sciences Europe. 2020;**2020**(32):5. DOI: 10.1186/s12302-020-00325-6

[48] Abdellatef E, Kamal NM, Tsujimoto H. Tuning beforehand: A foresight on RNA interference (RNAi) and in vitro-derived dsRNAs to enhance crop resilience to biotic and abiotic stresses. International Journal of Molecular Sciences. 2021;**22**:7687. DOI: 10.3390/ijms22147687

[49] Wang M, Wang Q, Zhang B. Response of miRNAs and their targets to salt and drought stresses in cotton (*Gossypium hirsutum* L.). Gene. 2013;**530**:26-32. DOI: 10.1016/j.gene.2013.08.009

[50] Fiaz S, Khan SA, Younas A, Shahzad K, et al. Application of CRISPR/Cas system for genome editing in cotton. In: Abd-Elsalam KA, Lim K-T, editors. CRISPR and RNAi Systems: Nanobiotechnology Approaches to Plant Breeding and Protection. Oxford: Elsevier Inc.; 2021. pp. 277-301. DOI: 10.1016/B978-0-12-821910-2.00010-2

[51] Kamburova V, Abdurakhmonov IY. Overview of the biosafety and risk assessment steps for insect-resistant biotech crops. In: Emani C, editor. The Biology of Plant-Insect Interactions. In A Compendium for the Plant Biotechnologist. USA: CRC Press; 2018. pp. 178-203. DOI: 10.1201/9781315119571

[52] Anees M, Shad SA. Insect pests of cotton and their management. In: Ahmad S, Hasanuzzaman M, editors. Cotton Production and Uses. Singapore: Springer Nature Singapore Pte Ltd; 2020. pp. 177-212. DOI: 10.1007/978-981-15-1472-2_11

[53] Chohan S, Perveen R, Abid M, Tahir MN, Saji M. Cotton diseases

and their management. In: Ahmad S, Hasanuzzaman M, editors. Cotton Production and Uses. Singapore: Springer Nature Singapore Pte Ltd; 2020. pp. 239-270. DOI: 10.1007/978-981-15-1472-2_13

[54] Deng Y, Ning Y, Yang D-L, Zhai K. Molecular basis of disease resistance and perspectives on breeding strategies for resistance improvement in crops. Molecular Plant. 2020;**13**:1402-1419. DOI: 10.1016/j.molp.2020.09.018

[55] Billah M, Li F, Yang Z. Regulatory network of cotton genes in response to salt, drought and wilt diseases (Verticillium and Fusarium): Progress and perspective. Frontiers in Plant Science. 2021;**12**:759245. DOI: 10.3389/fpls.2021.759245

[56] Hagenbucher S, Olson DM, Ruberson JR, Wackers FL, Romeis J. Resistance mechanisms against arthropod herbivores in cotton and their interactions with natural enemies. Critical Reviews in Plant Sciences. 2013;**32**:458-482. DOI: 10.1080/07352689.2013.809293

[57] Mitchell C, Brennan RM, Graham J, Karley AJ. Plant defense against herbivorous pests: Exploiting resistance and tolerance traits for sustainable crop protection. Frontiers in Plant Science. 2016;**7**:1132. DOI: 10.3389/fpls.2016.01132

[58] Fürstenberg-Hägg J, Zagrobelny M, Bak S. Plant defense against insect herbivores. International Journal of Molecular Sciences. 2013;**14**:10242-10297. DOI: 10.3390/ijms140510242

[59] Palanga KK, Liu R, Ge Q, Gong J, et al. Current advances in pathogen-plant interaction between *Verticillium dahlia* and cotton provide new insight in the disease management. Journal of Cotton Research. 2021;**4**:25. DOI: 10.1186/s42397-021-00100-9

[60] Chen JY, Huang JQ, Li NY, Ma X-F, et al. Genome-wide analysis of the gene families of resistance gene analogues in cotton and their response to Verticillium wilt. BMC Plant Biology. 2015;**15**:148. DOI: 10.1186/s12870-015-0508-3

[61] Shaban M, Miao Y, Ullah A, Khan AQ, et al. Physiological and molecular mechanism of defense in cotton against *Verticillium dahliae*. Plant Physiology and Biochemistry. 2018;**125**:193-204. DOI: 10.1016/j.plaphy.2018.02.011

[62] Diaz I. Plant defense genes against biotic stresses. International Journal of Molecular Sciences. 2018;**19**:2446. DOI: 10.3390/ijms19082446

[63] Bisht DS, Bhatia V, Bhattacharya R. Improving plant-resistance to insect-pests and pathogens: The new opportunities through targeted genome editing. Seminars in Cell & Developmental Biology. 2019;**96**:65-76. DOI: 10.1016/j.semcdb.2019.04.008

[64] Zhang J, Fang H, Zhou H, et al. Genetics, breeding, and marker-assisted selection for Verticillium wilt resistance in cotton. Crop Science. 2014;**54**(4):1289-1303. DOI: 10.2135/cropsci2013.08.0550

[65] Zhao J, Liu J, Xu J, et al. Quantitative trait locus mapping and candidate gene analysis for Verticillium wilt resistance using *Gossypium barbadense* chromosomal segment introgressed line. Frontiers in Plant Science. 2018;**9**:682. DOI: 10.3389/fpls.2018.00682

[66] Zhao Y, Chen W, Cui Y, et al. Detection of candidate genes and development of KASP markers for Verticillium wilt resistance by combining genome-wide association study, QTL-seq and transcriptome sequencing in cotton.

Theoretical and Applied Genetics. 2021;**134**(4):1063-1081. DOI: 10.1007/s00122-020-03752-4

[67] Abdelraheem A, Elassbli H, Zhu Y, et al. A genome-wide association study uncovers consistent quantitative trait loci for resistance to Verticillium wilt and Fusarium wilt race 4 in the US upland cotton. Theoretical and Applied Genetics. 2020;**133**(2):563-577. DOI: 10.1007/s00122-019-03487-x

[68] Wang C, Ulloa M, Duong T, Roberts PA. Quantitative trait loci mapping of multiple independent loci for resistance to *Fusarium oxysporum* f. sp. *vasinfectum* races 1 and 4 in an interspecific cotton population. Phytopathology. 2018;**108**:759-767. DOI: 10.1094/PHYTO-06-17-0208-R

[69] Said JI, Lin Z, Zhang X, Song M, Zhang J. A comprehensive meta QTL analysis for fiber quality, yield, yield related and morphological traits, drought tolerance, and disease resistance in tetraploid cotton. BMC Genomics. 2013;**14**:776. DOI: 10.1186/1471-2164-14-776

[70] Kumar P, He Y, Singh R, et al. Fine mapping and identification of candidate genes for a QTL affecting *Meloidogyne incognita* reproduction in upland cotton. BMC Genomics. 2016;**17**:567. DOI: 10.1186/s12864-016-2954-1

[71] ISAAA. Global Status of Commercialized Biotech/GM Crops in 2019: Biotech Crops Drive SocioEconomic Development and Sustainable Environment in the New Frontier. ISAAA Brief No. 55. New York: ISAAA; 2019. p. 20

[72] Razaq M, Mensah R, Athar H-ur-R. Insect pest management in cotton. In: Jabran Kh, Chauhan BS, editors. Cotton Production. USA: John Wiley & Sons Ltd; 2020. pp. 85-107. DOI: 10.1002/9781119385523.ch5

[73] Zhang T, Jin Y, Zhao JH, et al. Host-induced gene silencing of the target gene in fungal cells confers effective resistance to the cotton wilt disease pathogen *Verticillium dahliae*. Molecular Plant. 2016;**9**(6):939-942. DOI: 10.1016/j.molp.2016.02.008

[74] Xu J, Wang X, Li Y, et al. Host-induced gene silencing of a regulator of G protein signalling gene (*Vd RGS1*) confers resistance to Verticillium wilt in cotton. Plant Biotechnology Journal. 2018;**16**(9):1629-1643. DOI: 10.1111/pbi.12900

[75] Rodrigues TB, Figueira A. Management of insect pest by RNAi—A new tool for crop protection. In: Abdurakhmonov IY, editor. RNA Interference. Vol. p. London: IntechOpen; 2016. pp. 371-390. DOI: 10.5772/61807

[76] Zhang Z, Ge X, Luo X, Wang P, Fan Q, Hu G, et al. Simultaneous editing of two copies of GH14-3-3D confers enhanced transgene-clean plant defense against *Verticillium dahlia* in allotetraploid upland cotton. Frontiers in Plant Science. 2018;**9**:1-13. DOI: 10.3389/fpls.2018.00842

Chapter 7

Sustainable and Effective Management Strategies in Cotton Cultivation

Ertuğrul Karaş

Abstract

Cotton, which is one of the leading fiber and oilseed crops, consumes 16% of the total pesticides and about 24% of insecticides in the world. In arid climatic regions such as Turkey, most of the plant water consumption is met by surface irrigation methods, while a significant part of it infiltrates deep. During cultivation, a significant portion of pesticides and chemical fertilizers are consumed incorrectly, or unconsciously due to socioeconomic and cultural reasons such as the lack of education of farmers and low economic income. For this reason, it is necessary to understand the correct cultivation techniques from planting to harvest and to manage critical periods in practice. Owing to this, it is necessary to re-evaluate and sustain high-productivity and quality cotton cultivation together with human and environmental requirements. Especially for this purpose, the charts and figures prepared to give direction to experts are a tool for a correct and complete understanding of the topics covered. Considering the objectives and needs of agricultural production, the analysis of the most critical issues required for cotton cultivation from a different perspective will be an important stage for the next steps.

Keywords: sustainable management, cotton cultivation, irrigation, nutrient management, cotton yield

1. Introduction

Cotton (*Gossypium spp.*) is used to obtain fiber, oilseeds animal feed, cellulose, and biofuel [1]. It is among the plants that provide employment and income to millions of people for its production, processing, and marketing besides having an important share in the economy. The annual contribution of cotton to the textile industry is 600 billion dollars. More than 80% of the total cotton production of 25 million tons per year is produced in the top 10 countries, mainly India, China, the United States, Pakistan, Brazil, Pakistan, Uzbekistan, Turkey, Australia, Benin, and Greece [2]. Cotton is grown on almost 2.5% of the world's arable land by nearly 26 million farmers in 75 countries [3]. It meets 27% of the world's textile requirements and provides employment and income to almost 100 million families. Two-thirds of the total cotton produced in the world is obtained from 53% of the irrigated lands [4]. Cotton cultivation meets 27% of worldwide textile needs and supports jobs and income for roughly

100 million people. By 2030, it is estimated that world cotton production will reach 28 million tons with an annual increase of 1.5%. This growth is expected to come from global yield growth (1%) in addition to expanding the cotton field (0.5%) [5]. Where it is grown, cotton is an attractive environment for numerous insects, pests such as aphids, thrips, and spider mites [6]. The only solution that farmers use to fight pests is to use insecticides. About 16% of the pesticides consumed globally are used for cotton growing lands [7]. Chemical pesticides used on cotton are linked to everything from cancer to infertility to birth defects. With excessive irrigation, it is carried to groundwater, rivers, and oceans, but also threatens the ecosystem by polluting the soil, air, food, and drinking water [8]. It is 300 times stronger than the greenhouse gas CO_2, which is formed because of the conversion of nitrate-containing chemical fertilizers as nitrogen source, used per hectare, in cotton-grown lands [9]. Excessive use of chemicals threatens environmental resources as well as agricultural production. When the future production style is arranged according to the wishes and needs of ecology, progress will be made in the direction of sustainability.

2. Cotton cultivation

2.1 Climatic requirements

Cotton is a plant that needs temperature above freezing, a humid climate, and sunbathing during its growing period. In general, the seed sowing depth (4–5 cm) should be above 15°C for germination to occur [10]. It is desirable that the soil temperature in sowing is at least 18°C. According to the developmental periods, the optimum temperatures are around 21–27, 27–32, and 21–32°C in vegetative, reproductive, and maturity periods, respectively [11].

2.2 Phenological development

The development of cotton during the growing period can be examined in five stages [12]. These are (1) germination and emergence, (2) seedling/root establishment, (3) leaf area expansion and canopy closure, (4) flowering and boll formation, and (5) boll and fiber maturation (**Figure 1**).

The number of days of development periods of cotton from sowing to harvest is given in **Table 1**.

2.3 Planting

The ideal planting depth is 4–5 cm. Planting depth should be 2–3 cm when the soil temperature is low in early sowing and when tempering is high. As the sowing date progresses, the planting depth should be increased up to 5–6 cm to ensure germination by taking advantage of the soil moisture [14]. The harvestable number of plants for cotton should be between 70.000 and 140.000 per hectare. When determining the number of plants in sowing, 80–85% seed germination rates should be considered in order to obtain the number predicted to be harvested. Accordingly, for the conditions where the distance between rows is 70–76 cm, the ideal plant density for machine harvesting is 10 cm. The number of plants in the harvest should be around 140,000, and for this, the distance between the row spaces should be adjusted to 8–8.5 cm in the planter settings. If the harvest is done by hand, the ideal in-row distance after

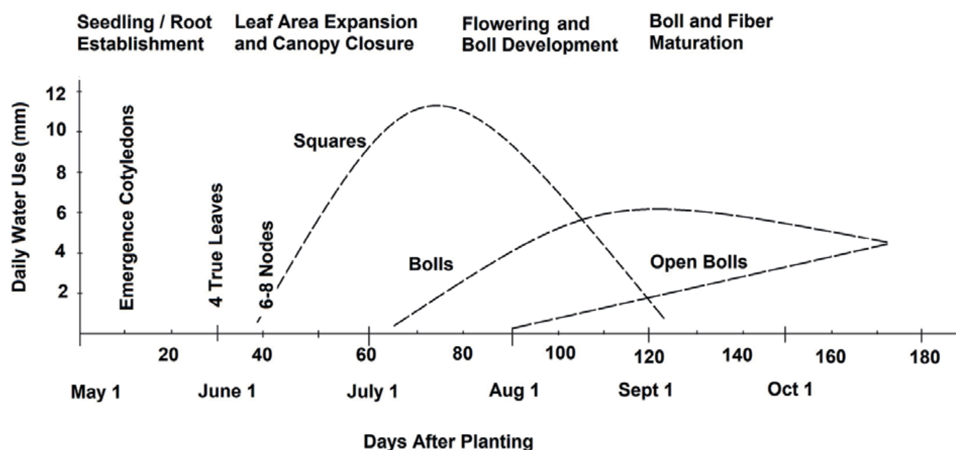

Figure 1.
Growth stages of cotton [12].

Event	Time required (days)	Average time required (days)	Cumulative duration (days)	Average date
Planting	0	1	1	May 1
Planting to emergence	4–14	7	7	May 7
Planting to first square	35–45	39	40	June 17
Planting to first bloom	55–70	62	62	July 6
Cut-out	8–10	9	71	July 28
Pinhead square to white flower	20–35	23	94	August 6
White flower to pink flower	1	1	95	July 29
Pink flower to open boll	50–60	55	112	July 30
Planting to 10% opening bolls	10–12	10	122	August 20
10% opening bolls to Harvest	160–180	51	170	August 30

**Assuming 20 main-stem nodes and 10–12 active fruiting branches.*

Table 1.
Growth and development periods of cotton [13].*

thinning is 17–20 cm. Cotton harvest starts when 60–65% of the bolls open (around September 15) and lasts until mid-December in Turkey.

3. Crop rotation

The purpose of the rotation is to provide sustainable management of the soils. Crop rotation also has purposes such as weed and pest control, disease management, utilizing the remaining nutrients from the previous plant, and improving the

accumulation of organic matter in the soil. The biggest problem with rotation in cotton cultivation is finding the rotation product that can generate the income of the main product. Other determining factors in this regard are the climatic conditions as well as how much the rotation product is affected by price changes. In addition to the market value of the product, farmer habits also affect the rotation.

In terms of sustainable management, organic matter accumulation is generally a major problem, especially in arid climatic regions where rainfall is insufficient, and irrigation is made. In such places, it is vital to adopt practices that will ensure the accumulation of organic matter in the soil in double, triple, and quadruple rotation applications. Prior cultivation of legume crops (such as soybean, peanut, beans, kidney beans, chickpeas, lentils) or legume fodder crop (such as Hungarian vetch, fodder pea, or vetch, broad bean) is an excellent choice for cotton planted land. In this way, since the physical, chemical, and biological characteristics of the soil will be enhanced, the organic matter content will also increase. More favorable soil conditions will be provided, especially in terms of plant nutrient intake, weed control, disease, and pest management.

In Şanlıurfa province in Turkey, the common practice in the rotation is wheat-corn in the first year and cotton in the second year. Farmers experience cotton yield decreases as high as 30–50% in wheat-cotton rotation in the same year. Another reason why wheat-cotton (Planting in May 15–30) rotation is not preferred is the increase in pest damage. In the same climatic region, an increase in cotton yield was observed in the lands of the farmers who preferred the first-year winter lentil (planting in October or November)—cotton (planting in June) rotation.

Another climatic region (İzmir and Muğla provinces) in the west of Turkey is wheat-silage corn or oat-silage corn in the first year and cotton in the second year of plant rotation. In the same area, it is silage maize for the first year consecutively and cotton for the second year. Some farmers in the region maintain a continuous cotton-cotton rotation. In Nazilli-Aydin province, first-year winter barley-cotton and second-year corn rations are maintained. In the alfalfa-cotton-wheat (W) rotation carried out by Nazilli Cotton Research Institute, a decrease in wilt loss disease was detected in cotton [15].

In rotation applications, corn-cotton and soybean-cotton are preferred in Mississippi, peanut-cotton in Arkansas, sorghum-cotton in Texas, and wheat-soybean-cotton rotations in Oklahoma. While practice yield increases range from 5 to 11%, research shows that rotation helps create a good balance and sustainability for farmers in matters related to disease and pest management, soil protection, soil, and plant health [13, 16].

It has been stated that the rotation of legumes with organic fertilizers is suitable for sustainable production in cotton and corn-growing areas in Burkina Faso [17]. In Pakistan, cotton-wheat rotation provided the highest yield, while sunflower-cotton rotation significantly reduced soil fertility and an allopathic effect [18]. Rotation schedules in India where cotton is grown in 10 states differ between states. In the north-western states of the country, Punjab, Haryana, and Rajasthan, there is a rotation of CW, C-Mustard, and C-Berseem (*Trifolium alexandrinum*), while most of them only cotton-wheat and Tamil Nadu Rice-C, Rice-Rice-C, C-GS. The states of Telangana and Andhra Pradesh practice C-Rice (Rank), C-Chilli, and C-Tobacco (two-year rotation).

4. Nutrient management

Dry matter formation in plants is the process of converting water and nutrients taken from the soil by photosynthesis [19]. Dry matter formation is adversely affected

in cases where plant nutrients are insufficient in the soil for various reasons, their uptake is reduced, or it is not possible. Chlorosis, malformation, stunting, and poor plant growth are common symptoms due to nutrient deficiencies, improper soil management practices, or climatic factors [20]. Nitrogen, phosphorus, potassium, and boron deficiencies are common in cotton production [21]. In addition to their inadequacies, plant growth may be adversely affected by the imbalances between each other (such as Ca-Mg and K) [22], antagonistic effects (such as phosphorus and zinc) [23], soil pH, nutrient application time, method and amounts, climate and environmental factors.

4.1 Nutrient requirement

Coordination of factors such as climatic conditions, drought, pH, macro and micro plant nutrient deficiencies, pest, soil, and water management directly or indirectly affects yield and quality characteristics [24]. Especially in arid climatic regions, the inadequacy of precipitation that can provide organic matter development is an important problem in the uptake of plant nutrients [25]. Soil pH is a problem not only in the uptake of macronutrients, but also in micronutrients, which are generally available in the pH range of 5.5–6.5 [26]. Deficiencies in the early stages of plant development are not a big deal in terms of their effect on plant growth. These elements, constituting only 1% of a bale, are removed after ginning, along with the seed and litter, along with macro and micronutrients [27]. **Table 2** gives the macro and micronutrient needs removed from the soil when 2500 kg of lint is taken per hectare.

The average daily intake rate of plant nutrients is given in **Figure 2**.

4.2 Nitrogen

Nitrogen (N) is an important nutrient needed for the healthy progression and physiological improvement of cotton, increasing fiber and photosynthesis efficiency [30]. It also affects the plant's ability to produce carbohydrates due to its role in chlorophyll production. N promotes vegetative growth and can be effective in increasing the number of bolls by increasing nodes and fruiting positions. The presence of nitrogen affects root morphology (root length, thickness, volume, and mass) and is associated with dry matter formation [31]. To maximize the effectiveness of N applied in cotton production, both pre-plant and side applications are recommended [32]. Inadequate N in early vegetative growth can be unfavorable to yield and quality [33]. Proper management of nitrogen is required in productive and high-quality cotton cultivation. To increase the efficiency of use (NUE), nitrogen should be given

Macronutrients (kg ha^{-1})					
N	**P_2O_5**	**K_2O**	**MgO**	**CaO**	**S**
220–230	50–60	270–280	70–75	280–300	70–75
Micronutrients (g ha^{-1})					
Fe	**Cu**	**Zn**	**Mn**	**B**	
400–420	75–80	270–280	800–850	600–650	

Table 2.
Nutrient requirement of cotton [28].

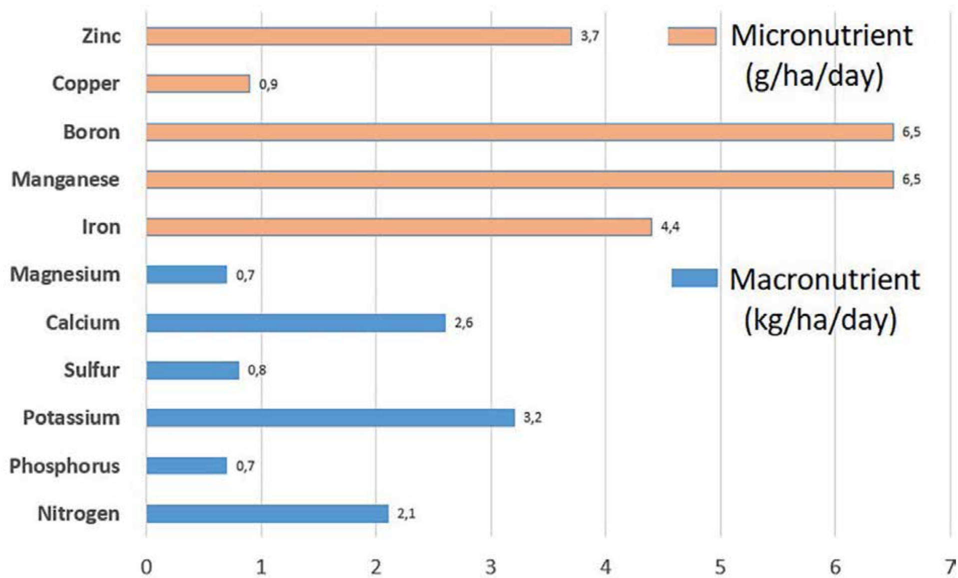

Figure 2.
Daily maximum nutrient uptake rates in cotton [29].

in the required amount, time, and form (such as NO_3^-, NH_4^+ or NH_2), to reduce leaching and evaporation losses with excessive irrigation, and to be given with partial and fertigation applications for effective management [34]. To reduce nitrogen losses, soil and tissue tests can be performed, and NDVI (Normalized Difference Vegetation Index) measurements can be applied using satellite or drone techniques [35].

4.2.1 Managing N fertility

Nitrogen is given in two periods, before planting (or with sowing) and before flowering. Pre-planting fertilization can be done according to the amount of nitrate-nitrogen to be determined in the soil test, considering the amounts given in **Table 3**.

The nitrogen fertilization system consists of two parts: (1) about 10–20% of the total nitrogen requirement before flowering (before or with planting), (2) the remaining nitrogen during the irrigation period of 60–75 days from the beginning of the flowering period. Additional nitrogen application may be required when needed against a sudden nitrogen deficiency to mature the crop later. However, it may be

NO_3^- N (ppm)	N/ha
0–5	30–50
5–10.	20–30
10–15.	0–20
> 15	0

**Source: [29]*

Table 3.
Nitrogen fertilization according to nitrate nitrogen level in the soil.*

advisable to give the last nitrogen fertilizer application in the flowering period with one of the nitrogen fertilizers produced as urea or slow-release nitrogen fertilizer. Because urea needs time to turn into ammonium and nitrate [36], it may be possible for the plant to benefit from nitrogen for a longer period with slow release. Measures such as reducing the losses of nitrogen under the root zone with surface irrigation methods (border or furrow) or preventing leaching with more controlled irrigation with pressure irrigation methods can be applied. It may be beneficial to increase the efficiency of nitrogen use by dividing the needs of the plant throughout the season into the growth period in climatic regions such as the Mediterranean where irrigation is used. In addition to these, utilizing the residual nitrogen from the plants entering the crop rotation may prevent the use of excess nitrogen. Considering that unnecessary or excessive nitrogen may delay maturation by encouraging leaf development by keeping the plant constantly green [9], it is necessary to be prepared for harvest with an optimum termination.

The base fertilization to be made before sowing is spread on the soil with fertilizer dispersing equipment, and then it is mixed into the soil at a depth of 10–12 cm with one of the appropriate tillage tools (such as a cultivator, rotavator), and the seedbed is prepared for planting. It is recommended to apply the fertilizers 5–6 cm below [37] and on the band so that they do not contact with the seeds, in the fertilization to be made on the seedbed with sowing.

Petiole sampling (**Figure 3**) is necessary for the health monitoring of nitrogen management in the plant [39]. The petiole test is done by plucking the first fully expanded leaf from the top of the plant 1 week before flowering. Repeat weekly for the following 8–9 weeks by breaking off the petiole of the first fully expanded leaf from the top of the plant [38].

For the first petiole analysis of the season, the petiole from the third node at the top of the plant should be taken and sampled from about 40 different locations in the field. As the results will show the nitrogen levels in the plant, it will be understood whether the fertilizer has been used adequately or insufficiently. The timely application of nitrogen prevents the reduction of leaf nitrate. If the level of nitrate-nitrogen drops to 4000 ppm or less before the first bolls open (**Figure 4**), it is recommended to apply nitrogen in the form of nitrate or urea.

Nitrogen in the form of nitrate in the soil solution is easily transported into the plant body. For this reason, the time required to eliminate the deficiency is shortened. In such a case, the source of nitrogen is not so important. Because the nitrification of

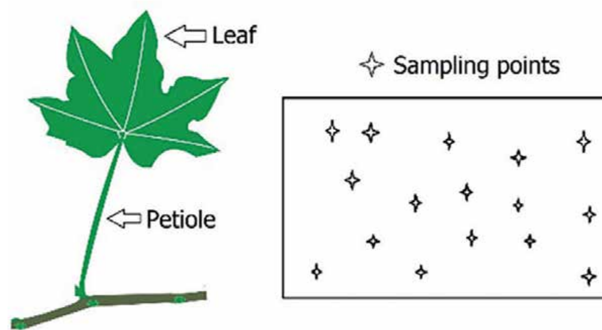

Figure 3.
Petiole sampling for analysis and location [38].

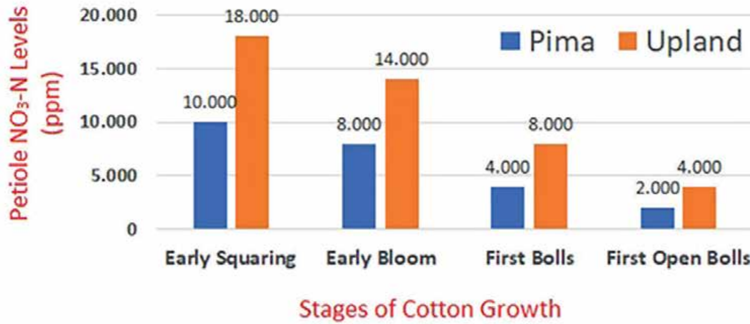

Figure 4.
Desired levels of petiole nitrate-nitrogen in cotton varieties [29].

ammonium sources can occur quickly enough to allow the resulting nitrate nitrogen to be transported to the root zone to meet the plant's needs [40].

4.3 Phosphorus

Phosphorus is effective in the energy transfer (ATP), transport, and storage of substances such as sugar and starch in plants [41]. It is necessary for flower and fruit formation, root development, and vital for the formation of new cells. In its deficiency, it causes lifeless, weak, and soft flower formation, weakening of the roots, lack of life and absorbent hairs, shortening of shoots, thinning and filamentous development, narrowing between the nodes, formation of small and amorphous fruits, decrease in the number of seeds, seed development disorders, and shortening in shelf life [42].

Phosphorus deficiency is commonly caused by conditions such as the presence of fully bound phosphorus in soils with a pH lower than 5.5 or higher than 7.5, with high clay content and low organic matter content, and soils with high aluminum or iron hydroxide content. In addition, plants need a large amount of phosphorus in their young stages, before the flowering period and during the seed formation periods. Phosphorus is also effective in metabolic activities such as energy production and gene transfer. In the excess of phosphorus in the soil, especially the intake of iron (Fe), zinc (Zn), and copper (Cu) is limited [43].

Cotton yield responds positively to the presence of phosphorus and affects dry matter accumulation in the soil [44]. Phosphorus (P) plays a crucial role in cotton by increasing reproductive growth and yield. Phosphorus contributes to root development by increasing root length, width, and diameter in plants [45]. Therefore, P deficiency inhibits cotton growth by reducing biomass accumulation and especially reduces seed cotton yield, stunted growth, and low yields have been detected [46].

4.4 Potassium

Potassium (K) affects vital metabolic, physiological, and biochemical functions in plants and increases product quantity and product quality. Studies show that potassium increases starch synthesis, helps to transport and store water and plant nutrients and photosynthesis products, prevents water loss in plants by regulating turgor, accelerating root development, promotes branching and lateral root formation. If there is enough potassium, the plants will form more branched roots. Root diameter expands, and root length and root growth rate increase [47]. Plants that do not get

enough potassium are mostly high in nitrogen and low in carbohydrates. Potassium affects the development of the cell wall in plants and accelerates maturation, increases the effectiveness of nitrogen, and positively affects the resistance against diseases and pests. In a study conducted, the effectiveness of potassium against diseases and pests in plants was determined to be 65% effective [48].

Heavy-textured soils in the clay-type illite group, which have insufficient K reserve and are rich in magnesium, have acid (low pH) character, light-textured sandy, are leached by excessive irrigation or precipitation, may show potassium deficiency [49]. Potassium concentrations in both the blade and petiole of fully expanded upper leaves on the main stem are good indicators of K deficiency. Potassium deficiency causes many disorders including a decrease in leaf area index, photosynthesis, and plant biomass in cotton, while the decrease in leaf photosynthesis and stomatal conductivity, low chlorophyll content, weak chloroplast structure, restricted saccharide translocation are attributed to K deficiency [50]. Potassium deficiency affects the seedling development of cotton and shows this with shortening of plant height, decrease in leaves and roots, and biomass. It also inhibits enzyme activities in cotton seedlings by affecting photosynthesis and respiration [51].

Potassium demand increases in cotton during boll formation, flowering, and fiber elongation periods [52]. Potassium deficiency manifests itself with interveinal chlorosis on old leaves 3–4 weeks after the first flowering; in addition to symptoms, leaves may begin to curl downward and thicken. If the deficiency is severe enough, the cotton plant may begin to shed leaves on its own. The high demand for potassium generated during boll formation in cotton can be greater than 3.4 kg per hectare per day [53]. Severe potassium deficiency caused by extremely hot and dry conditions can cause other undesirable effects, including fruit drop [54]. Drought, due to soil moisture deficiency, is a limiting factor for potassium uptake.

Potassium is an important nutrient that affects fiber length and quality in cotton. In a study conducted in an area with potassium deficiency in the soil, fiber strength and weight decreased up to 7.8% and 2.1%, respectively [50]. The most effective way to meet the potassium requirement is to regularly test the soil.

4.5 Secondary nutrients

4.5.1 Sulfur

The source of sulfur that can be taken up by plants is elemental sulfur and sulfur in organic matter. Plants take up sulfur in the form of the SO_4^{-2} ion. In addition, they can also take SO_3 and a little elemental sulfur with their sulfur leaves. In addition, plants can absorb this element in the form of SO_2 gas. Sulfur is an element that enters the structure of organic matter. Sulfur takes place in the structure of sulfurous amino acids such as cystine, cysteine, and methionine in plants and combines with them to form proteins. During this cycle, the sulfur takes part in many enzymatic activities [55]. Sulfur is easily transported (mobile) within the plant. However, it is less mobile than N, P, K, as it forms various compounds immediately after entering the plant body [56]. Sulfur deficiency shows symptoms very similar to nitrogen deficiency. Nitrogen deficiency is seen in old leaves, while sulfur deficiency is seen in young leaves [57]. It is found in the structure of some amino acids necessary for protein synthesis. It takes part in the synthesis of energy, hormones, and some enzymes, and accelerates nitrate and carbohydrate metabolism [58].

4.5.2 Calcium

Calcium is taken up by plants as Ca^{+2} ions through root tips. Calcium ions are transported to all plant tissues by xylem pipes due to transpiration [59]. Calcium is effective in the development of the endpoints of newly developing cell tissues, and the normal formation of roots and flowers. Calcium is located between the cell walls and is the building block of the cell wall [60]. Strengthening the cell wall, it helps cell growth and elongation. It is effective in the permeability of the cell membrane. It is necessary for normal flowering and root development. It helps in the uptake of nitrates. It increases the plant's resistance to diseases, drought, and stress [61].

Calcium is immovable within the plant. In other words, calcium, previously taken to the plant or in the leaf, does not pass to the fruit and newly formed leaves and shows the first signs of deficiency with the drying or upward curling of the leaf tips [62]. Dying at the shoot tips and stopping the growth of dead tissue, discoloration at the fruit tip, and brown-black rot (blossom end rot) in the following period are seen. To eliminate the calcium deficiency, Calcium Nitrate fertilizer should be applied regularly, if necessary, in each irrigation, or spraying calcium on the fruit 3–4 times is effective. The first calcium deficiency is seen on young leaves and shoots tips [57]. Young leaf margins dry up and die. Shoot tip growth stops, shoot tips dry out. Plant tissue and fruits are soft, shelf and storage life are short. Root development weakens, the durability of the root's decreases. The plant's resistance to diseases and pests is reduced [63].

Conditions reducing calcium uptake: Feeding with high ammonium (NH_4) nitrogen, insufficient water, or high salt concentration in the soil, conditions that prevent new root formation, such as low temperature and insufficient aeration, low pH soils, organic soils, or high organic matter added soils and giving too much potassium or magnesium with fertilizer [64].

4.5.3 Magnesium

Plants take magnesium in the form of Mg^{+2} ions. Magnesium is the central atom of chlorophyll and is vital in photosynthesis [65]. Therefore, in magnesium deficiency, the number of chlorophylls decreases and photosynthesis regresses. Due to mobility in plants, it accumulates most in the growing tips of plants and especially in young leaves. It is transported from these regions to the seed during seed formation [66]. Magnesium deficiency first manifests itself in older leaves. In magnesium deficiencies, older leaves become discolored, the main and secondary veins on the leaf are green, the thinner veins turn yellow, and local wavy round yellowing is seen between the veins [67]. Magnesium plays a very important role in the transport and placement of phosphorus and the conversion of amino acids into polypeptides. It is an enzyme activator and helps the function of many enzymes [68].

Conditions that cause decreased magnesium intake: Excessive potassium and calcium fertilization or high lime content, sandy soils with heavy rainfall, soil compaction, insufficient drainage, drought, and cold soils, and pH drop below 5. Toward the end of the development period, the deficiency increases due to the increase in the need for magnesium [69].

4.6 Micronutrients

Micronutrients are not needed in large quantities as macronutrients but are vital because of their role in plant development. Zinc (Zn) for plant hormone balance,

auxin activity, growth, cell division; iron (Fe) for photosynthesis with chlorophyll synthesis; boron (B) for flowering, maturation, sugar transport, cell division, and amino acid production; manganese (Mn) for chloroplast production and enzyme activation; copper (Cu) for stimulating enzymes necessary for photosynthesis; legumes need Molybdenum (Mo) elements for nitrogen fixation [70].

The deficiencies of these nutrients manifest themselves in the form of different symptoms. For example, in zinc deficiency, growth in plants stops, the internodes become shorter, and young leaves become smaller than normal, while chlorosis or yellowing occurs between the new leaf veins in iron deficiency. In boron deficiency, mild general chlorosis, death of the growing point, the color of the leaves fades and becomes deformed. Similarly, in Manganese (Mn) deficiency, chlorotic mosaic patterns occur on the leaves; in copper deficiency, mild general chlorosis, leaf tips are bent, and turgor loss is seen in young leaves; In molybdenum deficiency, like ordinary nitrogen deficiency, general chlorosis is seen in young plants [71].

EDTA (Ethylene Diamine Tetra-Acetic Acid) is a chelating agent that allows the molecular structure to bind to heavy metals, and chelation is a form of attachment of ions and molecules to metal ions. Chelates, on the other hand, are complex compounds called ligands in their structure and play a role in the uptake of micronutrients such as EDTA. Micronutrients are transported to the plant body mainly by applications such as soil, leaves, and irrigation water [72].

4.7 Foliar fertilization

In arid and some subtropical climatic regions where precipitation is insufficient, soils rich in lime are a problem in the uptake of microelements such as zinc, iron, and boron due to high soil pH [73]. Studies show a reduction in seed cotton yield in calcareous soils due to an imbalance of nutrients, especially micronutrients [74]. In general, high pH is a limiting factor for nutrient uptake in such lands with low organic matter and nitrogen content, and this type of soil is insufficient in terms of zinc, boron, and iron [75]. In addition, the imbalance between some elements and calcium can lead to nutritional problems, especially in terms of potassium and magnesium [76]. In such cases, foliar applications of micronutrients, whose uptake is limited by soil applications, are an inevitable need [77].

Foliar fertilization provides advantages such as low cost and rapid plant response in situations where soil problems arise, and root development is insufficient. Further, foliar feeding has disadvantages due to problems such as possible leaf toxicity and solubility [78], and therefore, it is not possible to fully meet the needs of the plant. Success in foliar fertilization can be achieved when the effects of many factors such as the development period of the plant to be applied, its need, the toxicity level, the number of applications, the number of repetitions, the method of application, the time of application, the compatibility with other pesticides and fertilizers are known. The quality of the nutrient to be applied to the plant is important. Because the dose and application stage to be used in the application of macro and micronutrients to be applied in foliar feeding can be different. The efficiency of foliar fertilization can be affected by the type of fertilizer, its solubility, the concentration and pH of the solution, and its incompatibility with some pesticides and chemicals used in foliar feeding [79].

Foliar fertilization of cotton is a convenient way of applying certain fertilizers that can complement conventional soil methods. Foliar fertilization can increase yield and fiber quality. The transport rate of plant nutrients used in foliar fertilization is given

Fast	Moderate speed	Slow	Very Slow
NH_2—Nitrogen	Phosphorus, P	Zinc, Zn	Boron, B
NH_4—Nitrogen	Sulfur, S	Copper, Cu	Magnesium, Mg
NO_3—itrogen		Ferrous, Fe	Calcium, Ca
Potassium		Manganese, Mn	
Sodium		Molybdenum, Mo	

Table 4.
Transition rates of nutrients to leaves [80].

Nutrient	The time required for 50% of the nutrient intake
NH_2—Nitrogen	0.5–2 hours
Phosphorus, P	5–10 days
Potassium, K	10–25 hours
Calcium, Ca	1–2 days
Magnesium, Mg	2–5 hours
Zinc, Zn	1–2 days
Manganese, Mn	1–2 days

Table 5.
The time required for the uptake of some nutrients into the plant [80].

in **Table 4**, and the times required for 50% of the applied plant nutrients to be taken are given in **Table 5**.

5. Water management

5.1 Critical stages for irrigation management

Although cotton varies according to the climatic regions where it is grown and the day length of the varieties, daily plant water consumption of cotton is different at plant growth stages. It increases from 2.5 mm at the seedling stage to the maximum value (6–10 mm) in the most intense flowering from the soil and gradually decreases in the boll development (5–8 mm), reaching the minimum value (2–4 mm) in the opening stage of the bolls. Even though susceptibility to water stress varies according to phenological periods, squaring, flowering, and boll development are critical stages in cotton [81]. Water stresses that will occur during these periods reduce the number of squares and cause yield and quality losses by shedding squares, flowers, and bolls [82].

The first square in cotton is seen approximately 50–60 days after sowing and the flowering period continues for 7 weeks. **Table 6** shows the effect of flowers formed during flowering on yield. As seen in the table, the bolls formed by the flowers formed in the first 3 weeks constitute 88% of the product. Considering the yield of flowers formed in the first 3 weeks, the importance of preserving the first bolls emerges spontaneously.

	Weeks of flowering						
	1	2	3	4	5	6	7
Total flowers (%)	8.1	23.5	29.4	25.6	9.8	2.3	1.3
Boll retention rate (%)	94.1	77.7	43.1	20.7	13.3	10.8	5.0
Total product (%)	21.0	43.0	24.0	8.5	2.5	0.9	0.5
Average boll weight (g)	5.3	4.6	3.7	2.8	3.1	3.2	—

Table 6.
The effect of flowering weeks on yield in cotton [83].

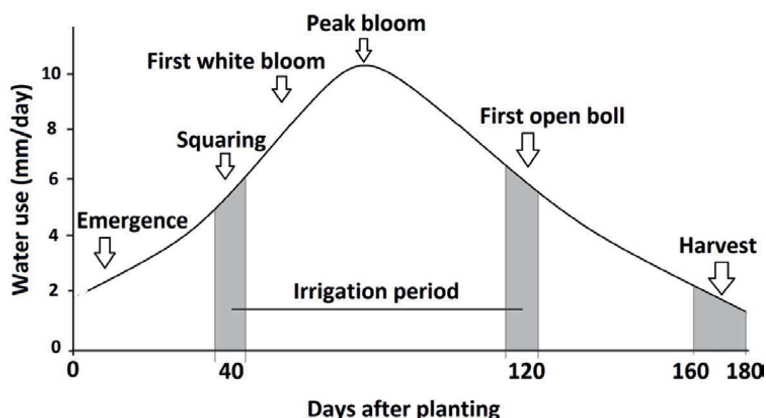

Figure 5.
Irrigation period of cotton throughout the growth stage (adapted from [84]).

The time between squaring and the opening of the bolls (between 40 and 120 days after planting) is considered the critical process for managing water in the root zone of cotton. The common practice in determining the irrigation period (**Figure 5**) is the 70–80 days period between the appearance of the first squares and the period when the rate of the first bolls to open reaches 10% [85].

Productive and high-quality cotton growth is important for the initiation and termination of irrigation [86]. Considering the sensitivity to water stress, it is necessary to start irrigation during the squaring period of the plant (35–45 days after planting) to prevent yield losses [87]. Because the water stress that may occur during this period can lead to boll shedding, resulting in significant losses in yield. Early termination of the irrigation period may result in yield losses, while delay may result in continued vegetative growth, delayed maturation of the bolls, increased pest management and harvesting costs, and reduced irrigation efficiency [88]. For climatic regions where the average daily plant water consumption is around 8–10 mm, the irrigation interval of 10–12 days is recommended in lands with medium and heavy-textured soil where surface irrigation methods such as furrows are applied [89]. Irrigation intervals can be selected as 6–8 days for lands where drip and sprinkler methods are used in lands with similar climates and as 4–5 days for sandy soils. While it is recommended to terminate the irrigation when the first opened bolls are seen in areas where surface irrigation methods are used, it can be delayed for 1–2 weeks in medium and heavy-textured fields where sprinkler and drip methods are used.

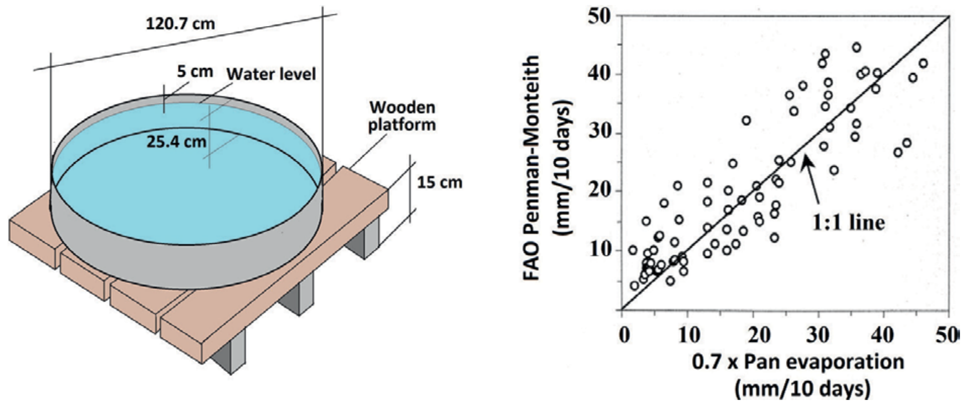

Figure 6.
Measuring of evaporation from Class A pan (left) and the relationship between FAO Penman-Monteith and pan evaporation (right) [90].

5.2 Crop water requirement

Plant water consumption is the sum of the water lost by evaporation and transpiration. Numerous methods have been developed for estimating plant water consumption. These are such direct measurements, estimating from climate data, plant and soil-based methods. The FAO Penman-Monteith method [90] is one of the most reliable methods in estimating using climate data. The results obtained by multiplying the evaporation volumes determined from Class A pan with a coefficient of 0.7 were analyzed together with the plant water depletion values estimated at 10-day intervals by the FAO Penman-Monteith method. In the results obtained, it was determined that the agreement was very high (**Figure 6**).

5.3 Effective root depth of cotton

Soil water potential is related to the soil texture and is important to determine the available water holding capacity (AWHC) at the effective root depth considered. The results of some studies on plant root morphology in recent years have revealed that a significant part of the cotton roots is concentrated at 0–30 cm and an average of 90% is at a depth of 60 cm [91]. For this reason, when determining the irrigation interval in cotton cultivation on lands with medium and heavy-textured soil, an effective root depth of 60 cm and allowing a maximum of 60% of the available water to be consumed is a suitable approach to ensure yield and quality without creating water stress on the plant.

The irrigation schedule is determined by considering the plant water consumption, the AWHC of the soil, and the part of the soil moisture that is allowed to be consumed. In terms of AWHC, soil texture is generally decisive and sandy soils have less capacity than clay soils. Sandy soils have an AWHC of 20–35 mm, medium-textured loamy soils 45–65 mm, and heavy-textured clayey soils 40–50 mm for each 30 cm soil profile.

5.4 Irrigation methods in cotton cultivation

The irrigation method is the way of applying water to the plant root zone. Today, two main methods are used, namely surface and pressurized.

5.4.1 Surface irrigation

Surface irrigation methods generally take place in the form of vertical and forward movement of water by gravity on land with no slope or very low slope (generally below 3%). These methods generally take place in the form of vertical and forward movement of water by gravity on land with no slope or very low slope (generally below 3%). In practice, furrow and border methods and their derivatives are widely used in cotton cultivation. Reducing in-field losses in the irrigation network is related to the irrigation method applied. The success of these methods depends on the reduction of water transmission in the channels and infiltration into the field and surface flow losses. Surface flow and infiltration losses are the most important problems in surface irrigation methods. These methods cause drainage problems by rising the groundwater in the field, the root zone remains wet for a long time, negatively affecting plant growth, and increasing fungal diseases, tillage, salinity, and sodium problems. Along with the deep infiltration, a significant part of the plant nutrients as fertilizer to the root zone is washed and pollutes the groundwater. The mixing of nitrogen losses, especially in the form of nitrates, into drinking and utility water is the biggest threat to human and environmental health.

5.4.2 Pressured irrigation methods

Pressurized irrigation systems use energy (such as electricity, diesel oil) to take water from a canal, stream, or well and transmit it to the land to be irrigated through lateral lines under a certain pressure. Irrigation water is directed to the lateral lines from the main canal at the head of the field and given to the root zone by various methods (such as drip or sprinkler methods).

5.4.2.1 Drip irrigation

Drip irrigation is the process of taking the water from a well, canal, or stream and giving the filtered water to the plant root zone in the field with a certain pressure (1–1.5 atm.) using plastic pipes called lateral and having drippers placed on it at certain intervals. With this method, it is possible to transmit irrigation water to the root zone in a controlled manner by the drip method and high WUE (85–95%). With successful planning, it is ensured that water, plant nutrients, and agricultural pesticides are supplied, and significant savings are achieved in labor, energy, and time. Thanks to the vertical and horizontal control of water, high efficiency and quality products can be obtained with an appropriate irrigation schedule.

Today, in the drip method, two types can be used by placing the lateral lines above ground and underground (30–35 cm from the surface). In the drip method, two types can be used by placing the lateral lines above ground and underground (30–35 cm from the surface).

The arrangement of the lateral lines in drip irrigation affects the irrigation water requirement and therefore the energy cost. In the application of one lateral line in two rows (**Figure 7a**), the required lateral line requirement per hectare is 7140 m. It may be necessary to irrigate for 24–36 hours, especially in the first irrigation, to bring soil moisture to the field capacity throughout the 60 cm effective root depth in medium and heavy-textured lands in hot and arid regions where cotton is grown in the Mediterranean climate. During the following 75–80 days irrigation period, half of the initially calculated water can meet the need if it is every 6–8 days. The condition

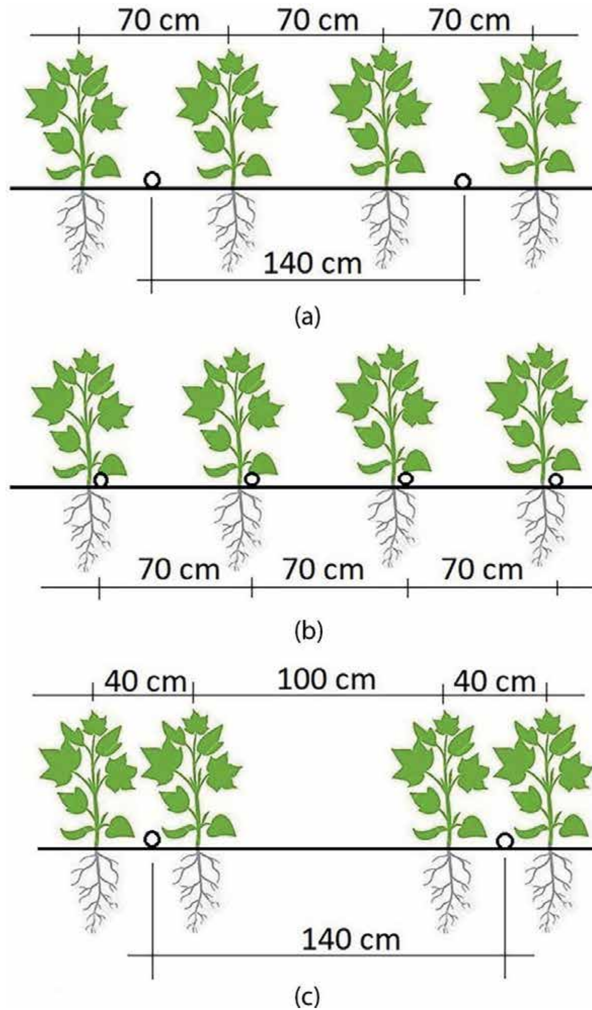

Figure 7.
Representation drawing of drip irrigation lateral positions in field systems. Systems are defined as follows: (a) surface one lateral line in two rows, (b) surface every row, and (c) surface twin rows (adapted from [92]).

of high yield and quality cotton cultivation in the calculated irrigation intervals depends on the application of the program that will not cause water stress in the root zone. This application, which will be repeated 12–14 times during the irrigation period, requires an average of 600–750 mm of plant water consumption. Generally, in dry climatic regions, the calculated amount of irrigation water may be needed to meet plant water consumption. With a motor pump with an average water application rate of 50 m^3/h, it is necessary to irrigate for an average of 120–150 hours per hectare throughout the year. During the irrigation period in the specified land, the diesel requirement per hectare is 204–255 liters and the average is around 265 US dollars for 2022 in Turkey.

Applying a lateral line to each row (**Figure 7b**), the lateral line requirement is 14280 m per hectare. In this application, lateral lines are placed in each row and adjacent to the plant. At the beginning of the irrigation period, it is sufficient to irrigate for a maximum of 10–12 hours in order to bring the soil moisture at 60 cm depth to

the field capacity in medium and heavy-textured lands. During the following 75–80-day irrigation period, it will be sufficient to irrigate for a maximum of 5–6 hours at intervals of 6–8 days on average. During this process, which will be repeated 12–14 times during the irrigation period, the annual irrigation water volume is an average of 3000–3500 m^3/year per hectare. With a motor pump with a water application flow rate of 50 m^3/hour, the annual irrigation period is 60–70 hours, and the energy consumption is approximately 102–119 liters of diesel fuel. The cost of irrigation energy per hectare of the land in question is an average of 122 US dollars in Turkey.

The cost of lateral line planting in twin rows (**Figure 7c**) in cotton cultivation is the same as when planting a line in two rows (**Figure 7a**). In contrast, the cost of irrigation water and energy will be close to or slightly higher than the line-up (**Figure 7b**) per row. Although this method is not a common practice yet, it will provide significant savings, especially in irrigation water. It is a method that can provide significant water savings without causing a decrease in yield and quality in sowing in narrow and wide intervals by adjusting the planter's feet with a telescopic planter in sowing. Especially with wide row spacing, it will be possible to use pesticides, foliar fertilization, and defoliants effectively with agricultural machinery until the end of the harvest period.

5.4.2.2 Sprinkler irrigation

This system is the process of applying the water supplied by pumping from a water source to the land by spraying it with sprinkler heads at a certain operating pressure. The water source can be a well, canal, or stream. In general, after the irrigation water is taken from the source, it is conveyed to the main lines under pressure varying between 1.5 and 5 atmospheres and then to the sprinkler heads on the lateral lines. The sprinkler method has a very high-water application efficiency (Ea, 60–95%) and uniform water distribution with proper planning. It is a method that can be used for

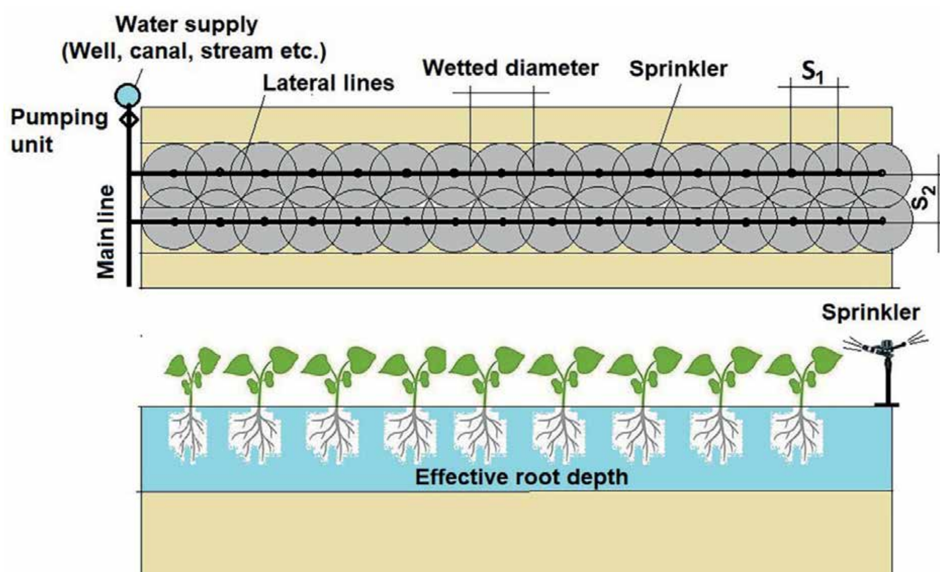

Figure 8.
Sprinkler irrigation system elements [93].

irrigation of all plants whose leaves are not sensitive to wetting in all kinds of field conditions. The factor limiting the application of this method is the wind. When placing the lateral lines on the land, it is essential to pay awareness to the fact that the effective winds in the region are perpendicular to the direction of movement.

The sprinkler system (**Figure 8**) can be immobile, mobile (linear or circular), and portable in the field.

In the design and operation of the sprinkler system, a higher precipitation rate than the infiltration rate of the soil is not desired. For this, the system's placement arrangement (S_1xS_2), nozzle (sprinkler) discharge, and operating pressure must be evaluated together. In practice, the fact that the precipitation rate is higher than the water intake rate of the soil causes the accumulation of water by ponding and drainage problems in flat topography, while surface runoff may occur in areas with slightly sloped topography. Therefore, it is crucial to pay attention to the selection of sprinkler heads with a high-water application efficiency in sprinkler irrigation.

6. Pesticide management

6.1 Weed control

Weed control in cotton is generally done in three periods: pre-sowing, pre-emergence, and post-emergence [94]. After the herbicides used before planting are applied to the field with a sprayer, they are mixed into the soil at a depth of 8–12 cm using a rotavator or cultivator. In this period, herbicides containing Pendimethalin, Metalochlor, and Benoxacor active ingredients can be used [95].

Pendimethalin, Alachlor, Fluometuron, Prometryne, and Linuron active ingredient herbicides can be used for weed control in the post-sowing and pre-emergence period. If there is no rain within 24 hours after the application, the effectiveness of the herbicides decreases due to evaporation. For this reason, it is useful to ensure that the herbicides infiltrate to a depth of 15–20 cm by making sprinkler irrigation after the pre-emergence applications [96].

Clethodim (116.2 g/L), Fluazifop-P-Butyl (125 g/L), Haloxyfop ethoxyethelester (125 g/L), Quizalofop + P/Ethyl (50 g/L), and Quizalofop/P/Tefuryl (40 g/L) active ingredients can be widely used in post-emergence herbicide applications. However, it is the best way to choose according to the weeds we want to control during this period [97].

6.2 Insect control and management

There are seven main issues that must be followed in order to be successful in the fight against pests. These are respectively, 1-diagnosis, 2- fighting method, 3-application time, 4-pesticide selection, 5-tool selection, 6-application, and 7-control [97]. In a plant protection process to be applied, it is easily possible to achieve success when these are applied in the order of the management. If someone makes a mistake, such as a wrong or incomplete application, not following the order, it brings failure or causes success to be caught by chance.

The periods in which the insects, which are commonly encountered in cotton and have the highest damage potential, are effective according to the development periods of the plant are given in **Table 7**.

Growth stages	Emergence	Seedling	The First Squares	The First Flowers	Green Boll	Opening Boll
Insects/Weeks	3	6	9	12	15	18
Agrotis spp	X					
Aphis gossypii		X	X	X		
Bemisia tabaci	X			X	X	
Empoasca decipiens		X	X			
Earias insulana					X	
Frankliniella intonsa				X		
Helicoverpa armigera			X	X	X	
Pectinophora gossypiella				X	X	
Spodoptera exigua			X			
Spodoptera littoralis		X			X	
Tetranychus cinnabarinus				X	X	
Thrips tabaci		X				

**Adapted from [98].*

Table 7.
Common pests and emergence periods in cotton during the growing season.*

6.3 Disease control and management

Disease management in agriculture is the practice of minimizing disease in crops to improve the quantity or quality of harvest yields [99]. Organisms such as fungi, bacteria, and viruses that cause infectious diseases can be found in plants. Effective disease management should be integrated into farm management, focusing on the host, potential pathogen, and environment. The way to reduce the risks of epidemics is through the adoption and implementation of disease management strategies. It is useful to have knowledge of the types and origins of diseases to implement solution-oriented management strategies [100]. Disease management in plants is based on several important principles. While disease control is often impractical or even possible, it may be possible to reduce disease progression and keep it at an acceptable level. Disease management has basic principles such as exclusion, eradication, protection, resistance, therapy, and avoidance [101]. Each of these principles helps in deciding the method and method of the application according to the course of the disease, its prevalence, severity, degree of effect, the area affected, the recurrence status, and risks. For example, exclusion includes applications to remove disease-causing pathogens from the disease-free area, so that the disease can be prevented from entering the area where the plants grow. Eradication is such as the destruction of infected plants, disinfection of storage areas, fumigation, and solarization. Cultural practices such as removing diseased plants from the

environment, destroying crops, and crop rotation can also be evaluated within this scope. Conservation practices include the protection of the elements that cause the spread of the disease with physical and chemical barriers. Cultural practices such as tillage, irrigation, and drainage are within this scope. In addition, applications such as determining the planting date or depth, distances between plants, pruning, and thinning due to disease risks, for example, are applications that help plants get away from infection and reduce the severity of diseases. Practices such as soil drainage and ridge planting, especially the cultural management of root diseases, are also conservation practices. Selecting and developing resistance, disease-resistant plants is a viable way to reduce losses. It has been determined that this application is especially successful in rust diseases, powdery mildew, and root rot. With some hybridization techniques in plant breeding, the effect of pressures on yield can be controlled by increasing the rust resistance of plants [102].

The main diseases in cotton cultivation are seedling root rot, verticillium, and fusarium wilt, leaf angular spot disease. Those diseases, which can lead to significant yield and quality losses if no precautions are taken, can be resolved with an integrated management approach. Integrated disease management is a concept that enables the application of one or more of the abovementioned applications in combination and should be applied in a coordinated, integrated, and harmonious manner to maximize the benefits of each component.

7. Plant growth regulators

Plant growth regulators (PGRs) affect many physiological events such as flowering, maturation, root development, bending and death of leaves, stems, and other organs, inhibition or advancement of stem elongation, the coloration of fruits, and prevention of foliation or defoliation, prevention of overgrowth, earliness, and plants themselves. Chemicals produced by the effects of PGR boll seed cotton weight, 100 seed weight, ginning yield, plant height, the number of fruit branches, 100 seed weight, the number of bolls, fiber fineness, and fiber rupture strength were determined by research [103].

It has been observed that the prevention of excessive grading in cotton, the lengthening of the distance between the nodes due to the sowing frequency, the increase in boll, boll holding, and yield [104]. Positive effects of Mepiquat Chloride (Pix) have been observed in terms of its effects on the abovementioned properties. Between the beginning of flowering and the peak of the flower or from the beginning of the boll, it is recommended to apply 3–4 applications at 25–50 ml/da with 10–15 days intervals. Mepiquat Chloride is 45–60 days from October when the first white flowers appear on a small number of plants. The first application should be made before the first watering in days. The second application is 20 days after the first application in 65–80 days. It is done on days to encourage the plant to produce mostly fruit, together with the green parts. The third application is 85–100 days after emergence for the plant to complete its development at an average of 14–16 nodes. Although it varies according to the regions and the variety, the estimated post-emergence is 100–120. The fourth and last application, which is recommended to be done on days, is aimed to complete the growth of green parts, maturing the bolls, and harvesting earliness. With this application, it is encouraged to increase the cotton core and fiber quality [105].

Active ingredient	Dosage (L/ha)	Effect
DE-4-Thidiazuron, 119 g/L+ Diuron, 60 g/L	0.6	Defoliant
Pyraflufen, 26.5 g/L	0.16	Defoliant
Carfentrazone-ethyl, 240 g/L	0.15	Defoliant
Ethephon, 480 g/L	3	Boll Opener and Defoliant
Ethephon 480 g/L+ Cyclanilide, 60 g/L	2	Boll Opener and Defoliant
Ethephon 720 g/L +Cyclanilide, 45 g/L	1.75	Boll Opener and Defoliant

**Compiled from [106, 110].*

Table 8.
Some chemicals used as boll opener and defoliant in cotton.*

8. Boll openers and defoliants in cotton

Manufacturers who will use chemical pesticides before cotton harvest must determine issues such as "chemical selection," "management time," and "management dose." There are three different methods to determine the most appropriate application time for defoliating and boll-opening chemicals. These are: (a) the boll opening rate should be at least 65–70%, (b) 85% of the bolls to be harvested should be mature, and (c) the number of knuckles on the cracked boll should be 4 or less [106].

The boll opening rate is determined by counting the bloomed and unopened bolls from 20 randomly selected plants representing the field [107]. Early and clean harvest with the use of defoliant or boll-opening chemicals, prevention of rotting of the lower bolls, especially in places where the humidity is high and high humidity, prevention of fiber contamination. It has benefits such as reduction. To get rid of the harmful effects of chemicals, the bolls must be at least 35–40 days old [108]. Depending on the nature of the plant, environmental conditions such as temperature and humidity, and the chemicals used, the leaves begin to fall 3–7 days after the application of the defoliant, and the defoliation process takes place after 10–14 days [109]. Early varieties can be harvested approximately 10 days after application. During the application of chemicals, the sprayer must be calibrated, 200–250 liters of water should be used per hectare; If there is an expectation of rain within 24 hours after the application, if the daily average temperature is below 17 ^{0}C, the application should not be made, and suitable conditions should be expected; care should be taken not to spread the chemical to the surrounding plants, it should not be used on other plants other than cotton. A suitable chemical should be selected according to the purpose. If only defoliation is aimed, a defoliant chemical should be used, if both defoliation and boll opening are aimed, defoliant + boll-opening chemicals should be used (**Table 8**).

9. Conclusion

In high-yield and quality cotton cultivation, the plant should not be exposed to any stress during the growing period. Problems such as extremely hot and cold climatic conditions, lack of water and nutrients in the soil, excessive nitrogen fertilizer applications, pesticide residues applied to the plant grown before, overdose in foliar fertilization, extreme and redundant use of pesticides, excessive and improper

irrigation due to the need for drainage owing to the root zone remaining wet for a long time can affect cotton yield, quality, and sustainable management. Considering these problems, applications to be made in a timely manner, with appropriate mechanization, in sufficient quantities and as needed are the key to controlling the stress conditions relatively. Success in sustainable cotton production depends on increasing controllable conditions. Effective management in cotton production is possible with the control of water, nutrient, and pesticide management. In this respect, maximum water and nutrient management in the root zone is ensured thanks to the high application efficiency, especially with drip and sprinkler methods. In this way, it is conceivable not only to control productivity and quality but also to protect environmental resources caused by deep infiltration due to excessive applications. Therefore, success in controlling the events in the rhizosphere will be the determining factor of sustainable production in the future.

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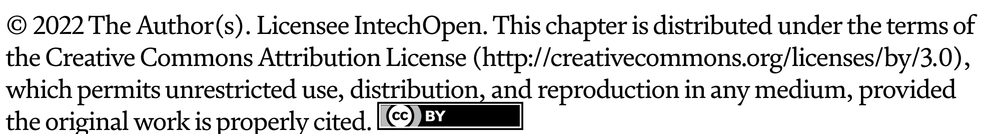

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References

[1] Hinze L, Kohel R. Cotton. In: Gupta S, editor. Technological Innovations in Major World Oil Crops. Vol. 1. New York, NY: Springer; 2012

[2] Khan MA, Wahid A, Ahmad M, Tahir MT, Ahmed M, Ahmad S, et al. World cotton production and consumption: An overview. In: Ahmad S, Hasanuzzaman M, editors. Cotton Production and Uses. Singapore: Springer; 2020. DOI: 10.1007/978-981-15-1472-2_1

[3] Park JH, Kyung WO, Hyung-Min C. Preparation and characterization of cotton fabrics with antibacterial properties treated by cross-linkable benzophenone derivative in choline chloride-based deep eutectic solvents. Cellulose. 2013;**20**(4):2101-2114. DOI: 10.1007/s10570-013-9957-8

[4] Soth J, Grasser C, Salerno R. The Impact of Cotton on Fresh Water Resources and Ecosystems: A Preliminary Analysis. Gland, Switzerland: Worldwide Fund for Nature; 1999

[5] OECD/FAO. Agricultural Outlook 2021-2030. Paris, France: OECD Publishing; 2021. p. 337. DOI: 10.1787/19428846-en

[6] Martini X, Kincy N, Vaughn K, Dever J, Nansen C. Positive association between thrips and spider mites in seedling cotton. Agricultural and Forest Entomology. 2013;**15**(2):197-203. DOI: 10.1111/afe.12004

[7] EJF. The Deadly Chemicals in Cotton, Environmental Justice Foundation in collaboration with Pesticide Action Network UK. London, UK: Emmerson Press; 2007

[8] Gill HK, Garg H. Pesticides: Environmental impacts and management strategies. In: Larramendy ML, Soloneski S, editors. Pesticides—Toxic Aspects. London: IntechOpen; 2014. DOI: 10.5772/57399

[9] Sainju UM, Ghimire R, Pradhan GP. Nitrogen Fertilization I: Impact on crop, soil, and environment. In: Rigobelo EC, Serra AP, editors. Nitrogen Fixation. London: IntechOpen; 2019. DOI: 10.5772/intechopen.86028

[10] Oosterhuis DM. Development of a cotton plant. In: Seagull R, Alspaugh P, editors. Cotton Fiber Development and Processing, an illustrated overview. Lubbock, TX: International Textile Center, Texas Tech University; 2001

[11] Freeland T, Pettigrew W, Thaxton P, Andrews G. Agrometeorology and cotton production. World Meteorological Organization. 2011;**10**(1):1-128

[12] Oosterhuis DM. In: Nitrogen Nutrition of Cotton. Miley WN, Oosterhuis DM, editors. Growth and Development of a Cotton Plant. Practical Issues, Chapter 1. 2015. p. 1-24. DOI:10.2134/1990.nitrogennutritionofcotton

[13] Gorman C. Mixing It Up—Crop Rotation In The Mid-South. 2020. Available from: https://www.cottonfarming.com/feature-story/mixing-it-up-crop-rotation-in-the-mid-south/

[14] Bowers CG, Collins CA, Harris EP. Low Soil Moisture Planting of Cotton for Optimum Emergence. Applied Engineering in Agriculture. 2006;**22**(6):801-808. DOI: 10.13031/2013.22248

[15] Aydemir M. Cotton Breeding, Cultivation Technique, and Fiber Properties (in Turkish). Ministry of Agriculture and Rural Affairs, General Directorate of Cotton Affairs. Nazilli, Aydın: Nazilli Cotton Research Institute, Publication No: 33; 1982

[16] Unger PW, Baumhardt RJ. Factors related to dryland grain sorghum yield increases 1939 through 1997. Agronomy Journal. 1999;**91**:870-875. DOI: 10.2134/agronj1999.915870x

[17] Traore O, Traore K, Bado VB, Lompo DJ. Crop rotation and soil amendments: impacts on cotton and maize production in a cotton-based system in western Burkina Faso. International Journal of Biological and Chemical Sciences. 2007;**1**(2). DOI: 10.4314/ijbcs.v1i2.39684

[18] Ahmad M, Khan MD, Sultan W, Hassan M, Austin N. Long-term cotton based crop rotation experimentation. In: Proceedings of the World Cotton Research Conference-2. Athens, Greece; 1998. pp. 377-378

[19] Kato M, Kobayashi K, Ogiso E, Yokoo M. Photosynthesis and dry-matter production during ripening stage in a female-sterile line of rice. Plant Production Science. 2004;**7**(2):184-188. DOI: 10.1626/pps.7.184

[20] Gadana DB, Sharma PD, Selfeko DT. Effect of soil management practices and slope on soil fertility of cultivated lands in Mawula Watershed, Loma District, Southern Ethiopia. Advances in Agriculture. 2020;**2020**(8866230):13. DOI: 10.1155/2020/8866230

[21] Ahmed N, Ali MA, Danish S, Chaudhry U, Hussain S, Hassan W, et al. Role of macronutrients in cotton production. Cotton Production and Uses. 2020;**2020**:81-104. DOI: 10.1007/978-981-15-1472-2_6

[22] Kopittke PM. Interactions between Ca, Mg, Na and K: Alleviation of toxicity in saline solutions. Plant and Soil. 2012;**352**:353-362. DOI: 10.1007/s11104-011-1001-x

[23] Santos E, Pongrac P, Reis A, White P, Lavres JJ. Phosphorus-zinc interactions in cotton: consequences for biomass production and nutrient-use efficiency in photosynthesis. Physiologia Plantarum. 2019;**164**(4):996-1007. DOI: 10.1111/ppl.12867

[24] Zhao C, Liu B, Piao S, Wang X, Lobell DB, Huang Y, et al. Temperature increase reduces global yields of major crops in four independent estimates. Proceedings of the National Academy of Sciences of the USA. 2017, 2017;**114**(35):9326-9331. DOI: 10.1073/pnas.1701762114

[25] Seddaiu G, Porcu G, Ledda L, Roggero PP, Agnelli A, Corti G. Soil organic matter content and composition as influenced by soil management in a semi-arid Mediterranean agro-silvopastoral system. Agriculture, Ecosystems and Environment. 2013;**167**:1-11

[26] Havlin HL, Beaton JD, Tisdale SL. Fertility and Fertilizers—An introduction to nutrient management. 7th ed. New Delhi: PHI Learning Private Limited; 2010

[27] Chaudhry MR, Guitchounts A. Cotton Facts, International Cotton Advisory Committee, Technical Paper No. 25 of the Common Fund for Commodities. New York: The United Nations; 2003

[28] Rochester IJ. Phosphorus and potassium nutrition of cotton: Interaction with sodium. Crop and Pasture Science. 2010;**61**:825-834. DOI: 10.1071/CP10043

[29] Silvertooth JC, Norton ER. Management of Fertilizer Nitrogen in Arizona Cotton Production. The University of Arizona Cooperative Extension; 2016. Available from: https://www.researchgate.net/publication/279500445

[30] Khan A, Wang L, Ali S, Tung SA, Hafeez A, Yang G. Optimal planting density and sowing date can improve cotton yield by maintaining reproductive organ biomass and enhancing potassium uptake. Field Crops Research. 2017;**214**:164-174

[31] Meng Q, Yue S, Chen X, Cui Z, Ye Y, Ma W. Understanding dry matter and nitrogen accumulation with time-course for high-yielding wheat production in China. PLoS One. 2013;**8**(7):e68783. DOI: 10.1371/journal.pone.0068783

[32] Xinhua Y. In-season side-dressing of urea and ammonium nitrate to cotton on no-till soils with high residual nitrogen and pre-plant nitrogen application. Open Journal of Soil Science. 2015, 2015;**5**: 276-286. DOI: 10.4236/ojss.2015.511026

[33] Leghari SJ, Wahocho NA, Laghari GM, Talpur KH, Wahocho SA, Lashari AA. Role of nitrogen for plant growth and development: A review. Advances in Environmental Biology (Jordon). 2016;**10**(9):209-218

[34] Groenveld T, Kohn YY, Gross A, Lazarovitch N. Optimization of nitrogen use efficiency by means of fertigation management in an integrated aquaculture-agriculture system. Journal of Cleaner Production. 2019;**212**:401-408. DOI: 10.1016/j.jclepro.2018.12.031

[35] Mahajan U. Drones for normalized difference vegetation index (NDVI), to estimate crop health for precision agriculture: A Cheaper Alternative for Spatial Satellite Sensors, Conference: International Conference on Innovative Research in Agriculture, Food Science, Forestry, Horticulture, Aquaculture, Animal Sciences, Biodiversity. Ecological Sciences and Climate Change (AFHABEC-2016). 2016

[36] Guan W. Effects of Nitrogen Fertilizers on Soil pH. Purdue: Purdue University Extension Service; 2016. Available from: https://vegcropshotline.org/article/effects-of-nitrogen-fertilizers-on-soil-ph/

[37] Wilfret GJ. Gladilolus. In: Larson RA, editor. Introduction to Floculture. Elsevier Inc., Academic Press; 1992. DOI: 10.1016/B978-0-12-437651-9.50001-4. ISBN: 978-0-12-437651-9

[38] Hickey MG, Stichler CR, Livingston SD. Using Petiole Analysis for Nitrogen Management in Cotton. Texas: Texas A&M University Extension Service; 1996. p. L-5156

[39] Havlin JL, Austin R, Hardy D, Howard A, Heitman JL. Nutrient management effects on wine grape tissue nutrient content. Plants. 2022;**11**:158. DOI: 10.3390/plants11020158

[40] Westcott M. Petiole Sap Analysis—A Ouick Tissue Test for Nitrogen in Potatoes. Montana State University Extension Service, Fertilizer Facts: June 1993, Number 5; 1992

[41] Kohlmeier M. Chapter 11—Minerals and trace elements. In: Second E, editor. Kohlmeier MBT-NM. San Diego: Academic Press; 2015. pp. 673-807

[42] Razaq M, Zhang P. Shen Hl (2017) Influence of nitrogen and phosphorous on the growth and root morphology of Acer mono. PloS one. 2017;**12**:e0171321

[43] Johan PD, Ahmed OH, Omar L, Hasbullah NA. Phosphorus transformation

in soils following co-application of charcoal and wood ash. Agronomy. 2021;**2021**:11. DOI: 10.3390/agronomy11102010

[44] Roy RN. Integrated plant nutrition systems—Conceptual overview. In: Proceedings of symposium on "Integrated plant nutrition management". Islamabad, Pakistan: National Fertilizer Development Center; 2000. pp. 45-48

[45] Lynch JP. Root phenes for enhanced soil exploration and phosphorus acquisition: Tools for future crops. Plant Physiology. 2011;**156**:1041-1049

[46] Singh V, Pallaghy CK, Singh D. Phosphorus nutrition and tolerance of cotton to water stress: I. Seed cotton yield and leaf morphology. Field Crops Research. 2006;**96**:191-198

[47] Rosolem CA, Helena R, Esteves SAF. Potassium supply to cotton roots as affected by potassium fertilization and liming. Pesquisa Agropecuária Brasileira. 2003;**38**:635-641. DOI: 10.1590/S0100-204X2003000500012

[48] Amtmann A, Troufflard S, Armengaud P. The effect of potassium nutrition on pest and disease resistance in plants. Physiologia Plantarum. 2008;**133**(4):682-691. DOI: 10.1111/j.1399-3054.2008.01075.x

[49] Krishnan K, Ngerong AA, Ahim K, Ahmed OH, Ali M, Omar L, et al. Mitigating potassium leaching from muriate of potash in a tropical peat soil using clinoptilolite zeolite, forest litter compost, and chicken litter biochar. Agronomy. 2021;**2021**:11. DOI: 10.3390/agronomy11101900

[50] Yang JS, Hu W, Zhao W. Soil potassium deficiency reduces cotton fiber strength by accelerating and shortening fiber development. Scientific Reports. 2016;**6**:28856. DOI: 10.1038/srep28856

[51] Fontana JE, Wang G, Sun R, Xue H, Pan X. Impact of potassium deficiency on cotton growth, development and potential microRNA-mediated mechanism. Plant Physiology Biochemistry. 2020;**153**:72-80. DOI: 10.1016/j.plaphy.2020.05.006

[52] Gormus O, Yucel C. Different planting date and potassium fertility effects on cotton yield and fiber properties in Cukurova region, Turkey. Field Crops Research. 2002;**78**:141-149. DOI: 10.1016/S0378-4290(02)00121-1

[53] Oosterhuis DM. Potassium management of cotton. In: Pasricha NS, Bansal SK, editors. Potassium for sustainable crop production. International Potash Institute Basel, Switzerland and Potash Research Institute of India; 2002. pp. 331-346

[54] Tiwari KN. Diagnosing potassium deficiency and maximizing fruit crop production. Better Crops. 2005, 2005;**4**:29-31

[55] Brosnan JT, Brosnan ME. The sulfur-containing amino acids: an overview. Journal of Nutrition. 2006;**136**(6 Suppl):1636S-1640S. DOI: 10.1093/jn/136.6.1636S

[56] Edwards PJ (1998) Sulfur cycling, retention, and mobility in soils: A review. Gen. Tech. Rep. NE-250. Radnor, PA: U.S. Department of Agriculture, Forest Service, Northeastern Research Station. 18

[57] Hawkesford MJ. Plant responses to sulphur deficiency and the genetic manipulation of sulphate transporters to improve S-utilization efficiency. Journal of Experimental Botany. 2000;**51**(342): 131-138. DOI: 10.1093/jexbot/51.342.131

[58] Singh R, Parul P, Prasad SM. Sulfur and calcium simultaneously regulate photosynthetic performance

and nitrogen metabolism status in as-challenged brassica juncea L. Seedlings, Frontiers in Plant Science. 2018;**9**:772. DOI: 10.3389/fpls.2018.00772

[59] Clarkson DT. Calcium transport between tissues and its distribution in the plant. Plant Cell Environment. 2006;**7**:449-456

[60] Thor K. Calcium—Nutrient and messenger. Frontiers in Plant Science. 2019;**10**:440. DOI: 10.3389/fpls.2019.00440

[61] Capaldi FR, Gratão PL, Reis AR. Sulfur metabolism and stress defense responses in plants. Tropical Plant Biology. 2015;**8**:60-73. DOI: 10.1007/s12042-015-9152-1

[62] Gao H, Wu X, Zorrilla C, Vega S, Palta E, Jiwan P. Fractionating of calcium in tuber and leaf tissues explains the calcium deficiency symptoms in potato plant overexpressing CAX1. Frontiers in Plant Science. 2020;**10**:1793. DOI: 10.3389/fpls.2019.01793

[63] Rahman M, Punja ZK. Calcium and plant disease. In: En Datnoff LE, Elmer WH, Huber DM, editors. Mineral Nutrition and Plant Disease. Saint Paul, MN: APS Press; 2007. pp. 79-93

[64] White PJ, Broadley MR. Calcium in plants. Annals of Botany. 2003;**92**(4): 487-511. DOI: 10.1093/aob/mcg164

[65] Shaul O. Magnesium transport and function in plants: the tip of the iceberg. Biometals. 2002;**15**(3):309-323. DOI: 10.1023/a:1016091118585

[66] Zhang B, Cakmak I, Feng J. Magnesium deficiency reduced the yield and seed germination in wax gourd by affecting the carbohydrate translocation. Frontiers in Plant Science. 2020;**11**:797. DOI: 10.3389/fpls.2020.00797

[67] Jákli M, Tränkner M. Critical leaf magnesium thresholds and the impact of magnesium on plant growth and photo-oxidative defense: A systematic review and meta-analysis from 70 years of research. Frontiers in Plant Science. 2019;**110**:776. DOI: 10.3389/fpls.2019.00766

[68] Ryan MF. The role of magnesium in clinical biochemistry: an overview. Annals of Clinical Biochemistry. 1991;**28**:19-26

[69] Cazzola R, Della Porta M, Manoni M, Iotti S, Pinotti L, Maier JA. Going to the roots of reduced magnesium dietary intake: A tradeoff between climate changes and sources. Heliyon. 2020;**6**(11):e05390. DOI: 10.1016/j.heliyon.2020.e05390

[70] Tripathi D, Singh S, Singh S, Mishra S, Chauhan D, Dubey N. Micronutrients and their diverse role in agricultural crops: advances and future prospective. Acta Physiologiae Plantarum. 2015;**37**:1-14. DOI: 10.1007/s11738-015-1870-3

[71] Hajiboland R. Effect of micronutrient deficiencies on plants stress responses. In: Ahmad P, Prasad M, editors. Abiotic Stress Responses in Plants. New York, NY: Springer; 2012. DOI: 10.1007/978-1-4614-0634-1_16

[72] Saleem MH, Ali S, Kamran M, Iqbal N, Azeem M, Tariq Javed M, et al. Ethylenediaminetetraacetic acid (EDTA) mitigates the toxic effect of excessive copper concentrations on growth, gaseous exchange and chloroplast ultrastructure of Corchorus capsularis L. and improves copper accumulation capabilities. Plants. 2020;**9**(6):756. DOI: 10.3390/plants9060756

[73] Pal D, Dasog G, Bhattacharyya T. Secondary calcium carbonate in soils

of arid and semi-arid regions of India. In: Lal R, Kimble JM, Eswaran H, Stewart BA, editors. Global climate change and Pedogenic Carbonates. London: Lewis Publishers; 2000. pp. 149-185

[74] Rafique E, Rashid A, Bhatti A, Rasool G, Bughio N. Boron deficiency in cotton grown on calcareous soils of Pakistan. Boron in Plant and Animal Nutrition. 2002:357-362. DOI: 10.1007/978-1-4615-0607-2_35

[75] Morgan JB, Connolly EL. Plant-soil interactions: Nutrient uptake. Nature Education Knowledge. 2013;**4**(8):2

[76] Kodama N, Nishimuta M, Suzuki K. Negative balance of calcium and magnesium under relatively low sodium intake in humans. Journal of Nutritional Science Vitaminology (Tokyo). 2003;**49**(3):201-209. DOI: 10.3177/jnsv.49.201

[77] Brown P, Fernández V, Sotiropuolus T. Foliar Fertilization: The Facts and the Fiction. Paris, France: International Fertilizer Industry Association (IFA); 2013. ISBN 979-10-92366-00-6

[78] Xie R, Zhao J, Lu L, et al. Penetration of foliar-applied Zn and its impact on apple plant nutrition status: in vivo evaluation by synchrotron-based X-ray fluorescence microscopy. Hortic Research. 2020;**7**:147. DOI: 10.1038/s41438-020-00369-y

[79] Aziz MZ, Yaseen M, Abbas T, Naveed M, Mustafa A, Hamid Y, et al. Foliar application of micronutrients enhances crop stand, yield and the biofortification essential for human health of different wheat cultivars. Journal of Integrative Agriculture. 2019;**18**(6):1369-1378. DOI: 10.1016/S2095-3119(18)62095-7

[80] Çolakoğlu H, Çiçekli M. Foliar Fertilization Guide (in Turkish). Istanbul: Toros Tarım; 2016. p. 115

[81] Zonta JH, Brandão ZN, Rodrigues JIS, Sofiatti V. Cotton response to water deficits at different growth stages. Engenharia Agrícola, Rev. Caatinga, Mossoró. 2017;**30**(4):980-990. DOI: 10.1590/1983-21252017v30n419rc

[82] Tariq M, Yasmeen A, Ahmad S, Hussain N, Afzal MN, Hasanuzzaman M. Shedding of fruiting structures in cotton: Factors, compensation and prevention. Tropical and Subtropical Agroecosystems. 2017;**20**:251-262

[83] Mart C. Integrated production in cotton (in Turkish). Journal of Turkish Seed Growers Association; 2017;(21):42-44

[84] Enciso J, Hillyer C, Porter D, Fipps G. Irrigation Timing During Drought. Texas AM Agri Life Extension; 2019. Available from: https://cdn-ext.agnet.tamu.edu/wp-content/uploads/2019/07/EBN-015_-Irrigation-Timing-During-Drought_-Corn-Cotton-and-Sorghum-Furrow-Systems.pdf. [Accessed: 2022-02-10]

[85] Hall D, Cloud C, Porter W. Cotton Irrigation Termination. University of Georgia Extensive Service; 2020

[86] Ale S, Omani N, Himanshu SK, Bordovsky JP, Thorp KR, Barnes EM. Determining optimum irrigation termination periods for cotton production in the Texas high plains. Transactions of ASABE. 2020;**63**:105-115

[87] Ahmad F, Din S, Perveen A, Afzal MN. Investigating critical growth stage of cotton subject to water deficit stress. Iranian Journal of Plant Physiology. 2013;**4**(1):873-880

[88] Tronstad R, Silvertooth JC, Husman S. Irrigation termination of cotton: an economic analysis of yield, quality, and market factors. Journal of Cotton Science. 2003;7:86-94

[89] Sankaranarayanan K, Praharaj CS, Bandyopadhyay KK. Water management practices to improve cotton production. Model Training Course on "Cultivation of Long Staple Cotton (ELS)" December 15-22, 2007. Central Institute for Cotton Research, Regional Station, Coimbarote. 2007;**2007**:171-182

[90] Allen RG, Pereira L, Raes D, Smith M. Crop Evapotranspiration: Guidelines for Computing Crop Water Requirements. FAO Irrigation and Drainage Paper 56. Rome, Italy: FAO; 1998

[91] Min W, Guo H, Zhang W, Ma L, Ye J, Hou Z. Root distribution and growth of cotton as affected by drip irrigation with saline water. Field Crops Research. 2014;**169**:1-10. DOI: 10.1016/j.fcr.2014.09.002

[92] Camp CR, Bauer BJ, Busscher WJ. A comparison of uniformity measures for drip irrigation. Transactions of the ASAE. 1997;**40**(4):1013-1020

[93] FAO. Irrigation Manual: Planning, Development Monitoring and Evaluation of Irrigated Agriculture with Farmer Participation—Volume II Module 7. Harare: The Food and Agriculture Organization of the United Nations; 2002

[94] McGinty J, Kimura E, Dotray P, Morgan G, Byrd S. Weed Management in Texas Cotton. 2017. Available from: http://cotton.tamu.edu/Weed%20Management/2017%20Cotton%20Weed%20Control%203-15-2017.pdf

[95] Tariq M, Abdullah K, Ahmad S, Abbas G, Rahman MH, Khan MA. Weed management in cotton. In: Ahmad S, HasanuzzamanM,editors.CottonProduction and Uses. Singapore: Springer; 2020. DOI: 10.1007/978-981-15-1472-2_9

[96] Pala F, Mennan H. Determination of activity preemergence herbicides for weed control in cotton. International Plant Protection Congress (September 5-8, 2008 Konya), In: Cotton Production Under Abiotic Stress. 2016. pp. 257-288

[97] Singh K, Singh H, Singh K. Weed management in cotton with pre-and post-emergence herbicides. Indian Journal of Weed Science. 2016;**48**:348. DOI: 10.5958/0974-8164.2016.00090.3

[98] TAGEM. Pamuk Entegre Mücadele Teknik Talimatı (in Turkish). Ankara, Turkey: General Directorate of Agricultural Research and Policies; 2017. p. 106

[99] Davara D, Rajani V. Integrated disease management of cotton. Journal of Cotton Research Development. 2020;**34**(2):261-266

[100] Haq İ, Ijaz S. Plant Disease Management Strategies for Sustainable Agriculture through Traditional and Modern Approaches. Berlin: Springer International Publishing; 2020. DOI: 10.1007/978-3-030-35955-3

[101] Pscheidt JW, Ocamb CM. PNW Plant Disease Management Handbook. Oregon State University; 2021. 998 p. ISBN-13: 978-1931979214

[102] Ogle H, Dale M. Disease management: Cultural practice. In: Brown J, Ogle H, editors. Plant Pathogens and Plant Diseases. Armidale: Rockvale Publishers; 1997. pp. 390-404

[103] Soliman AS. Plant Growth Hormones. In: Vikas B, Fasullo M, editors. Cell Growth. London: IntechOpen; 2019. DOI: 10.5772/intechopen.84350

[104] Biles S, Cothren JT. Flowering and Yield Response of Cotton to Application of Mepiquat Chloride and PGR-IV. Crop Science—CROP SCI. 2001;**41**:1834-1837. DOI: 10.2135/cropsci2001.1834

[105] Çopur O, Demirel U, Karakuş M. Effects of several plant growth regulators on the yield and fiber quality of cotton (Gossypium hirsutum L.). Not. Bot. Hort. Agrobot. Cluj. 2010;**38**(3):104-110

[106] Stewart A, Edmisten K, Wells R. Boll openers in cotton: Effectiveness and environmental influences. Field Crops Research. 2000;**67**:83-90. DOI: 10.1016/S0378-4290(00)00093-9

[107] Ren Y, Meng Y, Huang W, Ye H, Han Y, Kong W, et al. Novel vegetation indices for cotton boll opening status estimation using sentinel-2 data. Remote Sensing. 2020;**12**:1712. DOI: 10.3390/rs12111712

[108] Leon RG, Wright DL, Brecke J. 2020 Cotton Defoliation and Harvest Aid Guide. 2021. Available from: https://edis.ifas.ufl.edu/publication/AG188

[109] Gormus O, Kurt F, EL Sabagh A. Impact of defoliation timings and leaf pubescence on yield and fiber quality of cotton. Journal of Agricultural Science and Technology. 2017;**19**:903-915

[110] Beyyavaş V. The effect of different harvest aiding chemicals on yield and yield components of cotton (Gossypium Hirsutum L.). Applied Ecology and Environmental Research. 2019;**17**(2):2733-2743. DOI: 10.15666/aeer/1702_27332743

Section 3

Cotton Pests and Plant Protection Methods

Chapter 8

Pest Insects and Their Biological Control

Gozde Busra Eroglu

Abstract

Cotton is an industrial plant with a high commercial value. It is used in various fields such as textile, food (cotton oil), gunpowder industry, paper, and furniture production. One of the most important problems encountered during cotton production is insects that feed on cotton and cause economic loss. The intensive amount of pesticides is used by the producers for the control of pest insects. As insects gain resistance to pesticides over time, the amount of chemical pesticides applied is gradually increasing. Chemical products are quite harmful to both living things and the environment. For this reason, there is a need to popularize biological control methods instead of using pesticides to control pests. In this chapter, detailed information about insect species causing damage to cotton and biological control methods is given.

Keywords: cotton pests, damage, biological control, pesticide

1. Introduction

Cotton, *Gossypium hirsutum* (Linnaeus) is an important cultivated plant in the mallow family (Malvaceae), originated from India [1, 2]. Cotton is one of the oldest and most common agricultural products in the world. The fiber of cotton is used in the textile industry, cottonseed is used in the oil industry, and the pulp obtained after oil extraction is used in the feed industry [3]. The use of cotton in various commercial areas contributes to the economy of many countries and has an important place in both exports and employment [4]. It is an agricultural product that employs millions of people and earns money in the production, processing, and marketing, which is grown in temperate and subtropical regions of more than 60 countries. In addition, cotton is a very important economic base in developing and underdeveloped countries, and it is a product that provides foreign exchange income for these countries [1, 4]. Especially in recent years, organic cotton and organic textile products have become preferred by consumers [5]. However, factors affecting the economic importance of cotton in the field of plant protection are pests, diseases, and weeds. These factors reduce cotton yield by about 30% [6]. The use of plenty of water and fertilizer in the cultivation of cotton, which is a plant with abundant green parts, makes the plant attractive to harmful insects [3]. In cotton production, harmful insects are encountered in every period from sowing to the end of harvest. In cases where the pest population exceeds the economic damage threshold, the

yield loss in cotton is 15–20% [7]. There are 96 insect and mite species known as the main pest and other pests in cotton [8]. While chemical control should be the last method to be applied in the control against these pests, it is frequently referred to by the producers [9]. Since the fiber obtained from cotton is not a direct nutrient, the absence of pesticide residue problem allows the use of pesticides more widely than other herbal products in the fight against pests [10]. For many years, the most common method used by manufacturers to prevent product loss has been chemical control [11]. Although chemical control is seen as an easy-to-apply and successful method of controlling pests in the short term, it causes crucial problems in a long time. Chemical pesticides cause the insects to gain resistance over time, and the beneficial insects in nature die because they are not specific to the target organism [12, 13]. In addition, after application, it accumulates in the soil and mixes with the air and water, harming both plants and other vertebrates. Over time, it accumulates in the human body and causes many diseases. This situation causes the deterioration of the ecological balance and also harms the health of living things. In addition, chemical residues remaining on products, prepared for export, cause rejection of products by many countries. Thus, the need to develop biological control methods to be used as an alternative to chemical products in the control of agricultural pests has arisen.

Biological control is the use of predators, parasitoids, or pathogens to control the population of the target organism. In biological control, predators and parasitoids are methods based on the use of beneficial insects against the target organism, while pathogens consist of microorganisms that cause disease or death of the target organism. These microorganisms originate from fungi, nematodes, bacteria, protozoa, and viruses and are bioinsecticides that can reduce harmful insect populations below the economic damage threshold in a short time [14]. Studies on widespread use of these pathogens have gained importance because, unlike chemical substances, they are specific to the host, do not cause harm to nontarget organisms, do not leave residues in nature, and are environmentally friendly and reliable [15]. For this reason, as in other products, cultural measures and biological control should be the first preferred control methods in cotton [3]. Chemical control should be used as the last alternative. It is more important in terms of biological control to protect the natural enemies present in the grown cotton [16]. In order to keep pests below the economic damage threshold, natural enemies and friendly microorganisms should be given an opportunity.

In this chapter, harmful insects that feed on cotton plants and cause economic loss and biological control methods applied against them are given.

2. Cotton pest insects

The pest insects' variety and density vary according to the development stage of the cotton plant and the geography where it grows. In this section, insects that cause economic loss by feeding on cotton are classified under two headings as main pests and other pests.

2.1 Main pest insects

Insects that are the main pests of cotton are: cotton aphid (*Aphis gossypii*), cotton jassid (*Amrasca bigutulla*), tobacco thrips (*Thrips tabaci*), cotton leafhoppers (*Empoasca*

decipiens and *Asymmetrasca decedens*), two-spotted spider mite (*Tetranychus urticae*), and white tobacco fly (*Bemisia tabaci*) [9]. These insects cause great economic losses in cotton by invading cotton planted areas, especially in summer [17].

2.1.1 Cotton aphid, A. gossypii glover (Hemiptera: Aphididae)

Adult individuals of the pest, which have an average maturity of 7 days, have the ability to procreate offspring immediately. Since aphids reproduce by parthenogenetic reproduction, they have the ability to form large colonies in a short time [9]. This insect damages cotton in several different ways. Plant sap of cotton is rich in sugar, yet low in protein. For this reason, aphids need to take large amounts of sap to obtain sufficient protein. Excess sugar is secreted in the form of honeydew and makes the crop and fruit sticky. Black mold fungi (*Cladosporium* spp.) thrive in this plant sap, contaminating fruit and ornamental plants while making them unsuitable for the market. At the same time, photosynthesis in leaves decreases, which affects the production of cotton [18]. However, nymphs and adults take nutrients from the plant and disrupt the balance of growth hormones. As a result, plant growth is slowed by deformed leaves or pest infestation. In addition, being a vector of plant viruses, it causes different diseases to be transmitted to cotton [19]. This aphid species can transmit more than 70 different viruses, including the cucumber mosaic virus [18]. *A. gossypii* has many natural enemies and these are very effective in reducing the population of the pest. In the basic development period, it is very important for biological control that a large number of useful insects such as Coccinellid (bride beetles) pass to cotton after the wheat harvest. However, in order to preserve this existing natural enemy balance and to be effective, the field should be controlled very well during this period and care should be taken not to disturb the natural balance by avoiding unnecessary spraying. The most effective natural enemies of cotton aphids are especially *Chrysoperla carnea* and Coccinellid larvae. In addition, *Fusarium subglutinosa*, which is an entomopathogenic fungus, is effective in reducing the aphid population from time to time [9, 20].

2.1.2 Cotton jassid, Amrasca bigutulla *Ishida (Hemiptera: Cicadellidae)*

Amrasca bigutulla is one of the most damaging species to the cotton plant. It feeds on cotton in both nymph and adult stages by sucking the sap of the cotton plant due to its absorbent and piercing mouth structure. They cause damage to the plant with the poisonous saliva it leaves on the plant during feeding [21–25]. Intense infestation of *A. bigutulla* on cotton causes leaves to turn yellow, curl up, and fall off. In addition, the secretions that insects leave on cotton cause mold formation on the plant. In this case, it restricts the amount of light reaching the photosynthetic surfaces of the plant and reduces the yield [25]. These harmful species cause an epidemic in cotton plants almost every year [26]. Natural enemies (ladybugs, predatory lygaeid insects, and various mantises) and neem oil are widely used as a method of control [27].

2.1.3 Tobacco thrips, T. tabaci *Lindeman (Thysanoptera: Thripidae)*

T. tabaci grow in dry environments rather than moist environments, and in the years when the spring is dry, their density is quite high and the damage increases. It feeds on the underside of the leaves. Adults and nymphs tear the epidermis of the leaves and stems of cotton and tobacco plants with their mouthparts and suck the sap,

while also destroying the chlorophyll-bearing cells [28]. The places where the pest feeds on the plant take a silvery color after a while. In heavy contamination, the leaves of cotton seedlings curl, turn brown, and fall off. If the growth point of the plant is damaged, a forked plant occurs [29]. Reduction in fruit branches in the lower parts of the damaged plant causes a decrease in yield. In addition, delays in harvesting occur in heavy damage [30]. Tobacco thrips have many effective natural enemies. Natural enemies are effective in reducing the population of the pest. The *Orius* species (*Orius albidipennis*, *Orius niger*, *Orius horvathi*) are among the most effective natural enemies [9].

2.1.4 Cotton leafhoppers, Empoasca decipiens *Paoli and* Asymmetrasca decedens *Paoli (Hemiptera: Cicadellidae)*

Cotton leafhoppers, which are seen in dense populations in the early period in cotton fields, feed on the vegetative and generative parts of the cotton plant by sucking, affect the development of the plant negatively, and cause shedding especially in the generative organs [31]. It is known that hairless and broad-leaved cotton varieties are more adversely affected by the population growth of leafhoppers [32, 33]. In addition to the sucking damage, it gives to the plant, cotton leafhoppers are also harmful because of toxic secretions into the plant body. The toxic substances cause hypertrophy in the phloem tissue cells of the leaf and blockages in sap transport. Biological control of cotton leafhoppers is done with the use of natural enemies. Among these natural enemies, the most successful are: *C. carnea*, *Deraeocoris* spp., *Geocoris* spp., *Nabis* spp., and *Paederus kalalovae* [9].

2.1.5 Two-spotted spider mite, Tetranychus urticae *Koch (Acarina: Tetranychidae)*

Tetrancyhus urticae Koch (Acari: Tetranychidae), also called the two-spotted red spider, is an important polyphagous pest species that are frequently found in agricultural areas where crop production is carried out in the world [34, 35]. The two-spotted spider mite is found in all parts of the plant. However, it especially prefers fresh and strong leaves and lives under these leaves. It is densely located on the underside of the leaves, especially where the petiole and leaf blade meet, and passes from there to other parts of the cotton plant. As a result of the feeding of the pest, yellow spots interspersed on the upper surface of the leaves, which are its characteristics. Later, the yellow spots turn red due to the damage of the chlorophyll substance, which gives the leaf its green color. This redness increases and covers the entire leaf surface or a part of the leaf homogeneously, and the leaves dry out before time [36, 37]. Another feature of the pest is the nets they form due to the substances they secrete during their feeding. The abundance of the nets also indicates that the pest population is dense [9]. The economic loss caused by mites in the plant can reach significant dimensions depending on the population, and these mites can hardly be controlled even with the use of intensive pesticides. Although success can be achieved with biological control elements in the control of these mites in greenhouse cultivation in the world, producers in many places prefer chemical pesticides in the control of this pest. The extensive use of these chemical drugs has caused this mite to develop resistance primarily to organophosphorus, mitochondrial electron transport inhibitors, growth regulators, and many specific acaricides [34, 38]. In the biological control of *Tetrancyhus urticae*, ethanol extracts obtained from sage, rosemary, yarrow, and cumin plants are used to remove the

harmful species from the plant [39]. In addition, the two-spotted spider mite has many effective natural enemies. Of these species, *Scolothrips longicornis* and *Stethorus* spp. are specialized predators of the pest. For this reason, if pest control is required, specific acaricides should be used to protect beneficial species [9].

2.1.6 White tobacco fly, B. tabaci *Gennadius (Hemiptera: Aleyrodidae)*

B. tabaci has become one of the most important cotton pests due to its high reproduction rate and resistance to many chemical pesticides [40]. Whitefly larvae need a lot of protein to grow, so they consume large amounts of plant sap. Since the sap contains a large amount of sugar, the excess sugar is excreted as honeydew. As the larva grows, the amount of freshwater excreted also increases. The damages caused by whiteflies to cotton plants are as follows [41]:

- Since whiteflies feed by sucking the sap of the cotton plant during periods of high population density, plant leaves are greatly damaged. This damage to the leaves can affect fruit development and lead to a decrease in yield.

- Fruits become sticky due to the sweet juice left on them. Dirt sticks to the fruit, and the development of dark mold (Cladosporium spp.) accelerates, so the fruit becomes unsaleable. In severe cases, the fruit rots.

However, dark mold can also develop on the leaves, as a result of which the amount of photosynthesis and transpiration is reduced in cotton plants [41, 42]. The consumption of plant sap by whiteflies and the secretion of fresh juice also reduces the esthetic value of the crop. This is a very important problem, especially in ornamental plants. Besides, larvae inject enzymes into the plant, altering the plant's normal physiological processes [43, 44]. Many effective natural enemies are used in the control of *B. tabaci*. Natural enemies of this species include the predators such as *Amblyseius* spp., *Euseius rubini* (Acarina: Phytoseiidae), *C. carnea* (Neuroptera: Chrysopidae), and *Serangium parcecetosum* (Coleoptera: Coccinellidae); parasitoids such as *Encarsia fomosa*, *Encarsia lutea*, and *Eretmocerus mundus* (Hymenoptera: Aphelinidae); as well as entomopathogens such as *Aschersonia* spp., *Beauveria bassiana*, *Paecilomyces* spp., and *Verticillium lecanidae* [45–49]. In different studies conducted around the world, potential entomopathogenic bacterial species that can be used in pest control have also been determined. Among them, *Enterobacter cloacae*, *Acinetobacter radioresistens*, and *Erwinia persicinus* are promising bacteria for biocontrol of *B. tabaci* [50–52]. However, today there is no entomopathogenic bacterial species that is effective on whiteflies and can be converted into commercial form.

2.2 Other pest insects

Under this section of "other pest insects," information is given about the insects that cause significant damage to the cotton plant by causing epidemics in some years. These insects are cotton bollworm (*Helicoverpa armigera*), pink bollworm (*Pectinophora gossypiella*), Egyptian bollworm (*Earias insulana*), cutworms (*Agrotis ipsilon* and *Agrotis segetum*), beet armyworm (*Spodoptera exigua*), cotton leafworm, (*Spodoptera littoralis*), flower thrips (*Frankliniella intonsa* and *Frankliniella occidentalis)*, and plant bedbugs (*Creontiades pallidus, Lygus gemellatus, Lygus pratensis,* and *Lygus italicus*).

2.2.1 Cotton bollworm, Helicoverpa armigera *Hübner (Lepidoptera: Noctuidae)*

Helicoverpa armigera is an important group that causes millions of dollars of damage every year in the world [53]. Since the adults usually lay their eggs on fresh leaves, the damage starts on the leaves first. The larvae cause product loss by eating only the veins of the leaves and even eating some of the veins. In the following period, the larvae turn to the upper part of the plant and begin to feed on the flower bud, seed, and capsule. Since edible flowers generally cannot form seed capsules, crop yield is directly affected. After the seed capsules are formed, damage occurs as a result of the larvae feeding by piercing the capsules [54, 55]. In areas with high populations, they can cause significant damage, requiring replanting. *H. armigera* has a number of natural enemies found in the orders Hymenoptera, Diptera, Coleoptera, Hemiptera, and Neuroptera. Although parasitoids and predators have the ability to keep their hosts under pressure, they are not sufficient for the control of pests due to their insufficient number in nature [56]. There are 2 commercial preparations that are widely used in the world for the microbial control of *H. armigera*: *Bacillus thrungiensis* [57] and nucleopolyhedrovirus (NPV). These belong to the baculovirus group. However, it was reported that *H. armigera* developed resistance against *B. thrungiensis* [58, 59]. For this reason, studies on the development of baculovirus-derived products have been focused on the control of *H. armigera* [60–68].

2.2.2 Pink bollworm, Pectinophora gossypiella *Saund (Lepidoptera: Gelechiidae)*

The larvae of *Pectinophora gossypiella* feed on the comb, flower, and cocoon parts of the cotton plant, and the larvae eat pollen and anther, especially in the flower, preventing fertilization of the plant [69]. In addition, the larvae feeding on cotton seeds secrete a substance during feeding and this substance creates twin seeds by sticking 2 seeds together. In years when the pest density in the cocoon is high, blind cocoon formation is observed and the damage rate can reach up to 80% [70]. The small size of *P. gossypiella* eggs allows the pest to be easily suppressed by natural enemies. The most well-known natural enemies are: *Pyemotes ventricosus*, (Acarina: Pyemotidae), *Exeristes roborator* (Hymenoptera: Ichnoumonidae), Chrysocharis sp. (Hymenoptera: Eulophidae), Habrocytus sp. (Hymenoptera: Pteromalidae), and *Pediculoides ventricosus*. (Acarina: Piemotidae) [71, 72].

2.2.3 Egyptian bollworm, E. insulana *Boisduval (Lepidoptera: Noctuidae)*

E. insulana, which is an important pest in cotton, directly affects the yield and quality of a cotton plant. This pest causes damage to shoots, combs, flowers, and cocoons. The larva that emerges from the egg while the cotton plant is in its development period is fed by eating the bud. Then it pierces the shoot and enters the stem and continues to feed in the stem [9]. In the comb area of the cotton, the larvae generally penetrate the top of the comb and enter and cause damage. Larvae in more advanced stages can do their damage by piercing the comb from the side. Damaged combs are poured. *E. insulana* does its main damage in the cotton boll. The newly hatched larvae usually enter the lower half of the cocoon and expel the dung. The larva also feeds on undeveloped fiber and grains. More than one larva can be found in a cocoon. Cocoons damaged by prickly worms usually do not open, and the damaged bolls create a suitable environment for the growth of bacteria that cause angular leaf spot disease (*Xanthomonas malvacearum*). When there is no control during the epidemic years, it

can cause up to 90% damage [73]. Natural enemies are mainly used in the biological control of the *E. insulana*. Predatory insects, especially in the *Orius* genus, are quite successful in controlling the population density of *E. insulana* [9].

2.2.4 Cutworms, A. ipsilon *Hufnagel,* Agrotis segetum *Schiffer (Lepidoptera: Noctuidae)*

Cutworms larvae damage cotton seedlings by cutting them. It damages cotton plants by cutting from the two-leaf period, which is the basic development period, to the 6-8-leaf period, and cuts the young plants from the soil surface. However, they can also cut underground under conditions where the soil is soft and the soil moisture is low. Especially large larvae pull the cut plants under the ground and eat their leaves. They do damage by taking turns. Damage is greater in late planting areas and rainy spring months. Damage may occur to a degree that requires replanting [9]. Biological control agents, including fly and wasp parasites, disease organisms, and predatory beetles, continually reduce cutworm populations [74]. However, entomopathogenic nematodes are used successfully in the control of cutworms living under the ground [75, 76].

2.2.5 Beet armyworm, S. exigua *Hübner (Lepidoptera: Noctuidae)*

S. exigua larvae are mostly seen in cotton in the early period. Especially after the first hoe, it passes from weeds to cotton plants and its damage is important in this period. They are seen more intensely after the rainy spring months. The first instar larvae that have just hatched from the egg coexist collectively at first. Then, larvae consume the epidermis of the leaf, making it like a membrane. It prevents the growth of the plant by damaging the leaves and tip shoots of small cotton plants. The damage in the leaf is in the form of large holes with regular edges. If the plant is in the combing period, it will also be harmful to leaves, shoots, and combs. However, they do not eat the combs completely, and they gnaw them out from the outside, although they rarely get inside the comb. In addition, it can be damaged in the flower and cocoon of the cotton plant. However, this damage to the pest is not significant. During the epidemic years, it causes significant damage to the median by eating the top shoots and leaves of the cotton in a way that the middle vein remains or completely [9]. In its biological control, formulations originating from entomopathogenic bacteria *Bacillus thrungiensis* isolate, and toxin proteins produced by this isolation are used successfully [77–80]. However, baculovirus has been used successfully in commercial products [81, 82].

2.2.6 Cotton leafworm, Spodoptera littoralis *Boisduval (Lepidoptera: Noctuidae)*

Spodoptera littoralis larvae mostly damage the leaf part of the cotton plant. The newly hatched *S. littoralis* larvae feed in such a way that only the large veins of the leaf remain. They gnaw the lower surface of the leaf and eat the epidermis, making it like a membrane. In this case, the leaf takes on a sieve-like appearance. As it grows, it feeds on other leaves and punctures the leaves. In the following periods, they feed on buds and cocoons and cause these parts to shed or dry. Inside the cocoons, the insect's excrement and the holes they create can be seen. Predators (*C. carnea*, *Nabis pseudoferus*) and parasitoids (*Microplitis rufiventris*) are used successfully in biological control [83]. In addition, the use of the bacterial endochitinase enzyme from *Bacillus thuringiensis* has recently been used to control many bacteria-resistant *S. littoralis*

larvae [84]. However, Azadirachtin obtained from the neem tree is an effective herbal solution for the control of *S. littoralis* larvae [85].

2.2.7 Flower thrips, F. intonsa *Trybom,* F. occidentalis *Pergande Thysanoptera: Thripidae*

Flower thrips, especially in late planting cotton fields, in case the population is very high, adults feed on flowers and larvae feed mostly on the cocoons, causing shedding of flowers and newly formed bolls and early opening of mature bolls. However, no economic damage is caused in the cotton fields of our country. Species belonging to this genus are harmful, especially by sucking on the flowers and flower buds of the cotton plant. In addition, large and mature cocoons cause the formation of cocoons that do not fully open and are called "Crispy cocoons" as a result of the suction damage that occurs in dense populations [9]. In the biological control of flower thrips, predatory insects of the genus *Dicyphus* and *Orius* and the fungus *Metarhizium anisopliae* have been used successfully [86, 87].

2.2.8 Plant bedbugs, Creontiades pallidus *Rumb,* Lygus gemellatus *Herrich-Schaffer,* L. pratensis *Linnaeus,* Lygus italicus *Wagner (Hemiptera: Miridae)*

Plant bedbugs feed by sucking all the organs of the cotton plant due to their stinging and sucking mouth structures. The absorbed place deformed as a result of the toxic substance secreted and then turns black. If the damage occurs on the leaves, the leaf tissue dies over time in the place where it is absorbed. The leaves become perforated or segmented. These pest larvae do their main damage by feeding on generative organs [88]. Most of the scallops, flowers, and small bolls that are damaged by the suction are shed. As a result of casting, a decrease in the product occurs, as well as a delay in maturation. In sucked cocoons, the seed weight decreases. This reduces the seed yield [89]. In addition to generative organ casting, they also cause deformities such as abnormal comb formation, elongation of plant height, and an increase in the number of nodes on the branches. Predators (*C. carnea*, *Nabis pseudoferus*) and parasitoids (*Leiophron deciphiens*) are used in the biological control of plant bedbugs [9].

3. Conclusions

With the increasing importance of cotton plants both in commercial and domestic use, harmful insect species found in cotton fields and their damage to the product have started to gain more importance. Both the suitability of the leaf surface (especially the hairless cotton leaf) and the high irrigation rate of cotton attract harmful insects. For this reason, there are at least 20 agricultural pest insect species on the cotton plant. When cotton producers see the presence of harmful insects on the product, they prefer the use of chemical pesticides in terms of ease of application in a short time. However, the use of chemical products has long-term negative effects on natural enemies (predators and parasites), other nontarget invertebrates and vertebrates, the environment, nature, and human health. Besides, unnecessary and excessive use of chemical pesticides causes harmful species to resistance. Therefore, the use of chemical drugs should be reduced as much as possible, and biological control agents should be preferred instead. Predator and parasitoid species are used quite successfully for

the biological control of cotton pests. In addition, studies on the preparation and marketing of commercial formulations of entomopathogenic microorganisms continue all over the world. In recent years, consumers have started to prefer organic products for all products. In the food and clothing sectors, products containing organic cotton (especially baby clothes) are preferred. For this reason, the development of biological control agents and the cultivation of natural enemies should be supported, and producers should be encouraged to apply them in nature. In particular, the licensing procedures required for placing organic biopesticides on the market involve a very difficult process in some countries. Facilitation of this process by the relevant ministries of agriculture is one of the most important factors that will increase large-scale biopesticide production.

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References

[1] Kılıc S. Determination of population changes of some sıignificant cotton pests and natural enemies in after-crop cotton (*Gossypium hirsutum* l.) varieties of Aydın province [thesis]. Efeler/Aydın, Turkey: Adnan Menderes University; 2014

[2] Pranaya K, Bhat BN, Devi GU, Triveni S. In vitro evaluation of fungicides against Alternaria leaf spot of cotton. International Journal of Chemical Studies. 2020;**8**:3571-3575. DOI: 10.22271/chemi.2020.v8.i4as.10203

[3] Ozgur O, Tatli TA, Gunes M. Determination of damage status of first generation of cotton bollworm [Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae)] on cotton in Cukurova. Plant Protection Bulletin. 2018;**58**:17-23. DOI: 10.16955/bitkorb.328251

[4] Gencer O, Ozudogru T, Kaynak MA, Yılmaz A, Oren N. Cotton production and problems in Turkey. In: Agricultural Engineering of Turkey, VI. Technical Congress. Vol. 1. Ankara. Turkey: TMMOB, Chamber of Agricultural Engineers; 2005. pp. 459-480

[5] Karademir C, Karademir E, Ekinci R, Basbag S. Combining ability estimates and heterosis for yield and fiber quality of cotton in line x tester design. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 2009;**37**(2):228-233

[6] Oerke EC, Dehne HW. Safeguarding productionlosses in major crops and the role of crop protection. Crop Protection. 2004;**23**:275-285. DOI: 10.1016/j.cropro.2003.10.001

[7] Ullah Z. Population dynamics of insect pests of cotton in Dera Ismail Khan and chemical control of bollworms [thesis]. Pakhtoonkhwa, Pakistan: Gomal University;1992

[8] Yunus M, Yousaf M, Jilani G. Insect and spider mite pests of cotton in Pak. Monogr. PL-480, Department of Entomology University of Agriculture Faisalabad; 1980. pp. 256

[9] Anonymus TC. Ministry of Food, Agriculture and Livestock, General Directorate of Food and Control, Department of Plant Protection Products, BKU database. 2017. Available from: https://bku.tarim.gov. en/Search/Index [Accessed: November 11, 2021]

[10] Pimentel D. Estimated annual world pesticide use. In: Facts and Figures. New York: Ford Foundation; 1990

[11] Brooke E, Hines E. Viral biopesticides for heliothine control fact of fiction? Today's Life Science. 1999;**11**:38-45

[12] Fitt GP. Cotton pest management: Part 3. An Australian perspective. Annual Review of Entomology. 1994;**39**:532-562

[13] Haq SK, Atif SM, Khan RH. Protein proteinase inhibitor genes in combat against insects, pests, and pathogens: Natural and engineered phytoprotection. Archives of Biochemistry and Biophysics. 2004;**431**:145-159

[14] Ibaraa J, Castero N. Insect viruses diversity, biology and user as bioinsecticides. Tropical biology and conservation management. 2008;**5**:1-10

[15] Available from: http://www.agroziraat.com [Accessed: November 5, 2021]

[16] Goven MA, Efil L. Studies on the natural enemies and activities of the

harmful greenworm (Heliothis armigera Hübn.) (Lepidoptera: Noctuidae) in cotton fields of the Tigris valley. In: 3rd Biological Control Congress Proceedings. İzmir, Turkey; 1994. 449-457

[17] Al-Mizori HY. The Effects of Some Insecticides on Cotton Pests and Theirs Predators [thesis]. Sur/Diyarbakır, Turkey: Dicle University, institute of science; 2015

[18] Duzgunes Z, Tuatay N. Aphids of Turkey. Ankara Agriculture Control Institute Directorate. 1956;**4**:635

[19] Heneberry TJ, JECH LF. Cotton aphid Biology and Honey-Dew Production. United States of America: University of Arizona, College of Agriculture and Life Sciences; 2001

[20] Ebert TA, Cartwright B. Biology and ecology of *Aphis gossypii* Glover (Homotera: Aphididae). Southwestern Entomologist. 1997;**22**:116-153

[21] Borah PK. Insect pest complex in brinjal (*Solanum melongena* L.). Annals of Agriculture Research. 1995;**16**:93-94

[22] Petal Z, Petal JR. Resurgence of jassid, *Amrasca biguttula biguttula* in Brinjal. Indian Journal of Entomology. 1998;**60**:152-164

[23] Rafique MA, Shan HA. Cotton pest scouting of farmers fields at multan during 1996. Pakistan Entomologist. 1998;**20**:40-42

[24] Sudahlkar K, Punnalah KC, Krishnawa PC. Efficacy of certain selected insecticides on the sucking pest complex on Brinjal. Indian Entomology. 1998;**60**:214-244

[25] Singh A, Singh JJ, Singh K, Rani P. Host range and biology of *Amrasca biguttula biguttula* (Hemitpera: Cicadellidae). International Journal of Environment, Ecology, Family and Urban Studies. 2018;**8**:19-24

[26] Sharma HC. Biotechnological Approaches for Pest Management and Ecological Sustainability. London: CRC Press; 2014. p. 159

[27] Koul O, Dhaliwal GS, Khokhar S, Singh R. Biopesticides in Sustainable Agriculture Progress and Potential. India: Scientific Publishers; 2014. pp. 102-132

[28] Ali A, Bhatti MA, Ahmed KJ. Role of weather in fluctuating the population of Amrasca biguttula biguttula (Dist.) and Thrips tabaci (Lind.) proc. Pakistan Congress of Zoology. 1993;**13**:133-139

[29] Gupta MP, Sandeep S, Shrivastava SK, Sharma S. Population buildup of some sap sucking insects on cotton in Madhya pardesh. Journal of Insect Science. 1997;**10**:153-156

[30] Khan MA, Khaliq A, Subhani MN, Saleem MW. Insecticide and development of Thrips tabaci and Tetranychus urticae on field grown cotton. International Journal of Agriculture and Biology. 2008;**10**:232-234

[31] Bozkurt E. Studies on empoasca (fam. cicadellidae) species, distribution, hosts, type and degrees of damage in aegean region cotton. Ege University Agriculture Faculty Journals. 1970;**146**:71

[32] Ozgur AF, Sekeroglu E, Gencer O, Gocmen H, Yelin D, Isler N. Investigation of population developments of important cotton pests depending on cotton varieties and plant phenology. Turkish Journal of Agricultural and Natural Science. 1988;**12**:48-74

[33] Delvare G. Contribution to study of the insect fauna associated with cotton

crop. Report on a mission in Turkey. In: CIRAD. 1996. pp. 1-38

[34] Atalay E, Kumral NA. Biolocigal features and life tables of Tetranychus urticae (Koch) (Acari: Tetranycidae) on different table tomato varieties. Turkish Journal of Entomology. 2013;**37**:329-341

[35] Balcı MH, Ay R. The effects of some pesticides on life span and fecundity of tetranychus urticae koch adults. Süleyman Demirel University Journal of Natural and Applied Sciences. 2018;**22**:1010-1015. DOI: 10.19113/sdufbed.63244

[36] Toros S. Park and ornamental plants pests. Ankara University Agriculture Faculty Journals. 1992;**429**:165

[37] Saglam HD, Cobanoglu S. Determination of Tenuipalpidae (Acari: Prostigmata) species in parks and ornamental plants of Ankara, Turkey. Turkish Journal of Entomology. 2010;**34**:37-52

[38] Van Leeuwen T, Vontas J, Tsagkarakou A, Dermauwa W, Tirry L. Acaricide resistance mechanisms in the two-spotted spider mite Tetranychus urticae and other important Acari: A review. Insect Biochemistry and Molecular Biology. 2010;**40**:563-572. DOI: 10.1016/j.ibmb.2010.05.008

[39] Dogan M. The Effects of Some Plant Extracts on Some Biological Stage of Tetranychus Urticae (Acari: Tetranychidae) [thesis]. Selçuklu/Konya, Turkey: Selcuk University; 2019

[40] Anonymous. Insect Pest Management in Cotton. 2012. Available from: https://www.daf.qld.gov.au/plants/field-crops-and-pastures/broadacrefield-crops/integrated-pest-management/ipm-information-by-crop/cotton [Accessed: November 11, 2021]

[41] Brown JK, Frohlich DR, Rosell RC. The sweetpotato or silverleaf whiteflies: Biotypes of bemisia tabaci or a species complex? Annual Review of Entomology. 1995;**40**:511-534. DOI: 10.1146/annurev.en.40.010195.002455

[42] Ntawuruhunga P, Legg J. New Spread of Cassava Brown Streak Virus Disease and its Implications for the Movement of Cassava Germplasm in the East and Central African Region. USAID, Crop Crisis Control Project C3P; 2007. DOI: 10.13140/RG.2.2.29239.62887. Available from: http://c3project.iita.org/Doc/A25-CBSDbriefMay6.pdf

[43] Service AR. National Invasive Species Information Center: Silverleaf Whitefly. Washington, DC: United States Department of Agriculture. Available from: https://www.invasivespeciesinfo.gov/terrestrial/invertebrates/silverleaf-whitefly

[44] Schuster DJ, Thompson S, Ortega LD, Polston JE. Laboratory evaluation of products to reduce settling of sweetpotato whitefly adults. Journal of Economic Entomology. 2009;**102**:1482-1489. DOI: 10.1603/029.102.0412

[45] Lacey LA, Wraight SP, Kirk AA. Entomopathogenic Fungi for Control of Bemisia tabaci Biotype B: Foreign Exploration, Research and Implementation. In: Gould J, Hoelmer K, Goolsby J, editors. Classical Biological Control of Bemisia tabaci in the United States, A Review of Interagency Research and Implementation. Berlin: Springer; 2008. pp. 33-69

[46] Ulusoy MR. Whitefly Fauna of Turkey. Adana, Turkey: Baki; 2001. p. 96

[47] Karut K. Host Instar Suitability of Bemisia tabaci (Genn.) (Hom.: Aleyrodidae) for the Parasitoid Eretmocerus mundus (Hym.:

Aphelinidae). Journal of Pest Science. 2007;**80**:93-97. DOI: 10.1007/s10340-006-0157-2

[48] Curthbertson AGS. Update on the status of bemisia tabaci in the UK and the use of entomopathogenic fungi within eradication programmes. Insects. 2013;**4**:198-205. DOI: 10.3390/insects4020198

[49] Faria M, Wraight SP. Biological control of bemisia tabaci with Fungi. Crop protection. 2001;**20**:767-778. DOI: 10.1016/S0261-2194(01)00110-7

[50] Davidson EW, Rosell RC, Hendrix DL. Culturable bacteria associated with the whitefly, bemisia argentifolii (homoptera: Aleyrodidae). Florida Entomologist. 2000;**83**:159-171. DOI: 10.2307/3496151

[51] Dadasoglu F. Isolatıon and Identification of Bacterial Strains with Insecticidal Activities Against Greenhouse and Field Pests. Erzurum, Turkey: Atatürk University Journals; 2007. p. 92

[52] Ateyyat MA, Shatnawi M, Al-Mazra'awi MS. Culturable whitefly associated bacteria and their potential as biological control agents. Jordan Journal of Biological Sciences. 2009;**2**:139-144

[53] Moscardi F, Lobo de Souza M, Batista de Castro ME, Moscardi ML, Szewczyk B. Baculovirus Pesticides: Present state and future perspectives. In: Ahmad I, Ahmad F, Pachtel J, editors. Microbes and Microbial Technology. Berlin: Springer; 2011

[54] Ongoren K, Kaya N, Turkmen S. Studies on the morphology, bioecology and control of greenworm (Heliothis armigera Hüb.) that causes damage to tomatoes in the Aegean region. Plant Protection Bulletin. 1977;**17**:3

[55] Tunç İ. Garden Plant Pests, Akdeniz University Journals. Antalya, Turkey; 1988

[56] Uygun N, Ulusoy MR, Satar S. Biological Control. Turkish Journal of Biological Control. 2010;**1**:1-14

[57] Cherry A, Cock MJW, Van den Berg H, Kfir R. Biological control of Helicoverpa armigera in Africa. In: Neuenschwander P, Borgemeister C, Langewald J, editors. Biological control in IPM systems in Africa. Wallingford, UK: CABI International; 2003. pp. 329-346

[58] Luttrell RG, Jackson RE. Helicoverpa zea and Bt cotton in the United States. GM Crops. 2012;**3**:213-227. DOI: 10.4161/gmcr.20742

[59] Yang Y, Li Y, Wu Y. Current status of insecticide resistance in Helicoverpa armigera after 15 Years of Bt cotton planting in China. Journal of Economic Entomology. 2013;**106**:375-381. DOI: 10.1603/EC12286

[60] Ardisson-Araujo DM, Sosa-Gomez DR, Melo FL, Bao SN, Ribeiro BM. Characterization of Helicoverpa zea single nucleopolyhedrovirus isolated in Brazil during the first old world bollworm (Noctuidae: Helicoverpa armigera) nationwide outbreak. Virus Reviews Research. 2015;**20**:4. DOI: 10.17525/vrrjournal.v20i1.254

[61] Elamathi E, Cholan JR, Vijayakumar N, Ramamourti A. Formulation and optimisation of various nuclear polyhedrosis virus isolates and assessment of their insecticidal activity against Helicoverpa armigera (Hubner) (Lepidoptera: Noctuidae) larvae. Archives of Phytopathology and Plant Protection. 2012;**45**:750-765. DOI: 10.1080/03235408.2011.595908

[62] Eroglu GB, Nalcacioglu R, Demirbag Z. A new Helicoverpa armigera

Nucleopolyhedrovirus isolate from Heliothis peltigera (Denis & Schiffermuller) (Lepidoptera: Noctuidae) in Turkey. Turkish Journal of Biology. 2019;**43**:340-348. DOI: 10.3906/biy-1902-64

[63] Eroglu GB, Demir I, Demirbag Z. A novel alphabaculovirus isolated from the cotton bollworm, Helicoverpa armigera (Hubner) (Lepidoptera: Noctuidae): Characterization and pathogenicity. Biologia. 2018;**73**:545-551. DOI: 10.2478/s11756-018-0053-2

[64] Ferrelli ML, Taibo C, Fichetti P, Sciocco Cap A, Arneodo JD. Characterization of a new Helicoverpa armigera nucleopolyhedrovirus variant causing epizootic on a previously unreported host, Helicoverpa gelotopoeon (Lepidoptera: Noctuidae). Journal of Invertebrate Pathology. 2016;**138**:89-93. DOI: 10.1016/j.jip.2015.08.009

[65] Figueiredo E, Munoz D, Murillo R, Mexia A, Caballero P. Diversity of Iberian nucleopolyhedrovirus wild-type isolates infecting helicoverpa armigera (Lepidoptera: Noctuidae). Biological Control. 2009;**50**:43-49. DOI: 10.1016/j.biocontrol.2009.02.005

[66] Grzywacz D, Rabindra RJ, Brown M, Jones KAJ, Parnell M. Helicoverpa Armigera Nucleopolyhedrovirus Production Manual. Chatham: Natural Resources Institute. FAO; 2004. p. 107. Available from: www.fao.org

[67] Hughes PR, Gettig RR, McCarthy WJ. Comparison of the time mortality response of Heliothis zea to fourteen isolates of Heliothis nuclear polyhedrosis virus. Journal of Invertebrate Pathology. 1983;**41**:256-261

[68] Ibargutxi MA, Munoz D, Bernal A, Ruiz de Escudero I, Caballero P. Effects of stilbene optical brighteners on the insecticidal activity of Bacillus thuringiensis and a single nucleopolyhedrovirus on Helicoverpa armigera. Biological Control. 2008;**47**:322-327. DOI: 10.1016/j.biocontrol.2008.08.019

[69] Elmacı F. The Study and Identification of the Pink Worm *Pectinophora gossypiella* (Saunders) (Lepidoptera:Gelechiidae) Population Development Within the Region of Amik Plain [thesis]. Antakya, Turkey: Hatay Mustafa Kemal University; 2019

[70] Anonymus. Cotton Integrated Control Technical Instruction. 2017. Available from: https://www.tarimorman.gov.tr/TAGEM/Belgeler/yayin/009_pamuk.pdf [Accessed: November 11, 2021]

[71] Cheema MA, Muzaffar N, Ghani MA. Biology, host range and incidence of parasites of Pectinophora gossypiella (Saunders) in Pakistan. Pakistan Cottons. 1980;**24**:37-73

[72] Matthews GA. Pest management in cotton. In: Green MB, Lyon DJ d B, editors. Chichester. West Sussex, UK: Ellis Horwood Limited; 1989. p. 259

[73] Anonymus. Plant Protection Technical Instructions. Bollworm in Cotton (Earias insulana Boisd.) (Lep.: Noctuidae) Ministry of Agriculture and Rural Affairs, General Directorate of Protection and Control, Ankara; 1995. p. 435

[74] Available from: https://www.agric.wa.gov.au/pest-insects/cutworm-pests-crops-and-pastures [Accessed: November 5, 2021]

[75] Giannasi ADO, Brambila CR, Zart M, Guide BA, Alves VS. Assessment of entomopathogenic nematodes in Agrotis

ipsilon (Lepidoptera: Noctuidae) under laboratory and greenhouse conditions. Revista Colombiana de Entomología. 2018;**44**:25-31. DOI: 10.25100/socolen.v44i1.6533

[76] Goudarzİ M, Moosavİ MR, Asadi R. Effects of entomopathogenic nematodes, Heterorhabditis bacteriophora (Poinar) and Steinernema carpocapsae (Weiser), in biological control of Agrotis segetum (Denis & Schiffermuller) (Lepidoptera: Noctuidae). Turkish Journal of Entomology. 2015;**39**:239-250. DOI: 10.16970/ted.43220

[77] Xue JL, Cai QX, Zheng DS, Yuan ZM. The synergistic activity between Cry1Aa and Cry1c from Bacillus thuringiensis against Spodoptera exigua and Helicoverpa armigera. Letters in Applied Microbiology. 2005;**40**:460-465. DOI: 10.1111/j.1472-765X.2005.01712.x

[78] Zhu C, Ruan L, Peng D, Yu Z, Sun M. Vegetative insecticidal protein enhancing the toxicity of Bacillus thuringiensis subsp kurstaki against Spodoptera exigua. Letters in Applied Microbiology. 2006;**42**:109-114. DOI: 10.1111/j.1472-765X.2005.01817.x

[79] Hernández-Martínez P, Ferré J, Escriche B. Susceptibility of Spodoptera exigua to 9 toxins from Bacillus thuringiensis. Journal of Invertebrate Pathology. 2008;**97**:245-250. DOI: 10.1016/j.jip.2007.11.001

[80] Eski A, Demirbag Z, Demir I. Microencapsulation of an indigenous isolate of Bacillus thuringiensis by spray drying. Journal of Microencapsulation. 2019;**36**:1-9. DOI: 10.1080/02652048.2019.1572238

[81] Koul O. Microbial biopesticides: Opportunities and challenges. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources. 2011;**6**:1-26

[82] Elvira S, Ibargutxi MA, Gorria N, Muñoz D, Caballero P, Williams T. Insecticidal characteristics of two commercial Spodoptera exigua nucleopolyhedrovirus strains produced on different host colonies. Journal of Economic Entomology. 2013;**106**:50-56

[83] CABI. Spodoptera littoralis (cotton leafworm). 2020. [Accessed: November 9, 2021]

[84] Regev A, Keller M, Zilberstein A. Synergistic activity of a Bacillus thuringiensis delta-endotoxin and a bacterial endochitinase against Spodoptera littoralis larvae. Applied Environmental Microbiology. 1996;**62**:3581-3586

[85] Martinez S, Van Emden H. Growth disruption, abnormalities and mortality of Spodoptera littoralis (Boisduval) (Lepidoptera: Noctuidae) caused by Azadirachtin. Neotropical Entomology. 2001;**30**:113-125. DOI: 10.1590/S1519-566X2001000100017

[86] Ansari MA, Shah FA, Whittaker M, Prasad M, Butt TM. Control of western flower thrips (Frankliniella occidentalis) pupae with Metarhizium anisopliae in peat and peat alternative growing media. Biological Control. 2007;**40**:293-297. DOI: 10.1016/j.biocontrol.2006.12.007

[87] Shipp JL, Wang K. Evaluation of Dicyphus hersperus (Heteroptera: Miridae) for biological control of Frankliniella occidentalis (Thysanoptera: Thripidae) on greenhouse tomato. Journal of Economic Entomology. 2006;**99**:414-420. DOI: 10.1093/jee/99.2.414

[88] Hake SJ, Hake KD, Kerby TA. Prebloom decisions. In: Cotton Production Manual. University of California, Division of Agriculture and Natural Resources Publication; 1996. pp. 29-50

[89] Fournier A, Ellsworth PC, Barkley VM. Economic impact of lygus in arizona cotton: A Comparative Aproach. A College of Agriculture Report. No. 1437. University of Arizona, College of Agriculture, Tucson, AZ. Arizona Cotton Report; 2007. p. 151

Chapter 9

Influence of Abiotic Factors on Whitefly Population Abundance in Cotton

Abhijit Ghosal

Abstract

Whitefly started to infest cotton soon after planting in favourable weather condition. During November planting mean whitefly population were highest (6.9 whiteflies per 3 leaves) and slowly declined in successive planting dates. It was found that number of population were above ETL during the month of December, January and February. Maximum population were recorded in the month of February depending on the growth stage of the crop. Maximum temperature beyond 35°C, minimum temperature below 8°C and moderate to high rainfall was very much detrimental to successful population build up. The most favourable temperature was ranged in respect of min. temperature and max. temperature was ranged 12–30°C. Simple regression value reflects whitefly population were influenced to the tune of 70.8%, 69.5%, 35.3% and 75.4% in November, December, January and February month respectively. Whitefly population were negatively correlated with temperature (max. and min.), rainfall and relative humidity (max. and min.); while, positively correlated with sunshine hours, but during November planting relative humidity (max. and min.) was positively correlated and sunshine hours were negatively correlated. Thus adjustment of planting dates may be adjusted or suitable plant protection measure may be introduced according to the weather forecast.

Keywords: abiotic factors, cotton, population abundance, whitefly

1. Introduction

Crop productivity primarily gets highly influenced by biotic and abiotic stress. Several biotic fauna influence the growth of which insects are the most limiting factor to obtain the desired yield. On the other hand abiotic factors play an important role for the biotic stress abundance. Survival and thriving at extreme physical conditions require peculiar adaptations and plastic responses. Among abiotic factors, temperature and humidity stand out as the most important ones constraining abundance and distribution of insect. Furthermore, it is well documented that abiotic factors, regulate the ecology of insect communities. Although effects of temperature on survival, development, and reproduction of insects have been exhaustively explored over several decades, there is still a lot of interest on how temperature and other abiotic factors set the limits of distribution and define abundance of insect species.

Cotton, (*Gossypium hirsutum* L.) is the important cash crop in India due to its high industrial demand. Despite of huge share in areas the productivity of cotton (290 kg/ha) is still very lower than even the world average productivity. It is anticipated that this low production is mainly attributed due to infestation of pest problem. An array of insect pests has been reported to infest the crop rendering the low yield. About 162 species of insects has been known to occur in cotton at various stages of growth, of which 8 are key pests [1]. Among the sap feeders whitefly emerged as most dangerous due to its wide potency to act as vector of plant viruses.

Cotton leaf curl is suggested as a major factor in the decline in cotton production worldwide [2]. Whitefly is active throughout the year on different host plants depending upon the regional and ecological condition, though the activity of this pest is more in dry season. Likewise other insect pest biology of whitefly population is greatly influenced by abiotic factors both positively and negatively as explored by several workers [3]. Sing et al. [4] studied the effect of microclimate on population dynamics of whitefly in cotton and concluded that whitefly population were negatively associated with temperature but directly associated with relative humidity. It is important to understand the relation between the weather parameters and insect population fluctuation to predict and develop a strategic model of pest management in the changing climate. In search of that conclusion the present work has been oriented to study the impact of weather parameters on population of whiteflies in cotton in West Bengal condition.

2. Materials and methods

Field experiment was conducted in experimental plots of Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal, India during rabi season of 2012–2013 and 2013–2014 in randomised block design with three replication. Cotton (var. Bollguard-II) was raised in plots (10 m × 5 m) under recommended package of practices with 50 cm × 50 cm spacing in different days sowing at monthly interval starting from 1st November onwards till February [5]. The field was left as such without any plant protection intervention. Whitefly population were recorded from three leaves per plant top, middle and bottom canopies (randomly sampled tagged 10 plants per plot) from each plot were enumerated at weekly interval [6]. The meteorological data during the study periods was also recorded from the AICRP on Agro-meteorology, Directorate of Research, BCKV, Kalyani, Nadia to establish the correlation and regression co-efficient between whitefly population and weather factors.

The abiotic factors i.e. maximum temperature (X_1), minimum temperature (X_2), maximum relative humidity (X_3), minimum relative humidity (X_4), total rainfall (X_5) and sun shine hours (X_6) and population of whitefly (Y) were arranged as a weekly interval. The inter action between the population in one hand and meteorological data on the other hand had been worked out through correlation, regression and multiple regressions analysis. The data thus obtained were analysed statistically followed by Fisher's method of analysis of variance [7]. Simple and multiple regression analysis (X_1, X_2, X_3, X_4, X_5, X_6) were worked out and the data were detailed out based on spectrum of regression analysis and equation as $Y = a + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5 + b_6X_6$. Where, $b_1 \ldots b_6$ are the regression coefficient of $X_1 \ldots X_6$.

3. Result and discussion

The population build up of whiteflies in relation to abiotic factors were ascertained through the correlation studies along with simple and multiple regression analysis. The result showed that the population of whitefly was found in its first peak (9.7 whiteflies per three leaves) on 3rd week of December, when max. Temperature was- 26.1°C, min. Temperature was- 12.4°C, RH% was ranged between—61.5–91%, sunshine hour was—4.8 h and with zero rainfall. There were 10.2 whiteflies per three leaves were recorded in 3rd week of January and this was considered as the second peak. The population were greatly fluctuated with the fluctuation of mean temperature and relative humidity (RH%). Whitefly population stroked its highest and third peak during 2nd week of February (6th standard week) with max. Temperature 29.9°C and 13.4°C min. Temperature, relative humidity ranged 87% max.—43.5% min. and with sunshine hours—8 h. Gradually the population decreased with the subsequent advance of crop age (**Figure 1**). Population of whitefly illustrated non significant negative correlation with the max. Temperature (r = −0.18), min. Temperature (r = −0.30), rainfall (−0.003) and sunshine hours (r = −0.16); while positive correlation with max. Relative humidity (r = 0.54) and min. Relative humidity (r = 0.04). Maximum relative humidity showed significant correlation with the population load during November planting (pooled) (**Table 1**). Cumulative effect of weather parameters designated that 70.8% population (R^2 = 0.708) can be explained by the cumulative effect of the weather parameters (**Table 2**).

The number of whitefly population varied from 0.7 to 13.2 per three leaves during December planted cotton (Pooled) (**Figure 2**). Maximum whitefly population (13.2 per three leaves) was recorded during 2nd week of February; at this stage the max. Temperature was- 29.9°C, min. Temperature was- 13.4°C, max. Relative humidity was- 87%, min. Relative humidity was- 43.5%, sunshine hours was 8 h and without any rainfall. But, during 7th standard week the population suddenly lowered down (7.8 whiteflies per three leaves) which was apprehended due to sudden forms of torrential rain during that week. The population again started rebuilding from 8th standard week (9.4 whiteflies per three leaves) with a comfortable weather. After 2nd week of

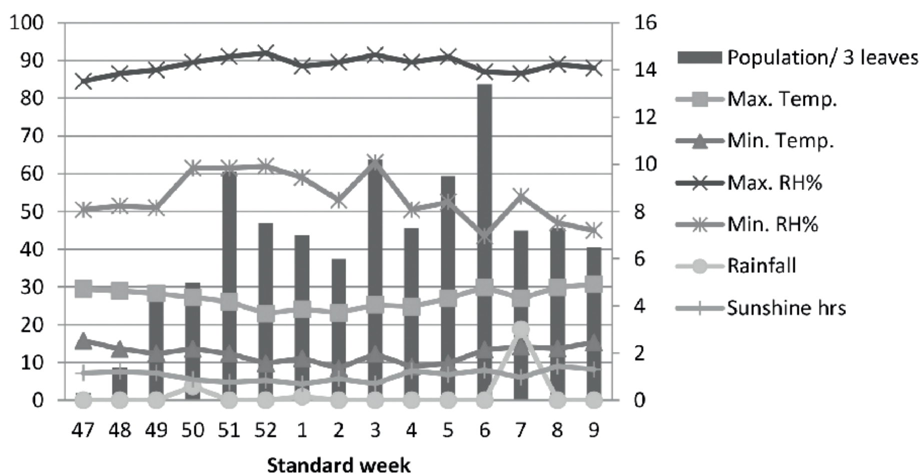

Figure 1.
Population dynamics of whitefly in relation with weather parameters in November planted cotton (pooled).

Meteorological parameters	(X)	Cotton			
		Nov.	Dec.	Jan.	Feb.
Max. temperature	(X_1)	−0.18	−0.03	−0.09	−0.38
Min. temperature	(X_2)	−0.30	−0.21	−0.19	−0.73**
Max RH (%)	(X_3)	0.54*	−0.16	−0.23	−0.41
Min RH (%)	(X_4)	0.04	−0.20	−0.23	−0.54*
Rainfall	(X_5)	−0.003	0.01	−0.25	−0.57*
Sunshine hour	(X_6)	−0.16	0.42	0.30	0.37

*Correlation significant at 0.05 level.
**Correlation significant at 0.01 level.

Table 1.
Correlation studies b/w incidence of whitefly and weather parameters.

Planting time	R^2	Regression equation
November	0.708	$Y = -112.86 + 0.600(X_1) - 0.032(X_2) + 1.859(X_3)^{**} - 0.800(X_4)^{*} + 0.207(X_5) - 2.831(X_6)^{*}$
December	0.695	Y = 106.548 + 3.103(X1) − 2.909(X2)* − 2.563(X3)* + 1.208(X4) + 0.013(X5) + 2.385(X6)
January	0.353	Y = 69.63 + 0.958(X1) − 1.045(X2) − 1.111(X3) + 0.261(X4) − 0.025(X5) + 1.042(X6)
February	0.754	Y = 23.731 + 0.126(X1) − 0.273(X2) − 0.200(X3) − 0.006(X4) − 0.012(X5) − 0.320(X6)

*Correlation significant at 0.05 level.
**Correlation significant at 0.01 level.

Table 2.
Regression equation showing quantitative relationship between B. tabaci (Y) and meteorological parameter.

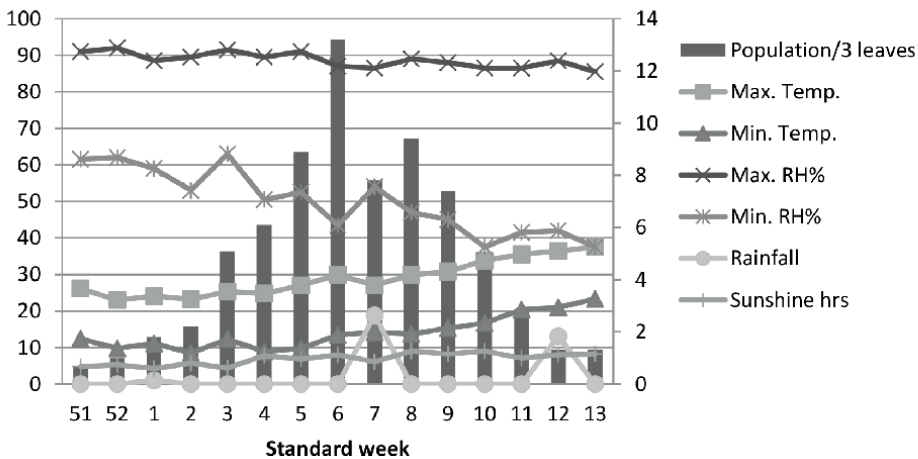

Figure 2.
Population dynamics of whitefly in relation with weather parameters in December planted cotton (pooled).

March the population were in a trend to decrease with steady increase of temperature up to the rest of the experiment. The correlation coefficient (r) showed negative trend with maximum and minimum temperature (r = −0.03 and − 0.21, respectively), max. and min. Relative humidity (r = −0.16 and − 0.20, correspondingly) with population of whitefly. Sunshine hours showed positive correlation (r = 0.42) with the whitefly population build up. The effect of weather parameters during the period of infestation failed to establish any significant correlation (**Table 1**). The combined contribution of the weather factors was 69.5% (**Table 2**).

Figure 3 depicts the incidence pattern of *B. tabaci* during January planted cotton (Pooled). Infestation was initiated (0.5 whiteflies per three 3 leaves) during 3rd standard week after the initiation of third leaf. It was noted that whitefly population were gradually in upward trend with the advances of crop stage and thereby hits its maximum peak (12.6 whiteflies per three 3 leaves) during 9th standard week (1st week of March), with max. temperature—30.8°C, min. temperature—15.3°C, max. RH—88%, min. RH—45%, sunshine hours—8.2 h and zero rainfall. During 10th standard week and afterwards the population were getting dwindled off with the increase of temperature. All the weather parameters were negatively correlated with whitefly population during December planting (pooled) except sunshine hours (r = 0.30) (**Table 1**). But none of the parameters showed significant relation. Only 35.3% population were influenced by the existing weather factors at this period (**Table 2**).

Effect of climatic factors on the incidence of *B. tabaci* in February planted cotton (Pooled) is presented in **Figure 4**. The number of whitefly population were varied from 0.5 to 4.2 per three leaves during the course of study. It was observed that max. Temperature above 35°C was unfavourable to the population load. Maximum whitefly population (4.2 per three leaves) was recorded during 1st week of March (max. temperature was—30.8°C, min. temperature was—15.3°C, max. relative humidity was—88%, min. relative humidity was—45%, sunshine hours was 8.2 h and zero rainfall). But, during 12th standard week the population suddenly fall down (1.8 whiteflies per three leaves) which was detained due to sudden forms of torrential rain during that week associated with high temperature. After that the population were in a trend to shrink with steady increase of temperature and rainfall up to the rest of the experiment.

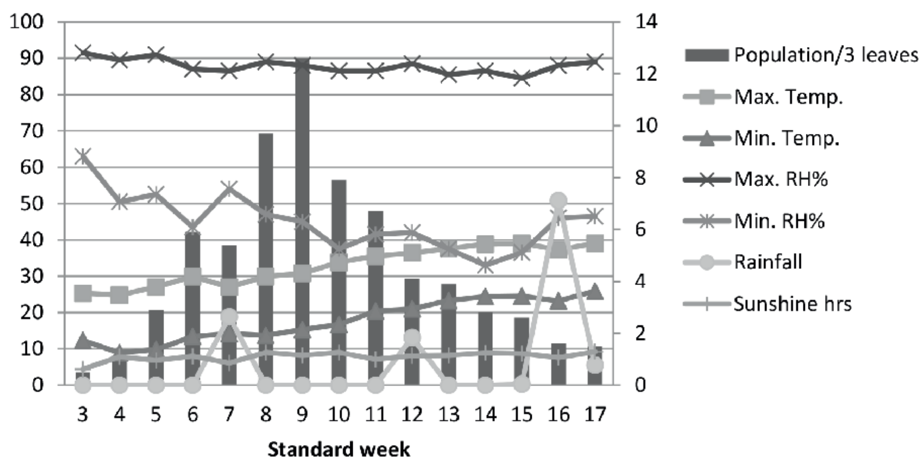

Figure 3.
Population dynamics of whitefly in relation with weather parameters in January planted cotton (pooled)

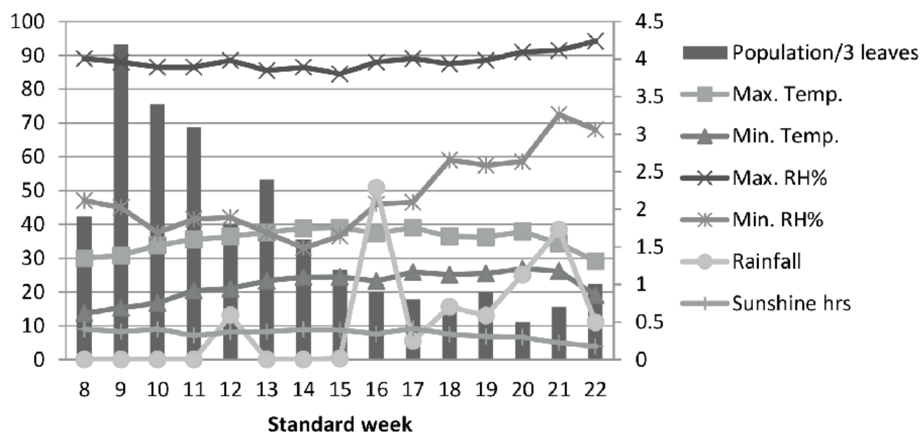

Figure 4.
Population dynamics of whitefly in relation with weather parameters in February planted cotton (pooled).

Lowest population were recorded in 20th standard week. Minimum temperature (−0.73), min. relative humidity (−0.54) and rainfall (−0.57) exhibited significant effect on the population of whiteflies (**Table 1**). 75.4% population of whitefly were influenced by the weather factors during this period of investigation (**Table 2**).

4. Effect of different dates of sowing on the incidence of whitefly

Wide fluctuation of population were noted in different dates after planting, which was varied from 0.9–7.5 mean whiteflies per three leaves. Lowest population were recorded in 21 DAS, and then slowly but steadily increased with the advance of crop age up to 63 DAS. These findings may be ascertained due to the favourable stage of the crop for successful multiplication of whitefly. It was recorded that population maintained uniform pattern of incidence during 49 DAS to 98 DAS. Accordingly the population were in decreasing trend and thus during 119 DAS only 2.6 numbers of whiteflies were recorded from three leaves (**Table 3**).

Each and every biological organisms are responsive towards the climatic factors. Biology of herbivore insects are greatly influenced by the weather parameters as these parameters exerted direct impact on the life cycle as well as the plant itself in which the insect used to feed and grow have potential impact on the population build up of the particular insects. It is apparent from this experimental findings that whitefly population were influenced by the weather parameters in different dates of planting of cotton. Population of whitefly in four different planting dates at monthly interval from November to February showed that the population of whiteflies decreased in successive planting. Population build up of whiteflies were high during spring season, as whitefly population were strongly affected by high temperature as well as low temperature; though the population of whitefly were greatly varied with the favourable growth stages of the crop. It was recorded that max. temperature beyond 35°C and min. temperature below 8°C was very detrimental for the population build up. The most favourable temperature was ranged in respect of min. temperature and max. temperature was 12–30°C depending on the favourable vegetative stage of the crop. Dry period greatly favoured the

Crop stage	Mean whitefly population/3 leaves on different dates of sowing (mean of two years)				Mean
	1st November	1st December	1st January	1st February	
21 DAS	0.3	0.7	0.5	1.9	0.9
28 DAS	1.4	1.0	1.1	4.2	1.9
35 DAS	4.8	1.8	2.9	3.4	3.2
42 DAS	5.0	2.2	5.9	3.1	4.1
49 DAS	9.7	5.1	5.4	1.8	5.5
56 DAS	7.5	6.1	9.7	2.4	6.4
63 DAS	7.0	8.9	12.6	1.6	7.5
70 DAS	6.0	13.2	7.9	1.2	7.1
77 DAS	10.2	7.8	6.7	0.9	6.4
84 DAS	7.3	9.4	4.1	0.8	5.4
91 DAS	9.5	7.4	3.9	0.7	5.4
98 DAS	13.4	4.9	2.8	0.9	5.5
105 DAS	7.2	2.8	2.6	0.5	3.3
112 DAS	7.3	1.3	1.6	0.7	2.7
119 DAS	6.5	1.3	1.5	1.0	2.6
Mean	6.9	4.9	4.6	1.7	-

Table 3.
*Effect of dates of sowing on the incidence of whitefly (*B. tabaci*) in cotton.*

population build up, while rainfall exerted negative effect on the population size because the population of whiteflies were washed out as well as mortality of adult population were noticed. Our result is in confirmation with the findings of Sing et al. [4] and Banjo et al. [8]. It was also observed that population of whiteflies were maximum in 63 days after sowing and maintained its uniform pattern of incidence up to 98 days after sowing, which suggests that whiteflies prefer to feed the crop at early growth stages of the crop. Similar findings were reported by Meena et al. [9]. Correlation matrix of whitefly population and weather factors showed few sort of inconsistency based on the weather parameters recorded on that growing period of the crop. Whitefly population were negatively correlated with maximum and minimum temperature, relative humidity and rainfall; while positively correlated with sunshine hours; which were in agreement with Kataria et al. [10] and Latif and Akhter [11]. During November planting (pooled) maximum and minimum relative humidity was positively correlated with the population dynamics of whitefly, whereas, rainfall showed non significant positive correlation with the whitefly population during December planted cotton (pooled); which was at par with the result of Dahiya et al., [12]. Fluctuation in correlation between weather parameters and whitefly population build-up in different planting dates may be due to inconsistency of weather parameters as an effect of global warming in recent days or may be associated with the other ecological factors influencing whitefly incidence.

5. Conclusion

In the changing climate it is much very difficult to manage the pest in field condition. The interaction of crop and herbivore are greatly influenced by the meteorological parameters. Now a days thus pest forecasting has gained an importance in world agriculture which strongly depends on the study of population of the biotic species build up in relation with the abiotic factors like temperature, rainfall, humidity etc. In our present study we have noticed that the population of *B. tabaci* were negatively correlated with maximum and minimum temperature, relative humidity and rainfall; while positively correlated with sunshine hours during November plating while the population showed non significant positive relation with the rainfall during December planting, which denotes that during winter season the light rainfall helps to increase the soil temperature as well as temperature of the micro climate of the field. Temperature is the key meteorological factor in relation to population abundance of whitefly. It is apparent that the population build up of whiteflies in Indian context was favourable in between the temperature range 12–30°C depending on the favourable vegetative stage of the crop, early growth stages of the plant favours the population build up. Thus plant protection measure should be adopted during the early growth phase depending on the temperature, humidity and rainfall. At high temperature and moderate to high rainfall as the population of the whiteflies get affected thus avoid spraying the crop.

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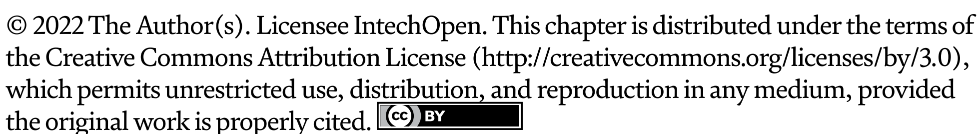
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References

[1] Dhawan AK. Cotton pest scenario in India: Current status of insecticides and future perspectives. Agrolook. 2000;**1**(1):75-85

[2] Singh, J., Sohi, A. S., Brar, D. S., Denholm, I., Russel, D. and Briddon, R. 1999. Management of cotton leaf curl viral disease in India. In: Proceeding of the ICAC-CCRI Regional Consultation, Insecticide Resistance Management in Cotton. Central Cotton Research Institute, Multan, Pakistan, pp-277-278

[3] Rameshbabu S, Meghwal ML. Population dynamics and monitoring of sucking pests and bollworms on bt cotton in humid zone of Southern Rajasthan. The Bioscanning. 2014;**9**(2):629-632

[4] Sing S, Niwas R, Saini RK, Khichar ML. Effect of microclimate on population dynamics of whitefly in cotton. Journal of Insect Science. 2006;**19**(2):209-212

[5] Selvaraj S, Ramesh V. Seasonal abundance of whitefly, *Bemisia tabaci* Gaennadius and their relation to weather parameters in cotton. International Journal of Food and Agricultural Veterinary Sciences. 2012;**2**(3):57-63

[6] Prasad NVVSD, Rao NHP, Mahalakshmi MS. Population dynamics of major sucking pests infesting cotton and their relation to weather parameters. Annals of the Plant and Protection Sciences. 2008;**18**(1):49-52

[7] Fisher RA. Statistical Methods for Research Workers. London: Oliver and Boyd (Edinburgh); 1934. p. 198

[8] Banjo AD, Hassan AT, Jackal LEN, Dixon AGO, Ekanayake IJ. Developmental and behavioural study of spiralling whitefly (*A. dispersus*) on three cassava (*Manihot esulanta* crantz) genotypes. Journal of Crop Research. 2003;**26**(1):145-149

[9] Meena NK, Kanwat PM, Meena A, Sharma JK. Seasonal incidence of jassids and whiteflies on okra, *Abelmoschus esculentus* (L.) Moench in semi-arid region of Rajasthan. Annals of the Agricultural Biological Research. 2010;**15**(1):25-29

[10] Kataria SK, Singh PB, Kaur J. Population dynamics of whitefly, *Bemisia tabaci* Gennadius and leaf hopper, *Amrasca biguttula biguttula* Ishida in cotton and their relationship with climatic factors. Journal of Entomology Zoological Studies. 2017;**5**(4):976-983

[11] Latif MA, Akhter N. Population dynamics of whitefly on cultivated crops and its management. International Journal of Bio-resources Stress Management. 2013;**4**(4):576-581

[12] Dahiya KK, Kumar D, Chander S. Influence of abiotic factors on leafhopper and whitefly population buildup in Bt-cotton hybrids. Indian Journal of Entomology. 2013;**75**(3):194-198

Chapter 10

Machinery for Plant Protection in Cotton Crop

Manjeet Singh Makkar and Santosh Kumar Gangwar

Abstract

Spraying is very tedious and time consuming operation. There is a need of an efficient, precise and high capacity machine for spraying. The pesticide with conventional sprayers is not so effective because non-uniform spray and of lesser width of coverage. Distribution of pesticide is not uniform especially at the underside of the leaves. The pest can be controlled effectively if pesticides are applied properly at the right rate, right time and on the target by right equipment. In field crops like cotton the pest attacks is on the lower side of the leaves during vegetative as well as reproductive stages of the cotton crop. The sprays with conventional sprayer do not enter at the bottom of the plant canopy and at lower side of the leaves. Different type of sprayers are being developed especially for the control of white fly in cotton crop. These sprayers can also be used for crops like sugarcane, potato etc. Air assisted electrostatic sprayer, Auto rotating gun type sprayer, Multipurpose high clearance sprayer and even drones are now a days used for spraying in cotton crop. Multipurpose high clearance sprayer mostly preferred by the farmers has three types of spraying arrangements namely auto rotate gun, boom type and drop up type nozzles can work at all stages of the crop and saves time, labor and cost of operation as well as it reduces drudgery of the operation. Selection of nozzle is also important for these type of sprayers. Automatic controller can be fitted on these sprayers for adjusting the discharge and to reduce missing or overlapping of spray.

Keywords: spraying, high clearance sprayer, auto rotate gun sprayer, electrostatic spraying, drone, weeding etc.

1. Introduction

Leading cotton producing countries worldwide in 2020–2021 are China with the production of 6.42 million metric tons followed by India with 6.16 million metric tons accounting for about 58% of the world cotton production [1]. Over the past several years, plantings of transgenic crops producing insecticidal proteins from the bacterium Bacillus thuringiensis (Bt) have helped to control several insect pests and reduced the need for insecticides. Broad-spectrum insecticides kill arthropod natural enemies that provide biological control of pests. The decrease in use of insecticide [2] sprays associated with Bt crops could enhance bio-control services [3]. Although, Bt. cotton provides effective protection against cotton bollworms, but sucking pests namely whitefly, jassid, aphid and mealy bug are the most serious in Bt. cotton and

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they cause maximum damage. Whitefly adults and nymphs suck sap from leaves and excrete honey dew on leaves which become sticky. Affected leaves and seed cotton turns black due to development of sooty mold on the plants. Regular, supervision of the crop is must for detection of whitefly incidence. If possible proper coverage of underside of leaves during the insecticidal spray may effectively reduce the whitefly population in cotton crop.

Chemical application by sprayer is a common field operation in crop production. Different types of sprayers namely; manual operated, tractor operated boom sprayer, auto-rotated gun sprayer, self-propelled high clearance boom sprayer, air assisted boom sprayer, air assisted electrostatic sprayer and unmanned aerial vehicle (UAV) based sprayer are available for spraying on cotton crop. Special devices, such as the portable knapsack sprayer, have been designed for manual operation has only one nozzle, which is fixed on a lance. Sprayers for crop protection can be divided as vehicle-mounted, trailed types and portable type sprayers. Vehicle-mounted sprayers generally use wider booms attached with nozzles for horizontal or vertical spraying. Horizontal boom sprayers are used to spray in field crops, while vertical boom sprayers are used to spray for vineyards and orchards. Both type of sprayers utilize air in which the nozzles spray into the air stream of a fan flow, which carries and distributes the droplets into the vineyards and orchards. Spray booms can also be mounted on drones, airplane or helicopter for application to spray on large fields.

Nozzle selection and efficient operation of sprayers is must for the better control of the pest on cotton crop. The success of control over insect-pest, diseases and weed depends upon use of proper spraying technologies (spray machine & spray method) [4]. Agrochemical may be applied at various crop growth stages of cotton during the entire crop seasons by different sprayers technology commercially available in the market. Sprayers may be designed as attachments to tractor or self-propelled power unit. Numerous types of sprayers are available i.e. knapsack sprayers, auto rotating gun sprayer, air assisted boom sprayer and tractor mounted boom sprayers, multipurpose high clearance spryer, and recent new advance developed sprayer i.e. air assisted electrostatics sprayer, Drone (UAVs) sprayer etc.

In China and some other countries, use of UAV spraying with low altitude and low volume is increasing for the application of chemical to control insect, pest and disease in different crops. It saves time, energy and drudgery of operation with less chance of contact chemical to operator skin also avoid soil structure damage by controlling traffic over field surface.

2. Sprayers

Sprayer is a machine dispersal of fluids chemical in the form of spray droplets by using hydraulic or gaseous or centrifugal energy are commonly known as sprayers.

Specification of all purpose sprayer are:

1. High clearance for tall crops.

2. Enough wide, light, flexible boom, adjustable in height.

3. Non corrosive construction to enable the sprayer to be used for all type chemical.

4. Boom section control valve.

5. Accurate ground speed indicator.

6. Flexible connections on the nozzles from the boom.

Different types of sprayers available for the protection of cotton crop are explained below;

2.1 Knapsack sprayer

Generally, knapsack sprayers are utilized for spraying on low height crops, vegetables and plant up to 1.5 meters in height (**Figure 1**). Different types of knapsack sprayers produce different impacts on agriculture in terms of the plant protection. Knapsack sprayers are indispensable agricultural equipment for small and marginal farmers for pest control because of affordability and ease of operation [5]. But this device has some limitations. It causes fatigue to operating person and hence cannot be used for longer time. The hand operated knapsack sprayer needs a lot of effort to move the lever up and down to generate the pressure inside the sprayer. The machine consists of lever-operated hydraulic pump to produce the desired pressure up to 3.0 kg cm^{-2}. It has hollow cone type nozzle mounted on a handheld lance of 1500 mm long with effective discharge of 1.3 $lmin^{-1}$ and having a 16-liter chemical spray tank. The field capacity of this sprayer is 0.08 hah^{-1}. The droplet size and percent coverage area of knapsack sprayer is as 347.85 µm, and 22.29%. The bio efficacy to control whitefly in cotton crop with the knapsack sprayer is varies between 65 and 70%.

2.2 Auto rotate gun type sprayer

An auto rotate gun sprayer was developed for the control of whitefly *(Bemisia tabaci)* in cotton crop. An auto rotate gun type sprayer [6] with two gun type nozzle (Make: Teejet) was developed in Department of Farm Machinery and Power, PAU, Ludhiana in collaboration with the industry (**Figure 2**). It has tractor mounted, boom with guns,

Figure 1.
Knapsack sprayer in cotton crop.

Figure 2.
Auto rotate gun type sprayer.

dc motor, hydraulic piston type pump and spray tank (600 liter). Spacing between the two guns is kept 9 m. Each gun rotates 120 degree of rotation to cover about 30 m of span or working width of sprayer at liquid pressure of 35–40 kg cm^{-2}. These guns can be operated independently if required. There are two rotation settings (30 and 40 RPM) for each gun. The auto rotate gun sprayer were control as 85–95% whitefly nymphs in cotton crop. The droplet of auto rotate gun type sprayer having size of 250–330 micron [7]. Auto rotate gun type sprayer was preferred by the farmer as it may be used for effective spraying at earlier stage of crop and saves time, labor and cost of operation as well as it reduces drudgery of the operation.

2.3 Multipurpose self-propelled high clearance sprayer

A high clearance self-propelled 4 wheel drive high clearances drive tractors with spraying system is developed (**Figure 3**) and popularized by Punjab Agricultural University (PAU), Ludhiana, India [8]. It has three types of spraying arrangements namely auto rotate gun, boom type and drop up type nozzles which is operated by a single pump. The spray machine consists of a hydraulic pump, spray tank (1000 liters), pressure gauge and hydraulic assistance for controlling the boom. The pump can be operated at 800 rpm to develop desired pressure up to 35 kg cm^{-2}.

Boom and drop-up nozzle mechanism consists of 14 hallow cone nozzles on boom and 13 hallow cone drop-up nozzles (Make: Teejet) mounted on foldable 9.8 m wide boom with 67.5 cm nozzle spacing. Boom nozzles are used to spraying on top side of plant canopy and other drop-up nozzles which is used to spray inside the crop canopy up to 65–75 cm below from the boom and within the row or underside of leaf through adjustable drop-up arrangement of nozzle to target whitefly residing locations. The height of boom can be adjusted up in the range of 30–250 cm according to the crop height with the help of a hydraulic assistance provided.

Figure 3.
Multipurpose high clearance sprayer.

The auto rotate gun type attachment has two guns (Make: Teejet) placed at 9.5 m apart on each end of its boom and it has coverage radius of 10 m per nozzle at limiting pressure of 35 kg cm^{-2} This gun performs 120^0 rotation to cover about 20 m of swath or working width. These guns can be operated independently if required. There is a provision for adjusting vertical height of boom from target which makes it suitable to spraying for different crops at different crop growth stage.

The auto rotate gun boom type nozzles and drop up nozzles performed batter as 92–95% whitefly nymphs are killed by these spraying attachments as compared to knapsack sprayer. Auto rotate gun, drop up nozzles and boom type nozzle performed batter as 65–75% whitefly adults are killed by these type of spraying attachments as compared to knapsack sprayer as control by which only 50–65% whitefly adults are killed. Droplet size (micron) is in the range by high clearance boom type (320–380), drop up (200–320), auto rotate gun type (250–330). High clearance was preferred by the farmer as it can work at all stages of the crop and saves time, labor and cost of operation as well as it reduces drudgery of the operation.

These multipurpose high clearance sprayer can be further modified to improve self-propelled high clearance sprayer with four-wheel drive system having narrow width tires and four-wheel steering system to facilitate the operation of these sprayers in other crops like rice, wheat etc. It has two types of spraying arrangements namely boom type and drop up type nozzles which is operated by a single pump.

Safety guide lines for tractor driver to operate PAU-Multi-purpose high clearance sprayer

1. Open and close the boom smoothly without any jerks.

2. Always engage the clutch gently.

3. Before machine putting in spraying field adjust the boom height according to the crop height, it should be 30 cm above the crop height.

4. Put the appropriate gear (Low/IV) of tractor at constant engine speed (1000 rpm) during the spraying in fields.

5. Check the pressure gauge reading if found low or high adjust it should be in pressure range 18–20 bar for boom with drop-up or single boom spraying system and 30–35 bar if gun is in working.

6. Reduce speed before making a turn or applying brakes.

7. Off the cut-off valve of outside boom, left and fold the boom during turning without crop damage.

8. After completion of spraying fold the boom and exit from field carefully without crop damage.

9. While driving on road it should insure that the boom supported on stand and locked properly.

10. Open the appropriate cut-off valve while refilling of spray water tank.

11. Important nuts and bolts should be checked if any of them are loose, it should be tightened.

2.4 Drop up with air-assisted boom sprayer

The sprayer machine is tractor operated and attached with three-point linkage system of tractor. Power requirement of drop-up air assisted boom sprayer (**Figure 4**) is 30–35 hp. and hydraulic pressure pump of sprayer is run by PTO power of tractor. The machine consists of water tank, hydraulic pressure pump, one blower fan, foldable and height adjustable boom with air assisted and drop-up type nozzles. High-density polyethylene made water tank which have enough water holding capacity of 600 liters to minimize frequent refilling of tank resulting improve field efficiency of machine. A hydraulic pump pressure in range 15–35 kg cm^{-2} bar is used to archive desire pressure range for efficient operation of drop-up and air assisted nozzle. Power from tractor PTO to hydraulic pump with gear ratio1:1.6 transmitted through a v-belt drive arrangement. The pump has one suction pipe diameter of 32 mm, three outlets port of

Figure 4.
Operational view of drop-up-air assisted boom sprayer.

diameters 12.7 mm and one overflow pipe with a pressure control lever and a pressure gauge. Suction pipe of pump is used to suck water solution from the water tank. Two pressure pipes with individual "on" and "off" valve were used to connect the outlets of pump to inlets pipe of drop-up and air assisted nozzle. Another one pressure pipe is used to fill water tank which connected with the suction pipe of water tank. The blower has two air stream discharge pipes diameter of 110 mm connected with air assisted nozzle boom through flexible PVC pipe diameter of 110 mm. The diameters of air discharge pipe were same as the diameter of air assisted boom pipe and this air stream opening around air assisted nozzle which improves the atomization of liquid for good spray pattern. A foldable and height adjustable boom is mounted on the rear side of spray frame which are made of 40 × 40 × 4 mm angle iron bar. The total width of boom is as 9500 mm which have five sections two each side left and right with one middle fixed section. The height of boom is adjusted from 1000 mm up to 2500 mm from the ground surface.

2.5 Back pack type air assisted electrostatic sprayer

Mobile Back Pack type (MBP) air assisted Electrostatic Sprayer powered by a 5.0 HP engine with an on-board compressor and spray gun can also be used for cotton crop (**Figure 5**). The engine power the air compressor and the compressor produces pressurized air which passes through conducting hose and used to atomize and propel the liquid spray. The electrostatic sprayer is equipped with a 15 liters tank which is hang on the operator's back. For charging the spray particles in the nozzle, two 9.0 V rechargeable batteries have been provided. Air and liquid enter separately at the outmost of nozzle. Just before leaving the nozzle, the air hit the liquid stream to atomize it into spray droplets that passed through the charging ring. Spray deposition on the upper side and underside of leaves by electrostatic sprayer is 80 and 85% more than knapsack sprayer respectively. Average drift loss of electrostatic sprayer is approx.

Figure 5.
Back pack type air assisted electrostatic sprayer.

50% lesser as compared to knapsack sprayer. Bio efficacy of the sprayer is given as percentage of insects killed by the operation of spraying [9]. Overall bio-efficacy of electrostatic sprayers i.e. 80–85% is comparatively higher than conventional sprayer.

2.6 Drone (unamend aerial vehicles)

In modern agriculture, Unmanned Aerial Vehicles (UAVs) have been used for field mapping, surveillance, farm management etc. It is also used for remote sensing, visual inspection of crop and soil conditions etc. This technology has utility in agriculture and forestry not only for taking observation and sensing but it can also be used in spraying application. Pesticides are applied in agricultural crop fields to increase output, improve quality and decrease cost of production. However, extended direct or indirect contact with these chemicals can cause various diseases to human such as cancer, complications in the respiratory system, neuro-logical diseases, asthma, allergies, hypersensitivity, and hormone disruption. According to World Health Organization (WHO) there are 3 million cases of pesticide poison every year and up to two lakhs twenty thousand deaths in developing countries. This problem may be reduced by the use of drone to carry out the task of spraying pesticides/herbicide.

The octa-copter type UAV with configuration of 8 propellers and its self-weight about 12 kg can be used for spraying as shown in **Figure 6**. The UAV have maximum take-off weight capacity up to 28 kg and its flying time 15–20 minutes with two lithium polymer batteries having capacity of 16,000 mAh. The UAV have two flight mode i.e. GPS and manual. A GPS receiver can locate UAV's exact location and altitude can be maintained by barometer. The range of remote controller has 1.5 km maximum transmission distance to control the UAVs. The UAV remote control system operates at 2.4 GHz radio wave frequency. Telemetry consisted of a radio modem and one ground control station which provide real time information during the flight. The UAV sprayer system consisted of 5–10 liters capacity tank and four flat fan nozzles having spray angle 110° were used in UAV sprayer and fitted beneath propeller. Swath width of UAV sprayer is upto 3 m with four nozzles. Transparent PVC pipes with an inner diameter of 8 mm is used; while a small independently 12 volts' electric power pump was used to develop desire pressure of 3.0 kg cm^{-2}. Remote control system is used to drive the pump, vary its speed and also autonomous of UAV through the electronic system and GPS. For this function, a pulse width modulation (PWM) system is used, in which the radio signal sent from the receiver adjusts the flow rate of the spraying system. Using this, the flow rate of the nozzles can be varied between 0.10–0.25 l/min at the minimum to maximum pump speed.

Figure 6.
UAVs sprayer in cotton crop.

The bio-efficacy of drone sprayer varying between 70 and 80% to control pest in cotton crops [10]. The water application rate also lies in range of 20–50 liter per hectare. Application of pesticides with the help of UAV has advantage of its use for any crop of any season also, in covering large areas quickly. UAV (drone) allows the farmer to take advantage of very small windows of opportunity such as weather conditions or pest growth cycle. UAVs do not cause soil compaction and crop damage. Now, many countries including India has their own regulations and guidelines for the use of drone in agriculture. Recently, Government of India has released standard operating procedures (SOP) for use of drone with pesticides for the crop protection and for spraying soil and crop nutrients in agriculture, forestry, non-cropped areas etc [11].

2.7 Cost on spraying

Actual field capacity of high clearance sprayer was found as 1.78 ha/h as compared to 0.80 ha/h for gun type sprayer and 0.08 for knapsack sprayer. Similarly cost, labour and time saving by using high clearance sprayer was 66, 95 and 95% respectively as compared to knapsack sprayer. Breakeven point for the multi-purpose high clearance sprayer was calculated 300 ha/year.

3. Selection of nozzles for various application

Most of the nozzle manufactures give discharge of nozzles at various operating pressures and on the basis of the purpose of use. However, nozzles should be selected on the basis of the type of spray job, i.e. spraying of insecticide, weedicide, fungicide

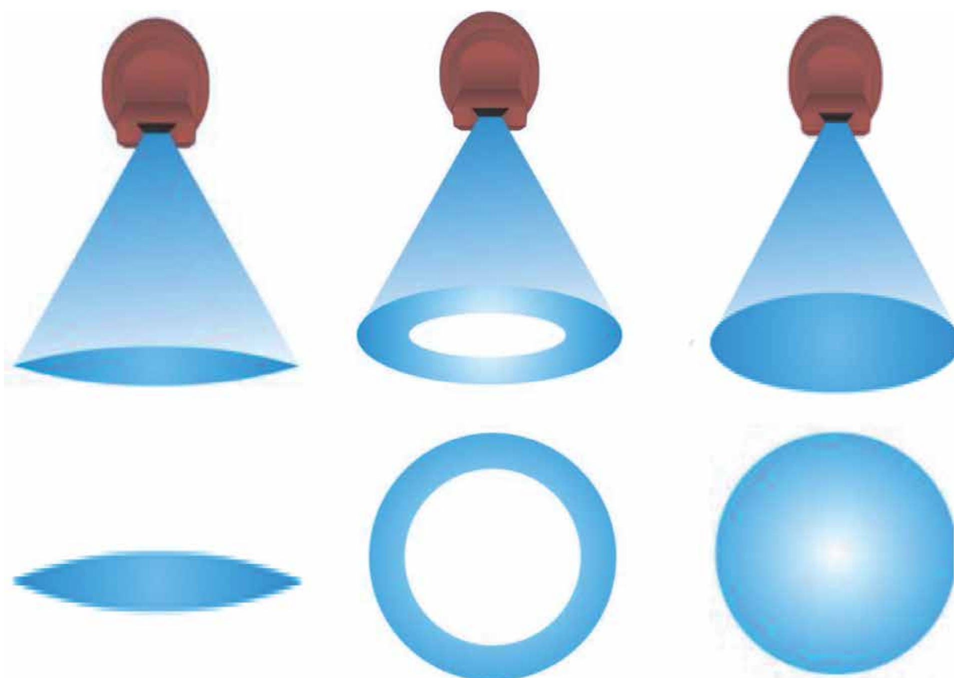

Figure 7.
Types of nozzle commercially available.

etc. Uniform distribution of chemical depends on the constant speed and proper nozzle selection and efficient operation of sprayers is must for the better control of the insect and pest on cotton crop. The success of control over insect-pest, diseases and weed may depends upon selection of appropriate spraying machinery (spray machine and spray method).

Flat fan nozzle is used for uniform coverage application such as for weed spraying (**Figure 7**). Hollow cone nozzles give a fine mist for complete coverage of plants being sprayed for insect control. Solid cone nozzles are used when a high pressure penetrating spray is required as for the control of whitefly in cotton. Use hallow cone nozzle which deliver 600 ml of spray material per minute for efficient pest control. Nozzle performance changes as spray materials erode the nozzle tip. Brass tips show wear about one third as fast as aluminum tip; stainless steel and some of the new plastic tips show wear only one-quarter as fast as brass. Nozzle wear is more significant in first 50 hours of use, depending on the abrasiveness of the spray material. Hence, nozzle performance should be periodically tested for changes in flow rate and spraying pressures used and for changes in spray pattern owing to nozzle tip wear.

4. Calibration of sprayers

Societal and environmental distresses as well as economics require precision application of only enough chemical to accomplish pest control. Conventional spraying technology depending on gravity force and spray droplet inertial forces often achieves less than 50 per cent deposit of the total spray volume on the plant targets and actual quantity reaching the insect or disease pest can be as low as 0.01% of the total spray volume. Hence, Air-assisted with electrostatic technology is better to achieve more penetration of spray and more uniform distribution on the plant canopies, particularly on the lower side of leaves. Calibration of the sprayer is very important to determine the effectiveness of spraying and elimination of the over-spraying. Sprayer requires too much care in calibration as various parameters affect the eventual spray concentration per acre are:

1. Pressure and delivery of pump

2. Speed of forward travel

3. Height of boom

4. Nozzle spacing

5. Concentration of spray materials

The insecticides recommended for control of sucking pests like whitefly, jassid etc., should be sprayed using 300–375 liters spray solution per hectare a with the manually operated knapsack sprayer. For calibration of a sprayer to control whitefly, let us suppose that dose of insecticides is recommended as 1500 ml/ha. Measure the nozzle discharge by collecting the liquid coming out from each nozzle in ml/min. Calculate total volume collected from 18 nozzles, let it was10 l/min. The sprayer travels at a forward speed of 4 kmh^{-1}. When the sprayer nozzles are spaced 67.5 cm apart on the boom and carried 50 cm above the crop canopy, the application will be uniform. Field efficiency of the sprayer is to be assumed as 50% (using Eq. (1)).

$$\mathbf{A} = \mathbf{D} * 10000 / \mathbf{F} * \mathbf{S} * \mathbf{N} * \mathbf{E} \tag{1}$$

Where, A = Application rate (l/ha) of spray, D = Pump Discharge (l/min), F = Forward Speed (m/min), S = Spacing between Nozzles (m), N = No. of Nozzles, E = Field efficiency.

To find the quantity of water required for spraying, fill the spray machine tank with measured quantity of water and spray in field. After spraying, measure the area sprayed and record amount of water consumed in that area. Calculate the amount of water required per hectare by the following equation (Eq. (2)):

$$\mathbf{Q} = \mathbf{V} * 10000 / \mathbf{A} \tag{2}$$

Where, Q = Amount of water required (l/ha), V = Volume of water consumed (liter), A = Sprayed area (m^{-2}).

5. Automatic spray control system

Various commercial spray controllers use section control technology to auto-turn boom-section valves to ON/OFF. This technology has a potential to reduce overlapping application resulting in savings on inputs. There are two type of spray control technology available first is Automatic Section Control (ASC) and second is Boom Section Control (BSC). The ASC technology has reduced over-application of pesticides to a large extent as compared to manual control system. Applying pesticides below a desired rate may lead to yield loss. Owing to its potential to save farm inputs, its ease of usage, and improved efficacy, the ASC technology has become increasingly popular. At this rate, this technology will gradually become a part of sprayer control systems. Based on this background, it is imperative to integrate factors such as speed and flow rate with the functioning of self-propelled sprayer in order to prevent the hazards of non-uniform spraying. Varios system and part of the technology are explained below.

5.1 Spray controller

The spray controller system consists of a manifold, electronic control unit (console), proximity sensor, pressure sensor, and water hose pipes (**Figure 8**). Manifold is composed of several components including flow meter, liquid strainer, electric regulating valve with bypass mode, and pressure relief valve (**Figure 2a**). These components ultimately control the spray application rate by adjusting the liquid flow rate. Flow meter is used to measure the actual rate of flow in a system and the value of system's real time flow rate is displayed on electronic control unit in a digital readout form. A liquid strainer is fitted to filter or separate out unwanted solid matter from the liquid stream. Regulating motor rated 3 rpm which is the most accepted motor used to regulate the liquid flow rate in automated systems was used. Motor opens the valve to the maximum flow in about 6 seconds and pressure relief valves in about 10 seconds. The pressure relief valve (PRV) is designed to open at a predetermined set pressure used to control or limit the pressure which otherwise may results in process upset or system failure. The pressure is relieved by allowing the pressurized liquid to flow from an auxiliary passage out of the system.

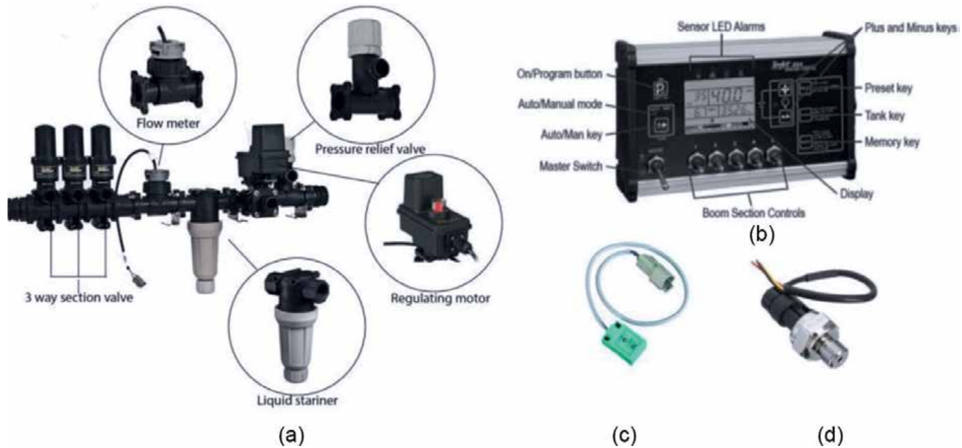

Figure 8.
Manifold with 3 way section and its components (b) electric control unit (ECU) (c) proximity sensor (d) pressure transducer (10 bar).

The function of the electronic control unit (ECU) was to control the spray boom sections during real-time applications and to switch on/off the spray. The selected ECU had a provision to control five boom sections in real-time. The number of active boom sections was set to three in ECU as only 3 boom sections were used. A small display provided on ECU delivered all real time information regarding sprayer. This system had a provision to run in auto and manual modes. The parameters like on-target application rate, number of tips on each boom sections, tip spacing, nozzle used were calibrated on ECU before the field operation.

A proximity transducer was fixed on rear tyre of high clearance boom sprayer to measure the forward speed. It works by sensing a metal object mounted in front of sensor face. The control system monitors the traveling speed and adjusts the amount of pesticide sprayed for a unit area accordingly for precise spraying applications. The analogue pressure transducer is mounted at valve manifold to monitor the overall liquid pressure. A pressure transducer generates an electrical signal which is displayed on electronic control unit (ECU) indicating the pressure imposed in the system. Pressure transducer used in this study is able to measure pressure up to 10 bar.

5.2 Installation and calibration of the automatic spray control system

A frame casing was fabricated using the angle-irons and G.I. sheet to house the manifold and sensors of the automatic spray control system. This complete unit was mounted on the high clearance sprayer such that the delicate parts were protected from the harsh operating conditions. The block diagram of the setup used to configure the automatic sprayer control system is shown in **Figure 9**.

Once the system was successfully installed, calibration was necessary to ensure its effective operation. To this end, different sensors and other electronic parts of the system were optimally calibrated. The proximity/speed sensor installed near the rear wheel of high clearance sprayer counts the number of wheel rotations (**Figure 10a**). It is calibrated by the spray controller (ECU) to provide the exact speed and spray area readings. For calibration, the tractor was made to run over a 100-meter distance i.e., point A to point B in the field after activating the calibration step of the automatic spray control system

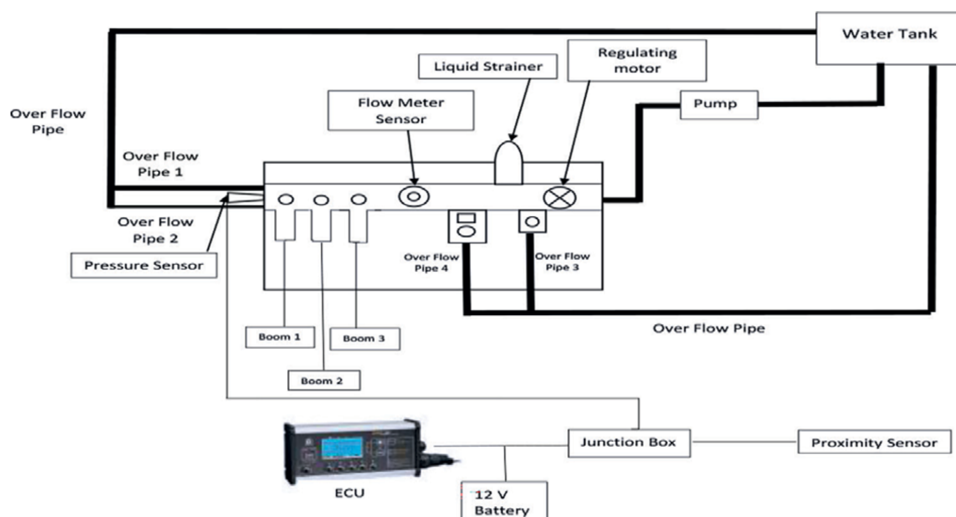

Figure 9.
Block diagram of an automatic spray control system.

Figure 10.
A view of (a) proximity sensor installed at rear wheel and (b) calibration step screen on electronic control unit.

and pulses generated by the speed sensor were counted. Once the desired distance was covered, the speed calibration number was displayed on the screen (**Figure 10b**). It was saved in console memory and calibration procedure was completed.

The pressure transducer was calibrated by adjusting the pressure displayed by the ECU to the actual pressure value. To this end, an accurate manual pressure gauge was placed in the spray line close to the spray nozzles to measure the actual pressure in the system. Then, in-auto mode calibration step was activated to calibrate the installed pressure transducer and the displayed pressure was adjusted. Once the actual pressure matched the displayed pressure, calibration was initiated. The newly recorded value of maximum rating of pressure transducer, which lies between "0–10", was displayed and saved in memory after calibration.

The calibration of flow meter sensor was performed by setting the console in auto mode and activating calibration step wherein the flow meter pulses are calculated on

the basis of a known volume of fluid passing through the flow meter. To achieve this, calibration step was activated and the sprayer pump was started. During calibration, a known volume of fluid (360 liters) was sprayed and monitored. Once the entire volume had been sprayed, the ECU was instructed to stop counting pulses. Based on the pulse count obtained, the flow meter was calibrated.

6. Spraying tips with safety precautions

Agro-chemicals are toxic to both humans and animals. However, the harm to humans and non-target animal species can be reduced, if necessary precautions are taken. Insecticides will cause maximum harm, if inhaled or ingested or if they are in direct contact with the skin. Pesticide particles can also be inhaled with the air, while they are being sprayed. Another risk is the contamination of drinking-water, food or soil with insecticide particles. Precautions should also be taken during transport, storage and handling of pesticides and spray equipment. Spray equipment should be regularly cleaned and maintained to prevent leaks. People who work with pesticides should receive proper training in their safe use. Some spraying tips along with safety precautions before, during and after spraying are mentioned below;

Before spraying:

1. Ascertain the insect, pest attack level and ascertain the damage done
2. Usage insecticide only if it has exceeded the Economical Injury threshhold Level.
3. Use only the recommended insecticide which is the less toxic.
4. Read guidelines booklet of the pesticide and equipment.
5. Check the spraying machine and fittings which are to be used.
6. Ascertain that all components are clean, especially filling and suction strainer, sprayer tank, cut off device and nozzle.
7. Replace worn out parts such as 'O' ring, seal, gasket, worn out nozzle tip, hose clamps and valves.
8. Test the sprayer and ascertain whether it pumps the required output at rated pressure.
9. Check the nozzle spray pattern and discharge rate. Make sure that appropriate protective clothing is available and is used.
10. Train all concerned with the application and also understand the recommendations.
11. Confirm that soap, towel and plenty of water is available at the spray site.
12. DO NOT transfer pesticides from original container and packing into another container.

13. Do not spray when high velocity of wind, high temperature and rain.

14. Never eat, drink or smoke when mixing or applying pesticides.

15. NOT EVER blow out blocked nozzles or hoses with your mouth.

16. Never allow children to be nearby during mixing.

17. NEVER leave chemicals unattended in the field.

18. Never spray if the wind is blowing towards grazing cattle or meadowlands regularly used or habitat.

During spraying:

1. Clean the spray tank and nozzles with clean water before and after the spray by running for 2–3 minutes.

2. Wear hand gloves for preparation of spray mix, full cover, full sleeve shirt and trousers during spraying.

3. Spray should be done on calm days in straight bands/strips. Avoid spraying in heavy wind, dry (low humid) and hot environment condition as possible.

4. Always use clean water for spraying to avoid clogging in the nozzles.

5. If rate of discharge of nozzle go above by 10–15% from the initial ejection rate, the nozzle is reflected as worn out. Change the worn out nozzle.

6. Only 15 liters of water volume per acre is needed for spraying with electrostatic sprayer.

7. In case of backpack type electrostatic sprayer, electrostatic nozzle should not be lifted above shoulder height.

8. Do not touches the nozzle tip while operating the backpack type air assisted electrostatic sprayer.

9. Keep the gap of the electrostatic nozzle tip about 1 to 1.5 feet above/away from the crop canopy.

10. Take only required pesticide for the day's application.

11. Make sure insecticides are mixed in the correct amounts

12. Liquid formulation should be poured carefully to avoid splashing.

13. Selecting right track of spraying to avoid drift

14. Fix nozzle and boom at a suitable height to avoid drift loss of spray.

15. Wear full and appropriate clothing to cover yourself.
16. Avoid infection of the skin mainly eyes and mouth.
17. Follow correct spray procedure.
18. Run sprayer at accurate speed and correct pressure.

After spraying:

1. Remaining pesticides left in the tank after spraying should be emptied and disposed off in pits dug on wasteland.
2. Store the tractor in a dry, well protected place.
3. Under no circumstances empty the tank into irrigation channels or pools.
4. Never leave unused pesticides in sprayers tank. Always wash sprayer machine properly. After use, oil it and then keep always in store room.
5. Do not use blank insecticide bottles for any other purpose.
6. Damage and bury the containers in the ground preferably in a land filled dump.
7. Clean various item used to prepare spray solution like buckets, sticks, measuring jars, etc.
8. Wash yourself, protective clothing and footwear after the operation.
9. Always keep the record of pesticide usage for each crop.
10. Stop peoples to enter spray treated areas
11. Mark the sprayed fields with an appropriate flag to identify.

7. Conclusions

The following conclusions have been drawn regarding the plant protection equipment used for cotton crop;

- Bt (bacterium B. thuringiensis) cotton provides effective protection against cotton bollworms, but sucking pests namely whitefly, jassid, aphid and mealy bug are the most serious in Bt cotton and they cause maximum damage

- Major specifications of all-purpose sprayer are that it should have high clearance for tall crops, boom adjustable in height, have boom section control valve, Accurate ground speed indicator and flexible connections on the nozzles from the boom.

- Multi-purpose high clearance sprayer has two types of spraying arrangements namely auto rotate gun, boom type with drop up type nozzles which is operated by a single pump.

- High clearance sprayer is preferred by the farmer as it can work at all stages of the crop and saves time, labor and cost of operation as well as it reduces drudgery of the operation.

- Bio-efficacy of electrostatic sprayers i.e. 80–85% is comparatively higher than conventional sprayers.

- Recently, Government of India has released standard operating procedures (SOP) for use of drone with pesticides for the crop protection and for spraying soil and crop nutrients in agriculture, forestry, non-cropped areas etc.

- Uniform distribution of chemical depends on the constant speed, proper nozzle selection and efficient operation of sprayers, must for the better control of the insect and pest on cotton crop.

- Calibration of the sprayer is very important to determine the effectiveness of spraying and elimination of the over-spraying.

- There are two type of spray control technologies available first is Automatic Section Control (ASC) and second is Boom Section Control (BSC). The ASC technology has reduced over-application of pesticides to a large extent as compared to manual control system.

- Special precautions must be taken during transport, storage and handling of pesticides and equipment. Spray equipment should be regularly cleaned and maintained to prevent leaks.

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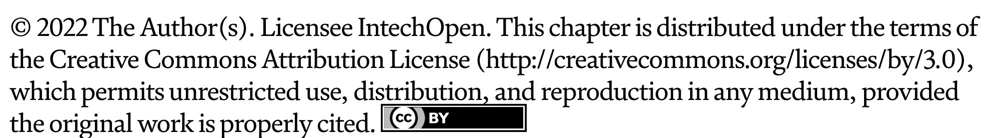

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References

[1] Leading cotton producing countries worldwide [Internet]. 2020/2021. Available from: https://www.statista.com/statistics/263055/cotton-production-worldwide-by-top-countries/ [Accessed: 03-01-2022]

[2] Consumption of chemical pesticides in various states/uts during 2016-2017 to 2020-2021 [Internet]. 2022. Available from: http://ppqs.gov.in/statistical-database [Accessed: 03-01-2022]

[3] Lu Y, Wu K, Jiang Y, Guo Y, Desneux N. Widespread adoption of Bt cotton and insecticide decrease promotes biocontrol services. Nature. 2012;**487**(7407):362-365. DOI: 10.1038/nature11153

[4] Kumar A, Kumar R, Aman VS, Karwasra N. Optimization of nozzle characteristics for different type of sprayers. International Journal of Pure & Applied Bioscience. 2020;**8**(2):273-281

[5] Narang MK, Mishra A, Kumar V, Thakur SS, Singh M, Mishra PK. Field evaluation of manual spraying technology against white flies on cotton crop in south-West Punjab. Agricultural Engineering Today. 2015;**39**(1):29-33

[6] Kumar S, Singh M, Manes GS, Singh NK. Development of auto rotate gun sprayer for the control of whitefly (*Bemisia tabaci*) in cotton crop. Journal of Cotton Research and Development. 2020;**34**(2):211-217

[7] Kumar S, Singh M, Arora J, Manes GS. Comparative field evaluation of auto-rotate gun sprayer for control of *Bemisia tabaci* in a cotton crop. African Entomology: Journal of the Entomological Society of Southern Africa. 2020;**28**(2):300-311

[8] Kumar S, Singh M, Manes GS, Pathania M. Development and evaluation of PAU multi-purpose sprayer to control whitefly *(Bemisia tabaci*) in cotton. The Indian Journal of Agricultural Sciences. 2020;**90**(6):1160-1165

[9] Singh M, Ghanshyam C, Mishra PK, Chak R. Current status of electrostatic spraying technology for efficient crop protection. AMA, Agricultural Mechanization in Asia, Africa and Latin America. 2013;**44**(2):46-53

[10] Parmar R, Singh S, Singh M. Bio-efficacy of unmanned aerial vehicle based spraying to manage pests. The Indian Journal of Agricultural Sciences. 2021;**91**(9):109-113

[11] Indra Mani. Standard Operating Procedures (SOP) for use of drone with pesticides for the crop protection and for spraying soil and crop nutrients in agriculture, forestry, non-cropped areas etc. Document released by Department of Agriculture and Farmers welfare, Ministry of Agriculture and Farmers Welfare, Govt of India in December 2021

Section 4

Cotton-Derived Products

Chapter 11

Development and Evaluation of an Extruded Balanced Food for Sheep Based on Cottonseed Meal *(Gossypium hirsutum)*

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Abstract

The objective of this research was to evaluate the effect of the content of cottonseed meal (*Gossypium hirsutum*) and the processing variables on the functional properties and the content of gossypol of an extruded feed for sheep *(Ovis aries)*. The diet was balanced according to the requirements of fattening Dorper sheep breed under 1 year. The extrusion process was optimized using a surface response methodology, with four independent variables: temperature in the last heating zone (120–160°C), moisture content (14–18%), screw speed (120 rpm–180 rpm), and cottonseed meal content (9 g^{-27} g 100 g^{-1}), in a single screw extruder. The optimal food had 27.25% crude protein, 4.24% crude fat, 12.21% crude fiber, 46.95% nitrogen-free extract, and 9.35% ash. The composition of essential amino acids in the optimal diet was 1.00 g kg^{-1} of lysine, 1.25 g kg^{-1} of phenylalanine, 2.04 g kg^{-1} of leucine, 0.87 g kg^{-1} of isoleucine, 0.98 g kg^{-1} of threonine, 1.15 g kg^{-1} of valine, and 0.65 g kg^{-1} of histidine. The fatty acids present in the highest concentration in the optimal diet were 2.14% linoleic acid, 1.11% oleic acid, and 0.81% palmitic acid. The gossypol content of the optimal diet was less than 0.1%, which ensures the safety of cottonseed meal as a protein source. The optimum conditions of the extrusion process were 120°C temperature, 120 rpm screw speed, 14.00% humidity, and 27 g 100 g^{-1} cottonseed meal.

Keywords: cottonseed meal, sheep, balanced feed, extrusion, gossypol

1. Introduction

Even though new livestock farming technologies are constantly being developed, worldwide many grazing animals feed on pastures, grasslands, crop residues, etc. due to their low input costs and better resilience to market fluctuations [1]. In Mexico and

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South America, the increased demand for sheep meat due to historical and cultural traditions generates an attractive market that has led to the intensification of sheep livestock production. In these regions, most producers use a grazing scheme for their animals while a small sector feeds them under stable weight-gain systems. In these conditions, sheep production requires high reproductive efficiency and low feeding costs. Balanced food is a necessity not only for the animal but also for the producer because it allows storage for long periods, provisioning in times of shortage, saving time in preparation, and ease of handling when feeding animals. Cottonseed meal is a by-product of cotton used for animal feed as it is rich in oil and protein. However, the gossypol content limits the use of cotton seeds in animal feed. High levels of free gossypol may be responsible for acute clinical signs of gossypol poisoning that include shortness of breath, decreased body weight gain, anorexia, weakness, apathy, and death after several days [2]. Gossypol is a phenolic compound with a molecular weight of 518.55 Dalton; it is a yellow, crystalline pigment, insoluble in water, soluble in acetone and chloroform, and is produced by the glands of the cotton plant [3]. Gossypol exists in the cotton plant as a defense agent and is responsible for toxicity problems associated with excessive feeding of cottonseed meals in animals [2]. In addition to animal toxicity, it has been reported to have anticancer, antiviral, and male infertility effects [2]. Ruminants can digest gossypol better than monogastric animals, so cottonseed meal is only used up to 23% in ruminant feed, due to the presence of gossypol, resulting in limited use [4]. Preventive procedures to limit the toxicity of gossypol involve the treatment of the cottonseed product to reduce the concentration of free gossypol with the most common treatment: thermal processing.

Extrusion has been described as a continuous-flow reactor capable of processing biopolymers and ingredient mixtures at high temperatures, pressures, and shear forces at low humidity. In addition, extrusion has a lower processing cost compared to other thermal processing, and being a continuous process, it has been used to modify functional properties [5]. The objective of this research was to produce an extruded feed for ruminants based on cottonseed meal and to evaluate its functional properties as well as the gossypol content.

2. Materials and methods

2.1 Diet formulation

A diet for fattening Dorper sheep breed (under 1 year, not castrated) was balanced using WinFeed 2.8© (1999–2004) program with the projected nutritional characteristics shown in **Table 1**. For the formulation of the treatments, cottonseed meal (*Gossypium hirsutum*) (CSM), dry molasses, soybean meal (44% protein), nixtamalized corn (*Zea Maize*) (NC), and dried distillers' grains with solubles (30% protein) (DDGS) were used, which were purchased from the main animal food shops in the municipality of Durango, Mexico. CSM, dry molasses, soybean meal, NC, and DDGS were subjected to grinding in a commercial coffee mill to reduce the particle size, which was sieved using a 40 mesh. The ratio of ingredients consisted of 12 g 100 g^{-1} of soybean meal, 15 g 100 g^{-1} of DDGS, 7 g 100 g^{-1} of dry molasses, and 30 g 100 g^{-1} of NC that were kept constant. Five different diets with different ratios of CSM and NC were evaluated according to the experimental design: 0:36, 9:27, 18:18, 27:9, and 36:0 g of CSM: g of NC 100 g^{-1}, respectively.

Nutritional characteristics	Composition [%]
Dry matter	211.905
Crude protein	28.65
Energy	1368.3 (Kcal/kg)
Neutral detergent fiber	6.48
Lysine	0.424
Methionine	0.144
Calcium	0.041
Phosphorus	0.162

Table 1.
Projected nutritional characteristics of the formulation using WinFeed 2.8©.

2.2 Extrusion processing

The extrusion of the treatments was performed using a single screw laboratory extruder (compression ratio 1:1) Brabender brand Model 20DN/8–235-00 (Duisburg, Germany), ¾" L/D – 25:1 ratio with the following characteristics: four heating zones (90, 100, and 110°C for the first, second and third zone, respectively, and the fourth one was adjusted according to the experimental design), screw compression ratio 1:1, screw diameter of 19 mm and exit die diameter of 6 mm i.d. Before extrusion, formulated mixtures were prepared, and moisture content was adjusted following the experimental design. The desired moisture level was adjusted by spraying distilled water onto the mix of ingredients, which was then hand-mixed for 15 min and conditioned for 12 h in closed plastic containers at 4°C. Three separate extrusion runs were carried out for each treatment. Extruded treatments were cooled down at room temperature for 1 h and stored in sealed polyurethane bags at 4°C for further analyses.

2.3 Experimental design and data analyses

A rotatable central composite experimental design (α = 2) with four independent variables was performed (**Table 2**) and 27 treatments were generated. The responses were expansion index (EI), bulk density (BD), penetration force (PF), water absorption index (WAI), water solubility index (WSI), and water activity (WA). Numerical optimization was performed using the superimposition of surface response for each

Variables	Levels				
	-α	-1	0	+1	+α
Temperature [°C]	100	120	140	160	180
Moisture content [%]	12	14	16	18	20
Screw speed [rpm]	90	120	150	180	210
Cottonseed meal content [g 100 g^{-1}]	0	9	18	27	36

Table 2.
Levels of independent variables.

treatment (Design Expert Version 13.0). Experimental data was adjusted to quadratic models, and regression coefficients were obtained. Statistical significances of the regressions' terms were examined by variance analyses (ANOVA) for each response ($p < 0.05$).

2.4 Determination of physical and functional properties

2.4.1 Expansion index and bulk density

Ten randomly selected extruded samples of each treatment were measured in diameter (d) and length (L). Each sample was taken three measurements of the diameter, the average value was calculated and then the extruded diameter was divided by the diameter of the hole of the exit die placed in the extruder nozzle using a vernier. Then each extruded (Pm) was weighed to determine the density using Eq. 1 [6].

$$\text{Density} = \frac{Pm}{\left(\pi\left(\frac{d}{2}\right)^2 L\right)} \tag{1}$$

2.4.2 Penetration force

The determination of the penetration force of the extrudates was performed using a Universal Texture Analyzer TA-XT2 (Texture Technologies Corp., Scarsdale, NY/ Stable MicroSystems, Haslemere, Surrey, UK) using a Warner Bratzler blade. A total of 15 samples were measured per treatment, at a speed of 1 mm s^{-1}, recording the average of the maximum penetration force.

2.4.3 Water absorption index and water solubility index

The extrudates of each treatment were ground in a commercial coffee mill to a particle size of mesh 40. In a pre-weighed centrifuge tube, 1 g of sample per treatment was weighed, and 10 mL of distilled water was added, stirred for 30 minutes, and centrifuged at 3000 rpm for 15 min. The supernatant was decanted and evaporated to dryness in a convective stove at 97°C; the residue was weighed, and the WSI was calculated using Eq. 2. After decanting the supernatant, the remaining sediment in the tube was weighed to calculate the WAI using Eq. 3 [7]. Both analyses were evaluated in triplicate.

$$WSI = \frac{Weight of the dry supernatant}{Weight of dry sample} x\ 100\ (\%) \tag{2}$$

$$WAI = \frac{Sediment weight}{Weight of dry sample}\ \left(g de H_2O g^{-1} of sample\right) \tag{3}$$

2.4.4 Water activity

The water activity was measured using HygroLab C1 equipment (ROTRONIC, Measurement solutions, Process sensing technologies), each treatment was evaluated in duplicate with an accuracy of ± 0.003.

2.4.5 Proximal chemical analysis

The moisture content was evaluated by drying the sample in a stove at 105°C until it reached constant weight (925.10, [8]). The ash content was determined by calcination of the sample in an oven-muffle at 550°C until obtaining constant weight (923.03, [8]). The protein content was determined from the composition of the total nitrogen in the samples, using the Kjeldahl technique, according to AOAC Method 910.87 (2019). The crude fat content of the sample was determined using the hot fat extraction method, Soxhlet equipment and petroleum ether (40–60°C), according to method 920.39, AOAC [8]. Neutral detergent fiber and acid detergent fiber contents were evaluated following the procedures proposed by Van Soest et al. [9]. The nitrogen-free extract was obtained by difference from the obtained values of moisture, crude protein, crude fat, and ash (Eq. 4).

$$\%NFE = 100 - (\%Moisture + \%Crude\ protein + \%Crude\ fat + \%Crude\ fiber + \%Ash) \quad (4)$$

2.5 Determination of gossypol concentration

The concentration of gossypol was obtained following the official Mexican standard NOM-Y-217-A-1982 to analyze free gossypol in cottonseed meal for animal feed using a factorial design where the variables were temperature (120, 140, and 160°C) and moisture content (14, 16, and 18%).

2.6 Mineral determination

From the optimal treatment, 500 mg were taken and washed at 150°C with 15 mL of concentrated HNO_3 and 2 mL of 70% $HClO_4$. The samples were dried at 120°C and the residues were dissolved in 10 mL of a 4.0% HNO_3–1% $HClO_4$ solution. The mineral content of each sample was determined inductively by argon plasma emissions by atomic spectroscopy.

2.7 Fatty acids determination

From the optimal treatment, samples were extracted using an ASE 200 system (Dionex, Idstein, Germany) using 11 mL extraction cells. An azeotropic mixture of cyclohexane and ethyl acetate was used as a solvent. The conditions used were temperature, 80°C; pressure, 10 MPa; preheating, 0 min; heating, 5 min; static, 10 min; flow, 60%; purging, 120 s; and 2 cycles. The remaining fraction was condensed with a broken evaporator (180 mbar, 30°C) and then evaporated using a stream of nitrogen [10]. The samples were analyzed in a Hewlett-Packard 5890 series II gas chromatograph. A capillary column covered with 100% cyanopropyl polysiloxane (CP-Sil 88, 50 m × 0.25 mm, 0.20 μm, Chrompack, Middelburg, The Netherlands), started at 60°C (waiting time of 1 min), increased in intervals of 7°C min-1 until reaching 180°C, then 3°C min^{-1} to 200°C (waiting time 1 min) and finally 10°C min-1 to 230°C (waiting time 10 min). Helium was used as carrier gas at a constant flow of 1.3 mL/min. Nitrogen was used as the makeup gas.

2.8 Determination of amino acids

The optimal treatment was analyzed to determine the amino acid content using an Agilent 1260 Infinity chromatograph equipped with a microdegassifier (G1379B), a 1260 binary pump (G1312B), a multiple wavelength standard detector (G1315C), and a Zorbax Eclipse-AAA column (150 mm × 4.6 mm, 5 μm, internal particle diameter, Agilent Technologies, Santa Clara, CA). The samples were freeze-dried, ground, and hydrolyzed. A total of 1 g of sample was weighed and HCl was added. The samples were hydrolyzed for 24 h to 110°C. After hydrolysis, the samples were vacuum evaporated, and the hydrolysates were reconstituted in 2 mL of HCl.

3. Results and discussion

3.1 Determination of physical and functional properties of extruded treatments

Table 3 shows the regression coefficients of the extruded treatments. The expansion index (EI) was negatively affected ($p \leq 0.05$) by the temperature in its quadratic term, possibly because high temperatures in extrusion cause degradation of starch molecules and result in reduced expansion [11]. The cottonseed meal content had a negative effect ($p \leq 0.05$) because the complexes that form proteins with starch and fiber disrupt the cutting force as a result of the interactions of the components; protein molecules could affect the gelatinization process in different ways depending on their ability to retain water and their ability to interact with starch molecules and surface granules [12]. The decrease in EI can be attributed to the fact that, during the extrusion process at high temperatures, starch undergoes further degradation and may become more dextrinized, reducing the EI values that are accentuated in mixtures with low starch content. The water absorption index (WAI) was positively affected ($p \leq 0.05$) by temperature, this being a property that indicates the amount of water retained by starch since a proliferation of hydrophilic sites allows greater accessibility of water to interact through hydrogen bonds [13]. The temperature-humidity interaction had a significant positive effect ($p \leq 0.05$) since water acts as a plasticizer during extrusion cooking, thus reducing the degradation of starch granules and resulting in a greater capacity for water absorption [14]; in addition, high temperatures increase the degradation and dextrinization of starch [15]. As the temperature increases, hydrogen bonds decrease just like the hydration of the ionic groups, so a denatured protein generally binds 10% more water than its native equivalent, in addition to increasing the surface area of proteins. However, it should also be borne in mind that the aggregation phenomenon may occur, increasing protein–protein interactions, and thus decreasing its water-binding capacity. For the water solubility index (WSI), humidity has a significant negative effect ($p \leq 0.05$). WSI measures the number of soluble components released from starch after extrusion and is related to the degree of polymerization of starch occurring within the extruder [16]. The low moisture content decreases the gelatinization of the starch because high temperatures decrease the humidity, lowering the availability of water for the starch granules. Sobuñola et al. [15] state that this is due to the interactions between starch, protein, fiber, and lipids. These interactions can increase the molecular weight of the complex formed causing a decrease in the solubility index. Pardhi et al. [16] report that high humidity levels result in low levels of WSI in the extrudate. Jong-Bang et al. [17] showed that a low humidity together with a high speed decreases the WSI, however, increasing the temperature increases the WSI, due to the depolymerization of the

	Intercept	Lineal				Quadratic				Interactions					
Response	b_0	X_1	X_2	X_3	X_4	X_1^2	X_2^2	X_3^2	X_4^2	$X_1 X_2$	$X_1 X_3$	$X_1 X_4$	$X_2 X_3$	$X_2 X_4$	$X_3 X_4$
EI	1.29E+00	1.85E-04	-1.42E-02	2.06E-02	-1.81E-03	−6.06E-02*	−3.00E-02	−4.93E-02	−5.22E-02*	−2.55E-02	−8.13E-03	−1.89E-02	−1.44E-02	4.75E-02	−1.86E-02
BD	1.56E+03	4.21E+02	−3.80E+02	−4.08E+02	4.43E+02	1.04E+02	1.55E+02	−2.22E+01	8.11E+01	−5.12E+02	−6.22E+02	6.49E+02	5.58E+02	−5.94E+02	−5.87E+02
PF	1.00E+02	3.54E+00	1.07E+00	−3.03E+00	2.22E+00	3.35E+00	−1.91E+00	−6.01E-01	2.72E+00	−1.55E+00	−5.01E+00	−9.44E-01	2.05E+00	1.59E+00	−1.18E+00
WAI	3.48E+00	3.69E-02*	−2.64E-03	−1.15E-02	−3.74E-03	1.40E-02	−6.49E-04	−1.82E-02	−3.19E-03	4.63E-03	−4.17E-02*	4.74E-02	−6.84E-05	−6.68E-03	−1.70E-02
WSI	1.05E+01	9.65E-01	9.78E-01	−1.10E+00*	5.63E-01	5.53E-01	4.46E-01	4.72E-01	5.47E-01	9.90E-01	−1.05E+00	6.13E-01	−1.09E+00	−2.26E-01	−1.05E-01
AW	6.65E-01	−1.86E-02*	3.04E-03	3.58E-02*	−1.49E-03	−1.55E-02	−2.35E-03	2.86E-03	−7.95E-03	−6.42E-03	2.17E-02	1.08E-02	−7.44E-03	−4.93E-03	−5.93E-04

*EI = expansion index, BD = bulk density, WAI = water absorption index, WSI = water solubility index, AW = water activity. X_1 = Temperature (°C), X_2 = screw speed (rpm), X_3 = moisture content (%), X_4 = cottonseed meal content (%), *indicates statistical significance ($p > 0.05$).*

Table 3.
Regression coefficients of the surface response models.

starch and other macromolecules present in the mixture, which leads to the reduction of the chain's amylose and amylopectin.

3.2 Numerical optimization

The numerical optimization was performed by a superimposition of surface response method, obtaining the following conditions: 120°C temperature, 120 rpm screw speed, 14% moisture content, and 27:9 g of CSM: g of NC 100 g^{-1}; the responses obtained for the optimal feed were: 150.75 N of penetration force, 3.48 g g^{-1} of water absorption index, 11.79% of water solubility index and 0.62 of water activity. The criteria used for the optimization of the factors were to minimize temperature, moisture content, screw speed and to maximize cottonseed meal content (by substituting soybean meal) to reduce processing and formulation costs.

3.3 Proximal chemical analysis

The optimal formulation of the diet consisted of 12 g 100 g^{-1} soybean meal, 15 g 100 g^{-1} DDGS, 7 g 100 g^{-1} molasses, 30 g 100 g^{-1} nixtamalized corn, and 27:9 g of CSM: g of NC 100 g^{-1} cottonseed meal, meet the nutritional requirements for sheep <1 year [18], and it was compared against some similar commercial foods (**Table 4**).

The high protein content of the food is desirable because the feeding of ruminants is supplemented by fodder in percentages of 60% balanced food and 40% fodder, since fodder usually has very low nutritional value, especially in developing countries during dry seasons. For instance, mature grasses only have crude protein levels of 3.5–8%. As an example, during the early dry season in Samoa (May, June, and July), crude protein content in batiki bluegrass dramatically decreases and ranges only between 3% and 9% [19]. Also, the supply of amino acids depends on the protein content in the diet, from the transfer through the rumen to the intestines as undegraded vegetable protein and microbial protein, and its absorption in the small intestine; furthermore, cottonseed meal has a better response compared to other protein sources, such as hay, for animal fattening [20]. National Research Council [18] indicates that energy is the most limiting factor in the nutrition of small ruminants, an energy deficiency will lead to low production, poor reproduction, high mortality, and susceptibility to diseases and parasites. Minerals play an important role in the functioning of the body's cells as they promote the health of the skin and promote growth. The type of carbohydrate found in the diet conditions the development of the type of flora suitable for fermentation and the pH adjustment to its ideal range. Thus, a starch-rich ration is fermented by an amylolytic flora that performs best at pH from 5.5 to 6.0. Fiber, as a nutrient,

Sample	Moisture [%]	Total dry matter [%]	g 100 g^{-1}				
			Crude protein	Crude fat	Crude fiber	Nitrogen free extract	Ashes
Optimal food	8.66	91.34	27.25	4.24	12.21	46.95	9.35
Commercial food	9.5	90.50	16.51	2.98	13.66	56.36	16.51
Requirement*	—	90	14.5	3	10	52	7.5

**Nutritional requirements for sheep less than 1-year-old [18].*

Table 4.
Proximal chemical analysis of balanced optimal diet for sheep.

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contributes to the maintenance of ruminal functioning, by acting as ruminal filling and stimulating ruminal physicochemical contractions and conditions.

3.4 Amino acids profile

Some vegetable proteins are deficient in sulfur amino acids (AA) compared to animal proteins and contain antinutritive factors. Nevertheless, by being supplemented with other proteins and physicochemical treatments, oilseed protein makes a significant contribution to human and animal dietary protein intake [21]. Extruded feeding increases the flow of AA to the duodenum of ruminants by 34% and increases the apparent absorption of AA in the small intestine by 58% [22]. The ruminant can synthesize arginine, although in insufficient quantities to meet the nutritional requirements, especially important during early growth or in reproductive stages. Aspartic acid and glutamic acid are rapidly metabolized and produce volatile fatty acids, histidine being one of the limiting amino acids in ruminants [23]. The optimal food meets most of the nutritional requirements of AA (**Table 5**). It was observed that both aspartic acid and glutamic acid are found in large amounts, which is desirable because they are metabolized very quickly and produce volatile fatty acids. Lysine contributes to weight improvement and its requirement in sheep is 2.78 g d^{-1}; since the food contains 1 g kg^{-1}, it covers the daily needs of the animal. The ruminant can synthesize arginine, although in insufficient quantities to meet body requirements: 2.01 g d^{-1} [18].

Amino acid	Sample [g kg^{-1}]	Nutritional requirement [g d^{-1}] *
Lysine	1.00	2.78
Phenylalanine	1.25	3.9
Leucine	2.04	6.03
Isoleucine	0.87	4.01
Threonine	0.98	3
Valine	1.15	3.5
Histidine	0.65	1.01
Arginine	1.95	2.01
Glycine	1.00	—
Aspartic acid	2.33	—
Serine	1.31	—
Glutamic acid	4.52	—
Proline	1.38	—
Hydroxyproline	0.06	—
Alanine	1.25	—
Tyrosine	0.79	2.7

Nutritional requirements for sheep less than 1-year-old [18].

Table 5.
Amino acid profile in the optimal food.

3.5 Fatty acids profile

Fatty acids are precursors that help the production of volatile fatty acids, being the main acetic, butyric, and propionic, which cover most of the energy requirements. Feed rations produce a lower concentration of volatile fatty acids compared to those based on concentrates with a high content of proteins or easily fermentable carbohydrates. The proportion of each of the volatile fatty acids in the mixture varies with the quality, quantity, and texture of the food ration components. Grain-based concentrates, which include heat and pressure treatment, are fermented more quickly and favor the production of propionic acid. This increase in digestibility occurs because during the previous treatment a certain degree of fragmentation of starch granules and partial hydrolysis of starch molecules occurs. The fatty acid profile of the optimal food consisted of 2.142% linoleic acid, 1.114% oleic acid, 0.122% stearic acid, 0.812% palmitic acid, 0.014% lauric acid, 0.015% myristic acid, and 0.016% palmitoleic acid. The optimal food provides an amount of linoleic acid above the nutritional requirements for sheep under 1 year (1.7 g d^{-1}, [18]), which can have a positive effect on the production of conjugated linoleic acid.

3.6 Minerals profile

The minerals profile of the optimal food meets the nutritional requirements of most micro- and macro-minerals (**Table 6**). For a ruminant, the main macro-minerals in his diet are Fe, Cu, Zn, and Mn. Iron represents 0.33% of the hemoglobin molecule, being necessary for the transport of oxygen by the blood to the tissues, in addition to being involved in the synthesis of myoglobin (muscle constituent) and ferritin. The optimal food had 475 ppm of Fe, being the maximum upper limit of Fe of 500 ppm. Cu intervenes in the fertility, enzymatic activation, and as a growth factor, being the upper limit of Cu of 50 ppm. Zn is a constituent of the hoof; in addition to reducing stress, somatic cells restore epithelial and are a fertility factor in adult animals; the minimum and maximum requirements are 22 and 150 ppm, respectively [18].

The food showed a concentration of 1.67% and 1.064%, for Ca and P, respectively. National Research Council [18] reports that Ca and P requirements are 0.51% and

Mineral	Concentration	Requirements[*]
Calcium	1.67%	0.51%[1]
Phosphorus	0.77%	0.24%[1]
Sodium	0.31%	—
Magnesium	0.349%	—
Manganese	74.9 ppm	—
Iron	475 ppm	500 ppm[2]
Zinc	58.1 ppm	22 ppm[2]
Copper	9 ppm	50 ppm[2]
Potassium	1.064%	—

[*]*Nutritional requirements for sheep less than 1-year old ([1]Molle and Landau, 2017; [2]National Research Council, 2007).*

Table 6.
Mineral content in the optimal food.

Sample	Moisture [%]	Temperature [°C]	Free gossypol [%]
1	14	140	0.2404
2	18	140	0.1320
3	14	160	0.1216
4	18	120	0.1278
5	14	120	0.0752
6	16	160	0.0895
7	18	160	0.1047
8	16	120	0.1096
9	16	140	0.0957

The safe level of gossypol in diets for ruminants less to 1 year old is 0.1% [25].

Table 7.
Content of free gossypol in the optimal food.

0.24%, respectively. These results cause possible hyperparathyroidism or urolithiasis to be questioned, since high levels of Ca reduce the use of other minerals, although it has been reported that if it is not exceeded 2% and 3%, for Ca and P, respectively, there would be no problem in the animals [24].

3.7 Gossypol

The free gossypol content in the analyzed samples of the optimal diet was below 0.1% (**Table 7**). It has been reported that it is not advisable to feed ruminants less than a year and a half with a diet with free gossypol content greater than 0.1%, while adult ruminants can feed with levels greater than 1% [25]. It is observed that samples 5, 6, and 9 are those with the lowest gossypol content, which are ideal for ruminants less than 1-year-old. Sample 5 has the same extrusion conditions as the optimal feed. It should be noted that sample 1, despite having a high temperature compared to sample 5, has the highest concentration of gossypol. It has been reported that the moisture content during extrusion helps the destruction of some aflatoxins, in addition to a high moisture concentration in the extruded product increases the loss of toxic factors [6]. According to Gomes et al. [4], cotton flour contains 0.1–0.4% free gossypol. The processing of cotton flour, especially at high temperatures, favors the reduction of gossypol and it has been suggested that diets containing up to 200 mg of free gossypol are safe for ruminants, while 400 mg is the limit for considering it toxic, and, at levels greater than 800 mg, causes death [4].

4. Conclusions

The obtained extruded optimal feed met the nutritional requirements of Dorper sheep <1 year, showing a good composition of amino acids, fatty acids, and minerals. Also, since the gossypol content was less than 0.1% in the diet, cottonseed meal might be a good alternative as a protein source to feed small ruminants at early development stages.

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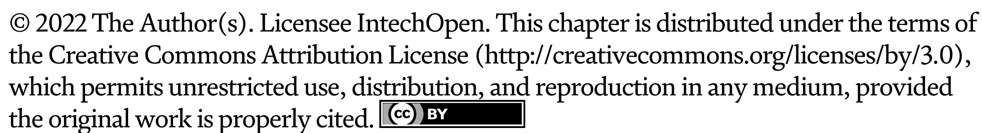

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References

[1] Odintsov Vaintrub M, Levit H, Chincarini M, Fusaro I, Giammarco M, Vignola G. Review: Precision livestock farming, automats and new technologies: possible applications in extensive dairy sheep farming. Animal. 2021;**15**(3): 100143. DOI: 10.1016/j.animal.2020.100143

[2] Gadelha ICN, Fonseca NBS, Oloris SCS, Melo MM, Soto- Blanco B. Gossypol toxicity from cottonseed products, The Scientific World Journal, Hindawi Publishing Corporation; 2014. pp. 1–11. DOI: 10.1155/2014/231635

[3] Ma M, Ren Y, Xie W, Zhou D, Tang S, Kuang M, et al. Physicochemical and functional properties of protein isolate obtained from cottonseed meal. Food Chemistry. 2018;**240**:856-862. DOI: 10.1016/j.foodchem.2017.08.030

[4] Gomes VS, Mano SB, Freitas MQ, Santos MD, Azeredo VB, Silva JM, et al. The effect of feed intake containing whole cottonseed on blood parameters of Nellore bulls. Arquivo Brasileiro de Medicina Veterinária e Zootecnia. 2017; **69**:551-558. DOI: 10.1590/1678-4162-9005

[5] Delgado E, Valles-Rosales DJ, Flores NC, Reyes-Jáquez D. Evaluation of fish oil content and cottonseed meal with ultralow gossypol content on the functional properties of an extruded shrimp feed. Aquaculture Reports. 2021; **19**:100588. DOI: 10.1016/j.aqrep.2021.100588

[6] Oke MO, Awonorin SO, Sanni LO, Asiedu R, Aiyedun PO. Effect of extrusion variables on extrudates properties of water yam flour – a response surface analysis. Journal of Food Processing and Preservation. 2012; **37**:456-473. DOI: 10.1111/j.1745-4549.2011.00661.x

[7] Oikonomou NA, Krokida MK. Water absorption index and water solubility index prediction for extruded food products. International Journal of Food Properties. 2012;**15**:157-168. DOI: 10.1080/10942911003754718

[8] AOAC. Official Methods of Analysis. 21st ed. Vol. I. Gaithersburg, Maryland: Association of Official Analytical Chemists International; 2019. p. 700

[9] Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science. 1991; **74**(10):3583-3597. DOI: 10.3168/jds.S0022-0302(91)78551-2

[10] Ribechini E, Pérez-Arantegui J, Colombini MP. Gas chromatography/mass spectrometry and pyrolysis-gas chromatography/mass spectrometry for the chemical characterization of modern and archaeological figs (Ficus carica). Journal of Chromatography A. 2011; **1218**:3915-3922. DOI: 10.1016/j.chroma.2011.04.052

[11] Olmolola OM, Faramade OO, Fagbemi TN. Effect of extrusion on protein quality, antinutritional factors, and digestibility of complementary diet from quality protein maize and soybean protein concentrate. Journal of Food Biochemistry. 2018;**42**:e12508. DOI: 10.1111/jfbc.12508

[12] Yousefi A, Razavi SM. Dynamic rheological properties of wheat starch gels as affected by chemical modification and concentration. Starch - Stärke. 2015; **67**:567-576. DOI: 10.1002/star.201500005

[13] Wani IA, Sogi DS, Hamdani AM, Gani A, Bhat NA, Shah A. Isolation, composition, and physicochemical

properties of starch from legumes: A review. Starch - Stärke. 2016;**68**:834-845. DOI: 10.1002/star.201600007

[14] Filli KB, Nkama I, Jideani VA. The effect of extrusion conditions on the physical and functional properties of millet – Bambara groundnut based fura. American Journal of Food Science and Technology. 2013;**1**:87-101. DOI: 10.1108/bfj-may-2010-0091

[15] Sobuñola OP, Babajide JM, Ogunsade O. Effect of brewers spent grain addition and extrusion parameters on some properties of extruded yam starch-based pasta. Journal of Food Processing and Preservation. 2013;**37**: 734-743. DOI: 10.1111/ j.1745-4549.2012.00711.x

[16] Pardhi SD, Singh B, Nayik G, Dar B. Evaluation of Functional Properties of Extruded Snacks developed from brown rice grits by using Response Surface Methodology. Journal of the Saudi Society of Agricultural Sciences. 2016;**18**: 7-16. DOI: 18.10.1016/j.jssas.2016.11.006

[17] Jong-Bang E, Fy-Hung H, Ok-Ja C. Physicochemical properties of rice-based expanded snacks according to extrusion conditions. Journal of the Korean Society of Food Science and Nutrition. 2014;**43**: 1407-1414. DOI: 10.3746/ jkfn.2014.43.9.1407

[18] National Research Council. Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids. Washington, DC: The National Academies Press; 2007. DOI: 10.17226/11654

[19] FAO. 2021. Food and Agriculture Organization of the United Nations. Consulted in December 2nd https://www.fao.org/ag/againfo/themes/documents/PUB6/P618.htm

[20] Detmann E, Valente EEL, Batista ED, Huhtanen P. An evaluation of the performance and efficiency of nitrogen utilization in cattle fed tropical grass pastures with supplementation. Livestock Science. 2014;**162**:141-153. DOI: 10.1016/j.livsci.2014.01.029

[21] Moure A, Sineiro J, Domínguez H, Parajó JC. Functionality of oilseed protein products: A review. Food Research International. 2006;**39**(9): 945-963. DOI: 10.1016/j. foodres.2006.07.002

[22] Duque-Quintero M, Rosero-Noguera R, Olivera-Ángel M. Digestion of dry matter, crude protein and amino acids of the diet dairy cows. Agron. Mesoam. 2017;**28**:341-356. DOI: 10.15517/ma. v28i2.25643

[23] Osorio J, Vailati-Riboni M, Palladino R, Luo J, Loor J. Application of nutrigenomics in small ruminants: Lactation, growth, and beyond. Small Ruminant Research. 2017;**154**:29-44. DOI: 10.1016/j.smallrumres.2017.06.021

[24] Molle G, Landau S. Husbandry of Dairy Animals. In: Sheep: Feeding Management, Encyclopedia of Dairy Sciences. United Kingdom: Academic Press; 2017. pp. 848-856. DOI: 10.1016/ B978-0-12-374407-4.00238-7

[25] Cope RB. Cottonseed Toxicity. In: Gupta RC, editor. Veterinary Toxicology. Third ed. United Kingdom: Academic Press; 2018. pp. 967-980. DOI: 10.1016/B978-0-12-811410-0.00068-4

Chapter 12

Cotton Based Cellulose Nanocomposites: Synthesis and Application

Patricia Jayshree Samuel Jacob

Abstract

Nanocellulose is a renewable natural biomaterial which has risen to prominence due to its biodegradability and physiochemical properties making it a promising candidate to replace non-biodegradable synthetic fibers. Due to its profound qualities, nanocellulose extracted from cotton fibers have tremendous application potential and have been intensively studied particularly in the generation of nanofillers and as reinforcement components in polymer matrixes. Deposition of inorganic nanoparticles on cotton fabric result in antimicrobial textiles with multifunctional use particularly in manufacture of PPE and as filtration devices against environmental pollutants and pathogens. This chapter compiles three main sections. The first section gives an overview of the extent of work done in the creation and application potential of cotton-based nanocomposites. The second section describes the in situ and ex situ methods of nanoparticle deposition and self assembly on cotton fabrics to generate multifunctional cotton-based nanocomposites with antimicrobial potential while the final section describes the incorporation of cotton nanofibers in polymer matrices, their reinforcing properties, as well as surface modification to assist their incorporation. Finally in the conclusion, a summary of the up-to-date challenges and progresses is presented postulating the undiscovered arenas and future undertakings of this venture.

Keywords: cotton nanostructures, cellulose nanocomposites, nanofibers, nanocellulose, antimicrobial textiles, reinforcement fillers

1. Introduction

With the dawn of the age of nanotechnology, there has been an intense scurrying and scavenging for nanomaterials with unique properties and specific molecular arrangements that allow it to find application in specific niches inaccessible to alternative forms. Nanostructured materials display unique physicochemical properties such as excellent electrical and thermal conductivity, solubility, porosity, surface interactions, density, band gap and surface electronic charge resulting in exceptional catalytic and optical activity and enhanced performance compared to their bulk counterparts [1]. Presently, nanoscale devices have widespread application in cell targeted therapeutic delivery, high resolution tissue imaging and in replacing damaged

tissue [2]. In agriculture, nanomaterials are being used to enhance crop production as nanofertilizers [3] and for crop protection as nanopesticides and nanobiosensors [4]. These active ingredients are encapsulated in nanocapsules, micelles, gels, liposomes, mesoporous silica nanoparticles or hollow nanoparticles to ensure controlled release, better solubility and for active stablity in the long-term [5]. To compensate for hazardous emissions to the environment, nanomaterials have been functionalized to remove contaminants through adsorption [6], immobilization, photocatalytic degradation, and electro-nanoremediation [7]. It is therefore undeniable that uncovering novel multifunctional nanosized materials is an elaborate pursuit with promising outcomes, yet filled with pressing concerns which are in dire need to be addressed.

One of the primary concerns of nanotechnology is the indiscriminate release of hazardous nanowaste, generated during the manufacturing and processing of engineered of nanomaterials, which could inevitably accumulate in the environment and inevitably end up in the food chain [8]. This has roused an overdrive in the hunt for sustainable nanomaterials from renewable bioresources such as cellulose, starch, chitosan, gelatin, alginate and chitin which are biodegradable, leave minimal implications on health and the environment and could be retrieved as value added waste in the production of a new generation of green nanomaterials [9].

Cellulose is a renewable feedstock with interesting properties such as biocompatibility and biodegradability. It is found to be chemically inert, displays excellent stiffness, high strength and dimensional stability, low density and easily functionalized surface chemistry [10]. Lignocellulosic biomass such as wood and agricultural residues such as tree trunks, rice straw, sugarcane bagasse, coconut husks, oil palm empty fruit bunches energy crops and grass are excellent feedstocks for green nanomaterials derived from cellulose or nanocellulose. This natural biopolymer is abundantly available and can be used as renewable feedstock in the generation of sustainable nanomaterials [11]. Reconstruction of lignocellulosic biomass waste residues into value added products such as nanostructures is an attractive, feasible option [12].

Cotton is an abundantly available fibrous crop grown for global commercial production with over 95% cellulose in its plant structure. Cotton stalk which is an overbearing agricultural residue generated in cotton-producing countries such as India, USA, China, Brazil and Pakistan, represents a semi-wood raw material made up of cellulose, hemicellulose, and lignin which could be utilized to fabricate value-added nanocellulose, paving an excellent way to maximize the utilization of waste [13]. Nanocellulose has exceptional properties such as high tensile strength, high Young's modulus, low weight, mechanical robustness, low coefficient of thermal expansion, biodegradability, surface functionality and hydrophilicity, biocompatibility and lack of toxicity [14]. In recent times, nanocellulose is used in energy storage, as aerogels, emulsion stabilizers, enzyme immobilization substrates, low-calorie food additives, reinforcing fillers, pharmaceutical binder, biomimetic materials and biosensors [15–17]. Nanocellulose derived from cotton feedstock can be broadly categorized as cellulose nanocrystals (CNC) and nanofibrillated cellulose (NFC). CNCs (as shown in **Figure 1**), also known as cellulose nanowhiskers or nanorods, are short (<500 nm) and narrow (<40 nm) rod shaped, rigid crystalline structures with diameters between 1 and 100 nm [18] with tremendous application potential in regenerative medicine [19], optoelectronics [20], automotive polymers [21] and as composite materials [22]. It is generated by eliminating the amorphous regions in cellulose fibers using acid hydrolysis [23]. CNC have been extracted from cotton fibers [24], processed cotton [25] and cotton linters [26], a byproduct of cotton processing. NFC or cellulose nanofibers (as shown in **Figure 2**) are longer (< 3000 nm) and wider

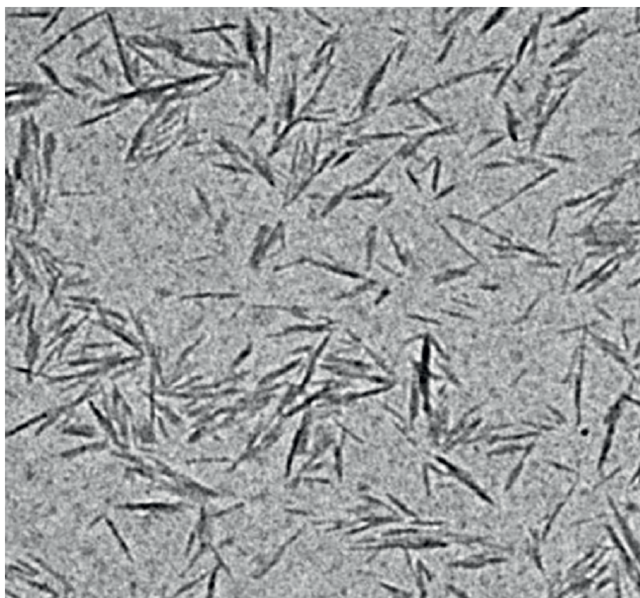

Figure 1.
TEM image depicting cotton-derived CNC.

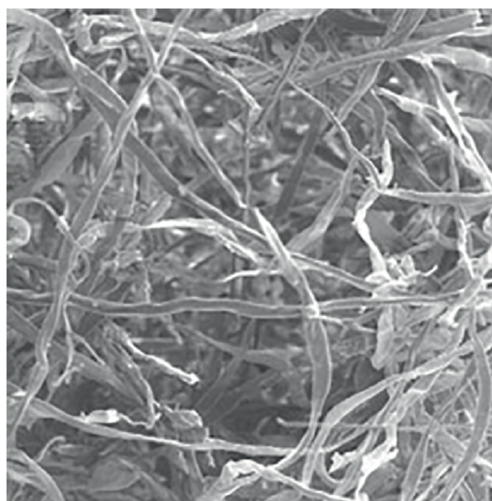

Figure 2.
FESEM images of the cotton based NCFs.

(< 100 nm) fibers with low crystallinity obtained by the mechanical disintegration of cotton biomasses using a high-speed ball grinder [27], ultrasonicator [28] or high-pressure homogenization [29].

Nanocomposites are materials made up of 2 or more constituent phases with at least 1 phase of nano-size particles (<100 nm) which creates a discontinuous phase over a matrix of standard material [30]. This unique multiphase structure that is reinforced by a stronger component of nanosized fillers [31] demonstrates greater mechanical and tensile strength and increased capacity for thermal expansion and

conductivity [32]. CNCs are interesting materials that could function as nanofillers owing to the abundance of the -OH groups, reactivity, high surface area, mechanical, thermal and optical properties, even at low concentrations [33] which enhances tensile strength and decreases elasticity due to the strong intermolecular linkages such as covalent bonds, van der Waals forces, mechanical interlocking and molecular entanglement between the fillers and its polymeric matrix [34]. Various methods have been developed to generate cellulose nanocomposites which include melt extrusion, ball milling, injection molding, compression molding, 3D printing, layered assembly, electrospinning, among others [35, 36]. Cellulose nanocomposites find vast application as packaging material, automotive and aerospace paints and coatings, adhesives, hydrogels, nanobarriers, fire retardants, construction materials, military defense and as emerging smart hybrids which display outstanding properties such as stretch ability, high mechanical strength, optical transparency, electrical and thermal conductivity, porosity and high adsorption [37]. Cotton based cellulose nanocomposites constructed with metals, metal oxides and non-metallic elements have exhibited innovative features due to its synergetic effects which are unattainable as pure nanomaterials [38]. Nanocomposites loaded with noble metal nanostructures have antibacterial properties and are used in biomedicine, enzyme immobilization, catalysis and as biosensors [39]. Rumi et al., 2021 observed that cotton-based CNC display high crystallinity, tensile strength and stiffness making it an attractive engineering nanomaterial for composite reinforcement [40]. In a separate study, Araujo et al., 2018 found that biopolymer nanocomposites reinforced with hydrolyzed cotton NFC extracted from cotton waste textiles resulted in a composite material with greater tensile strength and thermal capacity compared to the pure biopolymer [25]. Rafaella et al., 2019 constructed a cotton NFC/chitosan nanocomposite with collagen like properties which demonstrated increased surface roughness, improved cell adhesion, spreading and proliferation when used as scaffolds in tissue engineering [41]. Thus, surface modification of polymeric materials with cotton NFC for substrates used as scaffolds in tissue engineering would result in functionalized nanocomposites with novel physicochemical properties and large surface area which allow numerous contact points between cells and the nanocomposite surfaces for cell viability and growth. In a separate study, Li et al., (2013) generated cotton CNC through electrospinning and functionalized it into composites by surface coating it with CeO2 nanoparticles using the hydrothermal reaction. The resulting cotton based cellulose nanocomposite demonstrated excellent UV-shielding and enhanced photocatalytic properties making it of great value in medicine, military operations and optoelectronics [42].

Multifunctional cotton-based nanomaterials have been inadvertently thrust into the limelight with the recent Covid-19 pandemic through the design of various nanosensor devices for viral detection, surface decontaminants, antiviral compounds and nanocomposite fabrics which serve to prevent or annihilate the SARS-CoV-2. In this aspect, cotton nanocomposites have been constructed as nanosensors in the detection of the virus and as antimicrobial textiles for medical PPE (personal protective equipment). Eissa and Zourob, (2021) fabricated a cotton CNF-tipped electrochemical immunosensor as a one-step diagnostic tool for the detection of SARS-CoV-2 viral antigen [43]. Textiles embedded with antimicrobial nanoparticles such as Ag, ZnO and CuO have been tailored as a protective measure in PPE's for those on the frontline of defense against the SARS-CoV-2. An extensive research resulting in the design and manufacture of antibacterial cotton-based face mask embedded with CuO nanoparticles (CuONps) demonstrated that cotton

could be reconstructed as an antimicrobial nanocomposite and used as a PPV fabric to secure the protection of medical personnel embodying it [37]. In this work, Perelshtein et al., 2016 functionalized cotton fabric with CuONps using ultrasound-assisted deposition by an in-situ coating process on the surface of the fabric. The resulting nanocomposite material retained excellent antibacterial properties after 65 washing cycles at 75–92°C, making it an excellent material as a reusable medical PPE [44]. In a separate study, Adhikari et al., 2021 synthesized a nanoceutical cotton ZnO composite fabric using the hydrothermal method to filter viral particles without compromising on user's breathing mechanism [45]. The design of this nanoceutical fabric was constructed to find application as a one-way valve in a face mask that would facilitate breathing while trapping and filtering airborne viral pathogens and reducing transmission through droplets. It is therefore undisputable that cotton nanocomposite fabrics are the textiles of the future as a shield of protection in the war against the multitude of rising murderous pathogens of this millennia.

2. Synthesis of cotton based cellulose nanocomposites using *in situ* and *ex situ* methods

Cotton textiles are used widely in numerous applications and various industries particularly as sportswear and medical textiles due to its exceptional properties such as breathability, hypo allergenicity, hygroscopicity and low cost [46]. Some of the drawbacks of cotton include low tensile strength, UV-vulnerability, enhanced capacity for microbial growth and easily wrinkled [47]. Inserting nanoparticles into cotton as antimicrobial agents to form nanocomposites is a way forward to manufacture value-added fabric material [48]. These nanocomposites which are formed through the in situ or ex situ deposition of nanoparticles in the fabric material has endowed multi-functionalities to the cotton fabrics such as self-cleaning, UV protection and electric conductivity [49]. Cotton based textiles can actually be designed with self-cleaning features when hydrophobic surfaces are fabricated on these textiles to repel water in such a way that spherical droplets of water can remove stains through a mechanism known as easy roll-off. Wu et al., 2016 demonstrated that a sequential deposition of poly(ethylenimine), silver nanoparticles (AgNp) and fluorinated decylpolyhedral oligomeric silsesquioxane (F-POSS) on cotton fabrics resulted in a superhydrophobic surface entailing a 169° angle of water contact with a 3° sliding angle [50]. Cotton based nanocomposites embedded with ZnO, TiO_2 and reduced graphene oxides have also shown great promise in UV protection [51] and electromagnetic interference (EMI) shielding properties [52].

Fabrics with antimicrobial properties are sought after for the manufacture of healthcare textiles particularly as packaging material for drugs and syringes or medical tools, for the personal protective gear of medical personnel, in wound dressing, surgical aprons and hospital bedding [53]. While cotton is undoubtedly widely popular in the textile industry, its fibers are highly hydrophilic with a high tendency of water absorption and oxygen retention and with a large surface area causing it to be a breeding ground for bacteria and fungi [54]. Cotton nanocomposites have been designed to incorporate metallic nanoparticles for the demonstration of antimicrobial activity [55]. Incorporation of antimicrobial metallic nanoparticles into cotton to generate nanocomposites could be carried out via ex situ or in situ methods. An understanding of the interactions of the intramolecular forces in a cotton

nanocomposite architecture is critical in the selection of methods which appropriates its functionality.

2.1 *In situ* synthesis of cotton based cellulose nanocomposites

The *in situ* synthesis of cotton based nanocomposites is a key approach to generate composites with uniform dispersity using sol–gel or hydrothermal methods. In this method, nanoparticles such as Ag, TiO_2, CuO or ZnO are synthesized *in situ* using a precursor, such as the aqueous salt solution of the metal and a reducing agent which could be in the form of catalysts or plant extracts as in green synthesis. This would lead to the self-assembly of nanoparticles in a one-pot synthesis [56]. The advantage of using this method is that it initiates a unique assembly of nanostructures with enhanced control of particle size, morphology and aggregation. However, synthetic processes have to be designed meticulously to optimize appropriate assembly of nanoparticles into composite structure to ensure the serving functionality of the resulting nanocomposite [57].

The cellulose structure of cotton fiber constitutes complex chain conformations based on its chirality, length and morphology which varies consistently based on the high degree of polymerization of cellulose chains in its fiber which is about 15,000 [58]. One of the major challenges in the synthesis of cotton nanocomposites is to ensure uniform dispersion of nanoparticles without particle aggregation. Nanoparticles aggregate due to high surface area, high surface energy and strong inter-particle attractions [59] leading to lower Gibb's free energy making it detrimental to material performance [60]. The spatial distribution and nanoparticle assembly in a nanocomposite is primarily dependent on the delicate balance of intermolecular forces between nanoparticles within the matrix of its [61]. For proper particle dispersion, thermodynamic miscibility must be achieved [62]. Dispersion of the nanoparticles is highly dependent on the hydrogen bonding capacity of the cotton cellulose network. Where self-assembly of nanoparticles is strategically manipulated within a polymer matrix, it would result in a novel functionality of the forthcoming nanocomposite and expand its horizons for application due to its emerging properties such as water resistance, modulation of light, electrical conductivity and antibacterial sustenance [63]. It has to be noted that the ultimate performance of the nanocomposite is dependent on the interaction of the introduced nanoparticles and the cotton matrix which modulates the self-assembly architecture of the nanocomposite. Cotton fibers possess a backbone structure that is largely comprised of hydroxy groups which impart a strong affinity to water molecules, inducing microbial growth and raising the risk of contamination. The incorporation of the nanoparticle assembly however, renders the composite surface to be hydrophobic. Hydrogen bonding is the primary determinant in the spatial arrangement and self-assembly mechanism of molecules in cotton nanocomposites caused by the OH groups present in the glycoside backbones of the cotton cellulose fibers [57]. Hydrophobic interactions are also prevalent in cotton nanocomposites but are also responsible for the aggregation of nanoparticles in the structure [64]. Another force that participates in the self-assembly of nanoparticles in biopolymers during the in-situ synthesis of cotton nanocomposites is the van der Waals force, a short-ranged force, relatively weaker than hydrogen bonding, created by a transient dipole moment produced by an attractive force when nanoparticles move into close proximity [65].

Vajja et al., (2017) developed cotton nanocomposite material through the *in situ* generation of copper nanoparticles (CuNPs) using a one-step hydrothermal method

which demonstrated excellent antibacterial activity [49] while El-Naggar et al., (2016) incorporated Titanium oxide nanoparticles (TiO_2NPs) on cotton fabrics through in situ synthesis. The resulting nanocomposite demonstrated a microbial reduction of more than 95% which was sustained after more than 20 washing cycles [66]. In a separate work, Marnatha et al., (2018) generated bimetallic Ag and Cu nanoparticles in situ in cotton fabric polymer matrix using Aloe vera leaf extract and observed that besides demonstrating potent antimicrobial activity against *Escherichia coli*, Pseudomonas, Klebsiella, Bacillus and Staphylococcus, the agglomeration of these nanoparticles were also prevented through in situ synthesis [67]. ZnO nanoparticles (ZnO Nps) are hydrophobic, inert and cost-effective and well known for its photocatalytic activity, thermal stability, absorption in a broad range UV radiation and flame-retardant properties [68]. ZnO Nps coating on cotton textiles have demonstrated improved UV protective and antimicrobial properties [69]. However, most of the cotton ZnO nanocomposites are prepared with polymeric binders using the pad-dry-cure method [70]. The nanocomposite produced using this method produces fabrics of high stiffness, poor wash durability and low air permeability [71]. As ZnO Nps are deposited on the surface of the fabric, it does not form any chemical bonds as ZnO does not have any ionic interaction with the OH groups of the cellulose, causing it to only have a lose bond to the surface of the textile. Fabrics used as hospital bedding and PPV go through frequent vigorous washing and surface deposition of ZnO is not an appropriate method to generate cotton nanocomposites for applications that require high washing frequency. In situ generation of nanoparticles in these textiles are a better option. Verbic et al., 2021 demonstrated in situ synthesis of ZnO on cotton fabric using pomegranate peel extract as a reducing agent and wood ash as alkali with excellent UV protective properties due to the uniform dispersion of ZnONps [72]. In another investigation, the *in situ* synthesis of ZnO cotton nanocomposites was demonstrated using the one pot hydrothermal method which showed excellent antibacterial, UV protection, and photo catalytic performance [73].

2.2 *Ex situ* synthesis of cotton based cellulose nanocomposites

The *ex situ* synthesis of cotton based cellulose nanocomposites is carried out through a 2-step process. In the first step, metal oxide or metallic nanoparticles are prepared either through homogeneous precipitation, wet chemical and hydrothermal methods [74]. In the second step, the prepared nanoparticles are dispersed directly onto the cotton fabric to form a nanocomposite. One method to disperse the prepared nanoparticles in the fabric is through blending. In this approach, pre-synthesized nanoparticles are mixed in the biopolymer such as starch or cotton through solvent mixing or melting. Compared to the *in situ* method, a thorough dispersion of the nanoparticles are necessary before being added to the polymer to prevent aggregation of the nanoparticles due to high surface energy. This method of nanocomposite preparation is less tedious and flexible compared to in situ synthesis and can be scaled up for commercial scale due to its to lower investment cost. The drawback using this method is to prepare uniformly dispersed nanoparticles in biopolymers such as cotton and which could remain stable in the long-term without aggregation [74].

An ex situ method used to form cotton nanocomposites of added value was by surface coating the material with metallic or metal oxide nanoparticles. Daoud et al. (2004) reported the deposition of anatase TiO2NPs on cotton and observed that the coated cotton nanocomposite had enhanced UV protection, antibacterial potential and self-cleaning properties [75]. Uddin et al. (2021) showed ex situ deposition of

TiO_2NPs on cotton fabric using the sol–gel method which demonstrated similar properties [76]. A nanocomposite of AgNp loaded with SiO_2 nanoparticles was prepared using the sol–gel technique in which AgNps were generated using the *Ocimum lamiifolium* plant extract and the Stőber method used to obtain a SiO_2 impregnated Ag nanocomposite. When this nanocomposite was loaded onto cotton fabric, it demonstrated potent antimicrobial activity with no toxicity observed on mammalian cells [53]. In a separate method, ZnONps were applied on the surface of cotton fabric surface through layer-by-layer assembly [77]. This method otherwise known as multilayer decomposition was rarely used in textile coating. Here, cotton fabric was first cationised to generate positive charges on the surface of the fabric and then soaked alternately in anionic ZnO solution at pH 11, deionized water, cationic ZnO solution at pH 3 and deionized water repeatedly until 10–16 layers of ZnONps were deposited. Finally, the nanocomposite material was dried at 60°C and cured at 130°C for 3 min [78].

The *ex situ* synthesis of cotton nanocomposites using AgNP for antimicrobial activity have been carried out through the incorporation of silver salts and organic compound complexes of silver. However, this method entails a weak adhesion of this antimicrobial agent to the cotton fabric, allowing the rapid release of AgNp with increased washing resulting in lowered laundering durability. The release of Ag^+ ions from antimicrobial cotton nanocomposites also poses unnecessary duress to health and the environment due to its potent toxicity [79]. The weak adhesion of AgNps to cotton fabrics using the sorption process is a shortcoming in the production of antibacterial cotton nanocomposite material and surface modification is required to improve loading efficiency [80]. Shahidi et al., 2010 reported plasma-treatment of the cotton fabric prior to coating with AgNps which enhanced absorption and demonstrated increased quantity of AgNps on the surface of cotton. These nanocomposite fabrics showed 95% - 100% reduction in bacterial population which remained consistent after 10 times consecutive laundering [81].

3. Cotton based nanocomposites constructed from nanocellulose extracted from cotton cellulose nanofibers

Cotton nanofibers are natural fibers which mostly constitute holocellulose (cellulose and hemicellulose) and lignin and has several advantages such as lower density, availability, biodegradability and exceptional mechanical properties which make it an ideal candidate as a polymer nanocomposite. The valorisation of agro residues of cotton would result in novel materials that could be used as fillers or reinforcement materials to form nanocomposites of potent value. Unlike other plants such as jute, flax and kenaf which are made up of only 25% cellulose and wood-based trees which contain 40–50% cellulose, cotton fibers are made up of 90% cellulose [82]. The cellulose in the cotton fibers are among the highest in molecular weight among all plant fibers and the most crystalline and fibrillated [83]. Cotton fiber comprises cellulose with 1,4-d-glucopyranose structural units [84] which accumulate as microfibrils arranged in regular pattern with excellent mechanical properties such as the Young's Modulus and low thermal expansion [85]. Nanofibers generated from cellulose isolated from cotton fibers can be categorized as nanowires with aspect ratio beyond 1000, nanorods with aspect ratios between 3 and 5, nanoribbons and nanotubes with aspect ratios >10 [17]. Dried cotton fibers comprise large amounts of cellulose and hemi-cellulose which increase in tensile strength and durability when the impurities

are removed. These cellulose based fibers are usually added as reinforcement material to generate nanocomposites needed in construction, automotive and electronics industry, as membranes for ultrafiltration, ion exchange and fuel cells and as binders in pharmaceuticals and cosmetic fillers [86]. Cellulose nanofibrils gives greater tensile strength compared to natural fibers and it has exceptionally large surface to volume ratio compared to its bulk form [87].

The extraction of cotton nanocellulose can be carried out using mechanical methods such as high-pressure homogenization, ball grinding, ultrasonication or high-speed blending [88] or chemical methods using acid hydrolysis with strong acids such as sulfuric acid or hydrochloric acid, oxidation with TEMPO (2,2,6,6-tetramethylpyperidine-l-oxyl) [89] or a combination of both mechanical and chemical methods [90]. It is found that acid hydrolysis removes the amorphous regions in the cotton fiber and generates nanocellulose with high crystallinity and uniform size distribution [89]. Sulfuric acid generates a more stable colloidal suspension of cellulose nanocrystals [24] and is preferred to hydrochloric acid which causes mass aggregation of cellulose nanocrystals because of the minimal surface charge that causes a lack of electrostatic repulsion force between the crystal particles [91]. Also, the hazards of inorganic acids and their corrosive nature are detrimental to the environment [92]. Mechanical processes generate nanofibers at a high success rate but the strong mechanical shearing forces causes disruption of the fibers, depict excessive energy consumption and homogenizer obstruction after prolonged use [88]. To elude the shortcomings presented by both the mechanical and chemical processes of nanocellulose extraction, pre-treatment with cellulase or enzymatic hydrolysis has been considered. Enzymatic hydrolysis is an appropriate pretreatment method used to disrupt interfibrillar cohesive forces and facilitate the disintegration of cotton fibers, while decreasing the size and degree of polymerization of cellulose fibers [93]. This method has been found to be highly selective and carried out at conditions with lower energy requirements [14]. Additionally, it replaces harmful solvents with biodegradable enzymes such as cellulases, which does not release hazardous emissions to the environment [94]. Cellulose is comprised of highly ordered crystalline regions interspersed with disorganized amorphous regions. The amorphous regions of cellulose are more susceptible to enzymatic degradation compared to the crystalline area. Cellulase enzyme has the potential of selective hydrolyzation of the amorphous region while maintaining the crystalline region, making it a process of choice to isolate cellulose nanocrystals. Therefore, this route has become increasingly popular as a sustainable method to prepare cellulose nanocrystals because of its high selectivity, mild conditions, and weak changes in surface chemistry [93]. Moreover, it complies with the principles of green chemistry as it leaves no carbon footprint, generates no hazardous waste and poses less water and energy consumption [95].

The addition of nanocellulose extracted from cotton as a reinforcing agent to a polymer system such as plastic, rubber or concrete improves the mechanical, thermodynamic and adsorption properties of the composite without changing the original qualities of the parent material [94]. Cotton fibers with a diameter in the range of 10–30 nm and a high aspect ratio are observed to improve the mechanical properties in a polymer composite for non-food packaging applications [96]. These nanocomposites have been postulated to hold tremendous potential in biomedicine as scaffolds in tissue engineering and for encapsulation in drug delivery [97]. The advances in mammalian cell culture technology are astounding. Here, nanocomposite biopolymers perform as biomimetic substrates for cell adhesion and proliferation. The nanotopography of substrates constructed from biomolecules such as collagen which includes

surface roughness and porosity, influences interface interaction with mammalian cells or tissue that could improve cell adhesion and multiplication [98]. The incorporation of nanomaterials into these polymer matrixes can yield composites with the necessary properties for cell and tissue culture. Cotton based cellulose nanofibers (CCN) have a tremendous potential to be engineered for polymer composite reinforcement [91] as it mimics the structure of collagen in directionality and surface functionalization which is paramount to the adhesion, spreading and proliferation of cells [99].

Translating cotton based nanocellulose into polymer nanocomposites can be carried out using electrospinning, cast drying, freeze drying, vacuum assisted filtration, wet spinning, layer by layer assembly, micropatterning, melt blending, intercalated polymerization, sol-gel and solvent evaporation technique [100]. The solvent evaporation technique is the simplest method for nanocomposite synthesis which involves nanocellulose dispersion in polymer solution through energetic agitation followed by controlled evaporation of the solvent and composite film casting [101]. Li et al., (2014) prepared a nancomposite of cotton nanofiber in high density polyethylene (HDPE) using 2 different pretreatment methods. The first was blending the HDPE in a cotton CNF suspension, dehydrating and freeze drying the mixture followed by compounding and extrusion. This was a rapid, eco-friendly method as there were no chemical solvents involved in the process. In the second method, polyoxyethylene (PEO) was used as a dispersion agent to coat the cotton CNF before adding to HDPE granules and extraction. FESEM results revealed that both methods produced well dispersed CNF in HDPE and generated an excellent network structure of the cotton CNF/HDPE composites but the nanocomposite produced using the blending method was preferred as it demonstrated greater bending strength (MOR) and bending modulus (MOE) [102].

Nanocomposites have several advantages over conventional composites in their superior tensile strength, thermal capacity and barrier properties, biodegradability, recyclability and low weight [103]. Insertion of nanocellulose to biodegradable polymers to form bio-nanocomposites may improve the brittleness, poor barrier properties and low thermal stability of pure biodegradable polymers [104]. Much work has been carried out in recent times to explore the design of bionanocomposites en route to the development of higher quality bioplastics [105, 106].

A problem faced in generating cotton based cellulose nanocomposites is the limited dispersion of nanocellulose in polymers. This can be overcome by attaching a hydrophobic group to the surface of the cellulose matrix through esterification, acetylation or silanization which increases compatibility with the matrix. Solution casting is commonly used in the preparation of nanocomposite films but it its unsuitable for commercial scale production. Another method known as extrusion using melt processing has shown much promise for large scale production of cotton based cellulose nanocomposites [107]. However, for transforming research to industry and commercialization of cotton based cellulose nanocomposites, it is necessary to weigh the production costs, waste emissions, energy consumption, feasibility of the process and compliance to environmental ethics. Overall, the application prospects for nanocellulose appear to be very optimistic, but further research is needed to develop viable methods from laboratory to industrialization.

4. Conclusion

Nanocomposites are defined as multi-element materials with at least one element having a dimension of less than 100 nm [108]. In this chapter we have reviewed cotton

based cellulose nanocomposites which are constructed by adding multifunctional nanoparticles to the cotton fabric using in situ or ex situ processes or by extracting nanocellulose structures from cotton fibers and incorporating it into polymer matrices. This results in novel nanocomposites with enhanced antimicrobial activity, polymer reinforcement and enhanced adhesion and adsorption in inert matrixes.

The introduction of metallic nanoparticles into cotton textiles has resulted in high performance multifunctional cotton nanocomposites which demonstrate excellent antimicrobial activity, water repellency, UV protection and antistatic finishes. These nanocomposites are gaining much interest particularly in generating antimicrobial material for protection against emerging pathogens. It is projected that further research in nanocomposite technology would decipher the details of the functional properties and performance of existing and emerging cotton nanocomposites and to determine the toxicity and safety of the generated fabrics. Additionally, there is a pressing need that the discoveries in the laboratory should be translated to commercial applications through the design of fabrication processes that favor cost effective, large scale production.

The incorporation of cotton nanocellulose into polymers as fillers to form reinforced nanocomposites also shows much promise particularly in the creation of chemical and biodegradable polymers of increased strength and tensile modulus and as scaffolds and support substrates in biomedicine. Yet there are issues that need to be addressed prior to translation into commercial viability such as the influence of the size and morphology of cotton nanocellulose fillers in the polymer matrix and the structural compatibility of the resulting polymer, biocompatibility of the nanocomposites in biomedical applications and the poor dispersion of cotton nanocellulose in the polymeric domain structure [109]. It is believed that these issues will be addressed aggressively in the near future to pave the way for the birth of a new breed of nanocomposite material using cotton nanostructures.

Conflict of interest

The author declares no conflict of interest.

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References

[1] Jeevanandam J, Barhoum A, Chan YS, Dufresne A, Danquah MK. Review on nanoparticles and nanostructured materials: History, sources, toxicity and regulations. Beilstein Journal of Nanotechnology. 2018;**9**:1050-1074. DOI: 10.3762/bjnano.9.98

[2] Damodharan J. Nanomaterials in medicine – An overview. Materials Today: Proceedings. 2021;**37**(2):383-385, ISSN 2214-7853. DOI: 10.1016/j.matpr.2020.05.380

[3] Daghan H. Effects of TiO2 nanoparticles on maize (Zea mays L.) growth, chlorophyll content and nutrient uptake. Applied Ecology and Environmental Research. 2018;**16**:6873-6883

[4] Dwivedi S, Saquib Q, Al-Khedhairy AA, Musarrat J. Understanding the role of nanomaterials in agriculture. In: Singh DP, Singh HB, Prabha R, editors. Microbial Inoculants in Sustainable Agricultural Productivity. New Delhi: Springer; 2016. pp. 271-288

[5] Ojha S, Singh D, Sett A, Chetia H, Kabiraj D, Bora U. Nanotechnology in Crop Protection. In: Tripathi DK, Ahmad P, Sharma S, Chauhan DK, Dubey NK. Nanomaterials in Plants, Algae, and Microorganisms. London, UK: Academic Press. 2018. pp. 345-391

[6] Adeleye AS, Conway JR, Garner K, Huang Y, Su Y, Keller AA. Engineered nanomaterials for water treatment and remediation: Costs, benefits, and applicability. Chemical Engineering Journal and the Biochemical Engineering Journal. 2016;**286**:640-662. DOI: 10.1016/j.cej.2015.10.105

[7] Mukhopadhyay R, Sarkar B, Khan E, Alessi DS, Biswas JK, Manjaiah KM, et al. Nanomaterials for sustainable remediation of chemical contaminants in water and soil. Critical Reviews in Environmental Science and Technology. 2021;**2611-2660**. DOI: 10.1080/10643389.2021.1886891

[8] Bystrzejewska-Piotrowska G, Golimowski J, Urban P. Nanoparticles: Their potential toxicity, waste and environmental management. Waste Management. 2009;**29**:2587-2595

[9] Trache D. Nanocellulose as a promising sustainable material for biomedical applications. AIMS Mater. Science. 2018;**5**:201-205. DOI: 10.3934/matersci.2018.2.201

[10] Köse K, Mavlan M, Youngblood JP. Applications and impact of nanocellulose based adsorbents. Cellulose. 2020;**27**:2967-2990. DOI: 10.1007/s10570-020-03011-1

[11] Klemm D, Kramer F, Moritz S, Lindström T, Ankerfors M, Gray D, et al. Nanocelluloses: A new family of nature-based materials. Angewandte Chemie (International Ed. in English). 2011;**50**(24):5438-5466. DOI: 10.1002/anie.201001273 Epub 2011 May 20

[12] Klemm D, Cranston ED, Fischer D, Gama M, Kedzior SA, Kralisch D, et al. Nanocellulose as a natural source for groundbreaking applications in materials science: Today's state. Materials Today. 2018;**21**:720-748. DOI: 10.1016/j.mattod.2018.02.001

[13] Khan MA et al. World cotton production and consumption: An overview. In: Ahmad S, Hasanuzzaman M, editors.

Cotton Production and Uses. Singapore: Springer; 2020. DOI: 10.1007/978-981-15-1472-2_1

[14] Salimi S, Sotudeh-Gharebagh R, Zarghami R, Chan SY, Yuen KH. Production of nanocellulose and its applications in drug delivery: A critical review. ACS Sustainable Chemistry & Engineering. 2019;**7**:15800-15827. DOI: 10.1021/acssuschemeng.9b02744

[15] Seabra AB, Bernardes JS, Fávaro WJ, Paula AJ, Durán N. Cellulose nanocrystals as carriers in medicine and their toxicities: A review. Carbohyd. Polym. 2018;**181**:514-527. DOI: 10.1016/j.carbpol.2017.12.014

[16] Dufresne A. Nanocellulose processing properties and potential applications. Current Forestry Reports. 2019;**5**:76-89. DOI: 10.1007/s40725-019-00088-1

[17] Kim JH, Lee D, Lee YH, Chen W, Lee SY. Nanocellulose for energy storage systems: Beyond the limits of synthetic materials. Advanced Materials. 2019;**31**:1804826. DOI: 10.1002/adma.201804826

[18] Anju R, Kartik R, Raghavachari D. Preparation of nanofibrillated cellulose and nanocrystalline cellulose from surgical cotton and cellulose pulp in hot-glycerol medium. Cellulose. 2019;**26**:3127-3141. DOI: 10.1007/s10570-019-02312-4

[19] Fleming K, Gray DG, Matthews S. Cellulose Crystallites. Chemistry – A 565European Journal. 2001;**7**(9):1831-1183

[20] Revol JF, Godbout L, Gray DG. Solid self-assembled films of cellulose with chiral nematic order and optically variable properties. Journal of Pulp and Paper Science. 1998;**24**:146-149

[21] Dahlke B, Larbig H, Scherzer HD, Poltrock R. Natural fiber reinforced foams based on renewable resources for automotive interior applications. Journal of Cellular Plastics. 1998;**34**:361-379

[22] Jiang L, Hinrichsen G. Flax and cotton fiber reinforced biodegradable polyester amide composites, 1. Manufacture of composites and characterization of their mechanical properties. Die Angewandte Makromolekulare Chemie. 1 Jul 1999;**268**(1):13-17

[23] Soni B, Hassan EB, Mahmoud B. Chemical isolation and characterization of different cellulose nanofibers from cotton stalks. Carbohydrate Polymers. 10 Dec 2015;**134**:581-589. DOI: 10.1016/j.carbpol.2015.08.031

[24] de Morais TE, Corre^a AC, Manzoli A, de Lima LF, de Oliveira CR, Mattoso LHC. Cellulose nanofibers from white and naturally colored cotton fibers. Cellulose. 2010;**17**:595-606. DOI: 10.1007/s10570-010-9403-0

[25] Theivasanthi T, Anne Christma FL, Toyin AJ, Gopinath SCB, Ravichandran R. Synthesis and characterization of cotton fiber-based nanocellulose. International Journal of Biological Macromolecules. 2018;**109**:832-836. DOI: 10.1016/j.ijbiomac.2017.11.054

[26] Morais JP, de Freitas Rosa M, Nascimento LD, do Nascimento DM, Cassales AR (2013) Extraction and characterization of nanocellulose structures from raw cotton linter. Carbohydrate Polymers 91:229-235. doi:10.1016/j.carbpol.2012.08.010

[27] Ago M, Endo T, Hirotsu T. Crystalline transformation of native cellulose from cellulose I to cellulose II polymorph by a ball-milling method with a specific amount of water. Cellulose. 2004;**11**:163-167

[28] Zhao H-P, Feng X-Q, Gao H. Ultrasonic technique for extracting nanofibers from nature materials. Applied Physics Letters. 2007;**90**:073112

[29] Hideno A, Abe K, Uchimura H, Yano H. Preparation by combined enzymatic and mechanical treatment and characterization of nanofibrillated cotton fibers. Cellulose. 2016;**23**:3639-3651

[30] Sen M. Nanocomposite Materials. In: Sen M, editor. Nanotechnology and the Environment. London: IntechOpen; 2020. DOI: 10.5772/intechopen.93047

[31] Abdul Khalil HPS, Davoudpour Y, Saurabh CK, Hossain MS, Adnan AS, Dungani R, et al. A review on nanocellulosic fibres as new material for sustainable packaging: Process and applications. Renewable and Sustainable Energy Reviews. 2016;**64**:823-836,ISSN 1364-0321. DOI: 10.1016/j.rser.2016.06.072

[32] Pandey SH, Ahn CS, Lee AK, Mohanty MM. Recent advances in the application of natural fiber based composites. Macromolecular Materials and Engineering. 2010;**295**:975-989

[33] Chen Q, Liu Y, Chen G. A comparative study on the starch-based biocomposite films reinforced by nanocellulose prepared from different non-wood fibers. Cellulose. 2019;**26**:2425-2435. DOI: 10.1007/s10570-019-02254-x

[34] Pires JR, Souza VG, Fernando AL. Valorization of energy crops as a source for nanocellulose production–current knowledge and future prospects. Industrial Crops and Products. 2019;**140**:111642. DOI: 10.1016/j.indcrop.2019.111642

[35] Dufresne A. Cellulose nanomaterials as green nanoreinforcements for polymer nanocomposites. Philosophical Transactions of the Royal Society. 2018;**376**:20170040. DOI: 10.1098/rsta.2017.0040

[36] Thomas B, Raj MC, Joy J, Moores A, Drisko GL, Sanchez C. Nanocellulose, a versatile green platform: From biosources to materials and their applications. Chemical Reviews. 2018;**118**:11575-11625. DOI: 10.1021/acs.chemrev.7b00627

[37] Bacakova L, Pajorova J, Tomkova M, Matejka R, Broz A, Stepanovska J, et al. Applications of nanocellulose/nanocarbon composites: Focus on biotechnology and medicine. Nanomaterials. 2020;**10**:196. DOI: 10.3390/nano10020196

[38] Zhang Q, Zhang L, Wu W, Xiao H. Methods and applications of nanocellulose loaded with inorganic nanomaterials: A review. Carbohydrate Polymers. 2020;**229**:115454. DOI: 10.1016/j.carbpol.2019.115454

[39] Miyashiro D, Hamano R, Umemura K. A review of applications using mixed materials of cellulose, nanocellulose and carbon nanotubes. Nanomaterials. 2020;**10**:186. DOI: 10.3390/nano10020186

[40] Rumi SS, Liyanage S, Abidi N. Conversion of low-quality cotton to bioplastics. Cellulose. 2021;**28**:2021-2038. DOI: 10.1007/s10570-020-03661-1

[41] Zanette RSS, de Almeida LBF, Souza NLGD, de Almeida CG, de Oliveira LFC, de Matos EM, et al. Cotton cellulose nanofiber/chitosan nanocomposite: Characterization and evaluation of cytocompatibility. Journal of Biomaterials Science, Polymer Edition. 2019;**30**(16):1489-1504. DOI: 10.1080/09205063.2019.1646627

[42] Li C, Shu S, Chen R, Chen B, Dong W. Functionalization of

electrospun nanofibers of natural cotton cellulose by cerium dioxide nanoparticles for ultraviolet protection. Journal of Applied Polymer Science. 2013;**130**(3):1524-1529. DOI: 10.1002/app.39264

[43] Eissa S, Zourob M. Analytical Chemistry. 2021;**93**(3):1826-1833. DOI: 10.1021/acs.analchem.0c04719

[44] Perelshtein I, Perkas N, Gedanken A. Ultrasonic coating of textiles by antibacterial and Antibiofilm nanoparticles. In: Handbook of Ultrasonics and Sonochemistry. Singapore: Springer; 2016. DOI: 10.1007/978-981-287-278-4_20

[45] Adhikari A, Pal U, Bayan S, Mondal S, Ghosh R, Darbar S, et al. Nanoceutical fabric prevents COVID-19 spread through expelled respiratory droplets: A combined computational, spectroscopic, and antimicrobial study. ACS Applied Bio Materials 2021. 2021;**4**(7):5471-5484. DOI: 10.1021/acsabm.1c00238

[46] Huang S, Tao R, Ismail A, Wang Y. Cellulose nanocrystals derived from textile waste through acid hydrolysis and oxidation as reinforcing agent of soy protein film. Polymers. 2020;**12**:958. DOI: 10.3390/polym12040958

[47] Morais J, Rosa M, Souza FM. Extraction and characterization of nanocellulose structures from raw cotton linter. Carbohydrate Polymers. 2013;**91**:229-235. DOI: 10.1016/j.carbpol.2012.08.010

[48] Nandi S, Guha P. A review on preparation and properties of cellulose nanocrystal-incorporated natural biopolymer. Journal of Packaging Technology and Research. 2018;**2**:149-166. DOI: 10.1007/s41783-018-0036-3

[49] Sadanand V, Feng TH, Varada Rajulu A, Satyanarayana B. Preparation and properties of low-cost cotton nanocomposite fabrics with in situ-generated copper nanoparticles by simple hydrothermal method. International Journal of Polymer Analysis and Characterization. 2017;**22**(7):587-594. DOI: 10.1080/1023666X.2017.1344916

[50] Wu JD, Zhang C, Jiang DJ, Zhao SF, Jiang YL, Cai GQ, et al. Self-cleaning pH/thermo-responsive cotton fabric with smart-control and reusable functions for oil/water separation. RSC Advances. 2016;**6**(29):24076-24082. DOI: 10.1039/c6ra02252a

[51] Becheri A, Durr M, Nostro PL, Baglioni P. Synthesis and characterization of zinc oxide nanoparticles: Application to textiles as UV absorbers. Journal of Nanoparticle Research. 2008;**10**:679-689

[52] Chen J, Huang X, Zhu Y, Jiang P. Cellulose nanofiber supported 3D interconnected BN nanosheets for epoxy nanocomposites with ultrahigh thermal management capability. Advanced Functional Materials. 2017;**27**:1-9. DOI: 10.1002/adfm.201604754

[53] Amibo TA, Beyan SM, Mustefa M, Sundramurthy VP, Bayu AB. Development of nanocomposite based antimicrobial cotton fabrics impregnated by Nano SiO2 loaded AgNPs derived from Eragrostis Teff straw. Materials Research Innovations. 2021;**76**:1-10. DOI: 10.1080/14328917.2021.2022372

[54] Arenas-Chávez CA, Hollanda LM, Arce-Esquivel AA, Alvarez-Risco A, Del-Aguila-Arcentales S, Yáñez JA, et al. Antibacterial and antifungal activity of functionalized cotton fabric with nanocomposite based on silver nanoparticles and Carboxymethyl chitosan. PRO. 2022;**10**:1088

[55] Rajendran R, Balakumar C, Kalaivani J, Sivakumar R. Dyeability and antimicrobial properties of cotton fabrics finished with punica granatum extracts. Journal of Textile & Apparel Technology & Management. 2011;**7**(1):136-141

[56] Ahmed J, Arfat YA, Al-Attar H, Auras R, Ejaz M. Rheological, structural, ultraviolet protection and oxygen barrier properties of linear low-density polyethylene reinforced with zinc oxide (ZnO) nanoparticles. Food Packaging and Shelf Life. 2017;**13**:20-26

[57] Olson E, Liu F, Blisko J, Li Y, Tsyrenova A, Mort R, et al. Self-assembly in biobased nanocomposites for multifunctionality and improved performance. Nanoscale Advances. 2021;**3**(15):4321-4348. DOI: 10.1039/d1na00391g

[58] O'Sullivan AC. Cellulose: The structure slowly unravels. Cellulose. 1997;**4**:173-207. DOI: 10.1023/A:1018431705579

[59] Min M, Akbulut K, Kristiansen YG, Israelachvili J. The role of interparticle and external forces in nanoparticle assembly. Nature Materials. 2008;**7**(7):527-538

[60] Boles MA, Engel M, Talapin DV. Self-assembly of colloidal nanocrystals: From intricate structures to functional materials. Chemical Reviews. 2016;**116**(18):11220-11289

[61] Jancar J, Douglas JF, Starr FW, Kumar SK, Cassagnau P, Lesser AJ, et al. Current issues in research on structure–property relationships in polymer nanocomposites. Polymer. 2010;**51**:3321-3343

[62] Mackay ME, Tuteja A, Duxbury PM, Hawker CJ, Horn BV, Guan Z, et al. General strategies for nanoparticle dispersion. Science. 2006;**311**:1740-1743

[63] Xue C, Yan C, Wang T. From atoms to lives: The evolution of nanoparticle assemblies. Advanced Functional Materials. 2019;**29**:1807658

[64] Widom B, Bhimalapuram P, Koga K. The hydrophobic effect. Physical Chemistry Chemical Physics. 2003;**5**:3085-3093

[65] Bevan MA, Prieve DC. Hindered diffusion of colloidal particles very near to a wall: Revisited. The Journal of Chemical Physics. 2000;**113**:1228-1236. DOI: 10.1063/1.481900

[66] El-Naggar ME, Shaheen TI, Zaghloul S, El-Rafie MH, Hebeish A. Antibacterial activities and UV protection of the in situ synthesized titanium oxide nanoparticles on cotton fabrics. Industrial & Engineering Chemistry Research. 2016;**55**(10):2661-2668. DOI: 10.1021/acs.iecr.5b04315

[67] Mamatha A, Rajulu V, Madhukar K. In situ generation of bimetallic nanoparticles in cotton fabric using aloe Vera leaf extract, as a reducing agent. Journal of Natural Fibers. 2018;**17**:1121-1129. DOI: 10.1080/15440478.2018.1558146

[68] Baldwin S, Odio MR, Haines SL, Oconnor RJ, Englehart JS, Lane AT. Skin benefits from continuous topical administration of a zinc oxide/petrolatum formulation by a novel disposable diaper. Journal of the European Academy of Dermatology and Venereology. 2001;**15**:5-11

[69] Saif S, Tahir A, Asim T, Chen Y, Khan M, Adil SF. Green synthesis of ZnO hierarchical microstructures by Cordia myxa and their antibacterial activity. Saudi Journal of Biological Sciences.

2019;**26**(7):1364-1371. DOI: 10.1016/j.sjbs.2019.01.004

[70] Vigneshwaran N, Kumar S, Kathe AA, Varadarajan PV, Prasad V. Functional finishing of cotton fabrics using zinc oxide–soluble starch nanocomposites. Nanotechnology. 2006;**17**(20):5087-5095. DOI: 10.1088/0957-4484/17/20/008

[71] Gokarneshan N, Gopalakrishnan PP, Rachel DA. Characterization methods of Nano textile materials. Journal of Fashion Technology Textile Engineering. 2015;**3**:2. DOI: 10.4172/2329-9568.1000121

[72] Verbiˇc A, Šala M, Jerman I, Gorjanc M. Novel green In situ synthesis of ZnO nanoparticles on cotton using pomegranate Peel extract. Materials. 2021;**14**:4472. DOI: 10.3390/ma14164472

[73] Javed A, Azeem M, Wiener J, et al. Ultrasonically assisted In situ deposition of ZnO Nano particles on cotton fabrics for multifunctional textiles. Fibers and Polymers. 2021;**22**:77-86. DOI: 10.1007/s12221-021-0051-9

[74] Huang C, Cai Y, Chen X, Ke Y. Silver-based nanocomposite for fabricating high performance value-added cotton. Cellulose. 2022;**29**:723-750. DOI: 10.1007/s10570-021-04257-z

[75] Daoud WA, Xin JH. Nucleation and growth of Anatase crystallites on cotton fabrics at low temperatures. Journal of the American Ceramic Society. 2004;**87**:953-955

[76] Uddin MJ, Cesano F, Bonino F, Bordiga S, Spoto G, Scarano D, et al. Photoactive TiO2 films on cellulose fibres: Synthesis and characterization. Journal of Photochemistry and Photobiology, A: Chemistry. 2007;**189**:286

[77] Uğur ŞS, Sarıışık M, Aktaş AH, et al. Modifying of cotton fabric surface with Nano-ZnO multilayer films by layer-by-layer deposition method. Nanoscale Research Letters. 2010;**5**:1204. DOI: 10.1007/s11671-010-9627-9

[78] Montazer M, Maali AM. ZnO nano reactor on textiles and polymers: Ex situ and in situ synthesis, application, and characterization. The Journal of Physical Chemistry. B. 2014;**118**(6):1453-1470. DOI: 10.1021/jp408532r

[79] Gaillet S, Rouanet J-M. Silver nanoparticles: Their potential toxic effects after oral exposure and underlying mechanisms – A review. Food and Chemical Toxicology. 2015;**77**:58-63. DOI: 10.1016/j.fct.2014.12.019

[80] Huang C, Yu H, Abdalkarim SYH, Li Y, Chen X, Yang X, et al. A comprehensive investigation on cellulose nanocrystals with different crystal structures from cotton via an efficient route. Carbohydrate Polymers. 2022;**276**:118766,ISSN 0144-8617. DOI: 10.1016/j.carbpol.2021.118766

[81] Shahidi S, Rashidi A, Ghoranneviss M, Anvari A, Rahimi MK, Moghaddam MB, et al. Investigation of metal absorption and antibacterial activity on cotton fabric modified by low temperature plasma. Cellulose. 2010;**17**:627-634

[82] Singh SK, Khan S, Mishra RK. Extraction and characterization of Nano fibers from cotton fibers and its composite. Journal of Natural Fibers. 2022;**19**:1-15. DOI: 10.1080/15440478.2022.2069184

[83] Hsieh YL, Chemical structure and properties of cotton. In: Gordon S, Hsieh YL, editors. Cotton. Woodhead Publishing Series in Textiles, Vol 59. Cambridge,

England: Woodhead Publishing. 2007. pp. 3-34, ISBN 9781845690267. DOI:10.1533/9781845692483.1.3.

[84] Varghese MA, Vikas M. 5 - Surface modification of natural fibers. In: Shimpi NG, editors. Biodegradable and Biocompatible Polymer Composites. Woodhead Publishing Series in Composites Science and Engineering, Cambridge, England: Woodhead Publishing; 2018. pp. 115-155. ISBN 9780081009703. DOI: 10.1016/C2015-0-05524-1

[85] Abrahama E, Deepaa B, Pothana LA, Jacobc M, Thomasb S, Cvelbard U, et al. Extraction of nanocellulose fibrils from lignocellulosic fibres: A novel approach. Carbohydrate Polymers. 2011;**86**:1468-1475. DOI: 10.1016/j.carbpol.2011.06.034

[86] Sharma A, Thakur M, Bhattacharya M, Mandal T, Goswami S. Commercial application of cellulose nano- composites – A review. Biotechnology Reports. 2019;**21**:e00316. DOI: 10.1016/j.btre.2019.e00316

[87] Huang ZM, Zhang YZ, Kotaki M, Ramakrishna S. A review on polymer nanofibers by electrospinning and their applications in nanocomposites. Composites Science and Technology. 2003;**63**:2223-2253. DOI: 10.1016/S0266-3538(03)00178-7

[88] Jihua L, Wei X, Wang Q, Chen J, Chang G, Kong L, et al. Homogeneous isolation of nanocellulose from sugarcane bagasse by high pressure homogenization. Carbohydrate Polymers. 2012;**90**(4):1609-1613, ISSN 0144-8617. DOI: 10.1016/j.carbpol.2012.07.038

[89] Jiang F, Hsieh YL. Chemically and mechanically isolated nanocellulose and their self-assembled structures. Carbohydrate Polymers. 2013;**95**(1):32-40. DOI: 10.1016/j.carbpol.2013.02.022 Epub 2013 Feb 28

[90] Chen W, Yu H, Liu Y. Preparation of millimeter-long cellulose I nanofibers with diameters of 30-80 nm from bamboo fibers. Carbohydrate Polymers. 2011;**86**:453-461. DOI: 10.1016/j.carbpol.2011.04.061

[91] Wang J, Liu X, Jin T, et al. Preparation of nanocellulose and its potential in reinforced composites: A review. Journal of Biomaterials Science, Polymer. 2019;**30**(11):919-946

[92] Nel A, Xia T, Mädler L, Li N. Toxic potential of materials at the nanolevel. Science. 2006;**311**(5761):622-627

[93] Lee HV, Hamid SBA, Zain SK. Conversion of lignocellulosic biomass to nanocellulose: Structure and chemical process. Scientific World Journal. 2014;**2014**:1-20

[94] Mikkonen KS, Stevanic JS, Joly C, et al. Composite films from spruce galactoglucomannans with microfibrillated spruce wood cellulose. Cellulose. 2011;**18**:713-726. DOI: 10.1007/s10570-011-9524-0

[95] Zielińska D, Szentner K, Waśkiewicz A, Borysiak S. Production of Nanocellulose by enzymatic treatment for application in polymer composites. Materials (Basel). 2021;**14**(9):2124. DOI: 10.3390/ma14092124

[96] Montava-Jordà S, Torres-Giner S, Ferrandiz-Bou S, Quiles-Carrillo L, Montanes N. Development of sustainable and cost competitive injection-molded pieces of partially bio-based polyethylene terephthalate through the valorization of cotton textile waste. International Journal of Molecular Sciences. 2019;**20**:1378

[97] Dumanli Gumrah Ahu. Nanocellulose and its composites for biomedical applications. Current

Medicinal Chemistry. 2017;**24**(5):512-528. DOI: 10.2174/0929867323666161014124008

[98] Lee K-Y, Aitomäki Y, Berglund LA, Oksman K, Bismarck A. On the use of nanocellulose as reinforcement in polymer matrix composites. Composites Science and Technology. 2014;**105**:15-27, ISSN 0266-3538. DOI: 10.1016/j.compscitech.2014.08.032

[99] Pooyan P, Kim I, Jacob K, et al. Design of a cellulose-based nanocomposite as a potential polymeric scaffold in tissue engineering. Polymer. 2013;**54**(8):2105-2114

[100] Joshi, Chatterjee U. Polymer nanocomposite: An advanced material for aerospace applications. In: Rana S, Fangueiro R, editors. Advanced Composite Materials for Aerospace Engineering. Cambridge, England: Woodhead Publishing. 2016. pp. 241-264, ISBN 9780081009390. DOI: 10.1016/B978-0-8-100037-3.00008-0

[101] Rane AV, Krishnan Kanny VK, Abitha ST. Chapter 5 - methods for synthesis of nanoparticles and fabrication of nanocomposites. In: Bhagyaraj SM, Oluwafemi OS, Kalarikkal N, Thomas S, editors. Micro and Nano Technologies, Synthesis of Inorganic Nanomaterials. Cambridge, England: Woodhead Publishing. 2018. pp. 121-139, ISBN 9780081019757. DOI: 10.1016/B978-0-08-101975-7.00005-1

[102] Li J, Song Z, Li D, Shang S, Guo Y. Cotton cellulose nanofiber-reinforced high density polyethylene composites prepared with two different pretreatment methods. Industrial Crops and Products. 2014;**59**:318-328. ISSN 0926-6690. DOI: 10.1016/j.indcrop.2014.05.033

[103] Oksman K, Mathew AP, Bondeson D, Kvien I. Manufacturing process of cellulose whiskers/polylactic acid nanocomposites. Composites Science and Technology. 2006;**66**:2776

[104] Sorrentino A, Gorrasi G, Vittoria V. Potential perspectives of bio-nanocomposites for food packaging applications. Trends in Food Science and Technology. 2007;**18**:84-95. DOI: 10.1016/j.tifs.2006.09.004

[105] Petersson L, Kvien I, Oksman K. Structure and thermal properties of poly(lactic acid)/cellulose whiskers nanocomposite materials. Composites Science and Technology. 2007;**67**:2535

[106] Plackett D, Andersen TL, Pedersen WB, Nielsen L. Biodegradable composites based on l -polylactide and jute fibres. Composites Science and Technology. 2003;**63**:1287

[107] Chanda S, Bajwa DS. A review of current physical techniques for dispersion of cellulose nanomaterials in polymer matrices. Reviews on Advanced Materials Science. 2021;**60**(1):325-341. DOI: 10.1515/rams-2021-0023

[108] Ajayan P, Schadler LS, Braun PV. Nanocomposite Science and Technology. 1st ed. Weinheim: Wiley-VCH; 2004

[109] Mondal S. Review on Nanocellulose polymer nanocomposites. Polymer-Plastics Technology and Engineering. 2017;**57**(13):1377-1391. DOI: 10.1080/03602559.2017.138125310.1080/03602559.2017.1381253

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This book discusses the latest advances in cotton genetics and the biochemistry, physiology, bioinformatics, and genomics of the cotton plant. Chapters cover genomics and transcriptomics approaches to characterization and tagging of essential genes, novel transgenic tools to accelerate breeding against climate issues, abiotic and biotic stress pressures, biological control and machinery tools for cotton plant protection, cotton seed meal production, and sustainable and effective farming in the era of climate change and technological advance.

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