

Review

Resilience of cereal crops to abiotic stress: A review

Mushtaq Ahmad*, Gul Zaffar, S. M. Razvi, Z. A. Dar, S. D. Mir, S. A. Bukhari and Mehfuza Habib

Division of Genetics and Pant Breeding, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir Shalimar campus, Srinagar -191 121, India.

Received 3 December, 2013; Accepted 20 June, 2014

In the last century, conventional selection and breeding program proved to be highly effective in improving crops against abiotic stresses. Therefore, breeding for abiotic stress tolerance in crop plants should be given high research priority as abiotic stresses are the main factor negatively affecting crop growth and productivity throughout the globe. Advancement in physiology, genetics and molecular biology, have greatly improved our understanding of plant responses to stresses. Many studies show that salt tolerance is tightly associated with the ability to maintain ion homeostasis under salinity. Na⁺ transporter SKC1 unloads Na⁺ from xylem; plasma membrane N⁺/H⁺ antiporter SOS₁ excludes sodium out of cytosol, and tonoplast Na⁺/H⁺ antiporter NHX₁ sequesters Na⁺ into the vacuole. Silicon deposition in exodermis and endodermis of rice root reduces sodium transport through the apoplastic pathway. A number of transcription factors regulate stress-inducible gene expression that leads to initiating stress responses and establishing plant stress tolerance. Over expression of some transcription factors, including DREB/CBF and NAC, enhances salt, drought and cold tolerance in rice. A variant of one of ERF family genes, *Sub1A-1*, confers immersion tolerance to lowland rice. These findings and their exploitation will hold promise for engineering breeding to protect crop plants from certain abiotic stresses. Although, cereal crops are also quite sensitive to various abiotic stresses, hence in this short review, we will present recent progresses in adaptation of cereal crops to salinity, drought and cold tolerance are emphasized and the future potentials are highlighted.

Key words: Cereal crops, abiotic stresses, food insecurity, molecular breeding, quantitative trait loci (QTLs), salinity, water stress.

INTRODUCTION

Cereal crops assumes its cultivation under much diverse agro-climatic zones extending from truly sub-tropical to cooler temperate regions having altitudes above 7500 ft amsl. Therefore, inevitably, the crop remains open to

varied types of biotic as well as abiotic stresses, of which the temperature below 10°C are critical particularly at sowing and maturity in cold temperature areas (Zaffar et al., 2005). Abiotic stresses such as salinity, drought,

*Corresponding author. E-mail: sahilmuhtaqdar@rediffmail.com.

nutrient deficiency or toxicity, and flooding limit crop productivity and such situations become more problematic, where they cause food insecurity for large populations and poverty, particularly in rural areas. For example, drought stress has affected more than 70 million hectares of rice growing land world-wide, while salt stress and nutrient stress render more than 100 million hectares of agricultural land uncultivable thereby resulting in low returns, poverty and reduced educational and employment opportunities. Thus, abiotic stresses are the major factors of poverty for millions of people. Hence, it is widely urged that such strategies be adopted which may be used to get maximum crop stand and economic returns from adverse environments. Major strategies which may be used to overcome the adverse effects of such stressful environments may include development of new crop varieties, screening and selection of the well adapted existing germplasm of potential crops, production of genetically modified (GM) crops, exogenous use of osmo protectants etc. Abiotic stress one among the major causes of crop plant yield losses worldwide. Drought and salinity stress are the major environmental challenges faced by agriculture. Improving yield production and stability of crop plants under stressful environments is important to fulfill food demand of the ever-increasing world population. Though genes associated to plant response(s) to drought and salinity stress have been identified and characterized, in most cases, in the model plant *Arabidopsis*. However, while many of these genes are potential candidates for improving tolerance to abiotic stress, only a small proportion were transferred into crop plants. Further, transgenic crop plants over-expressing the genes of interest were, in most cases, tested under artificial conditions in the laboratory or controlled greenhouse. Thus, while many reports on drought and salinity tolerance in transgenic plants have been published, there is urgent need to test these traits under field conditions. In this chapter, we discuss recent advances in engineering drought and salinity tolerance in crop plants with emphasis on yield and the needs to close the gaps between the laboratory and the field conditions. Crop plants grown under unfavorable environmental conditions prevent the full expression of their genetic potential. The most frequently occurring abiotic stress conditions with adverse effects on crop yield are water, deficit or excess; ions, deficit or excess; temperature, low or high and light, deficit or excess. The ever-increasing human population, concomitant with loss of agricultural land (due to urbanization processes) and diminishing water availability (associated with climate change) pose serious challenges to world agriculture (Mittler and Blumwald, 2010). A significant increase in grain yield of major crop plants such as wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), and maize (*Zea mays* L.) is required to fulfill the food supply requirements for the projected population by 2050

(Godfray et al., 2010). Despite the progress with respect to economic yield already achieved, additional gains in agricultural productivity are demanded at faster pace due to population increase and by changing agricultural practices including biotic and abiotic stresses. Diseases, pests and weed competition losses account for 4.1 and 2.6% yield reductions, respectively, while the remaining reduction (69.1%) is attributed to unfavorable physico-chemical (abiotic) environments induced due to problematic soils and erratic climate patterns. Certainly, some of these losses are caused by inherently unfavorable environments and some by suboptimal management practices by farmers, often due to economic constraints or lack of training. Nevertheless, there is no doubt that a large fraction of potential crop productivity is lost to abiotic stress factors. Plants respond to abiotic stresses at multiple levels such as molecular, cellular, tissue, anatomical, morphological and whole-plant physiological levels (Witcombe et al., 2008) need a through/practical analysis and understanding. The response to stress depends on the duration and severity of the event, as well as the age and developmental stage of the plant, which varies at species and genotype level (Bray, 1997). Typically, early plant establishment (germination and seedling) and the reproductive stage are the most sensitive in determining yield under stress (Zaffar et al., 2005; Barnabas et al., 2008). However, a large segment of the research on abiotic stress credited to *Arabidopsis*, a model system in the past has focused primarily on the vegetative phase identify survival phenotypes, which limits our ability to readily translate the discoveries into improved yield in crop plants.

Abiotic stresses in agriculture are believed to be responsible for reducing crop growth and productivity. Because of their sessile nature, plants at different developmental stages of growth must endure adverse environmental conditions and consequently evolve a variety of responses to acclimatize to environmental stresses. During the course of evolution, plants have developed and acquired sophisticated mechanisms to sense the subtle changes of growth conditions, and trigger signal transduction cascades, which in turn activate stress responsive genes to produce proteins which ultimately lead to changes at the physiological and biochemical levels. A greater understanding of the physiology and molecular biology of stress tolerance may provide a useful platform to breed and develop improved stress-tolerant crop varieties in near future. In modern agriculture, abiotic stresses, especially salinity and drought are major factors limiting crop productivity worldwide. Drought affects plant growth, yield, membrane integrity, pigment content, osmotic adjustments, water relations, and photosynthetic activity (Balachandran et al., 2014). In addition to this, some other factors like low temperatures, heat shock, heavy metals, UV-radiation and pesticides are also affecting crop yields (Bohner et

al., 1995; Tardieu and Tuberosa, 2010). Abiotic stress is one of the primary causes of crop yield loss worldwide, causing average yield losses of more than 50% in major crops. Tolerance and susceptibility to abiotic stresses are very complex as plant traits that are associated with resistance mechanisms are mutagenic and thus difficult to control and engineer. Drought induces mechanical stress on roots due to soil hardness, osmotic stress because of cell dehydration and removal of water in the extra-cellular space, and oxidative stress by the accumulation of reactive oxygen species (ROS) (Kovtun et al., 2000). Genetic enhancements of cereal crops for improved performance under water-limited environment and high salt is of paramount importance to increase the per capita availability, were such conditions are of frequent occurrence. Developing improved lines for stressful environments requires the rigorous application of molecular breeding and biotechnology. To improve cereal crops further using marker-assisted selection (MAS) and map-based cloning, we require critical information on component traits, accurate phenotyping, the identification of candidate genes and quantitative trait loci (QTLs), the relationship between QTLs and genes, the contribution of individual QTLs to the phenotype, and their variability across different locations and different crop seasons. Another important tool to produce stress-tolerant rice varieties is genetic transformation, which offers a powerful means of incorporating exotic or even synthetic genes with a profound ability to up or down-regulate specific metabolic steps. Rapid progress has been made in developing transformation technologies for field crops and gene transfer need to be done regularly to harness the fruits of this improved technology. Isolation and characterization of novel genes/gene combinations and promoters and their successful transfer into rice will provide new avenues for metabolic engineering for stress tolerance. Such novel genes and promoters offer unique opportunities in genetic engineering of rice for stress environments. Marker aided selection and transgenic approaches are two powerful tools to accelerate plant breeding to produce varieties with improved drought and salt tolerance.

In case of drought, plants improve their water use efficiency either by dehydration avoidance or through dehydration tolerance (Blum, 2005). Due to global warming, it is believed that, India will suffer severe climatic changes in future, including longer drought, lesser amount of total rainfall that is distributed unequally with very heavy precipitation at shorter duration causing flooding, high temperature flux, and higher incidence of tropical storms. Selection among diverse germplasm types will therefore be difficult to meet by following individual characters because of the different response of the genotypes towards improvement for the component to cold tolerance traits. This necessitates the use of some balance selection criteria, which takes into consideration

all important cold tolerance attributes simultaneously, so as to end up with overall greater selection advances (Zaffar et al., 2005).

PLANTS RESPONSE TO WATER DEFICIT

Among the various abiotic stress conditions, water deficit is the most devastating factor affecting world agriculture (Araus et al., 2008). About one-third of the world's arable land suffers from chronically inadequate water availability for agriculture, and in virtually all agricultural regions, crop yields are periodically reduced by drought (Bruce et al., 2002). While currently 80% of the world's useable water resources are consumed by irrigated agriculture (Condon et al., 2004) within a few decades, the expanding world population will require more water for domestic, municipal, industrial and environmental needs (Hamdy et al., 2003). This trend is expected to accentuate due to global climatic change and increased aridity (Vorosmarty et al., 2000). Thus, to meet the projected food demands, more crops per drop are required (Condon et al., 2004).

PLANT RESPONSE TO SALINITY STRESS

Salinity is a major constraint on crop-plant productivity (Witcombe et al., 2008). More than 800 million hectares of land throughout the world are salt affected, which accounts for 6% of the world total land area (Munns and Tester, 2008). In most cases, salinity results from natural causes (salt accumulation over long periods of time). In addition, a significant portion of the cultivated agricultural land is becoming saline due to deforestation or excess irrigation and fertilization (Shannon, 1997). Current estimates indicate that 20% of the roughly 230 million hectares of irrigated land is affected by salinity. Given that one third of the food production comes from irrigated agriculture, salinity is becoming a serious problem for crop-plant productivity.

PLANT ADAPTATIONS TO ABIOTIC STRESS

Plant resistance to stress conditions may arise from escape, avoidance or tolerance strategies (Levitt, 1972). Escape relies on successful completion of reproduction before the onset of severe stress (that is, developmental plasticity), achieved by early flowering and/or short growth duration (Mooney et al., 1987). Avoidance involves the prevention or decreasing the impact of the stress on the plant, such as minimizing water loss and maximizing water uptake (Chaves et al., 2003) or exclusion of salt ions, a feature observed in halophytes (Munns and Tester, 2008). Tolerance relies on the inherent

ability of the plant to sustain growth (likely at a reduced rate) even when the conditions are unfavorable for the maintenance of basic plant processes. This strategy involves coordination of physiological and biochemical alterations at the cellular and molecular levels, such as osmotic adjustment (Morgan, 1984) and the sequestration of ion in the plants, in the vacuole or leaf sheath and/or older leaves (Mimura et al., 2003). In most cases, plants subjected to stress conditions combine a suite of responses, exhibiting a number of physiological and biochemical responses at the molecular, cellular and whole-plant level (Chaves et al., 2003). Salinity occurs through natural or human-induced processes that result in the accumulation of dissolved salts in the soil to an extent that inhibits plant growth.

NEW TECHNOLOGIES TO STUDY PLANT RESPONSE TO ABIOTIC STRESS

New technologies are providing opportunities to address the challenging problem of maintaining high-yield crop production under stressful and changing climates. The information provided by high-resolution transcript profiling, the identification of large-scale specific protein networks and their association with the plant responses to environmental perturbations are allowing the application of a systems-level approach to uncover the bases of plant responses to environmental changes. Model plants, such *thaliana* as *Arabidopsis*, *Brachypodium distachyon* and *Medicago truncatula*, have been and will continue to offer insights into the genetic and biochemical basis of abiotic stress adaptations (Bohnert et al., 2006; Hirayama and Shinozaki, 2010). Further, the identification of stress-related genes and pathways has been facilitated by introducing new tools and resources developed in these model plants. Numerous genes related to plant response to stressful conditions of drought and salinity stress have been identified and characterized (Ashraf, 2010; Pardo, 2010). Many of the genes so identified are considered as potential candidates for enhancing tolerance to abiotic stress. In the majority cases, these genes are over expressed in the target plant(s), whether with a strong constitutive promoter or a stress-responsive promoter. Early generations (T1-T3) are screened for responses to stresses to assess the efficacy of the construct. However, majority of these studies were conducted under laboratory conditions (that is, dehydration) in the vegetative phase (that is, seedling, or plate assays) using artificial stress (for example, PEG, mannitol), with very high concentration (that is, osmotic shock) and for short periods (that is, hours). Moreover, most of these studies showed stress tolerance and/or survival, but not the effects of the different stress conditions on plant productivity (Parry et al., 2005). Under rain-fed drought prone agriculture, water stress at the

reproductive stage is the most prevalent problem as in most rain-fed ecosystems, the crop season's rains diminish towards flowering and harvest time (Blum, 2009). Thus, more emphasis should be given to the study of the response of crop plants to abiotic stress at the reproductive stage that is under field conditions.

Stress-responsive gene expression

Under abiotic stress (drought, salinity) plants respond either with the activation of synthesis or inhibition of catabolism of osmolytes as sugars (saccharopine, trehalose, raffinose, galactinol, sorbitol, mannitol, fructans) (Cortina and Culianez-Macia, 2005), sugar alcohols (for example, polyols), amino acids as branched chain aminoacids (BCA), proline and agmatin, quaternary ammonium compounds and small dipeptides as glycinebetaine (Holmstrom et al., 2000). Polyamines (that is, putrescine) are involved in a wide range of plant processes including wounding (Groppa and Benavides, 2008). Besides these functions, such bio-molecules mediate the stress responses. There is a need to develop genotypes having enhanced or faster induction of expression of genes at the crossroad of permissive growth under stress condition. This group of permissive genes includes aquaporin isoforms able to optimize water fluxes (Javot et al., 2003). Several genes have been characterized for their role on stress protection, one among being ERECTA which regulate transpiration efficiency affecting stomatal closure, while the plant is able to maintain biomass production (Masle et al., 2005). Other important regulatory proteins involved in drought stress are proton antiporters as TNH_{X1} and a proton pyrophosphatase TVP₁ (Brini et al., 2005; Brini et al., 2007a) shown to improve salt and drought stress tolerance in *Arabidopsis*. Dehydrin (accumulate to a high level in the mature embryos) and its isoforms (LEA/DHN) of sizes 17, 30 and 40 kDa, function as intracellular chaperones for other proteins and nucleic acids. One dehydrin (DHN-5) was shown to be involved in salt and drought resistant phenotypes of durum wheat and its accumulation was shown to have a role in preservation of cell integrity during late embryogenesis and desiccation (Brini et al., 2007b).

Dehydrins are also known as late embryogenesis abundant (LEA) or early response to dehydration (ERD) proteins (Beck et al., 2007). It has been shown that LEA/DHNs exert chaperone activity on proteins and nucleic acids (Kovacs et al., 2008; Hara et al., 2009). DHN/LEAs which lack a fixed three-dimensional structure, even though remarkably stable at macromolecular crowding conditions, maintaining a disordered character under conditions (dehydration) in which unfolded states of several globular proteins would tend to collapse (Mouillon et al., 2008). Several LEA/DHN isoforms are

regulated by abscisic acid (ABA) (Jimenez et al., 2008). While the size of induced DHNs was large, ranging between 14 and 74 kDa, drought-tolerant Bermuda grass varieties showed to induce preferentially the 31 and 40 kDa isoforms (Hu et al., 2010). Roots grow and recover their function after environmental stresses through specific genes that adapt root development to these restrictive conditions. The recovery of *Medicago truncatula* roots after a salt stress is mediated by regulatory networks depending on TFIIIA-like transcription factors, involved in the control of root adaptation to salt stress. Those conditions induced synthesis of a novel RNA-binding protein, a small G-protein homologous to ROP₉, a receptor-like kinase, two TF IIIA-like and an AP2-like transcription factors (TF), MtZpt2-1, MtZpt2-2 and MtAp2, and a histidine kinase associated with cytokinin transduction pathways (Merchan et al., 2007). A full-length 1.1 kb cDNA, designated *O. sativa* Dehydrin 1 (OsDhn1), was isolated from the seed coat of rice. The deduced protein is hydrophilic and has three K-type and one S-type motifs (SK3-type), indicating that OsDhn1 belongs to the acidic dehydrin family, which includes wheat WCOR410 and Arabidopsis COR47. Expression of OsDhn1 was strongly induced by low temperature as well as drought. Its induction by cold stress was clear cut in the roots of seedlings and the epidermis of palea and lemma, while it was also up-regulated in UBI: CBF1/DREB1b transgenic plants indicating that it is regulated by the CBF/DREB stress signaling pathway.

In spite of a surge in literature on drought tolerance in crops during the past two decades, practical progress in breeding for drought tolerance has not been significant. Drought therefore requires an analytical approach of dissecting and studying the contribution of trait components using the quantitative trait loci (QTLs) model. This approach is particularly suited to crops like rice for which dense genetic linkage maps with a variety of DNA markers are already available. Most studies on drought tolerance deal with evaluating specific traits logically related to crop performance under drought. A molecular genetic analysis is more effective if conducted on individual traits (and even their components) before crop performance is considered. Thus, it is important to phenotype for specific traits or responses under managed nursery or greenhouse conditions, besides subsequent field evaluations under drought in target regions. A strong demonstrated linkage between traits in relation to crop performance in the target environment is a crucial step before advocating marker-assisted selection (MAS). A second approach is to create a novel and functionally known type of variability in plant stress response by genetic transformation.

The transgenic approach offers a powerful means of incorporating a broad spectrum of genes with profound ability to regulate specific metabolic paths associated with stress response. Although transformation with any

single gene or group of genes for a particular pathway may not be adequate for conferring drought tolerance as in majority of cases several pathways are required to be carried out and the outcome of these paths (products) are required to ensure drought tolerance (Ingram and Bartels, 1996). In general, many proteins and low-molecular-weight osmolytes accumulate under stress. It is not clear which factor(s) among many changes contributes to the development of stress tolerance because so many changes occur. Thus, transformation approach is useful, to obtain valuable information. By transferring a single gene into a plant, and then studying the response of the resulting plants to drought or salt stress, one can clearly understand whether or not a given gene has a well defined role in controlling a particular stress condition, and what related changes occur. This approach will thus be useful in identifying candidate genes for stress tolerance or its components with significant developments in gene transfer technologies for rice and rapid progress in gene isolation and manipulation.

Signalling pathway

Emergence of the novel "omics" technologies, such as genomics, proteomics, and metabolomics, allows researchers to identify the genetic bases behind plant stress responses. These technologies enable a direct and unbiased monitoring of the factors affecting plant growth and development and provide the data that can be directly used to investigate the complex interplay between the plant, its metabolism, and also the stress caused by the environment or the biological threats (insects, fungi, or other pathogens). Plant responses to stress are mediated via profound changes in gene expression which result in changes in composition of plant transcriptome, proteome, and metabolome (Alfocea et al., 2011).

Genomics

Functional genomics allows large-scale gene function analysis with high throughput technology and incorporates interaction of gene products at cellular and organism level. The information coming from sequencing programs is providing enormous input about genes to be analyzed.

The availability of many plant genomes nowadays (Feuillet et al., 2010) facilitates studying the function of genes on a genome wide scale. The lack of information from other plant genomes will also be compensated in part by the availability of large collection of expressed sequence tags (ESTs) and cDNA sequences. The basic interest behind these EST projects is to identify genes responsible for critical functions.

ESTs, cDNA libraries, microarray, and serial analysis of gene expression (SAGE) are used to analyze global gene expression profiles in a functional genomics program. Large mutant collections are tools that complement large-scale expression studies. Gene identification through physical and chemical mutagens has become amenable for large-scale analysis with the availability of markers (Lukowitz et al., 2000), but gene tagging is more promising for functional analysis on a wider scale. Moreover, the understanding of the complexity of stress signaling and plant adaptive processes would require the analysis of the function of numerous genes involved in stress response. Numerous investigations show that plant defense response genes are transcriptionally activated by pathogens and also by different types of abiotic stress. It has been described that the induction of specific defense genes, in the response against certain pathogens, is dependent on specific environmental conditions, suggesting the existence of a complex signaling network that allows the plant to recognize and protect itself against pathogens and environmental stress. Similar induction patterns of members of the 14.3.3 gene family (GF14b and GF14c) by abiotic and biotic stresses such as salinity, drought, ABA, and fungal inoculation have been documented in rice (Chen et al., 2006). The rice GF14 genes contain cis elements in their promoter regions that are responsive to abiotic stress and pathogen attack. The 14-3-3s family genes are also subject to the regulation by certain transcript factors. On the other hand, kinase cascades of the mitogen-activated protein kinase (MAPK) class play a remarkably important role in plant signaling of a variety of abiotic and biotic stresses, and it is an essential step in the establishment of resistance to pathogens (Pitzschke et al., 2009). It has been described that in *Arabidopsis*, MEKK1 and ANP1 act in the environmental stress response (Suarez-Rodriguez et al., 2007), and MPK3, MPK4, and MPK6, are activated by a diversity of stimuli including abiotic stresses, pathogens, and oxidative stress (Qiu et al., 2008). Elucidating the molecular mechanism that mediates the complex stress responses in plants system is an important step to develop improved variety of stress tolerant crops. Many crop traits are quantitative, complex, and controlled by multiple interacting genes. Recent progress in molecular biology provides the tools to study the genetically make-up of plants, which allows us to unravel the inheritance of all traits whether they are controlled by single genes or many genes acting together, known as the quantitative trait loci (QTL). The molecular marker technologies available since the 1980s allow dissecting the variation in traits. With the progress of QTL mapping, new breeding approaches such as marker assisted selection and breeding by design have emerged (Peleman and Voort, 2003). Advances in plant genomics research have opened up new perspectives and opportunities for improving crop plants and their productivity.

The genomics technologies have been found useful in deciphering the multigenicity of biotic and abiotic plant stress responses through genome sequences, stress-specific cell and tissue transcript collections, protein and metabolite profiles and their dynamic changes, protein interactions, and mutant screens.

Proteomics

The adaptation of plants to biotic or abiotic stress conditions is mediated through deep changes in gene expression which result in changes in composition of plant transcriptome, proteome, and metabolome. Since proteins are directly involved in plant stress response, proteomics studies can significantly contribute to elucidate the possible relationships between protein abundance and plant stress acclimation. Several studies (Bogeat-Triboulot et al., 2007) have already proven that the changes in gene expression at transcript level do not often correspond with the changes at protein level. The investigation of changes in plant proteome is highly important since proteins, unlike transcripts, are direct effectors of plant stress response. Proteins not only include enzymes catalyzing changes in metabolite levels, but also include components of transcription and translation machinery.

Metabolomics

The possibility of monitoring a complete set of metabolites could largely improve the understanding of many physiological plant processes. This systematic study, defined as "metabolomics," is intended to provide an integrated view of the functional status of an organism. Besides, its use as a breeding or selection tool, metabolomics techniques have also been used to evaluate stress responses in barley (Widodo et al., 2009), Citrus (Djoukeng et al., 2008), *M. truncatula* (Broeckling et al., 2005), and *Arabidopsis thaliana* (Fukushima et al., 2011).

ROLE OF JASMONIC ACID (JA) AND ABSCISIC ACID (ABA) IN STRESS SIGNALLING

Plant hormones play important role in all the developmental stages of plant, as they regulate all the important functions from the germination till the death of any particular plant/part. These bio-molecules regulate the commitment of plants to growth or senescence under abiotic stress. The plant copes with a specific stress through complex signals circulating from roots to shoots and leaves and delivering hormones and signalling back to the roots, activating the expression of protective proteins

or down-regulating unnecessary pathways.

These compounds sustain signals at short distance, between cells, or in a systemic way in the case of transport systems. In this review, we will try to highlight the role exerted by jasmonic acid (JA) and abscisic acid (ABA), as the former is the final product of the octodecanoic pathway which uses linoleic acid as substrate to produce oxylipins (Hughes et al., 2009). The octodecanoic pathway starts with the oxygenation of a polyunsaturated fatty acid (PUFA) by lipoxygenase (LOX), to form a fatty acid hydroperoxide. In plants, only the type-2 13-LOXs are believed to be associated with JA biosynthesis (De Domenico et al., 2007). In the biosynthesis of JA, the subsequent activities of 13-LOX, allene oxide synthase (AOS), allene oxide cyclase (AOC) lead to formation of *cis*- (+)-12-oxophytodienoic acid (OPDA), a JA precursor. JA is methylated by a specific JA-methyltransferase, which forms a volatile compound able to cross plasma membranes and exert its action at distance. Another effector in the lipoxygenase biosynthesis pathway is jasmonate-conjugated isoleucin (JA-Ile) that may accumulate stored in organelles and vacuoles. JA-Ile is able to translocate through membranes and move through xylem from roots to leaves and backward. Once the hormone has arrived at its destination in the target cell (locally or at distance), the priming of jasmonate-inducible genes is regulated by convergent pathways, linked to phosphoinositide and ABA-dependent signalling components.

ROLE OF ABSCISIC ACID IN ABIOTIC STRESSES

Abscisic acid (ABA) is a growth regulator involved in senescence, seed dormancy, plant development, drought tolerance and stress response, synthesized in the xylem and in the aerial parts of the plant, where it regulates stomatal movement and the activity of shoot meristems. ABA can flow in the root cortex across apoplastic barriers and play an important role in the regulation of signal intensity. The abscisic acid glucose ester (ABAGE) is a long-distance stress signal, stored in microsomes, and released by activated β -glucosidases, both in the apoplast and the cytosol of the mesophyll cells. ABA-GE transporters located on plasma membrane of the xylem parenchyma cells influence ABA mobility. Two intense sources of ABA absorption include internal and external sources. External ABA originates from root exudation and from ABA-producing soil organisms (predominantly fungi), whereas internal ABA comes from its biosynthesis at root shoots level and phloem import (Sauter and Hartung, 2000; Sauter et al., 2001). It has been reported that conjugated ABA (ABA-GE) also occurs in the soil, often in higher concentrations than ABA. The Casparian bands of the exodermis and endodermis are perfect barriers for ABA-GE. When an exodermis is absent

(*Fabaceae* and hydroponically cultivated plants), external ABA-GE enters into the apoplast of the root cortex. Apoplastic β -glucosidases can cleave the conjugated form and release free ABA, which is distributed to the symplast and/or transported across the endodermis into the xylem (Hartung et al., 2002).

SALT STRESS AND PHOSPHATE DEFICIENCY

Strong ABA synthesis and accumulation in the roots can be observed in plants affected by hemi parasites such as *Rhinanthus minor* (Jiang et al., 2004). ABA biosynthesis in the roots was reported to be 12-fold higher after attack, resulting in 14-fold higher ABA flows in the xylem. Plants regulate inorganic phosphate (Pi) homeostasis to adapt to environmental changes in Pi availability. Some degree of cross-talk between ABA and other signalling pathways was reported in phosphate limitation conditions. This mechanism involves phosphate uptake increase from the soil and phosphate mobilization from the leaf. Upon Pi starvation, up regulated miR399 cleaves its target gene, *PHO2*, in *A. thaliana*, an ubiquitin conjugating E₂ enzyme, thereby releasing several protein targets from ubiquitin-pathway dependent degradation and increasing Pi content in the shoots (Franco-Zorrilla et al., 2007).

TRANSCRIPTION FACTORS AND ABIOTIC STRESSES

Transcriptional regulation, also known as transcriptome reprogramming, is essential for plant adaptation to abiotic stresses. Till date, multiple transcription factors required for transcriptome reprogramming under abiotic stresses have been identified and functionally analyzed for several crop species. Among them, some have been well addressed in rice, for example, DREBs (dehydration responsive element-binding protein) /CBFs (C-repeat-binding factor) and NACs (NAM, ATAF, and CUC). These transcriptome reprogramming in rice needs to be utilized to improve adaptation of stress responsive traits in rice.

DREBs/CBFs

The DRE (dehydration-responsive element)/CRT (C-Repeat) were identified as a *cis*-acting element regulating gene expression in response to dehydration (salt, drought, and cold stresses) in *Arabidopsis* (Yamaguchi-Shinozaki and Shinozaki, 1994). Transcription factors DREB1/CBF1-3, CBF4 and DREB2, belonging to the ERF/AP2 family, were reported to bind to DRE/ CRT elements (Yamaguchi-Shinozaki and Shinozaki, 2005). While three *DREB1/CBF1-3* genes, *DREB1A/CBF3*, *DREB1B/CBF1* and *DREB1C/CBF2*, were induced by cold, but not by drought or salt stress (Medina et al., 1999), and were consequently believed to regulate the

expression of DRE/CRT genes under cold, *DREB2A* and *CBF4* were mainly responsive to drought and salt stresses (Haake et al., 2002). Interestingly, over expression of *DREB1s* in *Arabidopsis* increased freezing, drought, and salt tolerance, however, over expression of *DREB2A* in transgenic plants showed no increase in stress tolerance (Liu et al., 1998). These data suggest activation of *DREB2A* requires post-translational modification. Sakuma et al. (2006) found that the deletion of a region between residues 136 and 165 transformed *DREB2A* to a constitutively active form. Transgenic *Arabidopsis* plants expressing this active form exhibited improved drought tolerance, and slight freezing tolerance as well. It suggested the region between residues 136 and 165 of *DREB2A* is an inhibitory domain in the normal condition and is modified under salt/drought stress.

The functions of DREB orthologs have been demonstrated in rice, and five DREB cDNAs identified: *OsDREB1A*, *OsDREB1B*, *OsDREB1C*, *OsDREB1D* and *OsDREB2A*. Similar to their homologs in *Arabidopsis*, *OsDREB1A* and *OsDREB1B* were induced by cold; while *OsDREB2A* was regulated by salt and drought stress (Dubouzet et al., 2003). However, rice DREBs binding sites differed from their AtDREB counterparts as *OsDREB1A* showed much higher affinity binding to the DRE core sequence with GCCGAC than that with ACCGAC (Dubouzet et al., 2003), while AtDREBs bound equally to both sequences (Stockinger et al., 1997; Liu et al., 1998). Overexpression of *OsDREB1A* in *Arabidopsis* and rice induced expression of DRE/CRT genes (Dubouzet et al., 2003; Ito et al., 2006) and *OsDREB1A* overexpression lines showed phenotypes similar to AtDREB1A over expression lines, with improved stress tolerance as well as growth retardation under normal growth conditions (Dubouzet et al., 2003; Ito et al., 2006). These results demonstrate that the DREB1/CBF pathway was conserved in monocotyledons and dicotyledons. Contrary to the growth inhibition observed in cv. Kita-ake and Nipponbare rice, overexpression of *Arabidopsis* *DREB1A* in rice cv. Nakdong enhanced tolerance to abiotic stress without inhibiting growth or causing phenotypic alterations (Oh et al., 2005). The discrepancies observed between the different studies may have resulted from genotype differences, as observed by comparative analysis of their transcriptomes (Ito et al., 2006).

NAC genes

The NAC gene family encodes one of the largest families of plant-specific transcription factors and has not been found in other eukaryotes. There are 75 and 105 putative NAC genes in rice and *Arabidopsis* genomes, respectively (Ooka et al., 2003). Genes in the NAC family were found mainly to be involved in regulating plant development (Olsen et al., 2005). Their roles in abiotic

stresses were only discovered recently. A salt- and drought-induced gene, *ERD1*, was regulated in an ABA-independent manner (Kiyosue et al., 1993; Nakashima et al., 1997). However, no DRE/CRT element was found in its promoter region, suggesting a novel regulatory pathway for drought and salt adaptation (Kiyosue et al., 1993; Nakashima et al., 1997). Promoter analysis showed that an MYC-like site was necessary for induction of *ERD1* (Simpson et al., 2003). The MYC-like sequence was recognized by three transcription factors of the NAC family, ANAC019, ANAC055, ANAC072, and was named NACRS (NAC recognition sequence) (Tran et al., 2004). Consistent with *ERD1* expression patterns, the three NAC genes were induced under salt and/or drought stress, but were not remarkably regulated by cold (Tran et al., 2004). In addition to *ERD1*, many other salt and/or drought stress-induced genes were also regulated by the three genes, and consistently, over expression of these genes greatly enhanced drought tolerance in model plant *Arabidopsis* (Tran et al., 2004). In rice a similar set of NAC transcription factors may be used to regulation salt and/or drought responsive genes. In this direction, Chao et al. (2005) reported that multiple rice transcription factors, including a NAC gene, were induced in the early stage of salt stress. *OsNAC6*, a member of ATAF subfamily, was also induced by cold, salt, drought and abscisic acid (ABA) (Ohnishi et al., 2005). However, the precise functions of these NAC genes remain largely unknown, while recently (Hu et al., 2006) reported a NAC transcription factor significantly enhanced drought and salt tolerance in rice. The rice NAC gene *SNAC1* was up-regulated by drought and salt predominantly in guard cells. *SNAC1*- overexpressing plants showed greater sensitivity to ABA and increased stomatal closure to prevent water loss in rice. Drought resistance in transgenic plants was significantly improved under field conditions at the stage of anthesis, without phenotypic changes or yield reduction. However, although *SNAC* also triggered a series of salt and/or drought responsive genes including *OsERD1*, differences were noted in the regulation controlled by *SNAC* compared to ANACs, as the former could not interact with NACRS in the *OsERD1* promoter region. These data, in conjunction with differences noted between DREB/CBF regulons in rice and *Arabidopsis*, suggested that stress-related regulation pathways further evolved after the divergence of monocotyledons and dicots. Hence a thorough analysis and understanding in these systems is the need of hour to develop genotypes either with molecular or transgenic approaches to reach a productivity level, which can meet the dependant/increasing population on rice as stable food.

Other transcription factors

Although multiple transcription factors, including ICE

(inducer of CBF expression), CBFs/DREBs, AREB/ABF/ABI/bZip, MYC/ MYB and NACs, have been well characterized (Chinnusamy et al., 2004; 2006), we are far from fully understanding transcriptional reprogramming under salt/drought stress. It was estimated that about 8% of yeast genes were affected by salt stress (Zhu, 2002). If a similar percentage was assumed for rice, there would be about 4000 genes responsive to salt stress. To date, hundreds of salt responsive genes have been identified in cereal crops using high throughput technologies, such as microarray/gene chip (Chao et al., 2005; Wu et al., 2006). Although, these numbers are small compared with the potential 4000 genes, they cannot be fully explained by previously identified regulatory pathways. In *Arabidopsis*, a comparison of a transcriptome under cold and a CBF regulon revealed that only 12% of cold responsive genes were regulated by CBFs (Fowler and Thomashow, 2002). In addition, even in the CBF regulon, a few of the genes did not display DRE/CRT elements in their promoter region. It has consequently been hypothesized that subregulons control those genes without a DRE/CRT element, given that some transcription factors with DRE/CRTs in their promoters, for example *RAP2.1*, were represented in the CBF regulon (Fowler and Thomashow, 2002). A designed microarray has been used to analyze the response of transcription factors to biotic and abiotic stress, and demonstrated that more than 28 transcription factors were induced by abiotic stress (Chen et al., 2002). In rice, many transcription factors, including zinc finger, NAC, bHLH, MYB and WRKY, has also been reported to be induced by salt and drought stresses. Extensive research (Wu et al., 2006) also identified multiple transcription factors that were induced by stress and interestingly Chao et al. (2005) reported that transcription factors were rich in the earliest salt induced genes. These data suggest that multiple regulatory pathways under salt/drought stress remain to be characterized.

STRATEGIES FOR IMPROVING CROPS, AGAINST WATER AND SALT STRESSES

As mentioned earlier, both water stress reduces plant growth and crop productivity, so it is imperative to reduce yield gaps by increasing crop drought tolerance under these conditions, thereby ensuring food security for the increasing human population as well as for the benefit of poor farmers world-over. In this context, crop stress tolerance is defined in terms of yield stability under abiotic stress conditions. However, yield losses caused by abiotic stresses vary depending on timing, intensity and duration of the water stress, coupled with other environmental factors such as high light intensity and temperature. Based on this information, following means are suggested (Parry et al., 2005; Neumann, 2008) to

reduce/overcome the losses caused due to such stresses:

- (i) Water management practices that save irrigation water.
- (ii) Exploitation of the agronomic practices by which plants can perform well under water stress conditions.
- (iii) Selection of crop cultivars that require relatively lower quantity of water for their growth and crop productivity.

Strategies involving water saving irrigation technologies or cultural practices to alleviate drought stress, are expensive, inconvenient, and require specific knowledge for its implementation. On the other hand, use of drought resistant crop plants in drought prone environment, that is, biological approach is more feasible and efficient in achieving high crop productivity on drought hit areas. In addition, the biological approach involves, those methodologies which are used to enable plants that can effectively escape, avoid or tolerate drought.

SELECTION AND BREEDING FOR DROUGHT TOLERANCE

The development of drought-resistant cultivars/genotypes for any particular crop through conventional breeding method has been and still is of considerable economic value for increasing crop production in areas with low precipitation or with improper irrigation system (Subbarao et al., 2005). However, availability of genetic variation at inter-specific, intra-specific and intra-variety levels is of prime importance for selection and breeding for enhanced resistance to any stress (Serraj et al., 2005). In order to develop drought resistant/ tolerant cultivars, it is imperative to develop efficient screening method and suitable phenotyping criteria. Various agronomic, physiological and biochemical selection methods for drought tolerance are being employed to select drought tolerant plants, such as seed yield, harvest index, shoot fresh and dry weight, leaf water potential, osmotic adjustment, accumulation of compatible solutes, water use efficiency, stomatal conductance, chlorophyll fluorescence (Neumann, 2008). Development of drought tolerance in adaptation for a plant is the result of overall expression of many traits in a testing environment. Since many adaptive traits are effective only for certain aspects of drought tolerance and over a limited range of drought stress, there is no single trait that breeders can use to improve productivity of a given crop in a water deficit environment. Therefore, alternative potential systematic approach is to pyramid various traits in one plant genotype which can improve its drought tolerance. In this context, Subbarao et al. (2005) suggested that those traits, whether physiological or morphological, that contribute to reduce water loss through transpiration, and enhance water use

efficiency and/yield are traits of interest. While discussing prospects for crop production under drought, Parry et al. (2005) suggested some key traits to be keeping in consideration while breeding for drought tolerance (for example, phenology, rapid establishment, early vigor, root density and depths, low and high temperature tolerance, ^{13}C discrimination (a measure of the extent to which photosynthesis is maintained while stomatal conductance decreases), root conductance, osmoregulation, low stomatal conductance, leaf posture, habit, reflectance and duration, and sugar accumulation in stems to support later growth of yield components). However, they stressed that priority should be given to those traits that will maintain or increase yield stability in addition to overall yield, because traits for higher yield may in fact decrease yield stability (longer growth period). Thus, in order to improve crop productivity under water stress conditions, selection of a cultivar with short life span (drought escape), incorporation of traits responsible for well-developed root system, high stomatal resistance, high water use efficiency (drought avoidance), and traits responsible for increasing and stabilizing yield during water stress period (drought tolerance) should be given high priorities. Although a number of crop cultivars tolerant to drought stress have been developed through this method, this approach has been partly successful because it requires large investments in land, labor and capital to screen a large number of progenies, and variability in stress occurrence in the target environment. In addition, there is an evidence of marginal returns from conventional breeding, suggesting a need to seek more efficient methods for genetic enhancement of drought tolerance.

Molecular breeding

Now, it is well evident that water stress tolerant traits are mainly quantitative in nature and are controlled by multiple genes or gene complexes. The regions of chromosomes or the loci controlling these traits are called quantitative trait loci (QTLs). In QTL approach of plant breeding, parents showing extreme phenotypes for a trait are crossed to produce progenies with a capacity of segregation for that trait. This population is then screened for genetic polymorphism using molecular markers technique such as RFLP, RAPD, AFLP and SNPs. Genetic maps are being constructed and markers associated with a trait of interest are identified using computer software. Use of molecular markers to identify QTLs for physiological traits responsible for stress tolerance has helped to identify some potential sub-traits for drought tolerance (Hussain, 2006). Once molecular markers (that is, for a target QTLs) are linked to specific sub-traits of drought tolerance, it would be possible to transfer these various traits into other adapted cultivars with various

agronomic backgrounds under specific targeted environments through marker assisted breeding approaches.

Thus, identification of areas of a genome that have a major influence on drought tolerance or QTLs for drought tolerance traits could allow to identify the genes for drought tolerance. Thus, use of marker-assisted selection (MAS) seems to be a more promising approach because it enabled us to dissect quantitative traits into their single genetic components thereby helping in selecting and breeding plants that are resistant to any target trait like water stress (Chinnusamy et al., 2004; Hussain, 2006). The identification of QTLs for economically important traits has been achieved by developing linkage mapping to anonymous markers (segregation mapping) or through association studies (association mapping or candidate gene approach) involving candidate genes (Araus et al., 2003).

Although, most of the data for QTLs for drought tolerance available in the literature is based on segregation mapping studies (Cattivelli et al., 2008), association mapping or candidate gene approach is more vigorous than segregation mapping (Syvänen, 2005), because single genes controlling a trait such as flowering time, plant height, ear development and osmotic adjustment may have more important role in adaptation to drought-prone environment, as single candidate gene (*or* gene) conferring osmotic adjustment in wheat was mapped on the short arm of chromosome 7A (Morgan and Tan, 1996) and breeding for *or* gene improved yield in wheat under water deficit conditions (Morgan, 2000). While critically analyzed the reports on the application of QTL analysis (Cattivelli et al., 2008) pointed out that more efforts have been dedicated to understand the genetic basis of physiological traits responsible for drought tolerance, and little attention has been given to understand high yield stability in water deficit conditions. For example, more reports are available on genetic variation for osmotic adjustment, genetic basis of phenological traits, the ability of roots to exploit deep soil moisture, water use efficiency, limitation of non-stomatal water loss, and leaf elongation rate under varying degrees of water stress. Detailed information on QTLs for drought tolerance is available as GRAMENE (<http://www.gramene.org/>) or GRAINGENES. However, despite theoretical advantages of utilizing MAS to improve quantitative traits during the past decade, the overall impact of MAS on the direct release of drought-tolerant cultivars remains non-significant (Reynolds and Tuberosa, 2008). In view of the available information, identification of QTLs responsible for improving yield potential and drought tolerance will be main goal for the present and future research. Thus, it is suggested that deliberate selection for secondary traits related to drought tolerance is likely to achieve better results than direct selection for yield *per se* under stress conditions (Tuberosa et al., 2007). Marker assisted selection will

become more efficient if available markers are tightly linked to the genes of interest like stress related traits. Interestingly, Babu et al. (2003) while working with rice, found that QTLs for plant yield under drought were coincided with QTLs for root traits and osmotic adjustment. Likewise, Lanceras et al. (2004) found that favorable alleles for yield components were located in a region of rice chromosome 1 where QTLs for many drought related traits (root dry weight, relative water content, leaf rolling and leaf drying) were previously identified. However, in this strategy, parents of extreme contrasting traits (yield and drought tolerance) are required which may cause a cost on grain yield by decreasing yield component traits. From all this discussion, it seems that with the advent of this high throughput molecular biology technique, we are probably on the threshold of breakthroughs in our ability to understand and manipulate plant physiological responses to water deficit. Although use of molecular marker-assisted selection (MAS) seems to be more promising and meaningful, the contribution of molecular breeding to the development of drought tolerant cultivars has so far been marginal and a few reports are available in this regard (Cattivelli et al., 2008).

Another important application of molecular breeding is cloning of genes/DNA sequences associated with QTLs for drought tolerance. A number of strategies are being used to clone candidate genes/DNA sequences (Salvi and Tuberosa, 2005), which are evident from the available literature, by mapping of known stress responsive genes (Tondelli et al., 2006). For example, (Masle et al., 2005) cloned ERECTA gene in *A. thaliana*, a DNA sequence beyond a QTL for transpiration efficiency. However, there is no report available in the literature on cloning of genes underlying QTLs in any crop species. For identification of QTL corresponding gene (QTN-quantitative trait nucleotide), generation of molecular-linkage maps based on candidate genes (molecular function maps) is suggested to avoid time consuming fine mapping by a number of researchers. For example, this strategy has been applied to find genes for drought tolerance in barley and rice (Tondelli et al., 2006).

Molecular biology approaches to increase crop salt tolerance

Although, salt tolerance in plants is determined by a number of physiological and biochemical traits, but it is now well evident that salt tolerance is a complex trait involving the function of many genes (Munns, 2005; Munns and Tester, 2008). Furthermore, successful screening and selection of salt tolerant cultivars in conventional breeding program is limited by the significant influence of environmental factors affecting the expression of this polygene's (Ashraf et al., 2008). In view

of this argument, it is suggested to identify the molecular markers tightly linked to the genes governing salt tolerance which could indirectly be used to select plants in segregating populations as molecular markers are environment independent. Thus, the use of QTLs has improved the efficiency of selection, particularly, for those traits that are controlled by many genes and are highly influenced by environmental factors (Flowers, 2004).

Salt tolerance in plants varies with the change in growth stage that cause problem in selecting salt tolerant genotypes. Although, QTLs for salinity tolerance have been identified in a number of potential cereal crops such as rice, barley and wheat, robust markers that can be used across a range of germplasm are limited (Munns, 2008). Since 1993, a number of reports are available in the literature showing enhanced salt tolerance in different crop plants by over-expressing genes that are involved in controlling traits responsible for salt tolerance (Flowers, 2004; Munns, 2005; Ashraf et al., 2008).

Munns (2005) categorized these salt tolerant genes into three different categories (1) those that control salt uptake and transport; (2) that have an osmotic or protective function; and (3) that could make a plant grow more quickly in saline soil. However, large numbers of successful reports from transformation experiments have come from manipulating genes responsible for Na^+ exclusion or tissue Na^+ tolerance (Munns and Tester, 2008). These claims of improved salt tolerance were highly criticized because of poor experimental designs, inappropriate choices of methods to evaluate for salt tolerance (Flowers, 2004; Munns, 2005; Ashraf et al., 2008).

Which trait is useful, and which parent contributes?

One of the interesting revelations of the QTL analysis is that both parents (drought tolerant and -sensitive) contribute useful alleles for the trait of interest. Traditional grouping of drought-tolerance traits into four distinct classes (phenological, morphological, physiological and biochemical) may still be relevant for identifying QTLs. Because phenological and morphological traits can be scored easily in appropriately managed screening nurseries, the QTLs identified for these traits may be more reliable. Physiological traits such as osmotic adjustment or water-use efficiency are cumbersome to measure in the field, and can be relied on only under a carefully defined set of conditions. Biochemical traits, on the other hand, defy simple characterization, and most often they may only be the symptoms of stress. But they will have an increasing role in increasing our understanding of drought-tolerance mechanisms. The association between biochemical traits and QTLs is too speculative at the moment.

CONCLUSION AND FUTURE PROSPECTS

Although, it is widely recognized that salt and drought stresses are major constraints for crop productivity, knowledge about nature and magnitude of both stresses is scanty to develop an economically viable/sustainable agriculture. For example, a great gap exists in knowledge about the level of stress tolerance to be developed in crops intended to be grown on a targeted environment. Such kind of knowledge will certainly be helpful in prioritizing traits/selection criteria and developing screening techniques for improved stress tolerance. During the last two decades, plant breeders have been able to successfully develop cultivars with at least some tolerance for a number of abiotic stresses by exploiting genetic variation that exists among the cultivated varieties. Inter- and intra-specific genetic variation for stress tolerance in the present germplasm has resulted from long-term farmer selection or from wild relatives of crop plants that have evolved abiotic stress tolerance as a means to allow colonization of marginal and extreme habitats. However, desired diversity for improving stress tolerance is not available though small increase in stress tolerance feasible by exploiting existing genetic variation. In order to increase the extent of existing genetic variation for stress tolerance, use of wide hybridization, molecular breeding or transgenic approaches are suggested. Although wide hybridization can enhance the stress tolerance, it may cause a significant penalty in terms of yield. Development of transgenic plants for transcription factors, antiporter and compatible solutes resulted in enhanced stress tolerance in plants. However, such types of reports on enhanced stress tolerance are highly criticized due to adoption of poor evaluation methodology in carrying out such studies. At present, we are still unaware about stress-induced changes in metabolism in plants, and a major gap in our understanding of stress tolerance. With the advancement in functional genomics, it is possible to identify key genes and their immediate functions at cellular as well as at whole plant level. Thus, detailed analysis of underlying physiological and molecular mechanisms for salt tolerance using functional genomics is an important area of future research, which will eventually assist in developing transgenic plants for stress tolerance. Therefore, the improvement in abiotic stress tolerance in agricultural plants can only be achieved practically by combining traditional and molecular breeding approaches. In the meantime, it would be sensible to use shotgun approaches (exogenous application of compatible solutes, plant growth regulators, antioxidant compounds, inorganic salts) to increase salt tolerance in potential crops. Moreover, genetic modification should be combined with marker-assisted breeding programs with stress-related genes and QTLs, and ultimately, the different strategies should be integrated, and genes representing distinctive

approaches should be combined to substantially increase plant stress tolerance. Through more widespread application of forward and reverse genetic analyses in model plants and with the growing power of genomics and proteomics tools, progress in understanding abiotic stress signaling will certainly accelerate. With a better understanding comes more effective ways to improve plant tolerance to abiotic stress. A new world in modern agriculture is coming nearer and nearer.

Conflict of Interest

The author(s) have not declared any conflict of interest.

REFERENCES

- Araus JL, Slafer GA, Royo C, Serret MD (2008). Breeding for yield potential and stress adaptation in cereals. *Crit. Rev. Plant Sci.* 27:377-412.
- Araus JL, Bort J, Steduto P, Villegas D, Royo C (2003). Breeding cereals for Mediterranean conditions: ecophysiological clues for biotechnology application. *Ann. Appl. Biol.* 142:129-141.
- Ashraf M (2010). Inducing drought tolerance in plants: Recent advances. *Biotechnol. Adv.* 28:169-183
- Ashraf M, Athar HR, Harris PJ, Kwon TR (2008) Some prospective strategies for improving crop salt tolerance. *Adv. Agron.* 97: 45-110.
- Babu CR, Nguyen BD, Chamarek V, Shanmugasundaram P, Chezian P, Juyaprakash P, Ganesh SK, Palchamy A, Sadasivam S, Sarkarung S, Wade LJ, Nguyen TH. (2003). Genetic analysis of drought resistance in rice by molecular markers: association between secondary traits and field performance. *Crop Sci.* 43:1457-1469.
- Balachandran SM, Ravikumar G, Manimaran P (2014). GM Rice Engineered with AtDREB1A Gene for Improved Drought Stress Tolerance. *Plant Res.* 40:1-10
- Barnabas B, Jager K, Feher A (2008). The effect of drought and heat stress on reproductive processes in cereals. *Plant Cell Environ.* 31:11-38.
- Beck EH, Fettig S, Knake C, Hartig K, Bhattarai T (2007). Specific and unspecific responses of plants to cold and drought stress. *J. Biosci.* 32:501-510
- Blum A (2005): Drought resistance, water-use efficiency, and yield potential—are they compatible, dissonant, or mutually exclusive. *Austral. J. Agric. Res.* 56:1159-1168
- Blum A (2009). Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. *Field Crops Res.* 112:119-123.
- Bogeat-Triboulot MB, Brosché M, Renaut J (2007). Gradual soil water depletion results in reversible changes of gene expression, protein profiles, ecophysiology, and growth performance in *Populus euphratica*, a poplar growing in arid regions. *Plant Physiol.* 143(2):876-892.
- Bohnert HJ, Gong Q, Li P, Ma S (2006). Unraveling abiotic stress tolerance mechanisms—Getting genomics going. *Curr. Opin. Plant Biol.* 9:180-188.
- Bohnert HJ, Nelson DF, Jensen RG (1995). Adaptation to environmental stresses. *Plant Cell* 7:1099-1111.
- Bray EA (1997). Plant responses to water deficit. *Trends Plant Sci.* 2:48-54.
- Brini F, Gaxiola R, Berkowitz G, Masmoudi K (2005). Cloning and characterization of a wheat vacuolar cation/proton antiporter and pyrophosphatase proton pump. *Plant Physiol. Biochem.* 43: 347-354
- Brini F, Hanin M, Lumbreras V, Amara I, Khoudi H, Hassairi A, Pagès M, Masmoudi K (2007b). Overexpression of wheat dehydrin DHN-5 enhances tolerance to salt and osmotic stress in *Arabidopsis*

- thaliana*. Plant Cell Rep. 26: 2017-2026
- Brini F, Hanin M, Mezghanni I, Berkowitz G, Masmoudi K (2007a). Overexpression of wheat Na⁺/H⁺ antiporter *TNHX1* and H⁺-pyrophosphatase *TVP1* improve salt and drought stress tolerance in *Arabidopsis thaliana* plants. J. Exp. Bot. 58: 301-308
- Broeckling CD, Huhman DV, Farag MA (2005). Metabolic profiling of *Medicago truncatula* cell cultures reveals the effects of biotic and abiotic elicitors on metabolism. J. Exp. Bot. 56(410):323-336.
- Bruce WB, Edmeades GO, Barker TC (2002). Molecular and physiological approaches to maize improvement for drought tolerance. J. Exp. Bot. 53: 13-25.
- Cattivelli L, Rizza F, Badeck FW, Mazzucotelli E, Mastrangelo AM, Francia E, Mare C, Tondelli A, Stanca AM (2008). Drought tolerance improvement in crop plants: an integrated view from breeding to genomics. Field Crop Res. 105: 1-14.
- Chao DY, Luo YH, Shi M, Luo D, Lin HX (2005). Salt-responsive genes in rice revealed by cDNA microarray analysis. Cell Res. 15:796-810.
- Chaves MM, Maroco JP, Pereira JS (2003). Understanding plant responses to drought—From genes to the whole plant. Funct. Plant Biol. 30: 239-264.
- Chen F, Li Q, Sun L, He Z (2006). The rice 14-3-3 gene family and its involvement in responses to biotic and abiotic stress. DNA Res. 13(2):53-63
- Chen WQ, Provart NJ, Glazebrook J, Katagiri F, Chang HS, Eulgem T (2002). Expression profile matrix of *Arabidopsis* transcription factor genes suggests their putative functions in response to environmental stresses. Plant Cell 14:559-574.
- Chinnusamy V, Jagendorf A, Zhu JK (2004) Understanding and improving salt tolerance in plants. Crop Sci. 45: 437-448.
- Chinnusamy V, Zhu JH, Zhu JK (2006). Gene regulation during cold acclimation in plants. Physiol. Plant. 126:52-61.
- Condon AG, Richards RA, Rebetzke GJ, Farquhar GD (2004). Breeding for high water-use efficiency. J. Exp. Bot. 55:2447-2460.
- Cortina C, Culianez-Macia F A (2005). Tomato abiotic stress enhanced tolerance by trehalose biosynthesis. Plant Sci. 169: 75-82
- De Domenico S, Tsesmetzis N, Di Sansebastiano G P, Hughes R K, Casey R, Santino A (2007). Subcellular localisation of *Medicago truncatula* 9/13-hydroperoxide lyase reveals a new localisation pattern and activation mechanism for CYP74C enzymes. BMC Plant Biol. 7: 58
- Djoukeng JD, Arbona V, Argamasilla R, Gomez- Cadenas A (2008). Flavonoid profiling in leaves of citrus genotypes under different environmental situations. J. Agric. Food Chem. 56(23):11087-11097
- Dubouzet JG, Sakuma Y, Ito Y, Kasuga M, Dubouzet EG, Miura S. (2003). *OsDREB* genes in rice, *Oryza sativa* L., encode transcription activators that function in drought-, high-salt- and cold-responsive gene expression. Plant J. 33:751-763.
- Feuillet C, Leach JE, Rogers J, Schnable PS, Eversole K (2010).Crop genome sequencing: lessons and rationales. Trends Plant Sci.16(2):77-88.
- Flowers TJ (2004) . Improving crop salt tolerance. J. Exp. Bot. 55: 307-319.
- Fowler S, Thomashow MF (2002). Arabidopsis transcriptome profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold response pathway. Plant Cell 14:1675-1690.
- Franco-Zorrilla JM, Valli A, Todesco M, Mateos I, Puga M I, Rubio-Somoza I, Leyva A, Weigel D, García J A, Paz-Ares J (2007).Target mimicry provides a new mechanism for regulation of microRNA activity. Nat. Genet. 39(8):1033-7.
- Fukushima A, Kusano M, Redestig H, Arita M, Saito K (2011). Metabolomic correlation-network modules in *Arabidopsis* based on a graph-clustering approach. BMC Syst. Biol. 5:1.
- Godfray HC J, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM, Toulmin C (2010). Food security: The challenge of feeding 9 billion people. Science 327:812-818.
- Groppa M D, Benavides M P (2008). Polyamines and abiotic stress: recent advances. Amino Acids 34: 35-45
- Haake V, Cook D, Riechmann JL, Pineda O, Thomashow MF Zhang JZ (2002). Transcription factor CBF4 is a regulator of drought adaptation in Arabidopsis. Plant Physiol. 130:639- 648.
- Hamdy A, Ragab R, Scarascia-Mugnozza E (2003). Coping with water scarcity: Water saving and increasing water productivity. Irrigation Drainage 52:3-20.
- Hara M, Shinoda Y, Tanaka Y, Kuboi T (2009). DNA binding of citrus dehydrin promoted by zinc ion. Plant Cell Environ. 32: 532-541
- Hartung W, Sauter A, Hose E (2002). Abscisic acid in the xylem: where does it come from, where does it go to. J. Exp. Bot. 53:27-32
- Hirayama T, Shinozaki K (2010). Research on plant abiotic stress responses in the post-genome era: Past, present and future. Plant J. 61:1041-1052.
- Holmstrom KO, Somersalo S, Mandal A, Palva ET, Welin B (2000). Improved tolerance to salinity and low temperature in transgenic tobacco producing glycine betaine. J. Exp. Bot. 51: 177-185
- Hu HH, Dai MQ, Yao JL, Xiao BZ, Li XH, Zhang QF (2006). Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. Proc. Natl. Acad. Sci. USA 103:12987-12992.
- Hu L, Wang Z, Du H, Huang B (2010). Differential accumulation of dehydrins in response to water stress for hybrid and common bermudagrass genotypes differing in drought tolerance. J. Plant Physiol. 167: 103-109
- Hughes RK, De Domenico S, Santino A (2009). Plant cytochrome CYP74 family: biochemical features, endocellular localisation, activation mechanism in plant defence and improvements for industrial applications. ChemBioChem. 10: 1122-1133
- Hussain SS (2006). Molecular breeding for abiotic stress tolerance: drought perspective. Proc. Pak. Acad. Sci. 43(3): 189-210.
- Ingram J, Bartels D (1996). The molecular basis of dehydration tolerance in plants. Annu. Rev. Plant Physiol. Mol. Biol. 47:377-403.
- Ito Y, Katsura K, Maruyama K, Taji T, Kobayashi M, Seki M et al. (2006). Functional analysis of rice DREB1/CBF-type transcription factors involved in cold-responsive gene expression in transgenic rice. Plant Cell Physiol. 47: 141-153.
- Javot H, Lauvergeat V, Santoni V, Martin-Laurent F, Güçlü J, Vinh J, Heyes J, Franck KI, Schäffner A R, Bouchez D, Maurel C (2003). Role of a single aquaporin isoform in root water uptake. Plant Cell 15: 509-522
- Jiang F, Jeschke W D, Hartung W (2004). Water flows in the parasitic association *Rhinanthus minor*/*Hordeum vulgare*. J. Exp. Bot. 55: 2323-2329
- Kiyosue T, Yamaguchi-Shinozaki K, Shinozaki K (1993). Characterization of cDNA for a dehydration-inducible gene that encodes a CLP A, B-like protein in *Arabidopsis thaliana* L. Biochem. Biophys. Res. Commun. 196:1214-1220.
- Kovacs D, Agoston B, Tompa P (2008). Disordered plant LEA proteins as molecular chaperones. Plant Signal Behav. 3: 710-713
- Kovtun Y, Chiu WL, Tena G, Sheen J (2000). Functional analysis of oxidative stress-activated mitogen-activated protein kinase cascade in plants. Proc. Natl. Acad. Sci. USA 97: 2940-2945
- Lanceras JC, Pantuwan G, Jongdee B, Toojinda T (2004). Quantitative trait loci associated with drought tolerance at reproductive stage in rice. Plant Physiol. 135: 384-399.
- Levitt J (1972). Responses of Plant to Environmental Stress. Academic Press, New York.
- Liu Q, Kasuga M, Sakuma Y, Abe H, Miura S, Yamaguchi-Shinozaki K (1998). Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature- responsive gene expression, respectively, in *Arabidopsis*. Plant Cell 10:1391-1406.
- Lukowitz W, Gillmor CS, Scheible WR (2000). Positional cloning in Arabidopsis. Why it feels good to have a genome initiative working for you. Plant Physiol. 123(3):795-805.
- Masle J, Gilmore SR, Farquhar GD (2005). The ERECTA gene regulates plant transpiration efficiency in *Arabidopsis*. Nature 436: 866-870
- Merchan F, de Lorenzo L, Rizzo S G, Niebel A, Manyani H, Frugier F, Sousa C, Crespi M (2007). Identification of regulatory pathways

- involved in the reacquisition of root growth after salt stress in *Medicago truncatula*. *Plant J.* 51:1-17
- Mimura T, Kura-Hotta M, Tsujimura T, Ohnishi M, Miura M, Okazaki Y, Mimura M, Maeshima M, Washitani-Nemoto S (2003). Rapid increase of vacuolar volume in response to salt stress. *Planta* 216:397-402.
- Mittler R, Blumwald E (2010). Genetic engineering for modern agriculture: Challenges and perspectives. *Annu. Rev. Plant Biol.* 61:443-462.
- Mooney HA, Percy RW, Ehleringer J (1987). Plant physiological ecology today. *Bioscience* 37:18-20.
- Morgan JM (1984). Osmoregulation and water stress in higher plants. *Annu. Rev. Plant Physiol.* 35: 299-319.
- Morgan JM (2000). Increases in grain yield of wheat by breeding for a osmoregulation gene: relationship to water supply and evaporative demand. *Aust. J. Agric. Res.* 51: 971-978.
- Morgan JM, Tan MK (1996). Chromosomal location of a wheat osmoregulation gene using RFLP analysis. *Aust. J. Plant Physiol.* 23: 803-806.
- Mouillon J M, Eriksson S K, Harryson P (2008). Mimicking the plant cell interior under water stress by macromolecular crowding: disordered dehydrin proteins are highly resistant to structural collapse. *Plant Physiol.* 148:1925-1937
- Munns R (2005). Genes and salt tolerance: bringing them together. *New Phytol.* 167(3):645-663.
- Munns R, Tester M (2008). Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* 59:651-681.
- Nakashima K, Kiyosue T, Yamaguchi-Shinozaki K, Shinozaki K (1997). A nuclear gene, *erd1*, encoding a chloroplast-targeted Clp protease regulatory subunit homolog is not only induced by water stress but also developmentally up-regulated during senescence in *Arabidopsis thaliana*. *Plant J.* 12:851-861.
- Neumann PM (2008). Coping mechanisms for crop plants in drought-prone environments. *Ann. Bot.* 101: 901-907.
- Oh SJ, Song SI, Kim YS, Jang HJ, Kim SY, Kim M (2005). *Arabidopsis* CBF3/DREB1A and ABF3 in transgenic rice increased tolerance to abiotic stress without stunting growth. *Plant Physiol.* 138, 341-351.
- Ohnishi T, Sugahara S, Yamada T, Kikuchi K, Yoshida Y, Hirano HY, Tsutsumi N (2005). *OsNAC6*, a member of the *NAC* gene family, is induced by various stresses in rice. *Genes Genet. Syst.* 80:135- 139.
- Olsen AN, Ernst HA, Leggio LL, Skriver K (2005). *NAC* transcription factors: Structurally distinct, functionally diverse. *Trends Plant Sci.* 10: 79-87.
- Ooka H, Satoh K, Doi K, Nagata T, Otomo Y, Murakami K et al. (2003). Comprehensive analysis of *NAC* family genes in *Oryza sativa* and *Arabidopsis thaliana*. *DNA Res.* 10:239-247.
- Pardo JM (2010). Biotechnology of water and salinity stress tolerance. *Curr. Opin. Biotechnol.* 21:185-196.
- Parry MA, Flexas J, Medrano H (2005). Prospects for crop production under drought: Research priorities and future directions. *Ann. Appl. Biol.* 147:211-226.
- Peleman JD, van der Voort JR (2003). Breeding by design. *Trends Plant Sci.* 8(7):330-334
- Pitzschke A, Schikora A, Hirt H (2009). MAPK cascade signalling networks in plant defence. *Curr. Opin. Plant Biol.* 12(4):421-426.
- Qiu JL, Zhou L, Yun BW (2008). *Arabidopsis* mitogenactivated protein kinase kinases MKK1 and MKK2 have overlapping functions in defense signaling mediated by MEKK1, MPK4, and MKS1. *Plant Physiol.* 148(1):212-222.
- Reynolds M, Tuberosa R (2008) Translational research impacting on crop productivity in drought-prone environments. *Curr. Opin. Plant Biol.* 11: 171-179.
- Sakuma Y, Maruyama K, Osakabe Y, Qin F, Seki M, Shinozaki K (2006). Functional analysis of an *Arabidopsis* transcription factor, DREB2A, involved in drought-responsive gene expression. *Plant Cell* 18:1292-1309.
- Salvi S, Tuberosa R (2005). To clone or not to clone plant QTLs: present and future challenges. *Trends Plant Sci.* 10: 297-304.
- Sauter A, Davies W J, Hartung W (2001). The long-distance abscisic acid signal in the droughted plant: the fate of the hormone on its way from root to shoot. *J. Exp. Bot.* 52:1991-1997
- Sauter A, Hartung W (2000). Radial transport of abscisic acid conjugates in maize roots: its implication for long distance stress signals. *J. Exp. Bot.* 51:929-935
- Serraj R, Hash TC, Buhariwalla HK, Bidinger FR, Folkertsma RT, Chandra S, Gaur PM, Kashiwagi J, Nigam SN, Rupakula A, Crouch JH (2005). Marker-assisted breeding for crop drought tolerance at ICRISAT: achievements and prospects. In: Tuberosa R, Phillips RL, Gale M (eds) Proceedings of the International Congress "In the Wake of the Double Helix: From the Green Revolution to the Gene Revolution". Avenue Media, Bologna, Italy. pp. 217-238.
- Stockinger EJ, Gilmour SJ, Thomashow MF (1997). *Arabidopsis thaliana* CBF1 encodes an AP2 domain-containing transcriptional activator that binds to the C-repeat/DRE, a cis-acting DNA regulatory element that stimulates transcription in response to low temperature and water deficit. *Proc. Natl. Acad. Sci. USA* 94:1035-1040.
- Suarez-Rodriguez M C, Adams-Phillips L, Liu Y (2007). MEKK1 is required for flg22-induced MPK4 activation in *Arabidopsis* plants. *Plant Physiol.* 143(2):661-669.
- Subbarao GV, Ito O, Serraj R, Crouch JJ, Tobita S, Okada K, Hash CT, Ortiz R, Berry WL (2005). Physiological perspectives on improving crop adaptation to drought -justifi cation for a systematic component-based approach. In: Pessaraki M (ed) Handbook of Photosynthesis, 2nd edn. Marcel and Dekker, New York. pp. 577-594.
- Syvänen AC (2005). Toward genome-wide SNP genotyping. *Nat. Genet.* 37: S5-S10.
- Tardieu F, Tuberosa R (2010). Dissection and modelling of abiotic stress tolerance in plants. *Curr. Opin. Plant Biol.* 13: 206-212
- Tondelli A, Francia E, Barabaschi D, Aprile A, Skinner JS, Stockinger EJ, Stanca AM, Pecchioni N (2006). Mapping regulatory genes as candidates for cold and drought stress tolerance in barley. *Theor. Appl. Genet.* 112:445-455.
- Tran LS, Nakashima K, Sakuma Y, Simpson SD, Fujita Y, Maruyama K et al. (2004). Isolation and functional analysis of *Arabidopsis* stress-inducible *NAC* transcription factors that bind to a drought-responsive *cis*-element in the early responsive to dehydration stress 1 promoter. *Plant Cell* 16:2481-2498.
- Tuberosa R, Salvi S, Giuliani S, Sanguineti MC, Bellotti M, Conti S, Landi P (2007) Genome-wide approaches to investigate and improve maize response to drought. *Crop Sci.* 47(S3): S120-S141.
- Vorosmarty CJ, Green P, Salisbury J, Lammers RB (2000). Global water resources: Vulnerability from climate change and population growth. *Science* 289: 284-288.
- Widodo JH, Patterson E, Newbigin M, Tester A, Bacic, Roessner U (2009). Metabolic responses to salt stress of barley (*Hordeum vulgare* L.) cultivars, Sahara and Clipper, which differ in salinity tolerance. *J. Exp. Bot.* 60(14):4089-4103.
- Witcombe JR, Hollington PA, Howarth CJ, Reader S, Steele KA (2008). Breeding for abiotic stresses for sustainable agriculture. *Philos Trans. R. Soc. B Biol. Sci.* 363:703-716.
- Wu CQ, Hu HH, Zeng Y, Liang DC, Xie KB, Zhang JW (2006). Identification of novel stress-responsive transcription factor gene in rice by cDNA array analysis. *J. Integr. Plant Biol.* 48:1216-1224.
- Yamaguchi-Shinozaki K, Shinozaki K (1994). A novel *cis*-acting element in an *Arabidopsis* gene is involved in responsiveness to drought, low-temperature, or high-salt stress. *Plant Cell.* 6:251-264.
- Yamaguchi-Shinozaki K, Shinozaki K (2005). Organization of *cis*acting regulatory elements in osmotic- and cold-stress-responsive promoters. *Trends Plant Sci.* 10:88-94.
- Zaffar G, Shikari AB, Rather MA, Guleria SK (2005). Comparison of selection indices for screening maize (*Zea mays* L.) germplasm for cold tolerance. *Cereal Res. Commun.* (33):525-531
- Zhu JK (2002). Salt and drought stress signal transduction in plants *Annu. Rev. Plant Biol.* 53:247-273.