

Drought tolerance mechanisms and their breeding implications

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Abstract: An increase in atmospheric concentration of greenhouse gases and associated climatic variability is primarily responsible for inducing high heat waves and drought stress, affecting both plants and animals. Drought stress is one of the finest limitations to global agricultural production due to the problem of the water limiting environment and global climate change. It is critical to determine the plant response mechanisms to drought stress to reduce the impacts of drought stress on plants. Drought response mechanism includes morphological, physiological, biochemical, and molecular processes occurs in plants underlying drought stress. These processes include improvement in root structure, leaf structure, osmotic adjustment, relative water content, and stomata regulation. Moreover, calcium and phytohormone (Abscisic acid, Jasmonic acid, Salicylic acid, Auxins, Ethylene, and others) signaling pathways, as well as scavenging of reactive oxygen species are crucial mechanisms to cope with drought stress. To further elucidate and improve drought tolerance in plants, novel emerging approaches of plant breeding and biotechnological methods such as genome-wide association studies, mutational breeding, marker assisted breeding, double haploid production and CRISPR/cas genome editing serve as engineering tools for dissecting the in-depth physiological mechanisms. These techniques have well-established implications to understand plants' adaptations to develop more tolerant varieties and minimize energy expenditure in response to stress as well as to fill the void that would otherwise result in growth resistance and yield loss. Hereby, this review highlights various mechanisms of drought tolerance and it also discuss new cutting-edge mechanisms of breeding to cope plants with drought stress.

Keywords: Drought tolerance; CRISPR/Cas9; Antioxidant; Genetic engineering.

1. INTRODUCTION

Global food demand is continuously increasing, and it is expected to double in the next years. It is difficult to feed the world's population using current natural resources [1]. The genetic potential of the majority crops is not completely exploited, due to several biotic and abiotic stresses. Abiotic stresses such as severe drought, salinity, high temperature, and decrease in soil oxygen pollutants, excessive UV radiation, and lack of mineral nutrients are the major global food security constraints [2]. Drought is a global issue that affects both developing and developed countries [3]. As a result of anthropogenic activities, the balance between incoming solar radiation and outgoing radiation, as a result the Earth's energy in the form of heat increases, resulting in global warming [4]. Global warming increases water evaporation, turns water into vapor in the air, and consequently, leads to drought stress. It is predicted with a reasonable belief that at the end of twenty-first century, the wave of heat will be frequent and more intense [5]. Due to the increase in temperature, some crucial changes in atmospheric conditions could happen, including high temperature and drought [6]. These global climate changes affect trophic interactions, species distribution, abundance, and ecosystem function. With the rapid growth of the world population, drought and high temperature stresses are predicted to be increased in the next years. High temperature and water scarcity are two important interconnected stresses, having large impact on the growth and productivity of crops [7].

Drought is an abiotic factor that limits crop growth and adversely affects the crop from reaching its yield potential. The severity and duration of drought determine the rate and amount of its damaging effects. All phenological growth stages are affected by drought, especially germination and flowering stages are the most vulnerable stages. Understanding the mechanisms driving plant responses to drought stress is the most challenging mechanism, due to variations in the traits regulating the status of water availability in soil and the need for water at evaporation sites, responses to water status, and genetic variation across distinct crops. Droughts interfere with water relations, nutrient absorption and assimilation, photosynthetic activity, enzyme activity, assimilates partitioning, and ultimately cause significant damage [8, 9, 10]. Drought causes wilting, stomatal closure, and reduced cell expansion due to decreases in cell water content, turgor, and tissue water potential. These effects cause photosynthetic arrest, resulting in dramatic modifications in normal metabolism, which might result in growth halting or death. Under water stress, interruptions in the xylem water column or changes in the xylem vessel reduce the hydraulic conductivity of shoots and roots. Leaf hydraulic conductivity of the plant is controlled by aquaporins (membrane channels that facilitate the transport of water and small neutral molecules) [11]. Drought stress response involves metabolic and hydraulic regulation at both the cellular and whole plant level, connected with finely controlled signaling processes. Several significant metabolic and physiological changes of xero-halophytic plants in response to drought stress have been identified [12].

Drought stress causes physiological, biochemical, and molecular reactions at the cellular and molecular levels to counteract its damaging effects and sustain plant function. Drought stress tolerance is a complicated process controlled by several drought responding genes. In response to drought stress, plants activate their drought response mechanisms, such as morphological and structural changes, expression of drought-resistant genes, osmoprotection by osmotic adjustment, antioxidant scavenging defense system, water channel proteins, stress responsive proteins, transcription factors and signaling pathways actively participate in conferring drought tolerance in crop plants [13]. Moreover, drought upregulates several genes, including those involved in osmolyte metabolism, secondary metabolite synthesis, and hormone synthesis, allowing plants to respond to drought more quickly. Improving water loss by increasing diffusive resistance, creating a deep root system for improved water uptake, and developing small leaf size and succulent leaves to reduce water loss in the form of transpiration, are the most important strategies for drought stress tolerance plants [14, 15]. Mineral ions such as potassium and sodium help in osmotic balance, thereby contributing to the regulation of cellular water content, whereas silicon promotes water holding capacity through solidification of the endodermal cells. Low molecular weight solutes are very essential to protect the metabolic system of the plant.

Among several growth hormones, auxins, gibberellic acid, abscisic acid, salicylic acid, jasmonic acid, and ethylene are the most important modulating drought stress response. Polyamines and plant secondary metabolites have antioxidant properties that help to mitigate the severity effects of drought. Phytohormones, one is abscisic acid, which helps in regulating pathways that are either ABA-dependent or ABA-independent under drought stress conditions. As reported by Gouveia et al. [16] ABA mediated signal pathway increased water use efficiency and photosynthetic regulation of plants under drought stress. To better understand the mechanism of drought resistance of plants, based on a lot of previous work, we summarized the status and progress of studies on the morphological structure, physiological and biochemical mechanism changes, internal signal transduction system, molecular regulation mechanisms of plants under drought stress and their breeding implications available in recent years.

The potential of crop plants to maintain their growth and development under drought stress is termed as drought tolerance, yield stability is also related with drought tolerance under prevailing drought conditions. Tolerance is a very complex process and plants have evolved numerous adaptations at the morphological, physiological, and molecular levels to confer drought tolerance (Fig.1). Higher economic yield under drought stress is the characteristic feature of drought tolerant crops, survival is important at seedling stages, whereas, later on just survival without economic yield has no importance for breeders and farmers [17]. Plant growth and development, plant phenology, grain filling, plant leaf growth and anatomy, and translocation of photo assimilated reserves are important traits to be targeted for the improvement of drought tolerance in plants [18].

Several breeding approaches give an insight for drought tolerance successfully as reported in some major crops such as rice [19] and wheat [20]. Collaborative approaches incorporating comparative, functional, structural genomics and transcriptomics can enhance the traditional breeding approaches. In crop plants, genetic manipulation approaches have been used to identify genes associated with drought tolerance and their introgression [21]. The use of molecular tools in breeding

approaches is the most useful way of biotechnology [22]. However, there is still a significant gap between crop yields under stress condition and optimal conditions. Various traits have been identified in several researches for plant adaptability to drought stress environment. Genomic techniques and the physiology of the crop give new insights to plant breeders to overcome the drought problems using new emerging technologies for crop improvement [22]. Plants cope themselves with drought stress using various responsive mechanisms, and these mechanisms can be applied through various strategies and techniques. Hence, this paper discusses the drought tolerance mechanisms in plants, drought effects on growth and production, and adaptive strategies, and discusses new cutting-edge breeding techniques to cope with drought stress, where food security is the major Global concern.

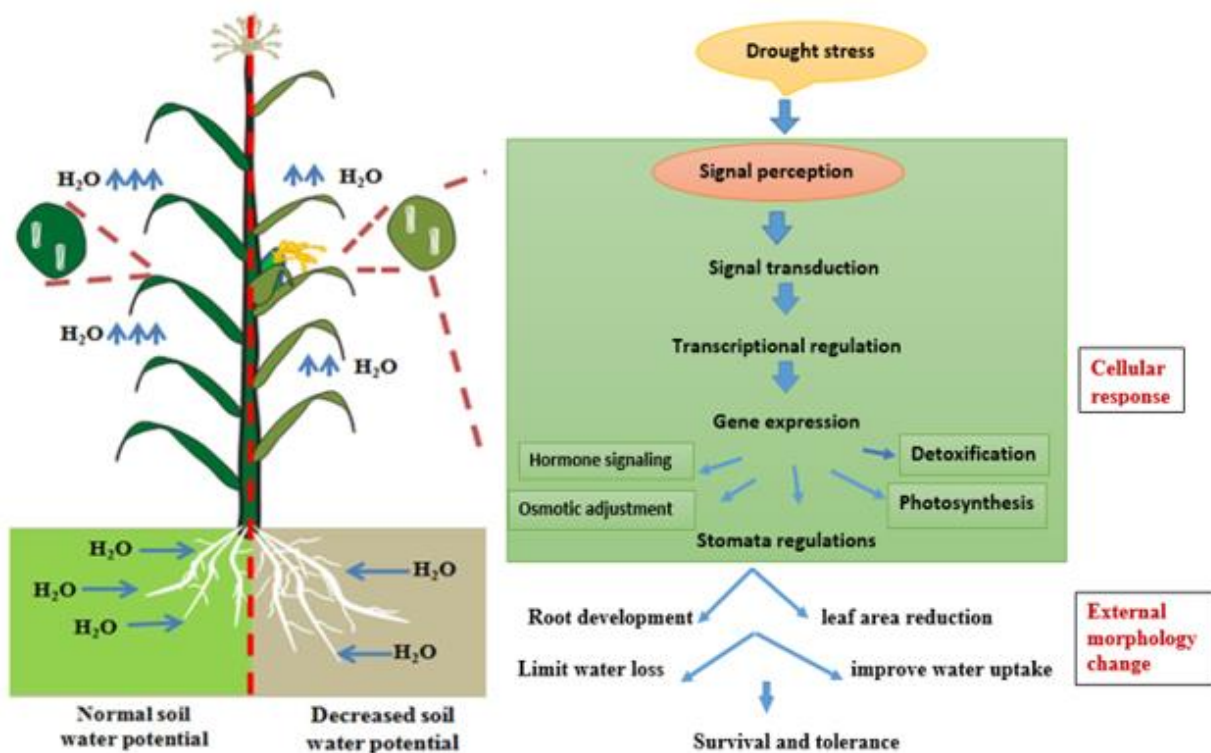


Fig. 1 dissecting mechanisms of drought tolerance in plants

2. STRUCTURAL AND PHYSIOLOGICAL ASPECTS OF DROUGHT TOLERANCE

Drought tolerance in crops varied according to the developmental stage of the plant, crop type as well as the duration. Plants show different kinds of response mechanisms (drought avoidance, drought escape, drought tolerance, and drought recovery) during drought stress [23]. Drought tolerance and drought avoidance are a critical plant responses during shortage of water. Drought tolerance is the ability of a plant to resist dehydration through various physiological activities, such as osmotic adjustment via osmoprotectants [24]. Drought avoidance is the continuity of physiological mechanisms, for example, stomata regulation, root system development, and other processes under water stress. Drought escape is the capacity of plants to adjust their life cycle (short life cycle to avoid drought stress). Drought recovery is the ability of a plant to restart growth and reproduction after exposure to severe drought stress [25]. Structural and physiological responses of plants to escape, avoid, recover, or tolerate drought stress have been listed below.

2.1 Leaf shape and structure

Reduction in transpiration rate is essential for plants under water scarcity condition, dropping and reduction in leaf size reduce transpiration, respectively [23]. Under the deficit of water, the leaves become wilt, the margin become yellow in color, suppress the shoot and leaf growth, affecting the crop's growth and productivity [26]. Plants reduce the rate of transpiration to respond to drought stress by developing a waxy and thick leaf cuticle layer [27]. Plant leaves develop some

xeromorphic characters and change their morphological structure to increase drought stress tolerance, such as smaller and less number of stomata openings, thick palisade tissues, large number of trichomes, thicker and tiny leaves and developed vascular tissues [28]. For example, expressing MtCAS31 gene improved drought tolerance in transgenic Arabidopsis by lowering stomatal density [29].

2.2 Root structure

Plant roots are critical for sensing and responding to diverse abiotic and biotic stresses, due to direct contact with nutrients and soil. Roots are possibly the first organ to detect drought stress, and the root system plays an important role in drought stress response. Plant roots respond to water stress in the upper soil profile causing deeper root penetration, while too much water in the upper layer reduces deep root penetration [30]. Longer roots are more favorable and better for drought tolerance compared to shorter roots. A considerable decrease in the number of roots is noted in drought stress, which is observed in *Helichrysum petiolare* [31]. Drought tolerant adaptive characters of plant roots including long roots, thick and denser root system. To maintain growth and productivity under water deficit, the plant needs long roots with high density, particularly when water is deeper [32]. Denser root system also absorbs larger amount of water than thinner roots because of the higher number of roots that may contact with more water vapor present in the soil layer [33]. In a case, overexpression of AtHDG11 gene enhanced drought tolerance in transgenic cotton with a longer and denser root system in addition to other drought tolerance characteristics such as reduced stomatal number and size, higher accumulation of malondialdehyde (MDA), proline content and ROS scavenging enzymes [34].

2.3 Stomata Regulation

In response to drought stress, stomata closure is the first reaction to reduce transpiration, stomata conductance indicating drought tolerance. Closing of stomata reduces transpiration which enhance drought tolerance capability of the plant, while its opening increases transpiration and subsequently reduce plant drought tolerance [35, 27]. Stomata regulation is involved in the maintenance of photosynthetic ability of plants under water stress condition [36]. Opening of stomata leads CO₂ to the mesophyll tissues and continues the photosynthesis process; however, closing of stomata reduces photosynthesis, respectively [37]. As stress becomes more severe, the dehydration of mesophyll cells slows photosynthesis, mesophyll metabolism is reduced, and water-use efficiency usually decreases. Results from many studies have shown that the relative effect of water scarcity on stomatal conductance is significantly larger than that on photosynthesis, a decrease in the number of stomata is another physiological response to drought stress. Moderate drought has an additive impact on the stomata number, while the number of stomata decreases under extreme drought conditions. The lower transpiration rate, thick cuticle, thick cell wall, and small stomatal aperture enhance drought tolerance in plants [38].

3. BIOCHEMICAL AND MOLECULAR ASPECTS OF DROUGHT TOLERANCE

Plants use a variety of biochemical mechanisms to respond to drought stress, like production of particular proteins, high levels of metabolites, and expression of different genes. Numerous findings regarding the metabolic changes due to drought stress are partial and a deep insight in the metabolic and biochemical mechanisms involving drought tolerance is essential for the engineering of plants for various abiotic stresses. For the adaptation to various stress conditions, plants have numerous mechanisms including the accumulation of solutes (like sugar and proline), enzyme activation (Peroxidase, Superoxide dismutase, Ascorbate and catalase), and non-enzymatic (reduced glutathione substances) [39]. In addition, phytohormones have a key roles in response to drought stresses. Drought stress conditions can also affects the production of endogenous levels of hormones, like Abscisic acid (ABA), Jasmonic acid (JA), Ethylene, Auxins, and Cytokinins (CK) [38]. These phytohormones have an important roles in regulating plant growth, development, and responses to drought conditions. During stress conditions, these phytohormones trigger various signaling pathways, consequently enhancing antioxidant enzyme production and secondary metabolites. To increase drought tolerance in plants, it is needed to study and develop numerous phytohormones related strategies [27]. These numerous mechanisms of biochemical and molecular response have been discussed below.

3.1 Abscisic Acid (ABA)

Abscisic acids regulates various physiological and developmental stages of plants including seed dormancy, plant development and growth, stomata opening, embryo morphogenesis, and the synthesis of storage lipids and proteins. It is a major phytohormone involved in the response to abiotic stresses, particularly osmotic stress [40, 27]. ABA, the plant stress

regulator, controls different biochemical and morph-physiological processes that help the plant to adapt to different abiotic stresses [41, 42]. Abiotic stresses such as salinity, drought, and cold increase the ABA production level in plants [43]. Abscisic acid synthesis increases during drought stress and consequently activates drought response signaling pathways in plants [44]. It triggers various drought stress-related genes that lead to closing of stomata, improve root architecture and structure, and increase the synthesis of drought tolerance substances and other drought-tolerant mechanisms in the plant [45]. The accumulation of ABA controls the closure of stomata to reduce transpiration [(46]. Stomata closure reduces the exchange of gases that decrease the rate of photosynthesis and transpiration [47]. Normally, light activate stomata opening, while ABA and raised CO₂ levels stimulate incomplete or complete closing down of stomata [48]. ABA inhibits the movement of ions in guard cells, causing the stomata to close and the rate of transpiration to slow [48, 49. 30] demonstrated that the plant performed better under drought stress, when the ABA either activated endogenously or added exogenously than nontreated plants. ABA dependent signaling pathways are ready to respond to drought stress upon the perception of stimulus. ABA dependent signaling pathways respond to drought stress via various morph-physiological and biochemical mechanisms. Under normal conditions, the amount of ABA phytohormone is low, the activity of SnRK2 protein is inhibited by PP2C (protein phosphatase 2C) which causes dephosphorylation [38]. However, under drought stress, the ABA level increases, which lead ABA binding to PYR/PYL/RCARs, which in turn bind and deactivate PP2Cs [27]. SnRK2s proteins are auto-activated, and phosphorylate a downstream targets and activate ABA signaling pathway, which triggers molecular and physiological responses to drought stress [50, 49].

3.2 Ethylene

Ethylene is a gaseous phytohormone that involves in numerous actions, such as seed germination, plant growth and development, flowering, fruit ripening, senescence of fundamental organs and response to different biotic and abiotic stresses [51]. It has been studied that ethylene regulate many biological mechanisms in response to various abiotic stresses [52]. Multiple number of Ethylene response factor (ERF), i.e., GmERF3, was induced by ABA, JA, ET, under salt and drought stress condition. The overexpressing GmERF3 tobacco gene showed higher tolerance to drought stress due to higher proline content and soluble sugars when compared to wild type plants [53]. Similarly, overexpression of another ERF gene, AtERF019, delayed flowering time, senescence, and lower transpiration rate, importantly responding to drought stress. Ethylene also interacts with other plant hormones such as ABA and jasmonic acid through ERFs to resist various biotic and abiotic stresses [52].

3.3 Auxin

Auxin is another crucial important plant hormone that controls the growth of the plant and responds to unfavorable abiotic conditions, such as drought [54]. Biosynthesis of auxin occur in leaf, primordial, juvenile leaves, and developing seeds [55]. Some pathways are proposed for the biosynthesis of Auxin, which include tryptophan dependent and *Trp*-independent pathways [56]. The pathway is extensively self-regulated through positive and negative feedback inhibition [57]. Auxin normally moves from the shoot tip to the root tip via the phloem, through cell-to-cell transport or via auxin transport proteins. Under drought conditions, the auxin levels in plants are increased to respond stress [58]. In a case, 31 aux/IAA genes were found in rice induced by drought tolerance and one of these genes (OsIAA6) was identified to respond to drought stress [59]. In another study, in transgenic potato, overexpression of AtYUC6 gene showed over production of auxin which increases drought stress tolerance than wild type potato [49]. Auxin hormones are essential in the development of plant roots [60]. While roots have a key roles in improving drought tolerance. For instance, primary drought stress promotes YUC7 gene function in roots and increases the auxin level in Arabidopsis, YUC7-1D stimulates root growth and development, and increases root architecture. As a result, plants with the YUC7-1D gene showed resistant to drought stress and controlled drought responsive genes [61].

3.4 Cytokinin

Cytokinin (CK) is a class of plant hormone that promotes cell division, root development, delays leaf senescence, regulates nutrient allocation, and plant response to various pathogen interactions. In addition, it also controls the drought stress responses of the plant [62]. Some CK related genes i.e., CKX1, CKX2, CKX3 and CKX4 were overexpressed in Arabidopsis, which decreases Cytokinin level and subsequently increase drought tolerance of plants [63]. Cytokinins enhances drought tolerance through; protection of photosynthetic apparatus, increase of antioxidant substances, regulate water balance, control plant growth, and regulate stress-associated hormones. Under drought stress conditions, cytokinin

regulates the genes that involve in CO₂ assimilation, electron transport rate, and increased photosynthesis rate [64]. CK works with abscisic acid to adapt the plant under drought stress conditions. It has been reported that CK has both negative and positive effects on plant growth and development during drought stress conditions [62]. Huynh et al. [65] identified increased level of cytokinin following overexpression of CK biosynthetic genes driven by the senescence-inducible promoter SAG12 enhanced drought tolerance in tobacco plants. This together with the reported role of CK in the sink-source polarization in tobacco [66] suggests that CK is involved in plant drought response.

3.5 Mitogen Activated Protein Kinase

Mitogen activated protein kinase (MAPK) participates in the signaling pathways in response to various stresses such as pathogen, drought, cold, salinity, ozone, and wounding of plants [67]. MAPK cascades are involved in several ABA responses including guard cell signaling, seed germination, and antioxidant defense system [68]. A transcriptomic study revealed that MAPKS are activated by multiple abiotic stresses, like cold, drought, salt, temperature, and pH [69]. As reported by Xu and Chua [70], in Arabidopsis, MAP kinase, MPK6 improves plant tolerance to drought stress conditions. In *Oryza sativa*, [71] reported raf-like MAPKKK protein, DSM1 (drought sensitive mutant1) functioned as a potential scavenging of ROS, and increased stress tolerance to water stress. In other study that reported by Zhang et al. (72) GhMPK2 has a role to adjust osmotic pressure and reduce water loss under water stress condition in cotton. GhMPK6 has also an important roles in H₂O₂ production and ABA-induced catalase expression [73]. MAPKs, GhMPK16 and GhMPK7 are participating in tolerance to various stresses and regulation of stress signaling pathways [72].

3.6 Antioxidants

Oxygen is a critical element in plants and is involved in a variety of energy-producing processes such as oxidative phosphorylation, metabolism, and mitochondrial respiration. During different metabolic process, oxygen is converted into damaging levels of reactive oxygen species (ROS), which must be normalized. ROS is a hydroxyl radical that is primarily responsible for the production of hydrogen peroxide, singlet oxygen, etc. The concentration of ROS increased under stress conditions resulting in cell death, damage to protein, irreversible organelle dysfunction results, and cytoplasmic membrane damage [74]. Reactive oxygen species (ROS), also known as reactive oxygen intermediate (ROI), are highly reactive chemical, formed from the incomplete reduction of atmospheric oxygen. There are four forms of ROS; hydroxyl radicle (HO•), hydrogen peroxide (H₂O₂), singlet oxygen (¹O₂) and super oxide anion radical (O₂⁻) [55]. These ROS are harmful to cellular components, such as nucleic acids, lipids, carbohydrates and proteins. To survive in drought stress conditions, plants have developed numerous interrelated signaling pathways to regulate a variety of stress-responsive genes for the production of different classes of proteins, such as protein kinases, enzymes, transcriptional factors and other functional proteins, that leads to diverse metabolic and physiological responses and enhance tolerance to abiotic. Plants need to keep ROS in balance, but abiotic stresses imbalance it and increase the production of ROS at cellular levels.

There are two major sources of reactive oxygen species during stress, i.e., metabolic and signaling ROS. Metabolic ROS directly change the redox status of rate-reducing enzymes and regulate the metabolic fluxes in the cell, thereby change the metabolic reactions to decrease the overall effects of stresses [75]. It can affect transcription and translation by altering the function of various regulatory proteins [76]. When stress sensor response, the signaling ROS are generated. The redox status of regulatory proteins, as well as transcription and translation, are all affected by ROS signaling, resulting in the activation of the acclimation response, which decreases the consequences of stress by lowering metabolically produced ROS. However, prolonged drought stress increases the production of ROS in the cell, such as cell wall, plasma membrane, chloroplasts, mitochondria, and nucleus [77]. Overproduction of ROS is harmful to cell function and affects other developmental processes. The oxidation of ROS may lead to cell death [23]. Hereby, enzymatic and non-enzymatic defense mechanisms have a crucial roles in detoxification and scavenging of the ROI and increase drought stress tolerance [78]. Multiply enzymes, sugars, and amino acids present in different parts of the plant cells participated in the scavenging of ROI, such as glutathione reductase (GR), glutathione peroxidase (GPX), ascorbic acid (AA), reduced glutathione (GSH), α tocopherol, carotenoids, osmolyte proline and flavonoids [79].

The enzyme, SOD convert O₂⁻ into hydrogen peroxide (H₂O₂), the CAT and PODs further break the hydrogen peroxide into molecular oxygen and water molecules [79]. MDAR, GR, and NADH along with APX remove the H₂O₂ via Halliwell-Asada pathway (Uzilday et al. 2012). Rboh (Respiratory Burst Oxidase Homolog) genes code for NADPH oxidases that are linked to the plasma membrane and play an important role in the transmission of ROS-related signaling pathways [80].

SOD act on $O_2^{\bullet-}$ and convert it into H_2O_2 , further APX, GPX, and CAT detoxify the H_2O_2 . Glutathione S-transferase (GST), peroxiredoxin (PRX), and GPX reduce the H_2O_2 into organic hydroperoxidase, through ascorbate independent thiol using glutaredoxin (GRX) or thioredoxin (TRX) [81]. Expression of OsAPX2 reduced MDA and H_2O_2 levels and increased the APX activity under water stress condition and consequently improves drought tolerance in transgenic plants [82]. Furthermore, overexpressing OsAPX2 plants were more resistance to drought stress than wild plants during abiotic stresses. In transgenic rice, the overexpression of OsAPX1, another gene of APX, enhanced fertility under cold stress [83]. Metallothioneins (MTs) are a class of low molecular weight proteins that have high cysteine accumulation and metal binding. In transgenic rice, overexpressing OsMT1a improves the activity of antioxidant enzymes such as CAT, APX, and POD, and increased drought tolerance of rice. SbMT-2 gene is also involved in maintaining cellular homeostasis by regulating ROS under drought stress [84].

3.7 Calcium signaling induces drought tolerance

Calcium (Ca^{2+}) regulates various physiological and cellular processes in plants and a crucial signaling molecule in drought tolerance. It has a great role in the perception and transduction of the overall signaling networks in plants that use calcium-dependent signaling networks [85]. Various stresses and phytohormones, like drought and ABA are involved in changing the cytoplasmic Ca^{2+} concentration [86]. Plant cellular Ca^{2+} signaling are detected by three classes of calcium sensor molecules; CBLs (calineurin B-like protein), CDPK (calcium-dependent protein kinase), and CaM (calmodulin) [55]. Li et al. [86] identified 41 CDPKs genes in the genome of diploid cotton (*Gossypium raimondii*) and reported that seven genes (GhCDPK2, GhCDK3, GhCDPK11, GhCDPK14, GhCDPK16, GhCDPK28, and GhCDPK35) are found to be involved in drought stress tolerance. CBLs interact with CBL interacting protein kinase (CIPKs) to transmit Ca^{2+} signaling molecule. The CBL-CIPK complex is combined with the plasma membrane [87]. GhCIPK6 gene was activated by ABA treatment, overexpression of GhCIPK6 in *Arabidopsis thaliana* significantly increased drought tolerance [88]. The Ca^{2+} attach to EF motifs, conformational change undergone in CaM that increase their catalytic activity or interaction with the downstream target proteins. Calreticulin (CRT) and Calnexin (CNX) have a great contribution in calcium binding protectors of Endoplasmic Reticulum and that responsible to regulate the folding of newly synthesized proteins [89]. The study identified that changes in Ca^{2+} ions concentration, transduced Ca^{2+} signals via CDPKs, CBLs and CaMs were involved in drought stress response.

4. BREEDING STRATEGIES FOR DROUGHT STRESS TOLERANCE

4.1 Mutation breeding

Mutation breeding is one of the most important tools for creating desirable genetic variation for crop improvement. An important point in mutation breeding is the selection of individual plants with a target trait requires two steps: screening and confirmation of mutants [90]. In mutation breeding, multiply number of seeds are exposed to different mutagen agents such as gamma rays, chemicals, X-rays and grown for segregation and the plants with desirable traits of interest are selected to grow for the next generation. Over-location trials are conducted for further selection and evaluation to introduce a new variety [91]. Mutation breeding has been successfully applied to improve genetic variability in many crops such as sugarcane, rice, wheat, barley, sorghum, legumes, cotton, and edible oil throughout the world [92]. Efforts have been done to screen drought tolerant mutants more productive under drought stress conditions. Recently, mutation breeding has regained popularity among geneticists due to the advent of various new emerging molecular approaches, such as targeted induced local lesions in the genome (TILLING) and the utilization of AC/DC transposons for tagging genes, useful for screening mutant populations and tagging genes with traits of interest [93]. Ten drought stress tolerant mutant lines of sorghum were identified in Indonesia. In drought seasons, these lines have higher biomass production and yields significantly higher than the original variety, Durra and the national check variety. Eight drought-tolerant pure soybean lines were selected in Malaysia. Four mutant lines (GH-7, I-209, M-220, and 60-MBB) were distributed for field evaluation in Malaysia and the Philippines [94]. In West Africa, sorghum is also undergoing irradiation treatment and, in field trials, some of the new mutants produced have demonstrated increases in yield of 30–50 %, higher protein content, and earlier maturation compared to previous local cultivars. Some varieties indicated an improved tolerance to drought stress and the new plants also maintained the important traits favored by farmers [94]. Adak and Kaynak [95] developed a mutant populations of strawberries with the aim of enhancing water stress in strawberries using ethyl methane sulphonate (EMS) chemical mutagenesis using tissue-cultured meristems of three cultivars of strawberry (Festival, Osmanli and Camarosa). The result showed that water deficit tolerance lines could be obtained from the variations.

4.2 Double Haploid (DH)

Double-haploid technique through gametic embryogenesis have emerged as an exciting tool for breeding techniques having the advantages of shortening of the breeding cycle, high selection efficiency, production of completely homozygous lines from heterozygous donor plants, and expression of recessive alleles suitable for breeding. Diploidization of haploid genomes can be produced either by artificial genome doubling or spontaneous genome doubling. DH technique was found to be efficient and effective for the fixation of important alleles that control important traits. In rice crop, it is utilized for the development of new varieties from photosensitive rice genotypes [96]. Double haploids may prove to be a useful technique for locating QTLs that control quantitative traits. More than 100 rice varieties have been developed in India, South Korea, USA, China and Japan by DH technique [97]. Several evaluations on DH technology offer insight to plant breeders in crop improvement, as it has a broader applicability in breeding and genetic research [98].

Screening haploid gametes under drought stress has proven to be an effective technique for increasing the frequency of homozygous drought stress-tolerant genotypes [99]. It has been reported that 65 percent of the genes important for cell structure and drought tolerance are expressed during the gametophytic stage, resulting in drought tolerance variation among gametophytes. The undesirable haploid cells or gametes are removed from the population within a single generation compared to field screening of plants where heterozygous plants continue to show segregation for drought tolerance in every next generation. In vitro screening has been carried out for the screening of drought tolerant microspores. Ambrus et al. [99] tested maize microspores for resistance to reactive oxygen species (ROS) induced by drought which reduces the callus induction and regeneration potential. However, drought stress only allowed tolerant microspores to proliferate and to be regenerated into complete plantlets.

4.3 Marker-assisted breeding (MAB)

Marker-based breeding is a kind of indirect selection in which the target trait is chosen based on markers rather than phenotypic selection. It is the use of molecular biotechnology, namely, molecular markers in combination with linkage maps and genomics, to improve genotypic assays in plants and animals. The success rate could be considerably increased by increasing the number of markers connected with QTL [100]. The marker to be applied should be highly linked to the gene of interest for efficient and accurate marker selection. Plant breeding and traditional genetics have both benefited from the use of molecular markers. In earlier time, development of molecular markers, QTL mapping, and fine mapping of the precise genes were considered difficult and time-consuming processes. However, with the emerging of next-generation sequencing, it has made the development of molecular markers easier methods. The emerging of these molecular markers has further facilitated the development of high-density genetic maps, which enabled the mapping of target genes. The identification of different genetic variations for various super traits is also done using molecular markers. Moreover, they allowed the detection of suitable parents for molecular breeding and used to identify the desirable offspring at the early developmental stages [101].

MAB is an efficient and accurate method of breeding technique for introgression of any line and it allocates selection in every breeding cycle for the introgression of the gene of interest in an accurate method. It has been applied in various crops such as wheat, rice, cotton, oil, seeds, and forage species and represents an additional tool in breeding techniques for enhancing yield and growth under a drought environment [93]. In rice, MAB has been employed in the mapping of QTLs which control abiotic tolerance such as drought, salinity, and submergence [20]. Prince et al. [102] conducted SSR-based QTL mapping on RIL population of rice derived from a cross of IR20 and Nootripathu for physiological and yield traits in drought environment. Moreover, Three QTLs mapped on chromosome 1 (RM8085), chromosome 4 (I12S), and on chromosome 6 (RM6836) can be appropriately utilized to transfer into elite rice lines for stable yield production and growth under drought stress conditions. Merchuk-Ovnat et al. [103] reported three QTLs for yield and biomass in RILs identified from a cross of wild emmer wheat (*Triticum turgidum ssp.*) and durum (*T. turgidum ssp.*) and bread wheat (*T. aestivum*) on chromosomes 1BL, 2BS and 7AS where wild emmer wheat used as source of drought resistance genotype. Li et al. (104) evaluated 5000 inbred lines of maize from a 30 joint linkage association mapping populations using 365 SNPs for genome-wide association, and these SNPs related with drought resistance traits, observed in 354 candidate genes in which Fifty-two of these genes indicated significant differential expression in the inbred line B73 under water-stressed conditions.

4.4 Genetic engineering for drought tolerance in plants

Genetic engineering techniques are one of the important tools to produce plants with enhanced abiotic stress tolerance. Drought tolerance is predominantly controlled by various genes as well as numerous physiological and molecular mechanisms that lead to upregulation of the expression of a number of genes in plants, which allow them to survive and growth in stress conditions [105]. Genes which response to drought provide a presumed postulation of the stress signal network for amplification and increasing the tolerance of plants to various abiotic stresses [106]. The technology of genetic transformation enabled scientists to attain the transfer of a gene from one organism to others in a precise manner. That is why scientist concern on the plant transformation to improve abiotic stress tolerance by operating the osmoprotectant channel by narrowing the lipid peroxidation, ROS scavenging and others [107].

Multiply genes regulating the response of Pima cotton (*G. barbadense*) under stressed environments have been selected from cDNA libraries [108]. These desired traits were transferred to upland cotton (*G. hirsutum*) through transgenic techniques. The transfer of the target genes from *Tellungiella halophila* overexpressing TsVP, H⁺-PPase into cotton, enhanced the overall growth and development of cotton plants than wild type. The transgenic line indicated better leaf chlorophyll level, photosynthesis efficiency, water content, and cellular thermos-stability. The transferred gene allows transgenic cotton to produce 51% more seed cotton than the wild type cotton [109]. Fan et al. [110] overexpressed a *CmWRKY1* transcription factor from *Chrysanthemum morifolium* in the chrysanthemum cultivar "Jinba." It showed that *CmWRKY1* regulates an ABA-mediated pathway by reducing the expression levels of various genes such as, *PP2C*, *ABII* and *ABI2* which in turn increases the expression of genes such as, *PYL2*, *SnRK2-2*, *ABF4*, *MYB2*, *RAB18* and *DREB1A* in favorable manner. In another work, overexpression of *MusaWRKY71* gene, a drought stress responsive *WRKY TF* gene cloned from *Musa* species Karibale Monthan, was found to provide various abiotic stress tolerance in banana [111]. Cheng et al. [112] transformed *choline oxidase* gene (*CodA*) obtained from *Arthrobacter globiformis*, which used in the synthesis of glycine betaine, into potato cv "Superior" under an oxidative stress-inducible *SWAP2* promoter to enhance drought stress tolerant. The results indicated that transgenic potato plants showed expression of *codA* gene and an accumulation of glycine betaine with a higher leaf water content compared to the nontransformed potato crop under water stress condition. Transgenic development efforts done in various crops for imparting drought stress resistance have been summarized in Table 1.

Table 1: Involvement of genes derived from various crops and their role in abiotic stress tolerance

Gene	Source of gene	Product of gene	Target plant	Target stress	Reference
ABP9	<i>Zea mays</i>	Transcription factor	<i>Arabidopsis thaliana</i>	drought and salt tolerance	[72]
Amyb2A	<i>Triticum aestivum</i>	Transcription factor	<i>Arabidopsis thaliana</i>	drought, salt and cold tolerance	[113]
HvCBF4	<i>Hordeum vulgare</i>	CBF transcription factor	Rice	drought and salinity tolerance	[114]
AtNHX1	<i>Arabidopsis</i>	Vacuolar Na ⁺ /H ⁺ antiporter	Groundnut	drought and salt tolerance	[115]
OsCIPK23m	<i>Oryza sativa</i>	CIPK	<i>Oryza sativa</i>	Drought	[116]
BdCIPK31	<i>Brachypodium distachyon</i>	CIPK	<i>Nicotiana tabacum</i>	Drought Salinity	[72]
GmCBL1	<i>Glycin max</i>	CBL protein	<i>Arabidopsis thaliana</i>	Drought Salinity	[117]
VrDREB2A	<i>Vigna radiata</i>	AP2-ERFBP	<i>Arabidopsis thaliana</i>	Drought Salinity	[118]
PtrABF	<i>Poncirus trifoliata</i>	bZIP	<i>Nicotiana tabacum</i>	Drought	[73]
TaNAC47	<i>Triticum aestivum</i>	NAC protein	<i>Arabidopsis thaliana</i>	Salt Cold Drought	[119]
ABF3	<i>Talaromyces purpureogenus</i>	Transcription factor	<i>Arabidopsis thaliana</i>	Drought	[120]

ROB5	Bromegrass	Late embryogenesis abundant proteins	<i>Solanum tuberosum</i>	Drought, cold & Salinity	[121]
Hva1	<i>Hordeum vulgare</i>	Late embryogenesis abundant proteins	<i>Morus indica</i>	Drought & Salinity	[122]
GhPIP27	<i>Gossypium hirsutum</i>	Aquaporin (plasma membrane intrinsic protein)	<i>Arabidopsis thaliana</i>	Drought	[82]
OsCIPK23	<i>Oryza sativa</i>	CIPK	<i>Oryza sativa</i>	Drought	[116]
GmCBL1	<i>Glycine max</i>	CBL protein	<i>Arabidopsis thaliana</i>	Drought & Salinity	[116]
GHSP26	<i>Gossypium arboreum</i>	Heat shock	<i>Gossypium hirsutum</i>	Drought	[123]

4.5 Genome editing for drought tolerance

Improvement of abiotic stress tolerance in plants through targeted gene editing with the advent of high-throughput technologies and its beneficiary use in agriculture are set as prime tasks by plant breeders at present. In recent years, a novel gene editing method known as CRISPR/cas technology has come in front with intense popularity for its versatile nature. It is a useful method for the efficient modification with deletion, addition, or replacement of small regions of DNA of interest. Clustered regularly interspaced short palindromic repeats (CRISPR), a family of DNA sequence found in the genomes of prokaryotic organism derived from bacteriophages which infected the prokaryote [124]. The endonuclease “Cas9” acts like “molecular scissors” and cuts DNA double strands at the specific site as directed by a guide RNA. Here, the gene knockout or substitution is carried out by the organism itself with desired traits, unlike the transgenic technique where a foreign gene is introduced into the original organism to incorporate new traits [125].

CRISPR/Cas9 is an emerging gene editing technique that uses the cas9 protein in conjunction with guide RNA from a complex to recognize target DNA sequences. The target DNA is cut by the Cas9 protein which consists of six domains (REC1, REC2, Bridge Helix, HNH, RuvC, and PAM) interacting. Nowadays, the CRISPR/cas9 technology has been used extensively in several major crops like maize, soybean, wheat, and sorghum [126]. By using CRISPR/Cas9, numerous genes in rice crop such as OsHAK1, OsERF922, OsPDS, TMS5, and Badh2 have been knocked out, and the results of the phenotype have been identified [127]. Some reported on elite rice showed direct gene editing of the genotype using the CRISPR/Cas9 technique. The gene OsERF922, which is an ERF transcription factor, was edited to enhance blast resistance in variety Kuiku131 having a normal phenotype [79]. Japonica rice cultivar WPB106 was resistant to drought stress and early maturity, although it was sensitive to salt stress. Its tolerance to salt has been improved by using Cas9-OsRR22-gRNA expression vector which knocks out the OsRR22 gene. In another study in upland cotton, a CRISPR/Cas9-mediated pooled sgRNA assembly was employed, providing a platform for sgRNA design for multiple target genes. The targeted genes, which were linked to male sterility in cotton, were successfully edited using the CRISPR/Cas9 technique. A total of 112 plant growth-related genes were knocked out using CRISPR/Cas9 gene editing system ([128]. Chen et al. [129]) successfully generated high-oleic and non-transgenic mutants in allotetraploid upland cotton by knockout of GhFAD2 gene via gene editing techniques. It suggested that GhFAD2-1A/D is an important gene which determines the fatty acid composition of cotton seed oil.

Paixao et al. [130] exploited a genetic trait ABRE1, to enhance drought stress in Arabidopsis as the abscisic acid responsive element binding protein 1/ABRE binding factor 2(ABRE1/ABF2) plays an important role in regulating drought stress tolerance. Catalytically inactive Cas9 (dCas9) was associated with HAT or histone acetyltransferase (correlated with gene expression) in this study. Application of CRISPR/dCas9^{HAT} in *Arabidopsis thaliana* has shown improved drought stress tolerance through the positive regulation of ABRE1 [130]. Osakabe et al. [131] utilized CRISPR/Cas9 in Arabidopsis to introduce novel alleles of OST2 that play an important role in stomatal movement, using co-expression of Cas9 and GFP for the improvement of water stress tolerance. High expression of OST2 in germ line cells with changed stress responses in Arabidopsis has indicated inheritable stress tolerance enhancement in that plant [131]. Genome editing efforts done in various crops for combating drought stress have been summarized in Table 2.

Table 2: CRISPR/Cas9 gene editing in plants for improving abiotic stress tolerance.

Name of the plants	Target gene	Abiotic stress	References
<i>Arabidopsis thaliana</i>	ABRE1	drought tolerance	[130]
<i>Heynh. (Brassicaceae)</i>	OST2	drought tolerance	[131]
<i>Zea mays L. (Poaceae)</i>	ARGOS8	drought tolerance	[132]
<i>Heynh. (Brassicaceae)</i>	OST2/AHA1	drought tolerance	[126]
Rice	OsSRL1	drought tolerance	[133]
Rice	OsmiR535	drought tolerance	[134]
Rice	DST	drought tolerance	[135]
Tomato	SINPR1	drought tolerance	[136]
<i>Arabidopsis thaliana</i>	AREB1	drought tolerance	[130]
Tomato	SIMAPK3	drought tolerance	[79]
<i>Arabidopsis thaliana</i>	MIR169a	drought tolerance	[137]
<i>Arabidopsis thaliana</i>	UGT79B3	drought tolerance	[137]

5. CONCLUSIVE REMARKS AND PROSPECTS

With the rapid growth of the population, climate change and drought stress are predicted to be increased in the next years, once again challenging our ability to feed the world. Therefore, there is an international interest in increasing yield and plant drought tolerance due to the severe decrease in crop production in the last few decades of drought. Drought response is a complex process that triggers many changes from morphological to molecular level. The morph-physiological mechanisms include changes in leaf anatomical structure, root architecture, and stomatal regulation. The biochemical processes are changes in phytohormonal levels such as ABA, JA, Auxins, Ethylene, and Cytokinin. The phytohormone level of plants increases upon drought stimulus and leads to the activation of morph-physiological and other biochemical pathways. These signaling pathways may include MAPK signaling, calcium signaling, regulation of transcription factors, and increased levels of antioxidant enzymes, to escape the effect of drought stress on plant species. On the other hand, scientists are also trying to further improve these mechanisms via exogenous application of substances, plant breeding, and transgenic approaches. Therefore, to be prepared for the upcoming food crisis, high yielding drought tolerant crops should be developed via integrating these approaches.

Moreover, new emerging breeding techniques and biotechnologies such as genetic engineering, marker-assisted breeding (MAB), double haploid production (DH), and gene editing technologies, particularly, CRISPR/Cas9 serve as techniques for dissecting the mechanism of breeding in plants that give an exciting outcomes to engineer plants for the search of novel drought resistance gene. In this regard, various technologies are available to determine the high yielding drought tolerant mechanisms and subsequently improve these processes. New technologies containing gene editing (CRISPR), microarrays, genomics, transcriptomics, metabolomics, next generation sequencing, and RT-PCR will enable scientists to understand and improve drought tolerance in plants.

Abbreviations

CRISPR: clustered regularly interspaced short palindromic repeat; *ABA*: abscisic acid; *SnRK2*: sucrose non-fermenting 1-linked protein kinase 2; *PP2C*: protein phosphatase 2C; O_2^- : superoxide anion radical; H_2O_2 : hydrogen peroxide; 1O_2 : singlet oxygen; *CAT*: catalyze; *SOD*: super oxide dismutase; *POD*: peroxidase; *SA*: salicylic acid; *gRNA*: guide RNA; *ROS*: reactive oxygen species; *ROI*: active reactive oxygen intermediates; $HO\cdot$: hydroxyl radical

Data Availability

All data are included in the manuscript.

Conflict of Interest

The authors confirm that this article content has no conflict of interest.

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