

## Battling arid adversity: unveiling the resilience of cotton in the face of drought and innovative mitigation approaches

Ayesha BIBI<sup>1</sup>, Wang LIHONG<sup>2\*</sup>, Athar MAHMOOD<sup>3</sup>,  
Muhammad M. JAVAID<sup>4</sup>, Basharat ALI<sup>5</sup>, Muhammad YASIN<sup>4</sup>,  
Kashif KAMRAN<sup>6</sup>, Bilal A. KHAN<sup>4</sup>, Adnan RASHEED<sup>7</sup>,  
Muhammad U. HASSAN<sup>8</sup>, Abeer HASHEM<sup>9</sup>, Mouna MECHRI<sup>10</sup>,  
Elsayed F. ABD\_ALLAH<sup>11</sup>

<sup>1</sup>University of Agriculture Faisalabad, Department of Botany, 38000 Faisalabad, Pakistan; [ayeshabibiuaaf@gmail.com](mailto:ayeshabibiuaaf@gmail.com)

<sup>2</sup>Baicheng Normal University, College of Tourism and Geographic Science, Baicheng 137000, Jilin, China; [wlb19721108@163.com](mailto:wlb19721108@163.com) (\*corresponding author)

<sup>3</sup>University of Agriculture Faisalabad, Department of Agronomy, 38000 Faisalabad, Pakistan; [athar.mahmood@uaf.edu.pk](mailto:athar.mahmood@uaf.edu.pk)

<sup>4</sup>University of Sargodha, College of Agriculture, Department of Agronomy, Sargodha, Pakistan; [mmansoorjavaid@gmail.com](mailto:mmansoorjavaid@gmail.com); [agrarianyasin@gmail.com](mailto:agrarianyasin@gmail.com); [bilalahmadkhan678@gmail.com](mailto:bilalahmadkhan678@gmail.com)

<sup>5</sup>Khwaja Fareed University of Engineering and Information Technology, Department of Agricultural Engineering, Rahim Yar Khan 62400, Pakistan; [basharat2018@yahoo.com](mailto:basharat2018@yahoo.com)

<sup>6</sup>University of Agriculture Faisalabad, Department of Physics, 38000 Faisalabad, Pakistan; [k.kamran@uaf.edu.pk](mailto:k.kamran@uaf.edu.pk)

<sup>7</sup>Hunan Agricultural University, College of Agronomy, Changsa 410128, China; [adnanrasheed@hunau.edu.cn](mailto:adnanrasheed@hunau.edu.cn)

<sup>8</sup>Jiangxi Agricultural University, Research Center on Ecological Sciences, Nanchang China; [muhassanuaf@gmail.com](mailto:muhassanuaf@gmail.com)

<sup>9</sup>King Saud University, College of Science, Botany and Microbiology Department, P.O. Box. 2460, Riyadh 11451, Saudi Arabia; [habeer@ksu.edu.sa](mailto:habeer@ksu.edu.sa)

<sup>10</sup>National Institute of Field Crops, Boussalem 8170, Tunisia; [mounassol.mm@gmail.com](mailto:mounassol.mm@gmail.com)

<sup>11</sup>King Saud University, College of Food and Agricultural Sciences, Plant Production Department, P.O. Box 2460, Riyadh 11451, Saudi Arabia; [eabdallah@ksu.edu.sa](mailto:eabdallah@ksu.edu.sa)

### Abstract

Climate change has had significant impacts on agriculture, particularly on cotton production, where drought has emerged as a major threat worldwide. Long and intense dry periods in cotton-growing regions have become more frequent and severe. Drought stress severely affects various aspects of cotton plants, including chlorophyll pigments, carbohydrate metabolism, and enzyme activities related to fiber development, such as vacuolar invertase and sucrose synthase. Furthermore, drought stress disrupts the movement of nutrients toward the reproductive tissues in cotton, resulting in compromised pollen function, propagative failure, and fiber characteristics. To tackle these issues, scientists have made advancements in creating drought-resistant cotton varieties through transgenic methods or molecular breeding techniques, genome editing, CRISPR/Cas9, utilizing quantitative trait loci (QTL). Moreover, the application of plant growth regulators and mineral elements has displayed the potential to improve cotton's ability to endure drought stress while also enhancing fiber yield and quality. These approaches activate stress-responsive signaling pathways, which could

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contribute to mitigating reproductive failure and improving fiber characteristics. While the impact of drought stress on cotton plants has been extensively studied, the variations in fiber quality resulting from drought stress are not yet completely understood. Current research has been focused on unraveling the mechanisms underlying these changes, including the physiological, biochemical, and molecular alterations during the multiplicative growth phase that contribute to poor fiber development. Understanding these mechanisms will facilitate the development of novel strategies to alleviate the adverse impact of worldwide weather changes on cotton growth and fiber quality. This research focuses on the drought stress in cotton cultivation and explores its different effects on cotton morphology, physiology, crop yield, and fiber characteristics as well as mechanisms by which cotton exhibits drought tolerance and highlights innovative strategies to mitigate drought stress.

**Keywords:** Crisper/Cas gene; drought stress; fiber quality; management strategies; source sink relationship

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## Introduction

Cotton (*Gossypium hirsutum*) is often referred to as 'White gold' due to its economic importance and India stands as an important participant in the world of cotton as a major producer, consumer, and exporter of cotton (Murali and Khan, 2022). Cotton holds significant importance in India as it is a widely cultivated fiber and appreciated cash crop. It performs an essential character in both the industrial and agricultural sectors, providing employment opportunities for approximately eleven million farmers. Additionally, the cotton industry indirectly employs around forty to fifty million individuals across various stages of cotton processing and trade. Due to its historical significance and global prominence (Nagargade *et al.*, 2023). In India, cotton cultivation covers a vast area of 12.90 million hectares, which accounts for nearly 41% of the international cotton cultivation area. Furthermore, during the 2019-20 period, the cotton cultivation area reached a new record of 13.40 million hectares, resulting in the production of 361 lakh bales. Each bale weighs around one hundred seventy kilograms (Masood *et al.*, 2022).

The rationale behind using Pakistan's cotton growth as a benchmark situation for studying manufacturing aspects, input issues, and efficiency relationships is to provide a perspective on non-food crops within the context of a developing country. This approach offers several reasons. Pakistan's proximity to India makes it a suitable comparative case study for analysing similar production factors and challenges in the region. Cotton, being a non-food crop, holds significant economic importance in developing countries like India and Pakistan. Therefore, studying Pakistan's cotton production can provide insights applicable to other developing countries with similar agricultural profiles. Both India and Pakistan share similar agro-climatic conditions, farming practices, and challenges in the cotton sector. Hence, using Pakistan's cotton production as a reference allows for meaningful comparisons and a better understanding of production dynamics in the region. By examining Pakistan's cotton production, researchers and policymakers can identify successful strategies, best practices, and potential improvements that can be implemented in the background of a rising country such as India (Arshad *et al.*, 2022).

Pakistan holds a prominent position among the top five largest cotton producers globally, and it is also the seventh largest cloth producer worldwide. The cotton industry performs an important part in Pakistan's budget, contributing almost 10% to the country's GDP (Gross development production). This contribution is noteworthy when compared to the overall GDP percentage of the agriculture sector, which stands at 18.9%. These statistics highlight the substantial economic significance of the cotton sector within Pakistan and its impact on the country's overall economic growth (Azumah *et al.*, 2019). Furthermore, the cotton sector in Pakistan is a significant contributor to employment, accounting for 42.3% of the labor force engaged in various activities related to cotton production. It serves as a crucial source of resources for numerous value additional

divisions, contributing to fifty-five of the country's overseas profits. This input is primarily driven by finished products derived from cotton (Sohaib and Jamil, 2017). Interestingly, despite being a developing country, Pakistan has traditionally focused on exporting raw cotton. However, there has been a shift in recent years. Since 2010, Pakistan has ceased exporting raw cotton and has instead become the fourth-largest country in terms of cotton finishing, according to the USDA Cotton Outlook Report of 2019. This transition reflects a strategic move towards adding value to the cotton sector and promoting the export of finished cotton products rather than just raw materials (Batoool and Saeed, 2018). One of the primary reasons for the increasing import of raw cotton in Pakistan is the declining trend in cotton yield per hectare over time. The cotton production per hectare in Pakistan has been progressively decreasing, and its numbers are amongst the lowest on a worldwide scale. Even nations with significantly smaller land areas have achieved to accomplish greater yields per hectare when compared to Pakistan (Arshad *et al.*, 2022). The average cotton yield in Pakistan stands at 730 kg/ha, resulting in a production of 10,671 million bales. This yield is comparatively lower, experiencing a decrease of around 1.5-2% when compared to the global average. Even in the irrigated regions of Pakistan, the cotton-growing areas fall behind unirrigated cotton cultivation zones worldwide in about the amount of lint produced per hectare. As a result, cultivating cotton has become less interesting to farmers in comparison to growing other types of crops. This has led to a reduction in the area dedicated to cotton crops. According to the International Textile Data Report of 2017 and the Economic Survey of Pakistan by the United States Department of Agriculture (USDA) (2019-2020). The reduction in the cultivated land area for cotton crops in Pakistan began in 2004-2005 and has worsened since 2013-2014. By the latest available data, the sowing area of cotton crops had decreased by 14.2% (Nadeem *et al.*, 2014).

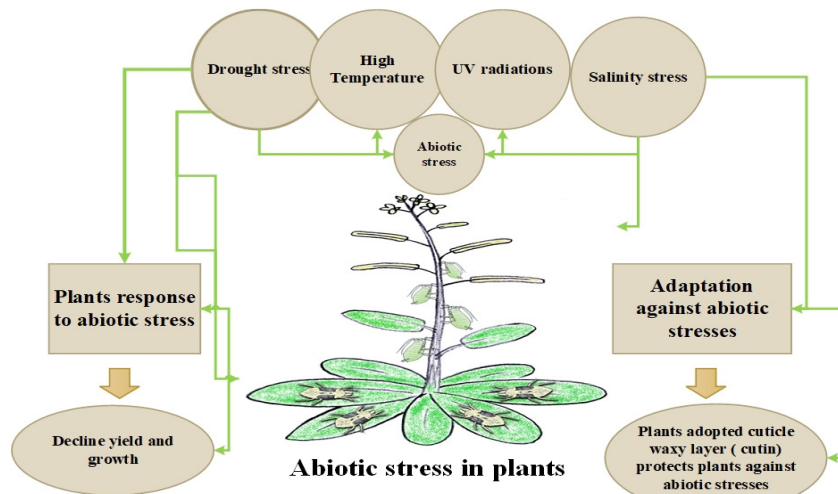
The minimum productivity of Pakistan's cotton harvest can be qualified to numerous aspects linked to the inefficient utilization of different resources. These factors encompass controlled amounts of water for agriculture production, plant thickness, disease management, plant nutrients, resource management abilities, and inadequate acceptance of progressive technology. As a result, a difference exists between the probable cotton output and the actual yield in Pakistan. To balance the low per hectare yield, Pakistani farmers often possibility to traditional performances like increase (raised use of inputs) or extensification (expanding cultivation to new lands). However, these practices yield adverse results, including the continuous degradation of the rural biological environment. They not only reduce the financial impact of agriculture but also harmful aspects to the resources and environment that agriculture depends upon for sustainability and progress. It is crucial to address these issues by promoting efficient and sustainable agricultural practices. This involves improving irrigation techniques, implementing disease management strategies, optimizing plant nutrition, enhancing resource management skills, and encouraging the adoption of advanced agricultural technologies. By adopting such measures, Pakistan can improve the productivity of its cotton crop while safeguarding the environment and securing the long-lasting feasibility of agriculture (Chen and Breedlove, 2020). In Pakistan, the production of cotton dropped by 34% to only 9.68 million bales, a significant decrease from the previous year's output of 14.4 million bales. This decline can be attributed to drought stress (Hussain and Mahmood, 2020).

The main objective of this article is to gather primary data regarding the impact of drought stress and its mitigation strategies on the cotton plant. The discoveries presented in this paper will support Pakistan in addressing its challenges. Additionally, we propose to explore the potential of different mitigation strategies and their roles in alleviating the influence of drought stress on the cotton plant. More prominently, this article will help identify alternative pathways for poor farmers growing different hybrid resistance tolerance cotton varieties and adopting agronomic practices that are essential to supplement their agricultural income. Furthermore, it will also help overcome the impact of drought stress by using different management strategies, agronomic practices, growth regulators, and irrigation management practices to enhance the characteristics of cotton yield, fiber quality, and overall production. In this review article, we comprehensively discuss various aspects connected to the effect of drought stress and its mitigation strategies on cotton. Section 2: Effects of Abiotic stresses, drought stress on cotton. Section 3: Assess the morphophysiological response of cotton plant

under drought stress. Section 4: Evaluate the effect of drought stress on cotton production, fiber quality, and overall plants growth. Section 5: analyse the mechanisms underlying drought tolerance in cotton plants, including. In Section 6: Drought stress changes carbon assimilation (Source and sink relationship). Section 7 Mitigation strategies of drought stress in cotton plants in Section 8, future perspectives, In Section 9 conclusions are presented. Cotton exhibits drought tolerance through strategies like drought tolerance mechanisms, although adverse effects of drought stress can reduce the morphophysiological factors of cotton. However, there is potential for innovative solutions through genome editing and genetic improvements

### Abiotic stresses

Terrestrial plants have faced challenging environmental conditions since their inception. A wide range of physical and chemical factors can impact them, including ultraviolet radiation, inadequate or excessive rainfall, heavy metal contamination, high salinity, extreme temperatures, and drought stress. Abiotic stresses reduce plant growth and yield which a severe danger to agriculture crops and ecosystems (Wani *et al.*, 2016). Throughout, plants have developed a range of biological, cellular, and morphological adaptations to cope with environmental challenges. One notable structure that helps plants withstand stresses is the cuticle wax layer. The cuticle wax layer is composed of a hydrophobic substance called cutin, which forms a protective covering over the epidermal surfaces of land plants. This layer serves as a barrier, preventing excessive water loss through transpiration and reducing the risk of desiccation (Singh *et al.*, 2018). This adjustment also aids plants in evading drought, which is a complex form of stress that impacts plants at various levels. Drought is caused by a decrease in soil moisture due to insufficient rainfall. The extent to which a plant is affected by drought depends on the duration or period involved in stress, the period of plant growth, and the severity of the stress (Kapoor *et al.*, 2020). Abiotic stress like drought stress can significantly reduce cotton plant growth and yield (Saleem *et al.*, 2016) (Figure 1).



**Figure 1.** The critical role of major abiotic factors in influencing plant growth, highlighting the environmental variables that significantly impact the development and well-being of plants

## **Impacts of water deficiency on cotton plant**

Cotton is widely recognized as a significant money crop on a global scale. It receives substantial contributions from major cotton-producing nations such as China, India, Pakistan, the US and Brazil. These countries have established themselves as key players in the cotton industry, with their production significantly influencing the global cotton market (Meyer, 2019). Climate change poses a significant threat to global agriculture, impacting both biotic and abiotic stresses.

Cotton production in states like China, India, the United States, Pakistan, Brazil, and other tropical regions is particularly vulnerable to these climate-related challenges. Some of the principal threats faced by cotton production in these regions are as follows climate change can lead to alterations in rainfall patterns, resulting in decreased water obtainability for irrigation, weather change can disturb rain patterns, producing changes in the frequency, intensity, and supply of rainfall, rising temperatures and prolonged drought conditions associated with environment change can enhance the regularity and harshness of drought and heat curses. These extreme weather events can have detrimental effects on cotton plants, impacting their growth, development, and yield potential, Climate change can lead to higher rates of evaporation and increased salt content in the soil, particularly in areas with limited freshwater availability. The salinization of soils can negatively affect cotton crops, making it hard for them to uptake essential minerals and water, thus hampering their growth and productivity (Haque *et al.*, 2018). Cotton, with its indeterminate growth habit, is indeed susceptible to environmental anomalies. Indeterminate growth means that cotton plants continue to grow and produce flowers and bolls over an extended period, making them more sensitive to environmental factors.

These environmental factors are such as temperature fluctuations, water stress, pests and diseases, and extreme weather condition like hurricanes, storms, or heavy rainfall (Rehman and Farooq, 2019). Cotton is considered a crop primarily suited for tropical and subtropical regions. While it exhibits moderate tolerance to drought stress during its vegetative, reproductive, stage cotton is highly complex to drought pressure. This means that water scarcity during the reproductive stage, which includes flower development, pollination, and boll formation, can have a significant negative impact on cotton harvest and characteristics (Niu *et al.*, 2018).

Indeed, drought is considered one of the greater significant abiotic stresses affecting agricultural systems worldwide. Approximately 20% of the worldwide land area is estimated to be facing modest to intense drought conditions. Drought poses an important task to crop growth, including cotton cultivation, as it disrupts the water balance necessary for plant morphology (Barichivich *et al.*, 2019). The overall quality of cotton fibers. Water deficiency during the development, and growth of cotton plants can have significant implications for fiber production of, fiber properties like fiber length, strength, maturity, and yield (Meyer, 2019). With global climate change and increasing water demands for sustainable agriculture, drought has emerged as one of the most essential abiotic stresses, leading to reduced cotton yield and growth. Drought stress affects plants by disrupting their water balance, hampering physiological processes, and hindering overall growth and productivity (FAO, 2018). Drought, as significant abiotic stress, hinders cotton growth and yield globally, particularly during the reproductive stage, impacting fiber properties and challenging sustainable agriculture in the face of climate change.

## **Impact of drought on cotton morphology**

### *Vegetative growth*

Cotton morphology can indeed be distributed into distinct phases, each with specific irrigation supplies. These stages provide a framework for understanding the crop's water needs throughout its lifecycle. Here are the five stages commonly recognized in cotton growth and their associated irrigation requirements such as establishing to appearance, development to first square, first square to first flower, first floret to top bud, peak blossom to first capsule open (Thorp *et al.*, 2020). At the planting stage, irrigation is indeed crucial for cotton

propagation and formation. Sufficient moisture in the soil helps to initiate seed germination and support early root development, ensuring a strong start for the crop. The stages from first square to first floret and from first floret to peak blossom are indeed the most delicate periods for cotton plants, particularly when it comes to abiotic stresses such as drought and heat stress. During these stages, the crop undergoes crucial reproductive processes that are highly vulnerable to water deficits and high temperatures (Zonta *et al.*, 2017). During the period from the top flower to the first capsule open, the water availability of the cotton crop is generally reduced compared to earlier stages. The plant has completed its reproductive phase, and bolls are maturing and progressing towards opening (Hussain *et al.*, 2020).

#### *Decrease in boll formation of cotton due to drought stress*

The effect of water deficiency in pollen tube growth was investigated, revealing that the carbohydrate contents in pistils during the primary development phase of the pollen tube were significantly reduced by 31% in comparison to non-stressed plants. The decrease in starch content in pistils of drought-stressed plants was qualified to lower the activities of AGPase (adenosine diphosphate glucose pyrophosphorylase) and SuSy (sucrose synthase). These reduced enzymatic activities ultimately led to the failure of fertilization, even after fruitful pollination, resulting in sprout shedding. Indeed, drought stress can significantly impact boll formation and lead to increased boll shedding in plants (Bakhsh *et al.*, 2019). Drought stress, along with temperature stress, is a dominant factor contributing to boll shedding in plants. Drought stress triggers the construction of damaging enzymes such as hydrolase, pectinase, and cellulase. These enzymes break down the structural components of the boll, including pectin and cellulose, leading to boll degradation and shedding (Zahoor *et al.*, 2017). when plants experience growth losses due to factors like drought stress, they often utilize reserved adapts to compensate for the reduction in development. Under drought stress conditions, the reduced assimilate accretion in bolls can lead to smaller capsule size and lower boll preservation. Drought stress affects the availability and transport of assimilates, as carbohydrates, to the developing bolls. With limited water availability, the plant's ability to produce and translocate assimilates to the reproductive structures is hindered (Wang *et al.*, 2016).

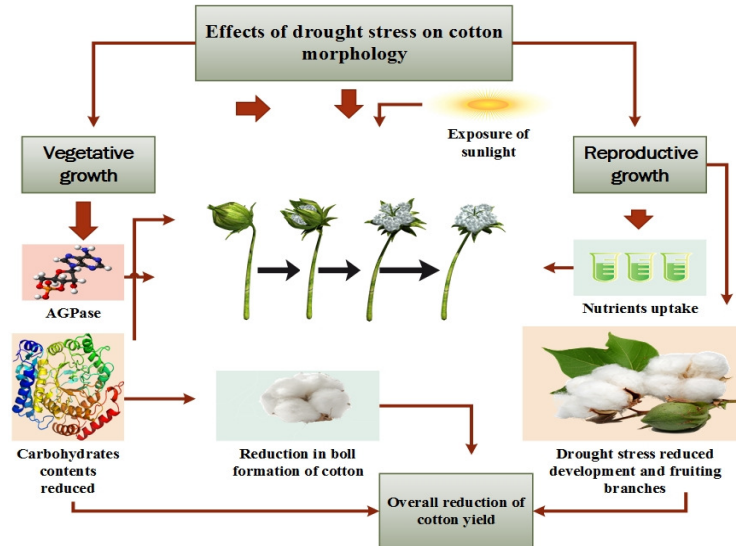
#### *Reproductive growth*

Fiber development in cotton involves a series of distinct stages that occur in a sequential and overlapping manner. These stages are essential for the transformation of protoderm cells in the ovule into elongated trichomes, which ultimately form the cotton fiber such as distinguishing from the protoderm, extending growth, Production of additional cell layers, and maturity (Rehman and Farooq, 2019). Drought stress can have adverse effects on various fiber characteristics in cotton. These effects are more distinct on the greater ripening branches compared to the lesser ones (Wang *et al.*, 2016). the accessibility of adequate water throughout all stages of fiber development is crucial for achieving the possible yield and quality of cotton fibers. Adequate water supply supports the various physiological and biochemical processes involved in fiber formation and maturation. Here's why water availability is important at every stage (Zhao *et al.*, 2019).

#### *Influence of drought on the progression of fruiting sites*

The arrangement of the cotton plant, the developing of the growth of fruit-bearing branches, and the distribution of flowering, plays a pivotal role in optimizing sunlight absorption, the spread of nutrients, and ultimately, attaining greater yield. The cotton plant is comprised of two primary categories of branches: monopodial and sympodial branches (Evers and Marcelis, 2019). cotton exhibits an unspecified growth pattern, where morphological stages occur simultaneously. This overlapping growth pattern is significant because it allows for continuous flowering and fruiting throughout the growing season. However, when drought conditions occur, the delicate balance between vegetative and reproductive development in cotton can be disrupted (Wang *et al.*, 2016). Cotton exhibits an indeterminate growth habit, where both vegetative and

reproductive growth stages occur simultaneously. This overlapping growth pattern is significant because it allows for continuous flowering and fruiting throughout the growing season. However, when drought conditions occur, the delicate balance between vegetative and reproductive development in cotton can be disrupted (Ergashovich *et al.*, 2020). Drought stress during reproductive stages decreases boll formation, and boll shedding, and affects vegetative and reproductive growth, ultimately impacting yield. (Figure 2).



**Figure 2.** Influence of drought stress on cotton morphology - comparative visual representation showcasing the structural alterations and physiological changes in cotton plants subjected to varying degrees of drought stress conditions

### Effects of drought stress on cotton physiology

#### *Drought stress enhanced reactive oxygen species*

Insufficient irrigation water can adversely affect the functions of plants, leading to inhibited growth, hindered development, and decreased crop productivity. Inadequate irrigation water can adversely affect the physiological processes of plants, leading to inhibited growth, impeded development, and reduced agricultural output (Bozorov *et al.*, 2018). When confronted with restricted water availability, the performance of leaves is particularly cooperated, giving rise to the creation of damaging compounds such as reactive oxygen species (ROS). This imbalance arises from the disparity between the absorption of light and its effective utilization by plant systems, resulting in the manufacture of hydroxyl radicals, superoxide anion, singlet oxygen, and  $H_2O_2$  (Foyer and Hanke, 2022).

#### *Reduced chlorophyll content*

Photosynthesis, the green pigment located within plant cell chloroplasts, plays an important role in the process of chlorophyll. It absorbs light energy and enables the synthesis of carbohydrates in plants. The quantity of chlorophyll present in plant tissues reflects their photosynthetic capacity. Among different cotton genotypes, *Gossypium barbadense* exhibited the highest levels of chlorophyll, followed by *Gossypium arboreum* in second place, *Gossypium herbaceum* in third place, and *Gossypium hirsutum* with the lowest levels. This demonstrates how drought stress affects the amount of chlorophyll in cotton genotypes (Babar *et al.*, 2023).

The reduction in soil water potential resulted in decrease in the overall quantity of chlorophyll in cotton plants (Arekhi *et al.*, 2023). Drought stress leads to a reduction in the size of leaf surfaces, resulting in reduced

photosynthetic productivity. This condition can lead to the incomplete dropping of important produce tissues like capsules, foliage, and blossoms (Zhao *et al.*, 2020).

#### *Decreased stomatal conductivity and ATP synthesis*

As drought sets in, plants respond by decreasing stomatal conductance to conserve water. This response declines the water potential of the leaves, subsequently affecting chlorophyll. Ultimately, the carboxylation efficiency of cotton is lowered due to these factors (Meeks *et al.*, 2019). Under stress conditions, such as drought, the levels of soluble carbohydrate metabolites within leaves diminish. This decrease is a result of reduced leaf area and decreased photosynthetic activity as a response to drought (Shareef *et al.*, 2018).

Drought-induced abnormalities in various physiological processes, including photosynthesis, stomatal conductance, ATP synthesis, carbohydrate cycling, and translocation, disrupt the availability of biomass-limited water supply through the reproductive phase. Under drought stress, there is a reduction in carbohydrate buildup and its subsequent damage into hexoses within the anthers of cotton plants. This reduction in starch availability limits the production of ATP (adenosine triphosphate) molecules, which are crucial for energy transfer and various physiological processes (Hu *et al.*, 2022). These disruptions have a significant impact on the characteristics of pollen due to decreased activity of carbohydrate synthesis enzymes, suppressed sucrose and invertase synthesis, and limited carbohydrate accumulation in pistils. Consequently, there is a negative effect on pollen tube growth, leading to reproductive failure, reduced fiber, harvest, and compromised characteristics of cotton (Wang *et al.*, 2020).

#### *Effects of drought stress on cotton crop yield and fiber characteristics*

Successful fertilization triggers the beginning of boll formation, which agrees with the growth of both seeds and fibers. The progression of fiber development involves four stages that occur simultaneously: fiber initiation, which commences approximately two days after anthesis; fiber cell elongation, taking place from 0 to Three weeks after flowering; the process of adding the secondary cell wall (Rehman and Farooq, 2019). Osmotic potential during fiber start and extension hinders the division of fiber cells, resulting in a decreased total count of fiber cells (Zhang *et al.*, 2020). Water stress induces a decrease in osmotic potential, subsequently resulting in the closure of stomata, limiting the process of photosynthesis and the assimilation of carbohydrates into the growing bolls. The significance of the carbohydrate synthesis gene in the growth of cotton fibers within the seeds has been extensively recorded (Ahmed *et al.*, 2018).

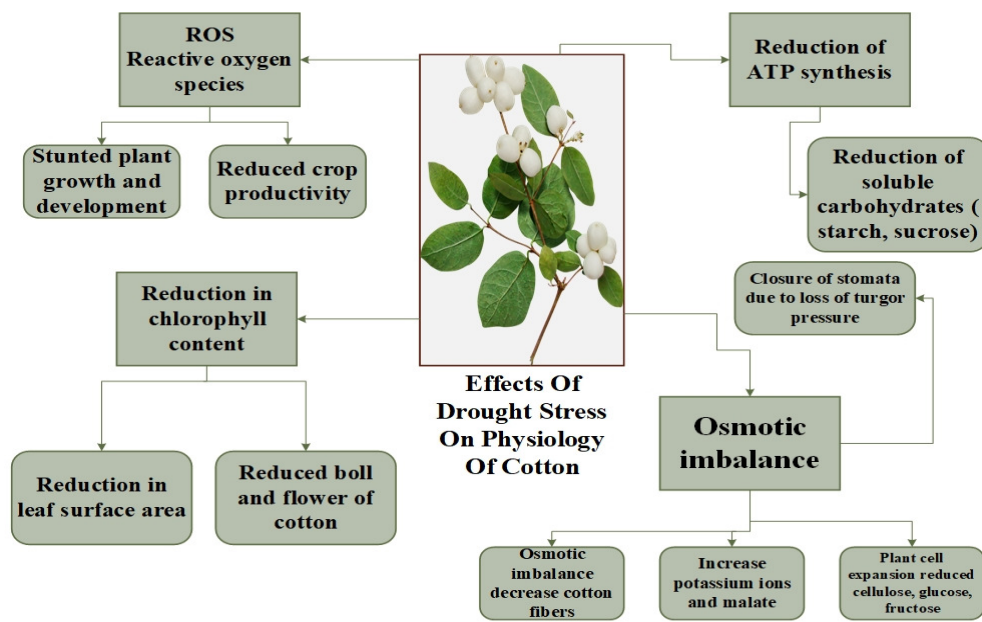
Drought-induced stress interferes with the turgor pressure of fiber cells (Wang *et al.*, 2016). Drought stress adversely disturbs various aspects of fiber quality in cotton. It results in a decrease in fiber length, consistency, and durability, while also triggering an elevation in fiber thickness. Consequently, assessing leaf water potential serves as a crucial marker of cotton fiber quality when cultivated under drought-stress conditions. Additionally, drought stress during the blossoming and boll development stages leads to a decrease in fiber height and the quantity of fibers per seed, while increasing their width (Hu *et al.*, 2018).

Research was shown to examine the impact of water-deficient stress on the characteristics of cotton fiber quality. The findings revealed a consistent reduction in fiber length and strength as the leaf water potential decreased. The investigation also proposed that the leaf water potential could serve as a dependable indicator for assessing cotton fiber quality under stressful conditions. The study further noted that both early and full bloom drought stress had comparable effects on the propagative growth of cotton. However, during the full bloom stage, the harmful impact on fiber length and quality was more pronounced compared to early bloom stress (Gao *et al.*, 2021).

The primary mechanisms responsible for fiber elongation involve the relaxation of cell walls and the elevation of cell turgor pressure. Around 80% of the overall osmotic adjustment is attributed to various solutes such as soluble sugars, potassium ions (K<sup>+</sup>), and malate. Among these solutes, sucrose performs a crucial character in facilitating osmotic potential (Yang *et al.*, 2016). Drought stress adversely effects on both the



mechanical and physiological aspects of cell expansion. Specifically, it inhibits the process of cell expansion by reducing the concentration of numerous carbohydrates, such as sucrose, starch, glucose, and fructose, in the fibers and also reduces the yield of the cotton plants (Tang *et al.*, 2017). Leaves respond to the occurrence of drought stress by engaging in osmotic adaptation by increasing the concentration of sucrose and potassium ions (K<sup>+</sup>) (Zhao *et al.*, 2019). Consequently, the reduced concentration of osmolytes in fiber cells hinders the proper development of fibers and yield. During the fiber extension stage, drought stress has an adverse influence on both the sucrose levels and the functionality of sucrose synthase within growing fibers. This, in turn, diminishes the turgor pressure of the fibers, resulting in a decreased final fiber length and reduced yield of cotton. For example, when drought stress conditions occur during flowering, it leads to a decline in fiber strength and enhancement in short fiber content. This is primarily attributed to decreased cell turgidity caused by drought, which impairs the enlargement of cells and the process of cellulose deposition in fiber development overall production yield of cotton plants is reduced (Abdelraheem *et al.*, 2020). Drought stress generates reactive oxygen species, reduces chlorophyll, and affects photosynthesis and biomass in cotton plants. It also disrupts fiber development, leading to poor fiber quality with shorter lengths and reduced yield. (Figure 3).



**Figure 3.** Effects of water deficit stress on cotton physiology, fiber development, and yield characteristics - illustration highlighting the repercussions of water deficiency on cotton plants, including diminished fiber growth, altered physiological responses, and compromised yield attributes

### Drought tolerance mechanism SMS in cotton

Developmental processes in plants have led to the development of various adaptive mechanisms that enhance their tolerance to the harmful impacts of drought stress. These accommodative mechanisms enable plants to cope with water scarcity and maintain their physiological functions (Batool *et al.*, 2020). Plants employ three significant strategies, namely stress avoidance, escape, and tolerance, to combat the challenges posed by drought stress. These strategies enable plants to respond and adapt to water scarcity at various levels, ranging from molecular processes to overall plant behavior (Galindo *et al.*, 2018).

#### *Evading drought escape*

Plants can undergo their entire life cycle to mitigate the adverse effects of negative impacts of drought stress on plant yield, certain plants utilize strategies that encompass swift growth, truncation of their life cycle, self-propagation, and seasonal development before to the initiation of the driest phase of the year. These strategies allow plants to finalize their life cycle and reproduce before the extreme drought conditions occur, maximizing their chances of survival and reproductive success (Álvarez *et al.*, 2018). Among these mechanisms, early flowering is considered one of the maximum effective resilient strategies in plants to escape the adverse effects of drought stress. While early flowering can be an effective adaptive mechanism for plants to escape drought stress, it is important to acknowledge that in some cases, it may result in a significant decrease in the overall duration of the plant's growth period and ultimately impact final plant production (Tekle and Alemu, 2016).

#### *Drought avoidance*

Under the escaping approach, plants continue a water potential by reducing stomatal transpiration losses and enhancing water uptake from the soil through a vigorous root system. In the context of the evasion strategy, plants sustain their water potential by decreasing losses through transpiration from stomata while bolstering water absorption from the soil through a robust root system. This involves the regulation of the stomatal aperture to minimize water loss through transpiration (Yang *et al.*, 2021). However, it is significant to distinguish that the overdevelopment of these avoidance mechanisms can come at a cost to the plant in terms of decreases in plant yield and a decrease in the average size of growth and development (Wasaya *et al.*, 2018).

#### *Drought tolerance*

At the photosynthetic machinery stage, plants employ an adaptive tolerance mechanism that involves decreases in the area of the leaf and restrictions in the growth of new leaves. These adjustments help plants cope with water deficits. Additionally, the production of trichomes, which are hair-like structures on the surface of the leaf, is an exomorphic trait that allows plants to bear drought stress in dehydrated environments (Zhang *et al.*, 2019). As a result, The pace at which water leakages through leaf transpiration is reduced. Trichomes, with their hair-like structures, create a barrier that reduces the direct exposure of the leaf surface to sunlight, which in turn lowers leaf temperature (Tiwari *et al.*, 2021). Indeed, it is widely recognized that adjustments in the root system play an important role in enabling drought-tolerant plants to manage drought stress. Different components of the root system, including root dimensions, length, proliferation, extension, and development rate, constitute fundamental elements of the primary approach utilized by drought-tolerant plants to adjust to inadequate water conditions (Tzortzakis *et al.*, 2020).

#### *Stomata regulation*

Minimizing water evaporation through leaves is an essential process in cotton plants when during periods of induced drought stress. The occurrence of flaccid and leaf progressing leads to a decrease in radiation exposure and consequently lowers the amount of water lost (Fang and Xiong, 2015). An illustration of this is observed in the cotton variability known as *G. hirsutum* YZ1, which exhibits fewer leaves in comparison to *G. hirsutum* Y668. The role of stomata, small openings on the leaf surface, perform a crucial role in facilitating vapor exchange amongst plant parts and the atmosphere. This mechanism is vital for energy production and the overall maintenance of cellular function in plants. It is worth noting that approximately ninety percent of water loss, referred to as transpiration, takes place through these stomatal openings in cotton plants, the initial response to minimize water loss during drought conditions, characterized by high transpiration rates, is the closure of stomata. Stomata conductance, which refers to the ability of stomata to facilitate gas exchange, holds promise as a probable marker of drought tolerance in cotton. An adverse association exists between stomata

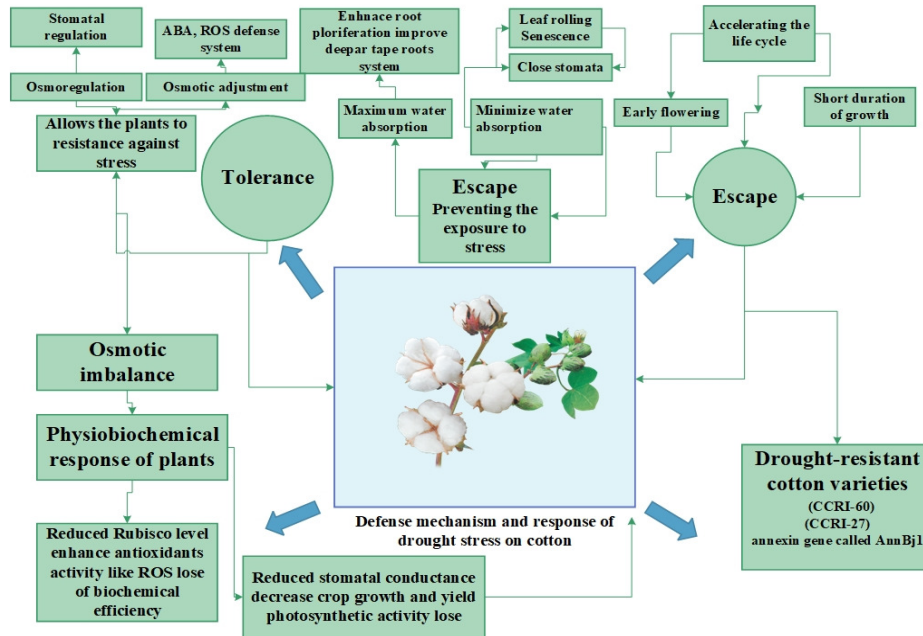
conductance and drought resilience, suggesting that lesser stomata conductance is related to advanced resistance to drought in cotton plants (Robertson *et al.*, 2021).

#### *Osmotic adjustment*

The transgenic cotton plants that were engineered to accumulate a maximum quantity of glycine betaine exhibited greater bear-to-drought stress related to the control plants. These transgenic plants demonstrated several advantageous traits, including enhanced chlorophyll pigments, greater RWC, enhanced osmotic potential, reduced breakdown of the lipid membrane, and a lesser percentage of ion leak. These characteristics collectively contribute to the enhanced capacity of drought-tolerance cotton plants to withstand and endure drought conditions (Ullah *et al.*, 2017). By introducing and expressing a mustard annexin gene called AnnBj1 in cotton plants, researchers observed an enhancement in the accumulation of proline and sucrose. This ectopic appearance of AnnBj1 consequences in increased drought tolerance in cotton. The elevated levels of proline and sucrose contribute to the better capability of the transgenic cotton plants to withstand drought conditions. In addition, when GhAnn1, an annexin gene derived from cotton, was overexpressed in cotton plants, it resulted in enhanced tolerance to both drought and salt stress (Zhang *et al.*, 2015).

#### *Antioxidants defense mechanism*

Plants have developed complex strategies for scavenging and controlling ROS, to maintain a balanced redox homeostasis within cells. Variations in the functioning of metabolic antioxidant enzymes can impact the capacity of cotton plants to bear periods of drought stress. A comprehensive review has extensively examined the defense mechanisms employed by plants to respond to the harmful impacts of ROS (Das and Roychoudhury, 2014). Glutathione, the resistance of cotton cultivars to drought stress depends on their varying antioxidative capacity. The cultivar M-503, known for its tolerance to drought, possesses antioxidative enzymes like APX, SOD, CAT, and POX, which are constantly active. These enzymes perform characteristics in reducing the oxidative stress caused by the breakage of lipids (Sekmen *et al.*, 2014). In cotton plants, the occurrence of drought led to the generation of ROS. However, concurrently, the activities of ascorbate peroxidase (APX) and glutathione reductase (GR) also rose, ensuring the continuous removal of ROS and sustaining the process until the plant regained its normal state after experiencing challenging situations (Zhang *et al.*, 2014). An experiment was conducted using two cotton cultivars, namely drought tolerance varieties (CCRI-27), (CCRI-60). The researchers discovered that the CCRI-60 cultivar exhibited drought tolerance due to several factors. Firstly, it displayed increased root length and vigor compared to CCRI-27. Additionally, CCRI-60 showed higher activity levels of antioxidant enzymes, particularly glutathione reductase (GR), along with an elevated proline content. This cultivar demonstrated the ability to effectively scavenge free radicals, providing superior protection against oxidative damage compared to CCRI-27. As a result, CCRI-60 exhibited enhanced resistance to drought and displayed improved growth. In contrast, when GbMYB5, a specific gene in *G. barbadense*, was down-regulated, the researchers observed a decrease in the function of antioxidant enzymes like SOD, POD, CAT, and GST. This down-regulation is controlled enhance oxidative stress under water-deficient situations. The findings suggest that cotton possesses multiple genes associated with antioxidant enzyme-related pathways, which warrant further investigation, particularly in drought-tolerant cultivars. Additionally, it is evident that other factors, such as zinc (Zn), also contribute to enhancing the antioxidant enzymes of cotton plants. Further investigations is required to better understand and harness these mechanisms to utilize them to enhance drought resilience in cotton varieties (Chen *et al.*, 2015). Plants employ diverse strategies (escape, avoidance, and tolerance) to manage drought, with adjustments in flowering, stomatal regulation, osmotic adaptation, and antioxidant defense mechanisms, crucial for cotton plant productivity. (Figure 4).



**Figure 4.** Unveiling the defense mechanisms and drought-resistant responses in cotton plants - visualization depicting the intricate defense strategies and adaptive responses deployed by cotton plants in combating drought stress, showcasing their resilience and survival mechanisms

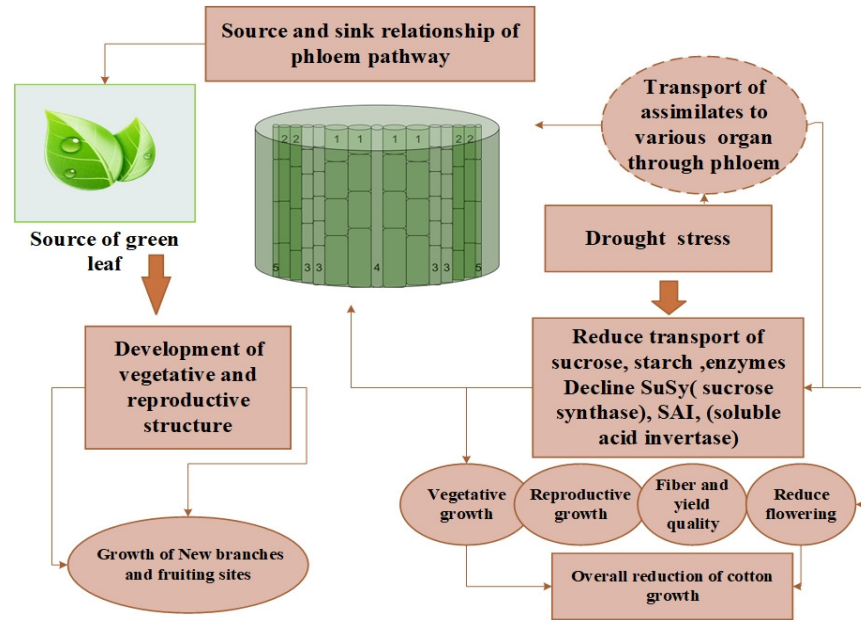
### Drought stress changes carbon assimilation

Plants have naturally evolved a complex mechanism to maintain a balance between sink, and source carbon assimilation activities. Cotton, as a crop with an indefinite growth period, stress disrupts this balance and results in decreased productivity. Cotton, being an indeterminate crop, is particularly susceptible to the influence of environmental stresses, such as drought, which can significantly affect the balance between Vegetative and propagative phases of cotton growth. Drought stress impacts the communication between sources, and sinks in the context of cotton. by altering the ability to assimilate construction in source tissues and their adaptation in various fruiting twigs of the cotton plant (Zhao *et al.*, 2019). During photosynthesis, starch and sucrose are produced in the source tissues of plants. These carbohydrates are then translocated to sink tissues, where they are converted into hexoses (simple sugars) and provide the vital energy required for vigorous plant development. This process of translocation and conversion of carbohydrates plays a vital role in supporting the overall development, and growth of plants (Rehman and Farooq, 2019). Moreover, drought stress results in a decrease in the accumulation of sucrose, starch, and hexoses in plants. This decline is primarily attributed to Diminished functioning of crucial enzymes engaged in carbohydrate processing, such as Wang *et al.* (2019) soluble acid invertase (SAI), sucrose synthase (SuSy), and sucrose phosphate synthase (SPS).

These enzymes perform critical characters in the synthesis and breakdown of sucrose and starch, and their decreased activity under drought stress disturbs the normal carbohydrate metabolism, contributing to the reduced accumulation of sucrose, starch, and hexoses (Loka *et al.*, 2020). As a consequence, drought stress promotes the preferential allocation of carbon assimilation towards older bolls in cotton plants (Zhao *et al.*, 2019). Under drought stress conditions, plants change their preference for translocating photo-assimilates (carbohydrates produced during photosynthesis). In the case of cotton plants, when faced with a limited water supply, there is a shift in the allocation of assimilates (Shareef *et al.*, 2018). In terms of assimilate partitioning

within bolls, there is a tendency for more assimilates to accumulate in the developing seeds rather than in the fiber of cotton plants (Tang *et al.*, 2017).

As a consequence of the preferential Buildup of absorbed nutrients in seeds rather than fiber within bolls, the activities of sucrose-cleaving enzymes may be lower than optimal, leading to Diminished fiber characteristics under conditions of water scarcity also affect the divisions of carbohydrates in flowers and leaves. When drought stress occurs following the beginning of the flowering phase, reproductive parts (such as pistils) and their subtending leaves undergo osmotic adjustment. This adjustment involves the accumulation of higher levels of sucrose in pistil cells to maintain cell turgor, ensuring their survival and development (Pilon *et al.*, 2019). Drought stress in cotton disrupts carbon balance, lowers carbohydrate accumulation, favors seed over fiber assimilation, and affects osmotic adjustment in reproductive parts, reducing productivity. (Figure 5).



**Figure 5.** Harnessing source-sink dynamics to mitigate drought stress in cotton - illustrating how managing carbon assimilation's source-sink relationship aids in alleviating drought effects on cotton plants

### Mitigation strategies for drought stress in cotton revolution

#### *Genome editing (GenEd)*

Cotton is one of the most important fibers, a good source for biofuel production, and an oil crop (Oliveira *et al.*, 2016). GenEd has brought about an innovative transformation in the field of life sciences. It is effectively employed for genetic manipulation in both plants and animals, representing equal levels of accomplishment. Scientists are exploiting genome editing techniques to achieve accurate alteration in the genetic makeup (Wen *et al.*, 2018). Fundamental investigations in the fields of biology and biotechnology can be undertaken using existing GenEd platforms.

#### *Various gene editing, tools in cotton plants*

In contrast to model plants such as *Arabidopsis*, rice, and maize, the whole genome sequencing of cotton has been covered. However, over the last decade, significant progress has been made in sequencing the genomes of tetraploid cotton species (Pei *et al.*, 2021). Cotton Gen stands out as a distinguished presented online knowledgeable source, conceding suitable entry to the available genomic and hereditary information related to

cotton. The source includes explained entire genomic sequences of diverse cotton species, singular genes, derived from ESTs, genetic maps, trait loci, markers, germplasm, and gene resources. In a similar vein, Cotton FGD also supplies a rapid and basic method to genome sequences, transcriptome details, functional explications, and genome re-sequencing data for all cataloged *Gossypium* genomes. Meanwhile, ccNET exposed co-expression networks and identified operational units from diploid and polyploid cotton species, presenting 1155 and 1884 modules within *G. hirsutum*, and *G. arboretum*, respectively (Song *et al.*, 2017).

The enhancing efficiency of the NGS method and the progression of experienced in silico approaches have allowed the generation of single nucleotide polymorphisms, and SNPs across the entire genome, even for complex genomes like the 2.5-Gb allotetraploid cotton genome. In the context of cotton, a SNP panel known as SNP63K has been created. This panel encompasses tests for 45,104 potential intraspecific SNP markers and 17,954 potential interspecific SNP markers (Ashrafi *et al.*, 2015). The primary attempt in creating the SNP63K array for cotton establishes a fundamental high-capacity genotyping instrument and a foundation for the genetic investigation of agriculturally and economically important features. Given that a considerable portion of the genome experiences copy number variations, SNPs, rather than CNVs these CNVs could prove appreciated in the discovery of many phenotypic differences that aren't described by SNPs. There exists a wealth of indications supporting the occurrence of CNVs in plant genomes, which can induce changes in gene structure, quantity, and gene regulation. Primarily, genes impacted by CNVs are closely related to essential traits. In the realm of cotton, a total of 989 genes affected by CNVs have been effectively identified, These genes are particularly interrelated to functions concerning cell wall organization, regulation of translation processes, and plant morphology (Fang *et al.*, 2017). In current times, the technique of transcriptome summarizing has occurred as a fundamental tool, exhibiting how insights extracted from sequence data can be translated into a comprehensive understanding of gene functionalities. Within this context, RNA-Seq has developed as a useful path for inclusive whole-genome transcriptome profiling, owing to its ability to directly sequence transcripts through high-throughput sequencing technologies. A notable example is the recent assembly of the *G. hirsutum* inbred line TM-1's transcriptome, along with a compilation of all publicly accessible expressed sequence tags, and ESTs (Ashrafi *et al.*, 2015).

Following this, a total of 519 cotton genes exhibits gene modifications when comparing domesticated and wild cotton cultivars. Remarkably, a subdivision of these genes is closely connected with important, agronomic and domestication-related traits (Song *et al.*, 2017). Within the field of cotton, alterations in DNA methylation are intricately tied to the seasonal fluctuations in fiber development, as a representation, the ever-changing function of methylation in the progression of ovules and fibers underlines that RNA-directed DNA methylation, RdDM mediated by CHH (where H is any nucleotide except G) methylation is linked to gene activation in ovules. In contrast, methylation reliant on chromomethylase 2 CMT2, is linked with the repression of genes in fibers (Yuan *et al.*, 2015).

Over the past few years, investigators have been exploiting various tools such as endonucleases, ENs ZFNs, zinc finger nucleases, transcription activator-such as effector nucleases (TALENs), meganucleases, and gathered regularly interspaced short palindromic repeats CRISPR-associated protein (CRISPR/Cas) and CRISPR. These tools are employed to occupy the cellular repair mechanisms by inducing DSBs, double-strand breaks at predetermined target locations, Creating a connection between an EN, reprogrammable endonuclease and the target sequence is vital to generate a DSB, double-strand break at the intended target location (Mahfouz *et al.*, 2014). ZFNs, mega nucleases, TALENs, and CRISPR/Cas9 are prominent genetic management tools that have been successfully exposed for precise gene alterations in plant species (Aziz, 2021). It is possible to modify and rearrange genome editing, tools to arrange with specific target sequences. Tools like TALENs, ZFNs, and CRISPR/Cas can be distinct in their design, expression vectors, cloning, and alteration techniques. However, they share a common initial principle, which involves inducing DSBs, at the designated target site (Gupta and Musunuru, 2014). Moreover, in the context of plants, this method has revealed lower competence. Additionally, the deletion of a gene can be talented by inducing two DSBs in the regions flanking

the gene. Numerous online and offline software tools are reachable for the design and virtual assembly of GenEd, tools. Plasmids or Clones can also be attained from various investigators or nonprofit plasmid sources like Add gene. Separately from the widely used TALENs, ZFNs, and CRISPR/Cas systems for genome editing, there are other Ens, available, such as Mega nucleases (DADGILAGLI), which have also been documented for targeted genome modifications (Khan *et al.*, 2018).

#### *Zinc finger nucleases (ZFNs)*

The early occurrence of a targeted gene mutation occurred in tobacco plants towards the close of the prior century, employing DSBs. A naturally occurring mega nuclease, specifically the I-Sei enzyme with an 18-base pair appreciation sequence, was applied to achieve the favorite modification (Urnov, 2018). At the seedling stage of Arabidopsis, precise mutation has been generated by inducing elevated temperature-dependent expression of ZFNs (Munaweera *et al.*, 2022). It was observed that 10% of the plants showed future mutations, which were passed on to consecutive generations. Following repair through homologous recombination and the addition of the NPTII reporter gene at multiple chromosomal locations, the function of a faulty GUS was detected across 10 distinct transgenic tobacco lines (Luo *et al.*, 2016). In maize, a gene conferring resistance to herbicides was intentionally directed to a specific location in multiple distinct instances. This was accomplished by co-expressing ZFNs, together with a complementary donor molecule. As a result, genetic modifications were introduced and continued across subsequent generations (Dong *et al.*, 2021).

#### *TALEs and TALENs for targeted genome modifications*

Making TALENs can be a difficult task due to the lengthy and repetitive nature of TALE, Transcription Activator-Like Effector DBDs. TALENs can be constructed through various methods, surrounding predictable cloning, the solid-phase techniques, and the Golden Gate approach (Kim and Kini, 2017). TALEs and TALENs have been working to target the genes of various plant species, including tobacco, Brachypodium, rice, barley, *Arabidopsis*, and various others (Z. Khan *et al.*, 2018). In short, natural TALE proteins are produced by plant pathogenic bacteria known as Xanthomonas (Teper and Wang, 2021).

While ZFNs and TALENs share the same nuclease domain, FokI nuclease, the binding domain character a more essential role in specificity and efficiency. This finally leads to a higher mutation rate. When compared to ZFNs, TALENs have been more commonly employed across various plant species for precise gene modifications (Shamshirgaran *et al.*, 2022). (Table 1).

**Table 1.** Various gene editing and modification of ZFNs and TALEs and TALENs in plants

<b>Plant species</b>	<b>Genes</b>	<b>Genes modification</b>	<b>References</b>
<i>Arabidopsis</i>	<i>ADH1, TT4</i>	NHEJ	(Shukla <i>et al.</i> , 2009)
Tobacco	<i>SuRA, SurRB</i>	NHEJ	(Townsend <i>et al.</i> , 2009)
<i>Petunia</i>	<i>mGUS</i>	NHEJ	(Marton <i>et al.</i> , 2010)
Maize	<i>IPK1</i>	NHEJ	(Zhang <i>et al.</i> , 2010)
Rice	<i>EBE (AvrXa7 and PtbXo3)</i>	NHEJ	(Li <i>et al.</i> , 2017)
<i>Brassica oleracea</i>	<i>FRIGIDA</i>	NHEJ	(Sun <i>et al.</i> , 2013)
Barley	<i>PAPhy_A</i>	NHEJ	(Gurushidze <i>et al.</i> , 2014)
Potato	<i>Vlnv, ALS</i>	NHEJ, HDR	(Butler <i>et al.</i> , 2016)
Maize	<i>Glossy2 locus</i>	NHEJ	(Char <i>et al.</i> , 2015)
Wheat	<i>MLO</i>	NHEJ	(Butler <i>et al.</i> , 2016)
<i>Arabidopsis</i>	<i>ADH1</i>	NHEJ	(Forner <i>et al.</i> , 2015)

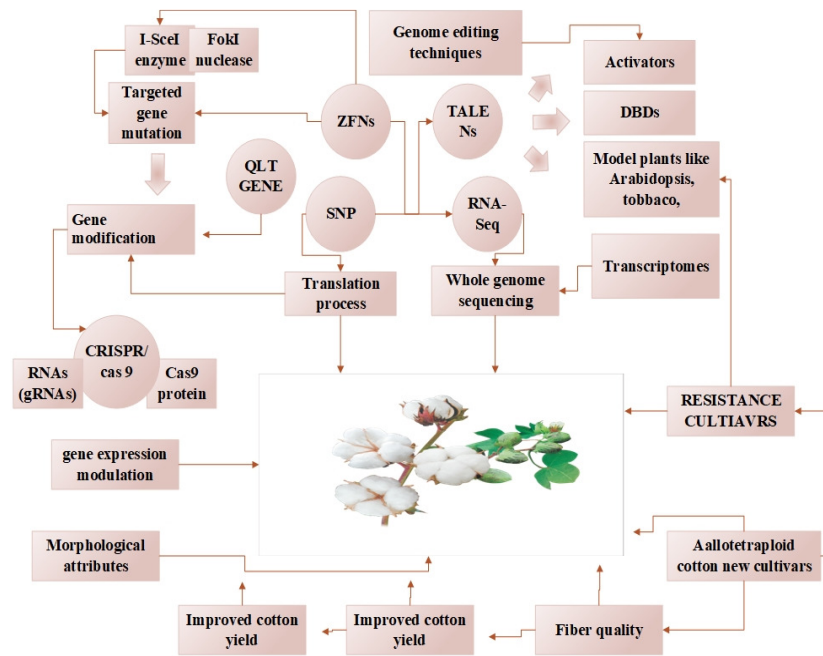
*CRISPR/ CAS Gene*

The importance of CRISPR/Cas9, shows the progressions in molecular tools like the CRISPR/Cas9 system have solved the potential to explore gene functions and influence fundamental biological visions to improve the agricultural performance and quality attributes of cotton. However, as of our present knowledge, there are no documented instances exposing, the utilization of these technologies for changing the cotton genome. At present, a majority of genome editing practices rely on elements of cell and tissue culture throughout and after the modification process to yield plants harboring the intended mutations. However, the renewal feature remains remarkably needful on the genotype and remains challenging for cotton (Janga *et al.*, 2017).

The role of CRISPR-associated protein 9 (Cas9) is created from *Streptococcus pyogenes* and is part of the collected habitually interspaced short palindromic repeat (CRISPR) system (Simón *et al.*, 2018). Indeed, CRISPR/ Cas9 is a rapidly advancing genome editing technology that has established positive applications in several model plant species. One notable distinguishing of CRISPR/Cas9 is that it identifies DNA cleavage sites based on the principles of Watson-Crick base pairing (Belhaj *et al.*, 2015). CRISPR-Cas9 functions through the teamwork of three essential components: the CRISPR RNA (crRNA), Cas9 protein, and trans-activating CRISPR RNA (tracrRNA) (Mei *et al.*, 2016). The application of the CRISPR/Cas9 system as a genome engineering tool gained distinction with the discovery that the target DNA sequence could be easily reprogrammed by modifying just 20 nucleotides within the CRISPR RNA (crRNA) Moreover, it was realized that employing various guide RNAs (gRNAs) with distinct sequences permitted the accomplishment of multiplex genome engineering, targeting multiple loci simultaneously. This groundbreaking research demonstrated that CRISPR/Cas9 is a simple, operative, cost-efficient, and adaptable tool for gene expression, gene mutation, modulation (both repression and activation), and genome editing (Javed *et al.*, 2019).

Scope of CRISPR In the field of plant biology, the initial application of CRISPR/Cas9-based genome editing (Zheng *et al.*, 2021). Demonstrated its extensive adaptability in the model species Arabidopsis and in the staple crop rice. Following this success, the technology has been attached to additional crop plants, including maize (Brutnell, 2015). In the field of cotton, the utilization of the CRISPR/Cas9 technology is still in its early stages. A distinguished current progression includes the attainment of multiple-site genome editing in allotetraploid cotton using the CRISPR/Cas9 system. By targeting genes like GhARG, arginase discosoma red fluorescent protein2, DsRed2, and GhCLA1, chloroplast development, this innovation highlights the high reliability and effectiveness of CRISPR/Cas9 for genome editing in cotton, Certainly, the potential and applications of CRISPR/Cas9 in cotton genome editing are composed to undergo significant advancement as time (Wang *et al.*, 2014). Moving forward, ongoing enhancements will progressively expand the scope of its application, evolving from mere mutant generation to accurate gene regulation within noncoding enhancer regions in cotton (Wang *et al.*, 2017). The development of precise and controlled CRISPR-Cas gene editing tools has been helped by the discovery of protein inhibitors, referred to as anti-CRISPRs or Acrs. These Acr proteins can effectively regulate unintended genetic changes and impede the editing functions of Cas proteins, impacting both on and off-target mutations (Choudhary *et al.*, 2023). (Figure 6).





**Figure 6.** Impact of gene editing and resistant cultivars on enhancing cotton growth and yield - visual representation highlighting the influence of diverse gene editing techniques and resistant cultivars in encouraging the growth and yield of cotton plants

#### *QTLs gene editing*

The genetic elements consist of both minor and major quantitative trait loci, QTLs with diverse genetic interactions, along with the maintaining of several genes. These molecular units exhibit the features of a genetic useful unit, resembling the breeding process that targets specific molecular attributes responsible for stress-resilient traits. Notably, a complex module has recently been proposed for the development of temperature-resilient crops (Zhang *et al.*, 2019).

Various genes and gene modifications have the ability of drought tolerance of different agricultural traits in cotton. In their study, utilized 261 restriction fragment length polymorphisms (RFLPs) to create a hereditary chart and identify QTLs, quantitative trait loci related to leaf morphology. They focused on an F2 population consisting of 180 plants derived from hybridization between *Gossypium hirsutum* and *Gossypium herbardense*. Through their analysis, the authors successfully mapped 40 QTLs, one of which is particularly significant as it is located on chromosome 6 and is related to drought stress. This specific QTL influences leaf trichome density and regulates the transpiration rate during conditions of water stress (Mahmood *et al.*, 2019). In this research, a population consisting of 188 F2:3 individuals were examined to detect quantitative trait loci linked to drought tolerance. The population was obtained through a hybrid mating between *Gossypium hirsutum* and Hawaiian cotton. The objective of the study was to determine QTLs specifically associated with drought tolerance in real field conditions (Zheng *et al.*, 2016).

Limited QTLs, related to drought have been recorded in cotton. To further investigate drought tolerance, a subsequent meta-analysis was conducted to identify areas of concentration (hotspots) and clusters of QTLs. Out of a total of 661 QTLs related to stress, 23 QTLs linked to drought tolerance were found across 15 distinct chromosomes. Significantly, two regions with a high concentration of quantitative trait loci linked to chlorophyll content were detected on chromosome 24 among the comprehensive set of 28 separate traits related with drought stress tolerance (Abdelraheem *et al.*, 2017). Drought induces the plant to close its stomata, leading to a decrease in the absorption of CO<sub>2</sub> and making the plant susceptible to photodamage (Zargar *et al.*, 2017). Meta-analyses allow for the convenient grouping of recently reported cotton QTLs from multiple

studies. These findings hold potential for MAS, to develop consistently drought-tolerant cotton varieties. Additionally, the identified quantitative trait loci collections and markers can aid in the discovery of new applicant genes linked with drought tolerance. Below is a list of various quantitative trait loci that have been reported for traits related to drought tolerance. Specifically, in cotton, molecular markers molecular markers like HY5-specific CAPS, PHYA, PHYB, and dCAPS, have been produced for MAS, aiming to alleviate the impact of drought stress. These markers enable the identification and selection of desirable traits related to drought resistance. Quantitative trait loci have been identified for multiple fruit characteristics such as harvest timing, skin color, fruit weight, and sugar content in fruits like apple, pear, peach, mango, avocado, papaya, and grapevine(Kuhn *et al.*, 2017). (Table 2).

**Table 2.** Presents the specific genetic regions such as QTLs, associated with drought resistance in cotton

QTLs	Attributes	Size, type of population	Quantity and varieties of indicator used	Reference
49	Fiber's production, cultivation of cotton seed, length fibers, fibers extension, bolls weight, leaf area, fresh shoots, weight plants height.	97 F5:9 RILs (TM-1 × NM24016)	RGA-AFLP, SSR and GBS-SNP (1004)	(Abdelraheem <i>et al.</i> , 2018)
59	Temperature of the canopy, index indicating vegetation difference, height of the canopy, and index indicating leaf area.	95 RIL (TM-1 × NM24016)	429, SSR	(Pauli <i>et al.</i> , 2016)
67	Height of plants, amount of chlorophyll present, number of leaves, area of leaves, DFW of leaves, no. of branches, no. of bolls, and bolls weight	188 F2:3 (CRI-12 × AD3-00)	1295, SSR	(Zheng <i>et al.</i> , 2016)
6	Height of plants, weight of fresh shoots, and weight of roots.	142 BILs (Pima S-7 × Sure-Grow747)	AFLP, RGA and RGA-AFLP (34)	(Abdelraheem <i>et al.</i> , 2015)
14	Amount of chlorophyll present, temperature of leaves, weight of fresh shoots and roots, Evapotranspiration rate and plant height.	140 RILs (Dan-dara × Giza-70)	SSCP (165)	(Abdelraheem <i>et al.</i> , 2015)
6	Water content relative to a reference point. Rate of moisture evaporation from separated leaves. stability of cell membranes, frequency of stomatal openings, and size of stomata.	100 F2 (FH-207 × FH901)	EST-SSR (2365)	(Amjid <i>et al.</i> , 2015)
3	Rate of moisture evaporate from separate leaves and water content relative to a reference point.	100 F2 (B-557 × FH1000)	SSR and EST-SSR (524)	(Saleem <i>et al.</i> , 2015)

Plant genome editing is hindered by a scarcity of efficient alleles, and the use of magnetic nanoparticles (NPs) and TALEs faces challenges in delivering them into reproductive cells. Although magnetoreception transformed cotton plants successfully, its dependence on pollen pores restricts its efficacy in targeting tissues with concealed germline cells and meristematic tissues (Mao *et al.*, 2019).

*Genetic improvements*

Enhancing the development of genotypes resistant to drought or employing genetic manipulation techniques to induce drought tolerance represents the most effective and sustainable approach to alleviate the effects of water-deficient stress. Drought tolerance is a multifaceted characteristic encompassing physiological factors such as water imbalances. These include numerous types of water (bound water, metabolic water, and cell sap), biochemical factors like the equilibrium of free amino acids, antioxidants, and starch, as well as structural features of plants and boll dropping patterns. Therefore, it is essential to hold these important apparatuses to determine continued growth in this field (Ergashovich *et al.*, 2020). Many genetic functioning has been exposed that grant genetic resilience to drought stress. Various genes and transcription factors have been predicted as pivotal controllers of these responsive mechanisms (Shinwari *et al.*, 2020).

Different cotton genotypes display differences in the presence of transcription genes and the appearance of these genes (Iqbal *et al.*, 2017). Out of the four cultivated cotton species, *Gossypium hirsutum* demonstrates restricted diversity in drought stress tolerance owing to consistent selection and breeding of nearly indistinguishable genetic strains. Conversely, *Gossypium barbadense* and *Gossypium arboreum* display distinguished flexibility to abiotic stresses, notably about drought stress. *Gossypium barbadense* and *Gossypium arboreum* are grown in controlled regions but own a wide genetic foundation. Multiple times, the effective addition of drought-tolerant genes into *Gossypium hirsutum* has been accomplished, increasing drought stress flexibility in this particular species (El-Esawi and Alayafi, 2019). (Table 3).

**Table 3.** Genes introduced to cotton, source species, principal function, and their impacts on reproductive growth and fiber maturation

Modified gene	Type	Source organism	primary function	Impact on reproductive growth and fiber development	References
<b>StDREB2</b>	Transcription factors known as Dehydration-Responsive Element Binding (DREB) Factors	<i>(Solanum tuberosum L.)</i>	Controlling routes related to stress tolerance	↑Increase in the number of bolls	(El-Esawi and Alayafi, 2019)
<b>IPT</b>	Gene encoding Isopentenyl Transferase	<i>Agrobacterium tumefaciens</i>	Control the production of cytokinins through regulation	↑Increase in boll count chiefs to an enhance in fiber yield.	(Zhu <i>et al.</i> , 2018)
<b>ScALDH21</b>	Gene belonging to the ALDH superfamily	<i>Syntrichia caninervis</i>	Metabolic processes in cells and the maintenance of balance in ROS, levels	↑A maximum in boll weight contributes to higher fiber production and improved fiber quality.	(Yang <i>et al.</i> , 2019)
<b>SNAC1</b>	Transcription factors belonging to the stress-related	<i>Oryza sativa</i> (Rice)	Promote or enhance the growth of roots	↑Enhance photosynthesis during the flowering stage, resulting in an	(Liu <i>et al.</i> , 2014)

	NAC superfamily			increased number of bolls.	
<b>AtEDT1/HDG11</b>	Transcription factor belonging to the Homeodomain-START family.	<i>Arabidopsis thaliana</i>	The gene encodes a transcription factor belonging to the homeodomain-leucine zipper (HD-ZIP) family.	↑ Increase in boll number, boll weight, and fiber harvest.	(Yu <i>et al.</i> , 2016)

Genetic engineering is an additional contemporary approach for introducing drought stress resistance in cotton through gene transfer (Table 2). Moreover, genetically engineered cotton, expressing specific genes, exhibits improved production related to non-transgenic cotton with regular gene expression. For instance, the introduction of an *Arabidopsis thaliana* transcript gene AtEDT1/HDG11 in transgenic cotton enhances drought stress tolerance by promoting the development of a wide root structure and robust enzymatic resistance strategies (Yu *et al.*, 2016). These studies have demonstrated improved drought stress resilience in transgenic cotton through the overexpression of genes associated with drought stress tolerance. These overexpressed genes play a crucial part in defending ROS, generated in response to drought stress (El-Esawi and Alayafi, 2019). Studies have reported that cotton genotypes engineered with an IAA, biosynthetic gene exhibit increased fiber production and improved fiber fineness. This is attributed to the part of IAA in the initiation of cotton fibers (Bajwa *et al.*, 2015). Research findings indicate that transgenic cotton plants overexpressing the GhEXPA8 gene display enhanced fiber length and strength. This improvement is achieved by producing the fiber content in the fibers (Han *et al.*, 2014). Genetic engineering techniques are employed for both the introduction of novel genes and the enhancement of the expression of prevailing genes. In the case of transgenic cotton, overexpression of the GhABF2 gene, which encodes a bZIP transcription factor, has been observed to confer drought tolerance. This is achieved through the regulation of ABA, a key hormone consist in drought response and stress signaling pathways (Mahmood *et al.*, 2019).

Resistance cultivars to improve cotton yield Cotton, a widely grown fiber plant, produced natural fibers used in the textile sector. *G. hirsutum* is responsible for over 90% of the production harvest. Many improved cotton types have been instrumental in expanding yields (Fang *et al.*, 2017). Breeder's goal to progress fresh cultivars by simultaneously enhancing involved genetic yield and quality factors and getting pliability against various challenges. However, the incomplete grasp of the genomic foundation of important agronomic traits currently constrains this effort (Consortium *et al.*, 2018). Progressive genome assembly for modern *G. hirsutum* strains, along with outdated varieties TM-1 and ZM24, with a focus on quality (Wang *et al.*, 2019). *G. barbadense* accounts for around 10% of the total yield and produces lint fibers of superior quality. A potential strategy for improving the fibers and disease resistance of *G. hirsutum* involves integrating favourable hereditary characteristics from *G. barbadense* into *G. hirsutum*. Nonetheless, there is a lack of clarity regarding the genomic differences between *G. barbadense* and the modern *G. hirsutum*. Pinpointing relevant single nucleotide polymorphisms SNPs, that are linked to these traits contributes to a better comprehension of the genetic basics sustaining cotton agricultural characteristics (Ma *et al.*, 2018). This research endeavor involved the creation of two accurately created genomes along with complete remarks for both the contemporary the *Gossypium hirsutum*, *barbadense* acc. Pima90 and *G. hirsutum* cv. NDM8. NDM8 holds prominence as a widely cultivated variety in the cotton-producing regions of China's Yellow River Valley, while Pima90 has played an important role as genetic material in the field of molecular breeding. Examination of the two genomes

and their resequencing visible essential genomic changes that transpired throughout the breeding process, thereby providing valuable possessions for improving cotton crop cultivation (Ma *et al.*, 2018).

### **Agronomic management**

#### *Applying external growth regulators to mitigate the effects of drought stress on cotton*

The exogenous treatment of PGRs has been found to improve the abiotic stress tolerance of plants, including drought stress resistance in cotton. Several PGRs have been reported to positively impact the drought stress response in cotton plants. These plant growth regulators have been described may including substances such as ABA, cytokinins, brassinosteroids, and SA, among others (Tanveer *et al.*, 2019). An example of the beneficial effects of exogenous treatment of PGR, in cotton under drought stress is the use of melatonin. Melatonin application has been shown to regulate the starch balance in reproductive development, specifically by improving carbohydrate synthesis through improved activities of SuSy, and ADP-glucose pyrophosphorylase (AGPase). This regulation is achieved by promoting higher ATP synthesis, resulting in increased productivity in drought-stressed cotton plants (Hu *et al.*, 2020).

Treatment of drought-stressed cotton seedlings with acetic acid has been found to have positive effects on boll development, resulting in increased fiber production and improved fiber characteristics. auxin treatment enhances the levels of ABA, and JA, through the upregulation of genes involved in their biosynthesis. This leads to improved soil RWT, which aids in the existence of cotton plants under drought-stress conditions. The application of acetic acid provides a potential means to mitigate the negative impacts of drought stress on cotton crops and enhance their flexibility in water-limited environments (Li *et al.*, 2021). The study investigated the effects of multiple plant growth regulators on biochemical traits, crop yield, and fiber quality in three cotton cultivars (Arekhi *et al.*, 2023). Similarly, the exogenous treatment of abscisic acid, SA, polyamines, and proline, has been shown to increase drought stress tolerance in cotton. These substances aid in osmotic potential regulating the turgor pressure of cells, and strengthening the plant's antioxidant defense mechanisms against oxidative stress (Zahoor *et al.*, 2017). PGRs face degradation from environmental factors, impacting their efficacy. Nanotechnology in agriculture addresses this challenge by providing nanoscale materials that enhance the stability and activity of active agents while reducing environmental impacts (Pereira *et al.*, 2017).

#### *Exogenous treatment of nutrients to alleviate drought stress in cotton*

Drought stress disrupts plant metabolism by impairing the absorption and movements of nitrogen to the beneath parts of the plant (Xiong *et al.*, 2018) The treatment of nitrogen to cotton plants under drought stress conditions can contribute to the enhancement of drought stress resistance. When N is supplied to water-deficiency -stressed cotton plants, it can stimulate the activation of antioxidant enzymes. These enzymes play a crucial role in reducing the accumulation of ROS (Iqbal *et al.*, 2020). The exogenous supply of nitrogen to cotton plants has been exposed to improve their performance under drought-stress conditions. When N is supplied to drought-stressed cotton plants, it enhances the uptake of nitrogen, leading to increased nitrogen availability for various metabolic processes, Furthermore, high N concentration in plants helps alleviate the stomatal limitations induced by drought stress. Stomata control the exchange of gases, including water vapor, between plants and the atmosphere. Under drought stress, plants leads to closure of stomata, limiting the uptake of carbon dioxide for photosynthesis but also reducing water loss (Iqbal *et al.*, 2020).

In arid regions, the cotton yield is negatively affected by limited phosphorus (P) availability and water scarcity. However, by applying phosphorus under water-deficient conditions, the uptake and efficient transmission of P are improved. This, in turn, improved the cotton plant's capacity to bear stress, leading to increased yield production and higher cotton harvest (Jun *et al.*, 2017). By applying phosphorus (P), the

availability of P in the soil is increased. This, in turn, leads to improvements in the growth traits of cotton plants and enhances the yield of seed cotton, especially in dry soil conditions. Underwater deficiency in harshly aeration soil (-1.5 MPa), the extreme availability of phosphorus (P) increased the leaf water content, including total, free, and bound water. This was primarily due to the supply of excessive osmolytes to the cells. As a result, there was an absorption of free and bound water within the leaves, leading to a rapid expansion of the leaf area (Ul-Allah *et al.*, 2021).

Potassium (K) is an important mineral for cotton plants, playing a crucial part in their water relations. It exerts a significant influence on the morphology of cotton by adapting various biochemical and physiological process (A. Khan *et al.*, 2018). The utilization of potassium has proven to be successful in enhancing the antioxidant defense mechanism, as well as N and sucrose metabolism, within the useful leaves of cotton plants experiencing drought stress. These improvements consequently contribute to an increased preservation of buds and ultimately result in higher fiber production. Furthermore, the application of potassium fertilizer also plays a role in maintaining the activities of PM, ATPase, and PEPC. These enzymes are closely associated with cotton length and strength of fibers (Zhao *et al.*, 2019). It has been revealed that the simultaneous foliar treatment of potassium nitrogen, and magnesium can enhance the characteristics of cotton fibers, in shortage of water conditions. Another study demonstrated that a moderate treatment of nitrogen enhanced the characteristics of cotton fibers in rainfed conditions. However, an excessive amount of nitrogen negatively impacted fiber quality in irrigated cotton (Sui *et al.*, 2017).

#### *Irrigation management techniques for optimizing water use in drought stress conditions on cotton*

Field management techniques can be implemented to prevent water loss through evaporation as part of drought management strategies in cotton cultivation. The incorporation of soil OM has the potential to improve the soil's capacity to absorb water (Minasny and McBratney, 2018). Preventing evaporation losses is a crucial aspect of effective drought management in cotton. It is widely acknowledged that weeds can create competition with crop plants for nutrients and water, which can exacerbate drought stress. Consequently, managing weeds can play a vital role in enhancing drought stress tolerance (Oldfield *et al.*, 2018). Studies have indicated that merging incomplete rhizosphere aeration with covering can enhance cotton productivity, and growth in conditions of shortage water supply. Furthermore, it has been observed that for limited water resources, opting for alternative irrigation methods and adjusting irrigation scheduling can lead to improved water use competence and water efficiency (Iqbal *et al.*, 2019). SWAP's optimization finds the minimum water for yield but doesn't address non-linear water stress effects. The new algorithm uses SWAP's seasonal water optimization to prevent cotton stress and follow farming practices (Polinova *et al.*, 2019).

#### *Integrated pest disease management strategies for drought-stressed cotton*

Various pest and insect diseases can disrupt the mechanisms of cotton plants, ultimately resulting in reduced yield and stunted growth. Cotton disease can be classified into two main groups based on the strategies through which they cause damage to plants: chewing insects and piercing-sucking insects (Imran *et al.*, 2019). The first group comprises insects that consume the plant biomass as part of their feeding behavior. These insects include the *Pectinophora gossypiella*, *Earias vittella*, *Helicoverpa armigera*, *Spodoptera frugiperda*, and *Alabama argillacea* (Kamburova *et al.*, 2022). Cotton pathogens encompass various types of microorganisms such as viruses, bacteria, and fungi. Among these, certain fungi belonging to the genera *Pythium*, and *Fusarium*, *Rhizoctonia*, *Thielaviopsis* are known to impact cotton seedlings, leading to the occurrence of seedling root rot (Dhamodaran *et al.*, 2023).

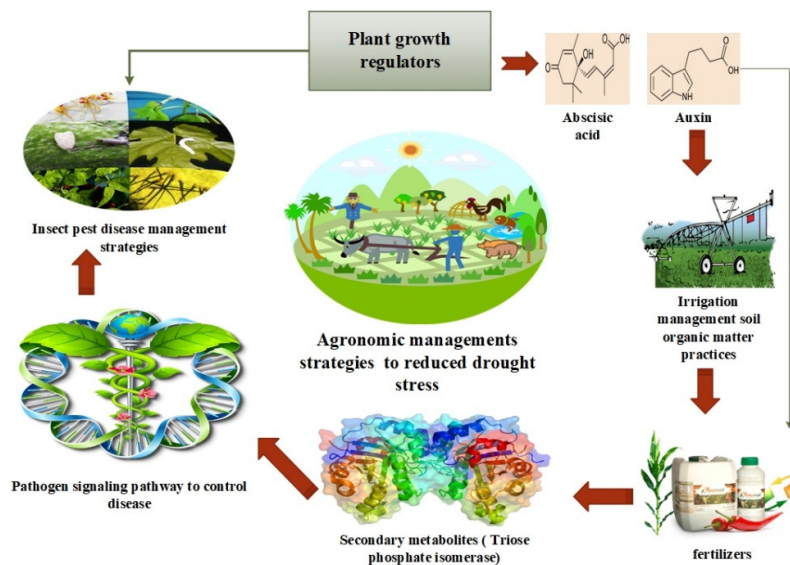
#### *Application of exogenous substances to induce drought tolerance*

several management strategies for insect pest control can help improve the production and fiber characteristics of cotton, particularly under drought-stress conditions. Some of these strategies include. The

extensive coevolution between cotton disease and pathogens has led to the development of strategies aimed at minimizing the harm caused by these biotic factors. At a molecular level, the activation of specific resistance genes (R-genes) occurs when cotton plants are exposed to pathogens. This activation sets off a series of internal processes that ultimately result in the production of defensive substances, which effectively decrease the damage inflicted by pathogens additionally, cotton has evolved morphological and chemical defense mechanisms as further means of mitigating the impact of pests (Deng *et al.*, 2020). Morphological defenses and chemical compounds, known as secondary metabolites, play a significant role in directly impacting insects, whether in their adult (imago) or larval stage. These defenses have a substantial influence on key parameters of the insect's life cycle. Among the morphological adaptations of cotton, trichomes are particularly noteworthy as they enhance the plant's resistance to insects. Trichomes serve as a physical barrier, preventing insect penetration, and they also secrete chemical repellents, toxins, or adhesive substances, further deterring insect damage (Kaczmarek and Boguś, 2021).

SM, such as flavonoids, terpenoids, tannins, and anthocyanins perform a crucial role in providing straight defense to cotton plants against insects. Among these metabolites, terpenoids have been extensively studied for their protective properties in cotton. Cotton plants synthesize various terpenoids, including hemigossypolone, and helicoides, hemigossypol, H1, H2, H3, and H4 gossypol, these terpenoids are predominantly found in small intracellular pigments, subepidermal within the cotton plant. The terpenoids present in cotton hurt a range of diseases, including *H. virescens*, *armigera*, *Estigmene acrea*, *P. gossypiella*, *E. insulana*, and *E. vitella*. Furthermore, compounds like gossypol-like substances exhibit toxicity towards the gall nematode species *Meloidogyne incognita* (Arce *et al.*, 2021).

It is valuable declare that when cotton plants are damaged by pests and pathogens, it triggers the synthesis of terpenoids through the activation of signaling pathways dependent on SA, JA, and ethylene. This activation occurs in response to specific elicitors, which interact with receptors and subsequently increase intracellular calcium levels. The elevated calcium levels, in turn, stimulate the activity of Ca<sup>2+</sup>-dependent protein kinases. CDPKs play a crucial role in the process by phosphorylating proteins and modulating gene expression patterns. This activity leads to the activation of MAPKs, mitogen-activated protein kinases (which then promote the biosynthesis of SA and JA, as well as activate the ethylene pathway (Kumar *et al.*, 2020). (Figure 7).



**Figure 7.** Agronomic Strategies Mitigating Drought Stress in Cotton - Diagram showing a range of agronomic management techniques employed to respond drought stress in cotton cultivation, showcasing diverse adaptive practices

## Future prospects

Additional investigation is unavoidable to examine these genes' behavior concerning drought stress in their natural environment, along with cotton protein kinases associated with drought. Further exploration is necessary to improve crop efficiency under water-deficient stress using RNAi, technology. Genome editing tools, CRISPR/Cas9 will be utilized to generate innovative alleles and necessary agronomic and characteristics attributes in engineered plants, enabling them to develop tolerance against drought stress and other challenging environmental conditions. Furthermore, this approach involves altering and discerning epigenetic patterns to establish a link between single-nucleotide polymorphisms (SNPs), which are examined for genetic characteristics, and genome-wide association studies. It is worth noting that this method was developed to attain a thorough understanding of the traits in the initial generation (T0) by creating a comprehensive mutant library across the entire rice genome. Mitigating the negative effects of drought on cotton can be achieved through the treatments of plant PGRs, osmoregulation mechanism, and essential mineral nutrients like potassium (K), and (N). These interventions aid in alleviating the negative impact of drought by enhancing cellular signaling, maintaining hormonal balance, regulating cell turgor potential, improving assimilate allocation to fruiting sites, and optimizing carbohydrate metabolism. Additionally, genetic methods such as gene introgression from relevant species, and genetic alteration of drought-related genes have exposed promising outcomes in inducing drought tolerance in cotton while maintaining fiber quality and production. Multi-Omics Approaches like Applying integrated methods like transcriptomics, genomics, metabolomics, and proteomics, to gain a complete understanding of the molecular responses of cotton to drought stress. Smart Farming advancements such as exploiting artificial intelligence, and machine learning sensor skills for real-time knowledge of crop responses to drought, enabling more adaptive and responsive agronomic interventions.

## Conclusions

To achieve substantial improvements in cotton production in drought-prone regions, it is crucial to test an integrated management approach that combines strategies such as the treatment of PGRs, and nutrient supplementation with effective field management practices. Furthermore, this approach should be complemented with genetic interventions to maximize its potential. It is essential to study the molecular and genetic mechanisms underlying PGR-induced drought tolerance to gain a comprehensive understanding of this process. Drought stress resistance is a complicated character influenced by multiple loci and genes. Currently, only an inadequate number of genes related to fiber characteristics under drought stress have been identified. Therefore, it is necessary to identify additional Quantitative Trait Loci (QTLs) genes accountable for enhanced fiber quality traits under drought stress conditions. By doing so, cotton cultivars with enhanced fiber quality characteristics in water-deficient environments can be developed. CRISPR/Cas9 helps to improve cotton yield and resistance tolerance varieties. Drought stress has a severe effect on the development and growth of cotton, particularly during reproductive stages, resulting in reduced fiber yield and inferior quality. It disturbs carbohydrate metabolism, translocation of nutrients to pollen function, sink tissues, and antioxidant defense mechanisms of the plant. This leads to smaller boll size and lower fiber quality due to insufficient assimilate supply. Cotton plants respond to drought by prioritizing vegetative growth at the expense of reproductive development, with most assimilates being directed towards roots and leaves instead of developing fibers and propagative parts. While numerous studies have examined the influence of water deficit on cotton reproductive development, further research is needed to understand the and molecular and physiological mechanisms underlying the effects of drought stress on fruiting branches and fiber development.



### Authors' Contributions

Conceptualization: AY and AA. Writing original draft: AB and AM. Reviewing and editing: WL, MNJ, MY, KM, BAK, BA, MN, AR, MUH, AH, MM and EFA. Funding acquisition AH and EFA. All authors read and approved the final manuscript.

### Ethical approval (for researches involving animals or humans)

Not applicable.

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### Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

### References

- Arekhi E, Ghasemi Bezdi K, Ajam Norozei H, Faghani E (2023). The effect of growth regulators on biochemical properties, yield, and fiber quality of different cultivars of cotton (*Gossypium hirsutum*) under different irrigation intervals. *Journal of Plant Growth Regulation* 42(9):5574-5586. <https://doi.org/10.1007/s00344-023-10937-w>
- Abdelraheem A, Adams N, Zhang J (2020). Effects of drought on agronomic and fiber quality in an introgressed backcross inbred line population of Upland cotton under field conditions. *Field Crops Research* 254:107850. <https://doi.org/https://doi.org/10.1016/j.fcr.2020.107850>
- Abdelraheem A, Fang DD, Zhang J (2018). Quantitative trait locus mapping of drought and salt tolerance in an introgressed recombinant inbred line population of Upland cotton under the greenhouse and field conditions. *Euphytica* 214:1-20. <https://doi.org/10.1007/s10681-017-2095-x>
- Abdelraheem A, Hughs S E, Jones D C, Zhang J (2015). Genetic analysis and quantitative trait locus mapping of PEG-induced osmotic stress tolerance in cotton. *Plant Breeding* 134(1): 111-120. <https://doi.org/10.1111/pbr.12228>
- Abdelraheem A, Liu F, Song M, Zhang JF (2017). A meta-analysis of quantitative trait loci for abiotic and biotic stress resistance in tetraploid cotton. *Molecular Genetics and Genomics* 292(6):1221-1235. <https://doi.org/10.1007/s00438-017-1342-0>
- Abdelraheem A, Mahdy E, Zhang J (2015). The first linkage map for a recombinant inbred line population in cotton (*Gossypium barbadense*) and its use in studies of PEG-induced dehydration tolerance. *Euphytica* 205:941-958. <https://doi.org/10.1007/s10681-015-1448-6>
- Ahmed M, Shahid AA, Akhtar S, Latif A, Din SU, Fanglu M, ... Xuede W (2018). Sucrose synthase genes: a way forward for cotton fiber improvement. *Biologia* 73(7): 703-713. <https://doi.org/10.2478/s11756-018-0078-6>
- Álvarez S, Rodríguez P, Broetto F, Sánchez-Blanco, MJ (2018). Long term responses and adaptive strategies of *Pistacia lentiscus* under moderate and severe deficit irrigation and salinity: Osmotic and elastic adjustment, growth, ion uptake and photosynthetic activity. *Agricultural Water Management* 202:253-262. <https://doi.org/10.1016/j.agwat.2018.01.006>

- Amjid M, W Malik, TA, Shakeel A, Wahid A (2015). QTL mapping for relative leaf water contents, cell membrane stability and excised leaf water loss under drought by using EST-SSR markers in *Gossypium hirsutum*. International Journal of Agriculture and Biology 17(4). <https://doi.org/10.17957/IJAB/14.0011>
- Arce CM, Besomi G, Glauser G, Turlings TC (2021). Caterpillar-induced volatile emissions in cotton: The relative importance of damage and insect-derived factors. Frontiers in Plant Science 12:709858. <https://doi.org/10.3389/fpls.2021.709858>
- Arekhi E, Ghasemi Bezdi K, Ajam Norozei H, Faghani E (2023). The effect of growth regulators on biochemical properties, yield, and fiber quality of different cultivars of cotton (*Gossypium hirsutum*) under different irrigation intervals. Journal of Plant Growth Regulation 1-13. <https://doi.org/10.1007/s00344-023-10937-w>
- Arshad M, Zhao Y, Hanif O, Fatima F (2022). Evolution of overall cotton production and its determinants: implications for developing countries using Pakistan case. Sustainability 14:840. <https://doi.org/10.3390/su14020840>
- Ashrafi H, Hulse-Kemp AM, Wang F, Yang SS, Guan X, Jones DC, ... Stelly DM (2015). A long-read transcriptome assembly of cotton (*Gossypium hirsutum* L.) and intraspecific single nucleotide polymorphism discovery. The Plant Genome 8(2). <https://doi.org/10.3835/plantgenome2014.10.0068>
- Aziz KJ (2021). Genome editing: new, emerging, and interesting developments for clinical applications. Journal of Biotechnology and Bioinformatics Research 3(1):1-4.
- Azumah SB, Donkoh SA, Awuni JA (2019). Correcting for sample selection in stochastic frontier analysis: insights from rice farmers in Northern Ghana. Agricultural and food economics 7(1):1-15. <https://doi.org/10.1186/s40100-019-0130-z>
- Brutnell TP (2015). Model grasses hold key to crop improvement. Nature Plants 1(5):1-3. <https://doi.org/10.1038/nplants.2015.62>
- Babar M, Khalid MN, Haq MWU, Hanif M, Ali Z, Awais M, ... Amjad I (2023). 12. A comprehensive review on drought stress response in cotton at physiological, biochemical and molecular level. Pure and Applied Biology (PAB) 12(1):610-622. <http://dx.doi.org/10.19045/bspab.2023.120063>
- Bajwa KS, Shahid AA, Rao A Q, Bashir A, Aftab A, Husnain T (2015). Stable transformation and expression of GhEXPA8 fiber expansin gene to improve fiber length and micronaire value in cotton. Frontiers in Plant Science 6:838. <https://doi.org/10.3389/fpls.2015.00838>
- Bakhsh A, Rehman M, Salman S, Ullah R (2019). Evaluation of cotton genotypes for seed cotton yield and fiber quality traits under water stress and non-stress conditions. Sarhad Journal of Agriculture 35(1):161-170. <http://dx.doi.org/10.17582/journal.sja/2019/35.1.161.170>
- Barichivich J, Osborn T, Harris I, van der Schrier, G, Jones P (2019). Drought: Monitoring global drought using the self-calibrating Palmer Drought Severity Index. Bulletin of the American Meteorological Society 100(9):S39-S40. <https://doi.org/10.1175/2019BAMSStateoftheClimate.1>
- Batool S, Saeed F (2018). Towards a climate resilient cotton value chain in Pakistan: Understanding key risks, vulnerabilities and adaptive capacities. Pathways to Resilience in Semi-Arid Economies (PRISE) Working Paper. Overseas Development Institute. <https://sdpi.org/sdpiweb/publications/files>
- Batool T, Ali S, Seleiman MF, Naveed NH, Ali A, Ahmed K, ... Alotaibi M (2020). Plant growth promoting rhizobacteria alleviates drought stress in potato in response to suppressive oxidative stress and antioxidant enzymes activities. Scientific Reports 10(1):16975. <https://doi.org/10.1038/s41598-020-73489-z>
- Belhaj K, Chaparro-Garcia, A Kamoun S, Patron NJ, Nekrasov V (2015). Editing plant genomes with CRISPR/Cas9. Current Opinion in Biotechnology 32:76-84. <https://doi.org/10.1016/j.copbio.2014.11.007>
- Bozorov TA, Usmanov RM, Yang H, Hamdullaev SA, Musayev S, Shavkiev J, ... Abdullaev AA (2018). Effect of water deficiency on relationships between metabolism, physiology, biomass, and yield of upland cotton (*Gossypium hirsutum* L.). Journal of Arid Land 10:441-456. <https://doi.org/10.1007/s40333-018-0009-y>
- Butler NM, Baltés NJ, Voytas DF, Douches DS (2016). Geminivirus-mediated genome editing in potato (*Solanum tuberosum* L.) using sequence-specific nucleases. Frontiers in Plant Science 7:1045. <https://doi.org/10.3389/fpls.2016.01045>
- Char SN, Unger-Wallace E, Frame B, Briggs SA, Main M, Spalding MH, ... Yang B (2015). Heritable site-specific mutagenesis using TALENs in maize. Plant Biotechnology Journal 13(7):1002-1010. <https://doi.org/10.1111/pbi.12344>

- Chen G, Breedlove J (2020). The effect of innovation-driven policy on innovation efficiency: Based on the listed sports firms on Chinese new Third Board. *International Journal of Sports Marketing and Sponsorship* 21(4):735-755. <https://doi.org/10.1108/IJSMS-12-2019-0136>
- Chen T, Li W, Hu X, Guo J, Liu A, Zhang B (2015). A cotton MYB transcription factor, GbMYB5, is positively involved in plant adaptive response to drought stress. *Plant and Cell Physiology* 56(5):917-929. <https://doi.org/10.1093/pcp/pcv019>
- Consortium IWGS, Appels R, Eversole K, Stein N, Feuillet C, Keller B, ... Distelfeld A (2018). Shifting the limits in wheat research and breeding using a fully annotated reference genome. *Science* 361:eaar7191. <https://doi.org/10.1126/science.aar7191>
- Dong H, Huang Y, Wang K (2021). The development of herbicide resistance crop plants using CRISPR/Cas9-mediated gene editing. *Genes (Basel)* 12(6):912. <https://doi.org/10.3390/genes12060912>
- Das K, Roychoudhury A (2014). Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Frontiers in Environmental Science* 2:53. <https://doi.org/10.3389/fenvs.2014.00053>
- Deng Y, Ning Y, Yang DL, Zhai K, Wang GL, He Z (2020). Molecular basis of disease resistance and perspectives on breeding strategies for resistance improvement in crops. *Molecular plant* 13(10):1402-1419. <https://doi.org/10.1016/j.molp.2020.09.018>
- Dhamodaran N, Konappa N, Chowdappa S, Jogaiah S (2023). Endophytic fungi: application in combating plant pathogens and sustainable agriculture. In: *Fungal Resources for Sustainable Economy: Current Status and Future Perspectives*. Springer, London pp 251-273. [https://doi.org/10.1007/978-981-19-9103-5\\_9](https://doi.org/10.1007/978-981-19-9103-5_9)
- El-Esawi MA, Alayafi AA (2019). Overexpression of StDREB2 transcription factor enhances drought stress tolerance in cotton (*Gossypium barbadense* L.). *Genes* 10(2):142. <https://doi.org/10.3390/genes10020142>
- Ergashovich K, Azamatovna B, Toshtemirovna N, Rakhimovna A (2020). Ecophysiological effects of water deficiency on cotton varieties. *Journal of Critical Reviews* 7(9):244-246. <http://dx.doi.org/10.31838/jcr.07.09.52>
- Evers JB, Marcelis L F (2019). Functional—Structural plant modeling of plants and crops. In *Advances in crop modelling for a sustainable agriculture*. Burleigh Dodds Science Publishing, Cambridge CB22 3HJ UK, pp 45-68. <https://www.taylorfrancis.com>
- Fang L, Wang Q, Hu Y, Jia Y, Chen J, Liu B, ... Zhou B (2017). Genomic analyses in cotton identify signatures of selection and loci associated with fiber quality and yield traits. *Nature Genetics* 49(7):1089-1098. <https://doi.org/10.1038/ng.3887>
- Fang Y, Xiong L (2015). General mechanisms of drought response and their application in drought resistance improvement in plants. *Cellular and Molecular Life Sciences* 72(4):673-689. <https://doi.org/10.1007/s00018-014-1767-0>
- FAO F (2018). The future of food and agriculture: alternative pathways to 2050. Food and Agriculture Organization of the United Nations Rome pp 60. [https://knowledge4policy.ec.europa.eu/publication/future-food-agriculture-alternative-pathways-2050\\_en](https://knowledge4policy.ec.europa.eu/publication/future-food-agriculture-alternative-pathways-2050_en)
- Forner J, Pfeiffer A, Langenecker T, Manavella P, Lohmann JU (2015). Germline-transmitted genome editing in *Arabidopsis thaliana* using TAL-effector-nucleases. *PLoS One* 10(3):e0121056. <https://doi.org/10.1371/journal.pone.0133945>
- Foyer CH, Hanke G (2022). ROS production and signalling in chloroplasts: cornerstones and evolving concepts. *The Plant Journal* 111(3):642-661. <https://doi.org/10.1111/tpj.15856>
- Galindo A, Collado-González J, Griñán I, Corell M, Centeno A, Martín-Palomo M, ... Memmi H (2018). Deficit irrigation and emerging fruit crops as a strategy to save water in Mediterranean semiarid agrosystems. *Agricultural Water Management* 202:311-324. <https://doi.org/10.1016/j.agwat.2017.08.015>
- Gao M, Xu B, Wang Y, Zhou, Z, Hu W (2021). Quantifying individual and interactive effects of elevated temperature and drought stress on cotton yield and fibre quality. *Journal of Agronomy and Crop Science* 207(3):422-436. <https://doi.org/10.1111/jac.12462>
- Gupta RM, Musunuru K (2014). Expanding the genetic editing tool kit: ZFNs, TALENs, and CRISPR-Cas9. *Journal of Clinical Investigation* 124(10):4154-4161. <https://doi.org/10.1172/jci72992>

- Gurushidze M, Hensel G, Hiekel S, Schedel S, Valkov V, Kumlehn J (2014). True-breeding targeted gene knock-out in barley using designer TALE-nuclease in haploid cells. *PLoS One* 9(3):e92046. <https://doi.org/10.1371/journal.pone.0092046>
- Han J, Tan J, Tu L, Zhang X (2014). A peptide hormone gene, Gh PSK promotes fibre elongation and contributes to longer and finer cotton fibre. *Plant Biotechnology Journal* 12(7):861-871. <https://doi.org/10.1111/pbi.12187>
- Haque E, Taniguchi H, Hassan MM, Bhowmik P, Karim MR, Śmiech M, ... Islam T (2018). Application of CRISPR/Cas9 genome editing technology for the improvement of crops cultivated in tropical climates: recent progress, prospects, and challenges. *Frontiers in Plant Science* 9:617. <https://doi.org/10.3389/fpls.2018.00617>
- Hu W, Cao Y, Lok DA, Harris-Shultz KR, Reiter RJ, ... Zhou Z (2020). Exogenous melatonin improves cotton (*Gossypium hirsutum* L.) pollen fertility under drought by regulating carbohydrate metabolism in male tissues. *Plant Physiology and Biochemistry* 151:579-588. <https://doi.org/10.1016/j.plaphy.2020.04.001>
- Hu W, Yang J, Wang S, Chen B, Zhou Z (2018). Effects of potassium deficiency on the enzymatic changes in developing cotton fibers. *Acta Physiologiae Plantarum* 40:1-12. <https://doi.org/10.1007/s11738-018-2674-z>
- Hu W, Zhang J, Wu Z, Loka DA, Zhao W, Chen B, ... Gao L (2022). Effects of single and combined exogenous application of abscisic acid and melatonin on cotton carbohydrate metabolism and yield under drought stress. *Industrial Crops and Products* 176:114302. <https://doi.org/10.1016/j.indcrop.2021.114302>
- Hussain B, Mahmood S (2020). Correction to: Development of transgenic cotton for combating biotic and abiotic stresses. In: Ahmad S, Hasanuzzaman M (Ed). *Cotton Production and Uses: Agronomy, Crop Protection, and Postharvest Technologies*. Springer, Singapore pp 537-555. [https://doi.org/10.1007/978-981-15-1472-2\\_31](https://doi.org/10.1007/978-981-15-1472-2_31)
- Hussain S, Ahmad A, Wajid A, Khaliq T, Hussain N, Mubeen M ... Awais M (2020). Irrigation scheduling for cotton cultivation. *Cotton Production and Uses: Agronomy, Crop Protection and Postharvest Technologies* 59-80. [https://doi.org/10.1007/978-981-15-1472-2\\_5](https://doi.org/10.1007/978-981-15-1472-2_5)
- Imran M. A, Ali, A, Ashfaq M, Hassan S, Culas R, Ma C (2019). Impact of climate smart agriculture (CSA) through sustainable irrigation management on Resource use efficiency: A sustainable production alternative for cotton. *Land Use Policy* 88:104113. <https://doi.org/10.1016/j.landusepol.2019.104113>
- Iqbal A, Dong Q, Wang X, Gui H, Zhang H, Zhang X, ... Song M (2020). High nitrogen enhances drought tolerance in cotton through antioxidant enzymatic activities, nitrogen metabolism and osmotic adjustment. *Plants* 9(2):178. <https://doi.org/10.3390/plants9020178>
- Iqbal M, Khan M A, Chattha W S, Abdullah K, Majeed A (2019). Comparative evaluation of *Gossypium arboreum* L. and *Gossypium hirsutum* L. genotypes for drought tolerance. *Plant Genetic Resources* 17(6):506-513. <https://doi.org/10.1017/S1479262119000340>
- Iqbal M, Khan M A, Naeem M, Aziz U, Afzal J, Latif M (2013). Inducing drought tolerance in upland cotton (*Gossypium hirsutum* L.), accomplishments and future prospects. *World Applied Science Journal* 21(7):1062-1069. <https://doi.org/10.5829/idosi.wasj.2013.21.7.222>
- Iqbal M, Ul-Allah S, Naeem M, Ijaz M, Sattar A, Sher A (2017). Response of cotton genotypes to water and heat stress: from field to genes. *Euphytica* 213:1-11. <https://doi.org/10.1007/s10681-017-1916-2>
- Janga MR, Campbell LM, Rathore KS (2017). CRISPR/Cas9-mediated targeted mutagenesis in upland cotton (*Gossypium hirsutum* L.). *Plant Molecular Biology* 94(4):349-360. <https://doi.org/10.1007/s11103-017-0599-3>
- Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* 337(6096):816-821. <https://doi.org/10.1126/science.1225829>
- Javed MR, Noman M, Shahid M, Ahmed T, Khurshid M, Rashid MH, ... Khan F (2019). Current situation of biofuel production and its enhancement by CRISPR/Cas9-mediated genome engineering of microbial cells. *Microbiological Research* 219:1-11. <https://doi.org/10.1016/j.micres.2018.10.010>
- Jun W, Ping L, Zhiyong L, Zhansheng W, Yongshen L, Xinyuan G (2017). Dry matter accumulation and phosphorus efficiency response of cotton cultivars to phosphorus and drought. *Journal of Plant Nutrition* 40(16):2349-2357. <https://doi.org/10.1080/01904167.2017.1346123>
- Kaczmarek A, Boguś M (2021). The metabolism and role of free fatty acids in key physiological processes in insects of medical, veterinary and forensic importance. *PeerJ* 9:e12563. <https://doi.org/10.7717/peerj.12563>. *eCollection 2021*

- Kim MS, Kini AG (2017). Engineering and application of zinc finger proteins and TALEs for biomedical research. *Molecular Cells* 40(8):533-541. <https://doi.org/10.14348/molcells.2017.0139>
- Kuhn DN, Bally IS, Dillon NL, Innes D, Groh AM, Rahaman J, ... Sherman A (2017). Genetic map of mango: a tool for mango breeding. *Frontiers in Plant Science* 8:577. <https://doi.org/10.3389/fpls.2017.00577>
- Kamburova V, Salakhutdinov I, Abdurakhmonov IY (2022). Cotton breeding in the view of abiotic and biotic stresses: challenges and perspectives. In: Ibrokhim YA (Ed). *IntechOpen* pp 6 <https://doi.org/10.5772/intechopen.104761>
- Kapoor D, Bhardwaj S, Landi M, Sharma A, Ramakrishnan M, Sharma A (2020). The impact of drought in plant metabolism: how to exploit tolerance mechanisms to increase crop production. *Applied Sciences* 10(16):5692. <https://www.mdpi.com/2076-3417/10/16/5692>
- Khan A, Pan X, Najeeb U, Tan DKY, Fahad S, Zahoor R, Luo H (2018). Coping with drought: stress and adaptive mechanisms, and management through cultural and molecular alternatives in cotton as vital constituents for plant stress resilience and fitness. *Biological Research* 51:0716-976 <http://dx.doi.org/10.1186/s40659-018-0198-z>
- Khan Z, Khan S H, Mubarik MS, Ahmad A (2018). Targeted genome editing for cotton improvement. Past, Present and Future Trends in Cotton Breeding 2:11. <http://dx.doi.org/10.5772/intechopen.73600>
- Kumar S, Abedin MM, Singh AK, Das S (2020). Role of phenolic compounds in plant-defensive mechanisms. *Plant Phenolics in Sustainable Agriculture* 1:517-532. [https://doi.org/10.1007/978-981-15-4890-1\\_22](https://doi.org/10.1007/978-981-15-4890-1_22)
- Li C, Kong X, Luo Z, Li W, Tang W, Zhang D, Ma C, Dong H (2021). Exogenous application of acetic acid improves the survival rate of cotton by increasing abscisic acid and jasmonic acid contents under drought stress. *Acta Physiologiae Plantarum* 43:1-10. <https://doi.org/10.1007/s11738-021-03202-8>
- Li C, Unver T, Zhang B (2017). A high-efficiency CRISPR/Cas9 system for targeted mutagenesis in cotton (*Gossypium hirsutum* L.). *Scientific Reports* 7(1):43902. <https://doi.org/10.1038/srep43902>
- Li J-F, Norville JE, Aach J, McCormack M, Zhang D, Bush J, ... Sheen J (2013). Multiplex and homologous recombination-mediated genome editing in *Arabidopsis* and *Nicotiana benthamiana* using guide RNA and Cas9. *Nature Biotechnology* 31(8):688-691. <https://doi.org/10.1038/nbt.265>
- Liu G, Li X, Jin S, Liu X, Zhu L, Nie, Y, Zhang X (2014). Overexpression of rice NAC gene SNAC1 improves drought and salt tolerance by enhancing root development and reducing transpiration rate in transgenic cotton. *PLoS One* 9(1):e86895. <https://doi.org/10.1371/journal.pone.0086895>
- Lo TW, Pickle CS, Lin S, Ralston EJ, Gurling M, Schartner CM, ... Meyer BJ (2013). Precise and heritable genome editing in evolutionarily diverse nematodes using TALENs and CRISPR/Cas9 to engineer insertions and deletions. *Genetics* 195(2): 331-348. <https://doi.org/10.1534/genetics.113.155382>
- Loka DA, Oosterhuis DM, Baxevanos D, Noulas C, Hu W (2020). Single and combined effects of heat and water stress and recovery on cotton (*Gossypium hirsutum* L.) leaf physiology and sucrose metabolism. *Plant Physiology and Biochemistry* 148:166-179. <https://doi.org/10.1016/j.plaphy.2020.01.015>
- Luo M, Gilbert B, Ayliffe M (2016). Applications of CRISPR/Cas9 technology for targeted mutagenesis, gene replacement and stacking of genes in higher plants. *Plant Cell Reports* 35:1439-1450. <https://doi.org/10.1007/s00299-016-1989-8>
- Mao Y, Botella JR, Liu Y, Zhu JK (2019). Gene editing in plants: progress and challenges. *National Science Review* 6(3):421-437. <https://doi.org/10.1093/nsr/nwz005>
- Ma Z, He S, Wang X, Sun J, Zhang Y, Zhang G, ... Sun G (2018). Resequencing a core collection of upland cotton identifies genomic variation and loci influencing fiber quality and yield. *Nature Genetics* 50(6):803-813. <https://doi.org/10.1038/s41588-018-0119-7>
- Maeder ML, Angstman JF, Richardson ME, Linder SJ, Cascio VM, Tsai SQ, ... Bernstein BE (2013). Targeted DNA demethylation and activation of endogenous genes using programmable TALE-TET1 fusion proteins. *Nature Biotechnology* 31(12):1137-1142. <https://doi.org/10.1038/nbt.2726>
- Mahfouz MM, Piatek A, Stewart Jr CN (2014). Genome engineering via TALENs and CRISPR/Cas9 systems: challenges and perspectives. *Plant Biotechnology Journal* 12(8):1006-1014. <https://doi.org/10.1111/pbi.12256>
- Mahmood T, Khalid S, Abdullah M, Ahmed Z, Shah MKN, Ghafoor A, Du X (2019). Insights into drought stress signaling in plants and the molecular genetic basis of cotton drought tolerance. *Cells* 9(1):105. <https://doi.org/10.3390/cells9010105>
- Marton I, Zuker A, Shklarman E, Zeevi V, Tovkach A, Roffe S, ... Vainstein A (2010). Nontransgenic genome modification in plant cells. *Plant physiology* 154(3):1079-1087. <https://doi.org/10.1104/pp.110.164806>

- Masood S, Khaliq A, Rauf H, Mahmood K, Ahmed I, Hussain N, ... Muhammad T (2022). Heat and drought forbearing, upland cotton (*Gossypium hirsutum* L.) variety; rh-668 for cultivation in semi-arid region. Biological and Clinical Sciences Research Journal 2022(1). <https://doi.org/10.54112/bcsrj.v2022i1.121>
- Meeks CD, Snider JL, Babb-Hartman, ME, Barnes TL (2019). Evaluating the mechanisms of photosynthetic inhibition under growth-limiting, early-season water deficit stress in cotton. Crop Science 59(3):1144-1154. <https://doi.org/10.2135/cropsci2018.07.0432>
- Mei, Y, Wang Y, Chen H, Sun ZS, Ju X-D (2016). Recent progress in CRISPR/Cas9 technology. Journal of Genetics and Genomics 43(2): 63-75. <https://doi.org/10.1016/j.jgg.2016.01.001>
- Meyer LA (2019). The World and US Cotton Outlook for 2019/20. [https://www.usda.gov/sites/default/files/documents/Leslie\\_Meyer](https://www.usda.gov/sites/default/files/documents/Leslie_Meyer)
- Miller JC, Tan S, Qiao G, Barlow KA, Wang J, Xia DF, ... Hinkley SJ (2011). A TALE nuclease architecture for efficient genome editing. Nature Biotechnology 29(2):143-148. <https://doi.org/10.1038/nbt.1755>
- Minasny B, McBratney A (2018). Limited effect of organic matter on soil available water capacity. European Journal of Soil Science 69(1):39-47. <https://doi.org/10.1111/ejss.12475>
- Munaweera T, Jayawardana N, Rajaratnam R, Dissanayake N (2022). Modern plant biotechnology as a strategy in addressing climate change and attaining food security. Agriculture & Food Security 11(1):1-28. <https://doi.org/10.1186/s40066-022-00369-2>
- Murali N, Khan M (2022). Determinants of production performance of cotton in different zones of India. The Mysore of Agricultural Sciences 56:231-235. <https://doi.org/10.54112/bbasr.v2022i1.22>
- Nadeem AH, Nazim M, Hashim M, Javed MK (2014). Factors which affect the sustainable production of cotton in Pakistan: a detailed case study from Bahawalpur district. Proceedings of the Seventh International Conference on Management Science and Engineering Management: Focused on Electrical and Information Technology 1:743-753. [https://doi.org/10.1007/978-3-642-40078-0\\_64](https://doi.org/10.1007/978-3-642-40078-0_64)
- Nagargade M, Tyagi V, Singh P, Kuma S (2023). Commercial Crops Other Than Vegetables for a Green Economy of India. <https://www.researchgate.net/publication/371580589>
- Niu J, Zhang S, Liu S, Ma H, Chen J, Shen Q ... Zhao X (2018). The compensation effects of physiology and yield in cotton after drought stress. Journal of Plant Physiology 224:30-48. <https://doi.org/10.1016/j.jplph.2018.03.001>
- Oldfield EE, Wood SA, Bradford MA (2018). Direct effects of soil organic matter on productivity mirror those observed with organic amendments. Plant and Soil 423:363-373. <https://doi.org/10.1007/s11104-017-3513-5>
- Oliveira M, Duarte J, Morello CDL, Suassuna N, Oliveira A (2016). Mixed inheritance in the genetic control of ramulosis (*Colletotrichum gossypii* var. *cephalosporioides*) resistance in cotton. Genetic and Molecular Research 15:1-6. <http://dx.doi.org/10.4238/gmr.15038667>
- Pereira AE S, Silva P M, Oliveira JL, Oliveira HC, Fraceto LF (2017). Chitosan nanoparticles as carrier systems for the plant growth hormone gibberellic acid. Colloids and Surfaces B: Biointerfaces 150:141-152. <https://doi.org/https://doi.org/10.1016/j.colsurfb.2016.11.027>
- Pauli D, Andrade-Sanchez P, Carmo-Silva, AE Gazave, French AN, Heun J, ... Strand RJ (2016). Field-based high-throughput plant phenotyping reveals the temporal patterns of quantitative trait loci associated with stress-responsive traits in cotton. G3: Genes, Genomes, Genetics 6(4):865-879. <https://doi.org/10.1534/g3.115.023515>
- Pei L, Li G, Lindsey K, Zhang X, Wang M (2021). Plant 3D genomics: the exploration and application of chromatin organization. New Phytologist 230(5):1772-1786. <https://doi.org/10.1111/nph.17262>
- Pilon C, Loka D, Snider JL, Oosterhuis DM (2019). Drought-induced osmotic adjustment and changes in carbohydrate distribution in leaves and flowers of cotton (*Gossypium hirsutum* L.). Journal of Agronomy and Crop Science 205(2):168-178. <https://doi.org/10.1111/jac.12315>
- Polinova M, Salinas K, Bonfante A, Brook A (2019). Irrigation optimization under a limited water supply by the integration of modern approaches into traditional water management on the cotton fields. Remote Sensing 11(18):2127. <https://www.mdpi.com/2072-4292/11/18/2127>
- Rehman A, Farooq M (2019). Morphology, Physiology and Ecology of cotton. Cotton Production 2346. <https://doi.org/10.1002/9781119385523.ch2>
- Robertson BC, He T, Li C (2021). The genetic control of stomatal development in barley: new solutions for enhanced water-use efficiency in drought-prone environments. Agronomy 11(8):1670. <https://doi.org/10.3390/agronomy11081670>

- Simón JE, Rodríguez ÁS, Santiago Vispo N (2018). CRISPR-Cas9: a precise approach to genome engineering. *Therapeutic Innovation and Regulatory Science* 52(6):701-707. <https://doi.org/10.1177/2168479018762798>
- Saleem MF, Sammar Raza MA, Ahmad S, Khan IH, Shahid AM (2016). Understanding and mitigating the impacts of drought stress in cotton-a review. *Pakistan Journal of Agricultural Sciences* 53(3):609-623. <https://doi.org/10.21162/PAKJAS/16.3341>
- Saleem M, Malik T, Shakeel A, Ashraf M (2015). QTL mapping for some important drought tolerant traits in upland cotton. *JAPS: Journal of Animal and Plant Sciences* 25(2).
- Sekmen AH, Ozgur R, Uzilday B, Turkan I (2014). Reactive oxygen species scavenging capacities of cotton (*Gossypium hirsutum*) cultivars under combined drought and heat induced oxidative stress. *Environmental and Experimental Botany* 99:141-149. <https://doi.org/10.1016/j.envexpbot.2013.11.010>
- Shareef M, Gui D, Zeng F, Ahmed Z, Waqas M, Zhang B, ... Fiaz M (2018). Impact of drought on assimilates partitioning associated fruiting physiognomies and yield quality attributes of desert grown cotton. *Acta Physiologiae Plantarum* 40:1-12. <https://doi.org/10.1007/s11738-018-2646-3>
- Shamshirgaran Y, Liu J, Sumer H, Verma PJ, Taheri-Ghahfarokhi A (2022). Tools for efficient genome editing; ZFN, TALEN, and CRISPR. In: Verma PJ, Sumer H, Liu J (Eds). *Applications of Genome Modulation and Editing*. Springer, US pp 29-46. [https://doi.org/10.1007/978-1-0716-2301-5\\_2](https://doi.org/10.1007/978-1-0716-2301-5_2)
- Shinwari ZK, Jan SA, Nakashima K, Yamaguchi-Shinozaki K (2020). Genetic engineering approaches to understanding drought tolerance in plants. *Plant Biotechnology Reports* 14:151-162. <https://doi.org/10.1007/s11816-020-00598-6>
- Shukla VK, Doyon Y, Miller JC, DeKelver RC, Moehle EA, Worden SE, ... Mitchell JC, Arnold NL, Gopalan S, Meng X (2009). Precise genome modification in the crop species using zinc-finger nucleases. *Nature* 459(7245):437-441. <https://doi.org/10.1038/nature07992>
- Singh S, Das S, Geeta R (2018). Role of cuticular wax in adaptation to abiotic stress: a molecular perspective. *Abiotic stress-mediated sensing and signaling in plants: An omics perspective* 155-182. [http://dx.doi.org/10.1007/978-981-10-7479-0\\_5](http://dx.doi.org/10.1007/978-981-10-7479-0_5)
- Sohaib M, Jamil F (2017). An insight of meat industry in Pakistan with special reference to halal meat: a comprehensive review. *Korean Journal for Food Science of Animal Resources* 37(3):329. <https://doi.org/10.5851/kosfa.2017.37.3.329>
- Song Q, Zhang T, Stelly DM, Chen ZJ (2017). Epigenomic and functional analyses reveal roles of epialleles in the loss of photoperiod sensitivity during domestication of allotetraploid cottons. *Genome Biology* 18(1):99. <https://doi.org/10.1186/s13059-017-1229-8>
- Sui R, Byler R, Delhom C (2017). Effect of nitrogen application rates on yield and quality in irrigated and rainfed cotton. *Journal of Cotton Science* 21(2):113-121. <http://journal.cotton.org>
- Tang F, Zhu J, Wang T, Shao D (2017). Water deficit effects on carbon metabolism in cotton fibers during fiber elongation phase. *Acta Physiologiae Plantarum* 39: 1-9. <https://doi.org/10.1007/s11738-017-2368-y>
- Tanveer M, Shahzad B, Sharma A, Khan EA (2019). 24-Epibrassinolide application in plants: An implication for improving drought stress tolerance in plants. *Plant Physiology and Biochemistry* 135:295-303. <https://doi.org/10.1016/j.plaphy.2018.12.013>
- Tekle AT, Alemu MA (2016). Drought tolerance mechanisms in field crops. *World Journal of Biology and Medical Sciences* 3(2):15-39. [www.sasjournals.com](http://www.sasjournals.com)
- Teper D, Wang N (2021). Consequences of adaptation of TAL effectors on host susceptibility to *Xanthomonas*. *PLoS Genetics* 17(1):e1009310. <https://doi.org/10.1371/journal.pgen.1009310>
- Thorp KR, Thompson A, Bronson K (2020). Irrigation rate and timing effects on Arizona cotton yield, water productivity, and fiber quality. *Agricultural Water Management* 234:106146. <https://doi.org/10.1016/j.agwat.2020.106146>
- Tiwari P, Srivastava D, Chauhan AS, Indoliya Y, Singh PK, Tiwari S, ... Agarwal L (2021). Root system architecture, physiological analysis and dynamic transcriptomics unravel the drought-responsive traits in rice genotypes. *Ecotoxicology and Environmental Safety* 207:111252. <https://doi.org/10.1016/j.ecoenv.2020.111252>
- Townsend JA, Wright DA, Winfrey RJ, Fu F, Maeder ML, Joung JK, ... Voytas DF (2009). High-frequency modification of plant genes using engineered zinc-finger nucleases. *Nature* 459(7245):442-445. <https://doi.org/10.1038/nature07845>

- Tzortzakis N, Chrysargyris A, Aziz A (2020). Adaptive response of a native mediterranean grapevine cultivar upon short-term exposure to drought and heat stress in the context of climate change. *Agronomy* 10(2):249. <https://doi.org/10.3390/agronomy10020249>
- Ul-Allah S, Rehman A, Hussain M, Farooq M (2021). Fiber yield and quality in cotton under drought: Effects and management. *Agricultural Water Management* 255:106994. <https://doi.org/10.1016/j.agwat.2021.106994>
- Ullah A, Sun H, Yang X, Zhang X (2017). Drought coping strategies in cotton: increased crop per drop. *Plant Biotechnology Journal* 15(3):271-284. <https://doi.org/10.1111/pbi.12688>
- Urnov FD (2018). Genome Editing BC (before CRISPR): lasting lessons from the “old testament”. *The CRISPR Journal* 1(1):34-46. <https://doi.org/10.1089/crispr.2018.29007.fju>
- Wang L, Wang G, Long L, Altunok S, Feng Z, Wang D, ... Mujtaba M (2020). Understanding the role of phytohormones in cotton fiber development through omic approaches; recent advances and future directions. *International Journal of Biological Macromolecules* 163:1301-1313. <https://doi.org/10.1016/j.ijbiomac.2020.07.104>
- Wang M, Tu L, Yuan D, Zhu D, Shen C, Li J ... Zhao G (2019). Reference genome sequences of two cultivated allotetraploid cottons, *Gossypium hirsutum* and *Gossypium barbadense*. *Nature Genetics* 51(2):224-229. <https://doi.org/10.1038/s41588-018-0282-x>
- Wang R, Ji S, Zhang P, Meng Y, Wang Y, Chen B, Zhou Z (2016). Drought effects on cotton yield and fiber quality on different fruiting branches. *Crop Science* 56(3):1265-1276. <https://doi.org/10.2135/cropsci2015.08.0477>
- Wang Y, Cheng X, Shan Q, Zhang Y, Liu J, Gao C, ... Qiu JL (2014). Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. *Nature Biotechnology* 32(9):947-951. <https://doi.org/10.1038/nbt.2969>
- Wang Y, Meng Z, Liang C, Meng Z, Wang Y, Sun G, ... Zhang R (2017). Increased lateral root formation by CRISPR/Cas9-mediated editing of arginase genes in cotton. *Science China. Life Sciences* 60(5):524. <https://doi.org/10.1007/s11427-017-9031-y>
- Wani S H, Kumar V, Shriram, V, Sah SK (2016). Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. *The Crop Journal* 4(3):162-176. <https://doi.org/10.1016/j.cj.2016.01.010>
- Wasaya A, Zhang X, Fang Q, Yan Z (2018). Root phenotyping for drought tolerance: a review. *Agronomy* 8(11):241. <https://doi.org/10.3390/agronomy8110241>
- Wen S, Liu H, Li X, Chen X, Hong Y, Li H, ... Liang X (2018). TALEN-mediated targeted mutagenesis of fatty acid desaturase 2 (FAD2) in peanut (*Arachis hypogaea* L.) promotes the accumulation of oleic acid. *Plant Molecular Biology* 97:177-185. <https://doi.org/10.1007/s11103-018-0731-z>
- Xiong X, Chang L, Khalid M, Zhang J, Huang D (2018). Alleviation of drought stress by nitrogen application in *Brassica campestris* ssp. *chinensis* L. *Agronomy* 8(5):66. <https://doi.org/10.3390/agronomy8050066>
- Yang H, Zhang D, Zhang D, Bozorov TA, Abdullaev AA, Wood AJ, ... Zhao J (2019). Overexpression of ALDH21 from *Syntrichia caninervis* Moss in upland cotton enhances fiber quality, boll component traits, and physiological parameters during deficit Irrigation. *Crop Science* 59(2):553-564. <https://doi.org/10.3390/agronomy8050066>
- Yang J, Hu W, Zhao W, Chen B, Wang Y, Zhou Z, ... Meng Y (2016). Fruiting branch K+ level affects cotton fiber elongation through osmoregulation. *Frontiers in Plant Science* 7:13. <https://doi.org/10.3389/fpls.2016.00013>
- Yang X, Lu M, Wang Y, Wang Y, Liu Z, Chen S (2021). Response mechanism of plants to drought stress. *Horticulturae* 7(3):50. <https://doi.org/10.3390/horticulturae7030050>
- Yu LH, Wu S J, Peng Y S, Liu R N, Chen X, Zhao P, ... Xu P, Zhu JB, Jiao GL, Pei Y (2016). Arabidopsis EDT 1/HDG 11 improves drought and salt tolerance in cotton and poplar and increases cotton yield in the field. *Plant Biotechnology Journal* 14(1):72-84. <https://doi.org/10.1111/pbi.12358>
- Yuan D, Tang Z, Wang M, Gao W, Tu L, Jin X, ... Zhu L (2015). The genome sequence of Sea-Island cotton (*Gossypium barbadense*) provides insights into the allopolyploidization and development of superior spinnable fibres. *Scientific Reports* 5(1):17662. <https://doi.org/10.1038/srep17662>
- Zahoor R, Zhao W, Abid M, Dong H, Zhou Z (2017). Potassium application regulates nitrogen metabolism and osmotic adjustment in cotton (*Gossypium hirsutum* L.) functional leaf under drought stress. *Journal of Plant Physiology* 215:30-38. <https://doi.org/10.1016/j.jplpb.2017.05.001>
- Zargar SM, Gupta N, Nazir M, Mahajan R, Malik FA, Sofi NR, Shikari AB, Salgotra RK (2017). Impact of drought on photosynthesis: Molecular perspective. *Plant Gene* 11:154-159. <https://doi.org/https://doi.org/10.1016/j.plgene.2017.04.003>



- Zhang F, Li S, Yang S, Wang L, Guo W (2015). Retracted Article: Overexpression of a cotton annexin gene, GhAnn1, enhances drought and salt stress tolerance in transgenic cotton. *Plant Molecular Biology* 87:47-67. <https://doi.org/10.1007/s11103-014-0260-3>
- Zhang F, Maeder ML, Unger-Wallace E, Hoshaw JP, Reyon D, Christian M, ... Peterson T (2010). High frequency targeted mutagenesis in *Arabidopsis thaliana* using zinc finger nucleases. *Proceedings of the National Academy of Sciences* 107(26):12028-12033. <https://doi.org/10.1073/pnas.0914991107>
- Zhang F, Wang P, Zou Y-N, Wu Q-S, Kuča K (2019). Effects of mycorrhizal fungi on root-hair growth and hormone levels of taproot and lateral roots in trifoliolate orange under drought stress. *Archives of Agronomy and Soil Science* 65(9):1316-1330. <https://doi.org/10.1080/03650340.2018.1563780>
- Zhang J, Li X-M, Lin H-X, Chong K (2019). Crop improvement through temperature resilience. *Annual Review of Plant Biology* 70:753-780. <https://doi.org/10.1146/annurev-arplant-050718-100016>
- Zhang L, Peng J, Chen T, Zhao X, Zhang S, Liu S, ... Yu S (2014). Effect of drought stress on lipid peroxidation and proline content in cotton roots. *JAPS: Journal of Animal and Plant Sciences* 24(6). <https://thejaps.org.pk/docs/v-24-6/23>
- Zhang M, Zhang X, Guo L, Qi T, Liu G, Feng J, ... Wang H (2020). Single-base resolution methylome of cotton cytoplasmic male sterility system reveals epigenomic changes in response to high-temperature stress during anther development. *Journal of Experimental Botany* 71(3):951-969. <https://doi.org/10.1093/jxb/erz470>
- Zhao W, Dong H, Zahoor R, Zhou Z, Snider JL, Chen Y, ... Wang Y (2019). Ameliorative effects of potassium on drought-induced decreases in fiber length of cotton (*Gossypium hirsutum* L.) are associated with osmolyte dynamics during fiber development. *The crop journal* 7(5):619-634. <https://doi.org/10.1016/j.cj.2019.03.008>
- Zhao W, Dong H, Zhou Z, Wang Y, Hu W (2020). Potassium (K) application alleviates the negative effect of drought on cotton fiber strength by sustaining higher sucrose content and carbohydrates conversion rate. *Plant Physiology and Biochemistry* 157:105-113. <https://doi.org/10.1016/j.plaphy.2020.10.014>
- Zhao W, Wang R, Hu W, Zhou Z (2019). Spatial difference of drought effect on photosynthesis of leaf subtending to cotton boll and its relationship with boll biomass. *Journal of Agronomy and Crop Science* 205(3):263-273. <https://doi.org/10.1111/jac.12320>
- Zheng J, Oluoch G, Mk RK, Wang X, Cai X, Zhou Z, ... Liu F (2016). Mapping QTLs for drought tolerance in an F2: 3 population from an inter-specific cross between *Gossypium tomentosum* and *Gossypium hirsutum*. *Genetics and molecular research: GMR* 15(3). <https://doi.org/10.4238/gmr.15038477>
- Zheng Y, Li Q, Ye M, Cehn A, Wang H (2021). Applications of CRISPR/Cas9-based genome editing in the plant biology. *Turkish Journal of Botany* 45(4):253-268. <https://doi.org/10.3906/bot-2103-50>
- Zhou Z, Oosterhuis M (2012). Physiological mechanism of nitrogen mediating cotton. *Figure Legends*. <http://www.SciRP.org/journal/ajps>
- Zhu X, Sun L, Kuppu S, Hu R, Mishra N, Smith J, ... Payton P (2018). The yield difference between wild-type cotton and transgenic cotton that expresses IPT depends on when water-deficit stress is applied. *Scientific Reports* 8(1):2538. <https://doi.org/10.1038/s41598-018-20944-7>
- Zonta JH, Brandao ZN, Rodrigues JIDS, Sofiatti V (2017). Cotton response to water deficits at different growth stages. *Revista Caatinga* 30:980-990. <https://doi.org/10.1016/j.fcr.2020.107850>



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