Plant drought stress tolerance: understanding its physiological, biochemical and molecular mechanisms

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ABSTRACT

Plants are vulnerable to many environmental constraints which include drought, salinity, extreme temperatures, heavy metals, etc., thereby posing damage to the development and yield of major crops. Of these stresses, drought represents a severe environmental threat to plant productivity in agriculture. Drought stress reduces the yield by affecting the key plant metabolic pathways. The capability of plants to switch on or off a series of genes result in alterations of the physiological and morphological attributes, thereby allowing plants to escape, tolerate or avoid drought stress. Different genes, transcription factors and signal transduction pathways are induced by drought stress. Advances in genome editing technologies have revolutionized the agriculture sector by using more accurate genome engineering techniques for targeted crop traits. Clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein 9 (Cas9) technology is a robust and efficient approach for developing plant/crop varieties tolerant to different climatic changes. The use of new genome editing technologies like CRISPR facilitates the creation of plant species with improved drought tolerance. Thus this review aims to elucidate the basic plant responses to stress and the mechanisms for the adaptation and attainment of tolerance.

Introduction

Water deficiency is one of the chronic abiotic crop stresses which impact plant growth and development, constituting about 70% of potential losses of crop yield and productivity globally [1,2]. Drought exists either due to extremely less rainfall or significant distinction in the quantity of moisture, and is considered as substantial abiotic stress hindering agriculture and forestry globally [3]. Predicted global warming and present climate scenarios are thought to have a highly significant impact on the moisture timeline, increasing the phenomenon and intensity of drought worldwide. Although plants are exposed to variable stresses during their growth and development, including drought, low light, temperature stress, soil salt stress, heat waves, and heavy metal exposure [4], repercussions of drought on plant morphology are detectable at all developmental stages specific to plant metabolism, resulting in a 30% productivity loss worldwide by 2025. With climate change, considerable drought constitutes a prime concern of agriculture as there is a prediction of the enhanced prevalence of water scarcity [5].

Crop plants combat drought through acclimatization and specific tolerance mechanisms to adapt to adverse conditions. Scientists can use these evolutionary mechanisms to combat yield loss and meet the growing food demands of a growing population. Many physiological biochemical and molecular processes that could occur at the cellular or whole plant level are modified in response to water deficit and play an indispensable part in mitigating stress [6,7]. In retortion to water deficiency, plant resistance can be accomplished...
via drought escape (e.g. early flowering time in drier environments), avoidance (e.g. transpiration regulator by stomata and development of expensive root systems), trait flexibility, maintenance of water management in tissues, antioxidant scavenging system, plant growth substances by plant growth regulators and osmotic regulation [8,9]. Drought stresses noticeably growth substances by plant growth regulators and elements in the promoter region of different stress-responsive genes [21–23], thus regulating downstream drought-responsive genes. Among the TF genes, a number of genes have been identified to counter drought stress in an ABA pathway dependent/ independent manner, respectively.

Advanced genome editing techniques have revolutionized the crop improvement programmes from the last decade. CRISPR/Cas9 has been used in crop improvement by enhancing crop resistance, improving tolerance to various biotic and abiotic stresses including drought and salinity [24,25]. The availability of these modern refined genome editing tools has offered new opportunities for discovery and enhancement of novel traits, resulting in targeted improvement of crop traits, especially towards abiotic stress tolerance [26,27].

In this review, we provide an overview of the most promising physio-biochemical and molecular responses that could be upgraded biotechnologically to obtain drought tolerant crops. In addition, we emphasize how modern genome editing technologies such as CRISPR could help to facilitate and manipulate novel genes that might help to develop resistance to drought in crop plants.

**Physiological and biochemical responses**

Drought is multifaceted stress for plants and can cause a critical impact on the metabolism of crop plants [8,28,29] and trigger remarkable limitation of crop production [30,31]. Water deficit conditions are the most common edaphic stress that is harmful to cellular homeostasis and obstructs plant development [32]. The water requirement for irrigation is ceaselessly expanding while there is a serious decrease in water accessibility. This is unfavorable in semi-arid and arid conditions [33]. Water scarcity in fields expressed by the absence of adequate moisture in the soil results in a delay and or failure of crop maturation and development. Water deficiency affects the biochemical, morphological and physiological processes in crop plants. Drought causes loss of turgor, disorganizes the activities of enzymes, and reduces the energy transfer from photosynthesis, which causes a negative impact on cell multiplication, elongation and specialization, thereby affecting plant growth and development [28,34–36].

Remarkable effects of drought on plants also include reduced cell expansion and division rate, decreased germination rate, reduced leaf area, disturbed stomatal
responses and reduced chlorophyll levels. However, plants can circumvent water deficiency by decreasing water depletion (e.g., closing stomata, reducing leaf surface area, etc.) or increasing uptake of water (by deep roots) [37]. An essential reaction of plants exposed to water deficit conditions is growth arrest. Restricted shoot development in the presence of drought stress diminishes the metabolic demands of the plant. It assembles the metabolites for the formation of defensive compounds, which are needed for osmotic adjustment. Constricted root development regulates the root meristem’s function and promotes root growth when the stress soothed [38]. The response of plants towards water deficit conditions is extremely intricate. It fundamentally fluctuates amongst different plants and between their development stages and extent of water deficiency [8,39]. Water scarcity causes a remarkable impediment of root and shoots development, and leaf area which is followed by reduced growth and development of plants [40]. Besides these alterations, plants engage various metabolic processes extending from photosynthesis to antioxidant and solute accumulation as constituents of water deficit resistance. Plants respond to drought stress by modifying the gene expression through complex transcriptional networks [41].

According to Joshi et al. [19], although efficient adaptation mechanisms are highly conserved among stress susceptible genotypes, the tolerant genotypes, however, evolved additional regulatory mechanisms that improve their ability to manage severe abiotic stresses. With the availability of diverse omics tools including genomics, transcriptomics and proteomics, major progress has been made for understanding the interaction and complexity of the stress adaptive mechanisms and their respective signaling pathways [42]. Many of the transcriptional network genes specified in several molecular studies have been noticed to contribute to drought stress tolerance in transgenic plants [43]. The signaling pathway of any abiotic stress comprises fundamental steps like signal perception, transduction, responsiveness, together with physiological and metabolic reaction activation [42,44].

Signaling processes require the perception of stress stimulus by plant cells via sensors or receptors that are located at the cellular membranes. The receptor then activates the second messengers such as calcium ions, inositol phosphate, reactive oxygen species (ROS), cyclic nucleotides (cAMP and cGMP), sugars and nitric oxide inside the cells. These second messengers then instigate signaling pathways and transduce the signals [28]. The modulation of phosphorylation and dephosphorylation of proteins by protein kinases and phosphatases, respectively, is an essential and noticeable mechanism in various signal transduction pathways, for example, the MAPKs and CDPKs, which have been established to play an essential role in drought stress signaling pathways [45]. After a phosphorylation cascade, TFs activation or suppression by protein kinases or phosphatases occurs, and TFs interact with distinct cis-elements in the promoter region and thus directly regulate the expression of a range of downstream genes [46]. Additionally, at the transcriptional level these TFs are modulated by other upstream components [47] and at the post-transcription level, their alteration by ubiquitination and SUMOylation, leads to the formation of a complex regulatory network which modulates the expression of stress responsive genes, regulating various physiological and metabolic processes [48]. Substantial work has been carried out towards the identification and characterization of different TFs that impart drought tolerance. Water deficit conditions, if persisting for longer duration, result in a notable decrease in growth and development, as reported in pepper (Piper nigrum) [49].

The effects of water scarcity on plant growth and development are outlined in Table 1. The following subchapters present the effects of drought stress on some physio-biochemical reactions of plants.

**Drought: physiological responses**

**Mineral nutrition**

Several environmental stresses like drought may cause deficiency of nutrients, even in agricultural fields, as

| Plant species       | Effect                                                                 | References |
|---------------------|------------------------------------------------------------------------|------------|
| *Thymus vulgaris*   | Decreased shoot and root fresh weight, and the ratio of root/shoot fresh weight. | [50]       |
| *Zea mays*          | Decreased plant height, reduced number of fresh shoot and dry weight and yield. | [51]       |
| *Phaseolus vulgaris*| Reduced dry mass, leaf area index, decreased number of pods per plant and seeds per pod and reduction in total crop production. | [52]       |
| *Zea mays*          | Significantly reduced shoot fresh and dry mass.                        | [53]       |
| *Piper nigrum*      | Reduced girth of stem, leaf number and area, fresh and dry mass of shoot and root. | [49,54]   |
the individual nutrient absorption depends on the physicochemical properties of soil [55, 56]. With the reduction in water availability, nutrient availability in the soil decreases so the nutrient concentration in the plant tissues decreases [57]. Water deficit conditions cause a significant impact on the uptake of nutrients by the roots and their translocation to the shoots. It has been noted that drought stress causes an enhancement of N, decreases P content, and generally has no consequence on the K content in plants [8]. Reduction in plant Ca levels under drought stress has also been revealed [28]. The stability of membranes in the roots had an important function in the proper mineral nutrition of plants. However cell membranes are the earliest subjects of various stresses including water stress. Hence, proper maintenance of the membrane stability is an essential element for drought tolerance in plants. Disintegrated cell membranes under drought stress are a key factor which causes disorganized ion balance in plants [8, 29, 57]. Under drought stress conditions, insufficient root functionality and slow water diffusion rates, make roots ineffective in absorbing nutrients from the soil [58]. Water deficit conditions also lead to stomatal closure as well as decreasing transpiration and limited translocation of nutrients from the root to the upper parts of plants [8, 59]. Hence, water deficit conditions result in lesser availability of soil nutrients and reduced nutrient translocation consistency in plants [56, 60, 61]. All these elements have a grave effect on plant development, impacting several physiological processes.

**Stomatal movement**

The closure of stomata is the chief physiological reaction of plants to water deficiency. By decreasing transpirational water losses, plants conserve water levels in cells and this is generated by either hydropassive or hydroactive processes [62]. Water deficit conditions can induce abscisic acid (ABA) generation, which leads to stomatal closure and stimulates the expression of drought stress-associated genes for the regulation of plant responses. Accumulation of ABA in plant cells leads to the ROS production [63, 64]. H₂O₂, an important ROS, participates in various plant metabolic reactions, stress responses and apoptosis [65, 66]. Additionally, H₂O₂ takes part in the modulation of stomatal movement. ABA and H₂O₂ relationship plays an essential part under water deficit conditions. ABA stimulates H₂O₂ production in guard cells through NADPH oxidase, and H₂O₂ imparts ABA-generated closure of stomata. H₂O₂ induced stomatal closure has been noticed in broad bean (Vicia faba) and tobacco (Nicotiana tabacum) epidermis.

**Photosynthesis**

Photosynthesis is an essential indicator of drought stress due to its severe susceptibility to numerous environmental stresses. Several reports describe that drought caused a decline in chlorophyll content, net photosynthetic rate, maximal quantum yield (Fv/Fm), stomatal conductance and transpiration, which ultimately reduces plant metabolism and production [52, 67, 68]. Under drought stress, stomatal and nonstomatal factors could be effectual in suppressing the photosynthetic rate [69, 70]. Water scarcity prompts the synthesis of ABA, which leads to the closure of stomata and thereby decreasing the intercellular carbon dioxide concentrations and hindering photosynthesis. However, this suppression is reversible so photosynthesis can resume upon the removal of stress [71]. Decreased CO₂ which occurred due to the closing of stomata, also reduces the activity of enzymes taking part in several dark reactions. Decreased activity of light-independent reactions might cause disparity in the light and dark reactions, leading to ROS accumulation in plastids [8, 28, 72], thus causing indiscriminate impairment to the photosynthetic apparatus. Under water-scarce conditions, there is a prompt fall in the amount and activity of RubisCO noticed in all the plants studied; however, the intensity of this reduction depends on the plant species [8, 67, 73]. Decreased activity of RubisCO results from the acidification of chloroplast stroma, reduced quantity and action of ATPase and ATP synthase, configurational changes in chloroplasts and RubisCO [8, 28, 73]. Additionally, under drought conditions, activities of several other enzymes that take part in carbon fixation, like phosphoenolpyruvate carboxylase, NADP-malic enzyme, fructose-1,6-bisphosphatase, NADP-glyceraldehyde phosphate dehydrogenase, phosphoribulokinase, sucrose phosphate synthase, and pyruvate orthophosphate dikinase, also reduce with decreased leaf water potential [8, 73]. Electron transport reactions of photosynthesis are also disrupted under water deficit conditions [28, 74]. An attempt has been made to summarize the important informative studies corresponding to the impact of water scarcity on photosynthesis and its associated attributes in Table 2.

**Drought: biochemical reactions of plants**

**Oxidative stress and antioxidant system**

Drought results in oxidation in plant cells by inducing the generation of ROS (superoxide radical, hydrogen peroxide, hydroxyl radical, and singlet oxygen). ROS generation in plants is detrimental to several physiological and metabolic processes like photosynthesis
Compared with other ROS, the relative stability of H\textsubscript{2}O\textsubscript{2} against pathogens during seed germination [90].

Elongation [88, 89] and also play a role in the defense of the membrane, and ion spillage [83,84]. Under normal conditions, a steady-state cellular ROS level occurs as a result of equilibrium occurring between ROS production and scavenging. However, this balance gets shifted under various stresses, including drought stress, where more production of ROS occurs than scavenged, hence generating oxidative stress. Moreover, an early rise in the generation of ROS, before reaching the stage when its production overcomes scavenging action, can act as a signaling system for defense reactions. The signaling function of ROS has been seen well in defense response against pathogens where the generation of oxidative stress trigger signaling mechanisms for defense [85]. Besides playing a negative role induced by stresses, ROS also play a role in growth and development. Therefore, ROS, e.g. H\textsubscript{2}O\textsubscript{2}, have been revealed to take part in radicle emergence during seed germination [86,87], growth of roots, and root hair elongation [88,89] and also play a role in the defense against pathogens during seed germination [90]. Compared with other ROS, the relative stability of H\textsubscript{2}O\textsubscript{2} and its potential to pass through the cell membrane makes it suitable for signaling. The site and level of production of ROS should be strictly under control for signaling, either under stress conditions or growth and development responses. The cell wall is one of the essential sites where defense response through ROS commences [84,91]. In this compartment, the most exposed enzyme for the generation of ROS is NADPH oxidase, which produces O\textsubscript{2}·, which spontaneously or by the action of cell-wall located SOD is dismutated to H\textsubscript{2}O\textsubscript{2} [89]. Apart from functioning as a ROS detoxifier, wall-bound peroxidases also play a role in ROS signaling by the production of ROS like O\textsubscript{2}· and H\textsubscript{2}O\textsubscript{2}. The latter may also function as the substrate for lignin, which is essential for cell wall composition. Cellular ROS signal peculiarity can be ascertained by the site of its production, control and transduction [92]. Therefore, different plant cell compartments will have distinct impact on the regulation of cellular redox signals under water deficit conditions. Downstream signaling of ROS or hydrogen peroxide occurs through calcium and reversible protein phosphorylation [93,94].

Under environmental stress conditions, alterations in cytosolic free calcium ([Ca\textsuperscript{2+}] cyt) have been described. To enhance the influx of Ca\textsuperscript{2+}, ROS (including H\textsubscript{2}O\textsubscript{2}) can stimulate plasma membrane-localized hyperpolarization-activated calcium channels (HACCs) [95]. Intracellular Ca\textsuperscript{2+} can also establish a positive feedback loop by prompting NADPH oxidase to generate ROS in the apoplast [96]. Reversible protein phosphorylation, on the other hand, is revealed to be required in downstream signaling after the production of ROS [94], and a number of protein kinases have been exhibited to be induced by H\textsubscript{2}O\textsubscript{2} [97]; however, such activation does not occur by Ca\textsuperscript{2+}, as no Ca\textsuperscript{2+}-dependent kinase has been described under H\textsubscript{2}O\textsubscript{2} regulation [94]. Moreover, various studies associate H\textsubscript{2}O\textsubscript{2} with mitogen-activated kinase (MAPK) signaling cascade, which regulates gene expression through stimulation of transcription factors [93,94]. Such modulated genes appear to play a role in cellular protection and repair process because some of the gene products are known for desiccation tolerance and DNA damage repair [98]. A cDNA microarray study in Arabidopsis has revealed the upregulation of 113 genes and downregulation of 62 genes by H\textsubscript{2}O\textsubscript{2} suggesting a key role of H\textsubscript{2}O\textsubscript{2} in governing plant drought responses regulating Ca\textsuperscript{2+} signaling, MAPK cascades and gene expression [99].

### Table 2. Impact of water scarcity on photosynthesis and its associated attributes.

| Plant/crop                  | Effects                                      | References |
|----------------------------|----------------------------------------------|------------|
| Phaseolus vulgaris         | Decreased net photosynthesis and its related parameters. | [52]       |
| Trifolium aestivum         | Reduced chlorophyll a, b, and a/b, diminished rate of photosynthesis (Pn), stomatal conductance (gS), transpiration rate. | [68]       |
| Acacia tortilis            | Decreased Water potential, photosynthetic attributes. | [75]       |
| Glycine max                | Decreased photosynthetic rates (Pn), stomatal conductance (gS), maximum quantum efficiency (Fv/Fm), photochemical quenching coefficient (qP) and non-photochemical quenching coefficient (qN) | [54]       |
| Brassica juncea            | Significantly reduced plant development and photosynthesis, ribulose 1,5-bisphosphate carboxylase (Rubisco) activity | [76]       |
| Saccharum officinarum      | Reduced chlorophyll content, disorganized chloroplast ultrastructure | [77]       |
| Zea mays                   | Decreased photosynthesis and transpiration | [78]       |
| Poa pratensis              | Diminished rate of photosynthesis, water use efficiency, decreased chlorophyll levels, Rubisco activity | [79]       |
| Plectranthus scutellariaoides | Reduced leaf Chl index, maximum photochemical efficiency and lateral reactivity | [80]       |
| Wheat                      | Reduced contents of chlorophyll a, b, total Chl, and carotenoids, decreased net photosynthetic rate, inhibited leaf transpiration rate | [81]       |
| Solanum lycopersicum       | Decreased levels of chlorophyll, Fv/Fm, chlorophyll stability index (CSI) | [82]       |
However, under water deficiency plants have developed several mechanisms to overcome this scarcity. Antioxidant system and osmotic regulation are chief defense systems that provide tolerance to crops against water stress conditions [100]. Catalase (CAT), peroxidase (POX), superoxide dismutase (SOD), glutathione reductase (GR), ascorbate peroxidase (APX), glutathione peroxidase (GPX) constitute enzymatic antioxidants, and ascorbate, glutathione and phenolic compounds constitute non-enzymatic antioxidants [83]. The magnitude of damage caused by ROS depends on the balance between the ROS generation and scavenging by the antioxidant system. SOD constitutes the first defense line in presence of ROS, which brings about dismutation of the $\text{O}_2^-$ radicals to $\text{H}_2\text{O}_2$ [101]. CAT and APX detoxify $\text{H}_2\text{O}_2$ and hamper its accretion. Several non-enzymatic antioxidants like flavonoids, tannins and lignin precursors take an active part in ROS detoxification [102], reducing oxidative stress. These antioxidants function cooperatively, engaging various redox reactions. Additionally, it has been noticed that phenolic compounds are crucial in detoxifying the hydrogen peroxide cascade in plant cells [103]. In rice (Oryza sativa), it was reported that total SOD activities and APX activities were enhanced with rises in water deficiency. Similarly, a rise in activities of SOD, CAT and APX was noticed in barley under drought stress [104]. In maize plants (Zea mays) [105] reported that drought stress enhances SOD and POD activities and treatment of AsA further increases the enzyme activities.

**Molecular mechanism for drought response in plants**

Plants challenged with drought undergo many adaptive mechanisms at molecular levels to modulate the soil water content. Under these conditions, up and down-regulation of many genes take place at the transcriptional level are induced in response to stress and the accumulation of stress proteins functions well in tolerance to drought. Signaling pathways during abiotic stress including drought shows the involvement of various dehydration-responsive element–binding genes [106]. Plants exposed to drought stress experience negative effects like ROS-mediated cell injuries, enhanced cellular temperature and viscosity of cellular contents, altered interaction, aggregation, and denaturation of proteins [107].

In order to tackle the toxic consequences of drought stress on crops, plant biotechnologists have developed new cultivars exhibiting high drought tolerance by using various molecular approaches, including transcriptomics, proteomics and metabolomics. Reports have revealed that in response to water stress conditions, plants modulate the expression of various drought-responsive genes whose regulation occurs via a complex transcriptome network [41]. A number of physiological, cellular and molecular processes are responsible for the enlargement of drought tolerance in plants, for instance, up-regulation or down-regulation of the expression of various genes accountable for the osmolyte accumulation, increased content of enzymatic and enzymatic antioxidant system, decreased rate of transpiration, growth of shoot and tillering [108]. Under water stress conditions, increased concentration of abscisic acid (ABA) plays a pivotal role in closing stomata. Also, it regulates the expression of various stress-responsive genes. However, the expression of drought receptive genes is also controlled by a system which is independent of ABA [109]. Numerous protein-coding key genes show drought-assisted expression and play a metabolic or regulatory role. For example, genes responsible for detoxification, biosynthesis of osmolytes, water channels, ion transporters, heat shock proteins, proteolysis of cellular substrates and proteins are related to late embryogenesis [17]. Furthermore, the genes having regulatory roles mainly comprise transcription factors (i.e. AReB, NAC, AP2/ERF, MYC, MYB and bZIP), mitogen-activated protein kinases (MAPK); responsible for different cellular signalling pathways, protein kinases related to ribosomes, receptors and transcriptional regulated system and proteins responsible for synchronization of signal transduction (phosphatases) [110]. Various osmotic stress-responsive genes have been identified to play a vital role in the expression of enzymes responsible for the induction of the ABA biosynthetic pathway [111] and synthesis of different osmoprotectants like glycine betaine (GB), ectoine, mannitol, trehalose and proline, thereby maintaining the osmotic balance under stressful conditions.

The signaling pathways of various abiotic stresses, including drought stress, involve some common key steps. Foremost is the perception of stress stimulus via sensors or receptors which are localized in the plasma membrane or exist freely in the cytosol. The second is the transduction of the signalling via secondary messengers. Once the perception of stress stimulus occurs, it leads to the activation of secondary messengers like calcium ions, cyclic nucleotides (cAMP, cGMP), nitric oxide, sugars, ROS, etc. Secondary messengers thereafter activate the signalling pathway via...
modulating the expression or repression of various genes responsive to different stresses [28,42,44]. Protein kinases and phophatases cause phosphorylation and de-phosphorylation respectively of the proteins involved in the signalling pathway (i.e. receptors, secondary messengers, transcription factors), and MAPKs and CDPKs are the two protein kinases that play a vital role in the regulation of the drought stress-mediated signalling pathway [45,112]. Some transcription factors (TFs) directly communicate with the cis-acting elements incorporated in the promoter region of a group of downstream genes, hence synchronizing their expression [46]. Moreover, there are various TFs which are regulated via enhancers present in the upstream region of their gene [46]. Ubiquitination and sumoylation, as modifications at the post transcriptional level, create a network of regulatory complex, which plays a pivotal role in the regulation of stress-responsive genes responsible for governing various plant physiological and metabolic processes [48]. Various TFs showing up-regulation in response to drought stress, such as AREB/ABF, AP2/ERF TFs, NAC TFs and Bzip, have been identified and characterized in several plants.

**Drought stress responsive transcription factor family AREB/ABF**

In response to drought-mediated osmotic stress, a key molecular complex known as AREB/ABFs (abscisic acid-responsive element-binding protein/ABRE binding factor) plays a significant role in the up-regulation of ABA responsive genes [113]. A conserved sequence PyA/CGTG/TC is present in the promoter region of ABA responsive genes, and the conserved cis-element functions in the expression of these genes in response to signalling switched on by ABA. Various ABRE or its coupling elements (CE) are involved in the regulation of ABA-dependent gene expression [114–116]. Four SnRK2 phosphorylation sites present in the AREB/ABFs and containing a conserved domain have an essential role in regulating ABA-mediated gene expression [114,117]. It has been found that vascular tissue and guard cells are the sources for the synthesis of ABA. Once ABA is synthesized, it is immediately transported to the target cells [118,119]. Researches have revealed the existence of five NCED (9-cis-epoxy carotenoid dioxygenase) coding genes in Arabidopsis. Among these genes, NCED3 has been shown to exhibit enhanced expression in response to dehydration. The AG-box recognition sequence acts as a regulation site of the gene located up to 2248 bp upstream to its transcription start site [120]. The ABA bound receptor complex PYL/PYR/RCArS then gets associated with PP2Cs and leads to the activation as well as the release of SnRK2 [115,121]. Activated protein kinase SnRK2, in turn, activates the AREB/ABF TFs via phosphorylation, which eventually recognizes and binds to ABRE cis-element of ABA-responsive genes. In the absence or under low concentration of ABA, PP2Cs play a pivotal role in the down-regulation of ABA signalling via dephosphorylating the SnRK2 protein kinase. Among the nine members of AREB/ABF transcription factor family, AREB1/ABF2 functions in the regulation of drought-mediated ABA signalling, particularly at the vegetative stage [17,122]. Increased transcriptome level of AREB1 has been reported to enhance drought tolerance in rice, soybean and Arabidopsis [116,123,124].

**Drought stress responsive transcription factor family AP2/ERF**

AP2/ERF, referred to as APETALA2/Ethylene Response Element Binding Factors, is a large family of TFs comprising of numerous plant-specific TFs. Purification and characterization of these TFs has revealed the presence of conserved AP2/ERF DNA binding domain as well as recognition of the GC-box of ethylene-responsive genes [125]. AP2/ERFBP TFs are considered to play a significant role in the regulation of various plant physiological and biochemical processes like development of vegetative and reproductive stages, cellular proliferation, biotic or abiotic stress, and phytohormone-mediated responses. Based upon the number and similarity in the domains of AP2/ERF, this TFs family is further divided into four subfamilies, including AP2, ABI3/VP1 related TF RAV, ERF and DREB (dehydration responsive element binding protein) [125–127]. Eleven genes of DREB1/CFB and six genes of DREB2 have been reported in rice whose expression under cold and dehydration respectively was ABA independent [128]. In Arabidopsis, the A/GCCGAC sequence has been found to be incorporated in the promoter region of drought-responsive genes, whose cis-element is recognized as DRE/CRT [129]. DRE/CRT is the specific binding site for DREB1/CFB TFs and governs the expression of various abiotic stress responsive genes, including drought stress [126,127,130,131]. Overexpression of DREB1/CFB TFs leads to increased transcriptome level of various stress responsive genes, ultimately increasing drought tolerance in rice (Oryza sativa) [132,133], tomato (Solanum lycopersicum), potato (Solanum tuberosum), chrysanthemum (Chrysanthemum indicum) [134], tobacco (Nicotiana) [135], peanut
(Arachis hypogaea) [136], sugarcane (Saccharum officinarum) [137], soybean (Glycine max) [138] and wheat (Triticum aestivum) [139].

**Drought stress responsive transcription factor family NAC**

NAC gene family is the largest plant-specific TF family, whose name is derived from the association of three proteins forming a specific NAC domain from Petunia and Arabidopsis, i.e. NAM protein (no apical meristem), ATAF1/2 protein and CUC2 protein (cup shaped cotyledon) [140]. Purification and characterization of NAC TFs uncovered the highly conserved N-terminal DNA binding domain and a variable C-terminal domain meant for transcriptional regulation. DNA binding NAC domain contains a nucleolus localization signal and interacts with other similar domains or different domains to form a homodimer or heterodimer, respectively [141]. NAC TFs interact with a CACG sequence known as NAC recognition sequence (NACRs) present in the promoter region of drought responsive eRD1 (RESPONSE TO DEHYDRATION1) genes. It has been found that SNAC1 TFs play a pivotal role in enhanced drought tolerance in rice via ABA independent regulation of downstream genes OsPP18 and aPP2C. Enhanced expression of various NAC genes has been reported in rice in response to early stages of drought and salt stress [142]. Drought stress increased the transcriptome level of about 40 NAC genes of rice and 38 NAC genes of soybean [143].

**Drought stress responsive transcription factor family bZIP**

Analysis of another TF family, bZIP (basic leucine zipper) revealed the presence of a conserved bZIP domain containing an N-terminal basic nuclear localization signal, a DNA binding region and a C-terminal leucine rich motif meant for dimerization. In addition to the regulation of plant growth and development, bZIP TFs play an essential role in the regulation of drought responsive genes [144]. Isolation and characterization of various members of bZIP TFs have been reported in various eukaryotes. For example, 55 in grapevine (Vitis vinifera) [145], 89 in barley (Hordeum vulgare) and rice (Oryza sativa) [146,147], 96 in Brachypodium distachyon [148], 92 in sorghum (Sorghum bicolor) 131 in soybean (Glycine max) [149] [144,154] and 125 in maize (Zea mays). It has been reported that bZIP TF-mediated regulation of abiotic stress related genes occurs in ABA-dependent manner after recognizing the ABRE specific promoter region [144].

**Role of CRISPR/Cas9 in stress tolerance**

Clustered regularly interspaced short palindromic repeats (CRISPRs)/CRISPR-associated 9 (Cas9), a newly discovered targeted genome editing tool, is a prokaryotic molecular immunity system against viral pathogens [150–154]. CRISPR and its associated complexes can be easily programmed to target any sequence of choice. This can be utilised and implemented for various crop improvement traits like drought stress resistance and other biotic stress tolerance and management within a short span of time [155–159]. Abiotic stress resistance has been achieved in plants by targeting and cleaving genes of interest using the CRISPR/Cas9 system.

Also, the host genome can be modified in order to introduce resistance in plants for abiotic and biotic stresses. Thus, CRISPR/Cas9 is a powerful tool to provide resistance to multiple abiotic stresses in plants [160,161]. It can be utilised and implemented for the amelioration of agriculturally important crop plants [158,159,162–164]. The trends of application of the CRISPR/Cas9 technology for amelioration of crop plants in tropical climates within the last 5 years are phenomenal, and it betokens a rapid immensely colossal-scale application of it in addressing emerging challenges in engenderment of crops in tropical climates [165]. This advanced CRISPR/Cas9 tool helped in precise and highly efficient gene editing even at a single base level. This in turn has brought about a revolution in crop improvement programs especially in abiotic stress tolerance [24,25]. Further, such techniques have widened the opportunities to discover new traits associated with different characters. Which could be used in targeted improvements in crops against different stresses [26,27]. Various genes, transcriptional factors and networks have been identified in the last decade that play a pivotal role in biotic and abiotic stress tolerance [166,167]. However, there has been a decrease in the genetic diversity of important and most cultivated crop plants because of the continuous and large use of a few ‘best’ cultivars, which has led to an increase in susceptibility towards various stresses [168]. Therefore, there is a need to develop more stress resilient crops with high yield potential under abiotic stress especially drought stress and salinity stress, which could be done by creating variation in gene pools where CRISPR/Cas9 based genome editing technology can play its role. So far many crop traits have been modified by knocking out species’ gene with the
help of CRISPR/Cas9 resulting in improvements in abiotic and biotic stress management and yield \[169,170\]. Mitogen-activated protein kinases (MAPKs) is an important group of signaling molecules in plants that play a vital role during drought stress, which helps in the regulation of transcription of many genes associated with drought stress. The *mitogen-activated protein kinases 3* (*slmapk3*) gene was knocked out using CRISPR/Cas9, assisting in understanding the mechanism associated with tolerance by tomato (*Solanum lycopersicum*) regulated by *SIMAPK3*. Some crops with improved tolerance for drought stress are given in Table 3 \[159,174,175\]. Using CRISPR/Cas9 promoter region *auxin regulated gene involved in organ size ARGOS8* in *Zea mays* was replaced by *GOS2* promoter, which led to constitutive expression of *ARGOS8*. The new variant had increased yield under drought conditions compared to the wild type \[164\]. Thus, genome editing tools like CRISPR/Cas9 can be utilized to improve the important crop traits and yield under drought conditions. Genes, regulatory sequences, transcriptional factors etc. are very important and suitable targets for genome editing tools like CRISPR/Cas9, helping in producing abiotic stress resilient and high yielding crops \[171,172\]. Under drought conditions, a complex network of genes/transcriptional factors might be regulating simultaneously, which could affect the selection of novel targets. Still, there is a need for better progress in CRISPR/Cas9 application for the development of highly efficient and stress-tolerant crops. 

### Conclusions and future prospects

Drought, which negatively impacts plant growth and yield, is a serious threat to food security worldwide. Drought stress affects many biochemical aspects, including morphological and physiological challenges causing severe effects on the growth and metabolism. To approach the adverse impact of the drought on crop plants, the plants embrace specific mechanisms that ameliorate drought resistance. Plants either acclimatize during drought stress or show avoidance mechanisms, while some tolerate the stress. Gene modulation and gene expression modification are important mechanisms of plants against drought stress. Using classical breeding strategies and biotechnological interventions together can help in improving drought tolerance in plants. Producing drought-tolerant genotypes can be another way to manage drought stress. Thus, there is a need to explore the untapped adaptation traits in different plants and their incarnation to the genotypes that may tolerate the adverse effect of drought stress without affecting its productivity. Omics applications with special approaches like genomics, transcriptomics, proteomics, metabolomics, glycomics and phenomics are of enormous importance to produce stress-resilient crop plants. Advanced genome editing techniques like CRISPR/Cas9 could dramatically improve plant tolerance to drought stress and enhance crop production. As CRISPR–Cas-mediated plant genome editing still faces many challenges, deciphering the regulatory mechanisms underlying drought stress tolerance in different plants using genomic strategies will aid the application of this system in various crops. These innovative strategies reduce the threat to global food security by providing a better understanding and potentially increasing plant productivity in arid environments.

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Authors declare that there is no conflict of interest.

### Data availability statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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### Table 3. Modified crops using CRISPR for drought stress tolerance.

| Plant                              | Target gene(s)                      | Abiotic stress                  | References  |
|------------------------------------|-------------------------------------|---------------------------------|-------------|
| *Solanum lycopersicum*             | SIMAPK3                             | Drought tolerance               | [159]       |
| *Arabidopsis thaliana*             | UGT79B2, UGT79B3                    | Drought stresses                | [159]       |
| *Arabidopsis thaliana*             | MIR169a                             | Drought tolerance               | [171]       |
| *Oryza sativa*                     | OsPDS, OsMPK2, OsBADH2, OsMPK5, OsAOX1a, OsBEL, OsAOX1b, OsAOX1c | Various abiotic stress tolerance | [163,172]   |
| *Oryza sativa*                     | OsDERF1, OsPMS3, OsMYB5, OLEPSPK, OSM3H1 | Drought tolerance               | [173]       |
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