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MINI REVIEW

Molecular responses to drought stress in plants

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Abstract

Drought is a severe environmental constraint to plant productivity. Being a multidimensional stress, it triggers a wide variety of plant responses ranging from physiological, biochemical to molecular levels. One of the inevitable consequences of drought stress is an increase in reactive oxygen species (ROS) production in different cellular compartments, namely the chloroplasts and mitochondria. This enhanced ROS production is, however, kept under tight control by a versatile and cooperative antioxidant system that modulates intracellular ROS content and sets the redox-status of the cell. Furthermore, ROS production under stresses functions as an alarm signal that triggers defence or acclimation. Specific signal transduction pathways involve, *e.g.*, H_2O_2 as a secondary messenger. ROS signalling under drought is linked to abscisic acid (ABA) and Ca^{2+} fluxes. At molecular levels, several drought-responsive genes, transcription factors, aquaporins, late embryogenesis abundant proteins, heat shock proteins, and dehydrins have been identified. This review discusses recent understanding on molecular responses and protective mechanisms of drought stress.

Additional key words: abscisic acid, aquaporins, calcium, dehydrins, late embryogenesis abundant proteins, reactive oxygen species.

Introduction

Among the abiotic factors that have shaped and continue shaping plant evolution, water availability is the most important (Nelson *et al.* 2014). From agricultural point of view, drought is a period with below average precipitation, less frequent rain events, or above normal evaporation often resulting in a decrease in crop productivity and growth (Rollins *et al.* 2013). The severity of drought is unpredictable as it depends on many factors such as occurrence and distribution of rainfall, evaporative demands, and moisture storing capacity of soils (Hayes *et al.* 2010).

Drought affects plant growth, yield, water relations, membrane integrity, pigment content, and photosynthesis (Praba *et al.* 2009). It is characterized by a decrease in water content, leaf water potential and pressure potential, closure of stomata, and a decrease in cell enlargement and growth (Anjum *et al.* 2011). It reduces plant growth by affecting various physiological and biochemical processes such as photosynthesis, respiration, translocation, ion uptake, sugar and nutrient metabolism, and also phytohormones (Farooq *et al.* 2009, Prasad *et al.* 2011). Severe drought may result in the arrest of photosynthesis and disturbance of metabolism finally leading to the death of plant (Jaleel *et al.* 2008). However, sensitivity of plants to drought depends on a degree and duration of stress, plant species, and their developmental stages (Demirevska *et al.* 2009). Various drought-related traits, including root traits, leaf traits, osmotic adjustment capabilities, water potential, abscisic acid (ABA) content, and stability of the cell membranes, have been used as indicators to evaluate drought resistance of plants. In the last decade, scientists have investigated genetic and molecular mechanisms of drought resistance to enhance it in various crops, and significant progress has been made with regard to drought avoidance and drought tolerance (Ha *et al.* 2012).

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Abbreviations: ABA - abscisic acid; ALA - 5-aminolevulinic acid; AOX - alternative oxidase; AREB - ABA-responsive element binding; DREB - dehydration responsive element binding; Hsps - heat shock proteins; LEA - late embryogenesis abundant; PGPRs - plant growth promoting rhizobacteria; PS - photosystem; ROS - reactive oxygen species; Rubisco - ribulose-1,5-bisphosphate carboxylase; RWC - relative water content; ZFP - zinc finger protein.

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Various molecular networks including signal transduction are also involved in drought stress responses (Nishiyama *et al.* 2013, Osakabe *et al.* 2014). Thus elucidation of these networks is essential for understanding stress tolerance of crops.

Responses to drought stress

Drought is a multidimensional stress affecting plants at various levels of their organization, *i.e.*, from phenological and morphological to molecular levels (Anjum *et al.* 2011). Under severe water deficit, cell elongation is inhibited by interruption of water flow to elongating cells. Relative water content (RWC), leaf water potential, stomatal conductance, rate of transpiration, leaf temperature, and canopy temperature are important characteristics that influence plant water relations. A decrease in the RWC in response to drought stress is commonly observed in a variety of plant species (Ings *et al.* 2013). Although plant growth is generally reduced when soil water supply is limited, shoot growth is often more inhibited than root growth and, in some cases, root/shoot biomass ratio of plants in drying soil may increase relative to that of well-watered controls (Anithakumari *et al.* 2012). Drought stress induces reduction in leaf area as ascribed by suppression of leaf expansion and reduction in photosynthesis (Avramova *et al.* 2015). It also causes impaired mitosis, cell elongation and expansion resulting in reduced growth (Potopová *et al.* 2016). The deficiency of water leads to a severe decline in yield traits of crop plants probably by disrupting leaf gas exchange due to stomata closure, phloem loading, assimilate translocation, and dry matter partitioning (Farooq *et al.* 2009, Akram 2011). Drought reduces not only plant growth but also development, leading to hampered flower production and grain filling; thus, smaller and fewer grains finally results in severe yield losses.

In the majority of the plant species, drought is linked to changes in leaf anatomy and ultrastructure (Rollins *et al.* 2013). Generally, changes include shrinkage in the size of leaves, a decrease in the number of stomata, submersion of stomata in succulent plants and in xerophytes, thickening leaf cell walls, cutinization of leaf surface, under-development of the conductive system, but an increase in the number of large xylem vessels, leaf rolling in cereals, and induction of early senescence (Anjum *et al.* 2011). Gloucousness is another feature that conserves water content under water deficit by reducing transpiration (Farooq *et al.* 2009).

Drought also affects the structures not only at the tissue and cellular levels but also at the sub-cellular level. At the sub-cellular level, major modification refers to the shape of chloroplasts and vacuole, leading to swelling of stromal lamellae, that change the structural organization of thylakoids and form antenna depleted photosystem (PS) II which thereby reduces photosynthetic and respiratory activities (Siddiqui *et al.* 2015). Water stress inhibits chlorophyll synthesis at four consecutive stages: *1*) formation of 5-aminolevulinic acid (ALA); *2*) ALA conversion into porphobilinogen and primary tetrapyrrol, which is transformed into protochlorophyllide; *3*) light-dependent conversion of protochlorophyllide into chlorophyllide; and *4*) synthesis of chlorophylls *a* and *b* along with their inclusion into developing pigment-protein complexes of the photosynthetic apparatus (Kaewsuksaeng 2011, Ashraf and Harris 2013). It causes a large decline in chlorophyll *a*, chlorophyll *b* and total chlorophyll content in sunflower (Manivannan *et al.* 2007, Zlatev and Lidon 2012), cotton (Mssacci 2008), and *Catharanthus roseus* (Jaleel *et al.* 2008). Drought stress inhibits Chl synthesis and decreases the content of Chl-binding proteins, leading to a reduction of the light-harvesting pigment protein complex associated with PS II (Anjum *et al.* 2011, Kannan and Kulandaivelu 2011). This decrease in chlorophyll is mainly the result of damage to chloroplasts caused by reactive oxygen species (ROS) induced by drought stress (Ashraf and Harris 2013). Unlike chlorophyll, an increase in xanthophyll pigments such as zeaxanthin and antheraxanthin in plants under water stress has been reported (Batra *et al.* 2014). Xanthophylls have a protective role in plants under stress, and some of these pigments are involved in the xanthophyll cycle which has inhibitory role on ROS production (Brestič and Živčák 2013).

Water deficit leads to progressive suppression of photosynthesis by disrupting all major components including the thylakoid electron transport, the carbon reduction cycle, and the stomatal control of the CO_2 supply, together with an increased accumulation of sugars (Farooq *et al.* 2009, Carmo-Silva *et al.* 2012). Severe drought limits photosynthesis by declining the activity of ribulose-1,5-bisphosphate carboxylase (Rubisco). This decline in Rubisco activity is accompanied by a decrease in maximum rate of carboxylation, rate of Rubisco regeneration, stromal fructose bisphosphatase, and the quantum efficiency of PS II (Zhou *et al.* 2007, Carmo-Silva *et al.* 2012). In addition to Rubisco, drought also reduces the activity of other photosynthetic enzymes to different extents such as NADP-dependent glyceraldehyde phosphate dehydrogenase, phosphoenolpyruvate carboxylase, NAD-dependent malate dehydrogenase, phosphoribulose kinase, fructose-1,6-bisphosphatase, and sucrose phosphate synthase (Kondrak *et al.* 2012). Moreover, the activity of photosynthetic electron transport chain is finely tuned to the availability of CO_2 in the chloroplast and often there is a decline in PS II activity under drought conditions (Joliot and Johnson 2011, Suzuki *et al.* 2014). Besides its negative effects on dark reactions of photosynthesis, drought also disrupts the cyclic and non-cyclic types of electron transport during the light reaction of photosynthesis (Obidiegwu *et al.* 2015).

The ROS production, known as the oxidative burst, is an early event of plant defense response to water-stress and acts as a secondary messenger to trigger subsequent defense reaction in plants (Miller *et al.* 2010). Under drought stress, ROS production is enhanced through multiple ways (Fig. 1). During photosynthesis, there is a higher leakage of electrons to O_2 by the Mehler reaction (Driever and Baker 2011). Under drought, one of the real threats towards the chloroplast is the production of the hydroxyl radical in the thylakoids through the Fenton reaction (Kar 2011). The hydroxyl radical is the ROS which has the shortest half-life (~1 ms) but it also has an extremely strong oxidizing potential reacting with almost every biological molecule (Queval *et al.* 2012). Furthermore, there is no enzymatic reaction known to eliminate the highly reactive hydroxyl radical, and its accumulation inevitably leads to damage of the thylakoidal membranes and the photosynthetic apparatus. Also, closure of stomata decreases CO_2 concentration in leaf mesophyll tissue and results in an accumulation of NADPH. Under such conditions, where NADP is a limiting factor, oxygen acts as an alternate acceptor of electrons from the thylakoid electron transport chain, resulting in the formation of superoxide radical (O_2^-) (Fischer *et al.* 2013). Further downstream reactions produce other reactive oxygen species such as H_2O_2. These reactive oxygen species react with proteins, lipids and DNA, thus causing oxidative damage and impairing the normal functions of cells.

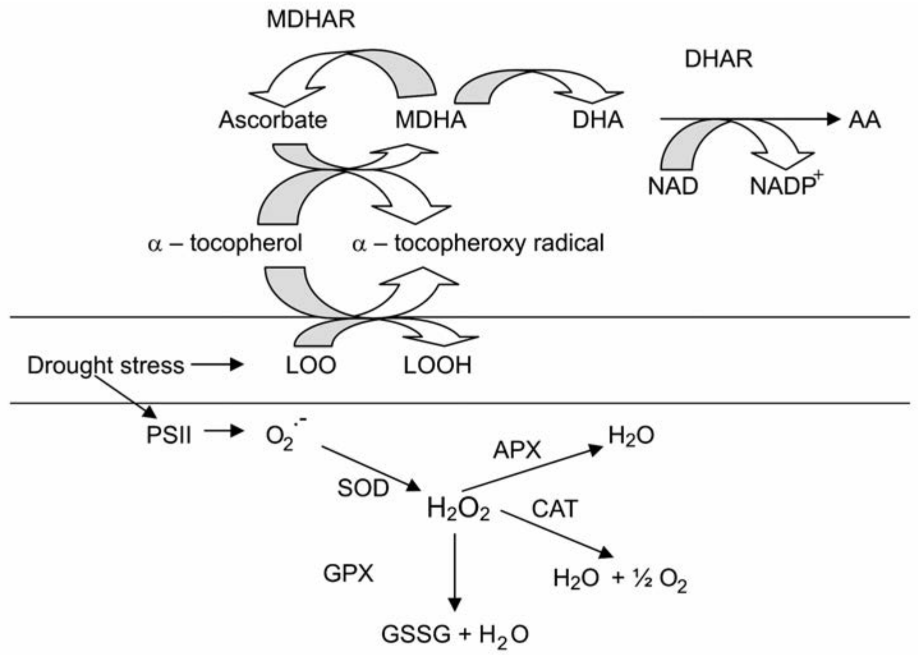

Fig. 1. Scavenging reactive oxygen species by enzymatic and non-enzymatic antioxidant systems. AA - ascorbate, APX - ascorbate peroxidase, CAT - catalase, DHA - dehydroascorbate, DHAR - dehydroascorbate reductase, GPX - glutathione peroxidase, GSSG - glutathione disulfide (oxidized), LOO - lipid peroxide radical, LOOH - lipid hydroperoxide, MDHA - monodehydroascorbate, MDHAR - monodehydroascorbate reductase, SOD - superoxide dismutase. Adapted from Gill and Tuteja (2010).

Drought resistance mechanisms

Osmotic adjustment has been considered as one of the vital processes in plant adaptation to drought as it helps sustaining the tissue metabolic activity. The osmotic compounds synthesized under drought include compatible solutes such as amino acids (proline, aspartic acid, and glutamic acid), glycine betaine, sugars (fructans and sucrose), and cyclitols (mannitol and pinitol). Of these, proline is one amongst the most important. Its accumulation in leaves under low water potential is caused by a combination of increased biosynthesis and slow oxidation in mitochondria (Hu *et al.* 2015). Many physiological roles have been assigned to proline including stabilization of macromolecules including enzymes and proteins, maintain membrane integrity, and scavenge reactive oxygen species (Verbruggen and Hermans 2008, Verslues and Sharma 2010, Kaur and Asthir 2015). Proline also acts as a source of energy, carbon, and nitrogen during post-drought recovery (Szabados and Savouré 2009). Transgenic plants overexpressing pyrroline-5-carboxylate synthase (P5CS) accumulate this osmoprotectant during drought and are thus resistant to osmotic stress (Khan *et al.* 2015).

Glycinebetaine, an amphoteric quaternary amine, also plays an important role as a compatible solute in plants under various stresses. Its protective role results either from a direct interaction with macromolecules or through formation of hydration shells around macromolecular complexes to prevent unfolding and denaturation (Giri 2011). Sugars have different functions in plants and plants use several sugar based strategies to adapt to environmental stresses (Krasensky and Jonak 2012). In several plants, polyols and cyclitols accumulate in leaves in response to water stress. Mannitol and sorbitol are the most frequent polyols found in plants. In some species, accumulation of these two polyols under drought may account for upto 80 % of the total solutes involved in the osmotic adjustment (Nezhadahmadi *et al.* 2013). Recent findings showed that trehalose plays a significant role in plant growth and development under drought stress (Nuccio *et al.* 2015). Heterologous expression of genes involved in the trehalose synthesis in *Escherichia coli* or *Saccharomyces cerevisiae* enhances tolerance to drought stress in several plant species (Iordachescu and Imai 2008). Over-expression of different isoforms of trehalose-6-phosphate synthase confers enhanced resistance to drought in rice (Li *et al.* 2011). Akram *et al.* (2016) highlights the role of trehalose in inducing drought tolerance in radish by enhancing the expression of superoxide dismutase and peroxidase.

In order to cope with continuous ROS production under stresses, plants have developed battery of enzymatic and non-enzymatic antioxidants that function as an extremely efficient cooperative system (Harba *et al.* 2015). The bulk literature showed an increased activity of the antioxidant enzymes under drought stress (Chugh *et al.* 2011, Chakraborty and Pradhan 2012, Marok *et al.* 2013). In a recent study, drought-tolerant barley genotypes exposed to progressive drought showed higher activities of catalase and superoxide dismutase than the drought-sensitive genotypes (Marok *et al.* 2013).

The avoidance of ROS production during drought stress is also an important strategy that enables plants to cope with water shortage without extensive damage. Mitochondria play an important role in the avoidance of ROS production by efficient energy dissipation mechanisms (Xu *et al.* 2011). The alternative oxidase (AOX) pathway is an alternative to the cytochrome pathway in the mitochondria that diverts electrons flowing through the electron transport chain to produce water by the reduction of O_2 (Fischer *et al.* 2013) (Fig. 2).

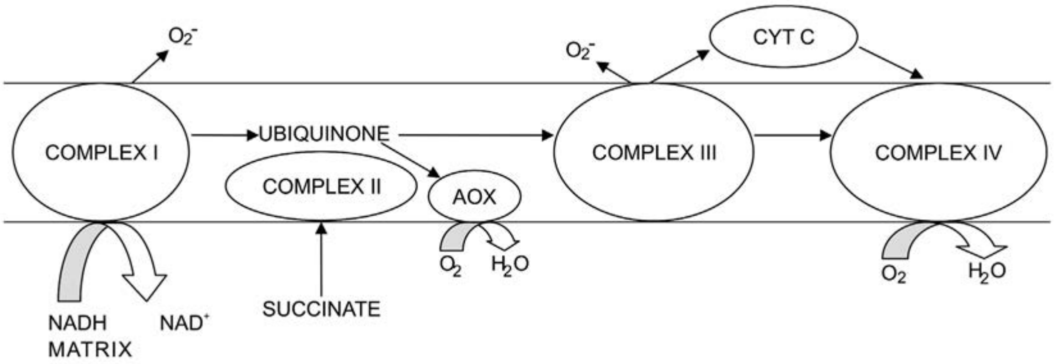

Fig. 2. Drought avoidance strategy of plants: alternative oxidase (AOX) diverts electrons that can be used to reduce O_2 into superoxide anion and uses these electrons to reduce O_2 to H_2O. CYT C - cytochrome C oxidase, NADH - nicotinamide adenine dinucleotide (reduced form). Adapted from Farooq *et al.* (2009).

Molecular mechanisms

The external drought stimuli are perceived and captured by sensors on the membrane which are still to be well-characterized, and then the signals are transmitted down through multiple signal transduction pathways, resulting in the expression of drought-responsive genes and drought adaptation. A variety of secondary messengers such as Ca^{2+}, ROS, ABA, phosphoglycerol, diacylglycerol, and transcriptional regulators play significant roles in various signal transmitting pathways (Fig. 3). Recent evidences suggest that drought-inducible genes are under complex governance including the transcriptional cascades (Todaka *et al.* 2015). The expression products of drought-responsive genes are mainly the proteins involved in the signalling cascades and transcriptional regulation (such as protein kinase, protein phosphatase, and transcription factors), functional proteins that protect the cellular membranes, and other proteins such as late embryogenesis abundant proteins, antioxidants, osmotin, proteins associated with the uptake and transport of water and ions such as aquaporins, and sugar transporters (Nakashima *et al.* 2014).

Drought triggers the production of ABA in roots which is transported to the shoots causing stomatal closure and eventually restricting growth (Mittler and Blumwald 2015). ABA is also synthesized in leaf cells and translocated around the plant. Recent evidence indicates that xylem/apoplastic pH influences ABA compartmentation and consequently the amount of ABA reaching stomata (Andrésa *et al.* 2014). In drought-stressed plants, alkaline pH in xylem/apoplast leads to a decrease in the removal of ABA from xylem and leaf apoplast to the symplast which is called as alkaline trapping of ABA, as a results that more ABA reaches the guard cells, enabling the modulation of stomatal aperture in response to a variety of environmental factors (Shatil-Cohen *et al.* 2011). Sugars translocated in the xylem of drought stressed plants likely exert an important influence on stomatal sensitivity to ABA (Le Gall *et al.* 2015).

Drought-responsive genes can be divided into two groups, ABA-dependent and ABA-independent, according to their dependency on ABA for induction (Kim 2014). Analyses of the expression patterns of genes induced by dehydration have revealed a broad variation in the timing of their induction and differences in their responsiveness to ABA. Among transcription factors depending on ABA, zinc finger proteins (bZIPs) are a large family with 75 members annotated in the *Arabidopsis* genome. Regarding water stress response, the ABA-responsive elements-binding (AREB) proteins respond at the transcriptional and post-transcriptional level to drought, increasing drought stress tolerance. ABRE is a major *cis*-acting element in ABA-responsive gene expression (Nakashima *et al.* 2014). Two ABRE motifs are important *cis*-acting elements controlling ABA-responsive expression of the *Arabidopsis* responsive to dehydration *(RD)29B* gene (Yang and Tan 2014). Induction of the drought-inducible *RD22* gene is mediated by ABA and requires protein biosynthesis for its ABA-dependent expression. A myelocytomatosis oncogene (MYC) transcription factor, AtMYC2 (RD22BP1), and a (MYB) transcription factor, AtMYB2, were shown to bind to *cis*-elements in the *RD22* promoter and co-operatively activate *RD22* (Singh and Laxmi 2015). The C-repeat/drought-responsive element binding factor (CBF4), a member of CBF/DREB1 family, was described as an ABA-dependent regulator of drought

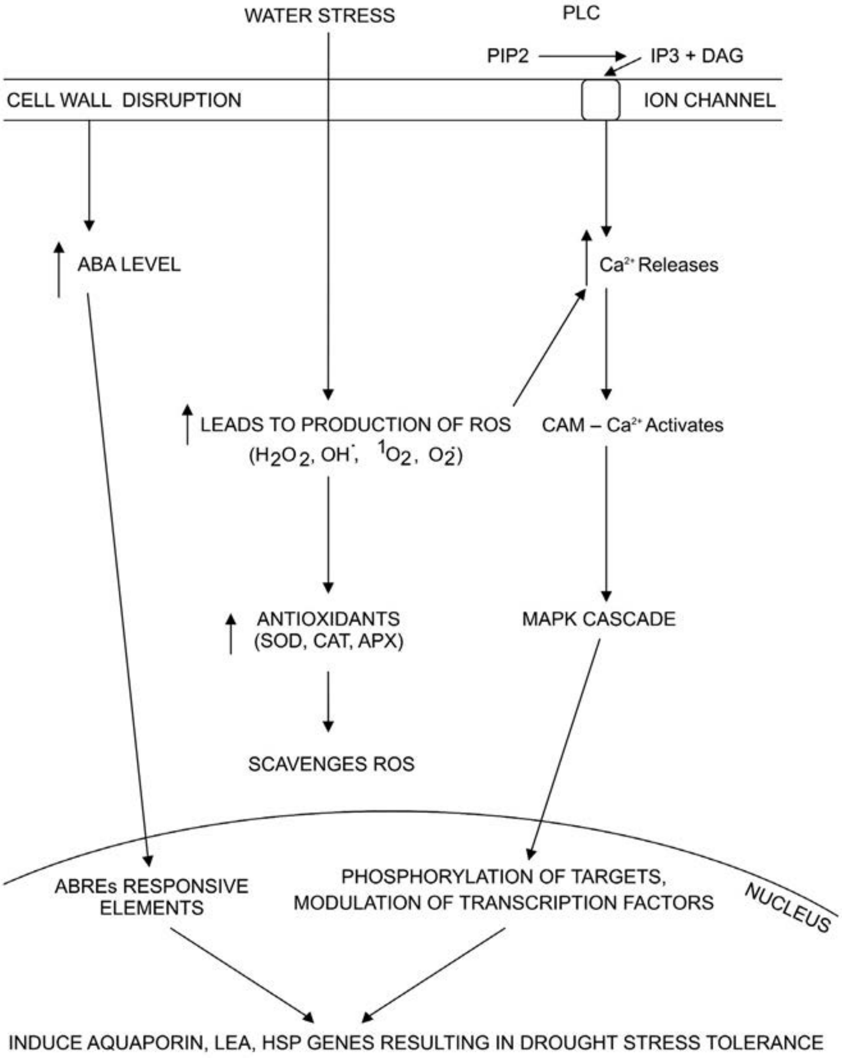

Fig. 3. Mechanism of drought stress tolerance. ABA - abscisic acid, ABRE - ABA-responsive element, APX- ascorbate peroxidase, CAM - calmodulin, CAT - catalase, DAG - diacylglycerol, HSP - heat shock protein, IP3 - inositol 1,4,5-trisphosphate, LEA - late embryogenesis abundant proteins, MAPK - mitogen-activated protein kinase, PIP2 - phosphoinositol-4,5-bisphosphate, PLC - phospholipase C, ROS - reactive oxygen species, SOD - superoxide dismutase. Adapted from Farooq *et al.* (2009).

adaptation in *Arabidopsis* (Zandkarimi *et al.* 2015). Recently, an active form of DREB2 was shown to transactivate target stress-inducible genes and to improve drought tolerance in transgenic *Arabidopsis* (Todaka *et al.* 2015). On the other hand, ABA-independent transcription factors acting in drought response are zinc finger homeodomain (ZFHD) proteins (Phuong *et al.* 2015). It was demonstrated that over-expression of the gene encoding 9-*cis*-epoxycarotenoid dioxygenase, a key enzyme in ABA biosynthesis, improves drought stress tolerance in transgenic petunia plants (Estrada-Melo *et al.* 2015). Recently, a cytochrome P_{450} CYP707A family member was identified as ABA $8'$-hydroxylase, an enzyme that degrades ABA during seed imbibition and dehydration stress (Liu *et al.* 2014). A T-DNA insertion mutant of CYP707A3, which is the most abundantly expressed gene amongst the four CYP707A members under stress conditions, exhibits elevated drought tolerance with a concomitant reduction in transpiration rate (Ng *et al.* 2014). The ABA-activated sucrose non-fermenting-related (SnRK2) protein kinase (OST1/SRK2E) functions in the ABA signal transduction pathway controlling stomatal closure (Kulik *et al.* 2011). The dehydration responsive element binding (*DREB2*) genes, belonging to ethylene responsive factor/Apetala2 (ERF/AP2) family, are induced by dehydration stress and may activate other genes involved in drought stress tolerance (Licausi *et al.* 2013). The AP2/ERF TFs family includes the ethylene-response factors, *e.g.*, a TaERF promotes drought tolerance in wheat accompanied with increased proline and chlorophyll content (Rong *et al.* 2014). Sucrose non-fermenting1-related protein kinase 2 family (SnRK2) consists of plant specific Ser/Thr kinases which are positive regulators of ABA signalling. SnRK2s were first reported to be involved in ABA signalling in wheat (Fujii and Zhu 2012).

The most represented functional category of proteins responding to drought contains various chaperones, chaperonins, heat-shock proteins, and other proteins that participate in protein folding. Aquaporin proteins act as vital regulators of plant-water relations and are potential targets in developing drought stress resistant crop plants (Afzal *et al.* 2016). Constitutive over-expression of an aquaporin gene (*GoPIP1*) in *Arabidopsis* led to an increased rosette/root ratio and decreased drought resistance due to stunted growth. A plant specific protein OsGRAS23 [named after GAI (gibberellic acid-insensitive) RGA (repressor of GAI) and SCR (scarecrow)] was found to be involved in expression of stress-responsive genes, particularly genes of large set of antioxidant enzymes which directly influence water stress related traits in rice (Xu *et al.* 2015). Veeranagamallaiah *et al.* (2011) have also suggested that LEA proteins could act as a special form of molecular chaperones that would prevent the aggregation of other proteins induced by water stress. A recent study indicated that *SiLEA14* overexpressing foxtail millet shows improved drought resistance (Wang *et al.* 2014). Karami *et al.* (2013) have

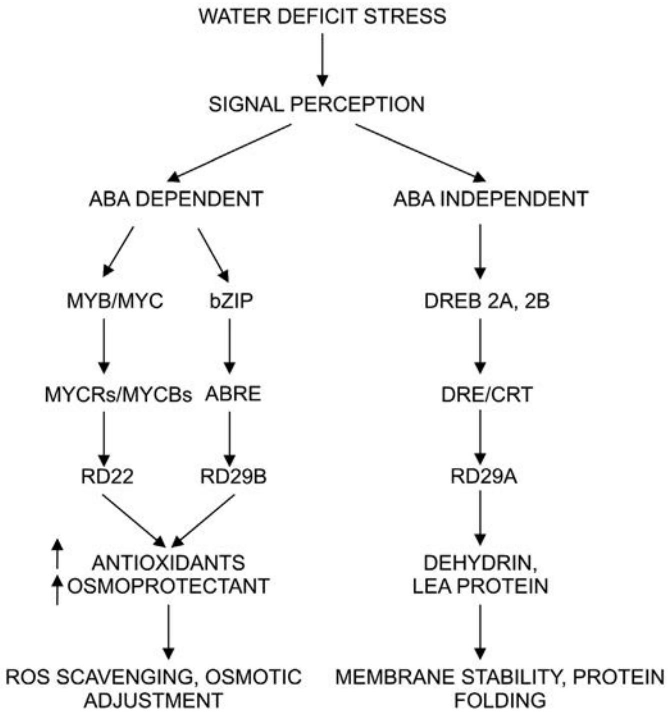

Fig. 4. Abscisic acid (ABA)-dependent and ABA-independent signalling cascades during drought stress. bZIP - zinc finger proteins, ABRE - ABA-responsive elements, DREB - dehydration responsive element binding, DRE/CRT - C-repeat (CRT)/dehydration-responsive element binding factor (GRE), LEA - late embryogenesis abundant proteins, MYB - myeloblastosis oncogene, MYCRs/MYCBs - MYC/MYB recognition sequence, ROS - reactive oxygen species, RD - responsive to dehydration. Adapted from Lata and Prasad (2011).

reported an induction of several dehydrin type proteins (Y_nSK_m) namely dehydrins (*Dhn1, Dhn3, Dhn5, Dhn7,*and *Dhn9*) in barley flag leaf under terminal drought. Relative expression of *Dhn3* and *Dhn9* revealed positive correlations with chlorophyll *a* and *b* content, osmotic adjustment, plant biomass, and grain yield, and negative correlations with malondialdehyde content and electrolyte leakage. In two barley cultivars with a differential tolerance to drought, both quantitative and qualitative differences in low-molecular dehydrins have been found when the plants were subjected to a decreased field moisture (Škodáček and Prášil 2011). *Hordeum vulgare* abundant protein 1 *HVA1* gene has been shown to confer tolerance against drought in transgenic maize (Nguyen and Sticklen 2013). Wang *et al.* (2014) have identified 54 dehydrin unigenes in common wheat genome by a search of wheat expressed sequence tag (EST) database. Recently, single nucleotide polymorphism (SNP) markers have identified *DREB-B1, DREB1A,* enhanced response to abscisic acid (*ERA1-B* and *ERA1-D),* fructan 1-exohydrolase (*1-FEH)-A, 1-FEH-B*), *WRKY1* (named after WRKY domain)*, TaSnRK2.8*, and high-affinity potassium transporter (*HKT-1*) genes for drought signaling and tolerance in wheat (Budak *et al.* 2015).

The effects of plant growth promoting rhizobacteria (PGPRs) were analyzed in relation to drought resistance. Recently, PGPRs have been found to play a significant role in alleviation drought stress in plants by producing polysaccharides, phytohormones, 1-aminocyclopropane-1-carboxylate deaminase, volatile compounds, and so inducing accumulation of osmolytes, antioxidants, up-regulation or down-regulation of stress responsive genes, and alteration in root morphology (Vurukonda *et al.* 2016). Induction of drought stress resistance by multi-functional PGPR *Bacillus licheniformis* in pepper has been observed (Lim and Kim 2013).

Conclusion

Under the influence of global warming all over the world, changes in the climatic condition are creating unusual weather phenomena often in the form of water stress. Moisture limitation affects almost every plant process, from membrane conformation, chloroplast organization and enzyme activity, at a cellular level, to growth and yield reduction in the whole plant. Drought stress induces stomatal closure, decreases transpiration and photosynthetic rates, and leads to earlier crop maturity and poor productivity. To minimize the negative effects of water stress, plants have various signalling pathways and respond by changing their growth pattern, up-regulation of antioxidants, accumulation of compatible solutes and by producing stress proteins and chaperones.

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