Journal of Cotton Research

REVIEW

Open Access

Multiomics approaches to explore drought tolerance in cotton

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Abstract

The situation of global warming imparts negative impacts on crop growth and development. Cotton is the most important fiber crop around the globe. However, frequent drought episodes pose serious threats to cotton production worldwide. Due to the complex genetic structure of drought tolerance, the development of a tolerant cultivar is cumbersome via conventional breeding. Multiple omics techniques have appeared as successful tool for cotton improvement in drought tolerance. Advanced omics-based biotechniques have paved the way for generation of omics data like transcriptomics, genomics, metabolomics and proteomics, which greatly expand the knowledge of cotton response to drought stress. Omics methodologies and have provided ways for the identification of quantitative trait loci (QTLs), gene regulatory networks, and other regulatory pathways against drought stress in cotton. These resources could speed up the discovery and incorporation of drought tolerant traits in the elite genotypes. The genome wide association study (GWAS), gene-editing system CRISPER/Cas9, gene silencing through RNAi are efficient tools to explore the molecular mechanism of drought tolerance and facilitate the identification of mechanisms and candidate genes for the improvement of drought tolerance in cotton.

Keywords Drought, Structural genomics, Functional genomics, Transcriptomics, Proteomics

Introduction

Global warming has become the most concerning issue, specifically in developing countries. The atmosphere is warming day by day with an expectation to raise the average temperature by 1.5–2.0 °C in 2050 globally (Armstrong McKay et al., 2022). The global warming is threatening the plant's growth and development by exposing different environmental stresses such as heat, drought, and salinity to plant, resulting in lower crop production. Drought stress is considered as the most devastating condition, as it alone affects 45% of the world's agricultural land area. It is predicted that the drought affected terrestrial areas would be doubled by the end of twenty-first century (Nagamalla et al., 2021).

Drought is defined as an imbalance between soil water intake and evapotranspiration rate (Wood et al., 2023). Drought stress occurrence is erratic as it depends upon several factors, e.g. the amount and distribution of rainfall, rate of evaporation, and moisture conservation ability of the soils (Panigrahi et al., 2021). Less precipitation along with increased evapotranspiration rate, low atmospheric and soil humidity and high ambient temperature also lead to drought stress. Several climatic models have predicted the increase in frequency and severity of

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drought in response to the ongoing global climate change scenario (Ma et al., 2018).

Cotton (*Gossypium hirsutum* L.) is the most important fiber crop and the second high-value oilseed crop which plays a key role in the world economy. It is cultivated as a commercial crop in more than 30 countries around the globe with the major share from China, India, USA and Pakistan (Fan et al., 2018). From the last fifty years, the drought stress has caused 67% of cotton lint loss in United States (Abdelraheem et al., 2019). Production of cotton fiber under drought and heat stress lead to the yield loss of 34% (Ullah et al., 2020). Cotton response to drought stress is growth stage dependent. From germination to fiber development, almost all stages are affected by drought stress. Cotton possesses moderate tolerance against drought stress at vegetative growth phase. However, the reproductive growth of cotton is highly sensitive to drought stress. Seed germination is considered as one of the earlier critical stage to drought stress, which creates metabolic imbalance (Khalequzzaman et al., 2023), and causing 26% reduction in the number of sympodial branches, 27% decrease in the number of bolls per plant, 14% decrease in the boll weight, 4% reduction in the ginning out turn rate and 27% reduction in seed cotton yield (Bakhsh et al., 2019). The reproductive stage from 1st square to 1st flower and from 1st flower to peak flowering are the most sensitive stages for cotton under heat and drought stress (Zonta et al., 2017). Drought stress during the 1st fourteen days after anthesis imparts detrimental effects and leads to the abscission of immature bolls; however, bolls are unaffected by drought or heat stress after this period. Water depletion during flowering, squaring, and boll-opening stages negatively correlates with yield, leads to 38.8%, 27.9%, and 7.6% yield reduction, respectively (Wang et al., 2017b).

At the early fiber developmental stage, water scarcity restricts the elongation of fiber length and uniformity by disturbing the physiological and molecular regulatory processes of cell expansion (Ul-Allah et al., 2021). Cotton at the fiber initiation stage is less sensitive to water scarcity, but profound influence of drought stress has been seen on fiber elongation via the down-regulation of genes involved in cell wall expansion and loosening process (Padmalatha et al., 2012). Plants under moisture-deficit conditions produce short, immature, and weaker fiber. Micronaire value of plants under severe water deficit conditions is increased (> 4.2) (Lokhande et al., 2014). Therefore, there is a dire need to develop drought tolerant cotton genotypes for ensuring the sustainable production under climate change scenario.

Plants adopt four types of strategies to protect themselves from drought stress: escape, avoidance, recovery, and tolerance (Ullah et al., 2017). In drought escape, plants shorten the developmental period and complete their life cycle before the environment becomes dry and hostile to avoid the damage. In drought avoidance, plants mainly make morpho-physiological adjustments, e.g. increased root length, less number of stomata and conductance, reduced leaf area, and increased leaf thickness to retain maximum water in plants (Clauw et al., 2016). In the recovery mechanism, plants resume their growth and yield after facing severe drought conditions; while tolerance refers to the ability of plant to grow and produce economical yield under restricted water supply (Abdelraheem et al., 2019; Rodriguez-Uribe et al., 2014; Ullah et al., 2017). Short-term and long-term responses of the plant under drought stress have been illustrated in Fig. 1.

In the past decades, efforts have been made to mitigate the effect of drought stress by untilizing four types of strategies (escape, avoidance, recovery, and tolerance) in cotton. But the success are very limited due to the complex genetic regulatory network of drought response. The drought response associated traits are regulated by quantitative genes, which have small effects and significant effect of genotype × environment (G × E) interaction. In the era of omics, the high-throughput data of genomics, transcriptomics, proteomics, and metabolomics techniques have offered better tools to understand the response of plants to stresses. The omics-based analysis on drought stress response provides insight into the respective molecular regulatory networks that in turn pave way for cotton improvement under drought stress (Wu et al., 2017; Shah et al., 2018; Jain et al., 2019).

Omics approaches for understanding drought response in cotton

Over the last few decades, omics approaches have become the valuable tool for exploring the plants' response to abiotic stress, by analyzing molecular dynamics on genomic, transcriptomic, proteomics and metabolism level in cotton (Yang et al., 2021). A general overview of omics approaches has been presented in Fig. 2 for its utilization in drought tolerance study in cotton.

Progress of genomics for drought tolerance in cotton

With the progress in DNA sequencing and genotyping techniques, genomic datasets in cotton are widely used for designing different sequence-based markers such as simple sequence repeats (SSRs), expressed sequence tags (ESTs), single nucleotide polymorphisms (SNPs), and so on.

Availability of a high-quality genome reference sequence for Gossypium species facilitate the development of microarrays, which has been widely used for

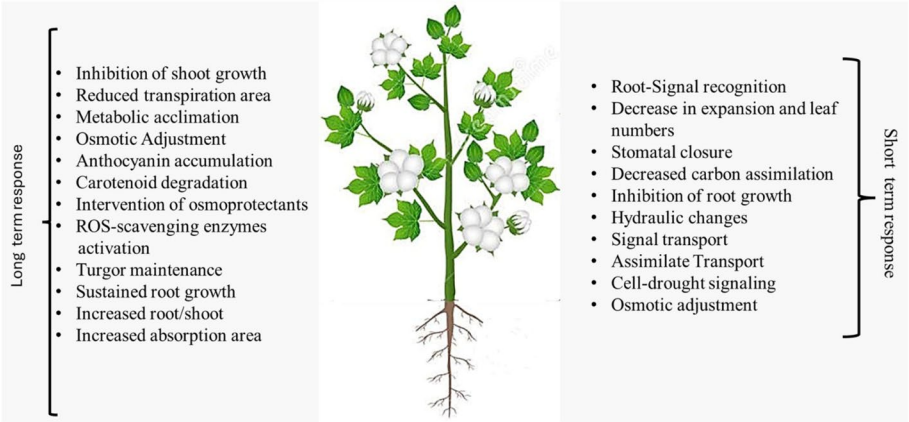

Fig. 1 An overview of short-term and long-term morphological, physiological, and biochemical changes in plants towards drought stress. The short-term changes include osmotic adjustment in roots, signaling transport, stomatal closure, decrease of carbon assimilation, assimilation of transport, decrease in expansion and leaf number and ultimately growth inhibition. Long-term changes include turgor maintenance, sustained root growth, increase of root/shoot and absorption area, osmotic adjustment and many more leads toward drought acclimation

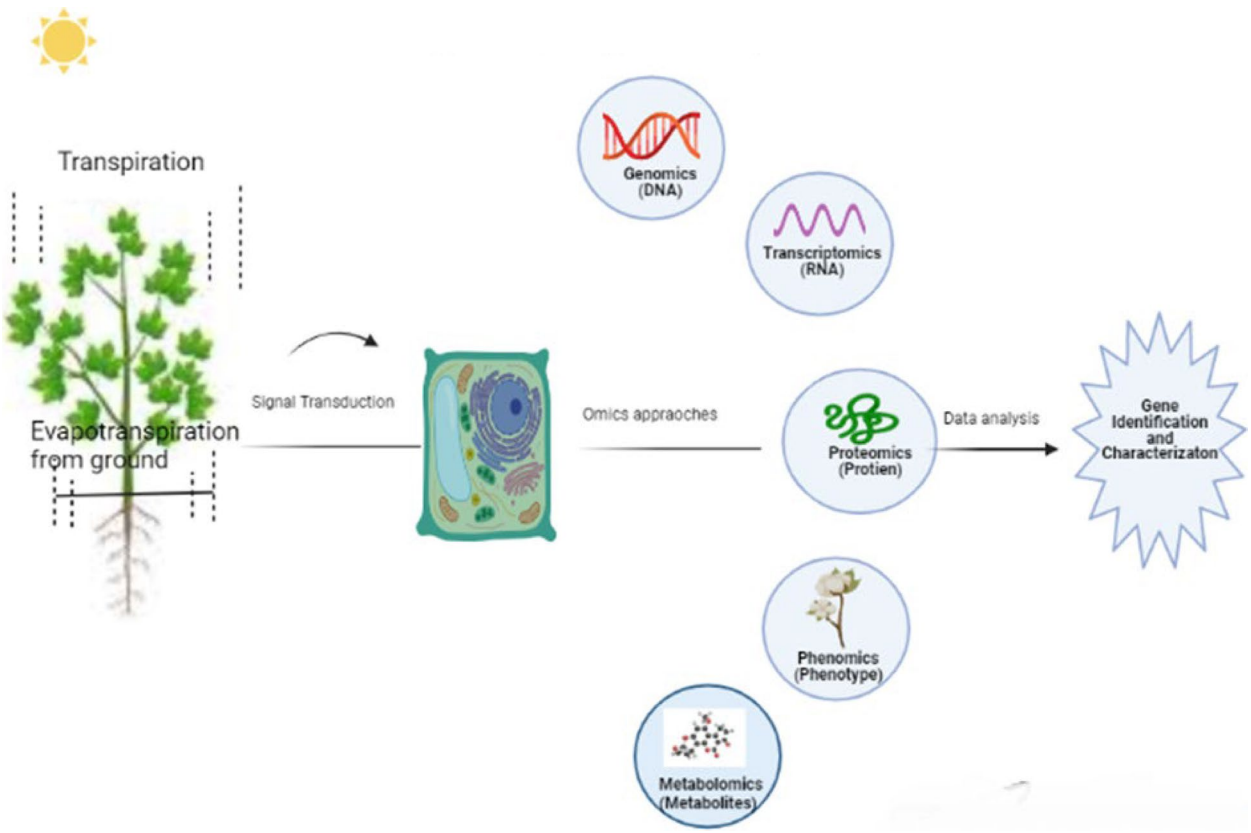

Fig. 2 Utilization of multi-omics approaches for investigating mechanism of drought response in cotton. Genomics characterizes variations at DNA level, transcriptomics describes the gene expression pattern, proteomics explores the protein abundant and their interaction, phenomics investigates the morpho-physiological parameters, and metabolomics profiles the metabolites in cotton. The data generated through these approaches are analyzed by different tools, and integrative analysis characterizes and identifies genes involved in drought response in cotton

the identification of large number of molecular markers (Hulse-Kemp et al., 2015). CottonSNP63K array, based on the Illumina technology, has been utilized for breeding and discoveries of regulatory networks in plant (Hulse-Kemp et al., 2015). Ulloa et al. (2020) reported that SNP markers are powerful for studying irrigation's effect on cotton and for marker assisted selection of drought tolerant and susceptible cultivars. Affymetrix GeneChip Cotton Genome Array was used in the development of single strand conformation polymorphism (SSCP) functional markers regarding drought tolerance. A total of fifty-two SSCP markers identified in cotton were found significantly correlated with different growth traits related to drought tolerance (Rodriguez-Uribe et al., 2014).

Genomic regions that are highly linked to drought stress tolerance traits could be used as molecular markers. A total of 4 QTLs for relative water contents, 2 QTLs for excised leaf water loss, 1 QTLs for cell membrane stability (CMS), stomatal size, and stomatal frequency have been detected using SSR and EST-SSR based marker data in upland cotton under drought stress (Amjid et al., 2015). There are 2 QTLs, one for osmotic potential and another for osmotic adjustment been identified in the intraspecific population of upland cotton, and 3 QTLs been mapped on each of chromosome 14, 16 and 25 against osmotic potential under drought stress in the interspecific population (*G. hirsutum* × *G. barbadense*) (Babar et al., 2009). Further research has also been carried out to study the genetic basis of drought tolerance in the backcrossed population of *G. tomentosum* and *G. hirsutum*, whereas 30 QTLs for CMS, chlorophyll content, saturated leaf weight, fresh and dry leaf weight, fresh and dry shoot biomass, fresh and dry shoot biomass ratio, ratio between fresh shoot biomass and fresh root biomass, total fresh and dry biomass, and the ratio between dry shoot biomass and dry root biomass have been mapped in this population. Most of the QTLs related to drought response traits have been mapped on At-genome rather than Dt-genome, in which 17 QTLs are mapped on At-subgenome and 13 on Dt-subgenome (Magwanga et al., 2020). Identified QTLs regarding fiber quality and yield contributing traits under drought stress have been enlisted in Table 1 and drought responsive physiological traits are listed in Table 2.

Genome-wide association study (GWAS) is an effective method, which can associate phenotypes with genotypes in natural populations and reveal vast natural variations and candidate genes (Edae et al., 2014; Kumar et al., 2015; Demirjian et al., 2023). Li et al. (2019) have explored the complex genetic architecture of drought tolerance in 316 upland cotton genotypes by using 81 675 SNPs, where 3, 6 and 8 SNPs show significant association with cotyledon wilting score, leaf temperature and euphylla wilting score respectively. Four candidate genes, *GhCIPK6, WRKY70, NET1A* and *SnRK2.6* identified in cotton may involve in drought stress response. In another study, 55 060 SNPs are used to identify potential candidate genes for drought tolerance in cotton, and four genes has been found, including *RD2*, *HAT22*, *PP2C* and *2C* (Hou et al., 2018). These genomics studies open a new window for marker assisted based breeding of cotton to produce better fiber quality and higher yield cultivars under drought stress.

Use of transcriptomic to explore drought tolerance mechanism in cotton

Transcriptome represents all types of transcripts produced by the organism, and in some studies from particular cell or tissue (Yang et al., 2021), which has been used to investigate the dynamic gene expression in response to a stimuli over a particular time period (El-Metwally et al., 2014). Previously transcriptome dynamics were explored by differential display-PCR (DD-PCR), cDNAs-AFLP and suppression subtractive hybridization (SSH), but they have the issue of low resolution (Nataraja et al., 2017). Nowadays with the invention of robust techniques, such as digital gene expression profiling, microarrays, and NGS, it has greatly simplified the gene expression profiling (Shah et al., 2018). Genes involved in starch and sucrose synthesis, carotenoid biosynthesis ABA precursor gene 9-cis-epoxycarotenoid dioxygenase (NCED), ABA pathway gene ABA 8'-hydroxylase, and aquaporin genes are found to be up-regulated in drought-challenged root tissue of cotton through RNA-Seq (Bowman et al., 2013). Further, higher expression of photosynthesis genes and chlorophyll a/b binding proteins have been found in roots and as compared to leaves (Ranjan et al., 2015). Transcriptional factors are important regulatory genes in drought stress signaling pathways, several TFs gene family have been up-regulated during drought stress (Fig. 3), such as genes from AP2/ERF, MYB, WRKY, bHLH, NAC, and bZIP family (Table 3).

High-throughput sequencing of small RNA libraries provided remarkable platform for the identification of microRNAs and their corresponding gene targets which respond to drought stress in crop (Jones-Rhoades et al., 2004; Xie et al., 2015; Ferdous et al., 2015). Through small RNA sequencing, four drought-responsive miRNA have been identified in cotton, including miR159–TCP3 and GRF1 present in roots and leaves, miR395–APS1 in roots and miR162–DCL1 in leaves (Wang et al., 2013).

Table 1 Published genomic regions/ QTLs associated with fiber quality and yield traits under drought stress in *G. hirsutum*

Traits	Chromosome location	Species	No. of QTLs	QTL identification model/analysis	Used marker system	References
Boll size	A1, A6, A7, A9, D15, D17, D19, D22, D23, D24, D26	*G. hirsutum*	14	Single-marker analysis	SNP	(Ulloa et al., 2020)
Number of seeds /boll	A6, A7, A9, A10, A11, D16, D17, D19, D23, D24, D26	*G. hirsutum*	13	Single-marker analysis	SNP	(Ulloa et al., 2020)
Seed index	A5, A7, A8, A9, A10, A12, D15, D16, D17, D20, D21, D22, D23, D25, D26	*G. hirsutum*	16	Single-marker analysis	SNP	(Ulloa et al., 2020)
Lint index	A2, A4, A7, A8, A9, A9, A10, A11, A13, D14, D15, D15, D19, D20, D21, D23, D24, D25, D25, D26	*G. hirsutum*	22	Single-marker analysis	SNP	(Ulloa et al., 2020)
Lint percentage	A2, A8, A9, A10, A10, A11, A13, D14, D19, D21, D22, D23, D25	*G. hirsutum*	15	Single-marker analysis	SNP	(Ulloa et al., 2020)
Fiber micronaire	A4, A5, A8, A9, A10, A13, D14, D16, D18, D19, D21, D22, D23, D24, D25	*G. hirsutum*	18	Single-marker analysis	SNP	(Ulloa et al., 2020)
Fiber strength	A8, A10, D14, D15, D17, D18, D19, D21, D23, D24, D25	*G. hirsutum*	14	Single-marker analysis	SNP	(Ulloa et al., 2020)
Fiber length	A3, A4, A8, A10, D14, D18, D19, D21, D23	*G. hirsutum*	13	Single-marker analysis	SNP	(Ulloa et al., 2020)
Fiber uniformity	A7, A8, A10, A12, A13, D14, D15, D17, D18, D19, D20, D21, D24, D25	*G. hirsutum*	15	Single-marker analysis	SNP	(Ulloa et al., 2020)
Boll size	A9, D19, D22, D26	*G. hirsutum*	4	MQM QTL model	SNP	(Ulloa et al., 2020)
Number of seeds /boll	A7, D26	*G. hirsutum*	3	MQM QTL model	SNP	(Ulloa et al., 2020)
Seed index	D23	*G. hirsutum*	1	MQM QTL model	SNP	(Ulloa et al., 2020)
Lint index	A2, A10, A13, D19	*G. hirsutum*	5	MQM QTL model	SNP	(Ulloa et al., 2020)
Lint percentage	A10, A11, A13	*G. hirsutum*	8	MQM QTL model	SNP	(Ulloa et al., 2020)
Fiber micronair	A8, A10, D14	*G. hirsutum*	7	MQM QTL model	SNP	(Ulloa et al., 2020)
Fiber strength	A8, A10, D14, D18, D24	*G. hirsutum*	7	MQM QTL model	SNP	(Ulloa et al., 2020)
Fiber length	A8, A10, D14, D21	*G. hirsutum*	6	MQM QTL model	SNP	(Ulloa et al., 2020)
Fiber uniformity	A10	*G. hirsutum*	2	MQM QTL model	SNP	(Ulloa et al., 2020)

Use of proteomics to explore drought tolerance mechanism in cotton

Most of the physiologic characters of the cell and phenotypic trait is determined by the protein rather than by nucleic acid (Deeba et al., 2012). Further, post-translational modifications of proteins i.e. phosphorylation, ubiquitination, methylation, acetylation, glycosylation, oxidation, and nitrosylation that profoundly affect their activities, which could be illustrated by omics studies (Deeba et al., 2012; Guerra et al., 2015).

Proteomic studies in cotton have been used to explore the role of different drought responsive proteins involved in signal transduction pathways, redox homeostasis for plant protection, acclimatization and activation of antioxidants under drought stress (Barkla et al., 2013). Proteomic analysis in cotton allowed the identification of 223 to 1 273 different expressed proteins under drought stress from seedling to cotton fiber development stage. Through matrix-assisted laser desorption/ionization (MALDI-TOF) and MALDI-TOF/TOF mass

Table 2 Published genomic regions/ QTLs associated with physiological traits under drought stress in cotton

Traits	Chromosome location	Species	No. of QTLs	QTL identification model/ analysis	Used marker system	References
Relative water contents	A12, D23	*G. hirsutum*	2	composite interval mapping (CIM)	SSR	(Saleem et al., 2015)
Excised water loss	D23	*G. hirsutum*	1	CIM	SSR	(Saleem et al., 2015)
Relative water contents	A5, A5	*G. hirsutum*	2	CIM	EST-SSR	(Amjid et al., 2015)
Excised water loss	A7	*G. hirsutum*	1	CIM	EST-SSR	(Amjid et al., 2015)
Cell membrane thermo stability (CMS)	A1	*G. hirsutum*	1	CIM	EST-SSR	(Amjid et al., 2015)
Stomatal frequency	A13	*G. hirsutum*	1	CIM	EST-SSR	(Amjid et al., 2015)
Stomatal size	A6	*G. hirsutum*	1	CIM	EST-SSR	(Amjid et al., 2015)
Osmotic potential	D20	*G. hirsutum*	2	CIM	SSR	(Saeed et al., 2011)
Osmotic adjustment	D20	*G. hirsutum*	1	CIM	SSR	(Saeed et al., 2011)
Osmotic potential	A6, D25, D14	*G. hirsutum* × *G. barbadense*	3	Single factor analysis	SSR	(Saeed et al., 2011)
CMS, Chlorophyll contents, and Saturated leaf weight	A1, A5, D15	*G. tomentosum* × *G. hirsutum*	5	CIM	SNP	(Magwanga et al., 2020)

spectrophotometry, proteins involved in metabolism, synthesis/ regulation of antioxidants, cellular transportation, and formation of cell structure have been found to play a key role in determining the leaf's growth and fine roots morphology. Proteins, i.e., 5 methyl tetra hydro pteroyl tri glutamate-homocysteine methyltransferase, ascorbate peroxidase, UDP-d-glucose pyrophosphorylase, the vacuolar H^+-ATPase catalytic subunit, ATP synthase CF1 alpha subunit, translation initiation factor 5A, 14–3-3 g protein, pathogenesis-related protein 10, Glycine-rich RNA-binding proteins, and GTP-binding protein G alpha subunit have been found as up-regulated in response to drought stress (Deeba et al., 2012; Xiao et al., 2020; Zhang et al., 2016; Zheng et al., 2014).

Regarding cell wall components of cotton fiber, sucrose synthase (SuSy), Uridine diphosphate glucose (UDP-Glc), and UDP-glucose pyrophosphorylas (UGPase) were enhanced in drought stress. Lignin synthetic, i.e., phenylcoumaran benzylic ether reductases (PCBER, 3203, 2705), and proteins involved in lignin methylation, i.e., S-adenosylmethionine, S-adenosylmethionine synthetase were overexpressed under drought stress leading to activate lignin biosynthesis pathway that effectively responds to avoid drought injury.

Online platform for integrative omics datasets

The era of omics has been progressing very fast with the development of automated sequencing-based techniques. In 2012, the genome sequence of a diploid cotton species (*G. raimondi*) has been released (Wang et al., 2012). Later, within two to three years, the genomes of one diploid (*G. arboreum*) and two cultivated tetraploid (*G. hirsutum* and *G. barbadense*) have also been sequenced (Du et al., 2018; Hu et al., 2019; Li et al., 2014, 2015; Wang et al., 2019a). With the availability of sequencing data, different databases, e.g., CottonGen, MaGenDB, CottonGVD, GRAND, CottonFGD and ccNet have been established for omics studies in cotton (Table 4) (You et al., 2017; Yu et al., 2014; Yang et al., 2023; Zhang et al., 2015; Wheeler et al., 2007; Zhu et al., 2017). These datasets have provided useful information for drought response analyses, such as CottonGen provides data including genome sequences, genes, markers, trait loci, genetic maps and germplasm resources (Fang et al., 2017; Yu et al., 2014); CottonFGD integrates genome sequences and annotations, genetic markers, and gene expression and sequence variation data for four *Gossypium* species (Zhu et al., 2017).

Table 3 Transcriptional factors and genes identified in cotton along with their expression and function in drought stress tolerance

Gene (TFs)	Expression	Tissue Specific Expression	Type of analysis	Type of protein	Function and Phenotype	Reference
GhABF2	Up-regulation under drought condition	Root, stem, leaves	Transcriptomic Analysis	Transcription factor	Regulation of genes linked to ABA, enhanced proline contents and the activities of CAT and SOD	(Liang et al., 2016)
GhWRKY59	Up-regulation under drought stress	Seedling	Transcriptomic Profiling	Transcription factor	Regulates drought responsive genes	(Li et al., 2017)
GhWRKY33	Up-regulation under drought stress	Leaves	Transcriptomic Analysis	Transcription factor	Negative regulator of drought responsive genes	(Wang et al., 2019b)
H^+-PPase	Up-regulation under drought stress	Root and shoot	Southern and northern blot analysis	Transporter Protein	Improved root and shoot growth, improved photosynthesis, higher chlorophyll contents	(Lv et al., 2009)
Gh_D06G0281	Coexpression under drought stress	Root, stem, leaves	Transcriptome analysis	Transcriptional factor	Reduce water loss through leaves	(Lu et al., 2019)
GhPYL9-11A	Up-regulation under drought stress	Leaf, root, stem, seed	Transcriptome analysis	ABA Receptor gene	Improved root length and up-regulate drought responsive genes	(Liang et al., 2017)

Table 4 Summary of omics databases and weblink in cotton

Database	Weblink	Description	Reference
Cottongen	https://www.cottongen.org/	Provides genomic, genetic and breeding resources of cotton	(Yu et al., 2014)
CottonFGD	https://cottonfgd.org/	Provides genetics and omics data, including genetic marker annotations, structural annotations, functional annotations, RNA-seq expression datasets, and population resequencing data	(Zhu et al., 2017)
GCGI	https://cotton.hzau.edu.cn/	Includes genomics data (genomic assembles and annotation files) of TM-1 accession of *G. hirsutum* and 3–79 accession of *G. barbadense*, SNP, and phenotypic data for GWAS in *G. hirsutum*	(Wang et al., 2019a, b)
NCBI	https://www.ncbi.nlm.nih.gov/genome/?term=cotton	A major source of bioinformatics and service tools also provides genome, transcript and protein data of different cotton species	(Wheeler et al., 2007)
GraP	http://structuralbiology.cau.edu.cn/GraP/	A source for functional genomics analysis in *G. raimondi*	(Zhang et al., 2015)
ccNET	http://structuralbiology.cau.edu.cn/gossypium/	A platform for comparative gene functional analysis across diploid and polyploidy species of cotton	(You et al., 2017)

Molecular regulatory network of drought response in cotton

Functional genomics is integrating the omics data with techniques of molecular biology and cell biology, to explore the function and regulation of genes. It relates the phenotype and genotype to analyze the molecular mechanism on levels of transcription, translation, protein–protein interaction and epigenetics. Gene editing tool CRISPR/Cas9 system and TALEN have being used to improve the crop genome without transgenes (Rinaldo et al., 2015). Particularly, CRISPER/Cas9 has been used successfully to study the drought stress tolerance by altering the drought responsive genes in rice (Wang et al., 2017a), papaya (Arroyo-Herrera et al., 2016), wheat (Kim et al., 2018) and wild tomato (Wang et al., 2017a).

When stress signal perceived by sensor proteins, i.e., RLKs and RLPs, which present in the plasma membrane activate the ABA-dependent and Independent signaling pathways, induce the drought stress response. In the ABA-dependent pathway, ABA level in the cell increases under stress condition, available ABA binds to receptor GhPYL9-11A which further dissociates the SnRK2/OST1 complex by phosphorylating SnRK2 and binds to protein phosphatase (PP2Cs) GhDRP1 (Chen et al., 2021a, b; Liang et al., 2017). Three PP2Cs, i.e., *GhHAI2*, *GhAHG3*, and *GhABI2*, have been identified (Shazadee et al., 2022). SnRK2 by interacting with multiple TFs stimulates the stomatal closure genes, i.e., *GhSLAC1*. Virus-induced gene silencing (VIGS) of *GhSnRPK2* in cotton compromise the drought tolerance in transgenic plants in contrast to wild type plants. Plants overexpressing *GhSnRK2* gene displayed less water loss, increased water content, turgor regulation, proline accumulation, and biomass in cotton under drought stress (Bello et al., 2014). Another set of PYR and PYL, i.e., *GhPYL9-5D* and *GhPYR1-3A* also have been identified in the cotton. MAPKK pathway also activates the drought-responsive TFs in the ABA-dependent manner. Increased ABA activates the MAP3Ks, i.e., GhMAP3K49 or GhMAP3K15, which activates MAKKs, i.e., GhMKK9 and GhMKK4, and then activates MAPK, i.e., GhMPK17, GhMPK7 and GhMPK6, and the cascade may activate ROS and other ABA-mediated drought stress response. GhWRKY59 is phosphorylated by MAPK cascade (GhMAP3K15-GhMKK4-GhMPK6), and the modified GhWRKY59 binds to the promoter of *GhDREB2* and regulates the expression of drought-sensitive genes (Li et al., 2017). Jasmonic acid (JA) is also involved in drought tolerance. Under water sufficient conditions, JA is absent, and jasmonate-insensitive/jasmonate-zim (JAI3/JAZ) protein interacts with several TFs, such as MYC2 (Myelocytomatosis), and reduces their activity. However, JA and its derivative are present under stress conditions and lead to the degradation of JAZ proteins resulting in the activation of MYC2. Activated MYC2 can regulate the expression of several other TFs that are important for drought tolerance, including DREB, AP2/ERF, NAC, and bZIP. VIGS-mediated silencing of *GbMYB5* transcription

factor decreases drought tolerance in cotton, with reduced proline contents, increased malondialdehyde (MDA), and antioxidants enzyme activities. Silencing of *GbMYB5* in cotton compromised the drought tolerance in cotton by reducing the recovery survival rate of post-rewatering to 50% ,in comparison to the 90% survival rate of wild type plants. These findings suggest the positive role of *GbMYB5* transcriptional factor in drought stress response (Chen et al., 2015). However, recently JAZ2 was also found to be involved in a cascade with OST1 in the ABA dependent control of the stomatal closure process, indicating that it is essential to coordinate the JA and ABA crosstalk under drought stress. Further, activation of JA-responsive genes leads to various morphological and physiological responses, such as stomatal closure, alteration of root architecture to enhance water uptake, synthesis of osmoprotectants such as proline and sugars, and reinforcement of cell walls aimed at mitigating the effects of drought stress in cotton. In cotton, similar as other plants, calcium (Ca^{2+}) signaling pathways play a crucial role in mediating responses to drought stress. Drought stress can induce the influx of calcium ions into the cytoplasm of cotton cells. This influx may be mediated by calcium channel protein GhCNGC (Chen et al., 2023). Calcium sensors such as calmodulin (CaM), calmodulin-like proteins (CMLs), Calcineurin B-like proteins (GhCBL2A1) and calcium-dependent protein kinases (GhCDPK4 and GhCDPK60) are activated in cotton cells during drought stress (Kong et al., 2023). Activated CBLs in cotton cells interact with CBL-interacting protein kinases (GhCIPK6A3, GhCIPK23), which phosphorylate target proteins involved in stress signaling pathways, ion transport, and gene expression regulation (Chao et al., 2022; He et al., 2013). Hence, calcium signaling pathways can regulate gene expression in cotton by modulating the activity of transcription factors and regulate reactive oxygen species (ROS) scavenging and antioxidant defense mechanisms, stomatal closure, and osmotic adjustment. Illustration of different signaling pathway in response to drought stress and VIGS system verified drought responsive genes have been summarized in Fig. 3 and Table 5.

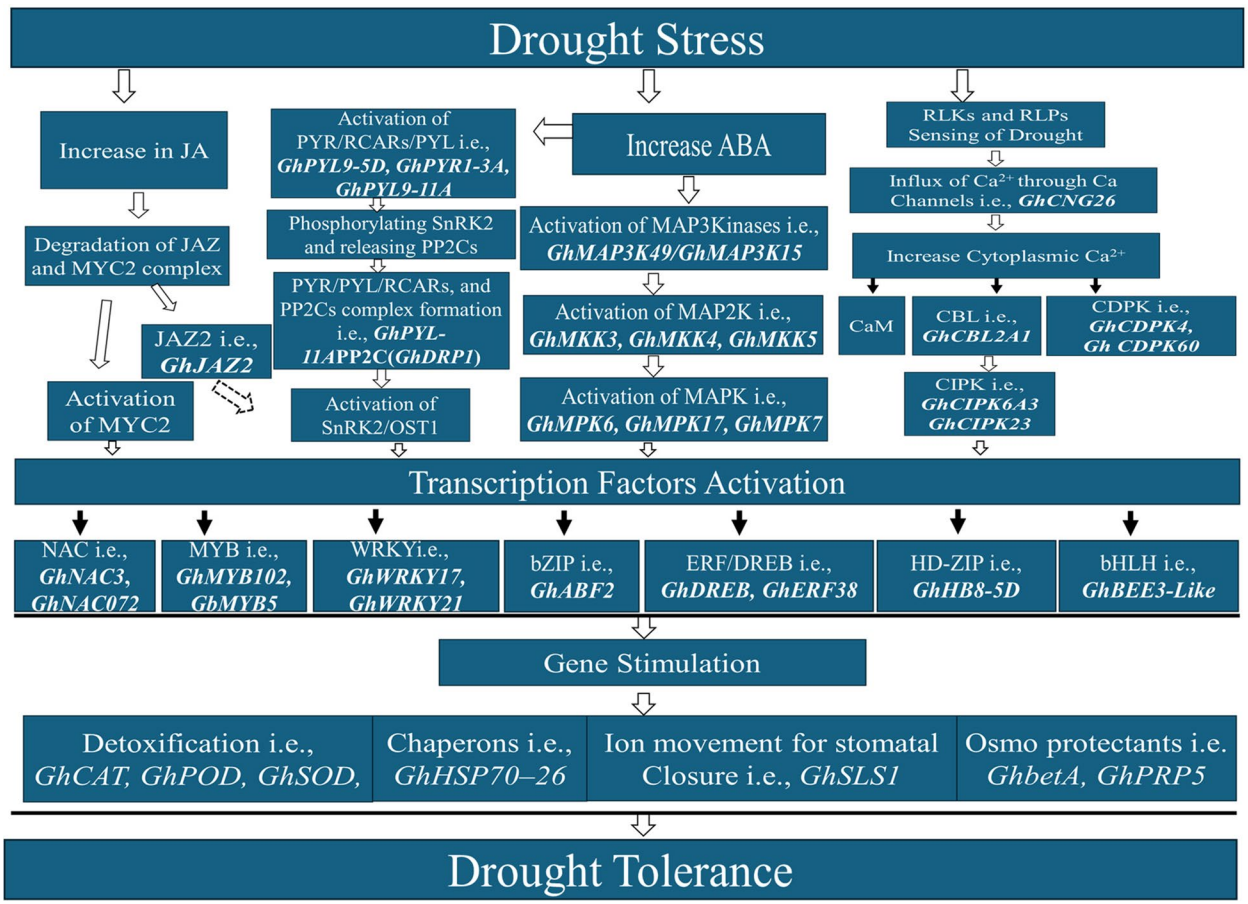

Fig. 3 Flow diagram shows activation of multiple signal transduction pathways in response to drought stress in cotton

Table 5 List of Genes involved in drought tolerance validated function through Virus induced gene silencing (VIGS) experiment in cotton

Genes	Phenotype in VIGS line	References
GhSnRK2 Sucrose non-fermenting 1-related protein kinase 2	Alleviated drought tolerance	(Bello et al., 2014)
GbMYB5 R2R3-type MYB transcription factor	Decreased drought tolerance	(Chen et al., 2015)
PHYA1	Improved drought, salt and heat tolerance	(Abdurakhmonov et al., 2014)
GhJUB1L1	Reduced drought tolerance retarded secondary cell wall (SCW)	(Chen et al., 2021a, b)
GhFAR3.1	Reduced wax contents and relative water contents of the leaves	(Lu et al., 2021)
GhNAC79	Overexpression led to early flowering and improved drought tolerance in cotton	(Guo et al., 2017)
GhWRKY46	Gene silencing led to reduced drought tolerance	(Li et al., 2021)
GhTRX134	Reduced drought tolerance	(Elasad et al., 2020)

Strategies to develop drought tolerance cultivars in cotton

Improvement of drought tolerance traits require exploring the diverse genetic resources adapting to harsh environment (Mickelbart et al., 2015; Valliyodan et al., 2017). Cotton plants are mainly discovered at water deficit areas, having greater genetic variability in drought tolerance (Pettigrew 2004). Different morphological (root, leaf, and stem) character, physiological parameters, and yield have been reported as key selection criteria of drought-tolerant cultivars in cotton (Valliyodan et al., 2017). Different drought tolerant genotypes identified/developed in cotton are presented in Table 6. Drought tolerance can be developed by using the gene editing tool to targete the negative regulators of abiotic stress responsive genes. CRISPER/Cas9 has been used successfully in cotton for genome editing in the target trait (Gao et al., 2017), but functional drought tolerant cultivars has not been reported yet. Transgenic cotton also paved the way for development of drought responsive cotton lines. For example, overexpression of *CaHB12* enhanced drought tolerance in cotton (Basso et al., 2021). Overexpression of *GhEXLB2* in cotton improved drought tolerance at germination, seedling and flowering stage (Zhang et al., 2021b). But to generate drought tolerant cultivars, there is a dire need to explore genetic diversity existed among germplasm using conventional and latest molecular approaches.

Table 6 Cotton drought tolerant genotypes identified/developed worldwide for different drought related parameters at different growth stages

Genotype Name	Criteria for Selection	Experimental Condition	Reference
IAC-13–1, Minas Sertaneja, IAC-RM4-SM5, Acala 1517E-1, and 4521	Survival rate at the seedling stage	Drought stress in growth chambers	(Penna et al., 1998)
MNH-552, SLS-1, MNH-806, 1021(Kivi), L.S.S, 841/52, MNH-6070, CIM-1100, MNH-636, MNH-812, FH-113, 4-F, MS-40, MNH-807, and FH-682	Higher boll retention capacity	Drought stress in field condition	(Dahab et al., 2012)
IUB-212, MNH-886, IR-3701, VH-144, NIAB-111, VH-295, AA-802, FH-113, NS-121, and FH-142	Stable yield performance	Drought stress in field condition	(Ullah et al., 2019)
Dexiamian 1	Physiological Parameters (root, stem, and leaf water contents, net photosynthetic rate	Drought stress in controlled condition	(Zou et al., 2020)
SPAN 837, 06K485, 06K486, FQMA	Morphological parameters	Drought stress in screen house	(Mvula et al., 2018)
DAK-66/3, GC 555, Delta Diomand, MS-30/1, Nieves, Nazilli M-503, Zeta 2, NIAB 999, and Eva	Geometric mean productivity, drought susceptibility index	Drought stress in field condition	(Sezener et al., 2015)
Delcerro, Zeta 2, DAK 66/3, and Nazilli 87	Water use efficient	Drought stress in field condition	(Baytar et al., 2018)

Conclusion

Development of drought tolerant genotypes is a major challenge for the cotton breeders because of the complex inheritance pattern of quantitative drought response related traits and the difficulty in accurate measurement of the polygenic trait. Omics approaches like genomics, transcriptomics and proteomics have been extensively used to investigate molecular regulatory networks in cotton in response to drought stress. However, metabolomics, phenomics, and epigenomics have been lagging. The combination of GWAS with transcriptomics and proteomics has been untilized as a powerful tool to reveal regulatory network in drought tolerance and cotton improvement. Meanwhile, the availability of online cotton databases facilitate integrative analysis of omics data.

Advanced multi-omics like genomics, transcriptomics, proteomics together with precise and accurate phenotyping of drought related traits exploring the mechanisms of cotton plant's response to drought stress is limited in laboratory setup so far, and has not been utilized in breeding. With the improvement of omic technique and data analytic tools, integration of conventional breeding and omics-based breeding is an efficient route to develop drought tolerant cultivars in the future.

Authors' contributions

Sharif I conceived the idea and compiled the manuscript, Aleem S contributed in collection of literature and write up, Junaid JA and Aleem M helped in finalizing the manuscript. Shahzad R, Farooq J, and Ellahi F reviewed and edited the paper. Arshad W and Khan MI revised it critically for important intellectual contents. All authors read and approved the final manuscript.

Funding

Not applicable.

Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 19 September 2023 Accepted: 29 July 2024
Published online: 11 September 2024

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