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Taming Drought Stress in Rice through Genetic Engineering of Transcription Factors and Protein Kinases

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ABSTRACT

The majority of the world's population depends on rice as the principal staple food crop. Water deficit or drought stress is one of the serious environmental threats and the main constraint to rice productivity. It affects rice at morphological, biochemical, physiological and molecular levels such as delayed flowering, reduced dry matter accumulation and decreased photosynthetic capacity as a result of stomatal closure, metabolic limitations and oxidative damage. Some of the physiological parameters that are affected during drought stress are root system, root/shoot ratio, stomatal frequency, leaf weight, leaf water potential, tissue water storage capacity, water permeability, leaf weight, thickness of cuticle, leaf chlorophyll content and finally, the yield. To withstand drought stress, plants need to be manipulated at the genetic level for improved metabolic processes like water absorption, stomatal conductance, transpiration, photosynthesis and finally seed development. Drought tolerance and adaptation in rice plants has been improved by engineering various genes related to transcription, signaling, accumulation of antioxidants and compatible solutes etc. In this review, we discuss the recent developments towards genetic engineering of transcription factors and protein kinases for enhancing drought stress tolerance in rice plants.

Keywords: ABA, cross-tolerance, signal transduction, transgenic, water-deficit stress, yield penalty

Abbreviations: ABA, abscisic acid; **ERF**, ethylene response factor; **EST**, expressed sequence tag; **PEG**, polyethylene glycol; **qRT PCR**, quantitative reverse transcription PCR; **ROS**, reactive oxygen species; **SOD**, superoxide dismutase; **TF**, transcription factor; **WT**, wild type

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INTRODUCTION

Rice is one of the most important cereal crops feeding more than half of the world population. There are many reasons for serious concern about its sustainable production, which include global climate change, less availability of arable land and water and the ever increasing world population (Takeda and Matsuoka 2008). The uneven distribution of rainfall and ground water shortage often create drought stress conditions in the environment (Luo and Zhang 2001), which eventually lead to enormous decrease in the grain yield potential of rice. Cell shrinkage and a subsequent decline in cellular volume are instant symptoms caused by dehydration. Upon exposure to this stress, plants experience multiple constraints including cell injury by producing reactive oxygen species (ROS) and increasing cellular temperature, which result into increase in the viscosity of cellular contents, alteration of the protein-protein interactions and protein aggregation and denaturation (Parry *et al.* 2002; Farooq *et al.* 2008). Besides, high accumulation of solutes causes toxicity and negatively affects functioning of some enzymes often leading to reduced photosynthesis and

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Invited Review

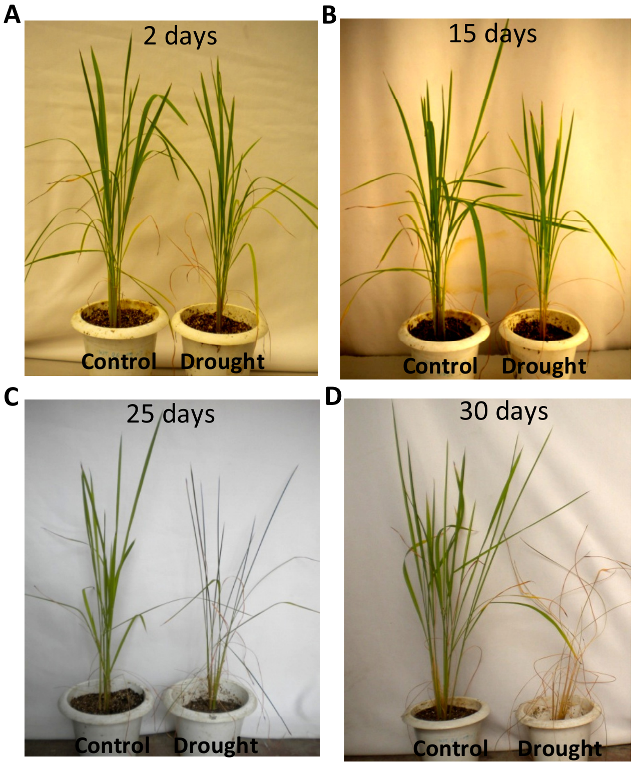

Fig. 1 Response of rice plants towards dehydration stress in a sensitive cultivar 'IR64'. Note that leaf rolling started to appear within 15 days (B) of water withdrawal which eventually causes complete wilting and bleaching of chlorophyll upon prolonged drought stress (i.e. 30 days, D) leading to the death of the plant (Sahoo *et al*., unpublished data).

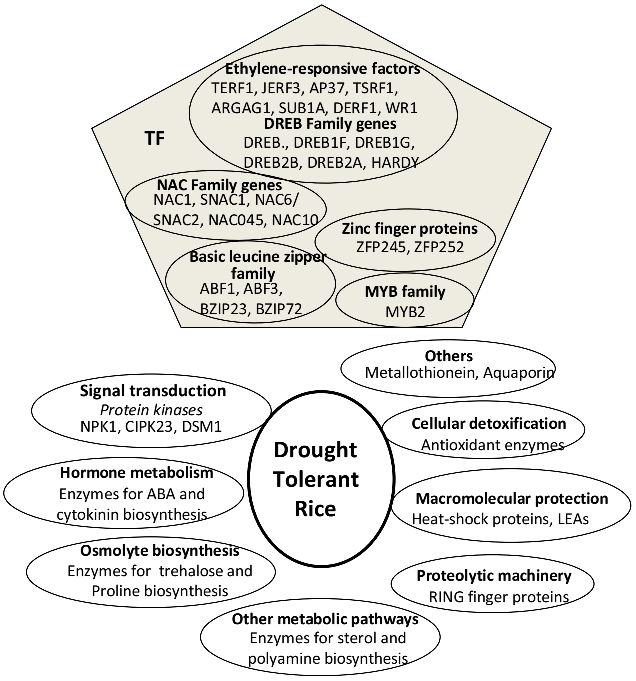

Fig. 2 Outline of genetic engineering targets employed to develop drought tolerant rice cultivars. The genes, functionally validated for their role in drought/water deficit tolerance and adaptation, belong to several classes on the basis of their cellular function viz. transcription (TF), osmolyte biosynthesis, signal transduction, hormone metabolism, cellular detoxification, proteolysis, macromolecular protection, and other metabolic processes. Genetic engineering targeting these genes has been shown to improve drought tolerance in various rice cultivars. See **Tables 1** and **2** for details.

water use efficiency. Under prolonged dehydration conditions, plants exhibit leaf rolling (the very first symptom), wilting and bleaching eventually leading to death of the plant (**Fig. 1**).

"Stress tolerance" and "stress avoidance" are two kinds of responses shown by plants under water deficit or drought conditions. The process of stress avoidance involves several physiological manifestations such as enhanced water retention and reduced span of vegetative and reproductive phases of life cycle (Zhang 2007). Drought tolerance is an outcome of a series of molecular, cellular and physiological processes including induction/repression of various genes leading to accumulation of various osmolytes, improved antioxidant system, reduced transpiration, efficient and deeper root system, inhibited shoot growth and decreased tillering. Besides, the phytohormone abscisic acid (ABA) is known to be produced more in water-deficit conditions, which further causes closure of stomata and induction of expression of various stress-related genes (Yang *et al*. 2010). However, it has also been shown that drought inducible gene expression is governed by ABA-independent regulatory system as well (Chinnusamy *et al*. 2004).

Considerable progress has been made utilizing genetics and functional genomics approaches such as transcriptomics, proteomics and metabolomics to identify and characterize various genes involved in drought responses in rice. These drought-responsive genes mainly code for proteins that have either metabolic or regulatory role. The former class includes genes involved in detoxification, osmolyte biosynthesis, proteolysis of cellular substrates, cellular transport and macromolecular protection (Shinozaki and Yamaguchi-Shinozaki 2007). The regulatory class majorly comprises of transcription factors and proteins involved in signal transduction such as kinases, etc. Moreover, many genes induced/repressed under drought conditions have functions yet to be ascertained.

Several studies have attempted the functional validation of drought-responsive genes using gene transfer methods and have found, in some cases, enhanced drought tolerance in transgenic rice plants (**Tables 1, 2**). In the present review, we summarize such studies related to functional characterization of drought-responsive genes for the development of rice varieties with improved drought tolerance ability using genetic engineering approaches. For the sake of brevity, we have discussed the studies related to the regulatory class of drought responsive genes only. Rice has been chosen for this study for obvious reasons but authors believe that most of these studies may be extended to other crops as well. The genetic and metabolic crosstalk between drought and other abiotic stresses have also been discussed.

Drought-responsive genes and their exploitation in developing drought tolerant rice cultivars

Many studies have been carried out to identify genes involved in drought stress tolerance and adaptation in the model plants rice and *Arabidopsis*. In rice, the main approaches utilized for such studies is transcript profiling via massively parallel signature sequencing (MPSS), ESTs profiling, RNA gel-blot analyses, microarrays and qRT-PCR (Rabbani *et al.* 2003; Kathiresan *et al.* 2006; Gorantla *et al.* 2007; Zhou *et al.* 2007; Rabello *et al.* 2008) and comparative proteome analysis (Xiong *et al.* 2010). Taken together, all these studies have identified hundreds of genes that are induced/repressed in response to drought stress. However, a very small fraction of these genes have been functionally validated for their role in enhancement of drought tolerance ability. Such genes can be grouped in several classes based on their cellular function viz. transcription, osmolyte biosynthesis, signal transduction, hormone metabolism, cellular detoxification, proteolysis, macromolecular protection, etc. (**Fig. 2**). The transcription factors constitute a major part (44%) of such genes followed by those involved in macromolecular protection (12%), signal transduction (8%), proteolytic machinery (8%), osmolytes biosynthesis (7%), hormone metabolism (3%), cellular detoxification (3%) and others (**Fig. 3**).

Table 1 Genetic engineering of transcription factors for enhancing drought tolerance in rice.

Gene/ protein	Name	Source	Promoter	Phenotype	Reference
NAC Family genes					
SNAC1	Stress responsive NAC1	*Oryza sativa*	CaMV35S	Drought and salinity tolerance	Hu *et al.* 2006
NAC6/ SNAC2	Stress responsive NAC2	*Oryza sativa*	OsNAC6, LIP9	Tolerance to cold, salt stress	Nakashima *et al.* 2007
NAC045	NAC045 transcription factor	*Oryza sativa*	CaMV35S	Drought and salt tolerance	Zheng *et al.* 2009
NAC10	NAC10 transcription factor	*Oryza sativa*	GOS2, RCc3	Tolerance to drought and low temperature	Jeong *et al.* 2010
Basic leucine zipper transcription factors					
ABF3	ABA responsive element binding proteins/factors3	*Arabidopsis thaliana*	Ubiquitin	Drought tolerance	Oh *et al.* 2005
bZIP23	Basic leucine zipper 23	*Oryza sativa*	Ubiquitin	ABA sensitivity, salinity and drought tolerance	Xiang *et al.* 2008
bZIP46	Basic leucine zipper 46	*Oryza sativa*	Ubiquitin	drought and osmotic stresses tolerance	Tang *et al.*2012
bZIP72	Basic leucine zipper 72	*Oryza sativa*	CaMV35S	ABA hypersensitivity, drought tolerance	Lu *et al.* 2008
HD-Zip	Homeodomain leucine zipper	*Oryza sativa*	CaMV35S	Plant development and drought stress adaptation	Agalou *et al.* 2008
MYB-Type transcription factor					
MYB2	MYB transcription factor	*Oryza sativa*	Ubiquitin	Salt, cold and dehydration tolerance	Yang *et al.* 2012
Zinc finger transcription factors					
ZFP252	TFIIIA Zinc finger protein 252	*Oryza sativa*	CaMV35S	Tolerance to salt and drought stresses	Xu *et al.* 2008
ZFP245	Zinc finger protein 245	*Oryza sativa*	CaMV35S	Cold, drought and oxidative stresses tolerance	Huang *et al.* 2009
Ethylene-responsive factors					
DREB1A/ CBF3	C-repeat/DRE element binding factors	*Arabidopsis thaliana*	Ubiquitin	Drought, salt and freezing tolerance	Oh *et al.* 2005
HARDY	AP2/ERF-like transcription factor	*Arabidopsis thaliana*	CaMV35S	Drought and salt stress tolerance	Karaba *et al.* 2007
TERF1	Tomato ethylene response factor	*Lycopersicum esculentum*	CaMV35S	Drought and salinity tolerance	Gao *et al.* 2008
DREB1F	DREB class1 protein	*Oryza sativa*	CaMV35S	Salt, drought,and low temperature tolerance	Wang *et al.* 2008
OsDREB1G, OsDREB2B	DREB class1 and 2 protein	*Oryza sativa*	CaMV35S	Water deficit stress tolerance	Chen *et al.* 2008
JERF3	Ethylene response factor	*Lycopersicum esculentum*	CaMV35S	Drought and osmotic stress tolerance	Zhang *et al.* 2010
AP37	AP2/ERF domain containing transcription factors	*Oryza sativa*	OsCc1	Tolerance to drought and high salinity	Oh *et al.* 2009
TSRF1	Tomato ethylene response factor	*Oryza sativa*	CaMV35S	Drought stress tolerance	Quan *et al.* 2010
ARAG1	ABA-responsive DREB gene	*Oryza sativa*	CaMV35S	Seed germination and drought tolerance	Zhao *et al.* 2010
SUB1A	ERF transcription factor	*Oryza sativa*	Ubiquitin	Drought and oxidative stress tolerance	Fukao *et al.* 2011
DERF1	Drought and ethylene-responsive factor	*Oryza sativa*	CaMV35S, Actin1	Drought sensitive	Wan *et al.* 2011
OsWR1	Rice wax synthesis regulatory gene	*Oryza sativa*	CaMV35S, Actin1	Drought tolerance	Wang *et al.* 2011
DREB2A	DREB class2 protein	*Oryza sativa*	4XABRC	Drought and salt stress tolerance	Cui *et al.* 2011
DREB2A	DREB class2 protein	*Oryza sativa*	RD29	Dehydration and salt stress tolerance	Mallikarjuna *et al.* 2011

Transcription factors involved in drought response and adaptation

Transcription factors possess the capacity to drive and alter the expression of a wide variety of genes. It is an established fact that drought response is a complex phenomenon, even at the cellular level, involving various components of the cellular machinery. Hence, it is not surprising that many transcription factors participate in this response. The drought responsive transcription factors can be grouped into either ABA-dependent or independent (**Fig. 4**). Such transcription factors can also be classified based on their different DNA binding domains into various classes as follows.

NAC family genes

NAC {an acronym for *NAM* (No Apical Meristem), *ATAF1-2* (Arabidopsis transcription activation factor), and *CUC2* (Cup-Shaped Cotyledon)} family includes plant-specific transcription factors like NAM, ATAF and CUC. Generally NAC proteins have conserved N-terminal DNA-binding domain, which contains a nuclear localization signal sequence and a variable C-terminal domain. It has been predicted that 140 and 75 NAC genes are present in the *Oryza sativa* and *Arabidopsis* genomes, respectively (Ooka *et al.* 2003; Xiong *et al.* 2005; Fang *et al.* 2008). Some NAC genes, such as AtNAC072 (RD26), AtNAC019, AtNAC055 from *Arabidopsis* (Fujita *et al.* 2004; Tran *et al.* 2004), and BnNAC from *Brassica* (Hegedus *et al.* 2003), were found to participate in response to various environmental stresses. Another NAC family gene from rice, SNAC1 (STRESS-RESPONSIVE NAC 1), was found to be highly upregulated in drought stress (Hu *et al.* 2006). SNAC1 showed strong localization in guard cells of stomata (Hu *et al.* 2006).

Table 2 Genetic engineering of other drought-responsive genes for enhancing drought tolerance in rice.

Gene	Encoded protein	Source	Promoter	Phenotype	Reference
Signal transduction					
CDPK7	Calcium dependent protein kinase	*Oryza sativa*	CaMV35S	Drought, salt and cold tolerance	Saijo *et al.* 2000
CIPK23	CBL- interacting protein kinase	*Oryza sativa*	Ubiquitin	Drought stress tolerance	Yang *et al.* 2008
NPK1	Nucleus and phragmoplast localized protein1	*Oryza sativa*	HVA22p, Actin1	Drought tolerance	Xiao *et al.* 2009
DSM1	Mitogen-activated protein kinase kinase kinase	*Oryza sativa*	Ubiquitin	Drought resistance	Ning *et al.* 2010
ITPK (ITPK2 or DSM3)	Inositol 1,3,4-trisphosphate 5/6-kinase	*Oryza sativa*	CaMV35S	Drought stress sensitivity	Du *et al.* 2011
OsPFA-DSP1	plant and fungi atypical dual-specificity phosphatase 1	*Oryza sativa*	CaMV35S	Drought stress sensitivity	Liu et al. 2012b
Hormone metabolism					
ABA3/LOS5	Molybdenum-cofactor sulfurase	*Arabidopsis thaliana*	HVA22	Drought stress tolerance	Xiao *et al.* 2009
IPT	Isopentenyl transferase	*Oryza sativa*	Psark	Drought tolerance and enhanced grain yield	Peleg *et al.* 2011
Osmolyte biosynthesis					
otsA and otsB	Trehalose-6-phosphate synthase and Trehalose-6-phosphate phosphatase	*Escherichia coli*	ABRC and rbcS	Salinity and drought stress tolerance	Garg *et al.* 2002
TPS and TPP	Trehalose-6-phosphate synthase and Trehalose-6-phosphate phosphatase	*Escherichia coli*	Ubiquitin	Drought, salinity, and low temperature	Jang *et al.* 2003
STRG	Salt tolerance-related gene encoding hypothetical protein	*Triticum aestivum*	Ubiquitin	Salt and drought tolerance	Zhou *et al.* 2009
TPS	Trehalose-6-phosphate synthase	*Oryza sativa*	CaMV35S	Cold, salinity and drought tolerance	Li *et al.* 2011
Proteolytic machinery					
COIN	Cold-inducible zinc finger protein	*Oryza sativa*	CaMV35S	Tolerance to chilling, salt and drought stress	Liu *et al.* 2007
OCPI1	Chymotrypsin inhibitor-like 1	*Oryza sativa*	CaMV35S	Drought resistance	Huang *et al.* 2007
SDIR1	Salt and drought induced ring finger 1	*Oryza sativa*	Ubiquitin	Drought stress tolerance	Gao *et al.* 2011
DIS1	Drought-induced SINA protein 1	*Oryza sativa*	Ubiquitin	Drought stress tolerance	Ning *et al.* 2011
RDCPs	RING domain-containing proteins	*Oryza sativa*	CaMV35S	Water deficit stress tolerance	Bae *et al.* 2011
Cellular detoxification					
MnSOD	Mn-Superoxide dismutase	*Pisum sativum*	SWPA2	Drought tolerance	Wanga *et al.* 2004
Cu/Zn-SOD	Cu/Zn- Superoxide dismutase	*Avicennia marina*	Ubiquitin	Salt and drought tolerance	Prashanth *et al.* 2004
Macromolecular protection					
HVA1	Late-embryogenesis abundant protein	*Hordeum vulgare*	Actin1	Water deficits and salt stress tolerance	Xu *et al.* 1996
HVA1	Late-embryogenesis abundant protein	*Hordeum vulgare*	Actin1, 4ABRC	Salt and drought tolerance	Rohila *et al.* 2002
HVA1/(PMA1 959), (PMA80)	Late-embryogenesis abundant protein	*Hordeum vulgare*	Actin1, CaMV35S	Dehydration tolerance	Cheng *et al.* 2002
HVA1	Late-embryogenesis abundant protein	*Hordeum vulgare*	Actin1	Dehydration tolerance	Babu *et al.* 2004
CSPA	Cold shock protein	*Escherichia coli*	CaMV35S	Drought resistance	Castiglioni *et al.* 2007
LEA3-1	Late-embryogenesis abundant protein	*Oryza sativa*	CaMV35, Actin1	Drought resistance	Xiao *et al.* 2007
sHSP17.7	Small heat-shock protein	*Oryza sativa*	CaMV35S	Drought stress tolerance	Sato *et al.* 2008
Other metabolic pathways					
adc	Adenine decarboxylase	*Datura stramonium*	Ubiquitin	Drought stress tolerance	Capell *et al.* 2004
DHODH1	Cytosolic dihydroorotate dehydrogenase	*Oryza sativa*	CaMV35S	Salt and drought tolerance	Liu *et al.* 2009
SAMDC	S-adenosylmethionine decarboxylase	*Datura stramonium*	Ubiquitin	Facilitation of 'drought recovery'	Peremarti *et al.* 2009
dsm2 (BCH)	β-carotene hydroxylase	*Oryza sativa*	CaMV35S	Drought and oxidative stress resistance	Du *et al.* 2010
SQS	Squalene synthase	*Oryza sativa*	Ubiquitin	Drought stress tolerance	Manavalan *et al.* 2011
Others					
RWC3	Aquaporin	*Oryza sativa*	SWPA2	'Drought avoidance'	Lian *et al.* 2004
SAP8	Stress associated protein 8	*Oryza sativa*	Ubiquitin	Salt, drought and cold stress	Kanneganti *et al.* 2008
MT1a	Type 1 metallothionein	*Oryza sativa*	Actin1	Zinc homeostasis and Drought stress tolerance	Yang *et al.* 2009
OsRIP18	Ribosome-inactivating protein	*Oryza sativa*	CaMV35S	Salt and drought tolerance	Jiang *et al.* 2011
hrf1	Harpin-encoding genes	*Xanthomon-as oryzae*	CaMV35S	Drought stress tolerance	Zhang *et al.* 2011

SNAC1 overexpressing transgenic rice plants improved drought resistance and salt tolerance under field conditions without affecting growth and productivity. Overexpression of SNAC1 increased ABA sensitivity and triggered upregulation of some genes known to be involved in stomatal movement such as the major intrinsic protein, calmodulin-binding protein, Rac-like GTP-binding protein and WWE domain containing protein (Hu *et al.* 2006). These results suggest that the observed drought tolerance was due to the increased stomatal closure and/or ABA sensitivity to pre-

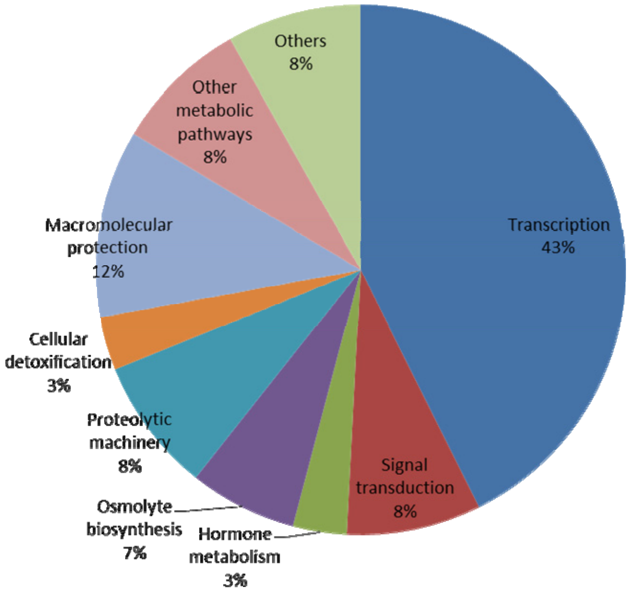

Fig. 3 Functional classification of genes involved in conferring drought-tolerance based on their cellular and physiological role. Pie chart depicts the distribution of the genes, shown to be involved in conferring drought-tolerance, in various classes based on their function. As shown, majority of such genes are transcription factors followed by those involved in macromolecular protection, signal transduction, proteolytic machinery, osmolytes biosynthesis, hormone metabolism, and cellular detoxification etc. See text for details.

vent water loss and hence improving water-retention.

Another NAC family gene, OsNAC10 was found to be induced by drought, high salinity and abscisic acid. Overexpression of OsNAC10, under the control of constitutive promoter GOS2 (GOS2:OsNAC10) and the root-specific promoter RCc3 (RCc3:OsNAC10) in rice was found to impart tolerance to drought and low temperature which was better in case of RCc3:OsNAC10 transgenic plants compared to GOS2:OsNAC10 (Jeong *et al.* 2010). Furthermore, grain yield of GOS2:OsNAC10 plants was similar to that of wild type plants under both normal and drought conditions whereas it was higher in RCc3:OsNAC10 transgenic plants under drought conditions. This may be explained as root-specific overexpression of OsNAC10 could not affect the development of reproductive organs while it did confer stress tolerance in the transgenic plants. Moreover, root diameter of the RCc3:OsNAC10 plants was more as compared to that of the GOS2:OsNAC10 and wild type plants due to the enlarged stele, cortex, and epidermis (Jeong *et al.* 2010).

Among other functionally validated NAC genes are OsNAC6 and ONAC045. Transgenic rice overexpressing OsNAC6 (or SNAC2) has been shown to have enhanced tolerance to cold, salt, and blast disease at the vegetative stage due to increased expression of stress-related genes (Nakashima *et al.* 2007; Hu *et al.* 2008). ONAC045 expression in roots and leaves was found to be induced by cold and drought stress. The transgenic rice plant overexpressing ONAC045 significantly improved drought tolerance during water deficit (Zheng *et al.* 2009), possibly due to high expression of OsLEA3-1 and OsPM1 genes (Xiao *et al.* 2007).

Basic leucine zipper transcription factors

The basic leucine zipper (bZIP) transcription factors play important roles in several biological processes such as flower development and stress responses in plants. In *Arabidopsis*, few members of a subfamily of the bZIP family called as AREB/ABF (ABA responsive element binding proteins/factors) have been studied for their roles in regulating ABA-mediated stress responses. Such bZIP transcription factors include ABI5 (Carles *et al.* 2002; Lopez-Molina *et al.* 2002; Bensmihen *et al.* 2005), ABF1 to ABF4 (Choi *et al.* 2000; Kang *et al.* 2002; Kim *et al.* 2004) and EEL (Bensmihen *et al.* 2002). In rice, more than 100 members of the bZIP family are present. Expression analysis has revealed that 33 of them are drought-responsive (Nijhawan *et al.* 2008). Out of these, 5 genes viz. ABF3 (Oh *et al.* 2005), OsbZIP23 (Xiang *et al.* 2008), OsbZIP46 (Tang *et al.* 2012), OsbZIP52 (Liu *et al.* 2012a) and OsbZIP72 (Lu *et al.* 2008) have been shown to confer drought tolerance when overexpressed in rice. ABF3 (ABRE-binding factor 3) was the first member of the bZIP class to be functionally validated for drought tolerance in rice. ABF3 overexpression has been shown to induce the expression of ABA-related genes that encode LEA (Rab21), Hsp70 and protein phosphatase 2C (PP2Ca), leading to

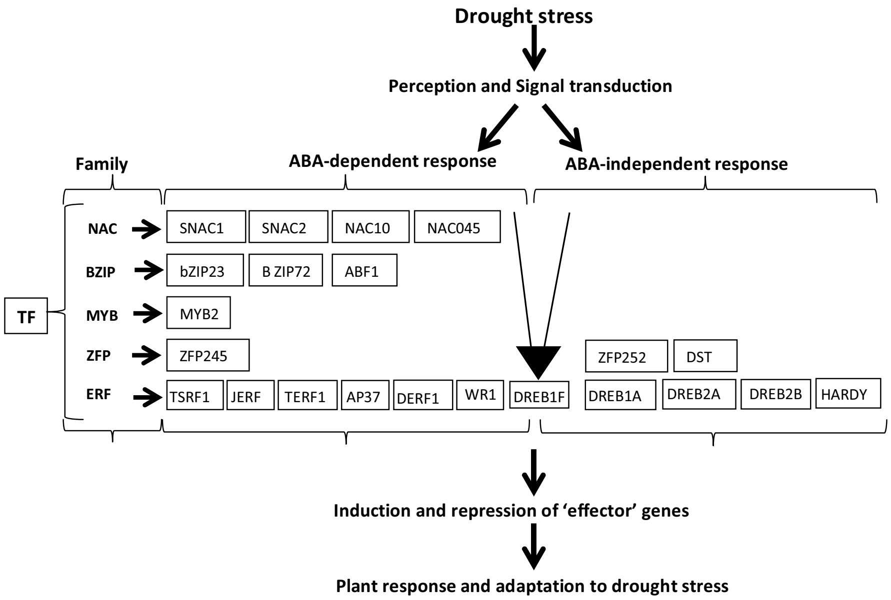

Fig. 4 Broad overview of drought response and the role of ABA-dependent and ABA-independent transcription factors in drought adaptation. Drought response is initiated with the perception of the stress and the signal is further relayed to either drive ABA-biosynthesis followed by activation of ABA-dependent transcription factors or expression of stress-responsive but ABA-independent transcription factors. The transcription factors induce/repress the expression of stress-related 'effector' genes, an eventual outcome of which is drought adaptation. Note that DREB1F functions in both the pathways and hence it has been predicted to act as a 'molecular bridge' between ABA-dependent and independent responses (see text for details).

drought tolerance (Oh *et al.* 2005).

OsbZIP23 plays diverse functions, including light signaling, developmental and physiological processes during floral transition, panicle development and abiotic stress tolerance (Nijhawan *et al.* 2008). OsbZIP23 expression is strongly induced by several abiotic stresses, including salt, drought, ABA and PEG treatments. OsbZIP23-overexpressing transgenic rice showed improved tolerance to drought and high-salinity stresses and were more sensitive to ABA (Xiang *et al.* 2008). OsbZIP23 overexpressing plants showed significant up-regulation of more than 800 genes involved in defense pathways. Such genes encode for proteins such as LEA proteins, dehydrins, transcription factors, seed storage proteins and transportation proteins, among others.

Recently, another bZIP protein - OsbZIP46 has been reported to be responsive to various abiotic stresses including drought, heat, hydrogen peroxide and ABA (Tang *et al.* 2012). Overexpression of the native OsbZIP46 rendered plants more sensitive to ABA but could not alter drought tolerance. However, overexpression of a deletion mutant of OsbZIP46 - OsbZIP46CA1, which was constitutively active, imparted increased tolerance towards drought and osmotic stresses. It was found via gene chip analysis that numerous stress-related genes were induced in OsbZIP46CA1-overexpressing plants. Many of such genes are predicted to be downstream genes of ABA-responsive pathways. These results suggest that OsbZIP46 may be a positive regulator of ABA signaling and drought tolerance in rice. Furthermore, the post-translational modification of OsbZIP46 might serve a regulatory role and may be required for its functions (Tang *et al.* 2012).

Another bZIP protein - OsbZIP52 has lately been shown to be enhancing drought and cold stress sensitivity (Liu *et al.* 2012a). qRT-PCR based expression analysis revealed that few abiotic stress-related genes, including OsLEA3 and OsTPP were down regulated in lines over expressing OsbZIP52. The findings of this study strongly suggested that OsbZIP52 could function as a negative regulator of drought and cold response (Liu *et al.* 2012a).

The other bZIP protein involved in conferring drought tolerance-OsbZIP72 was shown to bind to ABRE and transactivate a reporter gene downstream to the ABRE in yeast (Lu *et al.* 2008). This transactivation was dependent on its N-terminal region. The expression of OsbZIP72 was induced by ABA, cold, methyl jasmonate and polyethylene glycol treatment. Transgenic rice overexpressing OsbZIP72 showed enhanced drought tolerance and hypersensitivity to ABA. This observation was attributed to elevated levels of expression of ABA responsive genes such as *LEA*s (Lu *et al.* 2008).

MYB superfamily transcription factors

MYB transcription factors occur widely in plants, animals and fungi (Lippold *et al.* 2009). MYB proteins possess up to three imperfect repeats of 51–53 amino acids in their DNA-binding domain (MYB domain), and based on the number of repeats in the DNA-binding domain they are classified into three subfamilies - type R2R3, type R1R2R3, and MYB-related (Stracke *et al.* 2001; Chen *et al.* 2006). Among these, the MYB family with the two-repeat (R2R3) is the most common in plants (Stracke *et al.* 2001). 126 and 109 R2R3-type MYB proteins are present in *Arabidopsis* and rice, respectively (Chen *et al.* 2006). Findings of many studies support that some MYB proteins are involved in response and adaptation to cold stress (Vannini *et al.* 2004; Dai *et al.* 2007; Ma *et al.* 2009; Su *et al.* 2010). Recently, Yang *et al.* (2012) have reported enhanced tolerance of OsMYB2-overexpressing rice plants to multiple abiotic stresses viz. dehydration, salt and cold. Molecular characterization of the transgenic plants revealed higher expression of proline synthase and proline transporter leading to greater accumulation of free proline. Furthermore, many stress-related genes such as OsLEA3, OsRab16A, and OsDREB2A were highly upregulated in the overexpression plants, indicating the possible mechanism of stress tolerance.

Zinc finger transcription factors

The Cys-2/His-2-type (C2H2) zinc finger (also called as TFIIIA-type zinc finger) protein is an important class of eukaryotic transcription factors. Members of this family in plants have been shown to possess regulatory roles in stress responses and developmental processes (Sakamoto *et al.* 2004; de Lorenzo *et al.* 2007; Xu *et al.* 2008). Previous studies have shown that the *Arabidopsis* C2H2-type zinc finger proteins ZAT10/STZ and ZAT12 are involved in abiotic stress and ROS signaling (Rizhsky *et al.* 2004; Davletova *et al.* 2005; Mittler *et al.* 2006). A total of 189 C2H2-type zinc finger proteins have been identified in the *indica* rice genome and out of these, 26 genes were found to be upregulated by cold, drought or salt stress (Agarwal *et al.* 2007). Among these, ZFP252 (Xu *et al.* 2008) and ZFP245 (Huang *et al.* 2009) have been shown to improve drought tolerance in rice.

Overexpression of ZFP252 in rice resulted into accumulation of compatible osmolytes like free proline, soluble sugars and LEA proteins by regulating expression of stress-related genes, which ultimately led to increased tolerance to salt and drought stresses (Xu *et al.* 2008). Huang *et al.* (2009) have found that overexpression of ZFP245 increases the superoxide dismutase (SOD) and defense-related peroxidase (POD) activities in rice seedlings under cold or drought stress, indicating significant contribution to abiotic stress tolerance by activating the antioxidant system. It also promotes the accumulation of free proline by inducing the high expression of pyrroline-5-carboxylate synthetase and proline transporter genes, which ultimately enhances the ROS-scavenging ability of plant cells by activating the ROS-scavenging enzymes.

Ethylene-response factors

Ethylene-response factors are homeodomain containing proteins known to function partly in response to the plant hormone ethylene. Studies have demonstrated that ethylene-response transcription factors (ERFs) are involved in developmental regulation as well as biotic and abiotic stress responses (Nakano *et al.* 2006). Some rice ERF genes are inducible by environmental stimuli and are involved in responses to salt, drought, cold and submergence stresses (Dubouzet *et al.* 2003; Cao *et al.* 2006; Fukao *et al.* 2006; Xu *et al.* 2006; Liu *et al.* 2007; Hattori *et al.* 2009). One of the ERFs from tomato TSRF1 (tomato stress responsive factor) has been shown to increase the ABA-sensitivity under drought conditions, when expressed ectopically in rice (Gao *et al.* 2008). Transgenic rice plants overexpressing a jasmonate and ethylene response factor (JERF3) show improved tolerance to drought and osmotic stress via the upregulation of a network of stress-responsive genes such as genes for proline accumulation, OsSPDS2 (encoding Spd synthase for spermidine synthesis) and a bifunctional enolase involved in glycolysis-OsEnol (Jhag *et al.* 2010).

Another protein AP37, which is a member of the widespread APETALA2 (AP2/ERF) family of plant transcription factors has been implicated in drought tolerance and yield improvement under water stress conditions. Transgenic rice plants overexpressing AP37 under the control of constitutive promoter OsCc1 showed enhanced tolerance to drought and high salinity at the vegetative stage. Several antioxidant genes, such as thioredoxin, peroxidase and ascorbate-oxidoreductase were found to be upregulated in AP37 overexpressing plants indicating towards the activation of a ROS scavenging system (Oh *et al.* 2009). These AP37 transgenic rice plants showed significantly enhanced drought tolerance in the field conditions without yield penalty and exhibited no undesirable growth phenotypes.

Cuticular wax prevents non-stomatal water loss and is an important determinant of the water retention capacity of the plant and hence drought adaptation. Cuticular wax deposition is regulated both by developmental stages and environmental conditions and it involves induction of cascade of genes for several pathways (Suh *et al.* 2005; Shepherd and Griffiths 2006). OsWR1 (rice wax synthesis regulatory gene) is an ERF transcriptional activator and is a homolog of *Arabidopsis* wax/cutin synthesis regulatory gene WIN1/SHN1 that affects the expression of wax synthesis related genes in rice. OsWR1 is mostly expressed in leaves and induced by drought, ABA and salt stress condition (Wang *et al.* 2011). Overexpression of OsWR1 enhances wax production at the site of leaf cuticle by activating the expression of wax synthesis related genes, which resulted in reduced water loss leading to enhanced drought tolerance (Wang *et al.* 2011).

Another ERF gene-OsDERF1 (drought and ethylene-responsive factor) negatively regulates ethylene production through transcriptional activation of ERF repressors by directly interacting with the GCC box in the promoter of ERF repressors (OsERF3 and OsAP2-39) and was identified for its activation in response to drought, ethylene and ABA (Wan *et al.* 2011). Rice plants overexpressing OsDERF1 led to reduced tolerance to drought stress, while knockdown of OsDERF1 expression conferred improved drought tolerance. The molecular basis of this lies in the activation of expression of 'repressor' genes in the overexpression lines, which negatively regulate the expression of ethylene synthesis related genes (Wan *et al.* 2011) thus establishing a novel link between ERF transcriptional complex, ethylene biosynthesis and drought sensitivity.

The submergence tolerance regulator - SUB1A (SUBMERGENCE1A) is an ERF involved in submergence tolerance in plants (Xu *et al.* 2006). While SUB1A is absent in most japonica and indica rice cultivars, it confers submergence tolerance in varieties in which it is present. By comparative analysis of varieties with and without SUB1A, Fukao *et al.* (2011) found that the presence of SUB1A enhanced recovery from drought at the vegetative stage. Overexpression of SUB1A increased ABA responsiveness and activated expression of certain stress-inducible AP2/ERF transcription factors - DREB1A, DREB1E, and AP59, known to be key regulators of dehydration tolerance (Fukao *et al.* 2011).

1. DREB subfamily transcription factors

A major subfamily of ERFs is the DREB (Dehydration Response Element Binding) subfamily, involved mainly in the ABA-independent response. Members of this family bind to a dehydration response element (DRE) also known as C-repeat (CRT) element, having the consensus sequence A/GCCGAC in the promoter region of several drought-responsive genes. Accordingly, the DREB proteins have also been called CRT-binding factors (CBFs). DREB/CBF proteins were initially identified and isolated from *Arabidopsis* using a yeast-one-hybrid screening (Stockinger *et al.* 1997; Liu *et al.* 1998). They have been classified into two major classes, viz. DREB1 and DREB2. Rice has ten members in the class OsDREB1 and four in OsDREB2. While the expression of DREB1 class is strongly induced by cold (Thomashow 1999), DREB2 has been found to be more responsive to other abiotic stresses such as heat (Sakuma *et al.* 2006), salt and drought (Liu *et al.* 1998). Upon induction, they drive the expression of their targets many of which are involved in establishing stress tolerance. The DREB subfamily is one of the most well studied and exploited pathways for improving stress tolerance in various crops. In rice, various genes of the DREB1 class, such as OsDREB1A, OsDREB1B, OsDREB1G, AtDREB1A, AtDREB1B, and AtDREB1C have been overexpressed under the control of either drought responsive promoter OsHVA22p or various constitutive promoters (Hsie *et al.* 2002; Oh *et al.* 2005; Ito *et al.* 2006; Chen *et al.* 2008; Xiao *et al.* 2009; Yang *et al.* 2010). In the greenhouse soil-pot trials most of the transgenic plants overexpressing either of these genes showed improved tolerance to water-deprivation. However, under field conditions, expression of AtDREB1A driven by the constitutive OsActin1 promoter did not effect considerable change but the expression of the same gene driven by the drought-responsive promoter OsHVA22p enhanced the yield and spikelet fertility as compared to the wild type plants under drought conditions. Another member of the DREB1 class-OsDREB1F has been reported to be induced by drought or salt stresses in rice (Wang *et al.* 2008). Both rice and *Arabidopsis* transgenic plants overexpressing OsDREB1F gene resulted in improved tolerance to salt, drought and low temperature (Wang *et al.* 2008). OsDREB1F has been considered to be a 'molecular bridge' between ABA-independent and ABA-dependent pathways (Yang *et al.* 2010) as it is also induced by ABA-application but does not directly bind to the ABRE (abscisic acid response element). In the ABA-independent pathway, OsDREB1F activate the expression of genes containing DRE/CRT promoter sequence whereas in the ABA-dependent pathway, it interacts directly or indirectly with bZIP transcription factors, which subsequently activate the expression of RD29B and RAB18 genes containing ABRE element in their promoter region (Wang *et al.* 2008).

Among the DREB2 class members in rice, OsDREB2A and OsDREB2B show abiotic stress responsive gene expression (Matsukura *et al.* 2010). In contrast to the DREB1 class, the over expression of DREB2 genes in transgenic plants has not been shown to significantly improve stress tolerance (Yang *et al.* 2010). The basis for this observation has been explained as modulation of DREB2 activity via stress-regulated differential splicing, post-translational modifications or quick degradation by the cellular proteolytic machinery (Liu *et al.* 1998; Qin *et al.* 2008; Matsukura *et al.* 2010). Lately, however, OsDREB2A has been shown to confer drought and salt stress tolerance in rice; when overexpressed under the control of a drought-responsive promoter RD29A (Mallikarjuna *et al.* 2011). The other member that showed abiotic stress-induced regulation of expression viz. OsDREB2B when overexpressed under CaMV35S promoter conferred improved drought tolerance without any apparent morphological effect (Chen *et al.* 2008). On the contrary, Matsukura *et al.* (2010) have reported that constitutive overexpression of *OsDREB2B* did not show morphological changes or improved stress tolerance as compared to control plants. Hence, the role of members of DREB2 class in drought tolerance still remains enigmatic.

Although, there have been numerous attempts utilizing DREB1 and DREB2 class genes for conferring drought tolerance, the major drawback are the pleiotropic effects leading to growth defects such as short stature and delayed flowering, often observed by their constitutive overexpression. In order to avoid this side effect, many studies have been carried out placing such genes under RD29A promoter induced by cold and dehydration but not by ABA (Kasuga *et al.* 1999; Mallikarjuna *et al.* 2011). In such studies, it has been shown that stress-inducible overexpression of DREB genes confers substantial resistance to drought without apparent negative pleiotropic effects.

HARDY (HRD), a group III member of the DREB/CBF subfamily (Nakano *et al.* 2006) was identified by a gain-of-function experiment. *Arabidopsis* mutant *hrd-D* having roots with proper branching and cortical cells, showed drought resistance and salt tolerance. Higher expression of HRD gene is accompanied by enhanced expression of abiotic stress associated genes (Karaba *et al.* 2007). Constitutive overexpression of HARDY in *Arabidopsis* had negative effects on vegetative growth, forming small, thick green leaves and an abnormally dense root system. However, ectopic overexpression of the same gene in rice improved water use efficiency (WUE) by 50% (Karaba *et al.* 2007). The HRD-overexpression lines along with WT plants were subjected to less soil water as compared to controlled field conditions creating an artificial drought stress. The

HARDY-overexpressing lines showed increased biomass, increased leaf thickness whereas in contrast the WT plants showed typical stress-symptoms of leaf rolling and drying. The enhanced WUE has been explained by lower transpiration and higher photosynthetic carbon assimilation. Moreover, no reduction in growth and grain yield was observed though the transgenic rice had dark green leaves with more bundle sheath cells (Karaba *et al*. 2007).

Drought tolerance mediated by altering the expression of kinases

Signal transduction during stress begins with signal perception, followed by the generation of second messengers, which can alter intracellular Ca^{2+} levels initiating a protein phosphorylation cascade that finally targets transcription factors controlling specific sets of stress-regulated genes or proteins directly involved in cellular protection (Xiong *et al*. 2002). Abiotic stress signalling has been considered to be very complex and a large number of players are involved ranging from G-proteins, different kinases, membrane proteins, phytohormones and other second messengers. The genes shown to be involved in conferring drought tolerance encode proteins mainly belonging to kinase class, which phosphorylate different cellular substrates and thus modulate their activity. Such kinases include, mitogen activated protein kinases (MAPKs), Inositol 1,3,4-trisphosphate 5/6-kinase (ITPK) and 'calcium sensors' such as calcium-dependent protein kinases (CDPKs) and CBL-interacting protein kinases (CIPKs).

Mitogen-activated protein kinases

In eukaryotic cells, MAPK cascades function as important signaling modules. MAPK cascades consist of three protein kinase modules - MAPKK kinases (MAPKKKs), MAPK kinases (MAPKKs), and MAP kinases. Upstream signals can activate MAPKKKs, which then phosphorylate MAPKKs, which in turn activate a specific MAPK. The downstream targets of MAPKs can be transcription factors, phospholipases, or cytoskeletal proteins (Ning *et al*. 2010). In plants, MAPK cascades have been identified in signaling pathways including cell division, hormone responses, development, disease resistance, and abiotic stress (Tena *et al*. 2000; Nakagami *et al*. 2006).

NPK1 (nucleus and phragmoplast localized protein kinase 1), a MAPKKK was identified in tobacco plants, which plays crucial roles in cytokinin and auxin signaling transduction and responses to multiple stresses. A complete MAPK cascade consisting of NPK1-MEK1/ NQK1-NTF6/ NRK1 was identified using the yeast mutant *pbs2* to screen tobacco BY2 cDNA library (Soyano *et al*. 2003). It was reported that transgenic tobacco plants overexpressing NPK1 enhanced tolerance to salt, freezing and heat stresses (Kovtun *et al*. 2000). Genome wide analysis of rice NPK1-like (OsNPKL) gene family revealed that 21 OsNPKL genes are present in the rice genome. An OsNPKL gene cluster on chromosome 1 comprising of four OsNPKL genes co-localized with a previously uncharacterized QTL for drought resistance. Expression analysis of OsNPKL indicated their strong induction by drought, cold, and salt stresses. Recently, NPK1 from tobacco was ectopically expressed in rice under the control of both constitutive (OsActin1p) and drought-inducible (HVA22p) promoters (Xiao *et al*. 2009). Ectopic expression of NPK1 increased yield and spikelet fertility as compared to wild type under drought stress conditions. The drought tolerance was observed to be better with the use of HVA22p than OsActin1p (Xiao *et al*. 2009).

A member of the Raf family of MAPKKK, DSM1 has been found to have role in various abiotic stresses in rice. It was first identified in a drought sensitive mutant (*dsm*) in which the defected locus was designated as *dsm1*. The *dsm1* mutants lost water more rapidly than wild-type plants under drought stress, which was in agreement with the increased drought-sensitive phenotype of the mutant plants (Ning *et al*. 2010). Microarray analysis revealed that two peroxidase (POX) genes were highly down-regulated in the mutant compared to wild type, indicating that DSM1 may be involved in reactive oxygen species (ROS) signaling. Peroxidase activity, electrolyte leakage, chlorophyll content; and 3,3′-diaminobenzidine staining revealed that the *dsm1* mutant was more sensitive to oxidative stress caused by the reduced POX activity. Through expression analysis by qRT-PCR, it was found that the *DSM1* gene was induced by salt, drought, and abscisic acid, but not by cold. Overexpression of *DSM1* in rice increased the tolerance to dehydration stress at the seedling stage (Ning *et al*. 2010). These results suggest that DSM1 might function as an early signaling component in modulating responses to drought stress by regulating ROS metabolism.

Inositol 1,3,4-trisphosphate 5/6-kinase

IP_3 (D-myo-inositol-1,4,5-trisphosphate) is a second messenger generated in the phospholipid signaling pathway, mediating Ca^{2+} release from the endoplasmic reticulum to the cytosol (Taylor and Richardson 1991). IP_3 is phosphorylated by inositol 1,4,5-trisphosphate 3-kinase (IP3K) and inositol 1,3,4-trisphosphate 5/6-kinase (ITPK) to form inositol tetrakisphosphate (IP_4). IP_4 is another secondary messenger, which is responsible for mediating Ca^{2+} influx through the plasma membrane and intracellular Ca^{2+} mobilization by co-acting with IP_3 (Wilson *et al*. 2001). Hence, IP3K and ITPK play key roles in maintaining Ca^{2+} homeostasis by maintaining intracellular IP_3 and IP_4 concentrations. In plants, IP3K and IPTK play important role in response to environmental stimuli (Du *et al*. 2011). Out of the six ITPKs predicted to be present in rice, one viz. OsITPK2 (DSM3) has been shown to be stress responsive. Its transcript was found to be highly induced by drought, salt and ABA treatments and it was found that its optimal expression level is essential for drought and salt tolerance in rice (Du *et al*. 2011). OsITPK2 over expression lines showed increased salt and drought sensitivity, lower seed setting rate and biomass compared with WT after drought treatment at the reproductive stage; suggesting a regulatory role for DSM3 in drought response (Du *et al*. 2011).

Calcium sensors

Calcium is a ubiquitous second messenger in eukaryotic signal transduction cascades. In plants, intracellular Ca^{2+} levels are modulated in response to various signals, including phytohormones, light, mechanical perturbations, pathogen elicitors and abiotic stress (Evans *et al*. 2001; Rudd and Franklin-Tong 2001). Several families of Ca^{2+} sensors have been identified in higher plants, which include Calmodulins (CaM), CaM-related proteins, CDPKs (Calcium-Dependent Protein Kinases) and Calcineurin B-like (CBL) proteins (Snedden *et al*. 1995; Kudla *et al*. 1999; Cheng *et al*. 2004; Xu *et al*. 2006). Furthermore, the CBL proteins interact with a class of serine-threonine protein kinases called CIPKs (CBL-Interacting Protein Kinases) which serve as 'kinase effectors' (Batistic and Kudla 2004). Of all these sensors, CDPKs and CBL/CIPK have been shown to confer drought tolerance in rice.

1. Calcium-dependent protein kinases

Calcium-dependent protein kinases (CDPKs) have been identified throughout the plant kingdom (Ludwig *et al*. 2004; Harper and Harmon 2005) and in some protozoans (Ward *et al*. 2004), but not in animals (Harper and Harmon 2005). Each CDPK consists of a variable N-terminal domain and several functional domains, including a protein kinase domain, an autoinhibitory region and a calmodulin-like domain, which usually contains four functional EF-hands for calcium binding (Harper *et al*. 1991; Harmon *et al*. 2000; Cheng *et al*. 2002; Hrabak *et al*. 2003). CDPKs sense the intracellular calcium level and are activated by the binding of Ca^{2+} to the calmodulin-like domain, and upon activation CDPKs regulate downstream components of calcium signaling (Harper and Harmon 2005). CDPKs comprise a huge family of serine/threonine kinases in plants; consisting of 34 genes in *Arabidopsis* (Hrabak *et al.* 2003) and 29 genes in *Oryza sativa* (Asano *et al.* 2005). CDPKs are involved in various physiological processes in plants including development, abiotic and biotic stresses. Among *Arabidopsis* CDPKs, CPK4 and CPK11, which belong to group I of the CDPK family, have been shown to be involved in tolerance to abiotic stresses (Zhu *et al.* 2007). However, little is known about the functional role of rice CDPKs. Only one of the rice CDPKs viz. OsCDPK7 (Saijo *et al.* 2000, 2001) has been shown to improve tolerance to drought and other abiotic stresses.

OsCDPK7 has been shown to be a positive regulator involved in tolerance to cold, salt and drought (Saijo *et al.* 2000). Its expression was highly induced by cold and salt stresses. In the wild type plants it was found to be expressed predominantly in vascular tissues of crowns and roots where water stress occurs most severely (Saijo *et al.* 2000). Transgenic rice plants overexpressing OsCDPK7 were found to tolerate drought, salt and cold stress while those transgenics, which showed co-suppression of OsCDPK7 had lowered stress tolerance. Besides, the extent of tolerance to cold and salt/drought stresses of these plants showed positive correlation with the level of OsCDPK7 expression. Overexpression of OsCDPK7 enhanced induction of some stress-responsive genes such as RAB16a - a LEA protein. Moreover, broad substrate specificity has been observed for OsCDPK7 (Saijo *et al.* 2001), which suggests that OsCDPK7 may regulate multiple target proteins. In addition to those directly involved in the machinery of gene expression, a variety of transport proteins, e.g. aquaporins, ion channels and H^+-ATPases, which are responsible for cytosolic osmo-regulation, have been considered as its substrates (Sanders *et al.* 1999; Harmon *et al.* 2000). Moreover, constitutive induction of the above stress-inducible genes was not found upon OsCDPK7 overexpression suggesting that OsCDPK7 is normally kept inactive (Saijo *et al.* 2000). Therefore, overproduction of OsCDPK7 only, is not sufficient to trigger the downstream signaling, and stress stimuli are required to activate this CDPK. This indicates that the activity of OsCDPK7 is under some sort of post-translational control. In agreement with this, no significant pleiotropic effects were observed with regard to development, growth and fertility in overexpressing plants grown which is highly favorable for crop improvement (Saijo *et al.* 2000).

2. CBL/CIPK-pathway

Calcineurin B-like (CBL) proteins contribute to sensing calcium and decoding the signals by specifically interacting with a group of CBL-interacting protein kinases (CIPKs). CIPKs have a regulatory domain and a catalytic (kinase) domain. The kinase domain in CIPKs is separated from the less-conserved C-terminal regulatory domain by a junction domain (Batistic *et al.* 2008). Within the regulatory region of CIPKs, a conserved NAF domain, having the prominence of amino acids asparagine (N), alanine (A) and phenylalanine (F) is present. NAF domain mediates binding of CBL proteins and also functions as an auto-inhibitory domain (Albrecht *et al.* 2003). Binding of CBLs to the NAF motif removes the autoinhibitory domain from the kinase domain; thereby facilitating autophosphorylation and activation of the kinase, which can further phosphorylate range of cellular proteins. However, all the target proteins of the CBL/CIPK system are still not well defined.

CBL/CIPKs are known to function during the ABA response (Pandey *et al.* 2004; Hiroyama and Shinozaki 2007). The *Arabidopsis* genome encode as many as 10 CBLs and 26 CIPKs while the rice genome encodes 10 CBLs and 30 CIPKs providing a high level of diversity and flexibility with respect to CBL-CIPK interactions (Kolukisaoglu *et al.* 2004). Four rice CIPKs viz. OsCIPK03, OsCIPK12, OsCIPK15 and OsCIPK23 have been reported to be osmotic stress-responsive (Xiang *et al.* 2007; Yang *et al.* 2008). Overexpression of OsCIPK03 improved tolerance to cold, OsCIPK15 to salt and OsCIPK12 to drought (Xiang *et al.* 2007). Characterization of the OsCIPK12 overexpressing plants revealed that drought tolerance was mediated by significant increase in the proline and soluble sugar content after the drought stress was imposed (Xiang *et al.* 2007).

OsCIPK23, another CIPK has been shown to be induced during multiple stresses and mediates signaling pathways related to both pollination and drought stress responses in rice (Yang *et al.* 2008). Drought hypersensitive and tolerant phenotypes were obtained, by knocking down and overexpressing OsCIPK23, respectively. It has been predicted that OsCIPK23 acts in a signaling pathway commonly shared by pollination, phytohormones and multiple stress responses (Yang *et al.* 2008).

No pleiotropic effects have been reported in plants overexpressing CIPK genes. However, the genetic manipulation of CIPKs alone may not prove to be much useful for the obvious reason of the requirement of interaction with cognate CBLs required for their activation. For optimal utilization in crop improvement, it is imperative also to engineer the cognate CBLs. Besides, using constitutively active forms of CIPKs generated via mutations in the catalytic domain or by the deletion of the autoinhibitory motif-FISL in the CIPKs may serve the purpose (Yang *et al.* 2010), but then the concern of pleotropic effects needs to be dealt with.

Cross tolerance to other abiotic stresses

A host of studies aiming to understand abiotic stress responses in plants have supported the existence of cross-talk between various stress responsive pathways (Knight and Knight 2001; Chinnusamy *et al.* 2004). Moreover, the initial perception may be different for different stresses but the response pathways often converge. For instance, most of the stresses imposed to plant often lead to an oxidative damage requiring the anti-oxidant system. Thus, plants respond with stress-specific adaptive response as well as responses, which can protect the plants from multiple environmental stresses. Therefore, it is not surprising that attempts aiming to engineer plants to tolerate drought have succeeded in imparting tolerance to other abiotic and biotic stresses as well.

Various studies discussed above indicate that several transcription factors (**Table 1; Fig. 4**) play major role in regulating the expression of downstream genes responsible for plant response and adaptation during drought stress. Some of the transcription factors involved in conferring drought tolerance are able to impart cross-tolerance to other abiotic stresses indicating towards activation of 'effector' genes common to different stresses. Most of the ethylene-responsive factors (HARDY, TERF1, JERF3, and AP37) have been shown to confer tolerance to multiple abiotic stresses viz. salinity, cold and oxidative along with drought stress tolerance (**Table 1**). In addition, another ERF viz. SUB1A, which was initially identified as a positive regulator of submergence tolerance (Xu *et al.* 2006), was found to improve recovery from drought stress at the vegetative stage through retaining more leaf water and generating reduced level of lipid peroxidation and increased expression of genes associated with acclimation to dehydration (Fukao *et al.* 2011). Of the DREB family genes, overexpression of DREB1F under a constitutive promoter and that of DREB2A driven by either ABA/stress-responsive 4ABRC promoter or RD29A – another stress-responsive promoter, showed both salinity and water deficit stress tolerance in rice plants (Wang *et al.* 2008; Cui *et al.* 2011; Mallikarjuna *et al.* 2011). Amongst the NAC family members, overexpression of SNAC1, SNAC2 and NAC045 imparted tolerance to both salt and drought stress (Hu *et al.* 2006; Nakashima *et al.* 2007; Zheng *et al.* 2009). OsNAC10 overexpression led to drought as well as low temperature tolerance in rice plants (Jeong *et al.* 2010). Besides, OsMYB2 - a member of the MYB superfamily of the transcription factor was found to confer tolerance to salt and cold in addition to drought when overexpressed in rice (Yang *et al.* 2012).

Amongst the other drought responsive genes shown in **Table 2**, some of them confer cross-tolerance to other stresses as well. OsCDPK7, a calcium-dependent kinase has been shown to be a positive regulator involved in tolerance to multiple abiotic stresses including cold, salt and drought (Saijo *et al.* 2000). Also, a Raf-like MAPKKK gene DSM1 has been shown to be involved in oxidative stress tolerance in rice (Ning *et al.* 2010). Besides, genes responsible for accumulation of compatible solutes like trehalose (a soluble sugar) have been found to confer tolerance to both salt and drought (Garg *et al.* 2002; Karim *et al.* 2007; Kanneganti *et al.* 2008; Li *et al.* 2011).

CONCLUSION AND FUTURE PERSPECTIVES

Considering global climate change, crops tolerating environmental stresses, particularly drought and heat, will be in great demand as the world is getting hotter and drier (Battisti and Naylor 2009). Obtaining stress-tolerant cultivars requires painstaking efforts right from gene-discovery and proof-of-concept to commercialization. We have summarized various reports pertaining to functional validation and exploitation of drought-responsive regulatory genes, such as transcription factors and signaling proteins for engineering drought tolerance in rice. However, in our knowledge none of them have been successful up to the stage of commercialization. Drought, like any other abiotic stress, is a highly complex trait and different networks of genes are involved in drought response. Therefore, while several studies have shown that altering the expression of individual genes does improve tolerance to drought; development of drought-tolerant rice cultivars requires simultaneous engineering of multiple genes belonging to several pathways. Although changing the expression of transcription factors can, in turn, alter the intracellular levels of various targets, making transcription factors (TFs) the 'gene-class of choice' for drought tolerance; the pleiotropic effects often associated with the overexpression of TFs present great obstacle in this endeavour. On the contrary, such side-effects have not been found with the overexpression of stress-responsive kinases. Future studies should either target engineering multiple genes through gene pyramiding or overexpressing such drought-responsive genes that show minimal pleiotropic effects, under the control of specific stress-responsive promoters.

In the farmers' field, none of the stresses come alone and therefore developing rice plants, tolerant to multiple abiotic stresses, has become the need of the hour. Although few of the genes have been shown to confer tolerance to multiple stresses, more comprehensive studies are still required. Besides, even after considerable advancement in our understanding of the abiotic stress responses in plants, there are many unknown/uncharacterized components in the stress-related pathways. It is a serious challenge to identify and characterize the missing links. The advent of improved protocols for rice transformation (Hiei *et al.* 2008; Sahoo *et al.* 2011) has paved the way for functional genomics and easier genetic manipulation of rice. Future studies would utilize these to identify the undefined components in stress response pathways and aim to impart tolerance to multiple stresses, minimizing the 'yield penalty', via engineering the components involved in cross-tolerance.

In view of the critical problems that the present day world is facing including the increasing world population, diminishing arable land area and global climate change, the need is to develop crops with improved yield and resistance to biotic and abiotic stresses. Extensive efforts are required to develop such 'all-inclusive' crops and researchers working globally should be prepared to take on such challenging tasks.

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