

# Chapter 24

## Molecular Breeding of Cotton for Drought Stress Tolerance



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**Abstract** Availability of freshwater to crops is declining year by year, so there is a need to exploit genetic mechanism of drought tolerance in crops including cotton. Drought-tolerant cultivars may be tailored if generated data regarding inheritance of drought-related traits to tolerance is practically used. The complexity in inheritance of drought tolerance has been a main reason of slow progress. Although a lot of conventional and nonconventional research work has been conducted for the traits related to abiotic stress in cotton, fruitful field results have not been obtained. There is a need to understand drought stress and mechanisms adopted by cotton against drought stress. These include morphological, physiological, biochemical, and genetic responses in cotton. Identification of the important genes related to drought tolerance would also be a major contribution. The impactful genes and major QTLs could be stacked in a single cotton plant, using gene pyramiding, and this may produce the future cotton plant for upcoming adverse environment.

**Keywords** Gene pyramiding · QTL · Upland cotton

### Abbreviations

AA	Ascorbic acid
ABA	Abscisic acid
APX	Ascorbate peroxidase
CAT	Catalase
EL	Electrolyte leakage
GA	Gibberellic acid
GPX	Guaiacol peroxidase
GR	Glutathione reductase
GWAS	Genome-wide association study

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H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
HO <sup>•</sup>	Hydroxyl radical
IPT	Isopentenyltransferase
JA	Jasmonic acid
LEA	Late embryogenesis abundant
MAS	Marker-assisted selection
NO <sup>•</sup>	Nitric oxide
<sup>1</sup> O <sub>2</sub>	Singlet oxygen
O <sub>2</sub> <sup>•-</sup>	Superoxide anion radical
ROS	Reactive oxygen species
SA	Salicylic acid

## 24.1 Introduction

Origin of cotton is one of the most incredible stories in archives of crop origin, evolution and domestication. *Gossypium* comprises of more than 50 species (45 diploid, 5 tetraploid) distributed to tropical, subtropical, and semiarid regions worldwide with eight genomes (A, B, C, D, E, F, G, and K) with four cultivated species from family Malvaceae. *Gossypium hirsutum* and *Gossypium barbadense* are tetraploid (AADD) while *Gossypium herbaceum* and *Gossypium arboreum* are diploid (AA) cultivated species of cotton (Wendel and Cronn 2003).

The key purpose for cultivating cotton is fiber and oil (Ahmad et al. 2014, 2017, 2018; Abbas and Ahmad 2018; Ahmad and Raza 2014; Ali et al. 2011, 2013a, b, 2014a, b; Usman et al. 2009). Seed cotton is ginned to separate the fiber from seed. Separated fiber is a spinnable resource of yarn that is knitted into fabrics. Textile industry depends on fabrics for clothes, towels, and additional households. Cottonseed is composed of 45% meal, 28% hull, 17% crude oil, and 10% short fibers (Smith 1995). Cotton meal comprises of high content of protein (41%) and being used in animal feed. Cotton oil is used as edible oil, in plastics, and soaps.

Upland cotton is an important cash crop with a remarkable impact on economy of China, India, United State, Brazil, and Pakistan as these are top cotton-producing countries (Statista 2018). There are certain limitations to higher production of cotton because cotton is highly sensitive to abiotic stresses like salt, drought, and heat and to biotic issues such as diseases and pests. Among abiotic stresses drought and heat stress are causing frequent economic losses worldwide. Climate is changing due to global warming which may increase the atmosphere temperature by 1.8–4.0 °C in the next decade and may drastically affect cotton fiber parameters (Dai et al. 2017; Amin et al. 2017, 2018; Khan et al. 2004; Rahman et al. 2018; Tariq et al. 2017, 2018). Future climate change escalation will bring heat stress along with drought episodes in most parts of the world. Climate fluctuation accompanied with emissions of greenhouse gases in the atmosphere is predominantly responsible occurrence of abiotic stresses especially drought and heat stress. Among abiotic stresses, drought is

more limiting factor to crop productivity. Drought can adversely reduce yield and productivity depending upon severity, growth stage, and extent of occurrence of drought. Cotton plant handles drought stress by different built-in morphological, physiological, biochemical, and molecular responses to minimize lint yield reduction (Khan et al. 2018). Reduced fresh water availability and upcoming climate change effects are making condition more adverse for cotton around the world.

## 24.2 Drought Stress

As the moisture level in soil decreases the tissue water level in plants is also decreased. Drought stress begins with insufficiency of fresh water in plant to fulfill transpirational demands. Drought stress affects the physiology of plants by cellular and molecular mechanisms. Water contents in plants are linked to moisture contents in soil. Lesser water content and less leaf turgidity resulted in augmented stomatal resistance that lowers photosynthesis affecting growth of plants.

Tissue water plays a vital role in biochemical processes and standard functioning is extremely disturbed under drought (Burke and O'Mahony 2001). Under drought, because of lesser stomatal conductance, CO<sub>2</sub> uptake is decreased. This decrease in intercellular CO<sub>2</sub> concentration leads to reduced photosynthesis. Under severe water shortage, photosystem activity is affected leading to lower photosynthetic rate combined with photorespiration. The process makes way for the production of ROS that cause damage to ATP synthase, which reduces RuBP production in Calvin cycle and production of sucrose synthesis. Impaired photosynthesis hinders plants from normal growth (Lawlor and Tezara 2009). Drought affects productivity particularly when it happens at critical development stages (Salehi-Lisar and Bakhshayeshan-Agdam 2016).

Plants have natural ability to respond drought stress by some built-in physiological and molecular changes. These changes depend upon severity and extent of drought stress along stage of plant development. In general, plants adopt mechanisms (escape, avoidance, resistance, and tolerance) to endure in drought (Mitra 2001). Mechanism in plants which enables them to complete their life cycle in promising moisture circumstances before onset of meteorological drought is called drought escape. When the amount of water through transpiration exceeds than absorption, it causes dehydration. Plants can avoid dehydration (drought avoidance) by some physiological and morphological mechanisms to maintain balance between loss and uptake of water. These mechanisms are stomatal closure, leaf rolling, and changes in leaf angle, shedding of older leaves, cuticle thickness, and small canopy leaf area by reducing growth. However when drought prolongs, avoidance becomes difficult for plants. Plant can also resist drought stress by root enhancement and osmotic adjustment. Capability of crops to endure low water contents is termed drought tolerance (Ullah et al. 2017).

### 24.3 Impact on Cotton

Cotton is relatively more sensitive to drought compared to other field crops. In cotton, reduced water level in cellular tissues reduces photosynthesis rate due to increased production of reactive oxygen species. Amount of chlorophyll, carotenoids, proteins, and starch contents significantly reduced in water shortage condition (Parida et al. 2008). Severe drought stress increases the levels of glutathione-S-transferase, superoxide dismutase, and proline increase along with their gene expression while that of malondialdehyde content decreased (Singh et al. 2015). In cotton, drought stress affects photosynthesis due to stomatal resistance as well as causes non-stomatal limitations to photosynthesis in case of moderate to severe stress of water (Ennahli and Earl 2005).

Drought stress decreases leaf water content, cell membrane permeability, chlorophyll a, chlorophyll b, accumulation of biomass, leaf water content, and actual quantum yield of photosystem II (Wang et al. 2007). Low leaf water contents affects cell expansion (Schonfeld et al. 1988) and retards leaf, stem, root elongation as well as reduces the number of floral buds. The overall development of cotton plant, either vegetative or non-vegetative, halted under drought stress.

The affected plant may be recognized by reduced plant height, shedding of bolls, reduced number of nodes and leaf size. Drought stress at cotton boll development stage may seriously affect yield of cotton as yield has direct dependency on number of bolls per plant (Niu et al. 2018). However under drought stress the rate of root extension may be similar as in controlled conditions.

### 24.4 Morphological Response of Cotton Under Drought

Development and productivity of cotton is adversely affected due to drought stress. Plants respond to drought in different ways; it adjusts their growth period to mature early and avoid seasonal drought stress. Cotton tolerates, escapes, or recovers drought stress by adapting several morphological approaches (Khan et al. 2018). Cotton has a certain level of adaptations to drought stress owing to its perennial nature (Singh et al. 2018). In cotton drought stress limits root development, plant height, leaf growth, shoot growth, leaf area, vegetative growth, quality, and yield of fiber (Hasan et al. 2018). It has been found that water deficit limits 50% dry matter accumulation of *Gossypium barbadense* (Ullah et al. 2017). Under drought treatment, 60% reduction was observed in leaf area when compared to the control (Singh et al. 2018). Drought affects the root growth which in turn may leads to reduced biomass accumulation in cotton. Cotton undergoing water deficit explores moisture and nutrients by deeper root penetration (Fang and Xiong 2015).

Cotton showed some adaptations toward drought stress effect on roots. It enables increased root length and decreased shoot length; the enhanced root/shoot ratio indicates water assimilation and enhanced drought tolerance (Hasan et al. 2018).

The increased lateral roots enhance the root surface absorption area and increased root proliferation which are desirable traits for drought adaptation (Khan et al. 2018). Drought effect can be seen on cotton leaves which depict turgor loss, drooping, wilting, discoloration, yellowing, and premature senescence. Cotton exhibits leaf rolling, smaller and thicker leaves, abundant trichomes, smaller stomata and thick cuticle, and well-developed vascular bundle sheaths as stress adaptive traits (Fang and Xiong 2015). Drought stress at reproductive stage induces reproductive part abscission and boll size reduction. Reduced carbon assimilation leads to less biomass accumulation in cotton which causes large yield losses due to drought stress (Jawdat et al. 2018). Water deficit at flowering stage has been found to be most detrimental on seed cotton yield. Drought stress during fiber cell development affects the quality of the lint. Hence identification of more traits for drought tolerance under irrigated and water deficit agro-environment is needed to assist molecular breeding of for enhanced fiber quality and yield traits in cotton.

## 24.5 Physiological Response of Cotton Under Drought

Cotton maintains stomatal regulation and osmotic adjustment to tolerate stress (Ullah et al. 2017). Cotton leaves showed limited photosynthesis, decreased transpiration, low stomatal conductance and water potential when tested under drought conditions (Fang and Xiong 2015; Hasan et al. 2018). It has been established that 90% transpiration in plants occurs through stomata; hence stomatal regulation is an important mechanism through which plants maintain cellular function (Liang et al. 2016). In cotton, stomatal closure during water deficit helps in reducing water loss by limiting high transpiration rates. Negative correlation exists between stomatal conductance and drought tolerance which suggest stomatal conductance as a prospective indicator for drought tolerance in cotton (Fang and Xiong 2015).

Drought stress affects cellular structures by inducing osmotic stress and oxidative damage. This oxidative damage results from stomatal closure and decreased rates of gaseous exchange (Soomro et al. 2011). Damage to cell membranes due to drought stress causes electrolyte leakage (EL %). Electrolyte leakage is used as gauge to assess integrity and permeability of cell membranes and the subsequent leaking of intracellular contents. Sugars and amino acids generally termed as membrane compatible solutes protect the plasma membrane from desiccation-induced damage. Thus, osmotic adjustment and degree of membrane protection are linked; drought stress decreases membrane stability through lipid peroxidation caused by active oxygen species.

Drought stress affects cell turgidity and cellular homeostasis; hence osmotic adjustment is a crucial adaptation in crop plants against drought-induced damage (Wang et al. 2016b). Drought-tolerant genotype maintained higher relative water content in their leaves under water stress (Parida et al. 2007). Accumulation of osmolyte or osmoprotectants is a key strategy in plants to maintain cell homeostasis to withstand drought (Soomro et al. 2011). Proline, sugars, glycine betaine,

alkaloids, amino acids, and inorganic ions are the compatible osmolytes that regulate cell homeostasis and alleviate stress in cell membranes (Singh et al. 2018). These osmolytes also act as free radical scavengers and protective agent for enzyme function (Wang et al. 2016b). Drought-tolerant species of cotton accumulate higher proline content; a positive link between proline levels in leaves and drought stress resistance has been demonstrated by many researchers (Khan et al. 2018; Wang et al. 2016b). These solutes protect vital proteins, enzymes, and membranes from the damage due to oxidative damage and higher inorganic ion concentrations under drought stress. The exogenous application of phytohormones, osmoprotectants (proline plus glycine betaine), and inorganic substances has been proved to be effective in mitigating injurious effects of drought stress in cotton (Fang and Xiong 2015). Drought stress increases accumulation of osmolytes in cotton such as prolines, soluble proteins, soluble sugars, and betaines (Hasan et al. 2018; Parida et al. 2007). Transgenic cotton plants showed better tolerance to drought stress due to enhanced accumulation of glycine; these plants exhibited higher photosynthetic rate, enhanced osmotic adjustment, increased relative water content, less lipid membrane peroxidation, and less electrolyte leakage (Pilon et al. 2015).

Drought stress damages photosynthetic apparatus and alters chlorophyll content, thus impacting photosynthesis in plants (Fang and Xiong 2015). Chlorophyll a, b, total chlorophyll content, and a/b ratio are declined by drought stress in cotton (Soomro et al. 2011). A net 66% photosynthesis decline has been observed in mature leaves of cotton under drought stress (Ullah et al. 2017). Drought stress decreases protein, starch, chlorophylls, and carotenoid contents in cotton and an increasing tendency was observed upon recovery from stress (Parida et al. 2007; Soomro et al. 2011). Hence chlorophyll content, electrolyte leakage, membrane stability, and relative water content act as an effective criterion to screen cotton genotypes and select most efficient genotypes.

## 24.6 Biochemical Response of Cotton Under Drought

Cotton tolerates external stresses through certain adaptations. Drought tolerance mechanism is connected to numerous biochemical processes. These processes are controlled by hormonal interactions in plant body. Plant growth regulators, for example, abscisic acid (ABA), jasmonic acid (JA), gibberellic acid (GA), and salicylic acid (SA), play an important role in various physiological and biochemical processes in plant life cycle; specially during plant development, reproduction, and stress signaling (Pandey et al. 2003). ABA synthesis is promoted by osmotic stress which induces adaptive physiological changes and activates expression of stress-related genes. Signal perception occurs by the plasma membranes; this can be through ABA-dependent or ABA-independent pathways. ABA-dependent signaling plays a crucial role in the expression of stress-responsive genes (Mittal et al. 2014).

Drought treatments reduced the GA content of roots; upon rewatering GA content and CAT activity increases (Niu et al. 2018). JA and its active derivatives jasmonates play significant part in plant responses to drought stress; JA is associated in stomatal closure, root elongation, fruit development, and viable pollen production (Ullah et al. 2017). Drought stress breaks cellular physiological homeostasis by inducing accumulation of reactive oxygen species (ROS) in plants. These include superoxide anion radical ( $O_2^{\cdot-}$ ), hydrogen peroxide ( $H_2O_2$ ), singlet oxygen ( $^1O_2$ ), hydroxyl radical ( $HO^{\cdot}$ ), and nitric oxide ( $NO^{\cdot}$ ) (Wang et al. 2016b). These reactive oxygen species oxidize photosynthetic pigments, proteins, lipids, DNA, and RNA and increase the damaging processes in the cell (Pilon et al. 2015). Drought conditions enable the photorespiration and enhance RuBP oxygenation due to reduced  $CO_2$  fixation (Wang et al. 2016b). Plants have antioxidant defense mechanism against this oxidative damage, which controls cellular ROS levels during stress conditions (Fang and Xiong 2015). The antioxidant machinery has two arms: (1) enzymatic components, like catalase (CAT), SOD, ascorbate peroxidase (APX), glutathione reductase (GR), and guaiacol peroxidase (GPX), and (2) nonenzymatic antioxidants, for example, reduced glutathione (GSH), ascorbic acid (AA),  $\alpha$ -tocopherol, flavonoids, carotenoids, and proline. These two components work together to scavenge ROS (Pilon et al. 2015). Under drought stress, ROS production increases and reduces the activity of the photosynthetic apparatus. Superoxide dismutase (SOD) converts  $O_2^{\cdot-}$  into  $H_2O_2$ , which is further converted to water by ascorbate peroxidase (APX). Antioxidant response of cotton cultivars determines their resistance capability to drought stress. Drought-tolerant cultivar has active antioxidative enzyme mechanism, which decreases the oxidative stress induced by lipid peroxidation (Fang and Xiong 2015). Decreased antioxidant enzyme activities in transgenic *G. barbadense* resulted in increased oxidative stress under drought conditions. These results show the importance of antioxidant defense mechanisms. Genes and certain factors are involved in improving the antioxidant machinery of cotton plants, such as Zn, that further need to be explored (Khan et al. 2018).

## 24.7 Molecular Response of Cotton Under Drought

Modern tools of biotechnology and genetic engineering now holds leading role to dissect complex nature of abiotic stresses tolerance. Genetic tolerance to abiotic stresses are quantitative in nature, involving many genes and QTLs (Fang and Xiong 2015). High-density genome-wide association study by using SNP in cotton is being used to study quantitative traits in cotton. Five QTLs were identified on A-genome and nine QTLs on D-genome by using SNP markers for abiotic stress along with 12 putative key genes through GBS-SNP-based high-density genetic mapping (Diouf et al. 2017). Twenty QTLs associated with drought tolerance were identified along with number of candidate genes. Four important drought tolerance-related

genes, PP2C encoding a protein phosphatase 2C, HAT22 encoding a homeobox-leucine zipper protein, RD2 encoding a response to desiccation 2 protein, and PIP2 encoding a plasma membrane intrinsic protein 2, were potentially candidate genes for conferring drought tolerance in cotton (Hou et al. 2018). Transcriptomics and functional genomics have unveiled plenty of genes for drought resistance which include transcription factors, protein kinases, and some structural genes. Some NAC proteins regulate plant defense mechanism under drought stress. NAC protein *GhNAC2* reduced wilting and leaf abscission in cotton under drought stress. Overexpression of *GhNAC2* suppressed the ethylene pathway and activated the ABA/JA pathway which leads to longer roots, larger leaves, and hence higher yield in cotton under drought (Gunapati et al. 2016). Transcription factors are involved in biological processes. R2R3-MYB is the biggest family of transcription factors. The number R2R3-MYB transcription factor varies in different plant species. Cotton has narrow genetic pool due to constant breeding of same species. Other cotton species could be used to broaden the genetic base of cotton. For this purpose, R2R3-MYB transcription factor was investigated through genome-wide characterization and 205 putative R2R3-MYB genes were identified on D-genome in *Gossypium raimondii* which were distributed across 13 chromosomes in various densities. MYB genes were found to be expressed under drought stress at seedling stage in cotton (He et al. 2016). Transcription factors (TF) help plants to cope with abiotic stresses. The bZIP (basic leucine zipper) is important TF in plants to activate ABA accumulation. GhABF2 encodes for bZIP TF in cotton. Overexpression of GhABF2 improves drought resistance in cotton plant (Liang et al. 2016). Sixteen putative genes were identified by using SNPs linked with drought resistance and were found to be associated with ghr-miR169a and ghr-miR164 (Magwanga et al. 2018a). Late embryogenesis abundant (LEA) proteins play a key part in the mechanism of drought stress. LEA genes were identified in *Gossypium arboreum*, *Gossypium hirsutum*, and *Gossypium raimondii*. All the LEA genes contained W-Box, MBS, ABRE, and TAC elements in their promoters which are known to be functionally involved to confer drought stress in crop plants (Magwanga et al. 2018b). Overexpression of RAV TFs and bZIP helps cotton to cope drought (Mittal et al. 2014). ABP9 gene was introduced into *Gossypium hirsutum* L. Overexpression of ABP9 confers drought tolerance in cotton by better root systems, higher germination, reduced stomatal aperture, and stomatal density (Wang et al. 2017). Mitogen-activated protein kinase kinase kinase (MAPKKK) is associated in plant stress response. MAPKKK genes were identified in *Gossypium raimondii* genome and *Gossypium hirsutum*. Gene expression arrays discovered that MAPKKKs intricate in abiotic stresses (Zhang et al. 2018). The isopentenyltransferase gene (IPT) confers resistance against water deficit. The expression of IPT is critical about the time at which drought occurs. Occurrence of drought stress before flowering stage enhances cotton yield (Zhu et al. 2018a). Some other important genes against abiotic stresses are listed in Table 24.1.



**Table 24.1** Some important cotton genes for abiotic stress tolerance

Sr. No.	Gene	Trait	References
1	HSPCB	Peptide synthesis activated in drought-tolerant genotypes	Voloudakis et al. (2002)
2	GHSP26	Regulates cell metabolism, improves drought tolerance	(Maqbool et al. 2007)
3	KC3	Improves drought tolerance	(Selvam et al. 2009)
4	DREB 1 AND 2	Improves stress tolerance in crop plants	(Liu et al. 1998)
5	TPS (trehalose-6-phosphate synthase)	Protects proteins under water stress	(Kosmas et al. 2006)
6	<i>GhABF2</i> (bZIP)	Regulates genes related to ABA and increases the activities of SOD and CAT	(Liang et al. 2016)
7	<i>GhNAC2</i>	Lengthens roots, enhances drought tolerance	(Gunapati et al. 2016)
8	<i>GbMYB5</i>	Reduces stomatal size and rate of opening of stomata	(Chen et al. 2015)
9	<i>GhWRKY41</i>	Induces stomatal closure and higher antioxidant activity and lower malondialdehyde content	Chu et al. (2015)
10	<i>GhMKK3</i>	Enhances drought tolerance	Wang et al. (2016a)
11	<i>GhMAP3K40</i>	Enhances drought tolerance at the germination stage	(Chen et al. 2015)
12	<i>GhMPK17</i>	Increases salt and osmotic stress tolerance	Zhang et al. (2014)
13	<i>GbMPK3</i>	Increases drought and oxidative stress tolerance	Long et al. (2014)
14	<i>GhMKK1</i>	Increases drought and salt tolerance	Lu et al. (2013)
15	<i>GhMPK2</i>	Enhances drought and salt tolerance	Zhang et al. (2011)

## 24.8 What Is Next? Gene Pyramiding?

In cotton, drought being the major abiotic stress is significantly reducing the crop yield. Although scientists have made a lot of progress in developing the drought-tolerant varieties/cultivars through conventional breeding techniques, it is a tedious work which requires a lot of time, labor, and money. Alternatively, marker-assisted selection (MAS) is an efficient technique through which the genomic regions influenced by stress conditions are identified. Many QTLs have been identified for drought stress tolerance in different crops. Pyramiding the desirable genes from different sources into single genotype using the MAS technique may lead toward the development of drought-tolerant varieties/cultivars.

To develop future plant, resistant against drought stress, gene pyramiding could play pivotal role. The technique involves stacking important genes related to a particular trait, from different sources into a single genotype. The simultaneous expression of stacked genes may provide sufficient resilience against adverse conditions. Principally, the stacked genes are fixed into homozygous states that make plant with predictable performance. A number of examples are settled such as in maize; nine genes from different sources of two categories, plant defense response genes, and anti-apoptotic genes have been pyramided into a single maize line (Zhu et al. 2018b). Many of abiotic stress tolerance traits are quantitative in nature, confining breeders for conventional breeding approaches of plant improvement. However, scientists have reported many important genes for abiotic stress tolerance that could be stacked in a single genotype to make future plant resistant against drought stress. The cumulative expression of important genes in a single cotton plant may provide “stay green” type of ability under drought stress condition. The variety of genes would enable cotton to withstand adverse environmental conditions.

## 24.9 Conclusion and Future Perspective

Climate change is the consistent part of green planet but now its acceleration and adverse effect to agriculture has been phenomenal. Future breeding of cotton, a sensitive crop against drought, must imply modern techniques and knowledge to develop plant with some extra traits that could make cotton withstand unfavorable conditions. The mechanism of drought stress tolerance is complex in nature in cotton. QTL identification for drought tolerance in cotton is an ongoing strategy but the results have not been translated into a meaningful product. Many genes have also been identified that can overcome drought stress. A combination of stacked genes in cotton plant may provide sufficient empowerment against drought stress. Gene pyramiding could be future breeding of cotton for drought stress tolerance.

Abiotic and biotic tolerance, high yield, quality improvement, and production of special purpose cotton (long staple, medium long staple, color, and organic cotton) are new requirements in cotton production of the world. Currently genetic base of cotton is becoming narrow; that is why different exotic germplasm should be incorporated into different breeding programs to improve the cotton. Cotton genotypes from diverse origins should be assessed for the genomic regions conferring resistance through genome-wide association study (GWAS) to identify QTNs and genes by applying SNPs. Different genotypes conferring resistance may be incorporated in breeding programs. Identified genes may also be further validated using transcriptomics analysis to identify the expression of the said genes. For this purpose, the phenotypic data for abiotic stress-related trait can be investigated, and genome-wide SNP polymorphic loci can be studied using SNP chip. Based on phenotypic and genomic data, GWAS analysis is performed to study the genetic diversity across different cotton varieties/genotypes. The technique is useful to identify the elite alleles associated with the target traits, and to further investigate

the transfer of variation. These selected candidate genes will be functional confirmation and may provide genetic modification of traits in the future. The process of identifying useful genes followed by gene pyramiding, may lead to develop future cotton plant.

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