



RESEARCH ARTICLE

## Effect of Drought Stress and Response in Cotton

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### ABSTRACT

*Gossypium hirsutum* is one of the most vital economical crops. With climatic changes and global warming, its production is seriously affected and decreased. Among these climatic stresses, drought is the leading abiotic stress that lessens its yields. It affects negatively (directly or indirectly) on several processes including morphological, physiological, anatomical and biochemical processes in cotton plants that leads to down regulation of cotton plant's growth. Under drought stress, cotton plants reduce water loss through rolling of leaf margins, which decreases the surface area exposed to radiation. This response greatly inhibits the rate of photosynthesis, mostly because of reduced stomata conductance. The diminished photosynthetic activity results in a decline in dry matter, suggesting that flowers are somewhat protected from water scarcity compared to neighboring leaves. Various stress-responsive genes also participate in this process and aid the cotton plant in its ability to endure unfavorable conditions. In addition, drought stress decreases the expression of GhSUT-1, a sucrose transporter that is essential for exporting photosynthetic carbon assimilates. Drought stress affects important enzymes involved in starch production, including AGPase, GBSSase, and SSSase. During drought conditions, the activity of GBSSase, which is responsible for the synthesis of amylose, increases. However, the activity of AGPase, which is involved in the development of both amylose and amylopectin, is greatly reduced, thereby impeding the production of both starch components. In addition, a reduction in SSSase activity leads to a decrease in the generation of starch and a decrease in the amount of energy produced by the oxidation of glucose in cellular respiration.

**Key words:** Starch biosynthesis, Stress responsive genes, Amylose, Photosynthetic activity

### INTRODUCTION

Cotton (genus *Gossypium*) shows two different habits such as naturally it is a perennial plant and, commercially it is annual in different parts of the world (Egbuta et al. 2017; Zafar et al., 2024a). Cotton fiber development occur in four phases which includes protoderm differentiation, elongation, secondary wall synthesis and fiber maturation stage on last (Zou et al. 2016; Rehman et al. 2019; Zafar et al., 2024b). It produces an important textile fiber that fulfill about 35% of the world's fiber demand. It can be used as model system for the study of different mechanisms like plant polyploidization, cell elongation etc. *Gossypium hirsutum* produces about 95% of the total natural lint, mostly used by textile industries (Huang et al. 2021; Zafar et al.,

2024c). It is an important economic crop in different parts of world including Pakistan, China, India and Brazil etc (Meyer 2019). Its yearly economic impact was about 600 Billion dollars (Khan et al. 2020). In 2022-23 its production rate was 6.68 million metric tons in China, 5.66 million metric tons in India, 3.15 million metric tons in USA, 0.85 million metric tons in Pakistan and about 3.06 million metric tons in Brazil (Statista 2023). Ali et al. (2019), reported that about 15% of cultivated area in Pakistan is cultivated with cotton plants and 1.3 million Pakistani farmers cultivate cotton plants on their agricultural land which is about 3 million hectares which makes Pakistan third biggest exporter of raw cotton and fourth largest cotton producing country in the world. It is mainly cultivated in warm areas of Pakistan like Punjab (~80%) and remaining in Sindh.

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Cotton buds is utilized as raw material in different products like textiles related products, edible oil, as a fodder for life stock and many medicinal products as well (Blackwood 2002; Rogers et al. 2002; Hegde et al. 2004; Raju et al. 2008; Ezuruike et al. 2014; Zain-ul-Hudda et al., 2024). Cotton plant is also the source of precious by-product like terpenes, carbohydrates, lipids, proteins, fatty acids and phenolics etc (Bell & Memphis 1986; Shakhidoyatov et al. 1997; Perveen et al. 2001; Perveen et al. 2001; Essien et al. 2011; Hu et al. 2011; Zafar et al., 2023a). These compounds played functional role and are very essential for living organisms especially humans (Rogerio et al. 2009; Essien et al. 2011; Sánchez-Muñoz et al. 2012; Ku et al. 2013; Suijun et al. 2014). For example, the compound that plays the contraceptive effects in humans and animals is Gossypol, and it is phenolic compound (Han et al. 2007), The increase in globally cotton cultivation has resulted into the waste of millions of tons of residual after ginning (Buser 2001; Knox et al. 2006). These by-products can be used as nutritional supplements for animal (Knox et al. 2006; Kennedy et al. 2008; Wilde et al. 2010). It is also used as commercial bio-fuel applications (Jeoh 1998; Sharma-Shivappa et al. 2008; McIntosh et al. 2014).

The fiber quality of cotton depends upon many physical and morphological characteristics that enable it to flex and make it capable of being spun into fibers (Chee et al. 2005; Zafar et al., 2023b). The important physical characters of cotton fiber are its strength, fineness (micronaire) and length of fiber (Poehlman et al. 1995). The deposition of cellulose molecules inside cotton fiber is in positive correlation with the strength of fiber hence the cotton fiber will be strong with cellulose chains (Bradow & Davidonis 2000).

The production of cotton all over the worlds is severely affected by bad environmental conditions. The abiotic stress factors like flood, drought, heat waves, soil pH and salinity and global warming disturbing its growth and development. While the biotic factors like cotton pests and diseases, weeds and evolved herbicides lowers its yields (Mollae et al. 2019; Zafar et al., 2020). Among these stress factors, drought became the major threat for cotton because of its long and continuous spells all with climatic changes all over the world. Cotton fiber is badly affected by drought

because drought directly interferes the plant's internal mechanisms like photosynthesis, carbohydrate metabolism, vavoular invertase and starch synthase enzymes by producing water deficiency in cotton plants. Also under drought stress conditions, cotton plant shows reproductive failure, poor pollen fertility etc which ultimately affects cotton fiber development and its yields (Ul-Allah et al. 2021; Zafar et al., 2022). This review discuss effects of stress conditions mainly drought stress and response of cotton plant physiological, morphologically, genetically and biochemically at organism and cellular level against these stress conditions.

### Cotton Plant Physiological and Biochemical Responses to Drought Stress

Drought limits global crop yields by disrupting plant growth and water efficiency. It reduces photosynthesis through stomatal closure and affects enzyme functions crucial for energy production (Farooq et al. 2012). In cotton, drought during full bloom severely impacts fiber development (Snowden et al. 2014) and affects fiber thickness and strength during secondary cell wall formation, which are essential for lint weight (Gao et al. 2020). Mature cotton fibers are stronger due to their thicker secondary walls and smaller inner cavities (Zhang et al. 2019). Water scarcity disrupts cell expansion and carbohydrate metabolism, reducing fiber length, uniformity, and strength (Witt et al. 2020).

Drought affects fiber length, strength, and micronaire value, with upper fruiting bodies being more vulnerable than lower ones (Wang et al. 2016). Adequate water at all fiber development stages is crucial for optimal yield and quality (Rehman et al. 2019; Zhao et al. 2019). While drought early in the plant's life reduces plant height and node count, later water availability can compensate for yield (Ullah et al. 2017; Ibrahim et al. 2019).

Cotton's response to drought includes changes in photosynthesis, stomatal closure, root development, and production of abscisic acid (ABA) and jasmonic acid, alongside ROS scavenging (Ullah et al. 2017). Antioxidants, phytohormones, nutrient management, and other strategies can help mitigate drought stress (EL Sabagh et al. 2020).

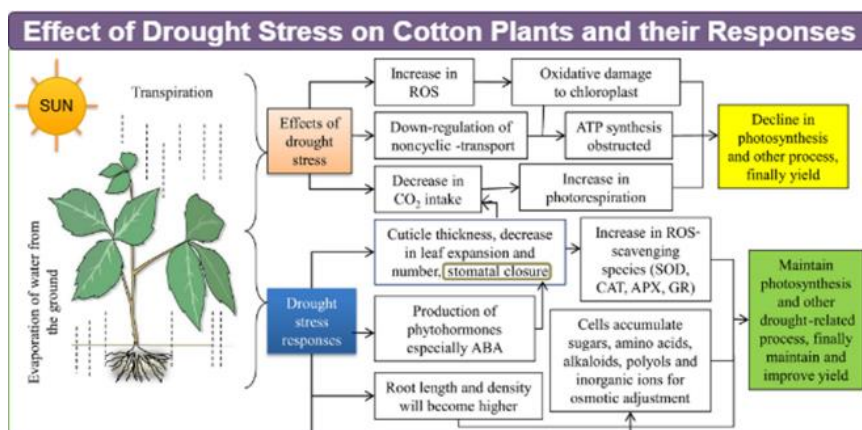


Fig. 1: Ullah et al. (2017), reported the general mechanism in cotton plants in response to drought stress

### Photosynthesis and Photorespiration under Drought Stress

Photosynthesis, a critical process for crop production, is significantly hindered by drought stress due to limited water availability and stomatal closure. Enhancing the photosynthetic rate ( $P_n$ ) is a key breeding strategy for improving crop yields (Richards 2000). It is widely recognized that genotypes capable of sustaining a higher photosynthetic rate in the flag leaf over a longer period tend to produce higher yields (Guóth 2009). Water use efficiency (WUE), which reflects a plant's ability to absorb higher concentrations of carbon and manage water loss through stomatal control, is crucial under water deficit conditions. Although WUE allows for efficient water use, it generally results in a reduced overall photosynthetic rate (Flexas et al. 2013; de Santana et al. 2015). Additionally, WUE is closely linked to photosynthetic activity and transpiration efficiency, both of which are influenced by water availability (Monneveux et al. 2006).

Studies on chlorophyll fluorescence in both irrigated and non-irrigated field plants during summer (Flexas et al. 1998) revealed that permanent photoinhibition, as assessed by pre-dawn photochemical efficiency ( $F_v/F_m$ ), was rare even under severe drought stress. While the rate of light-saturated electron transport (ETR), measured at midday, often decreased in non-irrigated plants, it did not decline as significantly as net  $CO_2$  assimilation ( $A_n$ ). This phenomenon is thought to reflect a relative increase in photorespiration, which is known to occur under drought conditions (Lawlor et al. 1975; Lawlor 1976; Lawlor et al. 1981) and is now well established (Wingler et al. 1999; Wingler et al. 2000).

In water-stressed plants, oxygen uptake increases due to heightened photorespiration activity (Flexas et al. 1999; Flexas et al. 2002). Photorespiration serves as an important photoprotective mechanism, as suggested for other species (Heber et al. 1996; Kozaki et al. 1996). A non-significant relationship between leaf water potential ( $\Psi$ ) and the rate of light-saturated electron transport indicates that reduced  $\Psi$  results in diminished thylakoid activity, which is a major factor contributing to decreased photosynthetic rates under drought (Flexas et al. 1999). The correlation between  $CO_2$  assimilation ( $A_n$ ) and stomatal conductance ( $g_s$ ) is well-documented in plants (Escalona et al. 2000; Flexas et al. 2002).

Additionally, drought stress leads to a decreased capacity for RuBP regeneration, as shown by the  $CO_2$ -saturated rate of photosynthesis (Von Caemmerer et al. 1984; Martin et al. 1992; Escalona et al. 2000). The RuBP content in leaves from water-stressed plants confirms that reduced RuBP regeneration is an early response to water deficit conditions (Gimenez et al. 1992; Gunasekera et al. 1993). Farquhar's model of photosynthesis suggests that this reduction may be due to decreased ETR. Tezara et al. (1999) reported that impaired ATP synthesis through ATPase

dysfunction could lead to reduced RuBP regeneration. Whether this impaired ATPase also affects ETR depends on the specific nature of the impairment.

### Regulation of Stomata under Drought Conditions

Stomata, small openings on the leaf surface surrounded by two guard cells, regulate the exchange of gases and water vapor (Brodribb et al. 2011; Henry et al. 2019; Sussmilch et al. 2019). They perform two main functions: opening to allow  $CO_2$  uptake for photosynthesis and facilitating the transpirational pull that delivers mineral nutrients from soil to plant tissues, and closing to limit water loss during harsh environmental conditions, such as drought (Fang et al. 2010; Brodribb et al. 2011; Assmann et al. 2016; Brodribb et al. 2017; Henry et al. 2019; Waseem et al. 2021).

Stomatal closure under water deficit conditions is regulated by both metabolic (active) and hydraulic (passive) processes (Pei et al. 2000; Brodribb et al. 2011; McAdam et al. 2014; McAdam et al. 2016; Brodribb et al. 2017; Sussmilch et al. 2017). In metabolic regulation, stomatal closure is achieved through complex processes, particularly ion trafficking (Pei et al. 2000; Geiger et al. 2010; Bauer et al. 2013; Abdul-Awal et al. 2016; McAdam et al. 2016). In contrast, passive stomatal closure is driven by a decrease in guard cell turgor pressure, which can be influenced by both exogenous and endogenous abscisic acid (ABA) (Brodribb et al. 2011; McAdam et al. 2012; Cardoso et al. 2019). There is a direct relationship between leaf water potential and stomatal conductance (Cardoso et al. 2019).

Water availability directly affects cotton growth, as cotton, derived from perennial plants, exhibits continuous growth characteristics (Wei et al. 2022). Stomatal responses to water potential are observed across all species. For instance, stomatal conductance can drop from 90% to 20% with a decrease in water potential of less than 1 MPa. Despite this rapid decline, some species maintain a continuous response. Specifically, when water potential falls below -1.65 MPa to -2.95 MPa, stomatal conductance decreases to below 20% (Pou et al. 2008).

### ABA, ROS, and $Ca^{2+}$ 's Roles in Regulating Stomatal Function under Stress

In response to abiotic stresses such as water deficit, cold, heat, and salt stress, plants use various signaling molecules including abscisic acid (ABA), reactive oxygen species (ROS), and calcium ions ( $Ca^{2+}$ ) (Agurla et al. 2018; Kollist et al. 2019; Gong et al. 2020; Hasanuzzaman et al. 2020; Pardo-Hernández et al. 2020). Stomatal regulation is crucial for plants to manage these stresses effectively (Agurla et al. 2018). During drought stress, plants rapidly close their stomata to minimize water loss (Gupta et al. 2020). This stomatal response involves various components, including receptors, protein kinases, transporters, and ion channels, which are regulated by cellular signaling mediated by ABA, ROS, and  $Ca^{2+}$  (Gong et al. 2020).

ABA is a key regulator of stomatal closure under drought conditions (Postiglione et al. 2020). It acts by triggering a rapid closure of stomata in response to water deficit signals. ABA is synthesized and accumulated in guard cells during water stress (Anfang et al. 2021) and promotes stomatal closure by activating downstream signaling pathways (Chen et al. 2021).

Reactive oxygen species (ROS) are harmful oxidants that can damage cellular proteins, lipids, and nucleic acids when present in excess. However, ROS also play crucial roles as signaling molecules in plant growth, development, and stress responses, including drought (Qi et al. 2018; Yang et al. 2018; Hasanuzzaman et al. 2020; Pardo-Hernández et al. 2020). ROS include singlet oxygen ( $^1O_2$ ), superoxide ( $O_2^-$ ), hydroxyl radicals ( $-OH$ ), and hydrogen peroxide ( $H_2O_2$ ) (Sierla et al. 2016; Singh et al. 2017; Qi et al. 2018). They are involved in regulating stomatal closure in response to osmotic stress to prevent water loss (Kwak et al. 2003; Singh et al. 2017; Postiglione et al. 2020). Exogenous application of  $H_2O_2$  to leaf epidermal layers can induce stomatal closure (Hua et al. 2012).

Under water stress, ROS production increases in various cellular compartments including cell membranes, chloroplasts, and peroxisomes (Cruz de Carvalho et al. 2008). Key factors involved in ROS generation have been identified (Qi et al. 2018), and several proteins regulated by ROS play crucial roles in controlling stomatal closure during drought (Singh et al. 2017; Qi et al. 2018). Recent studies have advanced our understanding of ROS signaling in stomatal closure, particularly through the identification of  $H_2O_2$  receptors (Wu et al. 2020).

ROS are also involved in ABA-mediated stomatal closure (Postiglione et al. 2020). The production and accumulation of apoplastic ROS depend on ABA signaling in plants (Wu et al. 2020), suggesting that both ABA and ROS are critical for stomatal closure during drought. Elevated ROS levels can cause oxidative damage, affecting protein activity and leading to reduced cotton fiber growth, biomass, chlorophyll content, and photosynthesis. Increased ROS during the reproductive stage can also degrade fiber quality (Anwaar et al. 2015).

Calcium ions ( $Ca^{2+}$ ) are important signaling messengers in stomatal regulation (Agurla et al. 2018). Stomatal movement correlates with cytosolic  $Ca^{2+}$  concentrations in guard cells, with higher levels inducing stomatal closure (McAinsh et al. 1996; Pei et al. 2000; Wu et al. 2020). Osmotic stress causes a rapid increase in cytosolic  $Ca^{2+}$ , activating calcium-dependent protein kinases (CPKs), calcineurin-B-like proteins (CBLs), and CBL-interacting protein kinases (CIPKs) (Gong et al. 2020). Many of these proteins are involved in stomatal regulation under osmotic stress. Additionally,  $Ca^{2+}$ -induced stomatal closure is also regulated by ABA and ROS, which can elevate cytosolic  $Ca^{2+}$  concentrations in guard cells. Many ABA signaling components are influenced by  $Ca^{2+}$ -mediated signaling

(Gong et al. 2020).

### Cotton under Drought Stress: Soluble Carbs and Starch Content in Leaves and Floral Parts

Under drought stress, cotton plants reduce water loss through rolling of leaf margins, which decreases the surface area exposed to radiation (Fang et al. 2015). This response significantly reduces the rate of photosynthesis, primarily due to decreased stomatal conductance. Although this reduction in photosynthesis enhances water use efficiency, it also lowers the stomatal index on both the adaxial and abaxial surfaces of the leaves. Effective stomatal regulation is therefore crucial for modulating water use efficiency at the leaf level (Bacon 2004; Parry et al. 2005).

Drought stress affects the concentration of soluble carbohydrates and starch in both the leaves and floral parts of cotton. The reduced photosynthetic rate leads to a decrease in dry matter, indicating that flowers are somewhat buffered from water deficit conditions compared to adjacent leaves (Pilon et al. 2019).

Furthermore, drought stress down-regulates the expression of GhSUT-1, a sucrose transporter that plays a crucial role in exporting photosynthetic carbon assimilates. This down-regulation results in a significant reduction in the amount of photoassimilates accumulated in the pistils of cotton (Hu et al. 2019). In addition to impaired sucrose transport, decreased starch synthesis limits starch accumulation. Key enzymes involved in starch biosynthesis, such as AGPase, GBSSase, and SSSase, are affected by drought stress. While GBSSase activity, responsible for amylose synthesis, increases under drought conditions, AGPase activity is significantly reduced, hindering the formation of amylose and amylopectin. Additionally, decreased SSSase activity results in lower starch synthesis and less energy production through glucose oxidation in cellular respiration (Wohl et al. 1942), impacting the tricarboxylic acid cycle (Nguyen et al. 2010).

The expression of GhSus and its orthologs, such as AtCYP78A6, AtGIF, and OsGL7, is down-regulated under drought stress. The Sus gene encodes an enzyme that reversibly converts sucrose into fructose and UDP-glucose, playing a role in cell wall formation and starch accumulation (Ruan 2014). Increased ABA concentrations due to water stress can further reduce Sus expression (Ruan 2014). In cotton, both suppression and overexpression of GhSus genes correlate with enzyme activity, seed growth, and fiber elongation (Ruan et al. 2003; Jiang et al. 2012).

Thus, fiber quality in cotton is highly dependent on water availability. During drought stress, reduced stomatal activity and photosynthesis lead to lower sugar content in the cells, down-regulating various genes that diminish fiber quality. Additionally, fiber length and strength are adversely affected by decreased leaf water potential, which impairs cell wall formation—a critical process in fiber development.

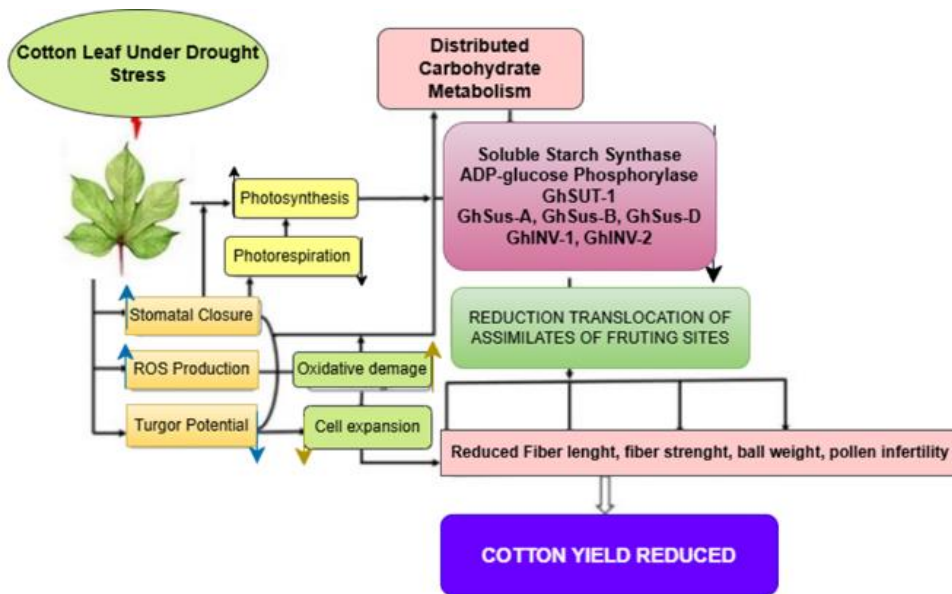


Fig. 2: Illustration of yield reduction pathway in cotton

### Biochemical Responses to Drought Stress in Cotton Plant

The plant under drought stress has several mechanisms that can eradicate both cellular hyperosmolarity and ion unbalance. Plants show biochemical responses to manage drought stress. These processes help in maintenance of water potential. This is done by accumulating low molecular weight compounds, such as sugars, glycine-betaine, sugar alcohols and proline (Pilon-Smits et al. 1995). Drought stress also increase the synthesis of osmolytes and specific proteins (Reviron et al. 1992). Moreover, water potential of cell is retained by the inward movement of water which is done due to the high concentration of solutes (Cosgrove 1997). There are some osmolytes that are also involved in fiber elongation, such as Soluble sugars, malate and potassium. Approximately 80% of sap osmolality of fiber is accounted by them (Dhindsa et al. 1975; Ruan 2005).

Under drought stress water deficiency cause the shorter fiber length at flowering stage (Loka et al. 2011). Longer length fibers are produced by longer elongation period (Quisenberry et al. 1975; Braden & Smith 2004). The synthesis of proline and glycine-betaine act as buffer in redox reaction of cell (Hare et al. 1998). Drought induces stomatal closure which cause the reduction of CO<sub>2</sub> uptake and due to this plants are more vulnerable to photo-damage (Cornic & Massacci 1996). Drought causes decrease in water potential required for photosynthesis in cotton (Krieg 1986), which is caused by the less production and activity of photosynthesis enzymes (Jones 1973) and production of photosystem II is also decrease due to increase of drought stress ((Wang et al. 2007). Hydroxyl radical, superoxide anion, H<sub>2</sub>O<sub>2</sub> and singlet oxygen are produced due to disorder in light capturing and utilization in cotton (Munné-Bosch et al. 2003). Polyphenol content rise in cotton plant under water stress and salt stress (Agastian et al. 2000; Muthukumarasamy et al. 2000).

### Reactive Oxygen Species (ROS) and Antioxidative Mechanism

Drought induces the overproduction of reactive oxygen species (ROS), which in turn cause the cellular damage and inhibition of physiological process in plants. Due to the this production leads to the oxidation of various biochemical compounds: lipids, proteins, DNA, and RNA (Tripathy et al. 2012). Overproduction of ROS can cause oxidative stress to the photosynthetic apparatus (Foyer et al. 2009; Dietz et al. 2011) and its threat to the cell, but they can play a functional role in secondary messengers involved in the stress signal transduction pathway (Foyer et al. 2009).

Drought disturbs the balance of reactive oxygen species (ROS) and antioxidant production, which further accumulates ROS in plant system. (Reddy et al. 2004). ROS has very vital role in the inter and intra cellular responses which control the growth and development process of plant (Van Breusegem et al. 2001). ROS degrades the lipids in protein, cell membrane and nucleic acid (Reddy et al. 2004). Not enough information is available about the effects of drought stress on agronomics crops like cotton, and other economical crops etc. Some studies show that there is decrease of linoleic oil, degree of acyl unsaturation, phospholipids and glycolipid of leaf tissue which undergone drought stress. These all due to the inhibition of phospholipids, glycolipid and polyunsaturated such as linolenic fatty acid synthesis. Due to water stress triacylglycerol is accumulated in leaf. The ROS production and membrane damage can be assessed by using the Malondialdehyde (MDA) (Singh et al. 2021). Plants produce antioxidant molecules such as peroxides, catalyses', reductases, mutases and these antioxidant are the scavengers of ROS (Xiong et al. 2002). Cotton plants have evolved the antioxidant mechanism against RSO production such as ascorbate peroxidase (APX), catalase (CAT), guaiacol peroxidase (GPX), glutathione reductase (GR), dehydro-ascorbate reductase (DHA), and monodehy-

droascorbate reductase (MDAR), these are belonged to enzymatic mechanism. There are some non-enzymatic mechanism such as ascorbic acid, reduced glutathione, flavonoids, a-tocopherol, and carotenoids (Zhang et al. 2014).

### Drought Stress Effects on Carbohydrates

Like other cellular constituents, drought stress effects the starch and sugar mostly (Prado et al. 2000; Abdel-Nasser et al. 2002). There are three vital enzyme such as Invertase (INV), starch synthase (StSy) and SPS. They play a vital role in carbohydrate metabolism in fiber cells. Invertase enzyme is an irreversible enzyme that hydrolyzes sucrose into glucose and fructose (Wang et al. 2010). Invertase enzyme increase the concentration of osmotic sucrose and play important role in expansion of cell fiber (Wang et al. 2010). When hexose level decrease then Invertase enzyme activities also declines which further stressed fibers sucrose synthase. (SuSy) is a glycosyl transferase, it catabolizes the sucrose into UDP-glucose and fructose (Sturm et al. 1999). Sucrose synthase (SuSy) provide solute such as hexoses and plays very important role in stages of development of fiber such as fiber instigation and elongation (Ruan 2007). Important sugars that are involved in the development of fiber are sucrose, fructose and glucose (Jaquet et al. 1982). Relative proportion of fructose and glucose are greater than the amount of sucrose present (Jaquet et al. 1982; Abidi et al. 2010; Tang et al. 2014) and both are considered osmotically active solutes in the fibers (Ruan et al. 2001).

Drought stress adversely effects the accumulation of starch and reduces it into hexose in the anther of cotton from which its restricts the ATP (Adenosine triphosphate) production (Hu et al. 2020). Due to this, energy currency level decreases which declines the production of pollen and leads to premature burst of flower and buds (Echer et al. 2014; Hu et al. 2020). and plant biomass is divided into different stages but reproductive biomass stages production is decline (Wang et al. 2016). Water stress effects the synthesis of ADP Glucose pyrophosphate (AGPase) and its activity runs downwards (Hu et al. 2019) which further suppressed the expression of gene of sucrose synthase (GhSusA, GhSusB, and GhSusD), sucrose transportation (GhSUT – 1) and Invertase (GhINV1 and GhINV2) in carpel (Hu et al. 2019; Pilon et al. 2019; Hu et al. 2020). Due to this unfertilized flower is produced (Hu et al. 2019). The complete fertilization is the only process through which bolls is formed, which overlaps to seed and fiber development (Chen et al. 2015; Rehman et al. 2019). The gene responsible for the development of fiber in cotton seed is well recognized (Ahmed et al. 2018; Chen et al. 2019) and its expression is mostly effected by the water stress (Gao et al. 2020; Loka et al. 2020)

Due to low carbohydrates assimilation in carpel of cotton this lead to the falling of flower and buds (Loka

et al. 2019) and due to this, boll cell will be destroyed (Wang et al. 2016). Some genetic response may stop the bolls shedding due to higher activities of callose and chitinase. This chitinase and callose regulate the assimilation of carbohydrates and its transportation in reproductive part (Ibrahim et al. 2019).

### Effect of Stress Environment on Cotton plant and its Genetic responses

DREB proteins, sub-family of ERF discovered in *Arabidopsis thaliana* (Sakuma et al. 2002) are transcriptional factors that participate to overcome several stresses in different plants (Nakano et al. 2006). DREB genes were identified 193 in *Gossypium barbadense* (AD2), 183 in *Gossypium hirsutum* (AD1), 80 in *Gossypium arboreum* (A2), and 79 in *Gossypium raimondii* (D5) respectively (Su et al. 2023). These transcription factors enhances tolerance in many plants against water and salt stress (Lata et al. 2011). For example, in cotton plants, transcription factors GhDREB1 expressed itself under low-temperature stress but over-expression of it may delay the flowering and decrease the height of the plant. However, its expression can be decreased by the treating it with gibberellins on cotton plants (Lata et al. 2011).

Floral organs were severely suppressed under water stressed conditions, it can cause lower levels of pyruvate under water stress in pistils, which is unable to provide enough amount of acetyl-CoA for TCA cycle. As a result, there is a low amount of ATP in water stressed plants (than control) thus a less amount of energy in the experimented plant pistils is available for the elongation of pollen tube. As a result there is reduction in the growth rate of pollen tube (Hu et al. 2019). Drought stressed cotton enhances GhSusC expression, but decrease the expressions of GhSusA, GhSusD and GhSusB in pistils. Thus, GhSusA, GhSusD and GhSusB have specific roles in degradation of sucrose under water stress. (Hu et al. 2019). The GhDREB2 also express under conditions of water stress (Agarwal et al. 2006).

Leaf water potential also affects cotton lint fiber quality. Water potential in leaves decreases due to scarcity of water, under water deficit condition such as drought. The cotton fiber is unable to elongate due to the less water turgor pressure in seed coat cells and hindrance in the cell wall formation due to the down regulation of genes under water deficit with the help of chemical signals in the form of ABA (Lokhande et al. 2014; Ruan 2014) Moreover, the turgor pressure is involved in the cell expansion of epidermal cells. In drought the less availability of water reduces turgor pressure and hence the lint fiber elongation decreases (Smart et al. 1998). In the metabolism of carbohydrates, the change in the expression of key enzymes' gene provide a good analysis of the impact of drought stress on cotton's reproductive units at molecular level. There is the identification of four discrete genes of sucrose synthase (GhSusA, GhSusB,

GhSusC and GhSusD) in cotton that regulate the breakdown of sucrose (Brill et al. 2011).

The main genes family of leucine zipper is another group of transcriptional factors that expressed in both living organism and abiotic stress conditions (Nijhawan et al. 2008). Kerr et al. (2017), reported the expression of GhABF in many biotic and abiotic stress conditions in cotton. While Liang et al. (2016), reported that enhanced expression of GhABF2 improves water and salinity stress tolerance in cotton. In the same way, the enhanced expression of GhABF2D increases drought and dehydration resilience by regulating stomata (Collin et al. 2021). Several genes are also reported in cotton plant that helps to tolerate stress conditions and promotes growth as well like the expression of GhNAC79 regulates the stomatal conductance under drought conditions and promotes flowerings and fiber elongation as well (Zhao et al. 2023), GhAnn1 expressed in response to water and salinity stress (Zhang Feng et al. 2015), GhNAC72 involves in water stress tolerance (Mehari et al. 2021), GhNAC12 promotes leaf senescence (Yang et al. 2023), GhCBF3 regulates stomatal closure and helps to tolerate drought and salt stress (NaCl) in cotton plant through ABA pathway (Ma et al. 2016), GhMKK3 regulate stomatal response, root hairs growth and enhanced drought tolerance (Wang et al. 2016), GhMAP3K40 enhance tolerance at the stage of germination against water and salinity stress (Chen et al. 2015).

GhMAPK4 expressed itself under high salt and osmotic stress conditions through ABA signaling pathway and negatively regulate the development of cotton (Wang et al. 2015), GhMKK4 negatively regulate the resistance of disease in cotton plants (Li et al. 2014), GhMPK17 expressed itself under salinity (NaCl), ABA signaling and osmotic stress (Zhang et al. 2014), GhMPK6a show its expression under several defense signal molecules and both types of stresses caused by living and non-living conditions (bacterial infection, salinity stress) (Li et al. 2013), GhMKK1 can be induced in cotton plants under H<sub>2</sub>O<sub>2</sub>, water and salinity stress (Lu et al. 2013), GhMKK5 show its expression under numerous stresses caused by abiotic factors like salinity and water deficiency stresses and pathogen infections (Zhang et al. 2012), GhMPK16 involves in defense mechanism and tolerance against many abiotic stress factors like temperature stress, salt (NaCl) and water deficiency stress (Shi et al. 2011).

The genetically modified cotton plants such as GhADF1-RNAi transgenic cotton (Qin et al. 2022), show an increase in tolerance against drought stress during seed germination, seedling development and the reproductive stage (Wang et al. 2009). These modified plants have enlarged root systems with longer primary roots. The root's biomass is also increased with more lateral growth of roots. They also have higher yield of fiber under both normal and drought conditions. So the regulation of the GhADF1 factor in cotton plants will improve their fiber yield and drought tolerance (Qin et al. 2022).

**Table 1:** Cotton genes that expressed in stress conditions.

Cotton Gene	Stress Condition	References
GhMKK3	Drought stress	(Wang et al. 2016)
GhCBF3	Drought and salt stress	(Ma et al. 2016)
GhMPK4	High salinity and osmotic stress	(Wang et al. 2015)
GhDREB1	Low temperature stress	(Lata et al. 2011)
GhMKK4	Bacterial and fungal pathogens stress	(Li et al. 2014)
GhSusC	Drought stress	(Hu et al. 2019)
GhMPK17	NaCl, mannitol, ABA	(Zhang et al. 2014)
GhDREB2	Drought stress	(Agarwal et al. 2006)
GhMPK6a	Salt stress, drought stress and bacterial infection	(Li et al. 2013)
GhABF2	Drought and salt stress	Liang et al. (2016)
GhABF2D	Drought and dehydration	(Collin et al. 2021)
GhMPK5	NaCl, drought, H <sub>2</sub> O <sub>2</sub>	(Lu et al. 2013)
GhNAC79	Drought stress	(Zhao et al. 2023)
GhNAC72	Drought stress	(Mehari et al. 2021)
GhMPK16	Low and high temperature stress, manitol, salt stress (NaCl), pathogens	(Shi et al. 2011)
GhAnn1	Drought and salt stress	(Zhang Feng et al. 2015)
GhMAP3K40	Drought and salt stress	(Chen et al. 2015)
GhADF1	Drought stress	(Qin et al. 2022)
GhMKK5	Salt stress, drought stress and pathogen infection	(Zhang et al. 2012)

## Conclusion

Drought is the most significant abiotic stress that reduces the yields of the plant. It has negative effects (either directly or indirectly) on a number of processes in cotton plants, including morphological, physiological, anatomical, and biochemical processes, which ultimately results in a reduction in the growth regulation of cotton plants. Different genes that are associated to stress also play a role in this process, which enables the cotton plant to survive in settings that are not necessarily beneficial. On the other hand, some genetically modified cotton plants exhibit satisfactory yield results when subjected to stressful conditions.

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