A comprehensive review on drought stress response in cotton at physiological, biochemical and molecular level

Mobeen Babar¹, Muhammad Nouman Khalid²*, Muhammad Waqar Ul Haq², Mamoonah Hanif³, Zeeshan Ali⁴, Muhammad Awais², Zaid Rasheed², Muhammad Faizan Ali⁵, Irfan Iftikhar⁶, Shahzad Saleem⁷ and Ifrah Amjad²

1. Centre for Excellence in Molecular Biology, University of the Punjab Lahore, Pakistan
2. Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan
3. Cotton Research Institute, Multan, Pakistan
4. Department of Agronomy, University of Agriculture Faisalabad, Pakistan
5. Department of Agronomy, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan
6. Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Pakistan
7. Department of Soil and Environmental Sciences, Muhammad Nawaz Shareef University of Agriculture, Multan, Pakistan

*Corresponding author’s email: noumankhalidpbg@gmail.com

Abstract

Cotton (Gossypium hirsutum L.) is a fiber crop cultivated in a variety of climatic settings around the world. Cotton and its by-products are in high demand because of increased usage in the textile sector and provision of edible oil. Drought events are projected to become more frequent in many areas due to erratic rainfall patterns and rising temperatures brought on by climate change. Growth, development, yield and fiber quality of cotton is greatly affected by drought. Plants, on the other hand, have evolved many cellular and molecular systems to deal with drought stress. Plant’s response to drought relies on various factors like time span and severity of stress, growth stage of plant and time to stress exposure. Different visible adaptations are closing of stomata, epicuticular wax deposition on aerial parts, greater root area, leaf rolling, membrane stabilization and osmotic adjustment. To respond against a stress for self-defense is an important function of cell. This protection is triggered by a change in gene expression patterns. As a result of stress different qualitative and quantitative alterations occur in proteins as a consequence which lead to modulation of various pathways involved in maintaining metabolism and defending cell. In this review article different physiological, biochemical and molecular characteristics of cotton under drought stress have been discussed.

Keywords: abiotic stress; cotton; drought; gene expression; plant responses; proline; universal stress proteins
**Introduction**
Cotton is the major cash crop of Pakistan belonging to family malvaceae and genus *Gossypium* [1]. In 2021-2022 (Fig. 1), cotton was grown on a total area of 1,937 thousand hectares producing 8,329 million bales with an average yield of 618 kg/ha while in the previous year average yield was 707 kg/ha from a total area of 2,079 thousand hectares producing a total of 7,064 million bales [2]. In spite of decline in growing area, cotton production enhanced because of improved yield. The enhancement in cotton yield was attributed to favorable climatic conditions, smooth input supplies, progressive crop management practices and praising cotton prices in international and domestic market [3]. But during the last decade or so, area under cotton cultivation has declined and replaced by its competing crops like sugarcane, maize, potato and rice primarily due to drought. That is the reason why so much extensive studies have been done for making cotton plant tolerant to drought stress [4]. All around the globe, 4 species of cotton are cultivated which are diploid *Gossypium arboreum* also known as ‘desi cotton’ and *Gossypium herbaceum*. The other two species are *Gossypium hirsutum* known as ‘American cotton’ and *Gossypium barbadense*. All these species are easy to be identified on the basis of their shape of leaves, flowers, bolls, fiber characteristics and growth habit [3-4].

![Figure 1: Area and production of cotton in Pakistan (Economy survey of Pakistan 2021-2022)](image)

**Abiotic stresses**
Terrestrial plants have been subject to severe environmental conditions since the very beginning. A big number of physical and chemical factors affect them such ultraviolet radiation, excessive or deficient rain fall, heavy metals, high salinity, low and high temperatures and some others (Fig. 2). These stresses are known as abiotic stress result in yield loss and are severe danger to agricultural crops and the ecosystem [5]. Because plants are sessile in nature, they come across various stresses and adapt potential tactics in order to survive and cope with these stresses and plants are prone to more than on stress at different stages of growth [6].

Over time, plants have developed various physiological, cellular and morphological systems and structures. One of such structures is cuticle wax layer which is composed of cutin and covers the epidermal parts of the land plants and saves them from desiccation and environmental stresses [7]. This adaptation also helps plants avoiding drought that is multidimensional stress which affects plants at variegated levels and is caused by depletion of soil moisture because of less rainfall. The degree to which a plant is influenced by drought relies on the time span...
of stress, stage of plant growth, severity of stress [8].

**Figure 2: Major abiotic stresses in plants**

**Responses of plants to drought stress**

Water is of much importance in a plant’s growth and development. Water is essential for all the operations in plants like germination, elongation, cellular division, different metabolic activities like photosynthesis, respiration and many more biochemical and physiological activities. It is water which is the salient substance from the very first stage of a plant, germination, up to its establishment in the soil [7]. Drought is the depletion of soil moisture level due to a period of less rainfall. Difference between drought and desiccation is that loss of water in drought is less on the other hand desiccation is far more considerable loss of water. Drought causes closure of stomata and gaseous exchange while desiccation causes damage at cellular level like cell structure and different metabolic reactions. Plants are immensely damaged by drought and to develop tolerance against drought has been the very objective of breeders [9]. Drought triggers many physiological, morphological and biochemical reactions in plants [10]. Drought affects both longitudinal and lateral plant growth and its affects have been vastly studied [11].

Plant’s response to drought relies on various factors like time span and severity of stress, growth stage of plant and time to stress exposure. Extent, to which a plant will respond under stress mainly depend on the stage of growth and water use efficiency [12]. Reproductive phase is thought to be the most critical stage in different crops susceptible to drought stress [13]. With evolution, plants have advanced in growing various modifications in order to respond to less available soil moisture. Different visible adaptations are closing of stomata, epicuticular wax deposition on aerial parts, greater root area, leaf rolling, membrane stabilization and osmotic adjustment [14]. Water deficiency limits plant growth as it decreases net carbon dioxide assimilation due to closing of stomata as gaseous exchange takes place through stomata. Studies have also found that photorespiration also decreases in response to drought stress [15]. Water shortage resulted in decreased stem length in soybean [16] and a decline in leaf respiration was also observed as a result of water deficiency [17]. In rice, number of tillers, cell division, grain weight, leaf area
and yield were decreased as a consequence of decreased moisture in soil [18].
Dry periods result in slowed production of plant shoots and leaves causing a decline in canopy development as a result of leaf senescence in sugarcane [19]. Shoot and leaf senescence as a result of water shortage in sugarcane [20]. A decrease in imbibition, sprouting and sapling growth was observed in maize [21]. Water stress caused 25% height reduction in citrus seedlings [22]. Hydrostatic pressure is around zero in soils with high moisture content. Soil moisture content reduction results in the decrease of hydrostatic pressure that becomes completely negative. As a result of which, water is tightly held to soil particles because of electrical charge on them and becomes unavailable to the roots [23]. In addition, the continuous uptake of water by roots leads to reduced moisture content in neighboring soil. On the one hand available water decreases and on the other hand there is continuous deprivation of water by transpiration, which results in plant experience drought stress (Fig. 3). When plant experiences water deficit, transformation of starch into soluble sugars occur and enhancement in the proportion of free amino acids also occur [24]. Proline content increases as a result of drought stress and it is crucial for a plant to adjust to drought stress [25] as it plays part as an osmoprotectant in plant species and is produced as a result of hydrolysis of proteins [26]. Proline also contributes in removal of free radicals and stabilizing membranes [27]. Undergoing drought stress results in the aggregation of solutes inside cells to balance the volume due to loss of water in these photosynthetic organisms [28]. It is evident that stresses such as drought has affected various physiological and biochemical activities. Different crops respond variably to drought stress. Conferring to various plant characters, response of different plants can be studied.

![Figure 3. Plant’s responses to drought stress](image)

**Leaf water potential**
To enhance drought tolerance in crops, water potential of leaves and osmotic adjustments (OA) are characteristics which can be utilized as selection criteria. Leaf water potential (LWP) is thought to be the indicator for water status of a whole plant [29] and to avoid dehydration, maintaining a high LWP is necessary [30]. The preservation of LWP is maintained by various mechanisms such as ornamentation of water uptake, approach to soil water, canopy size, stomatal
conductance, leaf rolling [31]. In wheat, water potential of leaves decreased dramatically when there was increase in temperature and the study also suggested that a relationship exists between mesophyll conductance and temperature [32]. Low stomatal density resulted in less stomatal conductance in drought tolerant rice and along with rice, wheat and *Oryza glaberrima* cultivars responded to drought by minimizing the decrease in mesophyll conductance through maintaining the ratio of chloroplast to exposed mesophyll cell walls high [33].

**Chlorophyll fluorescence**

Drought stress caused a decline in gaseous exchange, chlorophyll fluorescence and photosynthetic rate in dry beans [34]. Maximum to minimum ratio of chlorophyll fluorescence measured at 685 nm dropped due to water stress and it also blocked electron flow from photosystem II. Modulated chlorophyll fluorescence and fluorescence induction kinetics. Osmotic stress decreased the effectiveness of excitation energy captured by photosystem II and more significantly decreased photochemical quenching [35].

**Relative water content (RWC)**

Water deficiency brought alterations in the RWC of leaves in various varieties of wheat [36]. Experiment of drought on how effectively a plant uses its water and RWC [37]. It showed that drought has significant effect on the RWC. Reduction in RWC in cotton as a result of stress and when stress was removed, RWC recuperated after 48 hours [38]. Different genotypes of cotton act differently in RWC reduction levels as in *G. herbaceum* there is slow reduction in RWC as compared to *G. hirsutum* [39].

**Chlorophyll content**

A green pigment that occurs in chloroplasts of green plants cells is chlorophyll. In fact, these are required photosynthetic pigments which absorb light energy and are capable of synthesizing carbohydrates in plants. The chlorophyll content in a plant tissue is representative of photosynthetic ability of that plant. Three characteristics which are amount of chlorophyll, soluble sugar content and photosynthetic rate were highest in *G. barbadense*, after which *G. arboreum* ranks second, *G. herbaceum* comes in third place and *G. hirsutum* showed the least of them. Influence of drought stress on chlorophyll amount in cotton genotypes [39]. Decline in soil water potential also reduced the total amount of chlorophyll in cotton [40]. Nitrogen metabolism and osmotic adjustment in cotton in response to potassium application and evaluated that potassium contributed towards maintaining soluble proteins and chlorophyll contents which in result contributed towards drought tolerance [41].

**Cell membrane stability**

For a plant’s survival during stress integrity and stability of cell membrane is much essential as processes such as photosynthesis and respiration occur only when cell membranes are in good condition. Any disruption to these structures cause damage to plant’s all metabolic processes. kinetic energy and motion of molecules across membranes increase as a result of high temperature and water stress, thus slacking chemical bonds with cellular membranes. As a result of which lipid bilayer structure of these membranes disrupt either by protein denaturation or by enhancement in the amount of unsaturated fatty acids [42]. Integrity of all biological membranes can be prone to increased temperatures and drought stress because these alter the higher organization structures including protein’s tertiary and quaternary structures in membrane. The increased solute leakage, as a signal of declined cell membrane stability (CMS), has been utilized for long as an indirect method to asses tolerance against drought stress in various plant species, including tomato, potato, soybean, cotton, sorghum, cowpea and barley [43].
Electrolyte leakage depends on the plant species, plant age, growing season, tissue type, degree of hardening and developmental stage. Different plant species respond differently to stress and show different levels of RMP. Increased nitrogen application can be helpful in stabilizing cell membranes. RMP was increased with nitrogen application under drought stress [44]. In *Arabidopsis*, amount of total lipid in membranes declined and became about half. Along with it the ratio of saturated to unsaturated fatty acids also reduced to one-third of amount at usual temperatures of the plants subjected to high temperature [45].

**Total soluble sugars**

Environmental stimulus where affect the efficiency of photosynthesis it also affects other plant parts. In plants, some non-green parts that are not directly involved in photosynthesis such as roots, flowers and stems act as consumers of carbon during some parts in their life cycle. As leaves act as source of soluble sugars and roots and stem as sink, so when there is a decrease in photosynthesis it also causes reduction in the supply of soluble sugars. In such conditions, different physiological and biochemical changes occur in order to keep going certain metabolic processes. The early phases of plant’s life cycle like germination and seedling growth are highly dependent on carbohydrates mainly which are transferred from seed to other organs where these are required maintenance of homeostasis and growth. Studies have also suggested that sugar contents increase in plants as a consequence of various stresses like drought, flooding, salinity and cold. But later, it was also proved that variations in soluble sugars contents don’t follow the same pattern and vary with stress type and genotype [46]. In *Arabidopsis*, effects of drought and heat stress on source and sink transitions and CO$_2$ assimilation has been studied. Variations in the amount of total soluble sugars under different abiotic stresses also bring changes in carbon dioxide assimilation, carbon concentration present in source and sink and in production of specific proteins [47].

**Role of proline in stress tolerance**

Proline is a cyclic amino acid with low molecular weight and is proven to assist plants in osmotic adjustments when plants encounter stress. Proline is a substance released as a consequence of stress responses and plays role as an osmo-protectant. A positive correlation is present between amount of proline produced and drought tolerance [48]. Proline may also give a preservative effect by induction of such proteins which are stress responsive. In plants, a relative higher amount of proline helps in maintaining water potentials lower than normal. When water potential reduces the amount of osmolytes taking part in osmoregulation increases, it allows additional water to enter from the environment. This assists in minimizing the immediate effect of water deficit. Proline lessens the consequences of reactive oxygen species (ROS) by balancing antioxidant system by osmotic adjustments and saving the integrity of cell membranes [49]. The importance of enhanced proline level in various plants subjected to many stresses such as salinity, water deficiency, high temperature, low temperature, UV-B radiations and heavy metal stress has been described. There is no contradiction in that proline also increases the expression of some salt and drought tolerant genes [50].

Phytohormones are photoreceptors to detect light in plants and also act as chemical messengers which are linked to proline metabolism and help enhance stress tolerance. Available reports have presented that phytohormones are linked collaboratively with proline metabolism and stress tolerance. Yet, the part played by phytohormones in mandating of proline biosynthesis is still to be totally
known. Efforts have been done in order to spot the regulators which can perhaps link synergistically/antagonistically with proline metabolism and affect the tolerance to salt and drought. Enhanced proline accumulation as a result to drought stress has been described in various crops as *Sorghum bicolor*, *Triticum aestivum*, *Gossypium hirsutum* [51].

**Oxidative stress and reactive oxygen species (ROS)**

We can define oxidative stress as a disproportion between free radicals and antioxidants. Drought stress advances to oxidative stress in plant cell in response to more out flow of electrons to oxygen in the course of photosynthesis and respiration leading to boosting of ROS generation. The ROS including H$_2$O$_2$, OH and O$_2^-$ can attack lipids present in the membrane, inactivate basic metabolic enzymes and harm nucleic acids resulting in apoptosis. Photorespiration is among the main factors of ROS production in drought stress condition with a net production of H$_2$O$_2$ up to 70%. In order to adapt to ROS production, plants have succeeded in developing endogenic mechanisms and they are considered to counter water deficiency by reinforcing such protective mechanisms [52]. So, boosting the functions of antioxidant components, which are present naturally, may prove useful and can be used as one strategy for minimizing or avoiding damage caused by oxidative stress and increasing resistance against stress.

Certain evidences have shown the involvement of ROS acting as signals for various gene expressions. It is contemplated that ROS take part in signaling pathways and shielding responses of cells at low concentration, and if these ROS are occurring in higher concentrations, they may oxidize nucleic acids, proteins and lipids and bring oxidative damage which is irreversible. However, this opinion is oversimplified, as the position and timing of ROS and formation of antioxidants are leading elements for plant reactions. An enhancement in levels of ROS may initiate a semi or extreme oxidation of a cell’s components resulting redox status alterations therefore a constant control over the level of ROS is critical under stress conditions [21]. On the other hand, a majority of cellular compartments retain reducing surroundings, and when organism encounters stress it leads to higher concentration of ROS and cause oxidative stress, that is also described as a disturbance in redox signaling and redox check [26].

**Drought inducible gene expression**

In plants various types of genes are expressed when they encounter stress. Similarly, under water deficient conditions some genes are also expressed and these genes can be categorized into two categories on the basis of microarray analysis in *Arabidopsis* [7]. In the first group, such proteins are included which most commonly play role in making plants tolerant to abiotic stresses. Late embryogenesis abundant (LEA) proteins, antifreeze proteins, mRNA binding proteins, chaperones, enzymes playing role in biosynthesis of osmolytes, sugars, detoxification enzymes and different types of proteases and proteins of water channel [12]. Proteins that take part in regulation are included in second group. Such as proteins required in further signal transduction regulation and expression of genes which are responsive to stress. Different protein phosphatases, transcription factors, protein kinases, signaling molecules like calmodulin binding proteins and enzymes required in the metabolism of phospholipids. A number of transcription factor genes were inducible as a result of stress. It is suggested that different mechanisms that regulate transcription may play role in regulating drought, high salinity or cold stress signal transduction pathways as much more transcription factors of genes were induced as a result of stress. The expression of such genes as a result of stress
might be governed collectively or individually by above mentioned transcription factors [53].

**Role of universal stress proteins (USPs) in stress tolerance**

To respond against a stress for self-defense is an important function of cell. This protection is triggered by a change in gene expression patterns. As a result of stress different qualitative and quantitative alterations occur in proteins as a consequence which lead to modulation of various pathways involved in maintaining metabolism and defending cell. Abiotic stresses commonly result in dysfunction of proteins. They are capable of altering the organization levels of many proteins that are either soluble or structural in their function naturally [54].

Universal stress proteins (USPs) function in scaffolding, keeping the globular structure and avoiding the deterioration of globular macromolecules, and protein transport in cell. In addition to these, various USPs have the functions of repairing damaged DNA, DNA binding and refolding activities which are helpful to organisms in protecting their nucleic acids from the effects of external stresses. Along with all these multi-functional roles, USPs are also involved in many other roles such as functional motifs and that’s why USPs exhibit a vast degree of diversities in their structures. Flavoproteins are also included in protein groups which are USP-like, that play role in N-type protein phosphatase, electron transport and ATP [55].

On the basis of structural homology of USPA protein, from *Haemophilus influenza*, to X-ray crystals USPs are mainly categorized into two classes. USPs containing an ATP-binding motif present at their C-terminal region and have an alpha or beta-core structure comprising of five beta-strands and four alpha-helical structures are placed in the first group. While, in the second group such polypeptides are placed which are insufficient in the ATP-binding motif and are unable to adhere or utilize ATP. As a result of diversity in the structures and functions of USPs a number of orthologous proteins are put in USP groups, yielding a mega USP super family [56]. All the proteins in plants contain no less than one USP domain and on the other hand catalytic motifs that are expressed variably in certain tissues and organs at different developmental stages or under divergent stress circumstances [57]. In order to save plants from harms of different stresses brought by environment, they must evolve really promoted and sophisticated systems [58]. In *Arabidopsis* genome, all the USPs, which are 44 in number, have an ATP-binding site and display a large homology in sequence as compared to the protein family of 1MJH. In plants, the proteins exhibit various functions in shielding plants from various stresses like HRU1, USP present in *Arabidopsis*, controls hydrogen peroxide(H$_2$O$_2$) level inside cells under hypoxic circumstances and converting signal of oxygen deficiency to the defense signaling pathway present in the downstream [58, 59].

When a plant is exposed of water deficiency various cellular metabolisms and association between source and sink is disturbed, along with it certain elemental metabolisms are also affected. Inside cell various responses including water circulation regulation, osmotic adjustment, protecting cell’s internal environment from oxidative stress. When a certain plant encounters sudden high temperature which may lead to oxidative stress, redox status within the cell is altered to cause a move to USPs having low molecular weight to a high molecular weight complex [60]. The USP structure which occur as an inactive monomer or dimer in optimum conditions can be transformed into a highly oligomer complex in reaction to external stress, that enables the protein to obtain a new function of acting as ‘molecular chaperone’ acting on cytoplasm of plant cell.
After this, the holdase chaperone function of USP renders it to avoid denaturation of molecules within the cell from oxidative stress or heat shock. In addition to temperature-related ability of USPs, those present in *Salicornia brachiata* (SbUSP) has been studied to be involved in resistance to abiotic stress. There are more evidences supporting that along with salt stress, spUSP gene is activated when plant faces heat or cold shock, drought, wounding, treatment of paraquat and phytohormone such as abscisic acid, ethylene and gibberellic acid [9]. Transcriptional expression of both GaUSP1 and GaUSP2 in *Gossypium arboreum* is stimulated by drought stress, showing that these two GaUSPs have role in controlling water content within the cell. Treatment of plants with various stress-inducing elements like water scarcity, excessive salt concentration, insufficient light, phytohormones and heavy metals strongly enhance the function of USP promoters in cotton [60].

**Zinc finger (ZnF) proteins response under stresses**

Under environmental stress, various transcription factors and ZnF proteins have been proven to be playing role in regulating important responses in plants [57]. ZnF proteins involved in various stress responses have been discovered in rice. These proteins have been reported in many plant species like *Arabidopsis thaliana*, cotton, *Petunia*, rice, wheat and soybean [44].

As mentioned above many zinc fingers have been discovered in plants and investigations have been done in order to know about their role in growth, development and various abiotic stresses like cold, salinity, darkness and drought. A group of ZnF proteins known as C$_2$H$_2$ which are plant specific can be categorized into 3 classes on the basis of pattern and number of zinc fingers. First of them is tC$_2$H$_2$ proteins having 3 zinc fingers, second are the maC$_2$H$_2$ and third are spC$_2$H$_2$. C$_2$H$_2$ group of ZnF proteins are involved in tolerance against drought stress through ABA dependent and ABA independent pathways. Along with it, C$_2$H$_2$ proteins have the potential to increase endurance to drought by controlling levels of proline, ROS and other components of cells. In *Arabidopsis* drought stress enhanced the expression of ZAT18 that is C$_2$H$_2$ZnF protein. Similarly, increased expression of IbZFP1 also induced tolerance to drought and salinity in *Arabidopsis*, a gene involved in the up-regulation of such gene those are responsible for ABA signaling, ROS scavenging and proline synthesis. The expression of this gene also resulted an increase in chlorophyll, ABA, total soluble sugars, proline, abundant proteins in late embryogenesis and a decrease in MDA content and H$_2$O$_2$ [61].

ZFP245 is a C$_2$H$_2$ type ZnF protein which is expressed in rice plant as an outcome of drought stress and expression of this gene also resulted in tolerance to water deficiency as ZFP245 expression caused an enhanced sensitivity to ABA thus acting in a pathway which is ABA dependent. As a result of drought stress in cotton, proteins associated to stress tolerance having A20/AN1 zinc finger were identified through BLAST by using previously available stress associated genes of Maize, *Arabidopsis*, Potato as model sequences [62].

**Biotechnological applications of USPs in stress tolerant crops development**

The expression of specific genes in a plant initiate various biological signaling pathways causing changes in plant’s growth, physiology and yield by triggering metabolic cellular networks. It is evident that the complicated signaling pathways which are involved in the responses to stress regulate the activities of one another by different translational and post-translational alterations [63]. As mentioned, USPs are involved in a number of defense mechanisms against external stresses. Therefore,
controlling the expression of these genes may be of use in development of such crops which are tolerant to these stresses and success of this strategy needs a detailed molecular understanding of functions of USPs on the aspect of their stress tolerance abilities. Combined efforts are required from various fields like genetic engineering, molecular biology and plant breeding to combine various techniques in order to support this idea of manipulating the function of USPs to produce such crops those are stress tolerant and also give high yields under stress environment. Thus, developing crop varieties, under unfavorable conditions like controlled high temperatures and water scarcity, will result in the expression of USPs, can be helpful [64].

Authors’ contributions
All authors contribute equally.

References:


