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Chapter

Introductory Chapter: Recent Trends in "Cotton Research"

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1. Introduction

Cotton is one of the most important fiber crops in the world. It is cultivated in more than 100 countries contributing about 40% of the global market. About 350 million people are directly or indirectly linked with cotton production and industry. Cotton has major share in global agriculture economy; however, its production and yield are compromised due to various biotic, abiotic, and climatic factors. To meet the global need, innovative research has been carried out to enhance the cotton production. Starting from the conventional breeding strategies to the modern technologies, there is a long history of cotton research. In this chapter, the milestones of cotton research have been reviewed with recent and innovative results. The chapter briefly deals with cotton biotechnology, cotton cytogenetics, cotton genetics and genomics, genetic modification of cotton, genome editing, genome sequencing, etc. It contains the latest information in the field of cotton research.

Cotton is major economic crop in the world. However, its production is restricted by various biotic and abiotic factors. Its growth and efficiency are rigorously influenced by the stresses such as high temperature, salinity, less water availability, diseases, insects, etc. The chapter discusses the recent trends in cotton research to combat these biotic and abiotic stresses in crop plants.

2. Abiotic stress tolerance in cotton

Drought is a major abiotic stress which limits plant growth and fiber quality. Yield is badly affected under less water conditions. It is widely spread in many countries and is expected to increase further [1] due to sudden climate changes. Plant growth is decreased due to drought stress as photosynthetic rate and nutrient availability in soil are reduced. Similarly, salinity causes physiological dryness. High temperature can also cause stress [2], which is cause of reduced yield. A lot of research efforts have been done with a statement that the better understanding of these mechanisms would result in crop production under stress conditions. Desiccation stress results in the changing expression pattern of genes in plants, usually arbitrated with abscisic acid (ABA) [3] and physiology of plant is badly affected by the drought stress [4].

When a plant is under stress, the genes are turned on or off. Some proteins are regulatory in nature and regulate signal transduction and regulate expression of stress-responsive genes. Transcription factors also regulate gene expression and are grouped into large families, like WRKY, bZIP, AP2/ERF, Cys2His2, MYC, zinc finger, MYB, and NAC [5]. The DREB subfamily of proteins belongs to the CBF

(C repeat binding factor) proteins. ERF subfamily also has crucial role in plant gene expression under abiotic stress response and therefore received considerable attention in the recent past [6]. Drought causes significant damage to plants [7–9]. Plants have their self-defense mechanisms which respond to abiotic stresses at molecular, biochemical, and physiological levels [10–14]. Some genes are known to have role in the drought stress response at transcription level [8, 13, 15]. Few drought-tolerant genes have also been studied, which code for regulatory proteins, involved in further regulation of stress-responsive gene expression [14, 15].

Cotton expressing AtNHX1 was grown in the presence of salt, which produced more fiber and biomass. Cotton plants overexpressing the AtNHX1 gene resulted in the movement of salt (Na^+) into the vacuole, which leads to high concentration of salt in the vacuole, which leads to high salt tolerance in cotton. AVP1 gene from Arabidopsis introduced into the cotton resulted in enhanced salt tolerance and more fiber production [16]. CMO gene is a major catalyst in the production of glycine betaine and transgenic cotton expressing AhCMO was more tolerant to high salt concentration then non-transgenic cotton. Thus, it is clear that the salt tolerance in the cotton can be enhanced by the genetic engineering [17]. Cotton plants expressing GhDof1 gene resulted in an increased tolerance to cold and salt stress. GhSOD, GhMYB, and GhP5CS are the stress-responsive genes [18].

3. Biotic stress tolerance in cotton

GhWRKY3 is a gene which is expressed under biotic stress. Cotton seeds were analyzed with three fungi known as *Fusarium oxysporum*, *Rhizoctonia solani*, and *Colletotrichum gossypii* to understand the response of GhWRKY3. The level of GhWRKY3 protein was increased after infection with these three fungi clearly indicated that GhWRKY3 has an important role in fungal pathogenesis [19]. Increased pathogen tolerance can be achieved by the transgenic management of the expression of the GhLac1 gene in cotton (*Gossypium hirsutum*). Elevated tolerance to the fungal pathogen, *Verticillium dahliae*, and cotton aphid (*Aphis gossypii*) resulted in response to the up-regulation of GhLac1.

GhWRKY39 present in the nucleus and few cis-acting elements related to stress response were studied. GhWRKY39 gene was expressed by the fungal and bacterial attack and this gene resulted in the enhanced activity of the SOD, POD, and CAT antioxidants after pathogen attack. Thus, GhWRKY39 was found to be involved in the plant protection against pathogen attack. GhWRKY39 directs the reactive oxygen species (ROS) system, which positively results in regulation of plant protection against pathogens [20].

4. Cell and tissue culture technology

Cotton is included in the list of commercial plants because it can be generated from undifferentiated tissues. Tissue can be taken from plant which can be cultured on an appropriate media. By using tissue culture technology, cotton plants have been screened for disease resistance and embryonic plants produced from the callus young plants produced by suspension cultures from callus. Shoot tips were also used to regenerate whole cotton plants [21]. Cotton has successfully been regenerated from the shoot apex. Problem related to regeneration of plant from callus has been sorted out by using shoot apex as explant [21–23]. Cotton was regenerated by the use of shoot apex which was less expensive and required less labor to regenerate cotton plant.

5. Genetically modified cotton

Lots of efforts have been made to genetically modify cotton for insect tolerance. Bt cotton is the success story in this regard. It is being grown in more than 20 countries for the last more than two decades. Extensive studies on Bt cotton have been done in different countries like India [24], Mexico [25], China [26], and Argentina [27]. Bt cotton verities have good impact on environment and human health. Bt cotton has been produced by the transfer of genes from the bacterium known as *Bacillus thuringiensis* which produce the insecticidal proteins. Bt cotton also resulted in the production of high level of useful insects [28].

Naturally, 15% oleic acids are present in the cotton seeds. So, transgenic cotton was developed to contain high levels of oleic acids. By the subcloning of mutant allele of the Fad2 gene from the phaseolin (seed specific promoter), the level of Fad2 gene in cotton was decreased. Gas chromatography was used to analyze the fatty acids profile of seed lipids from the transgenic cotton varieties. About 21–31% oleic acids were seen in the transgenic lines. Progeny of some transformants showed high levels of oleic acid (47%). So, genetically modified cotton can be developed to produce high level of oleic acid [29].

6. CRISPR/cas9 system in cotton

Genome editing can be used for the functional studies and crop improvements. CRISPR/cas9 system has a sgRNA, which directs the break of double-stranded DNA but all sgRNAs are not equally good. So, in cotton it is necessary to decrease the use of less-efficient sgRNA which can be used in the production of genetically transformed plants without the preferred CRISPR/cas9-induced mutations. The transient expression system was used to improve the functions of sgRNA in cotton. This method was used to check the target sites for the genes known as GhEF1, GhPDS, and GhCLA1 and to analyze the nature of mutation induced by CRISPR/cas9 system. Most frequent mutations observed were deletions. So, it was confirmed that CRISPR/cas9 can generate the mutations in the cotton genes, which are very important for the allotetraploid plant. It was also shown that targeting of gene can be achieved by the expression of many sgRNAs. CRISPR/cas9 was used to generate the deletions in the GhPDS locus. Genetically modified cotton having gene editing mutations in GhCLA1 gene was produced by the CRISPR/cas9 system. Intense albino phenotype was produced by the mutation in the GhCLA1 gene [30].

Cotton is a significant crop for the production of fiber, oil and bio-fuel. Usually, *Agrobacterium tumefaciens*-mediated transformation into cotton takes 8–10 months to generate the T0 plants. Scientists used the transient expression system to validate the CRISPR/cas9 cassettes in cotton. Efficient CRISPR/Cas9 cassettes can be selected to get the better mutagenesis rate by the use of GhU6 promoter instead of Arabidopsis ATU6–29 promoter and GhU6 promoter. When CRISPR/cas9 expressed the sgRNA under the GhU6 promoter, CRISPR/cas9 mutagenesis rate was increased four to six times and expression level of sgRNA was increased from six to seven times, which was a great achievement in the targeted mutagenesis of cotton by the CRISPR/cas9 system [31]. CRISPR/cas9 system has been used to generate multiple sites in *Gossypium hirsutum*. Two genes GhCLA1 and DsRed2 were selected as targets. Plants containing edited DsRed2 gene reverted its character to wild type in T0 generation. Gene editing efficiency was 66–100% [32].

7. Cotton computational analysis

Genomic, genetic, and breeding data are available on the Cottongen (http://www.cottongen.org), which has information related to markers, gene maps, expressed sequence tags, whole genome sequence, and genetic map. Analysis tools like BLAST are also available on the Cottongen [33]. Drought-tolerant cDNA libraries have been made by suppressive subtractive hybridization (SSH) technique and many genes were characterized and expression level of the genes under drought stress was studied [34]. DREB gene was identified in cotton (*Gossypium arboreum*), which was cloned and sequenced. Further, it was characterized in silico to study its interactions with other genes. It was found to interact with MYB, NAC, ABRE, and AREB, which are involved in the drought stress tolerance pathways [35].

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Author contributions

PZ, RS, and HMA did the research work and wrote the first draft of manuscript. MR and MI designed and wrote the paper and BR and SAK assisted in writing the paper. All the authors read the manuscript and approved it for publication.

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