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The Application of Molecular Plant Biotechnology for Improvement of Drought Tolerance

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ABSTRACT: *Drought is the water deficit that impairs plants growth, development and yield compared with the normal water supply required for optimum growth. The drought which is an abiotic factor is one of the most common stresses that greatly hampered plants growth and development compared to other types of plant stresses. Plants tolerance to drought stress is a relevant issue that requires new improvement techniques like biotechnology to enhance stress-tolerant. The most common factors that influence plants tolerance to drought stress includes; the physiology of the plant, the extent of the plant stress, the growth stage, gene expression, the specie of the plant, etc.. Recent advances in plant biotechnology has seen remarkable progress in molecular markers selection processes and in developing transgenic plants with increased drought stress tolerant. These approaches have facilitated our understanding of underlying processes in plant responses to drought induced stress. Through plant genetic engineering and molecular marker techniques, drought stress induced genes have been identified and cloned. It therefore means that the applications of biotechnological and molecular approaches such as genomics, proteomics, and transcriptomic that can enhance a better understanding of plant water use efficiency and tolerance to improve yield under drought stress is very promising.*

KEYWORDS: Application, molecular, plant biotechnology, drought tolerance

INTRODUCTION

Drought is the water deficit that impairs plants growth, development and yield compared with the normal water supply required for optimum growth. The drought which is an abiotic factor is one of the most common stresses that greatly hampered plants growth and development compared to other types of plant stresses (Bismillah Khan et al., 2015; Shao et al., 2009). Drought induces metabolic changes in plants, such as increased levels of free sugars and free essential amino acids, which according to the "Plant stress hypothesis" causes the plant to have a higher nutritional value for herbivores and can play an important role in herbivore outbreaks (Ximénez-Embún et al., 2016). Drought stress is usually said to be an extremely dry condition beyond a threshold level which causes damage to plants. When plants lack adequate water supply, the resulting drought stress normally reduces growth more than all other plant abiotic stresses. Plant responds to lack of water by reducing the activities of photosynthesis and other plant processes. When plants experience drought stress and water loss progresses, leaves of some plant species appear to change colour (usually to yellow-brown) and drought stress also reduces crop productivity or yield (Araus et al., 2002; Boyer, 1982). Drought stress also plays a vital role in determining the availability of most plants species across different locations in the world. Naturally, drought stress in plants varies from species to species, a period of exposure and some environmental parameters. Plants tolerance to drought stress is a relevant issue that requires new improvement techniques like biotechnology to enhance stress-tolerant (Rizhsky et al., 2002). The most common factors that influence plants tolerance to drought stress includes; the physiology of the plant, the extent of the plant stress, the growth stage, gene expression, the specie of the plant, etc..

In this review, some important parameters about drought stress in plants such as drought tolerance mechanisms, plant responses to drought, gene regulation and major traits for drought stress tolerance in plants, effects of drought stress at different stages of plant growth and biotechnology methods in developing drought tolerance in plants was discussed.

LITRATURE REVIEW

Drought Tolerance Mechanisms in Plants

Plants exposed to drought stress can tolerate (adapt) to the stress depending on the plant species and period of exposure which the plants may survive under drought stress through the induction of diverse biochemical, physiological or morphological factors (Farooq et al., 2009). Phenotypic and morphological changes that often occur in plants are influenced by a spectrum of physiological and molecular interactions developed to acclimate to drought stress (Valdés et al., 2013). Drought stress tolerance is the ability of a plant to grow, develop and thrive with displayable economic yield and value under limiting or no water supply. Drought stress affects not only the water relations of plants at cellular and tissue levels but also at organ levels, which may result in explicit and/or relatively ambiguous interactions that can damage or acclimatize the plant (Beck et al., 2007).

Plants often respond to abiotic stresses through the expression of stress-regulated genes and protein production. The available data on stress related genes is still limited as their functions have not been thoroughly established. However, it has been established that plants' ability to tolerate drought stress is a complex event that involves a combination of some of the genes to express synergistically. The expression of genes may be triggered by stress-induced events or result from injury responses to the plant (Farooq et al., 2009). With the advent of genomics, some genes are known to be expressed when plants are drought-stressed to produce relevant drought stress-related proteins and enzymes including dehydrins (polypeptide), invertase, glutathione Stransferase, and late embryogenesis abundant; also, the expression of Abscisic acid (ABA) genes which is an essential phytohormone that regulates growth, development and adaptation to drought stress and the synthesis of macromolecules such as rubisco, helicase, proline, and carbohydrates are the molecular basis of drought tolerance. A polypeptide (dehydrins) was observed to be the most abundant among the accumulated macromolecules in response to loss that leaves water content in some plants (pea, maize, barley, Arabidopsis, etc.) and under drought-induced stress LEA proteins plays the protective role of plants. In extreme cases even though they are not plant specific, LEA proteins has been associated with cellular desiccation tolerance. Osmotin which is also a stress-responsive antifungal protein accumulates under both biotic and abiotic stress in several plant species (Radomiljac et al., 2013). Macromolecule such as phospholipids and glycolipids are the lipid components of the plant membrane layer, while triglycerides are primarily used to store CH4 and CO2 during the developmental stages of plants. 70%-80% of the total protein and lipid composition of leaf tissue are found in the chloroplasts. Lipids, which are one of the major components of the membrane, are likely to be affected by water stress.

The Molecular Biology of Drought

Plants have frequently evolved in habitats where drought occurs, and so have developed multiple strategies to cope with drought stress. Drought tolerance is defined as the ability of a plant to live, grow and reproduce satisfactorily with limited water supply. Tolerance strategies can be divided into resistance mechanisms, which enable plants to survive dehydration, and avoidance mechanisms, which are growth habits that prevent the exposure of plant to osmotic stress, such as deeper rooting or a shorter growth season. The capacity of a plant to tolerate drought depends largely on the drought adaptation mechanisms present within its genome, and how efficiently it can activate them. Unfortunately, the domestication of modern crops has greatly reduced the genetic diversity of elite cultivars, and may even have promoted accumulation of deleterious mutations in their stress response mechanisms (Schmidt, 2014). Exposure to drought stress in plants leads to cellular dehydration, causing decreased cytosolic and vacuolar volumes and osmotic stress. Drought responses of plants includes attenuated growth, altered gene expression, changes in hormone levels, accumulation of osmoprotective solutes and proteins, increased levels of antioxidants and suppression of core metabolism. Drought tolerance is a quantitative trait, with a complex phenotype including all of these responses and involving a number of genes.

Plant Responses to Drought

Physiological and Morphological Responses

Plant growth and development is a process that is usually accomplished through certain physiological and morphological complex interactions such as cell division, cell enlargement, and differentiation, as well as genetic interactions. The growth of a plant is regulated by these activities as well as the presence of organic and inorganic compounds required for the development of new protoplasm. The quality and yield of plant growth to a reasonable degree depend on these complex interactions which can be greatly reduced by drought stress (Farooq et al., 2009). The physiological response of plants to drought stress can include; interference with photosynthetic activity, stomatal regulation, oxidative stress which eventually leads to damage of the plant, generation of toxic metabolites which can cause plant death, water-retention level of leaf decreases, impaired growth rate, decrease in CO2 concentration, etc. Cell growth and differentiation alongside other physiological events are one of the most drought sensitive physiological events due to a decrease in turgor pressure which is one of the major plant responses to drought stress. Water is important in the maintenance of the turgor pressure which is necessary for cell enlargement, growth and for maintaining the plant as a whole. Turgor is equally vital in stomatal regulation and the motility of various differentiated plant structures. In extreme drought stress, cell differentiation and elongation of some plants are repressed through the ongoing interference of water and minerals flow from the vascular tissues to the other components of elongating cells (Schmidt, 2014). Severe drought stress mostly is accompanied by increased salt concentration which defined it as osmotic adjustment. Osmotic adjustment occurs when solutes gradually accumulate in the elongating cells of developing the plant as the water retention level decreases over time. Osmotic adjustment is one of the most essential events in plant acclimatization to water-limitation, for the reason that it maintained vascular tissue metabolic activity and enables re-growth upon water availability which of course varies greatly from species to species.

Plant response in relation to growth varies according to the tissue, mode/ severity/time scale of the stress and species of concern. Mild osmotic stress can cause growth arrest in leaves and stems, but no inhibition in root growth. The growth arrest can be a mechanism for either energy conservation with reduced metabolism for better subsequent recovery or a support for osmotic adjustment (Osório et al., 1998; Voetberg & Sharp, 1991; Westgate & Boyer, 1985). Several lines of evidence have supported the role of cyclin dependent kinases (CDKs) and cyclin-dependent kinase inhibitors (ICKs) in the regulation of cell division under drought conditions (Schuppler et al., 1998). There is also evidence linking ICKs with abscisic acid (ABA)-dependent mechanism of drought (Kang et al., 2002; Wang et al., 1998). In a recent study, it has been shown that Arabidopsis thaliana MYB, discussed further in this chapter, limits cell expansion since its constitutive expression results in a dwarf phenotype and small cells. However, drought triggered growth sustenance of roots can be an adaptive mechanism for water uptake. Expansin genes, involved in cell wall loosening, a parameter involved in cell expansion, were shown to alter their expression patterns in response to water deficit (Jones & McQueen-Mason, 2004).

Biochemical Responses

Reactive oxygen species are produced in different compartments of the plant cell, both under normal and stressful conditions. When plants are stressed by drought or other abiotic stresses, reactive oxygen species are generated as a result of the inhibition of photosynthesis and the preeminence of photorespiration. The generation of reactive oxygen species is one of the earliest biochemical responses of eukaryotic cells to biotic and abiotic stresses. The production of reactive oxygen species in plants, known as the oxidative burst, is an early event of plant defense response to water-stress and acts as a secondary massager to trigger subsequent defense reaction in plants. Reactive oxygen species, which include oxygen ions, free radicals, and peroxides, form as a natural byproduct of the normal metabolism of oxygen and have an important role in cell signaling. However, during environmental stress such as drought, reactive oxygen species levels increase dramatically resulting in oxidative damage to macromolecules such as proteins, DNA and lipids. Being extremely reactive, reactive oxygen species can severely damage plants by increasing lipid peroxidation, protein denaturation, nucleic acid fragmentation and finally cell death. Drought stress induces oxidative stress in plants by a generation of reactive oxygen species. Drought induced high production of reactive oxygen species and it increases the content of malondialdehyde. The content of malondialdehyde has been considered as an indicator of oxidative damage.

Reactive oxygen species are found to have a dual function in plants: they are needed as signaling molecules, but a high concentration it is detrimental. High reactive oxygen species concentration is therefore, a symptom of stress and plants have to maintain the reactive oxygen species within a certain level that is required for normal cellular homeostasis. Reactive oxygen species concentration in the cell is maintained by the antioxidant system, which is made up of the antioxidant molecules ascorbate, glutathione, and α-tocopherol in addition to the antioxidant enzymes peroxidases, catalases, and dismutases. In plants, reactive oxygen species are discharged through certain antioxidant molecules, polar and lipid-soluble molecule (Sharma et al., 2012), and the most effective antioxidant being the process that counteracts oxidative stress (Farooq et al., 2009). Most plants that are exposed to severe environmental stresses notably as drought have developed a mechanism to reprogram their metabolic pathways to tolerate the impending stress which often result in changes in the production and utilization of available metabolites. The advent of metabolomics has uncovered how plants subjected to abiotic stresses invest in the synthesis of essential macromolecules and metabolites that contribute to palliate stresses as osmoregulators, antioxidants and defense compounds. Drought induced stress can also alter the available content and composition of plants macromolecules such as proteins which eventually causes proportional changes of structural and soluble proteins.

Plants perceive drought prior to initiating a signaling cascade for appropriate response. A number of plant osmosensor candidates were proposed. A receptor-like protein, NTC7, was suggested in a study in which its transcripts were induced in response to osmotic stress and its overexpression induced osmotic stress tolerance (Tamura et al., 2003). Further, Arabidopsis homologue of SLN1, a plasma membrane nonethylene receptor histidine kinase, ATHK1, was shown to complement yeast SLN1 mutant (Urao et al., 1999).Recently another study showed that AHK1/ATHK1 positively regulates ABA-related drought response while other nonethylene receptor kinases called cytokine receptors (CK) including AHK2, AHK3 and CRE1 are involved in drought-related negative regulation. Further analysis of ATHK1 revealed that it is involved in drought response not only during early vegetative stages of growth but also during seed formation. This research showed that it is co-regulated with several Arabidopsis response regulators and its overexpression induced water deficit tolerance (Wohlbach et al., 2008). Recently, Oryza sativa receptor-like kinase (RLK), OsSIK1, was cloned, characterized in relation to kinase activity, and transgenic work has shown that it is involved in drought tolerance modulating stomata and activating antioxidative system (Ouyang et al., 2010).

Signaling pathways consist of signalling molecules and a network of protein interactions which are mediated by reversible phosphorylation in response to environmental factors including drought. Several components of the signal transduction have been identified although their interactions and positions along the pathway remain unknown. Differences in signalling between related genotypes can affect their drought response (Fig. 1).

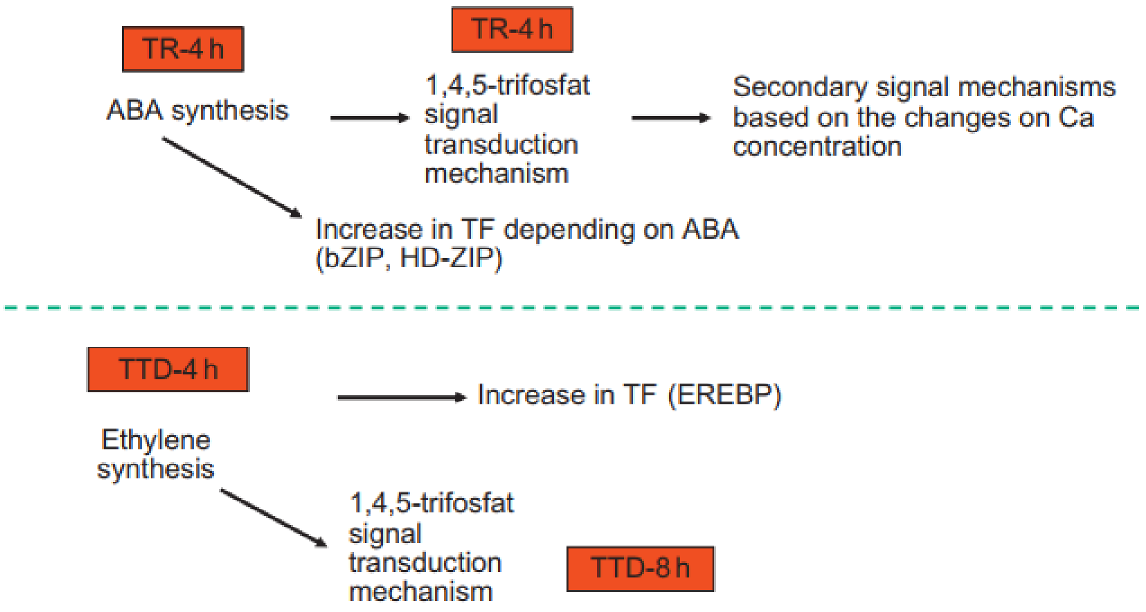

Fig. 1. A proposed model for the ABA and ethylene syntheses of wild emmer wheat, TRt (a tolerant genotype) and TTD (a sensitive genotype) under shock drought stress (4 and 8 h stress).

MAPKinases

Mitogen-activated protein kinase (MAPK) cascade includes three protein kinases (MAPK, MAPKK and MAPKKK) which are activated by serial phosphorylation, resulting in specific localization of the module in cell compartments, phosphorylating and regulating transcription factors and other proteins. In Arabidopsis, using sequence information, a number of MAPKinases were identified. Some MAPKinases were transcriptionally up-regulated and others were shown to be post-translationally activated by drought stress. Additionally, ADR1, a CC–NBS–LRR gene which is a homologue of serine/threonine protein kinases was shown to confer dehydration tolerance consistent with dehydration responsive gene expression(Chini et al., 2004). Recently, a rice drought-hypersensitive mutant (dsm1) of a putative MAP kinase (MAPKKK) was identified. DSM1 protein was shown to belong to Raf-like MAPKKK, localize in the nucleus, induced in response to water deficit/ABA and confer seedling drought resistance. It was also proposed as an early signalling component, a regulator of scavenging of reactive oxygen species (Ning et al., 2010).

Taking into consideration the above evidence that one MAPKinase can respond to different stress conditions and there are different numbers of proposed and identified MAPKinases from each of the three categories up to date, there should be a convergence in the signalling of MAPK cascade, possibly different stress factors activating MAPKinases to different levels.

SNF1-like kinases

SNF-1-like kinases, classified into three families, SnRK1, SnRK2 and SnRK3 are another family of protein kinases which are activated by the phosphorylation of their serine or threonines. In various plant species, several SNF-1 like kinases were predicted and shown to be expressed in response to dehydration or ABA, including Arabidopsis OPEN STOMATA1 (OST1) protein kinase. Arabidopsis OST1 protein kinase was shown to be involved as a positive regulator of ABA-induced stomatal closure and regulated negatively as a substrate of protein phosphatases 2C (PP2C) HAB1. There are lines of evidence on ABA-bound receptor inhibiting protein phosphatases resulting in activation of OST1(Mustilli et al., 2002). A recent study brings evidence for the involvement of distinct phosphorylation mechanisms in the activation of the two subgroups of SnRK2s. This can be related to their ABA responsiveness because members of SnRK2 are responsive to osmotic stress, but only some to ABA (Vlad et al., 2010). In line with these evidences, analysis of the phosphoproteome in response to ABA treatment leads to the identification of increases in the phosphorylation states of SNF1-related kinases after ABA treatment (Kline et al., 2010).

Phosphatases

Phosphatases are classified based on their substrates into two major groups phosphoprotein (serine/threonine) phosphatases (PPases) including PP1, PP2A, PP2B and PP2C; tyrosine phosphatases (PTPases), receptor-like, intracellular or dual specific. Phosphatases aid in counteracting the action of kinases as noted above. As noted above, there is intense research on the negative role of serine threonine PP2Cs including ABI1, ABI2 and HAB1 in ABA signalling. Studies with ABI1 and ABI2 mutants have shown in guard cells that ABA activation of Caþ-permeable channels requires intermediate steps of first ABI1 action, then ROS, finally ABI2 action. Two recent independent studies have revealed substrates of HAB1 as OST1 (as noted above) and PYL5 from the Bet v1-like superfamily, which was shown to be a cytosolic and nuclear ABA receptor that activates ABA signalling through direct inhibition of HAB1 and ABI enhancing drought tolerance (Santiago et al., 2009). In another study, PTPases were shown to be involved in stomatal closure, downstream of Ca2þ signalling, most probably aiding in dephosphorylation of an unidentified protein, resulting in subsequent ion flux from guard cells and stomatal aperture.

Phospholipid signalling

One recognized class of osmotic stress-signalling secondary messengers is phospholipid-derived signalling molecules of the phosphoinositide pathway which are cleaved from membrane phospholipids by phospholipases. Several phospholipid-derived secondary messengers, especially inositol 1, 4, 5- triphosphate (IP3), diacylglycerol (DAG) and phosphatidic acid (PA), were shown to be drought related. Phospholipases in the context of drought are phospholipase C (PLC) and phospholipase D (PLD). PLC cleaves phospholipid phosphatidylinositol 4,5-bisphosphate (PIP2), which is synthesized by a phosphatidylinositol-kinase, PIP5K, into IP3 and the membrane protein DAG. PIP5K, PIP, PLC and IP3 levels were shown to be induced in response to water deficit or ABA in several plant species (Kopka et al., 1998; Mikami et al., 1998; Takahashi et al., 2001). Two independent studies together support that phospholipid signalling involving this pathway is activated through both ABA-dependent and -independent mechanisms. The current hypothesis is that drought activation of PLC leads to higher IP3 levels, a subsequent release of Ca2þ from intracellular stores to cytoplasm, and activation of Kþ ion channels resulting in stomatal closure (Staxén et al., 1999; Takahashi et al., 2001). In addition, its initial synthesis, an additional regulatory mechanism for inositol phosphate levels, involves the action of 5-phosphatases (5Ptases) or inositol polyphosphate 1 phosphatases. Arabidopsis SAL1 belongs to the latter group mentioned and recently drought tolerant Sal1 mutants along with omics studies have supported it as a negative regulator of both ABA-independent and also -dependent drought-response pathways (Wilson et al., 2009). In another recent study, an inositol phosphate lacking transgenic plant was generated by the expression of inositol polyphosphate 5-phosphatase. These plants exhibited higher drought tolerance and ABA-induced stomatal closure. Moreover, SAL1 was suggested as a regulator of an ABA-independent pathway since expression of dehydration-responsive element binding protein (DREB), which will be discussed below, is induced in transgenic plants (Wilson et al., 2009). Another important secondary messenger is PLD which cleaves phospholipases producing PA which contains a Ca2þ-binding domain and can activate PLC. PLDs were shown to be drought or ABA induced in several plant species (Frank et al., 2000; Sang et al., 2001). Interaction of PLD with ABA effectors supports that PLD is involved in ABA-dependent pathway. There is supportive evidence of the role of PLD in stomatal closure such as its interaction with ABA effectors including ABI1(Sang et al., 2001).One study has also shown the simultaneous accumulation of PLD and PA in water deficit. Non-specific phospholipase C (NPC4) hydrolyses phospholipids in a calcium-dependent manner, producing DAG. In another recent study, transgenic studies of this messenger reveal that it is converted to PA and involved in ABA sensitivity, drought tolerance and stomatal closure (Peters et al., 2010).

Compatible Solutes

Compatible solutes are nontoxic molecules that accumulate in the cytoplasm in response to drought stress and do not interfere with metabolism. Major compatible solutes are sugars (sucrose, hexose, raffinose-type oligosaccharides, and trehalose); sugar alcohols including cyclic polyols (pinitol, D-ononitol); glycine betaine; and amino acids, most importantly proline. Current hypothesis on their mode of action ranges from conferring osmotic adjustment, scavenging ROS, stabilizing proteins and cell structures and adaptive value of metabolic pathways. The accumulation of several compatible solutes was observed to be drought induced and engineering the synthesis of compatible solutes has been relatively successful. Recent striking evidence is several truncated/recombinant transcripts of betaine aldehyde dehydrogenase (BADH), the enzyme involved in glycine betaine synthesis, were observed in monocots. Surrounding the deletion/ insertion sites of these transcripts, sequence similarities, named short, direct repeats (SDR), were detected (Niu et al., 2007). These can possibly be recognition sites for post-transcriptional silencing.

PROTECTIVE PROTEINS

Late embryogenesis-abundant proteins

LEA proteins are a diverse group of proteins expressed normally during embryogenesis, or in vegetative tissues, in response to ABA or drought stress. Accumulation of LEA proteins correlates with ABA levels and desiccation tolerance (Ergen & Budak, 2009; Galau et al., 1986). Evidence from expression profiles and overexpression studies supports a role for LEA proteins as protective molecules in water deficiency. LEA proteins were grouped based on conserved structural features (Hilhorst et al., 2018). Group 1 LEA proteins harbour high hydrophilicity and are thought to be soluble aiding in water binding or replacement. The group 2 (dehydrins) and group 4 LEA proteins may contribute to the maintenance of protein and membrane structures. Early response to dehydration proteins of the dehydrins family was recently shown to have disordered 3D structure enabling them to maintain in low water concentrations, probably enabling them to act as chaperones in high ionic strength (Kovacs et al., 2008). Additionally, a research on spatial and temporal accumulation patterns of group 4 LEA proteins was conducted. This was further followed by a bioinformatics analysis revealing origination of subgroups of group 4 LEA with gene duplication events. Generation of transgenic plants confirmed that the role of group 4 LEA proteins is indispensable in dehydration tolerance and recovery (Olvera-Carrillo et al., 2010). The group 3 and group 5 LEA proteins which harbour the most hydrophobicity are thought to sequester ions, which accumulate due to water deficit. Supporting evidence came from a recent study in which Arabidopsis LEA5 was shown to confer ABA dependent protection against oxidative stress by decreasing photosynthesis. Moreover, lately Medicago truncatula seed desiccation tolerance (DT) was linked to 11 mostly seed-specific LEA proteins from different groups (Boudet et al., 2006).

Aquaporins

Changes in water flow is crucial to drought and the rate of water flux into or out of cells can be determined either by diffusion resulting from water potential gradient or by aquaporin proteins facilitating osmosis by forming water-specific pores which increase water permeability of the membrane shown by aquaporin antisense experiments (Kaldenhoff et al., 1998; Siefritz et al., 2002). Aquaporins are members of a large superfamily of membrane spanning proteins called the major intrinsic proteins (MIPs) including tonoplast intrinsic proteins (TIPs) and plasma membrane intrinsic proteins (Weig et al., 1997). There are several reports that aquaporin genes are induced by dehydration enhancing water uptake (Fray et al., 1994; Guerrero et al., 1990; Weig et al., 1997; Yamaguchi-shinozaki et al., 1992) and some showing aquaporins are reduced by dehydration which can allow water conservation (Smart et al., 2001). Likewise, most classes of Arabidopsis MIPs are up-regulated in response to drought, but only some are down-regulated. A recent expression profiling study of Arabidopsis PIPs revealed that this regulation is consistent through accessions excluding the special case for three PIP genes. The relation of PIP genes to drought was further supported by linking variation of drought-related PIP expression to leaf water content and demonstrating the presence of drought stress response elements in the promoters of two PIPs (Alexandersson et al., 2010). Additional supportive evidence on the interspecies conservation of the aquaporin gating mechanism came from a recent work on structural dynamic simulations of spinach aquaporin SoPIP2;1.

Heat Shock Proteins

HSP are important for efficient cellular functions since they are chaperones that aid in folding and assembly of correctly structured proteins during synthesis, their maintenance by preventing aggregation by binding and stabilizing denatured proteins and in the removal and disposal of non-functional and degraded proteins. Low water content impairs protein structure and HSPs are usually only present in vegetative tissues under stress conditions. HSPs were shown to be dehydration induced in several plants (Alamillo et al., 1995; Campalans et al., 2001; Wehmeyer & Vierling, 2000). Their role in conferring tolerance was shown with transgenic studies and chaperone binding protein from HSP70 protein family is involved in targeting and was shown to be water deficit induced and shown to confer tolerance to drought (Alvim et al., 2001).

ANTIOXIDANTS

Drought stress leads to increased accumulation of ROS, generated mostly in chloroplast and to some extend in mitochondria, causing oxidative stress. Major ROS molecules are singlet oxygen, superoxide anion radicals, hydroxyl radicals and hydrogen peroxide (H2O2). To detoxify ROS, plants can intrinsically develop different types of antioxidants reducing oxidative damage and conferring drought tolerance. ROS scavengers are either non enzymatic (ascorbate (vitamin C), glutathione, tocopherol (vitamin E), flavonoids, alkaloids, carotenoids) or enzymatic containing superoxide dismutase, peroxidases and catalase. Free radical-mediated lipid peroxidation results in complex, highly reactive and toxic aldehydes, which are scavenged by either aldehyde dehydrogenases or aldose/aldehyde reductases. There are lines of evidence revealing the involvement of these enzymes in drought response (Mundree et al., 2000; Oberschall et al., 2000; Sunkar et al., 2003). The osmotic stress involvement of peroxiredoxins which detoxify toxic peroxides was also shown. Peroxiredoxin is also a potential target of DREB1A (Yamaguchi-Shinozaki & Shinozaki, 2001). Thioredoxins function as hydrogen donors, and their role in water deficit was studied (Pruvot et al., 1996; Rey et al., 1998). Peptide-methionine sulphoxide reductases (MsrA) can counteract the damage caused by the modification of methionine-containing proteins to methionine sulphoxide [Met (O)] making them vulnerable to protease degradation and causing loss of function. Their involvement in drought stress was confirmed with expression and transgenic studies (Kim et al., 2014).

Other Related Molecules

A number of other small molecules have putative roles in the drought response, although most of these await further confirmation. Recently, an Arabidopsis mutant was identified with lower content of dolichols, a type of polyisoprenoid, and increased drought tolerance (Zhang et al., 2008). Subtle differences were observed between tocopherol (vitamin E) mutant and controls in tolerance to drought stress, but the main focus of the study was on low-temperature stress (Maeda et al., 2006). Lipocalins are small ligand binding proteins and recently an A. thaliana Lipocalins AtCHL was functionally characterized. Its transcript and protein were found to be induced upon drought and ABA. With transgenic studies, it was shown that AtCHL aids in coping with stress conditions, especially damage upon photo-oxidative stress induced by drought (Levesque-Tremblay et al., 2009).

Major Traits Contributing to Drought Resistance

Early Flowering and Drought Escape

The molecular control of flowering time is complex, and has been highly studied in Arabidopsis as well as in many other plant species (Corbesier et al., 2007). During the developmental switch from the vegetative to the reproductive stage, the photoperiodic light signal from the environment is perceived by leaves, where the FLOWERING LOCUS T (FT) protein is synthesized. FT is loaded into the phloem and transported to the shoot apical meristem (SAM) where it initiates floral transition (Andrés & Coupland, 2012). It is now known that in the SAM, FT forms a complex with the bZIP protein FD in specific cells beneath the tunica layers, in which FD is expressed, with these cells then originating the floral primordia (Abe et al., 2019). When Arabidopsis is exposed to drought conditions, it can activate the DE response. DE is one of the main defense mechanisms against drought in Arabidopsis, and it integrates the photoperiodic pathway with drought-related ABA signaling (Conti, 2019). DE has mainly been studied in an evolutionary context in natural populations (Franks et al., 2007; McKay et al., 2003), and the molecular mechanisms that regulate it have only been unraveled recently. It is known that, to trigger DE, the key photoperiodic gene GIGANTEA (GI) needs to be activated by ABA (Riboni et al., 2013, 2016). A recent breakthrough was the discovery that the ABRE-BINDING FACTORS (ABF) 3 and 4, which act on the master floral gene SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 (SOC1) in response to drought, are involved in this process. The mutants abf3 abf4 are insensitive to ABA induced flowering and have a reduced DE response (Hwang et al., 2019). However, the precise molecular mechanisms that link ABA to GI and ultimately to DE are still rather obscure, and different crop species might have evolved unknown pathways that trigger DE in different environments (Figure 2).

From an agronomic perspective, DE and early flowering varieties with faster life cycles are interesting because an anticipated switch to the reproductive stage might allow grain filling before the onset of seasonal terminal drought. Furthermore, a shorter crop season reduces the need for agricultural inputs (e.g., fertilizers, pesticides) and might facilitate double cropping (i.e., the farming of two different crops in the same field within the same year). On the other hand, crops that switch too early to flowering will have their yield reduced. Despite DE being an emerging research field in crop science, there are not any biotechnologically improved crops that exploit DE as a drought resistance trait. Still, it has been proposed that DE can be used to obtain quick-growing, early-flowering cereal varieties, which would be especially useful in temperate regions like the Mediterranean area where terminal drought is expected to affect plants toward the end of the crop season (Shavrukov et al., 2017). Furthermore, it has been recently shown that OsFTL10, one of the 13 FLOWERING LOCUS T-LIKE (FTL) genes annotated in the rice genome, is induced by both drought stress and GA, and when overexpressed in transgenic rice plants confers early flowering and improves drought tolerance (Fang et al., 2019). However, as these transgenic rice lines were not tested in a field trial, it is unknown whether engineering FTL genes could deliver cereal varieties with superior drought performances and good yield in both dry and well-watered conditions. Nonetheless, the manipulation of the DE pathway could be an innovative and valid strategy especially in the context of highly variable water availability. As DE involves specific tissues (leaf, phloem) and cell types (phloem companion cells, FD-expressing SAM cells), it might be possible to devise strategies aimed at developing drought-resistant plants via manipulation of these plant components, adjusting DE to the different environmental conditions.

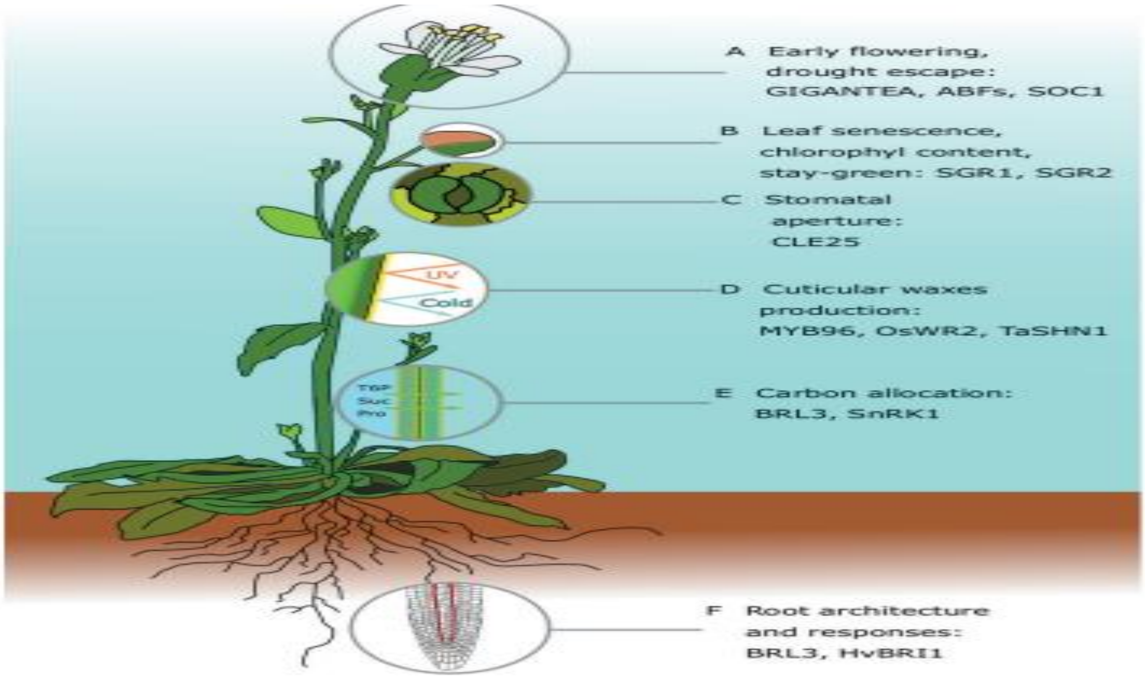

Figure2. Major traits contributing to drought resistance in Arabidopsis thaliana (Conti, 2019)

Leaf Traits: Senescence, Stay-Green, and Leaf Area

Senescence is a developmental stage of plant leaves that leads to the arrest of photosynthesis, the degradation of chloroplasts and proteins, and the mobilization of nitrogen, carbon, and other nutrient resources from the leaves to other organs. As most cereals are monocarpic annual species, these resources are directed to developing seeds, and senescence therefore plays a relevant role in crop yield. Environmental stresses like temperature, lack of nutrients, and drought might initiate senescence prematurely, affecting seed nutritional composition and crop yield(Buchanan-Wollaston, 1997; Distelfeld et al., 2014). In crops threatened by terminal drought, the ability to sustain photosynthetic activity longer by delaying or slowing down senescence could be an effective strategy to avoid yield losses. As such, leaf senescence has been extensively studied in crops (Figure 3). Plant breeders commonly refer to the trait that confers extended photosynthetic activity as stay-green, also defined as green leaf area at maturity (GLAM). This trait is well studied in sorghum [Sorghum bicolor (L.) Moench], a dry climate-adapted cereal in which a number of stay-green quantitative trait loci (QTLs) have been identified (Vadez et al., 2011). However, the genes underlying these QTLs have not yet been identified. Stay-greenness in sorghum is a complex trait, and it is also connected with the perennial tendencies of some varieties (Thomas & Howarth, 2000). Other plant species achieve stay-green characteristics via substantially different pathways that include disabling chlorophyll catabolism (like in the case of Gregor Mendel’s green peas), and altering the responses to plant hormones. Indeed, some stay-green genes have also been identified in Arabidopsis and rice, notably the Stay-Green Rice (SGR) genes and their homologs in Arabidopsis SGR1, SGR2, and SGR-like (SGRL). The respective molecular pathways have been elucidated, with the phytohormones ethylene, ABA, cytokinin (CK), and strigolactone (SL) having a prominent role in stress-induced leaf senescence (Abdelrahman et al., 2017). The connection between ethylene and leaf senescence is long known (Grbić &

Bleecker, 1995), and numerous attempts to improve photosynthetic activity and drought performance by manipulating ethylene biosynthesis have been published in dicots (Grbić & Bleecker, 1995) and cereal plants(Young et al., 2004) . The first biotechnologically produced plant ever to reach the market with improved drought resistance due to reduced ethylene sensitivity and delayed senescence was produced by Verdeca and named HB4® Drought Tolerance Soybeans. HB4 is a modified version of the homeodomain-leucine zipper (HD-zip) transcription factor (TF) HaHB4 from sunflower (Helianthus annuus). It is expressed under the control of the native soybean HaHB4 promoter, which is stressing inducible (Waltz, 2015). Although HaHB4 does not have conserved homologs in Arabidopsis, upon ectopically expressing HaHB4 in this model species, it was discovered that the TF acts at the intersection between the jasmonic acid and ethylene pathways (Dezar et al., 2005; Manavella et al., 2008). Interestingly, HB4-expressing soybean has increased yield in both water-limited and well-watered conditions. As shown in extensive field trials, this same gene confers similar drought tolerance properties without yield penalties when transferred to bread wheat (González et al., 2019), with the transgenic wheat having an unaltered quality and nutritional content when compared with its parental non transgenic variety Cadenza (Figure 3)(Ayala et al., 2019). As such, it is likely that the HB4 cassette could confer drought resistance to other cereals. It is worth pointing out that the success of HB4 is due to the exploitation of drought-responsive promoters rather than of constitutive strong promoters.

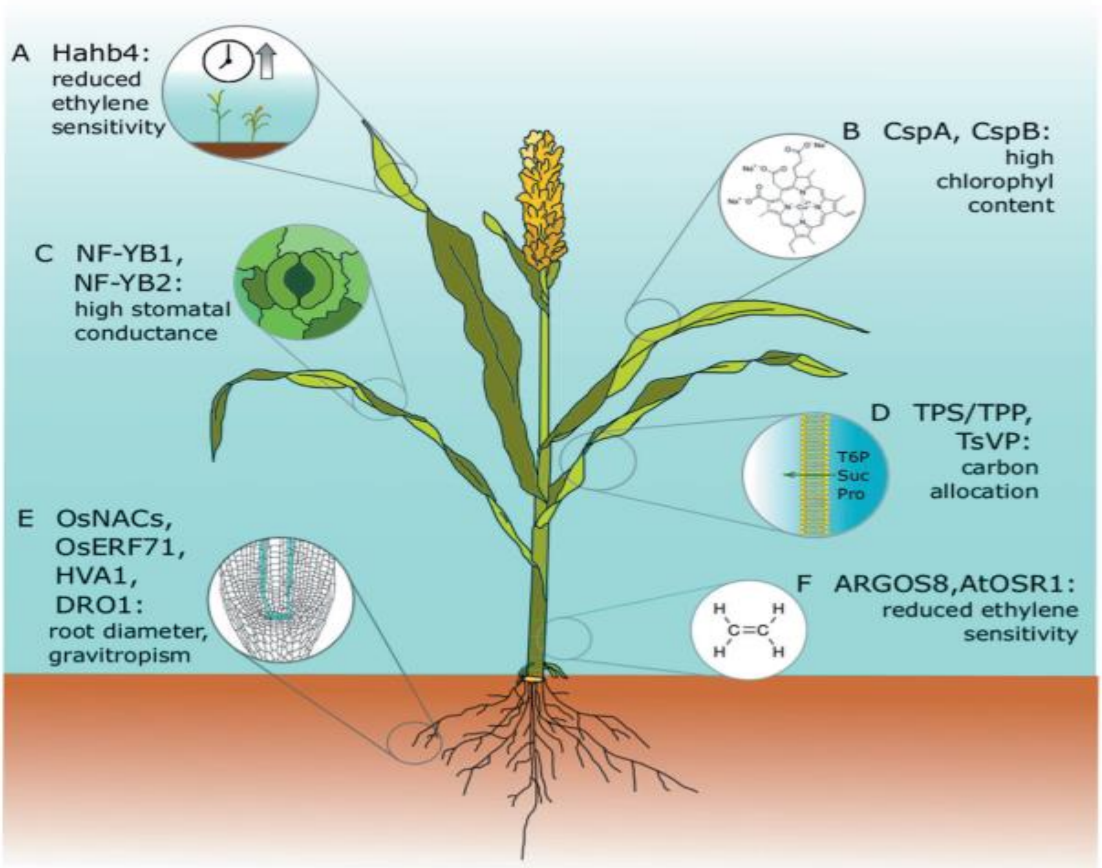

Figure3. Drought tolerance genes that have been discovered or tested in model species and translated successfully into crop species. All of these genes have been expressed in engineered

cereal crops and have been tested in field trials. Major agronomical traits, including yield, have been assessed, and conditions and drought performances have been successfully improved without negatively affecting plant growth or crop yield.

Root Traits

Roots are the main plant organ dedicated to the uptake of water, and are the first place where a lack of water is perceived. As such, an abundance of studies have examined root responses to dehydration. The most relevant root traits capable of improving drought tolerance and their biotechnological applications have recently been reviewed by (Koevoets et al., 2016) and by (Rogers & Benfey, 2015), respectively. Here, we will focus on the solutions offered by manipulation of the BR pathway, and will provide a brief overview on the most promising biotechnological strategies aimed at improving drought resistance through manipulating root-related traits. BRs are a class of plant hormones that are widely involved in plant growth and development, as well as in stress responses. Along with other plant hormones, BRs play a key role in root growth. As BR levels are finely regulated to permit proper root development, BR metabolism and signaling are clear targets for the manipulation of root responses (Planas-Riverola et al., 2019; Singh & Savaldi-Goldstein, 2015). Indeed, exogenous application of BRs has been extensively tested on a variety of crops with variable outcomes (Khripach et al., 2000). However, from a genetic perspective, the only BR-related mutant widely used in agriculture is the barley use mutant, which carries a single amino acid substitution in the BR receptor HvBRI1, homolog of the Arabidopsis BR receptor Brassinosteroid insensitive-1 (BRI1) and displays a semi-dwarf phenotype (Chono et al., 2003). Recently, the triple mutant of wrky46, wrky54, and wrky70—positive regulators of BR signaling Arabidopsis group III WRKY TFs—was shown to be drought resistant. Due to a significant up regulation and down regulation of dehydration-induced and dehydration-repressed genes, respectively, these TFs operate as negative regulators of drought tolerance (Jiani Chen et al., 2017). BR biosynthetic dwarf and semi-dwarf mutants were also shown to be drought tolerant (Beste et al., 2011). Somehow, contrasting with these results, it has recently been demonstrated that the overexpression of vascular-specific BR receptor BRI1-LIKE 3 (BRL3) increases the survival rate of Arabidopsis plants exposed to severe drought stress. Interestingly, these transgenic plants do not show reduced growth, which is typically associated with drought-resistant BR mutants, and retain the same RWC as wild-type plants. As previously mentioned, these transgenic plants displayed an osmoprotectant signature (proline, trehalose, sucrose, and raffinose) in response to drought, with the corresponding biosynthetic and metabolic genes unregulated in the root phloem.

Challenges in Breeding for Drought Tolerance

Recurrent drought has become the most prominent cause of reduced yield, grain quality and threat to food security and livelihoods (Havrlentová et al., 2021). Challenges in breeding for drought tolerance were reported. There are limited research efforts that identified key root traits in selecting and improving drought-tolerant wheat. Notably, improved root system attributes (e.g., deep and wide-spreading) are desirable for breeding drought-tolerant wheat cultivars (Siddiqui et al., 2021). Nevertheless, there is a need to identify key root traits to improve or develop cultivars with improved root attributes and aid in marker-assisted selection. Complementarily, there is a lack of simple and efficient phenotyping methods to improve root attributes as they are labor intensive and require destructive sampling (Abdolshahi et al., 2015; Khodaee et al., 2021). Developing new high-throughput phenotyping methods that will promote systematic phenotyping of root attributes is of paramount importance. There are limited research efforts that identified key agro-physiological traits in selecting and improving drought-tolerant crops. This is because most drought-adaptive and constitutive traits are controlled by polygenic epistatic and unstable QTL, which are highly influenced by genotype-environment interaction (Ashraf, 2010; L. Li et al., 2019; Mwadzingeni et al., 2016; Mwadzingeni, Shimelis, et al., 2017; Pinto et al., 2010; Tahmasebi et al., 2016). This renders low selection efficiency for superior genotypes (Langridge & Reynolds, 2015). Genotype–environment interactions are manifested through crossover ranking and rank inconsistencies when using different indices in identifying drought-tolerant genotypes (Semahegn et al., 2020). Identifying genes associated with drought stress tolerance and their expression and bridging the gap between theoretical research and applied crop breeding is another challenge for brought tolerance breeding. This can be tackled by establishing concerted research groups to reveal the genetic, epigenetic, transcriptomic and metabolomic bases of agro-physiological and root attributes associated with drought tolerance in wheat (Guo et al., 2020; Ma et al., 2017; Sun et al., 2021). The large genome size (17 GB) of wheat makes it comparatively more difficult to identify genetic loci controlling key agro physiological traits conferring drought tolerance in wheat due to its complex genetic background. In this regard, identifying stable QTL or establishing marker–trait associations under contrasting water regimes is crucial for improving drought tolerance using marker-assisted selection (MAS). Extending the genetic analysis research into applied breeding beyond QTL detection has been minimal due to the lack of robust phenotyping and the need for translational genetics. Furthermore, existing mapping populations are routinely used, needing the development of new drought-suited populations. Therefore, new mapping populations sourced from genetically and complementary genotypes will provide avenues for improved drought tolerance.

Opportunities for Drought Tolerance Breeding

Exploring Mechanisms of Drought Tolerance

There are different mechanisms of drought response, including drought escape, drought avoidance and drought tolerance (Farooq et al., 2014). Drought escape is an adaptive trait that enables the plants to grow and complete their life cycle before the beginning of severe drought (Shavrukov et al., 2017). Early heading, flowering and maturity, reduced plant height and short growth cycle are unique attributes to escape dry spells (Dolferus, 2014). In particular, early heading, flowering and maturity are major drought escape mechanisms that allow the completion of the life cycle before the onset of terminal drought stress, which is common in most rain fed agro-systems (Shamuyarira et al., 2019). Drought avoidance includes the ability for enhanced uptake of available water and nutrients by a longer or deeper root system. This mechanism is associated with a slow growth rate, small or closed stomata, decreased leaf area, reduced photosynthetic activity and low cell metabolism (Shavrukov et al., 2017). Root traits such as increased root biomass, root length density and rooting depth are key drivers of drought avoidance (Kashiwagi et al., 2005). Decreased leaf area is one of the drought avoidance attributes which results in reduced water loss through transpiration (Kapoor et al., 2020). Drought tolerance is the ability of the plant to maintain its growth, development and reproduction under drought stress conditions. Early maturity and reduced leaf area are common attributes in drought-adapted genotypes. Therefore, understanding plant response to drought tolerance at all growth stages is paramount for breeding.

Exploring Selection Indices for Drought Tolerance

Use of the target selection and production environments and water stress management remain fundamental approaches in drought tolerance improvement (Keneni et al., 2016; Monneveux et al., 2012). (Abdolshahi et al., 2015) reported three approaches for breeding drought tolerance. These include (1) breeding for higher yield under non-stress conditions, (2) breeding for maximum yield under drought prone environments and (3) breeding for drought tolerance using selection indices (traits). For enhanced selection efficiency under non-stress conditions aimed at improving performance under the target drought-prone environment, the procedure assumes the trait(s) measured in two different environments not as one but as two traits correlated genetically. This is because the physiological and genetic mechanisms and the genes required for superior performance may be different under these environments (Keneni et al., 2016). High genetic correlation of traits under complementary selection environments guarantees higher selection responses for yield and yield influencing traits. Traits with high heritability, genetic advance and genetic gains are essential for direct and indirect selection for better grain yield under different environmental conditions (Farshadfar et al., 2012; Isack Mathew et al., 2019). Indirect selection involves a selection of one trait via another, while direct selection involves the per se selection of the target trait (Keneni et al., 2016). The use of integrative traits accompanied by the development and application of new and advanced technologies could accelerate the phenotypic selection of drought adaptive traits and consequently improve yield in marginal/low-yielding environments. Agronomic traits such as early heading, anthesis, maturity, spike morphology and reduced plant height have been widely targeted in drought tolerance breeding programs (Mkhabela et al., 2019; Sheoran et al., 2019; Sobhaninan et al., 2019). These traits have been used in direct or indirect selection for grain yield and drought tolerance in wheat (Isack Mathew et al., 2019; Mkhabela et al., 2019, 2020; Sobhaninan et al., 2019). Drought response varies across the source populations. Hence evaluating each population is necessary for simultaneous improvement of yield and drought tolerance. Some physiological traits have been recognised as reliable, cost-effective and noninvasive methods for automated high-throughput phenotyping in crop breeding programs. According to (Monneveux et al., 2012), physiological traits can be used to select parental genotypes to be used in cross formation. Hence, physiological traits are useful as direct selection criteria for screening populations to eliminate undesirable segregants across generations. (Sallam et al., 2019) have extensively reviewed drought tolerance-related physiological traits and advances in breeding and genetics research. Key physiological traits are osmotic potential, stay-green, leaf area, relative water content, canopy temperature, normalised difference vegetative index (NDVI), leaf water status and stem water-soluble carbohydrates (WSC) (Afzal et al., 2017; Bayoumi et al., 2008; Bowne et al., 2012; Dong et al., 2016). Stay-green is the ability of a genotype to remain green and continue undertaking photosynthesis due to higher chlorophyll content compared with other genotypes under drought stress (John T. Christopher et al., 2016). Such genotypes have improved performance under drought conditions with higher grain yield and biomass production (Calderini & Reynolds, 2000; Junbo Chen et al., 2010). SeriM82, a high yielding cultivar released in 1982 exhibited a stay-green phenotype by maintaining green leaf area longer during the grain filling (J. T.

Christopher et al., 2008). NDVI is an indirect selection method for stay-green and yield potential (Babar et al., 2006), while CT denotes the plant's interaction with the soil and atmosphere whereby plants can mine water under water-restricted conditions (Lopes & Reynolds, 2010). Osmotic adjustments occur when molecular weight accumulates in lower levels of organic solutes. Leaf water status depends on the cell osmotic conditions and water transportation from plant shoots (Lawlor & Cornic, 2002). Canopy temperature (Afzal et al., 2017) and RWC (Sobhaninan et al., 2019) were major yield determinants. Grain yield was associated with CT at both vegetative and grain filling stages (Tahmasebi et al., 2016) and NDVI under rain fed conditions (Shi et al., 2017). Furthermore, stem WSC remobilization during grain filling contributes to grain yield under drought stress (M. Li et al., 2020). Relatively better yield under drought stress can be achieved by incorporating drought adaptive biochemical traits from genetically diverse and unrelated parents (Mkhabela et al., 2020). Some of the biochemical traits for drought tolerance include soluble sugar content, chlorophyll content, gas exchange, proline content, carbohydrate content, and superoxide dismutase concentration (Afzal et al., 2017; Mkhabela et al., 2020; Mwadzingeni et al., 2016). Proline content regulates nitrogen accumulation and contributes to membrane stability (Javadi et al., 2008). Gas exchange is among the key traits susceptible to drought stress. Gas exchange parameters include photosynthetic rate, stomatal conductance, chlorophyll content and water use efficiency. Drought stress tolerance in wheat was associated with high antioxidant enzyme activity, i.e., catalase, glutathione reductase and peroxidase and elevated S-metabolites, i.e., methionine cysteine and glutathione (Hassan et al., 2015; Islam et al., 2015). Synthetic derivatives (SYN-DERs) accumulated more soluble sugars, superoxide dismutase concentration, and proline content under drought stress (Afzal et al., 2017). Proline content was significantly correlated with grain yield suggesting selection efficiency of this trait under drought stress (Mwadzingeni et al., 2016). A reduction in chlorophyll content denotes decreased photosynthesis efficiency (I. Mathew et al., 2018).

Genetic Variation as a Source of Drought Tolerance

Genetic variation is the pillar for improving quantitative traits such as yield components and drought tolerance (Mwadzingeni, Figlan, et al., 2017). Genetic variation can be enhanced via the introduction of existing varieties, developing segregating materials through local or international nurseries, hybridisation and mutation breeding. The extent of natural variability changes with time and space due to evolution, natural selection, artificial selection, mutations, gene flow and genetic drift (Ren et al., 2012). The development of cultivars with improved adaptation to biotic and abiotic stresses, including drought stress, hinges on identifying suitable genetic resources with adequate and functional genetic variation for target traits. The use of parental lines of divergent genetic backgrounds, including unrelated and complementary genetic resources possessing suitable drought-adaptive and yield-enhancing traits, ensures the development of superior breeding populations (E. K. Liu et al., 2016; Mkhabela et al., 2019). Important sources of genes for economic traits in wheat include landraces, elite breeding lines, synthetics and wild relatives (Aberkane et al., 2020; Afzal et al., 2017; Bhatta et al., 2018). Among these genetic sources, landraces and drought-adapted varieties are ideal for use in pre-breeding and breeding programs. These genetic resources have high cross-compatibility, wide-adaptation and are rich in farmer- and consumer-preferred traits (Mwadzingeni, Figlan, et al., 2017). Furthermore, improved or breeding lines are essential for the creation of genetic variation with less linkage drag associated with undesirable genes or rare alleles. Some wheat genetic resources such as Dharwar Dry (originated from India), Drysdale, Excalibur and Gladius (from Australia) are widely used in developing genetic populations and drought-tolerant lines (Balouchi, 2010; Edwards et al., 2012; Kirigwi et al., 2007; Yadav et al., 2015). The reported genetic resources are divergent in transpiration efficiency, drought tolerance, stay-green and high water use efficiency. The major sources of genetic variation in wheat breeding programs are described below.

Landraces

Landraces are genetically heterogeneous breeding stocks adapted to their ecologies and farming systems. They are excellent sources of genes for drought tolerance (Blum et al., 1989; Wasaya et al., 2018). Aragon 03 was one of the landraces selected for its drought tolerance in the 1940s and is widely cultivated in Spain. This cultivar has exhibited durable drought tolerance and improved traits such as higher pre-anthesis biomass production in different conditions (Acreche et al., 2009). A Japanese variety, 'Norin10' had the *Rht* dwarfing genes (*Rht1* and *Rht2*) (Swaminathan, 2014) while the Aka Komugi landrace harboured the dwarfing *Rht8c* allele (Lopes et al., 2015) that contributes to drought tolerance. Landraces have not been widely used in breeding programs due to a lack of information on their utility and pedigree, limited availability of descriptors, loss of essential alleles due to evolution and domestication processes and the presence of undesirable alleles that may lead to linkage drag. The exploitation of landraces should be prioritized to deliver important alleles/traits in breeding programs, including drought tolerance.

Synthetics

Synthetic hexaploid wheats (SHWs) developed by artificial hybridization between tetraploid wheat (*T. turgidum*) and goatgrass (*A. tauschii*) are valuable sources of drought tolerance genes (C. Liu et al., 2019; Song et al., 2017). The major limiting factor in wheat is the narrow genetic variation in the D-genome, thus the SHWs were developed to increase diversity in D-genome for drought tolerance. Useful genes are identified and introgressed via synthetic derivatives or advanced synthetic backcross lines (SBLs).

BIOTECHNOLOGY METHODS IN DEVELOPING DROUGHT TOLERANCE PLANTS

Use of Classical Breeding

Conventional breeding for developing drought tolerance in plants involves the art of hybrid cross to develop new and improved cultivars. Retrospectively, filed crop breeding approaches have increased yields through selection and combination of identifiable characteristics. The breeding program requires the identification of genetic variants to drought stress and other abiotic stress among crop cultivars where the different genetic traits are introduced into varieties with the required features (Kiriga et al., 2018). This method has been used for ages in breeding programs of cereal crops. Due to the existence of tolerance variability to a large extent among plants to environmental stress, the existence of stress regulatory genes in plants to abiotic and biotic stress has been long accepted worldwide. Traditional breeding techniques have demonstrated the fact that heritable traits conferring stress tolerance are regulated by a spectrum of genes expression synergistically, which may explain why genetic engineering of plants with drought tolerance are cumbersome (Aguado-Santacruz et al., 2007). The expression of single gene encoding functional proteins like late embryogenesis abundant proteins, antifreeze proteins, and molecular chaperones, would normally confer some level of tolerance to stress but do not completely give sustained tolerance to the majority of environmental stresses. Nevertheless, as the plants develop and evolve, a composite of molecular interactions may lead to their sustenance in water limitation alongside other environmental stresses and in this way, a set of regulatory genes encoding regulatory proteins have been established. The expression of regulatory proteins among others is central to the expression of genes for defense. Marker-assisted selection is also a technique used in improving drought stress tolerance/resistance. In this technique, relevant quantitative trait locus for drought stress traits are usually added into plants with high yielding potential and thus developed mutant enhanced varieties that have only the major quantitative trait locus. Commonly known molecular markers such as random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism has helped to bring about the development of drought tolerance traits that their expression is independent of environmental effects (Kiriga et al., 2018).

Use of Genetic Manipulation

The response of a plant to drought stress has been studied at different levels such as the 9 40 ecological, cellular, physiological and molecular levels and research in these areas has established a bone technological basis now in use for developing plants with drought tolerance through genetic engineering. The limiting factor to this novel approach of plant modification for improvement is the availability of relevant genes and regulatory elements directly involved in tolerance to drought stress (Aguado-Santacruz et al., 2007). Increasing crop yield and value that are exposed to abiotic stress requires novel techniques to augment classical approaches which are often unable to a large degree prevent damage to a crop. One such novel approach is genomics where a whole genome sequence is analyzed to discover novel and functional genes. Loosely, with the aid of microarrays, 130 genes that are sensitive and may respond to drought stress have been established and these genes are directly or indirectly involved with transcription modulation, ion transport, transpiration control, and carbohydrate metabolism. With the advent of this technique, genes have been uncovered and functional genes for stress tolerance are established. The discovery paves room for another novel technique known as recombinant DNA technology where the genetic makeup of plants can be modified with relevant genes to tolerate environmental stress. Plant transgenesis in contrast with conventional breeding approaches ensures the incorporation of genes of interest into the target plant. de Campos et al. reported the transgenic 'Swingle' citrumelo induced with P5CSF129A gene that encode the key enzyme for proline biosynthesis and accumulation that is crucial in promoting drought tolerance in crops with higher osmotic adjustment (de Campos et al., 2011). With the era of recombinant DNA technology, the development of genetically engineered plants with improved value seems to be a viable approach of crop improvement in contrast to classical or marker-assisted breeding approaches. The development of transformation methods has resulted in an efficient generation of genetically modified plants to sustain crop productivity against abiotic stresses (López-Arredondo et al., 2015).

Through the Genes from Resurrection Plants

Resurrection plants are unique in that they can survive almost complete dehydration from their vegetative parts. They shut down their metabolic systems to tolerate dehydration and the plants are lifeless (Vicré et al., 2004). Plants species whose seeds and vegetative parts can survive severe water loss or are desiccation-tolerant are regarded as resurrection plants (poikilohydric), as opposed to dehydration sensitive plants (homohydric). Resurrection plants make up an outstanding group within the flowering plants. The plants have a unique ability to withstand thorough dehydration of their vegetative components (Xiao et al., 2015). The changes in water levels and cellular responses associated with dehydration in seeds are shown to be similar in resurrection plants that are exposed to metabolic stresses as a result of severe water loss. For plants to successfully withstand complete dehydration needs the concerted expression of thousands of genes that involved 63 metabolic pathways and the utilization of 64 biochemical defense mechanism to protect cellular biological integrity. The gene family of early light induced proteins (ELIPS) is generally over-expressed during dehydration in all studied resurrection plants and may play a central role in safeguarding against photo-oxidative stress of the photosynthetic machinery during extreme dehydration (Van Buren et al., 2019). One such dehydration associated gene (dsp-22) in resurrection plant Craterostigma plantagineum codes for a mature 21 kDa protein which accumulates in the vegetative parts and contrasts to other dehydration associated genes, light is crucial in regulating the expression level of dsp-22. Systemic studies of drought stress in the resurrection plant involve identifying a larger number of genes, metabolites, and proteins that usually respond to desiccation or drought stress. Some of these mechanisms that help cellular protection during extreme dehydration are peculiar to desert species (Bechtold, 2018). (Van Buren et al., 2018) reported that desiccated plant evolves from a combination of gene duplications and network level rewiring of existing seed desiccation pathways.

Through Protoplast Fusion

Protoplasts are cells without cell walls and cytoplasmic membrane forms the outermost layer in such cells. They can be obtained through the activity of some specific lytic enzymes such as cellulose, pectinase or macerozyme to degrade cell wall (Van Buren et al., 2018). Through protoplast fusion, scientists can circumvent mating type and incompatibility group limit to investigate mitochondrial genetics, performed inter-generic protoplast fusion. The fusion of isolated plant protoplasts by electrical stimulation has been studied and routinely employed as an experimental method. Nevertheless, the user of this method is faced with constraints by the composition of the suspension medium (Hayat & Christias, 2010). The protoplast fusion technique has remarkable possibilities for genetic variability and strain enhancement (Dimitrova & Christov, 1992). Protoplast technology is one of the promising techniques that can be used by plant breeders to improve crop varieties.

In vitro Selection Technique

The *in vitro* tissue culture approach which employ the use of a selective medium containing selective agents to select and improve plants with specific features. The technique has offer opportunity to regenerate and induce stress tolerance in plants through the use of selective agents such as NaCl, polyethylene glycol or mannitol, etc which allow preferential growth and survival of desirable features (Abaka et al., 2020; Rai et al., 2011). The explants are either exposed in a stepwise manner with gradual increase in the concentration of the these selecting agents or are exposed to shock treatment where the culture medium contain high concentration of the agents. Plants that survived such environmental exposure are eventually selected. These approach induces genetic variation among the exposed explants in cultured medium and regenerated plants called somaclonal variation which can result in genetically stable traits useful in crop improvement (Abaka et al., 2020; Rai et al., 2011). *In vitro* selection technique for explants demonstrating increased drought tolerance has been reported. Polyethylene glycol (PEG) has been utilized to induce drought stress in plants and the determination of plants that withstand water stress is based on accumulation of consistent solutes primarily proline as well as the presence of antioxidative enzymes such as peroxidases, catalases, and dismutases.

CONCLUSION

Plant drought stress is a major problem in the growth and development of plants. One of the major challenges of the plant biotechnologist is solving the problem of plant drought stress so that it can combat the problem of climatic change and increase in population growth. Recent advances in plant biotechnology has seen remarkable progress in molecular markers selection processes and in developing transgenic plants with increased drought stress tolerant. These approaches have facilitated our understanding of underlying processes in plant responses to drought induced stress. Through plant genetic engineering and molecular marker techniques, drought stress induced genes have been identified and cloned. It therefore means that the applications of biotechnological and molecular approaches such as genomics, proteomics, and transcriptomic that can enhance a better understanding of plant water use efficiency and tolerance to improve yield under drought stress is very promising.

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