

1 Drought Tolerance in Crops: Physiology to Genomics

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Abstract

More frequent and severe drought combined with high temperatures have been recognized as a potential impact of global warming on agriculture. Improving crop yield under water stress is the goal of agricultural researchers worldwide. Direct selection for yield under drought has been the major breeding strategy and was successful in some crops. Drought modifies the structure and function of plants. An understanding of the impact, mechanisms and traits underlying drought tolerance is essential to develop drought-tolerant cultivars. Identification and evaluation of key physiological traits would aid and strengthen molecular breeding and genetic engineering programmes in targeting and delivering traits that improve water use and/or drought tolerance of crops. There is an overlap between different osmotic stresses and the selection of appropriate drought evaluation methods. The benefits of genetic engineering have been realized in crop improvement for quality traits, and several promising genes have emerged in the last decade as candidates for drought tolerance. Combining the physiological traits that would sustain yield under drought, and incorporating elite quantitative trait loci (QTL) and genes underlying these traits into high-yielding cultivars, would be a successful strategy.

1.1 Introduction

Food production worldwide is affected by periodic droughts. Drought is an extended abnormal dry period that occurs in a region consistently receiving a below-average rainfall. Globally, agriculture is the biggest consumer of water, accounting for almost 70% of all withdrawals, and up to 95% in developing countries (FAO, 2007). Out of 1474 million hectares (ha) of cultivated land in the world, 86% comes under rain-fed cultivation (Kumar, 2005). Drought is classified into three major categories (Dai, 2010): (i) agricultural drought; (ii) meteorological drought; and (iii) hydrological drought. Meteorological drought is a period with less-than-

average precipitation, and is often associated with above-normal temperatures that precede and cause other types of drought. Meteorological drought is caused by constant changes in large-scale atmospheric circulation patterns such as high pressure. Agricultural drought is a period with below-average precipitation, less frequent rain events or above-normal evaporation, resulting in reduced crop production and plant growth. Hydrological drought occurs when there is a reduced supply of water or water levels from river streams and other water storage structures such as aquifers, lakes or reservoirs fall below long-term mean levels. A lack of rainfall triggers agricultural and hydrological droughts; but other factors, including

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high temperature, poor irrigation management and external factors like overgrazing and erosion, also cause drought. The proportion of the land surface globally in extreme drought is predicted to increase from 1% to 3% at present to 30% by the 2090s. The number of extreme drought events per 100 years and mean drought duration are likely to increase by factors of two and six, respectively, by the 2090s (Burke *et al.*, 2006). According to the World Bank, drought is the world's most expensive disaster, destroying the economic livelihood and food source for those dependent on the agricultural sector. Much effort is being made by agricultural researchers around the globe to reduce water use by crops to address the challenges that especially affect farmers in drought-prone environments across the developing world.

1.2 Global Impact of Drought On Crop Production

Global climate change, in the form of increasing temperature and fluctuating soil moisture conditions including drought and floods, is projected to decrease the yield of food crops over the next 50 years (Leakey *et al.*, 2006). By 2025, around 1800 million people will be living in countries or regions with absolute water scarcity, and two-thirds of the world population could be under stress conditions (FAO Water; www.fao.org/nr/water/issues/scarcity.html). It has been reported that 2000–2009 was the warmest decade since the 1880s (<http://www.nasa.gov/topics/earth/features/temp-analysis-2009.html>). Over the past 10 years, large-scale periodic regional drought and a general drying trend over the southern hemisphere have reduced global terrestrial net primary production (Zhao and Running, 2010).

The United States Department of Agriculture classified risk in agriculture into seven categories. Among them, yield risk is the most common and has a direct impact on agriculture, which is mostly influenced by water supply and temperature (Motha and Menzie, 2007). Drought has an extensive impact on agriculture as it disrupts cropping programmes, reduces breeding stock, and reduces the assets and farm

inputs. The National Climatic Data Center (NCDC) of the US Department of Commerce estimated that, from 1980 to 2010, a combination of drought and heatwaves caused a total loss of around US\$1825 million (<http://www.ncdc.noaa.gov/img/reports/billion/state2010.pdf>). It has been reported that about 50% of world rice production is affected by drought (Bouman *et al.*, 2005). Most of the 160 million ha of maize grown globally is rain-fed. The average annual yield losses to drought are around 15% of potential yield on a global basis and, as temperatures rise and rainfall patterns change, additional losses of maize grain may approach 10 million t/year, currently worth almost US\$5 billion (Edmeades, 2008). Total wheat production in the wider drought-affected regions of the Middle East and Central Asia is currently estimated to have declined by at least 22% in 2009 compared to 2008 (de Carbonnel, 2009). Drought stress after flowering is one of the most common and serious environmental limitations to yield in pearl millet, resulting in 50% yield loss (Mahalakshmi *et al.*, 1987). Drought is the most damaging abiotic stress to soybean production and, in the USA, dry-land soybeans yield approximately 60–70% less than irrigated systems (Egli, 2008).

Other than the socio-economic impacts, nearly every plant physiological process is directly or indirectly affected by water deficit. Cell enlargement depends on the level of cell turgor; photosynthesis is directly inhibited by insufficient water; and stomatal control of transpiration and CO₂ absorption rely on the water status of guard cells (Giménez *et al.*, 2005). The adverse effect of drought on plant structure and function such as xylem embolism, reduced carbohydrate pool size, leaf and fine root production, on the ability of plants to resist pathogen attacks, the impacts on soil microbial dynamics, decomposition and nutrient-supply processes, and shifting competitive abilities between plant species cannot be underestimated (Ciais *et al.*, 2005). While the negative impact of drought on crop yields is obvious, the adverse effects on crop quality are less recognized. Severe drought can result in a loss in food quality in terms of feed value, starch and lipid concentration, or physical/sensory traits (Wang and Frei, 2011). Crop improvement through conventional breeding and modern biotechnology

both offer potential for substantial progress (Cominelli and Tonelli, 2010).

1.3 Drought Resistance Mechanisms

Understanding the concept and components of drought resistance is a key factor for improving drought tolerance of crops. Drought resistance mechanisms for different crops have been extensively reviewed and summarized from crop physiology, plant breeding and molecular perspectives (Nguyen *et al.*, 1997; Turner *et al.*, 2001; Manavalan *et al.*, 2009; Shao *et al.*, 2009; Mittler and Blumwald, 2010; Todaka *et al.*, 2015). Drought resistance can be classified broadly into three categories (Taiz and Zeiger, 2002): (i) desiccation postponement (the ability to maintain tissue hydration or drought tolerance at high water potential); (ii) desiccation tolerance (the ability to function while dehydrated or drought tolerance at low water potential); and (iii) drought escape where the plants avoid drought by completing life cycles before the onset of a dry period to sustain some reproduction. These drought resistance mechanisms vary with the geographical area, based on soil and climatic conditions. For example, tolerance to extreme drought conditions (air < 0% relative humidity) exhibited by desert-adapted resurrection plants such as *Craterostigma plantagineum* (Bartels *et al.*, 1990) and *Tortula ruralis* (Oliver and Bewley, 1997), is achieved by limiting their metabolic functions. In contrast, most cultivated plants cannot withstand a water deficit less than 85% of relative humidity during the vegetative period (Bartels and Salamini, 2001), and these plants adapt to drought by either dehydration avoidance or dehydration tolerance mechanisms to maintain biological functions. Dehydration avoidance (a plant's capacity to sustain high water status by water uptake or a reduction of water loss in dry conditions) is achieved through the development of a large and deep root system to acquire water from the soil, as well as through the closure of stomata or a non-permeable leaf cuticle to reduce transpiration. Physiological traits such as leaf osmotic adjustment, proportion/quantity of ABA, chlorophyll, proline and soluble sugars; and toxic removal mechanisms such as peroxidase or superoxide dismutase activity contribute to dehydration tolerance (Luo, 2010).

1.4 Physiological Traits Affecting Crop Response to Drought

Effects of water deficit at the whole-plant level are manifested by effects on plant phenology, growth and development, source–sink relations and plant reproduction processes. An understanding of the various physiological traits controlling/regulating crop responses to drought is required for identifying natural genetic variation for drought tolerance. These traits can be broadly classified as shoot- and root-related traits.

1.4.1 Phenology

Plant developmental traits such as early vigour or phenology may be particularly significant in water-limiting conditions (Cairns *et al.*, 2009). Faster phenological development is particularly useful in drought situations where late season drought is prominent. The early planting system of soybeans in the USA is an example where short season cultivars are planted during March–April. The early maturing cultivars start flowering in late April and set pods in late May, thus completing the reproductive stage before the period of possible drought during July–August (Heatherly and Elmore, 2004). Seed size and early seedling vigour were found to be associated with drought tolerance in pearl millet (Manga and Yadav, 1995), wheat (Rebetzke and Richards, 1999), sorghum (Harris, 1996), cotton (Basal *et al.*, 1995), and rice (Cui *et al.*, 2008). While plants could potentially escape and avoid drought, theory and previous findings suggest that there are likely to be trade-offs between these strategies. For instance, early flowering is an adaptive strategy under drought conditions in *Brassica*, where a trade-off between drought avoidance and escape indicates that selection for drought escape through earlier flowering is more important than phenotypic plasticity (Franks, 2011).

1.4.2 Root system architecture

The role of the plant root system is to uptake water and nutrients from the soil through its highly responsive and plastic morphology, which

allows the plant to adjust and exploit the varying soil physical and chemical properties (Armengaud *et al.*, 2009). An increased depth and density of roots is considered a major mechanism for improving water uptake under drought conditions (Turner, 1986). Extensive information is available on the value of root traits in relation to drought avoidance in crops (Courtois *et al.*, 2009; Hochholdinger and Tuberosa, 2009; Hodge, 2009; Maurel *et al.*, 2010; Yamaguchi and Sharp, 2010). In addition, the alteration of root hydraulic conductance by different anatomical and biochemical traits provides the plants the ability to regulate plant water use for the critical crop stages (Vadez, 2014).

Screening for root architectural traits is one of the major bottlenecks in root research due to the difficulties associated with separation of a whole root system from the soil and the huge amount of time and labour requirements for field evaluation. In addition, most of the destructive analysis is done at the end of the experiment and hence monitoring root system development over a crop's life cycle is not feasible. Several encouraging assays using root observation chambers (Singh *et al.*, 2010), soil-less media (Bengough *et al.*, 2004; Manschadi *et al.*, 2008; Manavalan *et al.*, 2010), image-based phenotyping platforms (Hund *et al.*, 2009; Iyer-Pascuzzi *et al.*, 2010) and tools to analyse the images such as RootFlow (van der Wee *et al.*, 2003), EZ-RHIZO (Armengaud *et al.*, 2009) and RootTrace (French *et al.*, 2009) are showing exciting opportunities to understand the root traits and apply them in crop improvement.

1.4.3 Leaf water potential

Leaf water potential (LWP) is recognized as an index for whole-plant water status (Turner, 1982). When irrigated normally, plants transpire and create a negative LWP, which results in the uptake of water. Under water deficit conditions, LWP becomes more and more negative with no water to fill the xylem, resulting in cavitations and leading to the loss of turgor and wilting of plants. Significant differences between cultivars for LWP and its relation with plant performance and yield have been documented

in rice (Jongdee *et al.*, 2002), wheat (Winter *et al.*, 1988), maize (Cary and Fisher, 1971), sorghum (Jones and Turner, 1978), soybean (Djekoun and Planchon, 1991), sunflower (Boyer, 1968), cotton (Grimes and Yamada, 1982) and other crops. LWP can be measured in the field/greenhouse by using a pressure chamber apparatus. Another method is thermocouple psychrometry which is advantageous because of the flexibility to use small sample size and easy measurement of the components of water potential such as turgor pressure and osmotic potential (Boyer, 2010).

1.4.4 Leaf relative water content

Relative water content (RWC) is closely related to cell size and may strongly reflect the balance between water supply to the leaf and transpiration (Fischer and Wood, 1979). Estimation of leaf RWC is quite simple and certainly applicable to a large number of plants. It has been suggested that plant water status, rather than plant function, controls crop performance under drought. Therefore, those genotypes that can maintain higher LWP and RWC are drought resistant simply because of their superior internal water status (Kamoshita *et al.*, 2008). Genotypic differences that exist for RWC under drought are well documented in rice (Courtois *et al.*, 2000), wheat (Schonfeld *et al.*, 1988), soybean (Carter and Patterson, 1985; James *et al.*, 2008) and other crops. A positive relationship was observed between grain yield and RWC measured during the reproductive stage in wheat, where the high-yield selections maintained a significantly higher RWC than the low-yield selections (Tahara *et al.*, 1990). However, studies suggest that differences in RWC among cultivars are highly influenced by plant maturity, adaptation and severity of stress, and hence it may be used as a secondary selection trait (Lafitte, 2002). Boyer *et al.* (2008) found that osmotic adjustment causes discrepancies in RWC values (10–15% lesser values than the actual plant water status) and suggested possible alterations. If maturity differences can be feasibly monitored, with a suitable rehydration procedure, RWC should serve

as a practical and reliable indicator of drought resistance in mass selection programmes.

1.4.5 Stomatal conductance

Stomata, the specialized cells performing gas exchange in plants, account for water loss through transpiration. Transpiration rate is influenced by the diffusion resistance, provided by the stomatal pores and by the humidity gradient between the leaf's internal air spaces and ambient air. Stomata close when LWP decreases (Brodribb and Holbrook, 2003). The plant hormone ABA is involved in stomatal closure and appears to trigger stomatal closure even before significant decline in water potential occurs (Zhang and Davies, 1989; Liu *et al.*, 2003). When leaf RWC falls to around 70%, photosynthesis in most species becomes irreversibly depressed (Lawlor and Cornic, 2002), and thus the resistance of the photosynthetic apparatus to desiccation is also a potential trigger for stomatal closure. Plants exhibit adaptation to drought by stomatal closure (Vignes *et al.*, 1986; Muchow and Sinclair, 1989; Blum, 1996; Price *et al.*, 2002; Reynolds *et al.*, 2005). It is argued that stomatal conductance is not a desirable trait as it affects productivity under non-stress conditions. For regions that depend entirely on rain-fed agriculture, and for dry-land conditions where a conservative response to drying soils is needed, this trait should not be neglected. Successful application of stomatal regulation using partial root-zone drying (Loveys *et al.*, 2000) and regulated deficit irrigation (RDI) in maize (Kang *et al.*, 2000) and horticultural crops (Costa *et al.*, 2007) showed that reducing stomatal conductance by partial irrigation resulted in improved water use efficiency and productivity equivalent to non-stress conditions. Through genetic engineering and mutant approaches, several genes were manipulated to regulate stomatal closure downstream of ABA production (Schroeder *et al.*, 2001). Although non-invasive instruments are available for rapid measurement of stomatal conductance, they have drawbacks such as the control of humidity. Some of the improvements for detecting water stress-induced stomatal closure as a guide to irrigation scheduling are the use of infrared thermometry (Idso *et al.*, 1981) or thermography (Jones, 2004).

1.4.6 Anatomical modifications to reduce water loss (sunken stomata/glaucousness/epicuticular wax/leaf pubescence)

Plants such as *Nerium oleander*, *Ficus* spp., and modified leaves of certain plants (pine needles), avoid drought by sunken stomata, which is an anatomical adaptation. In these species, stomata are sunken below the epidermal plane (Fig. 1.1A). The guard cells are located in a depression, creating a more humid microclimate in the boundary layer. Air in the depression is slightly protected from wind, and any molecule of water that escapes from the stoma may remain in the depression long enough to actually bounce back into the leaf rather than evaporate (Fig. 1.1A, insert). Glaucousness is the waxy covering of the plant cuticle that renders a dull-white or bluish-green cast referred to as bloom in crops such as sorghum and wheat (Fig. 1.1B). Genotypes with low cuticular transpiration rates can conserve RWC in water-deficient conditions. Glossy leaf trait was found to be associated with seedling stage drought tolerance in sorghum (Maiti *et al.*, 1984). A positive association between water use efficiency and glaucousness was reported in wheat (Richards *et al.*, 1986), peas (Sánchez *et al.*, 2001) and maize (Ristic and Jenks, 2002). Leaf pubescence density is considered as an adaptive trait for drought tolerance in soybean (Fig. 1.1C). Pubescent hairs reflect excess radiation and reduce epidermal conductance. A significant negative correlation between epidermal conductance and water use efficiency in soybean (Hufstetler *et al.*, 2007) supports the importance of these traits.

1.4.7 Cell membrane stability

One of the cellular components that is intensively affected by water stress is the cell membrane. During a water deficit, membrane permeability increases, leading to disruption of the cell membrane and to the efflux of electrolytes. The measurement of ion leakage and further estimation of membrane stability have been used as criteria for selection for drought resistance in wheat (Blum and Ebercon, 1981) and rice (Tripathy *et al.*, 2000). A positive association between cell membrane stability (CMS)

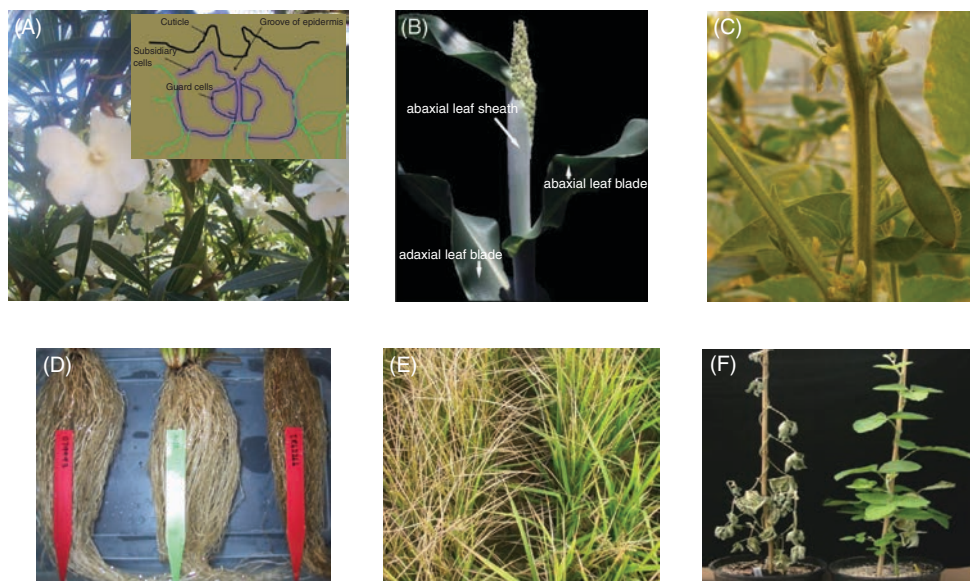


Fig. 1.1. Anatomical, morphological and genetic modifications in plants exhibiting drought tolerance. (A) Sunken stomata in *Nerium oleander*; insert: anatomy of sunken stomata. (B) Glaucousness in sorghum (Center for Plant Environmental Stress Physiology, Purdue University). (C) Pubescence in soybean. (D) Rice NIL (centre) with QTL for improved basal root thickness (Chamarark and Nguyen). Parents (IR62266 and CT9993) on both sides. (E) Transgenic rice with *sNAC1* gene (right) exhibiting greater drought tolerance than control (left) in field. Courtesy of Dr Lizhong Xiong, China. (F) Transgenic soybean with *AtDREB1* gene (right) exhibiting vegetative stage drought tolerance and control (left) in the greenhouse (Guttikonda *et al.*, 2014). NIL, near isogenic lines; QTL, quantitative trait loci.

and high phospholipid content was observed in drought-tolerant maize cultivars (Premachandra *et al.*, 1991). However, the ion leakage method is time consuming and needs many replications to capture genotypic differences.

1.4.8 Oxidative damage and reactive oxygen species scavenging indicators

Reactive oxygen species (ROS) are produced as by-products of various metabolic pathways localized in different cellular compartments. In plants, ROS are produced continuously, predominantly in chloroplasts, mitochondria and peroxisomes. The equilibrium between production and scavenging of ROS may be perturbed by a number of adverse abiotic stress factors, including drought (Apel and Hirt, 2004). Abnormal increase in ROS leads to irreversible damage to the cellular membrane and photosynthesis, and ultimately to cell death. ROS molecules such as hydrogen peroxide, superoxide and singlet oxygen are detoxified by

non-enzymatic antioxidants such as ascorbate and glutathione (GSH), as well as tocopherol, flavonoids, alkaloids and carotenoids. Enzymatic ROS-scavenging mechanisms in plants include superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione peroxidase (GPX) and catalase (CAT) (Vernoux *et al.*, 2002). Several methods are available for quantification of ROS in plants (Verslues *et al.*, 2006). The role of ROS in stomatal regulation in *Arabidopsis* (Pei *et al.*, 2000), auxin signalling and gravitropism in maize roots (Joo *et al.*, 2005), and maintenance of root elongation in maize under water deficit conditions (Zhu *et al.*, 2007) indicate that ROS may perform as positive signalling molecules to regulate the response of plant growth to water stress.

1.4.9 Osmotic adjustment

Osmotic adjustment (OA) is defined as the active accumulation of organic solutes intracellularly

in response to an increasing water deficit. OA is considered a useful trait because it provides a means for maintaining cellular turgor when tissue water potential declines. OA has been shown to maintain stomatal conductance and photosynthesis at lower water potentials, delayed leaf senescence and death, reduced flower abortion, improved root growth and increased water extraction from the soil as water deficit develops (Turner *et al.*, 2001). Consistent differences in OA exist among cultivars which can be associated with plant production under drought stress in *Brassica* (Kumar *et al.*, 1984), sorghum (Ludlow *et al.*, 1990), wheat (Blum *et al.*, 1999) and maize (Chimenti *et al.*, 2006). Beneficial effects of OA on root growth under water deficit conditions clearly show the value of this trait under water-limiting conditions.

1.4.10 Canopy temperature

Canopy temperature is considered as a sister/surrogate trait in relation to stomatal conductance, as they are directly related. Plants with high stomatal conductance transpire more and thus maintain a cooler canopy temperature. Canopy temperature and its depression relative to ambient air temperature indicate how much transpiration cools the leaves under the hot and humid climate that is typically associated with drought stress. Canopy temperature, measured with an infrared thermometer, has been used as a secondary trait to evaluate cultivars for drought tolerance in rice (Garrity and O'Toole, 1995), wheat (Reynolds *et al.*, 2007), sorghum (Blum, 1988) and maize (Araus *et al.*, 2012). New remote-sensing tools based on the use of thermal imaging to estimate plant water status at field level are gaining in importance. Use of thermography has been proposed for high-throughput phenotyping of tropical maize adaptation in water stress (Zia *et al.*, 2012).

1.4.11 Chlorophyll fluorescence and reflection indices

Drought affects the photosynthetic activity of leaves as a consequence of altered chlorophyll *a* fluorescence kinetics. The analysis of changes

in chlorophyll fluorescence kinetics provides detailed information on the structure and function of the photosynthetic apparatus, especially photosystem II (Strasser *et al.*, 1995). Measurement of chlorophyll fluorescence was used as a non-destructive measure of drought avoidance in wheat (Araus *et al.*, 1998), barley (Oukarroum *et al.*, 2007), rice (Pieters and El Souki, 2005) and maize (Earl and Davis, 2003). Use of spectral reflection indices and imaging for crop monitoring would allow us to detect stress at an early stage (Virlet *et al.*, 2015).

1.4.12 Effective use of water

Water use efficiency (WUE) has been used widely as a breeding target in water-saving agriculture (Condon *et al.*, 2004). High WUE, which is the ratio between the amount of dry matter produced per unit of water applied, could contribute to crop productivity under drought. The positive association between WUE and total biomass yield in a drought environment suggests that improvement of the WUE of a crop plant should result in superior yield performance if a high harvest index can be maintained (Wright *et al.*, 1996). Carbon-isotope analysis has been used as a tool in selection for improved WUE in breeding programmes for C_3 species (Farquhar and Richards, 1984). A surrogate method to determine WUE is the measurement of ash content and potassium concentration (Tsialtas *et al.*, 2002). Another relevant trait related to WUE is transpiration efficiency (TE), which is the assimilation or dry matter accumulation per unit of transpiration (Fischer, 1981). TE is under genetic control and excludes the amount of water lost by soil evaporation, and hence should be considered as a potential trait. However, under most dry-land situations where crops depend on unpredictable seasonal rainfall, the maximization of soil moisture use is a crucial component of drought resistance (avoidance), which is generally expressed in lower WUE (Blum, 2005). Blum (2009) suggested the term effective use of water (EUW), which implies maximal soil moisture extraction for transpiration (constitutive root traits) which also involves reduced non-stomatal transpiration and minimal water loss by soil evaporation (shoot adaptive traits).

1.5 Overlap Between Different Osmotic Stresses

The osmotic stresses (drought, salinity and cold) are often inter-related and result in the disruption of homeostasis and ion distribution leading to the arrest of shoot growth, cell division and expansion. Recent molecular and genetic studies have revealed that the signalling pathways induced in response to these osmotic stresses involve many signal components such as histidine kinases (HK), mitogen-activated protein kinases (MAPK), Ca²⁺-mediated salt overly sensitive signal transduction (SOS), calcium-dependent protein kinases (CDPK) and phospholipid signalling. These signalling compounds in turn trigger transcription factors such as dehydration-responsive (DREB) and ABA responsive elements (ABRE), and zinc finger proteins (ZFP) (Wang *et al.*, 2003). These transcription factors activate the synthesis and differential accumulation of osmoprotectants, late embryogenesis abundant proteins, antifreeze proteins, chaperones (heat shock proteins) and ROS-scavenging molecules (aldose/aldehyde reductases, thio redoxins, peroxiredoxins), which are common among these stresses (Bartels and Sunkar, 2005). Overexpression of dehydration-responsive DREB1/CBF also increased the tolerance of transgenic plants to freezing, drought and salt stresses, suggesting that the system is important for the development of stress tolerance in plants (Seki *et al.*, 2003). Recent studies indicate that cross-talk exists among cytokinins (CK), ABA and osmotic stress-signalling pathways, and that CK signalling and CK metabolism may play crucial roles not only in plant growth and development but also in osmotic stress signalling (Tran *et al.*, 2010).

1.6 Drought Evaluation Methods

In order to comprehend the balance between the different drought-tolerance traits and their values to plants, it is critical that drought evaluation studies include measurement of both plant growth condition (soil water status) and plant responses including tissue water status and its regulators such as leaf area and stomatal conductance (Jones, 2007). With the transgenic and mutant approaches to identify/characterize

gene function under stress, the most important requirement is reliable and repeatable drought evaluation methods. Specific physiological and biochemical conditions had to be met to test these plants in growth chambers, greenhouses or in field conditions (Boyer, 2010). Technologies such as automated plant phenotyping platforms to study the plant responses to soil water deficit under controlled conditions (Granier *et al.*, 2006), automated rotating lysimeter systems (Lazarovitch *et al.*, 2006), non-destructive measurement of plant water status over time using portable nuclear magnetic resonance equipment (Capitani *et al.*, 2009) and other precision equipment to quantify plant water use should be exploited. Rain-out shelter facilities provide a useful measure to evaluate germplasm in field conditions with precise control over irrigation. The emerging field of phenomics focuses on the characterization of the whole-plant phenotype. The Plant Accelerator (formerly the Australian Plant Phenomics Facility) is a world-leading plant growth and analysis facility based at the Waite Campus of the University of Adelaide. This facility utilizes digital imaging technologies, high capacity computing and robotics, which allow the dissection of traits that contribute to drought and salinity tolerance for large populations of plants (Furbank, 2009; Rajendran *et al.*, 2009; Berger *et al.*, 2010; Harris *et al.*, 2010; Munns *et al.*, 2010). Plant phenotyping has become an increasingly important tool to quantify the link between the genotype and the environment, and exciting new discoveries are paving the way to experimentally explore the entire genotype–environment matrix for individual factors and their interactions (Pieruschka and Lawson, 2015).

1.7 Molecular Breeding for Drought Tolerance

Molecular breeding approaches through identification of QTL and marker-assisted selection offer an opportunity for significant improvements in the drought tolerance of crops. To gain momentum in the progress of breeding for drought tolerance, two sets of cutting-edge tools are being actively utilized. One involves the use of molecular markers to better understand the genetic basis of drought tolerance and to select

more efficiently for this trait. For traits that are difficult to phenotype at a large scale, such as root traits and OA, molecular tagging with specific markers will facilitate the efficient identification of genes controlling these traits. The other tool, known as participatory plant breeding, offers a more active role to farmers, who make important contributions to selection for better drought tolerance as they closely observe plant performance. Identification of genetic hotspots in chromosomes through genome mapping across crop species will aid in prioritizing the set of genes to be used for crop improvement under drought. The combination of the above two approaches was employed successfully for breeding drought-tolerant rice (Steele *et al.*, 2006) and sorghum (Harris *et al.*, 2007). In rice, some of the many QTLs for roots are common across different genetic backgrounds (Li *et al.*, 2005). Marker-assisted back crossing has been used to introgress these QTLs into inbred hybrid parental lines for the subsequent production of improved hybrids (Hash *et al.*, 2000). The introgression of QTLs for yield under stress is proving to be a more successful strategy in pearl millet (Serraj *et al.*, 2005). Introgression of QTL for basal root thickness (BRT) improved the BRT by 25–40% in 18 near-isogenic lines of rice (Fig. 1.1D; Chamarark and Nguyen, unpublished data). High-density genetic maps and confirmed QTLs/genes, which are screened across various environments and across genetic backgrounds, are the most important criteria for developing drought-resistant soybeans through marker-assisted selection. The discovery of major QTL(s) representing 47% of the average rice yield under stress (Bernier *et al.*, 2007), 25% of variance in grain yield in pearl millet (Bidinger *et al.*, 2007), a major constitutive QTL for root and agronomic traits in maize (Landi *et al.*, 2010), QTL explaining 66% of phenotypic variance for deep rooting in rice (Uga *et al.*, 2011) and novel root QTL identified from the soybean interspecific population (Manavalan *et al.*, 2015) shows promising opportunities to improving drought resistance through molecular breeding.

QTL mapping using bi-parental populations has limitations because of limited allelic diversity and genomic resolution. A multi-parent advanced generation inter-cross populations (MAGIC) strategy has been proposed to interrogate multiple alleles and to provide increased recombination

and mapping resolution (Bandillo *et al.*, 2013). The increased recombination in MAGIC populations can lead to novel rearrangements of alleles and greater genotypic diversity. The limitation with multi-parental populations is mapping resolution limitations as it depends on meiotic events. In contrast, the genome-wide association study (GWAS) approach provides opportunities to explore the tremendous allelic diversity existing in natural germplasm (Deshmukh *et al.*, 2014). Recently GWAS was used successfully to identify loci associated with carbon-isotope discrimination in maize (Farfan *et al.*, 2015) and soybean (Dhanapal *et al.*, 2015).

1.8 Biotechnological Approaches to Improving Drought Tolerance

1.8.1 Genomics

Novel strategies for gene discovery based on germplasm screening and functional genomic research is needed for developing drought-resistant crops. Genomics research results will supply information on the biology of traits, especially for complex quantitative traits such as drought (Varshney *et al.*, 2005). Plant functional genomics has emerged as a new and rapidly evolving scientific discipline to study the functions of genes. Gene expression profiling through microarrays has been used successfully to identify genes regulating drought resistance in crops (Zinselmeier *et al.*, 2002; Hazen *et al.*, 2005; Wang *et al.*, 2011). Most of the drought-responsive genes identified from transcriptomics are classified into ABA-dependent, ABA-independent and DREB2A/ubiquitination-related mechanisms (Ahuja *et al.*, 2010). Genes associated with the production of osmolytes (raffinose family oligosaccharides, sucrose, trehalose, mannitol and sorbitol), amino acids (proline) and amines (glycine betaine and polyamines) are differentially expressed in response to drought stress (Umezawa *et al.*, 2006).

1.8.2 Proteomics

Proteomics, the systematic analysis of (differentially) expressed proteins, is a tool for the

identification of proteins involved in cellular processes (Jacobs *et al.*, 2000). Proteomics provides information on the amount of the gene products, their isoforms and which post-transcriptional modifications regulate protein activation. Several drought-responsive proteins have been identified by proteomics in different plant tissues (Salekdeh *et al.*, 2002; Hajheidari *et al.*, 2007; Alvarez *et al.*, 2008; Liu and Bennett, 2011; Sengupta *et al.*, 2011).

1.8.3 Metabolomics

Plant metabolism is highly altered in response to drought, and downstream transcript-level changes lead to the alteration in quality and quantity of various metabolites (Shulaev *et al.*, 2008). Metabolic profiling can give an instantaneous snapshot of the physiology and biochemical changes in the cell. Recent developments in the field of metabolomics with techniques such as mass spectrometry (MS), liquid chromatography mass spectrometry (LCMS), gas chromatography mass spectrometry (GCMS) and nuclear magnetic resonance spectroscopy (NMR) makes such comprehensive analysis possible, resulting in the identification of natural variants and phenotypic mutants (Keurentjes, 2009). Metabolomics is becoming a key tool in comprehensively understanding the cellular response to abiotic stress, in addition to techniques currently employed in genomics-assisted selection for plant improvement (Ruan and Teixeira da Silva, 2011). Current approaches are targeted to identify metabolic biomarkers that can predict the phenotypes associated with drought tolerance. Emerging techniques such as Transcriptome-To-Metabolome™ (TTM™) biosimulations (Phelix and Feltus, 2015) are interesting: the simulated results on metabolites of 30 primary and secondary metabolic pathways in rice (*Oryza sativa*) were used as the biomarkers to predict whether the transcriptome was from a plant that had been under drought conditions. The authors identified three metabolic markers including trehalose using this approach.

In addition to gene transcripts, proteins and metabolites, small RNAs (miRNAs, siRNAs) are reported to be involved in adaptive responses to abiotic stresses (Sunkar *et al.*, 2007). The next challenge is to incorporate these genes into the

genetic backgrounds of elite cultivars and hybrids and to evaluate their performance under field conditions.

1.8.4 Genetic engineering

The applications of genetic engineering of food crops have already led to examples of improved drought tolerance and increased yield under drought. Much genetic engineering work has been carried out in *Arabidopsis* with the dehydration-responsive element-binding (DREB) transcription factors of the ABA-dependent pathway IV that was first associated with improved cold tolerance (Gilmour *et al.*, 2004). Several transcription factors belonging to the MYB family were reported to play an important role in both stomatal and non-stomatal responses by regulation of stomatal numbers and sizes, and metabolic components (Saibo *et al.*, 2009). Many genes related to drought tolerance have been tested and characterized in the model dicot plant, *Arabidopsis thaliana*. Since the scope of this chapter is on drought resistance of crop plants, we will exclude the work on *Arabidopsis* and focus on the studies that are pertinent to crop plants. The transgenic/mutant approach resulted in the identification of several drought tolerance-related genes in crops. However, careful assessment of physiological mechanisms and meaningful slow progressive drought experiments is essential to confirm their function in drought tolerance. A comprehensive summary of genetic engineering for drought resistance in crops with known physiological mechanisms is presented in Table 1.1. Several studies showed promising drought tolerance in field screenings (Hu *et al.*, 2006, 2008 (Fig. 1.1E); Wang *et al.*, 2005, 2009; Nelson *et al.*, 2007; Xiao *et al.*, 2007; Oh *et al.*, 2009; Jeong *et al.*, 2010). Transgenic crops, with quantifiable drought-tolerance mechanisms were reported in controlled environmental studies in crops, including alfalfa (Zhang *et al.*, 2005), peanut (Bhatnagar-Mathur *et al.*, 2007), potato (Stiller *et al.*, 2008), rice (Manavalan *et al.*, 2012) and soybean (Guttikonda *et al.*, 2014; Fig. 1.1F). Surprisingly, only a few genetic engineering studies identified genes that showed a role in root growth. Considering the importance of roots

Table 1.1. Genetic engineering for drought resistance in crops with known physiological mechanisms.

Trait	Crop	Gene & promoter	Gene family	Function	Reference
Root architecture	Tobacco	<i>HDG11</i> - constitutive promoter <i>CaMV35S</i>	Homeodomain (HD)-START transcription factor	Overexpression of gene conferred drought tolerance associated with improved root architecture and reduced leaf stomatal density	Yu <i>et al.</i> , 2008
	Rice	<i>OsNAC10</i> - constitutive promoter <i>GOS2</i>	NAM (no apical meristem) ATAF1-2 and CUC2 (Cup-Shaped Cotyledon) (NAC domain)	Overexpression of gene enlarged roots and increased yield by 25–42% under field drought conditions	Jeong <i>et al.</i> , 2010
	Tobacco	<i>CKX1</i> - constitutive promoter <i>WRKY6</i>	Cytokinin oxidase/ dehydrogenase	Root specific overexpression resulted in improved root biomass up to 60% and high survival rate after drought	Werner <i>et al.</i> , 2010
	Rice	<i>DRO1</i> -	Deeper rooting 1-QTL	Under upland conditions, Dro1-NIL showed deeper rooting and maintained better grain filling under drought	Uga <i>et al.</i> , 2013
Relative water content	Rice	<i>RWC3</i> - stress-inducible promoter <i>SWPA2</i>	Aquaporin	Compared to the wild-type (WT) plant, the transgenic rice exhibited higher root osmotic hydraulic conductivity, LWP and relative cumulative transpiration at the end of 10 h PEG treatment	Lian <i>et al.</i> , 2004
	Tobacco	<i>TaPP2Ac-1</i> - constitutive promoter <i>CaMV35S</i>	Catalytic subunit (c) of protein phosphatase 2A	Maintenance of RWC	Xu <i>et al.</i> , 2007
Osmolyte accumulation/ turgor maintenance	Soybean	<i>p5cr</i> -inducible heat shock promoter <i>IHSP</i>	Δ 1-pyrroline-5-carboxylate reductase	Accumulation of proline was positively associated with maintenance of RWC and lesser degree of damage to phytochrome II	De Ronde <i>et al.</i> , 2004
	Potato	<i>TPS1</i> - drought-inducible promoter <i>StDS2</i>	Trehalose phosphate synthase	Maintenance of better water status and delayed wilting under drought	Stiller <i>et al.</i> , 2008
	Cotton	<i>TsVP</i> - constitutive promoter <i>CaMV35S</i>	Vacuolar H ⁺ -PPase gene from <i>Thellungiella halophila</i>	Enhanced drought tolerance and root biomass associated with higher solute content such as soluble sugars and free amino acids, and improved cotton fibre yield	Lv <i>et al.</i> , 2009; Pasapula <i>et al.</i> , 2011; Wei <i>et al.</i> , 2011
		<i>AVP1</i> - constitutive promoter <i>CaMV35S</i>	Vacuolar H ⁺ -PPase gene from <i>Arabidopsis thaliana</i>		
Maize	<i>TsVP</i> and <i>BetaA</i> - constitutive promoter <i>ZmUbi</i>	Vacuolar H ⁺ -PPase gene from <i>Thellungiella halophila</i> and <i>BetaA</i> , encoding choline dehydrogenase from <i>Escherichia coli</i>	Retention of high RWC, less anthesis silking interval and higher yield under drought		

Continued

Table 1.1. Continued.

Trait	Crop	Gene & promoter	Gene family	Function	Reference
	Tobacco	<i>GmERF3</i> - constitutive promoter <i>CaMV35S</i>	AP2/ERF transcription factor	Enhanced tolerance to drought and dehydration through accumulation of proline and soluble sugars	Zhang <i>et al.</i> , 2009
Membrane stabilization	Rice	<i>HVA1</i> - constitutive promoter <i>rice Act1</i>	Group 3 LEA protein	Maintenance of favourable water status and CMS leading to better recovery after drought	Babu <i>et al.</i> , 2004
	Maize	<i>betA</i> - constitutive promoter <i>CaMV35S</i>	Glycine betaine	Increased grain yield under drought due to protection of the integrity of the cell membrane and of the activity of enzymes	Quan <i>et al.</i> , 2004
	Maize	<i>ZmPIS</i> - constitutive promoter <i>ZmUbi</i>	phosphatidylinositol synthase	Overexpression of the phosphatidylinositol synthase gene conferred drought-stress tolerance by altering membrane lipid composition and increasing ABA synthesis	Liu <i>et al.</i> , 2013
Water use efficiency	Peanut	<i>AtDREB1A</i> -stress inducible promoter <i>RD29A</i>	Transcription factor	Increased TE under water stress	Bhatnagar-Mathur <i>et al.</i> , 2007
	Rice	<i>HARDY</i> - constitutive promoter <i>CaMV35S</i>	AP2/ERF-like transcription factor	Improved WUE and drought resistance by increase in adaptive root biomass under drought	Karaba <i>et al.</i> , 2007
	Tomato	<i>NCED</i> - super promoter <i>rbcS3C</i>	9-cis-epoxy-carotenoid dioxygenase	Increased accumulation ABA and improved transpiration efficiency	Thompson <i>et al.</i> , 2007
	Tobacco	<i>TaCRT</i> - constitutive promoter <i>CaMV35S</i>	Calcium binding protein	Better water status, WUE and membrane stability	Jia <i>et al.</i> , 2008
Stomatal regulation	Rice	<i>SNAC1</i> - constitutive promoter <i>CaMV35S</i>	NAC domain	22–34% higher seed setting than WT at reproductive stage	Hu <i>et al.</i> , 2006
	Rice	<i>DST</i> - constitutive promoter <i>CaMV35S</i>	Zinc finger protein	Loss of <i>DST</i> function increases stomatal closure and reduces stomatal density, consequently resulting in enhanced drought and salt tolerance in rice	Huang <i>et al.</i> , 2009
	Canola	<i>FTA</i> , <i>B-RD29A</i> and shoot specific HPR promoter	Farnesyl transferase	Downregulation through RNAi resulted in reduced stomatal conductance, increased yield under drought in field	Wang <i>et al.</i> , 2005, 2009
	Potato	<i>StMYB1R-1</i> - constitutive promoter <i>CaMV35S</i>	MYB transcription factor	Transgenic plants exhibited reduced rates of water loss and more rapid stomatal closing than WT plants under drought stress	Shin <i>et al.</i> , 2011

	Rice	<i>ZmSQS1</i> - constitutive promoter <i>ZmUbi</i>	Squalene synthase	RNAi mediated downregulation of squalene synthase-reduced stomatal conductance and conserved more moisture that led to increased grain yield	Manavalan <i>et al.</i> , 2012
Photochemical efficiency	Tobacco	<i>lpt</i> -senescence associated <i>SARK</i> promoter	Isopentenyl transferase	Delayed senescence and photosynthesis protection through overproduction of cytokinins	Rivero <i>et al.</i> , 2007
		<i>HvCBF4</i> - constitutive <i>maize ubiquitin1</i> promoter	Barley C-repeat DREB factor 3	Maintenance of photochemical efficiency	Oh <i>et al.</i> , 2007
	Maize	<i>CspB</i> -constitutive <i>rice Act-1</i> promoter	Cold shock protein	High photosynthesis, increased yield under drought	Castiglioni <i>et al.</i> , 2008
	Maize	<i>ZmPLC1</i> - constitutive <i>maize ubiquitin</i> promoter	Phosphatidylinositol-specific phospholipase C	High RWC, OA, photosynthesis, yield	Wang <i>et al.</i> , 2008
	Rice	<i>DSM2</i> - constitutive promoter <i>CaMV35S</i>	β -carotene hydroxylase	Drought and oxidative stress resistance by increased synthesis of xanthophylls and abscisic acid	Du <i>et al.</i> , 2010
Antioxidants	Rice	<i>AP37</i> - constitutive promoter <i>OsCc1</i>	Transcription factor encoding <i>Oryza sativa</i> cytochrome <i>c</i> gene	Increased expression of antioxidant genes and maintenance of cellular homeostasis resulting in increased grain yield under field drought	Oh <i>et al.</i> , 2009
	Potato	<i>codA</i> , <i>SOD</i> , <i>APX</i> -stress-inducible <i>SWPA2</i>	Choline oxidase, superoxide dismutase and ascorbate peroxide	Protection from ROS	Ahmad <i>et al.</i> , 2010
	Rice	<i>OsSIK1</i> - constitutive promoter <i>CaMV35S</i>	Receptor-like protein kinase	Reduced water loss through regulation of stomatal density and improved antioxidant activity	Ouyang <i>et al.</i> , 2010

CMS, cell membrane stability; DREB, dehydration-responsive element-binding; DST, drought and salt tolerance–zinc finger protein; LWP, leaf water potential; OA, osmotic adjustment; PEG, polyethylene glycol; ROS, reactive oxygen species; RWC, relative water content; TE, transpiration efficiency; WT, wild type; WUE, water use efficiency.

in drought avoidance and water uptake, more emphasis should be given to develop transgenic crop plants with improved root architecture. Although it is being argued that contribution of a single gene to a complex trait such as drought tolerance is questionable, recent evidence indicates that incorporation of a single gene does impart measurable drought tolerance to crops (Castiglioni *et al.*, 2008).

Another approach to improve drought tolerance by gene manipulation is by knocking down genes. Targeting induced local lesions in genomes (TILLING) is a powerful reverse genetics approach for functional genomics studies. Mutations induced in stress-related genes are being identified using this approach (Guo *et al.*, 2015). Since the technology is non-transgenic, and mutations are stably inherited, the alleles identified by TILLING can easily be incorporated into traditional breeding programmes.

1.9 Conclusion and Perspectives

The United Nations General Assembly declared the period from 2005 to 2015 the International Decade for Action, 'Water for Life', which officially started on World Water Day (22 March 2005) (<http://www.un.org/waterforlifedecade/background.shtml>). The Resolution stated that the main goal of the decade should be a greater focus on water-related issues at all levels and on the implementation of water-related programmes to achieve water-related goals that were internationally agreed upon. Identification of large-effect QTLs controlling yield under drought, targeting genes from those specific regions and pyramiding of a few genes in those regions and introgressing these genes into elite germplasm will be an effective strategy. For example, the maize hybrids AQUAmax® from Dupont Pioneer (Johnston, Iowa, USA) and

Agrisure Artesian™ from Syngenta (Greensboro, North Carolina, USA) were developed using this strategy. Also, recent advances in candidate gene approaches and genetic engineering of crops have shown promising improvements in drought tolerance of crops such as rice, maize, canola and soybean. As another possible strategy, given the wealth of genomics information and examples in model crop species, stacking of genes involved in a particular pathway related to dehydration tolerance should be considered. Recent results from Ramu *et al.* (2016) are encouraging, finding that pyramiding drought-adaptive traits by simultaneous expression of genes regulating drought-adaptive mechanisms resulted in abiotic stress tolerance in groundnut.

Production of crops with several genetically engineered traits may seem a logical step to follow and, as more locally adapted transgenic varieties become available and accepted on the market, these will become the source material into which novel genes will be further incorporated. However, difficulties associated with multiple gene inserts such as trait silencing due to similarities between transgene cassettes or epistatic interactions between transgenes should be addressed (Dhlamini *et al.*, 2005). The recent availability of genome editing tools such as clustered regularly interspaced short palindromic repeat (CRISPR) are opening new avenues to efficiently introduce targeted modifications in the genome. These will allow study of the functional aspects of various components of the genome in diverse plants and offer potential avenues for production of abiotic stress-tolerant crop plants (Jain, 2015). Such novel approaches to simplify and improve the process of precise introduction of single or multiple genes into crop varieties should be explored to enhance drought tolerance and subsequent crop improvement.

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