

General mechanisms of drought response and their application in drought resistance improvement in plants

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Abstract Plants often encounter unfavorable environmental conditions because of their sessile lifestyle. These adverse factors greatly affect the geographic distribution of plants, as well as their growth and productivity. Drought stress is one of the premier limitations to global agricultural production due to the complexity of the water-limiting environment and changing climate. Plants have evolved a series of mechanisms at the morphological, physiological, biochemical, cellular, and molecular levels to overcome water deficit or drought stress conditions. The drought resistance of plants can be divided into four basic types—drought avoidance, drought tolerance, drought escape, and drought recovery. Various drought-related traits, including root traits, leaf traits, osmotic adjustment capabilities, water potential, ABA content, and stability of the cell membrane, have been used as indicators to evaluate the drought resistance of plants. In the last decade, scientists have investigated the genetic and molecular mechanisms of drought resistance to enhance the drought resistance of various crops, and significant progress has been made with regard to drought avoidance and drought tolerance. With increasing knowledge to comprehensively decipher the complicated mechanisms of drought resistance in model plants, it still remains an enormous challenge to develop

water-saving and drought-resistant crops to cope with the water shortage and increasing demand for food production in the future.

Keywords Drought resistance breeding · Drought tolerance · Drought avoidance · Gene function · Genetic engineering

Introduction

The growth, development, and reproduction of plants require sufficient water. Approximately, one-third of the Earth's land area is arid and semi-arid, while periodically unexpected climatic droughts often occur in most of the other land areas. Water scarcity can be lethal to plants and lead to enormous social problems and economic losses. The development of the modern science and technology revolution, on one hand, has largely increased our capabilities in exploring the natural resources which have dramatically improved human life; on the other hand, the continuously growing world population, together with widespread water pollution and unpredictable climatic change, further aggravates the shortage of water resources [1–3]. It is estimated that total global water consumption has tripled over the last 50 years according to the United Nation Water Development Report (WWDR-3; <http://www.unesco.org/new/en/natural-sciences/environment/water/wwap/wwdr/wwdr3-2009/>), and the agricultural water demand which accounts for more than two-thirds of the global water use dramatically increased in the past few decades (<http://ceowatermandate.org/business-case/global-water-trends/increasing-water-demand/>) [4–6]. Drought not only inflicts heavy agricultural production losses, but also contributes to ecological damage, land desertification, and

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soil erosion. Therefore, water scarcity has been considered as an urgent global and environmental problem.

Water shortage has attracted great concern and stimulated more and more research inputs on the fundamental science of the drought resistance of plants and the application of the acquired knowledge for developing water-saving and drought-resistant crops. In recent years, a number of countries and international organizations have launched research projects on exploring the drought-resistance and water-saving mechanisms of plants to identify key genes or tools for the improvement of plant drought resistance. The Consultative Group on International Agricultural Research (CGIAR) organized the Generation Challenge Programme (GCP) as a time-bound 10-year programme in 2003 to improve crop breeding for drought-prone conditions (<http://www.generationcp.org>). The CGIAR has initiated programs for reducing rural poverty, improving human nutrition and health, increasing global food security, and sustainably managing of natural resources through agricultural research (<http://www.cgiar.org/our-research/>). Early in 1970s, the CGIAR was well-known for the contribution to the Green Revolution [7]. In recent years, CGIAR scientists integrated the progress in multiple disciplines (genome sequencing of major crop species, functional genomics, genetic markers, and genetic engineering, etc.) and application of information and communication technologies (ICTs) in their research [8]. They also adopted open source approaches for accelerating crop improvement research and increased the application of improved varieties of important crops such as rice, wheat, maize, sorghum, millet, potatoes, and so forth [8, 9]. In 2005, European and African countries initiated the Improving Water Use Efficiency in Mediterranean agriculture (WUEMED) project for technical improvements in the field of agriculture and the management of water resources under drought conditions (<http://www.distagenomics.unibo.it/wuemed/index.html>). Many countries including China have also provided special funds to support the basic study and genetic improvement of drought-resistance in crops.

For more than two decades, scientists have conducted vast amounts of research, including studies on morphological traits, and the structural, physiological, biochemical, and molecular regulation of above-/below-ground parts, to unravel the mechanisms of drought responses in plants. In recent years, with the rapid development of the theory and technology of modern biology (such as molecular genetics, genomics, proteomics, and metabolomics), researchers have made great strides in elucidating the biochemical, genetic, and signaling networks involved in plant drought responses, however, the underlying sophisticated mechanisms that differentiate resistance from susceptibility within a species, especially

for crops, remain largely unclear. When plants are confronted with drought or more commonly water deficit, they respond to the stress by integrating very diverse responses and adaptive mechanisms at the morphological, physiological, and molecular levels, but different plant species or genotypes within a species often have large variations in the utilization of these mechanisms. At the early stage of water deficit, plants usually have an ability to absorb water from the underground efficiently through root system, partially close stomata to reduce water loss from transpiration, and alter the metabolism to match with the available carbon resource [10, 11]. As stress conditions increase, some osmolytes such as prolines, soluble sugars, spermines, and betaine accumulate in plant cells to maintain the cell turgor pressure [12]. Variations in the activities of numerous oxidation-protective enzymes such superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), and glutathione reductase (GR) are also frequently observed in drought-stressed plants [10, 13]. More general responses of plants to drought stress include the altered expression of numerous genes, such as those related to stress signal transduction, and the transcription and regulation of thousands of functional proteins, which collectively contribute to the molecular control of drought resistance.

In this review, we mainly focus on the major morphological and physiological traits associated with the mechanisms underlying drought resistance in plants, and then we briefly summarize the advances in characterization of the effective genes for drought response in plants. Finally, we provide an overview on the progress on drought resistance improvement in crops. This review aims to provide general mechanisms of plant drought response and their potential application in drought resistance breeding to a broad spectrum of scientific communities, especially for plant biologists and breeders who are not working in this field.

Dissecting drought resistance in plants

With respect to adaptation to water-deficit, plants can be roughly divided into three types: xerophytes (mainly in arid areas), mesophytes (mainly in semi-arid and sub-humid areas), and hydrophytes which are mainly distributed in environments with sufficient moisture or water [14]. The majority of model plants and crops belong to the mesophytes. Meteorologically, drought refers to the condition caused by a sufficiently long period of dry weather which results in plant injury [15, 16]. May and Milthorpe defined drought resistance as “the ability of plants to grow satisfactorily when exposed to water deficits” [17, 18]. Later, crop breeders defined drought resistance in terms of

“relative yield of genotypes” or “the ability of a crop plant to produce its economic product with minimum loss in a water-deficit environment relative to the water-constraint-free management” [16, 19]. Plants have evolved several different types of drought resistance strategies which allow them to adapt to specific habitats for the benefit of their growth and development. Drought resistance is defined as the integrated capability of plants in response and adaptation to the harsh environment caused by drought stress conditions. This capability is a sophisticated trait and is related to the adaptations at different levels, ranging from plant morphology and anatomical structures to physiological and biochemical reactions [20].

Plant drought resistance involves four major mechanisms: drought avoidance (DA) (or “shoot dehydration avoidance” in some literature), drought tolerance (DT), drought escape (DE), and drought recovery [15, 21–26]. Among the four components of drought resistance, DA and DT are the two major mechanisms for drought resistance conferred by plants [25]. DA is the capability of plants to maintain fundamental normal physiological processes under mild or moderate drought stress conditions by adjusting certain morphological structures or growth rates to avoid the negative effects caused by drought stress [23, 27, 28]. DA is principally characterized by the maintenance of high plant water potentials in the presence of a water shortage [16, 23]. Plants generally adopt three strategies to accomplish DA: (1) reducing water loss via rapid stomatal closure, leaf rolling [29], and increasing wax accumulation on the leaf surface in many plant species such as alfalfa, tobacco, and rice [30–32]; (2) enhancing the water uptake ability through a well-developed root system (especially increased rooting depth, rooting density or root/shoot ratio) and enhancing the water storage abilities in specific organs (such as fleshy water-storing tissues of cacti, the trunk of candlenut, earthnuts or tubers of some plants, etc.) [29, 33, 34]; (3) accelerating or decelerating the conversion from vegetative growth to reproductive growth to avoid complete abortion at the severe drought stress stage [16, 23]. DT refers to the ability of plants to sustain a certain level of physiological activities under severe drought stress conditions through the regulation of thousands of genes and series of metabolic pathways to reduce or repair the resulting stress damage [16, 23, 35]. Plants commonly exert protoplasmic tolerance by increasing osmoregulatory molecules in the cells to maintain the cell turgor pressure, and adjusting the activities of cell defense enzymes to reduce the accumulation of hazardous substances. The term DE refers to natural or artificial adjustment of the growth period, life cycle, or planting time of plants to prevent the growing season from encountering local seasonal or climatic drought [16, 36]. Farmers usually choose crop varieties with short life cycles which complete their life

cycle by avoiding the seasonal drought stress in agricultural production. Drought recovery refers to the plant capability to resume growth and gain yield (for crops) after exposure to severe drought stress which causes a complete loss of turgor pressure and leaf dehydration [23].

The drought resistance of plants is quite complex. For a given plant species, plants often combine different categories of mechanisms to confer drought resistance at different developmental stages. At a particular developmental stage, plant drought resistance is associated with a series of events (such as stomatal movement, photosynthesis, cell osmotic regulation, synthesis of protective macromolecules and antioxidants, etc.) in every conceivable facet at the morphological, physiological, and molecular levels. In addition, natural drought stress is dynamic and unpredictable. Therefore, it is rather difficult to comprehensively and accurately evaluate the overall drought resistance of a given plant species. Levit [22] pointed out that the determination of drought resistance is much more difficult than that of other stress resistances. Researchers often use a specific trait or several combined indicators to assess plant drought resistance depending on the purposes of their study. These indicator traits can be generally classified into three types: DA-related, DT-related, and integrative indicators [11, 23].

The indicators associated with DA are usually related to the moisture maintenance and water uptake and use efficiency. DT-associated indicators mainly cover physiological parameters related to osmotic adjustment (OA) (such as osmotic potential, proline content, soluble sugar content, ABA content, etc.) and alleviation of drought damage (such as the activities of protective enzymes and chlorophyll content, etc.). Some complex traits relevant to biomass or economic yield under stress conditions are also used to evaluate the drought resistance of crops in agricultural production. These traits include fresh or dry weight, survival rate, stay-green capability, seed-setting rate, spikelet fertility, grain weight, and so on. These traits are more meaningful and effective in breeding for drought resistance, although they have seldom been used to unveil the mechanisms of drought resistance at the physiological and molecular levels.

Drought avoidance-associated mechanisms

Modulation of root system architecture

Plants constantly obtain water (and nutrients as well) from the soil through their roots. Hence, the root system plays a critical role in response to water deficit stress. Some plants have the robust ability to increase root growth at the early stage of drought stress to absorb the water in deep soil [11].

A positive correlation has been found between penetration ability of roots and the degree of drought resistance in *Phaseolus acutifolius* [37]. The length, weight, volume, and density of plant roots were also reported to be associated with the drought resistance in crops [38–41]. Nevertheless, other research showed a lack of perceptible association between root traits with single plant and plot yield under reproductive stage stress in rice [42]. In dry areas, woody plant seedlings have vertical roots with ten times the length of the above ground height [43]. With this extensive root system and rooting depth, plants are able to maintain a higher water potential and a longer duration of transpiration under drought conditions, which provides further advantages for their growth and development [44]. Rooting depth, volume, and distribution are mainly influenced by the depth and range of soil moisture. In cases of soil water deficit, plants dynamically adapt and modify their root system architecture by changing their root growth in diverse manners depending on the species [45–47]. It is evident that severe soil water deficit can reduce root elongation, branching, and the formation of the cambium layer, and root tips of plants growing in arid soil become suberified [48]. Root growth is also influenced by the water or nutrient status of the aerial portion of the plant [49]. Increased root/shoot ratios are often observed under water stress conditions [50, 51]. For a long time, the root/shoot ratio has been used as a criterion to describe the plant capacity for drought resistance [52–55].

Leaf traits

The morphological and physiological responses of leaves to drought stress are crucial to reduce water loss and promote water use efficiency. When plants sense severe water deficiency, their leaves droop or roll because of the loss of cell turgor pressure and this phenomenon is called wilting [56]. High rates of transpiration temporarily induce an insufficient water supply, and some plants wilt around the middle of the day, while a decline in transpiration relieves the water deficit at night, and the rolled leaves slowly re-expand. Wilting is a passive movement of plant leaves to prevent excess water consumption under drought stress conditions. Apart from this, some plants can actively adjust the orientation of leaf blades to keep them parallel to the direction of incident solar radiation by rolling. The phototropic movement of plant leaves can regulate the interception of solar radiation [57, 58]. When the leaf blades expand in a direction perpendicular to the direction of solar radiation, the single blade receives the largest amount of radiation, while deviation from the vertical direction will reduce the amount of radiation. Upright leaves under water stress conditions will receive less radiation, resulting in reduced water loss and better overall

water status, indicating that erect leaves are an effective mechanism of DA [59–61]. Leaf rolling is a common response of plants to water deficit, and it is a mechanism to reduce water consumption when water stress is present [57]. Leaf rolling is a drought-adaptive trait induced by turgor pressure, and osmotic adjustment can delay leaf rolling [62]. Both passive and active leaf movements have a role in reducing incident solar radiation and thus reducing leaf surface temperature, protecting plants from excess water loss.

Plants with increased drought resistance often have xeromorphic structures such as smaller and thicker leaves, more epidermal trichomes, smaller and denser stomata, a thicker cuticle epidermis, thicker palisade tissue, a higher ratio of palisade to spongy parenchyma thickness, and a more developed vascular bundle sheath [63]. Leaf epidermal trichomes reduce plant transpiration under intense light conditions and help to reflect light [64]. Lipids accumulate in the epidermis to form wax and increase the reflectivity of sunlight to prevent plants from excessive transpiration and high leaf surface temperatures [65]. The fortified sclerenchymas (mechanical tissues) can reduce the damage from wilting and protect the plants from direct light irradiation [66]. Palisade tissues and vascular bundles ensure transportation and retention of water and nutrients [67]. These features effectively reduce excess water loss and enhance the water-holding ability to avoid damage from exposure to drought stress conditions.

Stomata are pores which formed in the leaves of terrestrial plants during a long-term evolutionary process. As the vital organs for exchanging gas and water between the plant and the external environment, stomata play critical roles in the activities of plant life by ensuring maximum absorption of CO₂ for photosynthesis, and meanwhile controlling the optimal transpiration. The stomatal density and aperture are closely related to the plant drought resistance [68]. Guard cells which in pairs surround the stomatal pores are extremely sensitive to environmental conditions. After receiving the environmental stimuli, changes of water potential and turgor movement in guard cells control the opening and closure of stomata, and further regulate pivotal physiological processes in plants such as transpiration and photosynthesis. Under water-limiting conditions, the function of stomata in adjusting transpiration is particularly important. Stomata of water-saving plants (which avoid dehydration by reducing transpiration) are sensitive to water deficit, and the leaf stomata close before the leaf water status approaches wilting, thereby exerting a DA function. Stomata respond to water stress mainly in two ways: (1) as a direct response to the air humidity in which guard cells and adjacent epidermal cells directly evaporate moisture to induce stomatal closure and prevent leaf water deficit, and (2) stomata respond to the water potential

changes in the leaves in which stomata close when the leaf water potential falls below a certain threshold.

Stomatal movement is controlled by osmotic potential changes in guard cells, and K^+ is one of the major ions affecting the osmotic potential in guard cells [69]. The influx or efflux of K^+ from the guard cells plays a critical role in changing the osmotic potential and the turgor pressure, which leads to the opening or closure of stomata [70]. The outward- and inward- K^+ channels on the plasma membrane of guard cells are vital to the transmembrane transport of K^+ . There are various specific proteins such as substrate-binding proteins (including ABA-binding proteins, acetylcholine receptors, GTP-binding proteins, and light receptors), pumps, and channels on the plasma membrane of guard cells which are involved in the control of stomatal movement [71–74]. These proteins are crucial for receiving and transducing stress signals in guard cells, and constitute the fundamental basis of the opening and closure of stomata under drought stress conditions. Furthermore, numerous studies suggest that stomatal movement is also controlled by the ABA signaling which is triggered by roots in drying soil profile [75, 76]. Stomatal closure is an important strategy to support water conservation by plants during drought stress. Thus, better understanding of the stomatal movement mechanisms is crucial for optimizing water use efficiency related to drought resistance in plants.

Besides, other leaf-associated traits such as epidermal hairs, cuticular wax, along with leaf water potential, relative water content, water loss rate, and canopy temperature are also used as criteria for appraisal of DA [11].

Photosynthesis

Plant production is mainly determined by photosynthesis, and plant photosynthesis is governed mainly by stomata for CO_2 /water exchange and photosynthetic activity in mesophyll cells. Water stress affects not only the light reactions, but also the assimilation efficiency of the dark reactions, thereby reducing the contents of the photosynthetic products [10, 77–79]. Plants have evolved three photosynthetic pathways including C_3 , C_4 , and crassulacean acid metabolism (CAM) to assimilate atmospheric CO_2 . Generally, plants utilizing C_4 and CAM photosynthetic mechanisms can better adapt to drought-prone climate [80]. C_3 plants open their stomata during the day for CO_2 absorption and fixation and close their stomata at night. This mechanism is deficient when C_3 plants confront water limitation because it does not retain moisture under drought stress conditions. C_4 plants have evolved a metabolic pump to concentrate CO_2 in the bundle sheath cells, and perform the fixation of CO_2 in mesophyll cells and the bundle sheath cells separately [81]. This particular mechanism contributes to higher

water use efficiency than that of C_3 plants and provides more chance for C_4 plants to survive in arid areas [81]. In the CAM cycle photosynthetic pathway, plants open their stomata for CO_2 absorption and fixation at night, and close their stomata to reduce transpiration water loss during the day. Therefore, CAM metabolism can dramatically increase the water use efficiency and is proposed to be a plastic photosynthetic adaptation to extremely arid environments [82]. When challenged by water stress, some plants considered as facultative CAM species are capable of switching their photosynthetic pathway from the C_3 cycle to the CAM cycle mode [83, 84]. Researchers have found that the key enzyme in the CAM metabolic pathway, phosphoenolpyruvate carboxylase, is transcriptionally regulated by water stress conditions [85].

Drought tolerance-associated mechanisms

Osmotic adjustment

Plants accumulate a variety of organic and inorganic substances (such as sugars, polyols, amino acids, alkaloids, and inorganic ions) to increase their concentration in the cytochylema, reduce the osmotic potential, and improve cell water retention in response to water stress. This phenomenon is defined as osmotic adjustment (OA) [86, 87], a significant strategy for plant drought tolerance. OA has been documented to sustain cell structure and photosynthesis at low water potentials, and to delay leaf senescence and death and improve root growth as water deficits become severe [26]. Substances currently known to be involved in OA encompass several types of organic compounds such as mannitol, proline, glycine, betaine, trehalose, fructan, inositol, and inorganic ions [88–92]. These organic substances can regulate the plasma osmotic potential, and protect the enzymes and plasma membranes. In addition, changes in the ion and water channels control the export and import of ions and moisture for plant cells, which also contributes to OA. The inorganic ions mainly regulate the osmotic potential of the vacuole to maintain turgor pressure; however, a high concentration of inorganic ions is likely to cause metabolism disorders in plant cells. Hydration of the membrane and the surface layer of intracellular proteins are also important for stabilizing the structures of biological macromolecules. The OA substances are capable of stabilizing the surface-bound water of and sustaining the spatial structure of biological macromolecules [93–96].

Proline has very strong hydration abilities. Its hydrophobic part is able to bind to proteins while its hydrophilic part is able to bind to water molecules, allowing proteins to access more water to enhance their solubility and to

prevent protein denaturation from dehydration under osmotic stress conditions [97]. Proline acts not only as a cytoplasmic protective agent for enzymes and cell structure, but also for adjusting the redox potential and reducing cell acidity [98–100]. Trehalose is a reducing disaccharide. Under drought stress conditions, the intercellular trehalose content increases rapidly to block the transformation of the phospholipids bilayer membrane from the liquid crystal state to the solid state, and stabilize the structure of proteins, nucleic acids, and other biological macromolecules [88]. Betaine is a metabolic intermediate belonging to the water-soluble alkaloid quaternary ammonium compounds, and functions as one of the nontoxic OA substances in higher plants [101]. Betaine also helps to stabilize the structures and activities of photosynthesis, including protective enzymes, and helps in the maintenance of membrane integrity against the pervasive damage under drought stress conditions [102–104].

Late embryogenesis abundant (LEA) proteins, aquaporins (AQP), and molecular chaperones have also been demonstrated with crucial roles in OA in plant cells. LEA proteins are formed during the process of seed development. They are low-molecular weight proteins which are usually between 10 and 30 kDa, and consist of basic amino acids rich in lysine, glycine, and serine, and commonly lack cysteine and tyrosine residues [105]. LEAs are hydrophilic proteins with extremely high thermal stability, and they can remain in the aqueous state even under boiling conditions. LEA proteins can protect biological macromolecules, redirect intracellular water distribution, bind to inorganic ions to avoid the damage attributed to the accumulation of high concentrations of ions under drought stress conditions, prevent excessive dehydration of plant tissues, and control the expression of other genes by binding to nucleic acids [106]. Aquaporins (AQP) are channel proteins which are responsible for water transportation. AQPs form selective water transport channels in plant cells and regulate the rapid transmembrane transport of moisture during the processes of seed germination, cell elongation, stomatal movements, and abiotic stress responses [107, 108]. AQPs are divided into three groups according to their subcellular location: plasma-membrane-intrinsic proteins (PIPs), tonoplast-intrinsic proteins (TIPs), and nodulin-26-like major-intrinsic proteins (NLMs) [109]. AQPs mediate passive water transport along the osmotic pressure gradient inside and outside the membrane, and they are capable of regulating the moisture balance for the entire plant under drought stress conditions. This is achieved by maintaining the water potential balance between the xylem parenchyma cells and the transpiration current, regulating water transport across cells and tissues, as well as adjusting the cell turgor and volume [108]. Further characterization of key genes controlling the

biosynthesis and metabolism pathways of these OA substances may provide useful candidate genes for improving drought tolerance by transgenic approach.

Antioxidant defense systems

Oxidative stress commonly occurs along with drought stress. Antioxidant defense system is one of the drought response mechanisms. Aerobic metabolism which provides energy for plant growth and development is often accompanied by the generation of reactive oxygen species (ROS) as by-products such as $^1\text{O}_2$, H_2O_2 , O_2^- , and $\text{OH}\cdot$. Under normal circumstances, the intracellular generation and removal of ROS is under dynamic equilibrium. When plants suffer from exposure to drought stress conditions, the dynamic equilibrium is broken and the excessive accumulation of ROS injures cells, and the oxidative deterioration may ultimately lead to cell death [110]. The membrane phospholipids and fatty acids which are sensitive to the over-accumulation of ROS are damaged, resulting in the peroxidation of membrane lipids [111]. Under ROS stress, the spatial configurations of various membrane proteins or enzymes are disturbed, leading to increased membrane permeability and ion leakage, chlorophyll destruction, metabolism perturbations, and even severe injury or death of plants [112].

Plants produce ROS in chloroplasts, peroxisomes, mitochondria, endoplasmic reticulum, plasma membrane, and the cell wall due to the imbalance between the generation and utilization of electrons under drought stress conditions [113]. ROS attack the most sensitive biological macromolecules in plant cells to induce lipid peroxidation, protein carbonylation, and DNA damage, and impair their functions to result in a catastrophic cascade of events [114]. To protect cells against the deleterious effects of excessive ROS, plants have evolved a series of sophisticated enzymatic and non-enzymatic antioxidant defense mechanisms to maintain the homeostasis of the intracellular redox state. The protective enzymes include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase (GPX), glutathione reductase (GR), glutathione S-transferase (GST), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDAR), thioredoxin peroxidase (TPX), alternative oxidase (AOX), peroxiredoxin (PrxR/POD), etc. [115, 116]. SOD, the H_2O – H_2O cycle, the AsA–GSH cycle, GPX, and CAT cooperatively build up major ROS-scavenging pathways [113, 117, 118]. The balances among the SOD, APX, and CAT activities are pivotal to maintaining the H_2O_2 homeostasis in plants. The non-enzymatic antioxidant system is comprised of several reducing substances, such as ascorbic acid (AsA), glutathione (GSH), carotenoids (CAR), α -tocopherol (vitamin E),

cytochrome *f* (Cyt f), flavanones, anthocyanins, and so on [112, 115, 119]. To date, quite a few instructive cases have been reported to achieve enhanced drought tolerance by eliminating the excessive accumulation of ROS in cells or promoting the ROS-mediated signal transduction in plants [110, 120, 121]. Further characterization of genes controlling the antioxidant defense system may also provide useful candidate genes for improving drought tolerance by transgenic approach.

Phytohormones and chlorophyll content

Endogenous phytohormones are also responsive to environmental cues, and several phytohormones have indispensable roles in coordinating the responses to drought stress through rather complicated crosstalk mechanisms [122, 123]. ABA was regarded as the phytohormone which is most closely related to drought stress responses in plants. Soil water deficit is perceived as a distant signal by root cells which then triggers a huge increase in the *de novo* synthesis of ABA [124]. ABA is transported mainly to leaves as an intercellular messenger and recognized by guard cells which trigger stomatal closure via intracellular signal transduction, and weakening the metabolic activities related to plant growth [125–127]. The influences of ABA have multiple effects on the drought response, encompassing the regulation of stomatal closure, channel activities in guard cells, transcriptional levels of the calmodulin protein, and the expression of some ABA-responsive genes [128–131]. As a key chemical messenger of drought signals, ABA is located at the central position in the regulatory network of stomatal closure [132, 133]. ABA has also been shown to promote the synthesis and accumulation of proline by affecting the activity of pyrroline-5-carboxylate reductase (P5CR) and enhancing the synthetic activity of glutamoyl-phosphate [134–136]. The expression of Δ^1 -pyrroline-5-carboxylate synthetase (P5CS) can be induced by drought and ABA [137, 138]. ABA is also the most important signaling molecule for drought signal transduction. There are at least three relatively independent signal transduction pathways which function in drought stress responses in plants. Two pathways are ABA-dependent, and the other pathway is ABA-independent [139]. To date, ABA signaling in the drought response has been well-illustrated. Drought stress induces the biosynthesis of intracellular ABA which activates the corresponding transcription factors, and then promotes the expression of downstream drought-related genes [140].

Thylakoid membranes are the structural foundation for light absorption, transmission, and transformation in chloroplasts. Many types of pigment–protein complexes in charge of light absorption, transmission, and transformation are located on the thylakoid membranes. Under

drought stress conditions, the membrane system in plant cells including the membrane structure associated with photosynthesis is destroyed due to the deficit of moisture, nutrients, and energy, resulting in the disruption of physiological processes. These factors are likely to directly or indirectly affect the chlorophyll content. It has been reported that the chlorophyll content significantly declines with decreasing soil water content, and the decrease of total chlorophyll content is mainly due to the decrease of chlorophyll *a* [141–144]. The plants which can maintain higher chlorophyll contents under water stress conditions are considered to use light energy more efficiently, and therefore are thought to have increased drought resistance.

Molecular basis of drought resistance

With the growing knowledge of modern biology and power of biotechnical tools, the mystery of the molecular mechanism of plant drought resistance has been gradually unveiled in the past decade. The response of plants to drought stress is a complex process involving many genes and signaling pathways. The transcript abundance of a large number of genes with diverse functions alters under drought stress conditions [145]. The external drought stimuli are perceived and captured by sensors on the membrane which have not been well-characterized, and then the signals are passed down through multiple signal transduction pathways, resulting in the expression of drought-responsive genes and drought adaptation [146]. A variety of secondary messengers (such as Ca^{2+} , ROS, phosphoglycerol, ABA, and diacylglycerol) and transcriptional regulators play significant roles in various signal transmitting pathways [147]. Emerging evidence suggests that drought-inducible genes are under complex governance, including the transcriptional cascades. The expression products of drought-responsive genes are mainly divided into three categories: (1) the proteins involved in the signaling cascades and transcriptional regulation (such as protein kinase, protein phosphatase, and transcription factors); (2) functional proteins that protect the cellular membranes and other proteins (such as late embryogenesis abundant protein, antioxidants, and osmotin); and (3) proteins associated with the uptake and transport of water and ions (such as aquaporins and sugar transporters) [139, 146, 148, 149].

Much effort has been justifiably dedicated to identifying the key components in elucidating the genetic and molecular bases of drought resistance by quantitative trait locus (QTL) mapping and cloning, mutant screening, expression profiling, and verification of candidate genes. Many QTLs for root traits, leaf traits, and physiological traits related to drought resistance have been mapped, and some of them

have been validated for plant performance under drought stress conditions [11]. The research has largely accelerated the progress of understanding the complexity of genetic and environmental interactions under drought stress conditions, and provided valuable references for drought resistance improvement. Meanwhile, thousands of genes responsive to drought stress have been identified by Affymetrix GeneChip technology and RNA sequencing [150–153].

Numerous candidate genes derived from the mutant screening or expression profiling studies have been further characterized for their functions in drought response. Regulatory proteins have been proven to play crucial roles in the responses of plants to drought stress conditions. The phosphorylation and dephosphorylation of proteins in plants are common events induced by drought stress conditions. Several types of kinases such as calcium-dependent protein kinases (CDPKs), CBL (calciurein B-like) interacting protein kinase (CIPK), mitogen-activated protein kinases (MAPKs), and sucrose non-fermenting protein (SNF1)-related kinase 2 (SnRK2) have been reported to participate in drought response. In *Arabidopsis* the CDPK gene CPK10 was reported to mediate stomatal movement via the ABA and Ca^{2+} signaling pathways in response to drought stress conditions [154]. OsCDPK7 was shown to positively regulate drought and salt stress tolerance in rice [155]. OsCIPK23 was found to be induced by various abiotic stress conditions and phytohormones, and *OsCIPK23* RNAi suppression transgenic plants were more sensitive to drought stress conditions [156]. MAP kinase signaling cascades in plants, such as *OsMPK5* and the MAPK kinase kinase (MAPKKK) gene *DSM1* in rice, have also been implicated with roles in regulating drought resistance [157–159]. A SnRK2 gene *SnRK2C* is thought to confer drought tolerance by regulating the expression of stress-responsive genes in *Arabidopsis* [160]. Transcription factors are another indispensable group of regulatory proteins which modulate gene expression to respond to drought stress at the transcriptional level. Many members from diverse transcription factor families such as APETALA2/Ethylene-responsive element binding protein (AP2/EREBP), basic leucine zipper (bZIP), MYB, NAM-ATAF1/2-CUC2 (NAC), and zinc finger have been reported to be involved in the drought responses. An AP2/EREBP domain transcription factor SHN was reported to confer enhanced drought tolerance by activating wax biosynthesis to alter cuticle properties in *Arabidopsis* [161]. Some dehydration-responsive element-binding factors (DREB) with the AP2 domain such as OsDREBs and ARAG1 have also been reported to participate in the drought responses in rice [162, 163]. The ABA-responsive element-binding proteins/factors (AREBs/ABFs) belong to a subfamily of

bZIP transcription factors, and they are well-known for their roles in ABRE-dependent ABA signaling under drought stress conditions. Three members from the AREB/ABF subfamily, AREB1, AREB2, and ABF3, are up-regulated by ABA and their full activation requires ABA [164]. The *areb1 areb2 abf3* triple mutant displays enhanced ABA resistance and reduced drought tolerance, indicating that the three factors coordinately govern ABRE-dependent gene expression under water stress conditions [164]. Several rice bZIP proteins such as OsbZIP23 and the constitutive active form of OsbZIP46 were also characterized to have a high potential in drought resistance improvement in rice [165, 166]. Cominelli et al. [167] reported a R2R3-MYB gene *AtMYB60* which is specifically expressed in guard cells to modulate the physiological responses of stomata. The T-DNA insertion mutant *atmyb60-1* showed constitutively increased stomatal closure and was more resistant to water stress. *OsMYB2*, with a multiple stress-induced expression pattern, was found to be related to the increased tolerance to salt, cold, and dehydration stresses in transgenic plants by regulating the accumulated amount of H_2O_2 and malondialdehyde and the expression of genes encoding proline synthase and transporters in rice [168]. NAC transcription factors are involved in almost every aspect of plant activities throughout the entire plant life cycle. A stress-induced rice NAC gene *SNAC1* was proven to have high potential in engineering drought resistance improvement in rice, wheat, and cotton [169–171]. Over-expression of *OsNAC10* driven by a root-specific promoter *RCc3* remarkably enlarged the root diameter in transgenic rice and consequently resulted in enhanced drought tolerance at the reproductive stage, and improved grain yield in the field under both normal and drought stress conditions [172]. Zinc finger factors belong to another super family of transcription factors. Constitutive over-expression of a Cys₂/His₂ (C₂H₂)-type zinc finger protein encoding the *ZPT2-3* gene in transgenic petunia improved tolerance to dehydration stress [173]. Another zinc finger protein DST was documented to function as a negative regulator of drought and salt tolerance by controlling the genes involved in H_2O_2 -mediated stomatal movement in rice [174]. Reduced stomatal density and increased stomatal closure was observed in the *dst* mutant, and the mutant showed enhanced tolerance to both drought and salt stress conditions [174].

Phytohormones are documented to have different roles in the response to drought stress. Among the various phytohormones, ABA is the one that is most closely related to drought stress [76]. In recent years, the ABA biosynthesis and metabolic pathways in higher plants have been clarified through the biochemical and genetic analyses of ABA biosynthesis-deficient and ABA-insensitive mutants

[175, 176]. ABA is synthesized de novo from an indirect carotenoids (C40) pathway in which zeaxanthin epoxidase (ZEP), 9-*cis*-epoxycarotenoid dioxygenase (NCED), and ABA-aldehyde oxidase (AAO) are key enzymes for the ABA biosynthesis pathway in higher plants [177]. Notable progress has been made to improve drought tolerance in plants by manipulating the ABA biosynthesis and metabolism pathways. Over-expression of *AtZEP* in *Arabidopsis* led to more vigorous growth of transgenic plants under drought treatment conditions compared to wild-type plants [178]. Over-expression of the *AtNCED3* and *PvNCED1* genes resulted in significantly more accumulation of endogenous ABA in transgenic plants and enhanced drought resistance [179, 180]. The *LOS5/ABA3* gene encodes a molybdenum cofactor (Moco) sulfurase which produces a cofactor (the sulfurylated MoCo) of AAO, which functions in the final step of ABA biosynthesis in *Arabidopsis* [181]. A *los5* mutant showed more severe drought-induced damage compared to the wild-type, and over-expression of *AtLOS5* increased ABA accumulation and drought resistance in both transgenic tobacco and cotton [181–183]. Xiao et al. [184] overexpressed several well-characterized genes including *NCED2* and *LOS5* in rice for testing drought resistance under field conditions, and the results showed that some of these genes might be potential candidates for drought resistance breeding.

ROS generation and scavenging has been recognized to influence the drought resistance of plants [110, 185]. Some ROS genes have been utilized to engineer drought-resistant plants. Over-expression of a pea manganese superoxide dismutase gene (*MnSOD*) under the control of an oxidative stress-inducible promoter *SWPA2* in rice chloroplasts enhanced the drought tolerance of the transgenic rice [186]. Cytosolic *APX1* was revealed to play an important role in response to a combination of drought and heat stress conditions [187]. *ATGPX3*, a gene encoding an *Arabidopsis thaliana* glutathione peroxidase, was found to act as a scavenger and an oxidative signal transducer in ABA and drought stress signaling, playing a distinctive role in H₂O₂ homeostasis [188]. Several other genes such as *OsSKIPa* and *OsSROIc* have been reported to modulate drought resistance by controlling ROS metabolism and regulating ROS homeostasis in plants [121, 189].

LEA proteins are expressed at specific stages of late embryonic development and play critical roles in desiccation tolerance by capturing water, stabilizing and protecting the structure and function of proteins and membranes, as well as acting as molecular chaperons and hydrophilic solutes to protect cells from the damage of water stress [190]. Xiao et al. [191] identified *OsLEA3-1* and tested the performance of transgenic rice over-expressing *OsLEA3-1* under drought stress conditions in

the field. The results indicated that the transgenic families exhibited higher grain yields than the wild-type under drought stress conditions. Transporter proteins have been demonstrated to function as regulators of drought resistance [192]. Transgenic maize co-transformed with *betA* and *TsVP* genes showed significantly improved drought resistance [193]. A guard cell plasma membrane ABCC-type ABC transporter gene *AtMRP4* was investigated for its role in stomatal aperture regulation in *Arabidopsis* [194]. The *atmrp4* mutant plants exhibited larger stomatal apertures than the wild-type plants, and consequently also exhibited reduced drought resistance [194]. AQPs are involved in OA and regulation of water movement across cellular membranes in plants. Transgenic rice expressing an AQP gene *RWC3* controlled by a stress-inducible *SWPA2* promoter displayed enhanced root osmotic hydraulic conductivity, leaf potential, and relative cumulative transpiration under PEG treatment [195]. Over-expression of another AQP gene *PgTIP1* increased biomass production and water-deficit tolerance in *Arabidopsis* [196].

Accumulation of osmoprotectants (such as trehalose, betaine, and proline) is a widespread adaptive strategy for plants to retain the water potential, cell turgor, and membrane stability to avoid drought stress-induced damage [197]. *TPS1* encodes trehalose-6-phosphate synthase, which is a key enzyme for trehalose biosynthesis in yeast. Yeo et al. [198] reported that constitutively over-expressing *TPS1* in potato significantly improved drought resistance. Over-expression of a betaine aldehyde dehydrogenase encoding gene *BADH* from spinach under the control of a stress-induced *rd29* promoter enhanced the tolerance of transgenic potato to drought and salt stress conditions [199]. *P5CS* functions as a rate-limiting enzyme in proline biosynthesis. Kishor et al. [200] successfully raised proline production/yield and enhanced osmotolerance in transgenic plants by over-expression of the mothbean *P5CS* gene in tobacco.

Plenty of other metabolic-related proteins have also been demonstrated to be closely associated with the drought response in plants. The rice *Drought-Induced Wax Accumulation 1 (DWA1)* and *Glossy 1 (GLI-2)* genes were proposed to be involved in leaf wax deposition, and both the *dwa1* and *osgli-2* mutants were impaired in cuticular wax accumulation under drought stress conditions and exhibited increased drought sensitivity [32, 201]. Ornithine δ -aminotransferase (δ -OAT) is a proline and arginine metabolism-related enzyme, and over-expression of *Os-OAT* which is a target gene of *SNAC2* resulted in improved resistance to drought, osmotic, and oxidative stresses, indicating that *OsOAT* conferred drought tolerance by enhancing pre-accumulation and ROS-scavenging in rice [202].

Genetic improvement of drought resistance in crops

Beside the importance of understanding the general mechanisms of drought response in plants, the exploration of genetic variations in diverse traits related to drought resistance within species is also important for genetic improvement of drought resistance in crops. Here, we briefly summarize some successful efforts or promising approaches related to drought resistance breeding in the last two decades. Delayed silking is a commonly observed phenomenon in maize when it is subjected to water stress at flowering stage [203]. The anthesis silking interval (ASI), a trait of intermediate heritability that generally exhibits a negative correlation with grain yield under drought conditions, provided a valuable and effective selection target for drought resistance in maize [204]. Root angle dramatically influences root distribution in the soil and is thus considered as a proxy for selecting deeper roots varieties to increase drought resistance [205]. Kato et al. [206] documented that root growth angle is useful for preliminary estimation of genotypic variation in root distribution in rice. Mace et al. [207] identified a putative association between root angle QTL and grain yield in sorghum. It has been demonstrated that modulation of the partitioning of metabolically important carbohydrates is a satisfying strategy for breeding drought-resistant cultivars in some crops [208, 209]. Storage and remobilisation of water soluble carbohydrates (WSC) in stems and leaves is an essential contributor to grain filling, especially under the water-limiting conditions [210]. Phenotype analysis was performed in three mapping populations for WSC mass per unit area (WSC-A) in wheat, indicating that WSC-A plays a significant role in assuring stable yield and grain size [211, 212]. Transpiration efficiency has been also reported to have a strong correlation with water use efficiency and yield under drought conditions, and therefore used as a selection criteria for drought resistance identification [213]. In maize, the stay-green trait was found to be closely related to grain yield, while in sorghum it is associated with maintenance of water status [214, 215]. Four QTLs which control stay-green have been mapped in sorghum [215]. Improved symbiotic nitrogen (N_2) fixation was evidenced with effect in increasing the soybean yield by enhancing dry-matter accumulation under drought [216]. Sinclair et al. [217] screened one hundred lines of filial generation derived from a cross between Jackson and KS4895, and identified two soybean lines with decreased sensitivity of N_2 fixation to water deficit and increased yield. In bermudagrass, rhizome production was proven to be an important trait which was closely linked to high productivity and drought resistance [218, 219]. Zhou et al. [219] comprehensively investigated the association between rhizomes and drought resistance by assessing eighteen

bermudagrass genotypes (*Cynodon* spp.) from four climatic zones under drought. Canopy temperature depression (CTD), which can be measured by thermal imaging, was indicated to be a significant predictor of yield performance under drought stress in bread wheat [220, 221], while GA sensitivity was claimed to be implicated in drought resistance in wheat [222]. Probing and establishment of the morpho-physiological criteria which could precisely assess the degree of drought resistance is a crucial aspect in studying drought resistance in plants.

Conventional drought resistance breeding largely depends on genetic variation of the DR-related traits in a specific species, and it is a labor-intensive and time-consuming process [223, 224]. Genetic transformation or engineering provides an alternative or complementary approach for developing desired traits more efficiently [224]. Numerous genes, as introduced in the previous section of this review and other reviews [11], have shown to be effective in improving drought resistance based on experiments mostly conducted in green house. However, compared to other simple traits, genetic improvement of drought resistance in crops by the transgenic approach is still facing many challenges. The understanding of the general molecular mechanisms of drought responses and the genetic variation in a given plant species is rather limited, which is a major obstacle for the molecular breeding of drought resistance and/or the selection of appropriate candidate genes for transformation. Furthermore, some other stresses such as high temperature are usually co-incident to drought stress. Therefore, it is foreseeable that the outcome of transferring one single gene to increase drought resistance is unsatisfactory in most instances. Present transgenic studies commonly use constitutive promoters including *CaMV35S*, *ubiquitin*, and *actin* to make overexpression constructs [191, 198, 225]. These promoters efficiently cause high expression levels of the target genes for improving drought resistance, but constitutive expression of some target genes frequently lead to adverse side effects on plant growth, development, or production under normal conditions, some of which are likely owing to the unnecessary consumption of energy or metabolic disturbance [226, 227].

To promote the application of transgenic technologies to drought resistance breeding, the following considerations may be taken into account in the future. First, multidisciplinary approaches may be adopted to decipher the elaborate genetic control of drought resistance mechanisms, which is also essential to unveil the genetic and molecular mechanisms of drought response and the cross-talk between different stresses. Second, compared to the single gene transformation, multi-gene transformation strategy that combines several major functional or regulatory genes or a series of genes in a signaling cascade

contributing to drought resistance may be more reasonable or promising for improving drought resistance in plants. Third, attention should be paid to exploiting and identifying suitable promoters which could ensure stable and accurate transcription of genes in an anticipated pattern under specific stress conditions [223]. Drought-inducible promoters generally maintain low expression levels of the driven genes under unstressed conditions and activate the genes under water scarcity to minimize the potential adverse effect on normal growth [227]. Using promoters with specific temporal-spatial pattern to control the location and time of expression should also be considered. Furthermore, combination of traditional breeding (such as cross and/or recurrent backcross of wild relatives and elite cultivars) and transgenic technology may provide a great potential to pyramid the desired traits especially for improving resistance to multiple stresses.

Conclusion and perspective

Drought resistance in plants is an extremely complex trait. Plants adopt a suite of strategies encompassing morphological, physiological, cellular, and molecular changes to survive under drought stress conditions. Different plants adapt to drought stress via diverse and integrated mechanisms. In recent years, researchers have identified numerous genes related to the drought response of plants, and have made substantial progress in the genetic improvement of drought resistance. However, our understanding of the regulatory networks and the crosstalk between the signaling pathways under drought-prone conditions is still fragmentary. Current knowledge on the molecular basis of drought resistance in plants is merely the tip of the iceberg. We have a long way to go to comprehensively and intensively elucidate the significant biological functions involved in drought response, and place them in their accurate locations in the refined regulatory network to obtain a clear picture.

Drought is a major constraint of agricultural development. It is an enormous challenge to integrate modern genetics, genomics, molecular biology, and proteomics, along with the metabolomics approaches to investigate the crosstalk and fill the vacancies in the regulatory networks of plant drought response, and better apply the fundamental theories and experience into practice for plant drought resistance breeding. Integration of the molecular approaches, morpho-physiological analysis, and conventional breeding strategies will substantially accelerate the progress of cultivating drought-resistant plants for agricultural production.

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