

Chapter 2

Genetics of Drought Stress Tolerance in Crop Plants

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2.1 Introduction

Ensuring stable crop yields in an era where climate change threatens traditional agricultural practices through altered rainfall patterns and increased urban consumption has become a vital concern in global food security. Projected freshwater availability for irrigation indicates that between 20 and 60 Mha of irrigated cropland may have to be reverted to rainfed management [57]. Formerly irrigated crops would become entirely dependent on rainfall and vulnerable to yield loss due to drought. Biotechnology and mining of germplasm of numerous crop species has resulted in discovery of traits that control water use efficiency (WUE) and improve drought tolerance, but only a few of these traits have been implemented in the field [116]. “We need a Blue Revolution in agriculture that focuses on increasing productivity per unit of water—more crop per drop,” Secretary-General Kofi Annan called for a “Blue Revolution” in agriculture [153]. The Green Revolution drastically increased agricultural productivity by remediating nutrient-poor soils with fertilizer, developing irrigation methods and systems, instigating integrated control strategies for weeds and pests, and selected crops that responded to these changes with high yields. The Blue Revolution proposes to address decreasing water availability and climate change. This requires not just drought-tolerance traits, but a high level of control of WUE while still maintaining high yields required by both industrial agriculture and smallholders. An integrated research approach has emerged as a valuable strategy to tackle these necessities. One component of this approach is the mining and introduction of traits from landraces and wild relatives in order to improve drought

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tolerance and WUE in crop species. In some cases, this has been combined with modern biotechnology in an integrated approach. Recently, drought-tolerant maize hybrids have been released in the United States as a result of biotechnology and introduction of traits from distant relatives [140].

This chapter gives a broad overview of the genetic basis of developmental and cellular responses known in crops to enhance drought tolerance. A number of key processes and genes have been discovered in model systems that show great promise for use in crop species. Furthermore, this chapter also addresses the potential for wild relatives of five major crop species (tomato, potato, wheat, rice, and corn) as sources for genetic improvement. These landraces and wild relatives are often remarkably well adapted to their environment through natural selection and traditional breeding. These relatives provide a resource for mining novel traits for the genetic improvement of cultivated crops that are vulnerable to environmental stress.

2.2 Cellular Mechanisms and Traits Conferring Drought Tolerance

2.2.1 *Genes Controlling Primary and Secondary Metabolism*

Plants respond to environmental stresses through various physiological and biochemical changes. Exposure to drought, high salinity, and low temperature leads to cellular dehydration. This removal of water from the cytoplasm results in a decrease of cytosolic and vacuolar volumes. In response, plants increase the production of specific sets of primary and secondary metabolites that act as osmoprotectants, osmolytes, antioxidants, and stress signals. The net accumulation of these solutes lowers the cellular osmotic potential and draws water into the cell to maintain turgor pressure. Osmoprotectants preserve the cellular apparatus from the damage caused by dehydration, without interfering with the normal metabolic processes at the cellular level. These solutes include amines (polyamines and glycinebetaine), amino acids (proline), soluble sugars (glucose, sucrose, trehalose), and polyols (mannitol, sorbitol and inositol; [172]). Because some crops have low levels of these compounds, the manipulation of genes involved in osmoprotectant biosynthesis pathways is one of the strategies to improve stress tolerance in plants [158].

Polyamines (PAs) are small aliphatic nitrogen compounds that are ubiquitous in all organisms. The biological function of PAs is associated with their cationic nature. In plants, PAs act as regulatory molecules implicated in fundamental cellular processes, including embryogenesis, floral development, and pollen tube growth [185]. Significant accumulation of the three most common PAs, putrescine (Put), spermidine (Spd), and spermine (Spm), occurs during biotic and abiotic stress [198]. Modulation of the PAs biosynthetic pathway by overexpression of ornithine and arginine decarboxylases (*ODC*, *ADC*), S-adenosyl-methionine-decarboxylase

(*SAMDC*), and Spermidine synthase (*SPDS*) resulted in enhanced tolerance to different environmental stresses in rice (*Oryza sativa*; [37, 54]), tobacco (*Nicotiana tabacum*) and tomato (*Solanum lycopersicum*; [7]).

Glycine betaine (GB) is a quaternary ammonium derivative of glycine and is considered a major osmolyte involved in cell membrane protection. In response to various abiotic stresses such as drought and salinity, GBs are accumulated in chloroplasts and other plastids of many plant species. One of the principal roles of GB is that it encourages water influx into cells for maintaining the intracellular osmotic equilibrium and regulates the cascade of signal transduction [156]. The overproduction of GB in various plants including maize (*Zea mays*) [155] and cotton (*Gossypium hirsutum*) [131] by modulation of two key genes involved in GB biosynthesis, *betA* (encoding choline dehydrogenase) and *CMO* (choline monoxygenase), results in improved yield production under stressful field conditions.

In addition, the accumulation of proline under stress conditions in many plant species has been correlated with stress tolerance. Proline (Pro) is a versatile amino acid that is essential both as a component of protein and as a free amino acid. To avoid cellular dehydration, proline facilitates water uptake and reduces the accumulation of Na^+ and Cl^- [12]. In plants, the Pro biosynthesis takes place in the cytosol and in the plastids. The principal precursor, glutamate, is converted to proline by two consecutive steps catalyzed by pyrroline-5-carboxylatesynthetase (*P5CS*) and P5C reductase (*P5CR*). The degradation of proline occurs in mitochondria by the reverse action of proline dehydrogenase (*PDH*) and pyrroline-5-carboxylate dehydrogenase (*P5CDH*; [113]). The modulation of expression of these genes significantly enhances endogenous levels of proline and increases drought stress tolerance in wheat (*Triticum aestivum*; [193]) as well as in rice [177], promoting growth, antioxidant defense, and decreasing uptake of Na^+ and Cl^- .

The manipulation of osmoprotectant accumulation was also successfully used with the reduced forms of sugars such as glucose, sucrose, fructose, and trehalose. Sugars provide carbon for cellular metabolism and regulate growth and development in plants. During salinity and drought stresses, sugars and sugar alcohols act as osmoprotectants regulating the osmotic adjustment, protecting the membrane by interacting with protein complexes and enzymes, and scavenging toxic ROS [191]. The regulation of trehalose activity occurs by expression of trehalose synthesis-related genes: trehalose phosphate synthase (*TPS*) and trehalose-6-phosphate phosphatase (*TPP*), improve tolerance to abiotic stresses in rice [66] and alfalfa (*Medicago sativa*; [178]), decreasing aggregation of denatured proteins [13, 112]. Similarly, sugar alcohols including mannitol, sorbitol, and inositol improve stress tolerance of plants. Mannitol is the most common polyol in nature and is synthesized in mature leaves from mannose-6-phosphate by the combined action of mannose-6-phosphate reductase (*M6PR*) and a mannose-6-phosphate phosphatase. It is then translocated through the phloem and oxidized to mannose by mannitol dehydrogenase (*MTD*) or stored and used as a carbon source. During abiotic stress, mannitol is accumulated in the cytosol to act as a scavenger of hydroxyl radicals and to stabilize macromolecules [47].

Metabolic plasticity as well as biosynthesis and accumulation of osmoprotective compounds are promising mechanisms of plant acclimation to stress. Polyamines,

glycine betaine, amino acids, polyols, and some classes of sugar are active participants in the response to drought and salinity. Therefore, their biosynthetic pathways represent interesting targets for future breeding applications.

2.2.2 *Stress-Induced Regulatory Genes*

Plants respond and adapt to water deficit at both the cellular and molecular levels, by accumulating osmolytes and proteins specifically involved in stress tolerance. Drought induces the biosynthesis of the phytohormone abscisic acid (ABA), which in turn causes stomatal closure and induces expression of stress-related genes. There are two important transcriptional networks activated under abiotic stress conditions in *Arabidopsis*: an abscisic acid (ABA)-dependent signaling pathway and an ABA-independent regulatory network. Transcription factors (TFs) activated by ABA include the AREB/ABF (ABA-responsive *cis*-element binding protein/ABA-responsive *cis*-element binding factor). The AREB/ABF TFs have a bZIP domain and four conserved domains containing SnRK2 phosphorylation sites. Upon phosphorylation, AREB/ABFs are activated and bind to the ABA-responsive *cis*-element (ABRE; PyACGTGG/TC), enriched in the promoter regions of drought-inducible genes. AREB/ABFs function as master transcriptional activators regulating ABRE-dependent gene expression in ABA signaling under drought stress conditions.

Other important transcriptional regulators, such as the MYC and MYB proteins, function as activators in the ABA-dependent regulatory systems [2, 190]. A MYC TF, RD22BP-1 (AtMYC2), and AtMYB2 have been shown to bind *cis*-elements in the RD22 promoter and cooperatively activate RD22 [1]. These MYC and MYB proteins are synthesized after the accumulation of endogenous ABA, indicating that their role is in a late stage of the stress responses. SnRK2 functions upstream of ABA-responsive expression of RD22 and RD29B. In particular, two ABRE motifs are important in the expression of RD29B. Instead, ABRE-like motifs are not involved in the ABA regulation of RD22. Several drought-inducible genes do not respond to ABA treatment, suggesting the existence of an ABA-independent pathway in the dehydration stress response. This pathway is mediated by dehydration-responsive element-binding (DREB)-type TF. DREB2 proteins are members of the AP2/ERF family of plant-specific TFs; they bind to dehydration-responsive element/C-repeat (DRE/CRT) and their conserved DNA-binding motif is A/GCCGAC. Among the eight DREB2 genes in *Arabidopsis*, DREB2A and DREB2B are highly induced by drought, high salinity, and heat stress. Evidence for interaction between the AREB/ABFs and DREB/CBFs has been reported. Lee et al. [119] showed that the DREB1A/CBF3, DREB2A, and DREB2C proteins interact physically with AREB/ABF proteins. These data suggest crosstalk between elements of the ABA-dependent and -independent response pathways.

It has also become clear that changes in gene expression patterns and in RNA processing are involved in stomatal movement. The first TFs for which a role in

stomatal opening/closure has been clearly demonstrated were the Arabidopsis AtMYB60 [46] and AtMYB61 proteins [122]. AtMYB60 is specifically localized in guard cells and its expression is upregulated by signals that induce stomatal opening, such as white and blue light, and downregulated by darkness, desiccation, and ABA treatment, signals that promote stomatal closure. In contrast to AtMYB60, the AtMYB61 gene is mainly expressed in guard cells in the darkness, when stomata are closed.

Two other Arabidopsis MYB have been described for their involvement in stomatal movements: AtMYB44 [103] and AtMYB15 [53]. AtMYB44 expression was induced by ABA and by different abiotic stresses. It was highly expressed in guard cells. AtMYB44 negatively regulates the expression of genes encoding a group of serine/threonine protein phosphatases 2C (PP2Cs) that have been previously described as negative regulators of the ABA signaling. The AtMYB15 gene has a role in the regulation of stomatal closure. In Arabidopsis three other TFs involved in stomatal movements have been characterized: AtERF7 [175], NFYA5 [121], and NPX1 [106]. AtERF7 belongs to the APETALA2/ethylene-responsive element binding proteins (AP2/EREBP) family. This protein binds to the GCC box located in the promoter of its target genes and acts as a repressor of transcription. NFYA5 is a member of the Arabidopsis NF-YA family. Nuclear factor Y (NF Y) is a TF that binds to the CCAAT box, a *cis*-element present in about one fourth of eukaryotic gene promoters. The expression of NFYA5 is upregulated by ABA and drought and the gene is highly expressed in vascular tissues and guard cells.

A novel Arabidopsis transcriptional regulator involved in stomatal movement is nuclear protein X1 (NPX1). This protein is a nuclear factor, without a functional DNA binding motif. It acts as a negative regulator of transcription, probably through the interaction with other proteins that bind DNA.

Two TFs, SNAC1 and DST, involved in the regulation of stomatal movements have been identified in rice [83, 86]. Stress responsive NAC1 (SNAC1) is a member of the plant-specific NAC (NAM, ATAF, and CUC) family of TF that includes 149 members in rice. SNAC1 expression is induced in response to abiotic stresses and is predominant in guard cells under drought conditions. Drought and salt tolerance (DST) is a C2 H2-type zinc finger-containing protein. DST is unique in that its single zinc finger motif is required for both its DNA-binding and transactivation. Huang and colleagues [86] found that DST is involved in a novel H₂O₂-mediated pathway for stomatal closure that is ABA-independent.

2.2.3 Regulation of ROS

An important cellular mechanism conferring drought tolerance is the regulation of reactive oxygen species (ROS). ROS are chemically reactive molecules containing oxygen and they are formed in the metabolism of oxygen and have important roles in cell signaling and homeostasis. It has been established that 1–2 % of oxygen absorbed by plants is used to produce ROS in plants [24]. Overaccumulation of

ROS from abiotic stress contributes to major losses of crop productivity and is an important economic problem for cultivated plants worldwide [69]. Accumulation of ROS causes oxidative stress, which in turn results in oxidative damage to proteins, DNA, and lipids [69]. Increased levels of ROS have been reported during biotic and/or abiotic stresses, such as pathogen attack, wounding, UV irradiation, high light, drought, salinity, and chilling [170]. Acclimation of plants to drought and salinity is often associated with increased levels of ROS, such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (HO), and singlet oxygen (O_2), which are toxic for the cell [74]. These products are produced in chloroplasts by the electron transport chain when CO_2 is limited, in mitochondria during overreduction of the electron chain transport and in peroxisomes when glycolate is oxidized to glyoxylic acid during photorespiration [144]. In addition, the plasma membrane together with the cell wall and apoplast can make an important contribution to drought-induced ROS production. During nonstress conditions, ROS are efficiently eliminated by nonenzymatic and enzymatic antioxidants. However, during drought and salt stress, the production of ROS exceeds the capacity of the antioxidative systems to remove them, causing oxidative stress [192]. In these conditions, the elimination of ROS is a key response to tolerate drought stress. This is mainly achieved by antioxidant compounds such as ascorbic acid, glutathione, thioredoxin, and by oxyreductant enzymes as glutathione peroxidase, superoxide dismutase, and catalase (Table 2.1).

The antioxidant compounds play different roles: ascorbic acid (AsA) is used as a substrate by ascorbate peroxidase to reduce H_2O_2 to H_2O in the ascorbate–glutathione cycle and generates monodehydroascorbate, which further dissociates to ascorbic acid and dehydroascorbate [65]. α -Tocopherol is a lipid soluble antioxidant that acts as lipophilic antioxidant and interacts with polyunsaturated acyl groups of lipids. This in turn reduces the deleterious effects of ROS by stabilizing the membrane and acts as a modulator of signal transduction [60]. Glutathione (GSH) is a tripeptide (γ glu-cys-gly) that reduces disulfide bonds formed within cytoplasmic proteins to cysteines by serving as an electron donor. It has been reported that the conversion ratio of reduced glutathione to its oxidized (GSSG) form during the detoxification of H_2O_2 is an indicator of cellular redox balance. This has been widely reported in plants under various abiotic stresses [81]. The defense against ROS is maintained by detoxifying enzymes such as superoxide

Table 2.1 ROS scavenging antioxidant enzymes, their substrates and products

Enzymatic antioxidant	Reaction catalyzed
Superoxide dismutase (SOD)	$O_2^- + O_2^- + 2H^+ \rightarrow 2H_2O_2 + O_2$
Catalase (CAT)	$H_2O_2 \rightarrow H_2O + \frac{1}{2} O_2$
Ascorbate peroxidase (APX)	$H_2O_2 + AsA \rightarrow 2 H_2O + DHA$
Monodehydroascorbate reductase (MDHAR)	$MDHA + NAD(P)H \rightarrow AsA + NAD(P)^+$
Dehydroascorbate reductase (DHAR)	$DHA + 2GSH \rightarrow AsA + GSSG$
Glutathione reductase (GR)	$GSSG + NAD(P)H \rightarrow 2GSH + NAD(P)^+$

dismutase, ascorbate peroxidase, glutathione peroxidase, and catalase. Superoxide dismutase converts superoxide to H_2O_2 , and ascorbate peroxidase and glutathione peroxidase detoxify H_2O_2 to water, and catalase converts H_2O_2 to oxygen [9]. Several studies have been published about tolerant transgenic plants overexpressing scavenging antioxidant enzymes. Transgenic rice plants overexpressing *OsMT1a*, a gene coding for superoxide dismutase, showed enhanced tolerance to drought together with an increase in catalase and ascorbate activity [203]. Overexpression of ascorbate peroxidase in tobacco chloroplasts enhanced plant tolerance to salt stress and water deficit [15]. Tobacco plants overexpressing *Prosopis juliflora* glutathione S-transferase (*PjGSTU1*) had increased survival over controls under 15 % PEG stress [68]. Tobacco cells silenced in the PDH gene showed an accumulation of proline and enhanced osmotolerance with respect to the wild-type cells [182].

2.3 Developmental Mechanisms and Traits Conferring Drought Tolerance

2.3.1 Genes Controlling WUE: Stomatal Sensitivity and ABA

Stomata are the key organs regulating plant gas exchanges with the environment, responsible for controlling over 98 % of the CO_2 and H_2O exchanged by plants with the outside air [117]. Several endogenous and environmental cues regulate stomatal movements, including hormonal stimuli, atmospheric CO_2 concentrations, presence and wavelength of light, and pathogen attack [46]. Integration of all these signals determines stomatal conductance and therefore photosynthesis and transpiration rate, resulting in plant growth and control of dehydration. WUE is defined by the ratio of water loss to carbon gain [105]. At the leaf level, WUE is determined by the net CO_2 assimilated by photosynthesis divided by the water lost through transpiration [180]. Changes in climatic conditions result in a modulation of WUE. For example, the recent rise in atmospheric CO_2 has resulted in increased CO_2 uptake and reduced transpiration in temperate and boreal forests of the northern hemisphere [105]. An increased WUE is a main target of breeding programs for several important crops and genetic variation has been used to identify quantitative trait loci governing WUE, often using carbon isotope discrimination as a simple measure to quantify WUE. This approach has been used in tomato [137], alfalfa [101], and sunflower [4]. In some instances, quantitative trait loci (QTL) isolation has been followed by identification of candidate genes. This was the case in a recent study in *Pinus*, where the first WUE QTL for which the responsible gene identified was an orthologue of the *Arabidopsis Erecta* gene [50]. This gene encodes a receptor-like kinase involved in the determination of stomatal density and patterning [138]. The identification of genes involved in regulation of stomatal movements has extensively relied on *Arabidopsis*. Several components of the signal transduction cascades activated by high/low CO_2 concentrations and ABA have

been identified and signaling pathways have been elucidated. Responses appear specific to the different stimuli in the early stages and subsequently merge in the downstream transduction pathways [107].

Several plasma-membrane-associated ion transporters are responsible for membrane polarization and depolarization events necessary to induce water exit/entrance in the guard cells that cause stomatal closure/opening, respectively. Slow anion channel-associated 1 (SLAC1) is an S-type anion channel, which, upon activation, transports anions such as malate to the apoplast [67, 189]. Membrane depolarization in turn stimulates the efflux of K^+ ions, followed by osmosis of H_2O . In an opposing role to SLAC1, ABC transporter B family member 14 (AtABCB14) transports malate into the guard cells, thereby preventing stomatal closure [118]. Gated outwardly-rectifying K^+ channel (GORK) is a K^+ outward-rectifying channel expressed in guard cells and largely responsible for the K^+ efflux caused by ABA [56, 82]. Knockout mutants in which *GORK* expression is abolished display an increased water loss caused by defects in stomatal closure [82]. Potassium channel in *Arabidopsis thaliana* 1 (KAT1) is a hyperpolarization-activated inward-rectifying potassium channel that mediates potassium influx into guard cells leading to stomatal opening [21]. Another essential component of stomatal opening is AHA1/OST2 (open stomata 2), the guard-cell plasma membrane H^+ -ATPase responsible for the plasma-membrane hyperpolarization, which initiates stomata opening [142]. Plasma membrane located NADPH oxidases AtRBOHD/AtRBOHF (respiratory burst oxidase homologue D and F) determine the ABA-triggered production of second messenger ROS [115, 173] that activate plasma membrane Ca^{2+} channels, and cause ABA-induced stomatal closure [115].

Regulation of these effectors to induce stomatal closure when water scarcity conditions are perceived is achieved through an increase in ABA concentration within guard cells. This intracellular increase is the result of biosynthesis, translocation from other tissues, or the mobilization of inactive, glycosylated forms. ABA is perceived by the pyrabactin resistance (PYR)/PYL (PYR1-like)/regulatory component of ABA response (RCAR) family of intracellular ABA receptors [133, 151]. Initially identified in *Arabidopsis*, the ABA receptors have since been described in many species such as tomato [179, 71], beechnut [164], strawberry [39], rice [108], sweet orange [163], and soybean [16]. Upon binding to ABA, the receptors undergo a conformational change that enables them to bind to and inactivate protein phosphatase 2C (PP2Cs), a family of major negative regulators of ABA responses including ABA insensitive 1 (ABI1), ABA insensitive 2 (ABI2), homology to ABI1 (HAB1), and PP2CA [64]. When the PP2Cs are bound to the ABA receptors, their activity is inhibited and downstream components are released from PP2C-operated inactivation. In guard cells, the main target of PP2C inhibition is OST1 (open stomata 1, also known as SnRK2.6/SRK2E), a Ser/Thr kinase that constitutes a major hub for the regulation of immediate and transcriptional responses to ABA and is also involved in CO_2 responses. When OST1 is released from PP2C inhibition, several downstream effectors such as TFs (see section above) and proteins located on the plasma membrane get phosphorylated, resulting in the activation of stomatal closure promoters such as SLAC1 and AtRBOHF [67, 109, 173]. By contrast,

OST1-induced phosphorylation results in the inactivation of inhibitors of stomatal closure. For example, phosphorylation at Thr306 results in reduction of the activity of KAT1 [168].

Early signaling components of the CO₂ response pathway have also been isolated. The first identified negative regulator of CO₂-induced stomatal closure is the kinase high temperature 1 (HT1) [76]. *ht1-2* mutants show a constitutive high CO₂ response but still retain the ability to respond to variations in light wavelengths and to ABA, placing HT1 upstream of the merging point of these different signal transduction cascades. Stomatal closure induced by high CO₂ concentrations is promoted by two β-carbonic anhydrases expressed in the guard cells, CA1 and CA4. Analysis of *ca1ca4* double mutants, which retained the ability to respond to shifts in light wavelength and ABA, indicated that carbonic anhydrases act early in CO₂ perception [84]. Triple mutants in which *HT1*, *CA1*, and *CA4* were inactivated showed a constitutive high CO₂ response phenotype similar to that of *ht1-2*, indicating that *HT1* is epistatic to *CA1/CA4*. An upstream regulator of HT1 kinase was recently identified in resistant to high carbon dioxide 1 (RHC1), a mate-like protein [184]. By examining genetic interactions and biochemical properties of the different CO₂ sensing and signal transduction machinery, Tian and colleagues [184] proposed that RHC1 senses carbonic anhydrase-generated increases in carbonic acid and undergoes a conformational change that enables the inhibition of HT1. Inactivation of HT1 removes inhibition of stomatal closure by releasing OST1 from inhibition through phosphorylation.

2.3.2 Architectural: Roots, Stomatal Density, Cuticle and Waxes

2.3.2.1 Roots

The root is an important organ providing water, nutrients, and hormones to the aboveground tissues as well as mechanical support. Root architecture is a term that describes the distribution of roots within the soil profile through space and time [132] and defines the zone of water and nutrient availability to plants. This plays an important role in abiotic stress tolerance, crop performance, and yield. The interactions between developmental programs and the responses to abiotic and biotic environmental stimuli determine the architecture of the roots through which the plant explores the soil [135]. In response to environmental changes, root architecture is modified to increase water uptake efficiency. Therefore, understanding the development and architecture of roots has potential for the exploitation and manipulation of root characteristics to optimize growth in unfavorable environmental conditions [51]. Drought has a major effect on root architecture, with many plants preferentially increasing primary root elongation and suppressing lateral root branching in response to stress. Many plants adapted to drought, such as sorghum, have a naturally more vertically oriented root structure [171]. Recent studies have

identified many genetic components that contribute to root architecture; some of these have the potential to limit crop loss due to adverse environmental conditions [102].

In rice, several genes related to root architecture that confer a yield advantage in conditions of water deficit have been identified [41, 42, 93, 98]. In particular, the NAC family of TFs was characterized with regard to root architecture and some members of this family were overexpressed [211]. One of these, OsNAC9, alters the root architecture enhancing drought resistance and grain yield under field conditions [157]. The authors evaluated the overexpression of the TF OsNAC9 under the control of a constitutive or root-specific promoter in transgenic rice under both normal and drought conditions. In suboptimal water availability, grain yield was found to be improved in both transgenic lines, which showed a reduced lateral root density. Zhan et al. [208] reported that maize recombinant inbred lines with few, but long, lateral roots had substantially deeper rooting, greater leaf relative water content, greater stomatal conductance, and 50 % greater shoot biomass than lines with numerous short roots. In water stress conditions, these recombinant lines had 144 % greater yield than controls.

Hormone balance also plays an important role in the definition of root architecture. Seo and colleagues [169] reported that an Arabidopsis R2R3-type MYB transcription factor, MYB96, regulates lateral root meristem activation under drought conditions via ABA-auxin signaling crosstalk. In this signaling scheme, the MYB96-mediated ABA signals are incorporated into an auxin signaling pathway that involves a subset of *GH3* genes encoding auxin-conjugating enzymes. The activation-tagged mutant overexpressing *MYB96* had a dwarf phenotype and reduced lateral root formation while exhibiting enhanced drought resistance. Expression of the *GH3* genes was significantly elevated, which is consistent with the reduced lateral root formation. In contrast, the *MYB96*-deficient knockout mutant produced more lateral roots and was more susceptible to drought stress. The authors speculate that *MYB96* is a molecular link that integrates ABA and auxin signals that auxin homeostasis during lateral root development, especially under water-deficit conditions. Cao and Li [36] showed that autophagic programmed cell death (PCD) happens in the region of the root apical meristem in response to severe water deficit. They emphasized that ROS accumulation may trigger the cell death process of the meristematic cells in the stressed root tips. Analysis of the Arabidopsis mutant *atbi1-1*, BAX inhibitor-1 (AtBI1), under severe water stress revealed that AtBI1 and the endoplasmic reticulum stress-response pathway modulate water stress-induced PCD. These mutants develop thick and short lateral roots that result in increased tolerance to the water stress. Under severe drought conditions, plants activate the PCD program in the root apical root meristem, so that apical root dominance is removed. In this way, plants can remodel their root system architecture to adapt to the stress environment.

In conclusion, aboveground components of plants are well studied because they are accessible. Roots are less well studied because they are not readily visible and replicating the conditions in which they grow can prove difficult. Root traits, especially root length, density, and depth, have long been seen as critical traits in

order to improve crop adaptation to water stress. It is important to note that drought tolerance is a complex composite resulting from the interaction of root and shoot traits [130]. The size and activity of the root system determines the rate at which the shoot system can produce photosynthates. It is evident that improving yield under drought conditions will require a whole plant growth and functioning approach. Drought adaptation must be evaluated in relation to the timing and severity of drought stress. This may vary according to soil water-holding capacity, moisture availability at crop sowing, timing and quantity of in-season rainfall, and in association with other major abiotic stresses, such as high temperature and salinity. Drought stress frequently occurs along with high-temperature stress, and crosstalk occurs between the responses to these stresses at various levels.

2.3.2.2 Stomatal Density

Stomata are pores on leaf epidermis for both water and carbon dioxide fluxes and play a crucial role in photosynthesis and transpiration processes. The best compromise between photosynthesis and transpiration would maximize CO₂ uptake and minimize water loss, and ultimately achieve the possible maximal WUE. Stomata are, therefore, the primary determinants of plant drought tolerance because crop water loss directly involves stomata [206]. Leaf gas exchanges are greatly affected by stomatal density, defined as pore size or number. Environmental factors, such as light, CO₂, temperature, humidity, and drought, can affect plant stomatal density during the development of plants. Recent advances have identified a number of genes regulating stomatal density and this has made it possible to generate plants with modified stomatal densities and analyze the effect of stomatal density on plant WUE. In *Arabidopsis* it has been demonstrated that *angustifolia3* (*AN3*) functions as a focal regulator of water stress tolerance and WUE by a mechanism that involves transcriptional repression of *YDA*, a MAPKK kinase gene that negatively regulates stomatal development, stomatal density, and transpiration [141]. *Arabidopsis* plants lacking *AN3* activity have high drought stress tolerance because of low stomatal densities and improved root architecture. Such plants also exhibit enhanced WUE through lower transpiration without a reduction in biomass accumulation. The *AN3* was associated with a region of the *YDA* promoter *in vivo*. Mutation in *YDA* significantly decreased the stomatal density and root length of *an3* mutant, thus proving the participation of *YDA* in *an3* drought tolerance and WUE enhancement. These components form an *AN3*–*YDA* complex, which allows the integration of water deficit stress signaling into the production or spacing of stomata and cell proliferation, thus leading to drought tolerance and enhanced WUE.

Also in *Arabidopsis*, Franks et al. [63] found that a reduction in stomatal conductance via reduced stomatal density in epidermal patterning factor (*EPF2*)-overexpressing plants increased both instantaneous and long-term WUE without altering significantly the photosynthetic capacity. Conversely, plants lacking both *EPF1* and *EPF2* expression exhibited higher stomatal density, higher stomatal conductance, and lower instantaneous and long-term WUE. *Arabidopsis* plants with

lower stomatal densities that have reduced transpiration and greater drought tolerance have been found to have little or no loss of nutrient uptake [78].

In rice it has been reported that an Arabidopsis homeodomain–leucine zipper transcription factor enhanced drought tolerance/homeodomain glabrous11 (EDT1/HDG11) was able to confer drought tolerance and increase grain yield in transgenic plants. The improved drought tolerance was associated with a more extensive root system, reduced stomatal density, and higher water use efficiency [207]. Heterologous expression in tobacco of *SIERF36*, a tomato EAR motif-containing transcription factor, leads to a 25–35 % reduction in stomatal density but without any effect on stomatal size or sensitivity [187]. Reduction in stomatal density leads to a marked reduction in stomatal conductance (42–56 %) as well as transpiration and is associated with reduced CO₂ assimilation rates, reduction in growth, early flowering, and senescence. *SIERF36* overexpressing plants have constitutively high nonphotochemical quenching (NPQ) that might function as a protective measure to prevent damage from high excitation pressure. The high NPQ leads to markedly reduced light utilization and low electron transport rates even at low light intensities. Taken together, these data suggest that *SIERF36* exerts a negative control over stomatal density and modulates photosynthesis and plant development through its direct or indirect effects. In Arabidopsis, the expression of *Medicago truncatula* cold-acclimation specific protein 31 (MtCAS31) in response to NaCl, ABA, cold, and drought stress was analyzed [200]. MtCAS31 was significantly upregulated following drought stress. Overexpression of MtCAS31 markedly increased drought tolerance and decreased stomatal density of transgenic plants.

In rice, Liu et al. [123] observed that phytochrome B (*phyB*) mutants exhibited enhanced drought tolerance, suggesting that *phyB* may be involved in the regulation of tolerance to drought stress. They demonstrated that *phyB* mutants exhibited reduced stomatal density and length and showed a decreased transpiration per unit leaf area that contributed to the improved drought tolerance. In these plants, the expression of genes related to stomatal such as Erecta and Expansin gene families were upregulated in the *phyB* mutants by comparison. This suggests that this increased expression in the leaves of the *phyB* mutants probably resulted in the enlarged epidermal cells and therefore the reduced stomatal density without changing the stomatal index. The Arabidopsis GT-2 LIKE 1 loss-of-function mutations (*gtl1*) result in increased water deficit tolerance and higher integrated WUE by reducing daytime transpiration without a reduction in biomass accumulation [206]. The *gtl1* plants had higher instantaneous WUE that was attributable to about 25 % lower transpiration and stomatal conductance but CO₂ assimilation. Lower transpiration was associated with higher expression of stomatal density and distribution1 (SDD1) and an about 25 % reduction in abaxial stomatal density. GTL1 expression occurred in abaxial epidermal cells where the protein was localized to the nucleus, and its expression was downregulated by water stress. GTL1 interacts with a region of the SDD1 promoter that contains a GT3 box, necessary for the interaction between GTL1 and the SDD1 promoter. These results establish that GTL1 negatively regulates WUE by modulating stomatal density via

transrepression of *SDD1*. Using two cultivars with contrasting responses to salinity has been able to demonstrate that reduced stomatal density increased salinity tolerance and WUE under salt stress. Constitutive low transpiration fluxes associated with reduced stomatal density may uncouple plant adaptation and yield reduction under saline stress in a specific agricultural context [18, 150]. It is plausible that through genetic modification of stomatal density, by breeding selection and/or genetic methods, improving crops for better WUE and drought tolerance is achievable. Understanding how multiple signals contrast with the components of stomatal development will be the next challenge. Knowledge of such molecular interactions will elucidate the significance of stomata to whole-plant growth, development, and physiology. Lastly, as genome sequence information of more plant species becomes available, it will also become possible to understand the conservation and uniqueness of the evolution of gene regulatory networks specifying stomatal development.

2.3.2.3 Cuticle and Waxes

The plant cuticle is a hydrophobic coating composed of a cutin polyester membrane impregnated and overlaid with free waxes that provides the last barrier over essentially all aerial plant organs [72]. The cuticle is synthesized by the epidermal cells and it can protect plants from nonstomatal water loss, dust deposits, pollen, and air pollutants as well as biotic and abiotic stresses such as UV radiation damage and bacterial and fungal pathogens [97, 114, 161, 162]. The mechanical structure and chemical composition of cuticle lipids vary considerably between plant species, and in response to environmental stimuli and stresses. Several studies have indicated that drought can induce increased wax deposition on the leaf surfaces of different plant species, including *Arabidopsis* [204], cotton [28], peanut [166], and tree tobacco [34]. The importance of cuticle function is highlighted by studies using mutants defective in cuticle biosynthesis, which often do not survive when germinating under normal conditions but can be rescued by high humidity [203].

Increased levels of cuticular waxes have been associated with enhanced drought tolerance in oat [22], rice [92], and sorghum [100]. A mutant of wild barley, *eibil*, with a very thin cutin layer, was hypersensitive to drought [40]. Breeding for greater tolerance and yield under drought conditions led to increased amounts of cuticle waxes, further confirming the connection between drought tolerance and cuticle properties [70]. Thus, the activated biosynthesis of cuticle waxes appears to be an established plant response to dry conditions.

Many genes coding for enzymes involved in the biosynthesis of cuticle components have been isolated and characterized [99, 167, 169].

Transcription factors (TFs) are involved in the regulation of biosynthesis and accumulation of cuticle components. Most of these belong to one of three different families: ethylene responsive factors (ERFs), myeloblastosis family (MYB) TFs, and homeodomain–leucine zipper class IV (HD-Zip IV) factors [5, 31, 45, 96, 169, 209]. Overexpression of these TFs leads to changes in cuticle accumulation and/or

composition often increasing stress tolerance. In many cases, overexpression of these TFs negatively affects plant growth and yield [5, 209]. Recently, Wang et al. [195] isolated a *CER1* homologue *CsCER1*, a gene involved in alkane biosynthesis, in cucumber. They showed that abnormal expression of *CsCER1* in transgenic cucumber plants had dramatic effects on very-long-chain (VLC) alkane biosynthesis, cuticle permeability, and drought resistance. In Arabidopsis, the mutant, *shine* (*shn*) displays characteristics of plant surface defects [5]. When compared with the wild type, leaves of *shn* show a deep shiny green appearance, with a curled structure. They also have altered cuticle permeability, cuticular wax load and structure, and epidermal differentiation. The *SHN* gene encodes an AP2/EREBP transcription factor, and the characterization of two of its homologues suggests that this clade of genes acts in the regulation of lipid biosynthesis required for protection of plants from the environment, including organ separation processes and wounding. The tomato orthologue, SISHN1 transcription factor, was also isolated and the expression analysis indicated that it is induced in response to drought conditions [6]. Overexpression of SISHN1 in tomato produced plants that showed mild growth retardation with shiny and dark green leaves. Expression analysis indicated that several wax-related synthesis genes were induced in transgenic lines overexpressing SISHN1. Transgenic tomato plants showed higher drought tolerance compared to wild-type plants; this was reflected in delayed wilting of transgenic lines, improved water status, and reduced water loss.

Zhou et al. [212] conducted a functional analysis of OsGL1-6 in rice. OsGL1-6 is homologous to *CER1* in Arabidopsis and *Wdal* in rice, universally expressed in vegetative and reproductive organs, and especially highly expressed in leaf epidermal cells and vascular bundles. A phenotypic characterization and drought sensitivity experiments on OsGL1-6 antisense-RNA transgenic plants indicated that OsGL1-6 is involved in cuticular wax accumulation and drought resistance. The drought susceptibility was in agreement with their deficient cuticles and positively correlated with the reduced accumulation of the leaf cuticular wax, implying its role in drought stress resistance. Thus, genetic modification of OsGL1-6 may have great potential for improving the drought resistance of rice. Studies on mutation of the *eceriferum9* (*CER9*) gene in Arabidopsis showed extreme alteration in the cuticular wax profile (especially on leaves) toward the VLC free fatty acids tetracosanoic acid (C24) and hexacosanoic acid (C26; [129]). Relative to the wild type, *cer9* mutants exhibit elevated cuticle membrane thickness over epidermal cells and cuticular ledges with increased occlusion of the stomatal pore. *CER9* is the first described cuticle biosynthesis gene whose deficiency improves both plant response to water deficit and WUE, indicating that *CER9* may encode an important new cuticle-associated drought tolerance determinant. These studies provide evidence that the *CER9* protein is a negative regulator of cuticle lipid synthesis via its putative role as an E3 ubiquitin ligase, similar to Doa10 in yeast. Due to its novel impact on plant water status, elucidation of *CER9*'s cellular function may reveal new molecular breeding and transgenic strategies to improve the drought tolerance and WUE of crop plants.

Systematic studies of the large collection of diverse wax mutants now available should highlight the specific contribution of single wax compounds in

plant/environment interactions as well as in the organization of waxes, together with cutin, in the highly structured cuticle. Clearly, a coregulation of cutin and waxes is required for both environmental and developmental purposes of the cuticle. The transcriptional regulators controlling the deposition of both lipophilic materials throughout plant development must be investigated [23].

2.4 Landraces and Wild Relatives as Sources for Drought Tolerance Traits

2.4.1 Tomato

Global demand for tomato is steadily increasing, particularly in developing areas prone to drought cycles [174]. Therefore, it is necessary to develop more drought-tolerant tomato varieties to address this need. Although there are a growing number of studies designed to investigate the mechanisms of drought response in tomato, most modern varieties are sensitive to a wide range of abiotic stresses [62]. The efforts of the International Solanacee genome project (SOL) and of the 100 Tomato Genome Sequencing Consortium et al. [183], have recently provided a tremendous genomic resource for the research community and the possibility successfully to exploit for tomato breeding for stress tolerance the enormous reservoir of adaptive traits present in wild species and in some *S. lycopersicum* landraces locally adapted to arid environments. There are a number of tomato relatives, including *Solanum chilense* and *S. peruvianum*, adapted to growth under water restriction imposed by their habitat, that are known to be very drought tolerant, but only a few studies have examined in detail drought tolerance-related morphophysiological traits [181, 199]. Here, we focus on two promising wild relatives that have been already extensively characterized for their adaptive features and for which a wide collection of genetic and genomic tools are available. The wild relative *Solanum pennellii* is native to the Andean area of South America and is evolutionarily adapted to arid conditions [160]. Comparative transcriptomics between *S. pennellii* and *S. lycopersicum* showed distinct patterns of evolution. Domesticated tomato was selected for a number of fruit traits and postharvest quality, however, the wild relative retained a number of gene expression patterns more suited for environmental response and stress tolerance [110]. For example, several genes involved in wax deposition were highly expressed in *S. pennellii*, possibly accounting for the thicker cuticle of the wild species compared to cultivated tomato. By contrast, one developmental regulator contributing to the definition of the stomatal index had a lower expression in *S. pennellii*, which has a different stomatal density compared to *S. lycopersicum* [110]. This selective pressure driven by adaptation to an arid environment has resulted in rapidly evolving genes not selected for in the artificial selection that shaped domesticated tomato. The recently completed genome of *S. pennellii* makes it an ideal source for mining novel traits [27]. A number of introgression lines (ILs) are available, where the whole genome of *S. pennellii* is represented in the genetic

background cultivar, M82 of *S. lycopersicum* [58]. Some of these segments have been shown to increase remarkably the agronomic performance of *S. lycopersicum* and some lines showed increased yield and brix units under drought stress [75]. Adaptation to arid conditions in *S. pennellii* is also accompanied by increased salt tolerance over cultivated tomato. It appears that the antioxidative systems in *S. pennellii* are more robust than cultivated tomato, particularly under salt stress [145]. Genomic studies, between *S. lycopersicum* and *S. pennellii*, of QTLs associated with drought or salt tolerance have correlated these QTLs with gene copy number, allelic polymorphisms, and polymorphisms within the promoters of key genes [27]. Traits from *S. pennellii* have already been shown to increase drought tolerance in cultivated tomato. The universal stress protein (USP) is involved in ABA responses to abiotic stress but is not well characterized. Transgenic tomato plants expressing the *S. pennellii* USP gene were more tolerant to drought stress as seedlings and adults [128]. It is certain that *S. pennellii* will provide a number of traits for the genetic improvement of tomato under adverse stress conditions.

The second species of interest is *Solanum habrochaites*, which is highly tolerant to drought and low temperatures [41, 42]. Comparative transcriptomic studies between *S. habrochaites* and *S. lycopersicum* have been done on secondary metabolism [25], freezing tolerance [41, 42], glandular trichomes [139], and disease resistance [165]. As with *S. pennellii*, near isogenic lines and backcross recombinant inbred lines have been developed in the *S. lycopersicum* background [61, 146]. These lines provide a valuable resource for discovery of novel traits and map important quantitative trait loci. Under root chilling stress *S. habrochaites* exhibits tight control of stomatal closure and retention of water whereas *S. lycopersicum* is unable to prevent water loss in these conditions [11]. This implies that *S. habrochaites* exhibits tighter control in water limiting conditions. Introduction of the *S. habrochaites* cold-induced SK3-type dehydrin gene increased both cold and drought tolerance in cultivated tomato. These transgenic plants also grew better under osmotic and salt stress and were more tolerant to oxidative stress [125]. Adaptations to the environment on slopes of the Andes of Ecuador and Peru have already provided novel traits for cold tolerance and will doubtlessly provide more for the improvement of drought tolerance in tomato.

2.4.2 Potato

Potato production worldwide is strongly affected by water stress, either because of insufficient rainfall or due to inadequate irrigation. Improving drought tolerance is consequently becoming a priority for potato breeders, particularly in the perspective of climate change. Modern potato varieties are highly sensitive to drought stress [197]. In contrast, landraces of Andean potato species and wild potatoes occurring in the Americas, from the United States to Chile and Uruguay [79] are better adapted to harsh environments and regularly exposed to water-deficit conditions. Moreover, primitive forms of cultivated potato and their wild relatives provide a rich, unique,

and diverse source of genetic variation, which could be a source of various traits for potato breeding. This may be because of their adaptation to a broad range of habitats and niches varying in latitude, altitude, habitat, soil, and precipitation regimes.

The many wild relatives and primitive cultivars of potato have proven to be valuable in breeding programs for improvement of disease resistance, abiotic stress tolerance, and other agronomic traits and qualities of interests [17, 19, 49, 80, 95, 149, 176]. As potato has gained importance as a food source in developing countries [90], the breeding has shifted to adaptation to the conditions of these countries, generally hot and dry environments.

Therefore genes from wild relatives and landraces should be explored to the improvement of potato tolerance [79]. The major problem in this case is undesirable effects of genes linked to the introgressed trait or gene [91, 136, 32]. In addition, photoperiod requirements of modern varieties compared with native potato are different, which could explain why plants from interspecific crosses and backcrosses often have lower yield, small tuber number, late maturity, poorer foliage, and altered tuber appearance when grown under nonsuitable photoperiods [104].

Drought-tolerant accessions identified in Andean potatoes [188] have been barely used in breeding programs because of their adaptation to the short day conditions prevalent in the low latitudes. In this context, some attempts have been made to transfer drought-tolerance genes from wild to cultivated potato species via traditional breeding. In addition, potato breeders have used somatic fusion, embryo rescue, and bridging strategies to overcome the natural barriers from interspecific crossing between wild and cultivated species. Screening for drought tolerance in potato landraces has been performed by Cabello et al. [33, 32]. A high proportion of accessions combining drought tolerance with high irrigated yield were found in Andean landraces, particularly in the species *S. curtilobum* in the *S. tuberosum* L. cultivar groups *Stenotomum*, *Andigenum*, and *Chaucha*. Watanabe et al. [196] identified *S. chillonanum*, *S. jamesii*, and *S. okadae* as potential drought-tolerant species by screening 44 accessions of wild species selected based on their drought habitats derived from GIS information. Climate change and other factors that additionally increase pressure on ecosystems are threatening the existence of many wild relatives. The establishment and maintenance of gene banks is intended to narrow the loss of this diversity in varieties. The genetic resources of the potato are preserved in the form of true potato seeds, vegetative tubers, and in vitro seedlings. In particular, conservation under in situ conditions is considered an important strategy to preserve the genetic resources. In situ conservation involves exposing the varieties in question to natural conditions in the field. Sustainably increasing productivity in a changing climate is one of the most important challenges for people conducting research on potato worldwide to ensure food security. Primitive cultivars and wild relatives of potato have been used as sources of desirable traits, such as resistance or tolerance to diseases, pests, and environmental stresses, and of tuber qualities, for potato breeding. Tools for incorporating useful alleles from its wild relatives into cultivated potato have been developed so that there remains a

broad gene pool to be more effectively exploited. Currently, large amounts of potato germplasm containing useful alleles are available in gene banks around the world; however, re-collection may reveal novel genes. Precise identification of species is essential for making decisions for effective utilization of germplasm collections; therefore, taxonomic research and updating taxonomical descriptions of the gene bank collections in potato are indispensable [134].

2.4.3 *Wheat*

The Green Revolution resulted in high-yielding semi-dwarf wheat (*T. aestivum*) and rice (*O. sativa*) cultivars with improved responsiveness to fertilizer and irrigation [29]. This selection for aboveground traits and focus on yields under optimal environments may have overlooked traits that enhance growth under limited water [194]. Over the previous decades, a number of studies have examined the drought tolerance of wheat landraces [26, 52, 55, 152]. Later studies used DNA fingerprinting to reveal diversity and divergence among wild relatives and landraces [159]. Larger high-throughput studies of these wheat relatives have been able to assess over 9000 SNPs amongst 2994 accessions comprising both modern cultivars and landraces [38]. Such studies that establish genomic diversity maps are valuable tools for finding relevant traits in landraces and wild relatives.

Allelic diversity in wheat has been increased with landrace accessions from extreme environments through crossing or interspecific hybridization. This hybridization uses ancestral genomes to produce synthetic hexaploid-derived wheat lines (SYN-DER) [159]. Synthetic hexaploid wheats (SHWs) and their synthetic derivative lines (SDLs) provide a way of introducing genetic diversity from ancestor genomes into cultivated wheat varieties [127]. These SYN-DER lines have been used to study novel drought tolerance alleles from wheat relatives [8, 48]. Landraces of wheat can also be used to improve traits controlling root architecture and drought avoidance. Many modern wheat varieties have smaller root masses, optimized for shallow irrigation and absorption of added fertilizers, compared to some landraces. Some of the landraces have mapped traits, such as 1RS that can improve root architecture and drought avoidance [194]. When the chromosome segment 7DL from the wild relative *Agropyron elongatum* was introduced into cultivated wheat, the translocation line showed improved root and shoot biomass, improved water stress adaptation, and enhanced access to water for growth [154].

Nutrient assimilation and growth under limiting conditions is an additional target for genetic improvement. Landraces and wild relatives have the potential to improve assimilation, particularly under limiting conditions. Studies with *Brachypodium distachyon* have revealed heritable traits that maintain growth, even in limiting conditions [89].

Proteomic studies using contrasting wheat landraces, N49 and N14, under drought stress revealed key proteins that are involved in oxidative stress response, senescence, and mobilization of carbohydrate reserves [20, 59]. Further studies of drought-tolerant landraces with proteomes and subproteomes under stress can elucidate the role of drought-responsive proteins and their expression, abundance, and posttranslation modifications [85].

2.4.4 Rice

O. sativa is thought to have been domesticated over 6000 years ago, but since domestication introgression of wild germplasm from cross-compatible species has been a natural and ongoing process [14]. Over 120,000 genotypes of *O. sativa* and *Oryza glaberrima* exist in gene banks, but very little genetic diversity exists within these accessions [14]. More than 22 wild species of *Oryza* are known and the genetic diversity within these wild relatives can provide novel traits for drought tolerance. Transfer of genes from wild relatives into cultivated rice has had several impediments to making crosses, such as low crossability and limited recombination between chromosomes [30]. With the discovery of the killer–protector system at the S5 locus encoded by three tightly linked genes, open reading frame 3 (ORF3), ORF4, and ORF5, it may be possible to overcome reduced fertility in hybrids [205]. The research community is striving to build tools to utilize the existing diversity. Two of the goals of the International Oryza Map Alignment Project are to sequence reference genomes and transcriptomes for all species and generate advanced mapping populations for functional and breeding studies [94]. Sequencing of 517 rice landraces revealed approximately 3.6 million SNPs that were used to construct a high-density haplotype map of the rice genome [87]. Genomewide association studies using such resources can identify novel traits that contribute to agronomic improvement. Such association studies can reveal the role of known drought tolerance genes, such as OsDREB1F, and the presence of variant proteins within drought-tolerant wild relatives [172]. Generation of introgression lines using elite cultivars and a wild rice Dongxiang accession (*O. rufipogon* Griff.) was used to generate and identify a drought-tolerant introgression line [210]. Comparative analysis of cultivated rice and the drought-tolerant landrace, Nagina 22 (N22), revealed differential regulation of both primary and secondary metabolism genes [120]. Of the three major cereal crops, rice is the most sensitive to drought stress, primarily due to the shallow roots of most cultivars [111]. Improvement in root architecture and root depth is a key focus in rice improvement [73]. The locus deeper rooting 1 (DRO1) has been definitively shown to contribute to drought avoidance in rice. DRO1 alters root system architecture by controlling root angle growth [186]. DRO1 was successfully introduced into IR64, a commonly grown shallow-rooting cultivar, and the near isogenic line containing DRO1 demonstrated deeper rooting and improved drought avoidance [10].

2.4.5 Corn

When researchers analyzed field-level data for 17 years of maize productivity in the American mid-west, they found that absolute yields have increased over that time period. However, sensitivity of maize yields to drought stress had increased. Selection and breeding for traits clearly increased overall yield, but failed to decrease yield sensitivity to drought stress [126]. Although yield potentials have drastically increased in maize through traditional and molecular breeding, much less emphasis has been placed on breeding for WUE or drought tolerance. QTLs associated with tolerance have been identified, however, they may be of limited utility for applied breeding due to their dependency on genetic background and a lack of understanding of the biophysical basis of these traits [35].

In maize, tropical landraces and inbred lines possess numerous potential traits for increasing WUE and drought tolerance. A number of these lines have been assessed for their drought tolerance [3, 88, 143, 147, 148, 202]. Traits controlling root anatomy and morphology can also play a key role in maize drought tolerance. When lines with contrasting cortical cell sizes were subjected to water-stress conditions, those with large cortical cells showed 21 and 27 % deeper rooting, 50 % greater stomatal conductance, and 59 % greater CO₂ assimilation [43]. The group who reported these findings proposes that the increased tolerance is due to a reduction in the metabolic cost of soil exploration by roots and facilitates greater exploration to increase water acquisition. The same group found that lines with a reduced cortical cell file number have a similar benefit under water stress [44]. Recombinant inbred lines with contrasting root number and length have been assessed in a number of water-limiting conditions. Lines with fewer, but longer roots showed greater stomatal conductance and 50 % more shoot biomass and up to 144 % greater yield under water-limiting conditions [208]. Identification of hormonal regulators associated with QTLs, such as members of the CYP707A subfamily responsible for ABA catabolism and ARR, a negative regulator of cytokinin signaling, have revealed control points in regulating hormonal responses to drought stress [201]. Genomewide analysis of 368 varieties was used to evaluate DREB transcription factors in conjunction with the cloning of 18 *ZmDREB* genes present in the maize B73 genome. Analysis indicated a significant association between *ZmDREB2.7* and drought tolerance at early developmental stages. Natural variation in the promoter region of *ZmDREB2.7* correlated with varying levels of drought tolerance [124]. Tolerant landraces demonstrated higher stomatal conductance and rates of photosynthesis under drought stress. Upregulation of *ABI3* and *HVA22* exclusively in drought-tolerant lines indicated ABA-responsive genes may play a key role. The investigators also found a number of other differentially regulated genes, *AP2*, *bHLH*, *C2C2*, *C2H2*, *C3H*, zinc finger, *CCAAT* binding factor (*HAP2*), and *WRKY* gene families, expressed in tolerant lines, but not in the sensitive line [77].

2.5 Conclusions

As discussed in this chapter, there are a number of key genes that control or contribute to drought responses in crop species. Understanding the mechanisms behind these traits is essential for the genetic improvement of crops. Although new genomes and transcriptomes emerge daily for these species, model systems, such as *Arabidopsis thaliana*, remain our best system for dissecting these traits. Wild relatives and landraces represent a vast pool of traits that can be utilized for novel stress tolerance traits, but a thorough functional characterization is necessary in order to take advantage of the benefits they offer. The challenges posed by drought and water deficit are not insurmountable; they can be overcome with sufficient understanding of the genetic basis of tolerance and the resources available to the research community.

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