

Inducing Drought Tolerance in Upland Cotton (*Gossypium hirsutum* L.), Accomplishments and Future Prospects

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Abstract: Cotton (*Gossypium hirsutum* L.) is dreadfully drought sensitive crop causing incentive reduction in yield. Although conventional breeding has been successful in the past in developing drought tolerant cotton cultivars by modifying the morphological and physiological traits, yet the approach is time consuming and labor intensive. Furthermore the polygenic nature of drought tolerance slows the progress. Considerable efforts have been made to know the drought tolerant mechanisms and figure out the traits related to drought tolerance. The integration of conventional breeding with genetic and genomic tools such as quantitative trait loci (QTL), microarrays and transgenics offer new opportunities for improving drought resistance in cotton. Aim of this review is to integrate current knowledge of physiology, molecular genetics and functional genomics approach which might influence the consolidation of genetic engineering approaches of cotton for drought tolerance.

Key words: Drought tolerance • Conventional breeding • Marker assisted selection • Quantitative trait loci and transgenic plants

INTRODUCTION

Millions of people in the world are directly or indirectly associated with Cotton (*Gossypium hirsutum* L.) business, i.e. production, transport, ginning textile and other allied industries thus it is livelihood to the millions of people. Cotton fiber, occupies a pivotal position in the international economy. Considerable quantity of its production is exported in form of yarn, cloth and value added ready made garments to earn foreign exchange needed for the development of the country [1]. Since world population is increasing at alarming rate, the sustainability and improvement of cotton yield will be the prime objective of cotton breeders. But, unfortunately efforts are seriously being hampered by a number of abiotic stresses among those drought is the major factor limiting its productivity [2].

Drought stress or water deficit is a complex phenomenon affecting the physiology [3], growth and productivity of cotton plant [4]. Earlier [5] reported that water stress decreased seed cotton yield, in part because of decreased flowering and in part because of decreased boll retention. The detrimental effects

of drought can be minimized by the development of drought tolerant cotton cultivars. However there are limited reports on this aspect due to complex nature of drought tolerant mechanisms explaining slow progress in drought prone areas [6]. This review article aims to provide an overview of the cotton breeding progress with reference to drought tolerance, highlighting future perspectives in plant breeding that could result from the integration of the recent advances in physiology and genomics.

Mechanism of Drought Tolerance in Cotton: Drought tolerance is a multigenic trait [7-9] associated with morphophysiological characters [10] moderating the genetic improvement on morphophysiological based selection of crop plants. These are mainly distance of 1st main lateral root from transition zone, seedling vigor, rate of increase in root system, root / shoot ratio [11], increased size of Tap roots [12], lower excised leaf water loss, lower transpiration rate, lower stomatal size and frequency and high relative leaf water contents [13,14], stomatal conductance, Rate of photosynthesis [15] and canopy temperature [16].

In genetic sense, drought tolerance mechanisms can be grouped in to three categories viz, drought escape, drought avoidance/postponement and dehydration tolerance [17]. Drought escape is the capability of the plant to complete its lifecycle i.e vegetative and reproductive growth before the onset of drought season. Plants that are better prepared to escape drought tend to close their stomata, thus reducing CO₂ diffusion but this ultimately leads to decreased rate of photosynthesis resulting in reduced growth and economic yield. Only those plants adopt this strategy that are surviving under extremely stressful condition that allow them to redirect absorption and energy which are usually used in metabolic processes of growth and production for the synthesis of protective molecules [18,19].

In drought avoidance/postponement the plant becomes capable of maintaining tissue water potential, this is done by increasing the water uptake from soil through their deeper and vigorous root system and reducing transpiration by stomatal closure [20, 21].

Dehydration tolerance is the ability of crop plants to maintain a favorable water balance through the expression of morphological traits i.e. earliness, reduced leaf area, leaf rolling, wax content, efficient rooting system etc., physiological traits i.e. reduced transpiration, high water-use efficiency, stomatal closure and osmotic adjustment and biochemical traits i.e. accumulation of proline, polyamine, trehalose, etc. traits [22].

Conventional Breeding for Drought Tolerance in Cotton:

Drought stress is a complex phenomenon that affects the physiology of cotton plant. Cotton is classified as a drought sensitive crop as it is not an efficient water consumer. Significant efforts have been made in the past to improve drought tolerance in cotton cultivars through conventional breeding. Most of the drought related breeding program concentrate on selection of those cotton cultivars that yield well under drought stress. This selection is generally based on identifying those traits that can be utilized for screening to drought tolerance. Many of such attributes including anatomical traits (root characters), physiological traits (gaseous exchange, osmotic adjustment) and plant water status measurement (leaf water potential, relative water contents, cell membrane stability) are recognized as important components of drought tolerance in cotton [23, 24]. But for a plant breeder to evaluate a large populations by these parameters is too tedious and time consuming.

Qtls Associated with Drought Tolerance in Cotton:

There are many minor genes (poly genes) with additive effect in their expression which control drought stress [25-27]. Natural variability that exist in the crop can be utilized for selection under stress environment, it might be natural or stimulated using mapping of QTLs (polygenes) and following the marker assisted selection [28].

Quantitative trait loci (QTL's) which are responsible for improved productivity of crops under water stress condition have been identified via genetic mapping techniques [29-31].

It is also reported that physiological variation due to QTLs is considered to be coupled with tolerance against stress such as osmotic adjustment [32].

The so called QTLs providing the path towards marker assisted selection (MAS) [33] and following their cloning are further manipulated through genetic engineering [34]. QTL mapping is very much important as it pave the way for the mechanism of gene action, identification of location and number and total phenotypic effect [35].

QTLs analysis play a key role in controlling drought stress implementing their wide assessment via traditional ways in past and through advanced mapping approaches and DNA marker based examination in detail [36].

From a segregating population using a variety of genetic markers, a large number of plants are evaluated phenotypically for QTL analysis and then the whole population or part of it is genotyped and finally statistical analysis is done to discover exactly "the loci" controlling drought trait [37].

Saranga *et al.*, [38] reported 33 QTLs, identified under water stress condition in cotton, using F₃ families which were derived from the cross between *Gossypium barbadense* cv. F-177 and *Gossypium hirsutum* cv. Siv'on. Out of these 33, 5 identified for some of the important physiological traits, 11 QTLs identified for plant productivity potential and 17 are linked with fiber quality. From the cross of *G.barbadense* L. and *G.hirsutum* L. two generations of progeny were obtained and their QTL analysis was performed, to evaluate the association of genetic control and productivity and physiological parameters under irrigated and drought condition respectively. A total of 33 QTLs found for variability for physiological parameters (osmotic potential OP, carbon isotope ratio $\delta^{13}C$ (which is an indicator of water use efficiency), canopy, temperature and chlorophyll a and b. About 46 QTLs were also detected which were associated

with productivity measures like dry matter, seed cotton yield, harvest index, boll weight and boll number. QTL for carbon isotope can be associated with either low or high productivity and indicate the high water use efficiency [39].

A polygenetic approach was used to study the impact of selection from different environment (dry and irrigated) on the genetic basis of selected cotton lines from F6 population it is thought that these lines have already evolved various adaptations to drought stress condition due to their selection environment and it is assumed that under each environmental condition different introgressions have stabilized. There were some QTLs which were mapped for drought under each selected environment (dry or irrigated). One QTL (BNL1693) found for seed cotton on chromosome 1 and 15, while two more QTLs (BNL1153 and BNL2884) for seed cotton were identified on chromosome 6. Similarly QTLs, (BNL3259, BNL1153 and BNL2884) for osmotic potential were mapped on chromosome 14, chromosome 25 and chromosome 6 respectively. QTLs for drought resistant trait and productivity under drought were identified which can be utilized for marker assisted selection program of cotton [40].

Marker Assisted Selection: The conventional breeding relies on selection of desirable genotypes from the segregating populations. However the selection efficiency is greatly reduced if the trait under study is polygenically controlled (like drought tolerance). Whereas DNA markers such as random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), microsattellites (SSRs) etc, prove very beneficial in quest of breeding for drought tolerance.

One of the valuable tools in cotton breeding investigation is the effective utilization of these molecular markers for molecular dissection of complex traits and characterization of genome organization [41]. A revolution came in last twenty years in all the areas of conventional breeding due to the vast study of molecular biology. The duration of the breeding program is reduced by the use of such molecular markers. The utility of the molecular marker in the conventional breeding brings improvement of accuracy of crosses and gave chance to the breeders to produce strains or cultivars with combined characters [42]. Selection process in the breeding programs could be improved by utilizing DNA markers linked to a gene of interest. MAS is helpful for phenotypic screening of expensive as well as complex polygenic traits.

Gene Conferring Drought Tolerance: In Many crop species the number of stress related genes have been drawn out and characterized in the last two decades [6]. However due to very low genetic variability in cotton, there are few reports about genes conferring drought tolerance [43, 44].

Under high water deficit conditions, HSPCB gene responsible for peptide synthesis become activated in leaves of cotton drought tolerant genotypes [45]. An alpha crystalline small heat shock protein gene (*GHSP26*) was identified from *Gossypium arboreum* L. *GHSP26* gene activated in stress condition and improves drought tolerance by regulating cellular metabolism as it assists in protein folding and prevent protein denaturation [46]. Selvam *et al.* [47] identified a novel drought resistant gene KC3. Physiological and biochemical studies proved that KC3 gene improves drought tolerance in cotton. Under drought stress condition in cotton, dehydration responsive element binding gene (DREB) I and II were activated and their function is to assists DNA binding. It also act as transcription activator which controls the expression of number of stress related genes thus it improves stress tolerance of crop plants by interacting with specific cis acting element named DRE/CRT [48]. By using a chromosome-walking technique, a gene TPS (trehalose-6-phosphate synthetase) identified during stress which participate in biosynthesis of trehalose. Trehalose is an osmolyte that help in protection of proteins under water stress [49].

Molecular and Functional Genomic Approach

Proteomics: Studies of entire complement of proteins include modifications made to particular set of proteins produced under different stress conditions. Proteome is blend of protein and genome. It was coined by Marc Wilkins in 1994. Multiple of transcriptional factors and stress proteins enhance tolerance to abiotic stress like drought by dehydration of cellular structures because most of stress proteins are soluble in water [50, 51].

Abiotic stress like drought leads to differential expression of genes. Drought stress responsible genes function in three main ways. (I) those that assist in signaling cascades and control of transcription such as MyC, MAP kinases and SOS kinase [18, 52, 53], phospholipases [54, 55]. (II) Those that are involved in protection of membranes and proteins such as Heat shock proteins (HSPs) and chaperons, late embryogenesis abundant (LEA), proteins [56-58]. (III) Those that function

in uptake of water and ions and act as transporters of aquaporins and ions [59]. Set of proteins, HSPs function in response to drought conditions. HSPs function as molecular chaperons has also been documented. Molecular chaperons are proteins that enhance correct folding of denatured substrate protein by controlling irreversible aggregation [50, 58, 60, 61].

Using differential display of PCR, A4B1, a novel *Gossypium* transcript of 855 base pairs was reported that was induced by drought stress which had a very similar function as small heat shock proteins (SHSPs) of plants [46].

ESTs: An expressed sequence tag (EST) is short sub-sequence of a transcribed cDNA sequence. ESTs used to identify gene transcript gene discovery and gene sequence determination. ESTs are sequenced by sequencing several hundred base pairs from an end of a cDNA library. ESTs represents portions of expressed genes because these consists of DNA that is complementary to mRNA, the ESTs represents portions of expressed genes.

From multiple tissues and organs under different conditions, 185,000 *Gossypium* ESTs were organized from 30 cDNA libraries which contain more than 94,800,000 nucleotides including drought stress tolerance [62]. It has been reported that in drought stress condition, photo system IPsaH protein and H (+)-ATPase related gene expressed reducing evaporation adjusting photosynthesis, hence help to utilize water efficiency. Increase expression level of glyceraldehyde-3-phosphate dehydrogenase, alcohol dehydrogenase and drought induced cysteine protease [63, 64] was analyzed under drought conditions (These enzymes assists in cellular functions during stress and maintain chloroplast membrane, chlorophyll contents by catabolic actions).

Microarray: Microarray is a multiplex technology being widely used in molecular biology. It includes mapping of thousand of features i.e. microscopic DNA oligonucleotide spots, each feature contains probes i.e. pico moles of a specific DNA sequence. Microarrays are being used in genomic research including expression of gene, discovery of gene, mutation assay, gene profiling, genetic mapping and quantitative trait loci analysis. These microarrays have been developed from cotton ESTs to facilitate cotton genomics research. Udall *et al.* [62] obtained 51,107 unigenes by analyzing approximately 185,000 ESTs from both fiber/ovule (124,299ESTs) and non-fiber source (608,99ESTs) of *Gossypium hirsutum*,

Gossypium arboretum and *Gossypium raimondii*. In cotton, several additional batches of ESTs or cDNA-based microarray have reported [62, 65, 66] that there is no question that these microarrays of cotton will act as a key tool for genomic studies in cotton.

Transferring of desirable genes from a species in to a target organism is possible through genetic engineering. It is a good technique to transfer a desirable gene responsible for controlling certain traits which determine the resistance/tolerance to biotic and abiotic stress without facing the consequences of undesirable genes of source species [28].

A drought tolerant cotton having “stay green” like phenotype was obtained by introducing *Arabidopsis* gene GF14 lambda encoding a 14-3-3 protein which have good stomatal conductance, response well under drought condition by higher rate of photosynthesis [67]. Lv *et al.* [68] reported that drought stress was obtained by transferring H (+)-PPase gene TSVP from *Thellugiella halophilla* in cotton. Transgenic cotton have better shoot and root growth, higher photosynthesis, greater stability of cell membrane and higher relative water content (RWC) under water deficit conditions. This research was quite similar with the work of [69] who reported that increase efficiency of cotton under drought stress condition, when *Arabidopsis* vacular H (+)-pyrophosphaatase gene (AVP1) was introduced in cotton. That facilitated ions and sugar movement into roots resulting greater absorption of water and reducing water potential hence greater fiber yield then wild type cotton.

Certain organic osmolytes like glycinebetaine (GB), proline and trehalose play key role in dehydration stress [28, 70, 71]. So genes controlling synthesis of such organic solutes can be engineered in transgenic plants [28, 72].

CONCLUSION

Drought tolerance improvement is probably one of the challenging tasks of cotton breeders. Keeping in view the drought losses in cotton, there is a dire need to develop cotton cultivars that will produce acceptable yields in both water-limited and favorable environments. Although conventional breeding has its limitations, yet it has contributed considerably in developing drought tolerant cotton cultivars. Many traits are known in cotton that contribute towards drought tolerance. Attention should be focused to know the physiological and genetic basis of these traits in cotton. The future contribution will depend on our ability to map QTLs and their effective

incorporation in to marker assisted breeding programs. Transgenic approach will have its role in future as through this we can get substantial improvement in traits concerned in shortest possible time. Through cotton genomics research, potentially new tools like microarrays, ESTs and proteomics are being introduced allowing the identification of multiple genes and QTLs for drought tolerance in cotton. However concerted efforts are required to further refine these modern tools to make them readily usable in applications in order to exploit their full potential in cotton genetic improvement. The integration of these novel approaches with conventional system of crop genetic improvement should provide exciting results to breed for drought tolerance in cotton in near future.

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