Chapter 16

FUNCTIONAL GENOMICS FOR TOLERANCE TO ABIOTIC STRESS IN CEREALS

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1. INTRODUCTION

The world food grain production needs to be doubled by the year 2050 to meet the ever growing demands of the population (Tilman *et al.*, 2002). This goal needs to be achieved despite decreased arable land, dwindling water resources, and the environmental constraints such as drought, water logging, excess heat, frost, salinity, metal toxicity and nutrient imbalances, which cause major losses in cereal grain production. Drought, salinity and cold stress alone are known to cause nearly 35% of cereal crop losses throughout the world (Quarrie *et al.*, 1999). The effectiveness of traditional breeding approaches to deal with the problem is limited due to complex nature of stress tolerance traits and due to incompatibility barriers encountered during transfer of genes from wild species to cultivated ones. Therefore, newer strategies need to be used for developing crop plants that are tolerant to abiotic stresses. Such strategies will include molecular breeding and genetic engineering based on our fast increasing knowledge in genetics, genomics and molecular physiology.

Abiotic stress conditions cause changes in plant metabolism, involving generation of reactive oxygen species (ROS), membrane disorganization, inhibition of photosynthesis and altered nutrient acquisition (Bray, 1993; Ingram and Bartels, 1996; Hasegawa *et al.*, 2000). These changes in turn lead to alterations in development, growth and productivity. Severe stress

P.K. Gupta and R.K. Varshney (eds.), Cereal Genomics, 483-514.

even threatens plant survival. However, tolerant plants can adjust themselves in a number of ways by changing their phenology, morphology, anatomy and physiology. At the molecular level, protection can be achieved by diverse mechanisms. Accumulation of osmoprotectants, water channel activities, production of chaperones, superoxide radical scavenging mechanisms, exclusion or compartmentation of ions by efficient transporter and symporter systems are some of the factors that determine tolerance against salinity, drought and cold (for reviews see Ingram and Bartels, 1996; Ishitani *et al.,* 1997; Thomashow, 1999; Hasegawa *et al.*, 2000; Zhu, 2001; Apse and Blumwald, 2002; Iba, 2002; Shinozaki *et al.,* 2003). Each response involved in stress tolerance is regulated and coordinated by multiple genes, such that the alterations in gene expression profiles of stress-responsive genes are integral parts of stress resistance mechanisms.

The genomic tools and methods that have become available recently provide new opportunities to characterize the gene networks involved and to gain a more holistic view of abiotic stress responses. Expressed sequence tags (ESTs) from abiotic stress-treated libraries of various crop plants, complete genome sequence information for rice and Arabidopsis and the development of new bioinformatics tools allow us to identify the key stress-responsive gene-pools. Furthermore, use of multi-parallel techniques such as expression profiling by microarrays, random and targeted mutagenesis, complementation and promoter-trapping strategies provide important clues for functional characterization of stress responsive genes and stress tolerance mechanisms (Bohnert *et al.*, 2001). Recent genomic studies show considerable overlap of plant responses to cold, drought and salinity stresses (Knight and Knight, 2001; Kreps *et al.*, 2002; Chen *et al.*, 2002; Seki *et al.,* 2002b; Abe *et al.,* 2003) underlining the complexity and provide opportunities to engineer new stress-resistant crop varieties. However, to successfully deal with this complexity, genomics, genetics, physiology and breeding disciplines need to join together to manipulate the genome with precision for abiotic stress tolerance (for reviews see Cushman and Bohnert, 2000; Bohnert *et al.*, 2001). The role of different disciplines and a broad outline of experimental strategies in crop improvement for stress tolerance are indicated in Fig. 1. At first, sources of genetic variation have to be identified and used in strategies to develop new cultivars with greater yield potential and stability over seasons and ecogeographic locations. With the advent of newly developed genomics, two major approaches could be used in exploiting the gene-pool for imparting abiotic stress tolerance: *firstly*, identification and introduction of genes imparting stress-tolerance into crops of interest, and *secondly*, development and identification of molecular markers associated with genes or QTLs (quantitative trait loci) conferring tolerance to stress in germplasm collections and their use in marker-assisted

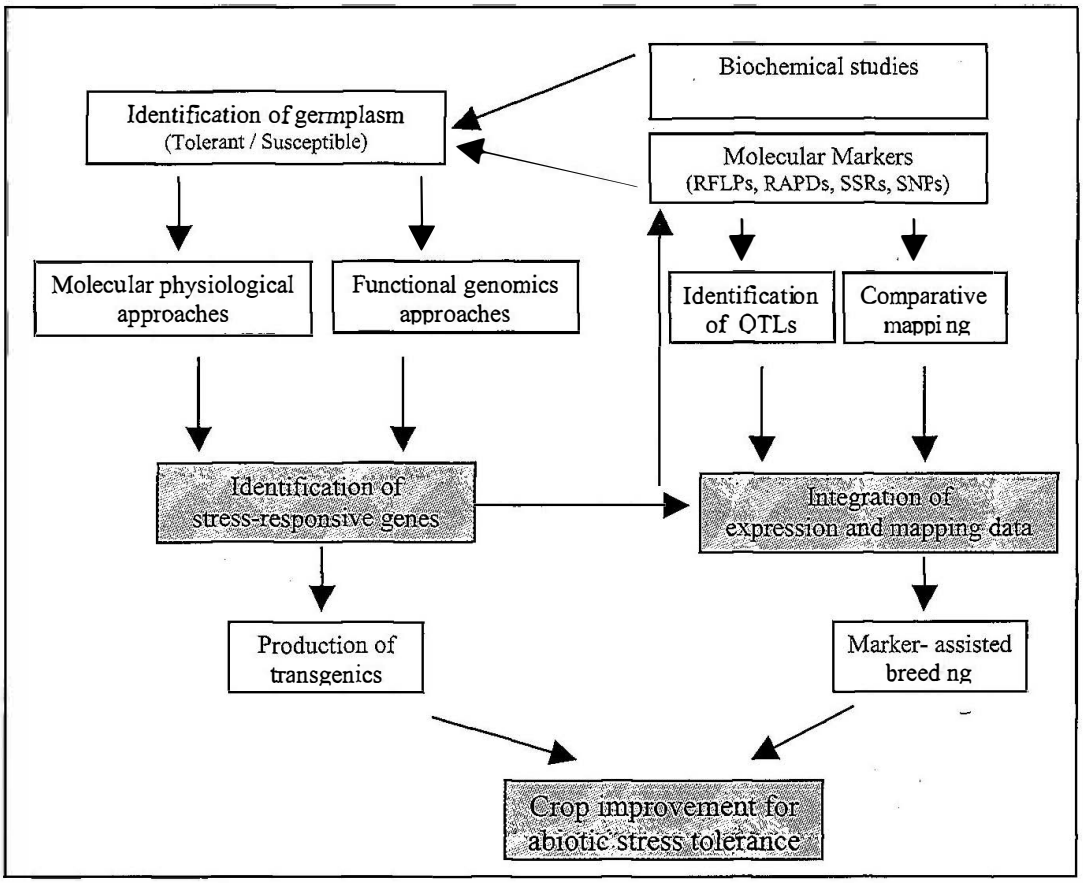

Figure 1. Integrative functional genomic approaches for abiotic stress tolerance.

breeding programs. A complete overview on prime abiotic stress tolerance QTLs is given in detail by Tuberosa and Salvi in Chapter 9 of this book. In the following sections, we review the results of functional genomic approaches to analyze and manipulate abiotic stress tolerance.

2. DISCOVERY OF STRESS-RESPONSIVE GENES FROM CEREALS BY FUNCTIONAL GENOMICS APPROACHES

Functional genomics approaches for abiotic stress tolerance include discovery of novel genes, determination of expression levels of genes induced in response to abiotic stress, studies to understand the functional roles of abiotic stress-responsive genes and generation of stress tolerant

transgenic plants. The strategies of functional genomics approaches to identify abiotic stress-associated mechanisms are discussed in this section.

2.1. Tracing Genes Responsible for Abiotic Stress Tolerance through ESTs

In cereals, large numbers of ESTs have been generated which have great potential to provide functional genomics information (refer web pages http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html; http://www.tigr.org/tdb/tgi/plant.shtml) including that on abiotic stress tolerance. Projects based on this approach were started in rice, barley, wheat, maize and sorghum (http://stress-genomics.org/stress.fls/dbase/dbase.html). Based on the search results (gene index of TIGR database; dated 20.09.03) a total of approximately 13,022 abiotic-stress related ESTs were reported from *Hordeum vulgare*, 13,058 from *Oryza sativa*, 2,641 from *Secale cereale*, 17,189 from *Sorghum bicolor*, 20,846 from *Triticum aestivum* and 5,695 from *Zea mays*. However, the number of ESTs generated so far solely from stress-treated libraries is low, as compared to total ESTs. Therefore, there is a need to enforce sequencing programmes from stress-tolerant genotypes of cereals (treated with different abiotic stresses) covering a wider range of tissue types and developmental stages. We surveyed all the publicly available EST collections from cereal species (barley, maize, rice, and wheat) and identified drought- and salt stress-responsive genes (Table 1). Since we used an EST dataset from non-normalized libraries, the EST clustering results provide information on relative expression levels of stress-responsive genes belonging to different pathways. The ESTs are selected from non-normalized cDNA libraries of cereals and subjected for clustering analysis by software StackPACK v2.1.1. As a result of clustering, homologous sequences were grouped together to identify abundantly expressed gene sets. Among them, genes associated with stress-relevant pathways were found commonly expressed in drought- and salt-treated cereal plants (Table 1). Recently, Reddy *et al.* (2002) used normalized cDNA libraries from drought-stressed seedlings of rice to select novel stress-responsive genes. They identified genes metallothionein-like proteins, glyceraldehyde-3-phosphate dehydrogenase, aldolase, rd22, glycine-rich protein, glutathione-S-transferase, catalase, LEA and HSP, and several transcription factors (DREB, MYB, MYC, AP2, Zinc finger protein) as well as kinases (mitogen activated protein kinases, calcium-dependent protein kinase) that were abundantly expressed upon drought stress.

Table 1. ESTs that are abundant in cDNA libraries from drought, salt-treated cereals

Functional catalogue	Putative gene identification	Drought No.ESTs /cluster	Salinity No.ESTs /cluster
Aquaporins	Tonoplast intrinsic protein, (gamma tip)	***	**
	Plasma membrane intrinsic protein 1	**	***
	Plasma membrane intrinsic protein 2	***	**
Antioxidants	Glycine-rich RNA-binding protein	******	*****
	Glutathione s-transferase	****	***
	Glutathione peroxidase (PHGPX), chloroplast	**	**
	Phenylalanine ammonia-lyase	****	****
	L-ascorbate peroxidase, cytosolic	**	**
Growth regulators	Indole-3-acetic acid induced protein arg 2	**	***
	Abscisic acid induced protein	*	**
Osmoprotectants	Proline-rich protein	**	***
	Glyceraldehyde-3-phosphate dehydrogenase	**	**
	Mannitol dehydrogenase	**	*
Protein destination	Metallothionein-like protein 1	******	******
	Ubiquitin	***	*****
	Cysteine proteinase	***	****
	Cysteine protease inhibitor	****	*****
Photosynthetic	Chlorophyll a-b binding protein 3c	******	******
	Rubisco small subunit	******	*****
	Rubisco small subunit c	******	*****
	Photosystem I reaction center subunit psak	*****	*****
	Rubisco activase a	****	****
	Rubisco large subunit	*****	****
Stress responsive genes	Non-specific lipid-transfer protein	*****	****
	Glycine-rich-protein	****	***
	Osmotin-like protein	**	*
	Thaumatin-like protein	**	*
	Late embryogenesis abundant protein 3	**	*
	Late embryogenesis abundant protein 2	**	*
	Late embryogenesis abundant protein 1	*	*
	Heat shock protein 81	**	**
	Heat shock protein 70	**	*
	Heat shock protein 17	**	**

EST mining approach: The consensus sequences of abundantly expressed transcripts were subjected to similarity search (BLASTX) against public protein database (SWISSPORT) for functional annotation using the arbitrary criteria expectation value less than 1.0×10^{-15}. The genes (represented by sequence clusters) with their putative function assigned to the functional catalogues are listed in the table. Number of ESTs present in each sequence cluster reflects the relative expression level of the corresponding gene (*2 to 5 ESTs; **6 to 10; ***11 to 15, ****16 to 25, *****26 to 50, ****** 51 and above) in different cereals (barley, rice, wheat and maize).

2.1.1. Transcription Profiling using ESTs from Abiotic Stress Related cDNA Libraries

The cDNA macro/microarray technology for transcript profiling has been established, based on EST programmes, in cereal species such as barley (Ozturk *et al.,* 2002; Sreenivasulu *et al.*, 2002, 2004a), maize (Lee *et al.*, 2002) and rice (Kawasaki *et al.*, 2001) after generating non-redundant unigene sets. Transcript profiling based on micro/macro-arrays was carried out in cereal crops (Kawasaki *et al*·, 2001; Ozturk *et al.,* 2002; Sreenivasulu *et al.,* 2004b) as well as in Arabidopsis (Seki *et al.*, 2001, 2002a, 2002b)·to analyse gene expression in response to a variety of stresses. Array technology provides a powerful tool (i) to compare the relative expression levels between tolerant and sensitive cultivars within the same species under stress and control conditions and (ii) to identify stress-specific transcriptional responses as well as cross-talks between different stress responses.

2.1.1.1. Expression levels in tolerant and sensitive cultivars

In rice, microarrays based on 1,728 stress-regulated transcripts (obtained from seedlings of the salt-tolerant rice variety Pokkali) were used for large-scale gene expression profiling in salt-tolerant Pokkali as well as in the salt-sensitive variety IR29 during 15-minutes to 7-day time intervals under control as well as high salinity treatments (Kawasaki *et al.,* 2001). In the tolerant cultivar, changes in transcript levels were observed first as early as 15 min after salt stress. Upregulated genes could be assigned to signaling pathways (calcium-dependent protein kinases, nucleoside diphosphate kinase), cell division processes (40S ribosomal proteins and elongation factor-1α, glycine rich proteins), protease inhibitors and hormonal induced genes (see Table 2). As a corollary, these genes were generally downregulated in the sensitive cultivars. During long-term salt stress (24 h and 7 days), tolerant rice plants showed upregulation of antioxidant transcripts (glutathione-S transferase, ascorbate peroxidase), aquaporins (water channel protein I and IV), protease inhibitors (subtilisin inhibitor, trypsin inhibitor), hormonal induced and some unknown genes. Similar transcript profiling studies were carried out in 3-week old barley seedlings, where salt as well as drought-responsive genes were identified (Ozturk *et al.* , 2002). The upregulated gene set in salt-stressed leaf and root tissues encodes antioxidants and osmoprotectants and in addition contains genes for protein destination, and regulatory and stress-response processes (see Table 2). In drought-stressed barley leaves, transcripts encoding proteins of

jasmonate biosynthesis (allene oxide synthases) and several jasmonate-induced proteins were upregulated along with amino acid metabolism genes, osmoprotectants, protein destination and stress responsive genes (see Table 2). Since extensive synteny exists between different grass genomes (Gale and Devos, 1998), cDNA arrays developed from one grass species can be used for the analysis of other species. Therefore, we explored the possibility to use barley cDNA arrays for examination of gene expression patterns in tolerant and sensitive seedlings of foxtail millet (*Setaria italica* L.) exposed to 250 mM NaCl. The upregulated 14 transcripts in the salt-tolerant line includes protease inhibitors, antioxidative enzymes and some unknown genes, which are similar to salt-responsive genes already identified in rice and barley (Sreenivasulu *et al.,* 2004b).

Scientists at Pioneer Hi-Bred performed cDNA microarray analysis in a maize breeding population that showed improved tolerance to water stress during ear growth (c.f. Bruce *et al.,* 2002). They reported that synthesis of water channel aquaporins and β-glucosidase transcripts was down-regulated during stress, whereas during the recovery period these transcripts were up-regulated. Their results indicated that a family of cell cycle genes exhibit three different gene expression patterns: (a) increasing mRNA levels during drought stress; (b) decreasing mRNA levels during stress followed by a subsequent increase during recovery; (c) increasing mRNA levels only during recovery. These results suggest specific functions of different members of the cell cycle gene family. Recently, transcript profiling was performed for placenta and endosperm of maize kernels grown under water deficit (Yu and Setter, 2003). Only eight out of 70 genes upregulated under water stress in the placenta were also upregulated in the endosperm. The related proteins have expected roles in stabilization of proteins as well as membrane structure during stress (for instance, 70 kD heat shock protein, DNAJ and lipid transfer protein), show aquaporin function (plasma membrane intrinsic protein) or are involved in trehalose synthesis (trehalose-6-phosphate synthase) expected to stabilize macromolecule structures during stress (Garg *et al.*, 2002). Microarrays based on 11,000 unique, full-length cDNA sequences (outcome of the Rice Genome Research Program) were used to study responses of rice seedlings to UV-B and gamma irradiation (Kikuchi *et al.*, 2002). Although both types of irradiation induce similar physiological effects, very few genes were induced in parallel, including those for polygalacturonase inhibitor (PGIP), major intrinsic protein, beta tubulin, eukaryotic initiation factor, lipid transfer protein and metallotionein-like protein.

Table 2. Genes upregulated by abiotic stress – an index from microarray analysis

Functional class	Genes
I. Barley seedlings (3 week-old) exposed to dehydration: 6 & 10 h Ozturk *et al.* (2002)	
Amino acid metabolism	Arginine decarboxylase 2, Arginine decarboxylase SPE2 Asparagine synthetase, Tryptophan synthase beta chain 1
Jasmonate biosynthesis	Allene oxide synthase
jasmonate induced proteins	Lipoxygenase 2 (methyl jasmonate-inducible) Jasmonate-induced protein (jip) 60 kD, jip 23kD, jip 1 and jip 6
Osmoprotectants	Delta-1-pyrroline-5-carboxylate synthetase
Protein destination	Metallothionein-like protein type 2
Stress responsive genes	Dehydrin 9, Late embryogenesis abundant protein 14-A
II. Barley seedlings (3 week-old) exposed to 150 mM NaCl: 24 h Ozturk *et al.* (2002)	
Antioxidants	Glutathione-S-transferase (auxin-induced)
Jasmonate biosynthesis	Allene oxide synthase
Osmoprotectants	Proline rich protein, Delta-1-pyrroline-5-carboxylate synthetase
Photosynthetic	Photosystem II 10 K protein
Protein destination	Metallothionein-like protein type 2, Aspartic proteinase
Regulatory	Transcription factor POU3A, Acidic ribosomal protein 60S Replicase associated polyprotein
Stress responsive genes	Heat shock protein DNAJ, Lipid transfer protein cw18, Late embryogenesis abundant like protein
Unknown	6 unknown genes
III. Rice seedlings exposed to 150 mM NaCl: 15 min, 1h, 3h and 6h Kawasaki *et al.* (2001)	
Hormonal induced	Gda-1 (gibberellic acid-induced gene) Asrl (ABA and stress-induced protein) Osr40c1 (ABA and salt-induced protein)
Protein destination	Subtilisin-chymotrypsin inhibitor 2, Trypsin inhibtor 1
Regulatory	Calcium-dependent protein kinase, Nucleoside diphosphate kinase Calmodulin, Protein phosphatase 2C homologue, Elongation factor 1 40S ribosomal protein S4, 40S ribosomal protein S7
Stress responsive genes	Glycine/serine-rich protein (grp) 1, grp 2
Unknown	5 unknown genes
IV. Rice seedlings exposed to 150 mM NaCl: 24h and 7 days Kawasaki *et al.* (2001)	
Antioxidants	Glutathione-S-transferase, Ascorbate peroxidase, cyt
Aquaporins	Water channel protein I, Water channel protein IV
Hormonal induced	Gda-1 (gibberellic acid-induced gene) Osr40c1 (ABA and salt-induced protein), Osr40g2
Protein destination	Trypsin inhibtor 1, Metallothionein-like protein
Unknown	3 unknown genes

Table 2. Continued

V. Foxtail millet seedlings exposed to 250 mM NaCl: 7 days Sreenivasulu *et al.* (2004b)	
Antioxidants	Glutathione peroxidase, L-ascorbate peroxidase, cyt, Catalase
Protein destination	Trypsin inhibitor, Subtilisin-chymotrypsin inhibitor
Regulatory	Kruppel-like transcription factor, Argonaute protein, Cyclophilin
Unknown	1 unknown gene
VI. Maize developing kernels exposed to drought stress Yu and Setter (2003)	
Aquaporins	Plasma membrane intrinsic protein
Protein destination	20S proteasome beta subunit PBD2
Regulatory	Calcium-dependent protein kinase, TATA binding protein, Small nuclear ribonucleoprotein, Histone H2A, Cyclophilin
Stress responsive genes	Heat shock protein 70 kDa, Lipid transfer protein
Unknown	4 unknown genes

Significantly upregulated transcripts in barley, rice, maize and millet were considered (2.5 fold deviation from the control plant expression values, includes repeat experiments)

2.1.1.2. Stress specific responses

Since only few transcriptional profiling studies were conducted in cereals, we here include studies on Arabidopsis in order to gain deeper insights into functional genomic aspects of multiple stress interactions. Using 1300 full-length clones (Seki *et al.,* 2001) and 7,000 full-length clone inserts (Seki *et al.,* 2002a, 2002b) multistress interactions of abiotic stress treatments were studied to identify genes of potential interest to salt, drought and cold responses. By using 1,300 full-length clones, Seki *et al.* (2001) identified a set of only 44 genes, which are induced either by drought or cold stress response. Among them, 12 were identified as stress-inducible target genes of the DREB1 transcription factor family. By using 7,000 full-length inserts, 299 drought-inducible genes, 213 high-salinity-stress-inducible genes, 54 cold-inducible genes and 245 ABA-inducible genes were identified (Seki *et al.,* 2002a, 2002b). Multistress interactions of abiotic stress treatments were studied by Kreps *et al.* (2002) using a larger array containing oligonucleotides for about 8,100 Arabidopsis genes, to identify genes of potential interest to salt, drought and cold responses. They identified changes in gene expressions (more than 2-folds over control) for 2,409 out of 8,100 genes as part of cold, drought and salt responses. Above differences in the lists of stress-inducible genes found by using the full-length cDNA array or the oligonucleotide gene chip array might be due to the presence of different sets of genes on the respective arrays (only 1919 genes are common between both arrays) and different plant growth conditions as well as stress treatments used for experiments. Shinozaki *et al.*

(2003) analysed the complex cascades of gene expression in drought and cold stress responses and made an attempt to demonstrate the regulatory network of gene expression in drought and cold stress responses. Recently, Chen *et al.* (2002) identified approximately 21 transcription factors preferentially induced by abiotic stress conditions such as salinity-, osmotic-, cold- and jasmonic acid treatment. These transcription factors include DRE/CRT binding factors (shown to be activated by cold stress by Liu *et al.*, 1998), CCA1 and Athb-8 (shown to be regulated by hormones by Baima *et al.*, 2001), Myb proteins, bZIP/HD-ZIPs and AP2/EREBP domain transcription factors.

Comparative analysis of the response to abiotic stresses among diverse tolerant species can lead to the identification of evolutionarily conserved and unique stress defense mechanisms. By applying clustering algorithms to large-scale gene expression data of abiotic stress responses, stress-regulons, i.e. sets of genes regulated in a similar fashion, can be identified. This approach also enables the identification of new promoter elements/transcription factor binding sites in co-expressed gene sets and further helps to explore regulatory networks controlling abiotic stress responses (Aarts *et al.*, 2003). However, mining information will not reveal the complete functions of stress-regulated genes. Other approaches are necessary as, for instance, activation tagging. In Arabidopsis (ecotype C24) 43,000 T-DNA insertion lines were generated (Weigel *et al.*, 2000; http://stress-genomics.org/stress.fls/tools/mutants.html), of which about 30,000 lines were screened for stress-related gene regulation mutants (Xiong *et al.*, 1999); details of these results are available on web (http://stress-genomics.org/stress.fls/tools/mutants/arabid/T_DNA_ mutants/table1.html).

2.2. Functional Aspects of Abiotic Stress Tolerance Mechanisms Identified Through Molecular-Physiological Studies and Transgenics

In silico mining and transcription profiling led to the discovery of a larger number of genes involved in abiotic stress responses (Tables 1, 2 and 3). These genes can be used in functional studies, preferentially by transgenic approaches (Table 4 and Grover *et al.*, 2003). The results of these studies will be discussed in the following this section.

Table 3. Genes encoding enzymes/proteins associated with abiotic stress response in cereals

Gene category	Gene	Species	Cellular response	Reference
Antioxidants				
Superoxide dismutase	*FeSOD*	Maize	Cold stress	Van Breusegem *et al.* (1999)
	cyt Cu/ZnSOD	Rice	Drought, Heat stress	Sakamoto *et al.* (1995)
	chl Cu/ZnSOD	Rice	Abiotic stress	Kaminaka *et al.* (1997)
	MnSOD	Millet	Salt stress	Sreenivasulu *et al.* (2000)
Catalase	*CAT*	Maize	Cold stress	Prasad *et al.* (1994)
Ascorbate peroxidase	*APX*	Maize	Cold stress	Prasad *et al.* (1994)
		Millet	Salt stress	Sreenivasulu *et al.* (2000)
Osmolyte compounds				
Proline	*P5CS*	Wheat	Salt stress	Sawahel and Hassan (2002)
Glycine betaine	*Chlcod*	Rice	Salt, Cold stress	Sakamoto *et al.* (1998)
	bet A	Rice	Salt, Cold stress	Takabe *et al.* (1998)
	BADH	Sorghum	Osmotic stress	Wood *et al.* (1996)
Mannitol	*ADC*	Rice	Drought stress	Capell *et al.* (1998)
	ADC	Rice	Salt stress	Roy and Wu (2001)
Regulatory genes				
bZIP	*OSBZ8*	Rice	ABA	Nakagawa *et al.* (1996)
	OsZIP-1a	Rice	ABA	Nantel and Quatrano (1996)
	EmBP1	Wheat	ABA	Hobo *et al.* (1999)
	TRAB1	Wheat	ABA	Choi *et al.* (2000)
Stress-responsive genes				
LEA proteins	*HVA1*	Rice	Salt, Drought stress	Xu *et al.* (1996)
	HVA1	Barley	Abiotic stress	Hong et (1992)
	HVA1	Barley	Cold stress	Sutton *et al.* (1992)
	HVA1	Wheat	Freezing tolerance	Sivamani *et al.* (2000)
	DHN1-DHN12	Barley	Salt, Drought stress	Choi *et al.* (1999)
	DHN	Wheat	Drought stress	Labhilili *et al.* (1995)
COR or BLT genes	*COR14b*	Barley	Cold stress	Cattivelli and Bartels (1990)
	BLT4, BLT14	Barley	Cold stress	Pearce *et al.* (1998)
	BLT63	Barley	Cold stress	Dunn *et al.* (1993)
	BLT801	Barley	Cold stress	Dunn *et al.* (1996)
Thaumatin-like protein	*TLP-D34*	Rice	Osmotic stress	Datta *et al.* (1999)
Low temperature induced protein	*LIP5, LIP9, LIP19*	Rice	Cold stress	Aguan *et al.* (1991)
RAB genes	*RAB16A*	Rice	Drought stress	Mundy *et al.* (1990)
	RAB17	Wheat	Drought stress	Vilardell *et al.* (1990)

Table 3. Continued

RAB genes	*RAB28*	Maize	Drought stress	Pla *et al.* (1993)
WCS genes	*WCS120*	Wheat	Cold stress	Oullet *et al.* (1998)
	WCS19	Wheat	Cold stress	Chauvin *et al.* (1993)
Heat shock protein	*HSP90*	Rice	Heat stress	Pareek *et al.* (1995)
	HSP104	Rice	Heat stress	Singla and Grover (1994)
	HSP16.9	Rice	Heat stress	Tzeng *et al.* (1992)
Transporters				
Na^+-K^+-symporter	*OsHKT1*	Rice	Salt stress	Horie *et al.* (2001)
	OsHKT2	Rice	Salt stress	Horie *et al.* (2001)
Na^+-H^+-dependent K^+ transporter	*EcHKT2*	Barley	Salt stress	Rubio *et al.* (1999)

2.2.1. Genetic Engineering for Osmolyte Biosynthesis in Cereals during Stress

Many monocotyledonous plants including cereals evolved different mechanisms for balancing osmotic strength of cells under salt/water stress conditions. Cereals like wheat, sorghum, maize and pearl millet can avoid dehydration by synthesizing different organic osmolytes that are compatible with cellular functions and can help as osmotic balancing agents, if accumulated in large quantities. A majority of the compounds can function as osmoprotectants. Almost all cereals accumulate proline albeit to a lesser extent relative to other osmo-tolerant plants; some cereals (wheat, maize, sorghum, barley) accumulate glycine betaine in response to salt and drought stresses. It seems that cereals do not accumulate sugars such as trehalose and sugar alcohols like ononitol, pinitol, etc. during exposure to abiotic stresses. Genes associated with the accumulation of various osmoprotectants have been the target for genetic engineering studies for more than a decade to develop genotypes tolerant to salt and water stresses. In most of the cases, introduction of a single gene into a plant (mostly dicots) resulted in only a moderate increase in tolerance with a modest accumulation of osmoprotectants. In the following we describe molecular physiology and genetic engineering work related to the synthesis of osmoprotectants such as proline, glycine betaine and sugar alcohols in cereal crops. Transgenic cereals that accumulate various compatible solutes and could sustain moderate abiotic stress treatments are listed in Table 4.

2.2.1.1. Proline

Proline accumulates in plants exposed to many abiotic stresses, due to upregulation of the gene for pyrroline 5-carboxylate synthetase (P5CS), a key enzyme that converts glutamate to Δ^1-pyrroline-5-carboxylic acid (P5C)

Table 4. Transgenic cereal plants developed for abiotic stress tolerance

Gene category/Gene	Species	Cellular response	Reference
Antioxidants			
Mn-superoxide dismutase	Rice	Salt tolerance	Tanaka *et al.* (1999)
Mn superoxide dismutase	Rye grass	Winter hardiness	McKersie (1999)
Fe- superoxide dismutase	Maize	Cold tolerance	Van Bruesegem *et al.* (1999)
Catalase	Rice	Chilling tolerance	Matsumura et al. (2002)
Osmolyte compounds			
Pyrroline carboxylate synthase *(p5cs)*	Rice	Drought, Salt tolerance	Igarashi *et al.* (1997) Zhu *et al.* (1998)
p5cs	Rice	Oxidative, Osmotic tolerance	Hong *et al.* (2000)
p5cs	Wheat	Salt tolerance	Sawahel and Hassan (2002)
Choline dehydrogenase	Rice	Drought, Salt tolerance	Takabe *et al.* (1998)
Choline oxidase	Rice	Cold, Salt tolerance	Sakamoto and Murata (1998) Mohanty *et al.* (2002)
Trehalose-6-P-synthase Trehalose-6-P-phosphatase	Rice	Salt, Drought, Cold tolerance	Garg *et al.* (2002) Jang *et al.* (2003)
Mannitol dehydrogenase	Wheat	Drought, Salt tolerance	Abebe *et al.* (2003)
Glycerol-3-phosphate acyltransferase	Rice	Cold tolerance	Yokoi *et al.* (1998)
waxy gene	Rice	Cold tolerance	Hirano and Sano (1998)
Glutamine synthetase	Rice	Salt, Cold tolerance	Hoshida *et al.* (2000)
Arginine decarboxylase	Rice	Drought tolerance	Capell *et al.* (1998)
Regulatory genes			
Calcium depdendent protein kinase	Rice	Salt, Drought, Cold tolerance	Saijo *et al.* (2000)
DREB1A	Wheat	Drought tolerance	Pellegrineschi *et al.* (2002)
Stress-responsive genes			
Late embryogenesis protein group 3 *(HVA1)*	Oat	Drought tolerance	Maqbool *et al.* (2002)
HVA1	Wheat	Drought tolerance	Sivamani *et al.* (2000)
HVA1	Rice	Drought, Salt tolerance	Xu *et al.* (1996) Rohila *et al.* (2002)
Thaumatin-like protein	Rice	Osmotic adjustment	Datta *et al.* (1999)
Heat shcok protein 101	Rice	High temperature tolerance	Katiyar-Agarwal *et al.* (2003)
Ferritin	Rice	Enhanced iron storage	Deak *et al.* (1999)
Pyruvate decarboxylase1	Rice	Submergence tolerance	Quimio *et al.* (2000)
Alcohol dehydrogenase	Rice	Flooding tolerance	Minhas and Grover (1999)
Transporters/symporter			
Potassium transporter *(HKT1)*	Wheat	Salt tolerance	Laurie *et al.* (2002)
Na+/H+ antiporter	Rice	Salt tolerance	Ohta *et al.* (2002)

in the proline biosynthetic pathway. Proline is formed from P5C by P5C reductase (P5CR) both in prokaryotes and eukaryotes. Initially, Kishor *et al.* (1995) overexpressed a mungbean P5CS gene in transgenic tobacco and reported accumulation of proline up to 18-fold over control plants resulting in enhanced biomass production under salt stress. Similarly, a P5CS gene isolated from rice was transferred back into rice (Igarashi *et al.,*1997), where over expression resulted in enhanced root biomass and flower development under water and salt stress conditions. In another set of experiments, a P5CS gene from *Vigna aconitifolia* was introduced into wheat plants using *Agrobacterium*-mediated gene transfer (Sawahel and Hassan, 2002). Transgenic analyses proved the expression of the transferred gene, and salinity tests indicated increased salt tolerance (Table 4) supporting the notion that proline acts as an osmoprotectant in transgenic wheat plants also. Proline is synthesized not only from glutamate but also from arginine/ornithine. Ornithine is transaminated to glutamic semi-aldehyde (GSA) by ornithine δ-aminotransferase (δ-OAT), which subsequently gets converted to proline via P5C (Delauney *et al.*, 1993). However, this gene has not been transferred yet into cereals though it was introduced and conferred salt stress tolerance in other plants (Madan *et al.,* 1995; Roosens *et al.*, 1998). A proline transporter (ProT) cDNA was isolated from *Oryza sativa* cv. Akibare (Igarashi *et al.*, 2000) and was shown to specifically transport L-proline in a transport assay. Although mRNA levels of ProT2 were observed throughout the plant, its transcript levels were found to be strongly induced by water or salt stress (Hare and Cress, 1997), suggesting an increase of proline transport during osmotic stress conditions.

Proline has also been shown to reduce enzyme denaturation caused by abiotic stress treatments such as salt, water, heavy metal, and UV radiation. (Iyer and Caplan, 1998). Under stress, it is mainly synthesized in chloroplasts and protects photosystem II against photodamage. Intermediates in proline biosynthesis and catabolism, such as glutamine and P5C also increase the expression of several osmotically regulated genes in rice, including *salT* and *dhn4* (Iyer and Caplan, 1998).

2.2.1.2. Glycine betaine

Betaines have been found to stabilize the quaternary structure of proteins and membranes. They also protect photosystem II from salt induced inactivation (Papageorgiou and Murata, 1995). Glycine betaine (GB) is a dipolar, electrically neutral molecule. It is synthesized from serine, which gets converted to choline *via* a series of steps that are not characterized properly. Unlike bacteria, plants possess choline monooxygenase (CMO), a ferridoxin dependent soluble Rieske-type protein, which oxidizes choline to

betaine aldehyde. CMO is a stress inducible iron-sulphur enzyme localized in the chloroplast stroma (Russell *et al.*, 1998). Betaine aldehyde dehydrogenase (BADH) is a soluble NAD^+ dependent enzyme that converts betaine aldehyde to glycine betaine. A positive correlation was found between the accumulation of betaines and tolerance to salt and cold, respectively, in maize and barley (Kishitani *et al.*, 1994). The gene coding for BADH is upregulated under high salt or drought conditions in wheat plants (Guo *et al.*, 2000).

Pathways for production of glycine betaine vary between organisms. In some bacteria choline gets converted to betaine directly by choline oxidase (*codA*), but the gene encoding the enzyme was not found in higher plants. Genetically engineered rice with the ability to synthesize GB was established by introducing the *codA* gene from the soil bacterium *Arthrobacter globiformis*. Levels of GB were high in two types of transgenic plants in which *codA* was targeted either to the chloroplasts (ChlCOD) or the cytosol (CytCOD). Inactivation of photosynthesis, used as a measure of cellular damage, indicated that ChlCOD plants were more tolerant than CytCOD plants to photoinhibition under salt and low-temperature stress. These results indicate that the subcellular compartmentalization of GB biosynthesis is a critical element in the enhancement of tolerance to stress in the engineered plants (Sakamoto and Murata 1998). Rice plants that produced bacterial choline dehydrogenase (CDH) targeted to the mitochondria were also generated (Takabe *et al.*, 1998). These transgenics accumulated GB at levels similar to those in transgenic rice that produced COD and showed enhanced tolerance.

2.2.1.3. Sugars and sugar alcohols

Sugars play an essential role as osmolytes and function also in signal transduction during development and under stress (Smeekens, 2000). Based on molecular genetic approaches, a link between hexose-sugar sensing and ABA signal transduction was found in Arabidopsis (Smeekens, 2000), and it was shown that an ABA-dependent signal transduction pathway is involved in the induction of stress genes (Zhu, 2002). This complex situation suggests that genes involved in carbohydrate metabolism and those involved in ABA biosynthesis can also be used to engineer abiotic stress tolerance.

Accumulation of a variety of polyhydroxylated sugar alcohols (polyols) such as trehalose, sorbitol, mannitol, ononitol, pinitol, etc. was reported in organisms osmotically stressed by drought and salinity (Csonka and Hanson, 1991) but not in cereals. While mannitol is synthesized from fructose 6-phosphate, other sugar alcohols like sorbitol, ononitol and pinitol are synthesized from glucose 6-phosphate. Sorbitol accumulates under

drought and salinity stress conditions and plays an important role in abiotic stress tolerance. The enzyme aldose-6-phosphate reductase involved in sorbitol synthesis was identified in barley. This enzyme was shown to be transcriptionally regulated under osmotic stress conditions (Bartels and Nelson, 1994) and hence could be an important candidate for overexpression. Mannitol also provides enhanced tolerance in response to high salinity or water stress. Recently, Abebe *et al.* (2003) demonstrated that ectopic expression of the *E. coli* gene for mannitol-1-phosphate dehydrogenase (mtlD) involved in mannitol biosynthesis improves tolerance to drought and salinity stress in wheat.

Trehalose as a compatible solute, might be involved in the stabilization of biological structures under abiotic stress conditions. Trehalose accumulation is reported in *Escherichia coli* but not in plants. Trehalose biosynthesis is controlled by the *ots*A and *ots*B loci in *E. coli*, which encodes trehalose 6-phosphate synthase (*ots*A) and trehalose 6-phosphate phosphatase (*ots*B). *Ots*A catalyzes the formation of trehalose 6-phosphate from UDP-glucose and glucose 6-phosphate. Further, *ots*B catalyzes the formation of trehalose from trehalose 6-phosphate (Kaasen *et al.,* 1994). Garg et al. (2002) reported the overexpression of *E. coli* trehalose biosynthetic genes (*ots*A and *ots*B) in Pusa Basmati rice as a fusion gene by using tissue-specific and stress-dependent promoters. In this study, comparison to control plants, several transgenic rice lines accumulated increased amounts of trehalose and exhibited sustainable plant growth under salt, droght and low-temperature stress conditions. Also, the transgenic plants in this study exhibited improved photosystem II function.

2.2.2. Genetic Engineering of Detoxification Pathways for Abiotic Stress Tolerance

All cereal crops that grow under a variety of adverse environmental conditions are prone to oxidative damage. Therefore, they have to deal with the highly reactive nature of oxygen derivatives such as superoxide radicals, hydrogen peroxide, hydroxyl and lipid radicals. Higher plants possess an array of antioxidant molecules (vitamin C, vitamin E, carotenoids, flavonoids) and antioxidant enzymes such as superoxide dismutase (SOD), catalase, ascorbate peroxidase (APX), glutathione peroxidase (GPX), glutathione S-transferase (GST) and glutathione cycle enzymes. It is evident that radical-induced damage (oxidative damage) is typically found in stress situations such as heat, cold, ultraviolet light, drought, salinity and heavy metals. A variety of physiological studies showed correlations between levels of antioxidants and stress tolerance among diverse cereal varieties

and biotypes (see, for instance, Sreenivasulu *et al.*, 2000; Prasad *et al.*, 1994). Comparisons between heat and drought-tolerant maize inbreeds displayed a correlation between antioxidant defense enzymes and heat as well as drought stress (Malan *et al.*, 1990). Similarly, comparative studies between salt tolerant and salt sensitive millet (*Setaria italica*) for general peroxidases, ascorbate peroxidase and superoxide dismutases revealed higher expression of these antioxidative transcripts/enzymes in the salt-tolerant cultivar during salt exposure (Sreenivasulu *et al.*, 1999; 2000). Since the antioxidant enzymes are important in protection against a variety of environmental stresses, production of transgenic cereals with genes encoding modified antioxidant enzymes is very promising. When rice was transformed with the yeast mitochondrial MnSOD gene, the transgenics displayed resistance to H_2O_2. Also, these transgenics were more resistant to salt stress than the control plants (Tanaka *et al.*, 1999). A *Nicotiana plumbaginifolia* MnSOD gene was used to generate transgenic maize plants overproducing MnSOD. To target this mitochondrial enzyme into chloroplasts, the MnSOD-coding sequence was fused to a sequence encoding a chloroplast transit peptide from a pea ribulose-1,5-bisphosphate carboxylase/oxygenase gene and engineered behind the CaMV 35S promoter. Transgenic MnSOD activity contributed to 20% of the total SOD activity and had clear effects on foliar tolerance to chilling and oxidative stresses. The results suggested that overproduction of MnSOD in the chloroplasts increased the antioxidant capacity of the maize leaves (Van Breusegem *et al.*, 1999). Similarly, overexpression of MnSOD as well as Cu/ZnSOD conferred freezing and drought tolerance in alfalfa (McKersie *et al.*, 1999). Among hydrogen peroxide scavenging enzymes, wheat catalase gene was overexpressed in rice and the transgenic rice plants exhibited reduction of hydrogen peroxide levels under chilling stress (Matsumura *et al.,* 2002). Likewise, transgenic overexpression of GST and GPX in tobacco led to accumulation of higher levels of glutathione and ascorbate relative to wild type seedlings, which in turn resulted in reduced oxidative damage and a higher degree of salt tolerance (Roxas *et al.*, 2000).

2.2.3. Stress Responsive Genes from Cereals and Their Effect on Stress Tolerance in Transgenic Plants

Stress-related genes were also isolated from cereals, which could be broadly classified into Late Embryogenesis Abundant (*LEA*) genes, Dehydrin genes (*DHN*), Cold Responsive genes (*COR*), Early Light Inducible Protein genes (*ELIPs*), etc. *LEA* genes are induced in vegetative tissues during dehydration, salinity, cold, ABA treatments and also in seeds during the desiccation phase (Dure, 1993). They are grouped into three classes (1, 2

and 3), and many of them were cloned from Triticeae species also. Among the *LEA* class 2 proteins, dehydrins are characterized by lysine rich amino acid sequences at the C-terminus. Dehydrins (*DHN*; *LEA D11*) are water-soluble lipid-associating proteins that are exclusively expressed during dehydration conditions, and are thought to play a role in freezing and drought tolerance in plants (Close, 1997; Ismail *et al.,* 1999). Choi *et al.* (1999) identified 11 unique *DHN* genes and estimated a total of 13 *DHN* genes in the barley genome. In addition, *DHN* genes were characterized in a wheat drought-tolerant cultivar (Labhilili *et al.,* 1995). The *LEA* class 3 gene, *HVA1* was isolated from barley and transferred to rice (Japonica). The transgenics exhibited enhanced accumulation of the *HVA1* protein, increased tolerance to water deficit and salt stress, higher growth rates, delayed stress-related damage symptoms as well as faster and improved recovery after stress removal (Xu *et al.*, 1996). Transgenic wheat plants containing the constitutively expressed *HVA1* gene also resulted in improved growth characteristics under water-deficit conditions. As compared to the control, the transgenics produced more biomass and showed higher water use efficiency (Sivamani *et al.*, 2000).

Cold responsive genes such as *COR* or *BLT* form a small gene family shown to be involved in cold and frost tolerance (Grossi *et al.,* 1998; Cattivelli *et al.,* 2002). Cattivelli and Bartels (1990) isolated the cold induced chloroplast localized *COR14b* gene from barley. Constitutive expression of *COR15a* gene of *Arabidopsis thaliana* results in a significant increase in the survival of isolated protoplasts frozen at –7 °C (Steponkus *et al.,* 1998). The *BLT* genes found to be induced under low temperaurtes encode *BLT4* (non-specific lipid transfer protein), *BLT63* (elongation factor 1α), *BLT801* (RNA binding protein) and *BLT14* (Dunn *et al.,* 1993; Dunn *et al.,* 1996; Pearce *et al.,* 1998). High temperatures cause high membrane fluidity and plants adapted to high temperatures contain a high proportion of saturated fatty acids in the membranes. Exposure to high temperatures causes synthesis of heat shock proteins (*HSP*) that play a defensive role by stabilizing proteins and membrane structures. Recently it was reported that transfer of the *HSP*101 gene from *Arabidopsis thaliana* mediates enhanced tolerance to high temperature stress in rice (Katiyar-Agarwal *et al.*, 2003).

2.2.4. Engineering Ion Transport and Homeostasis Genes

Salt stress causes both osmotic and ionic effects. Different factors including Na^+/H^+ antiport are known to be involved in maintaining ion homeostasis in plants exposed to salt stress (Zhang *et al.*, 2001). Arabidopsis salt overly sensitive (*sos*) mutant 1 was shown to encode a plasma membrane Na^+/H^+

antiporter with sequence similarity to plasma membrane Na^+/H^+ antiporters from bacteria and fungi (Shi *et al.*, 2000). A vacuolar Na^+/H^+ antiporter gene (AtNHX1) from Arabidopsis was transferred into *Brassica napus* (Zhang *et al.*, 2001) and *Lycopersicon esculentum* (Zhang and Blumwald, 2001). The transgenic Brassica and tomato plants were able to grow, flower, and produce seeds in the presence of 200 mM NaCl. Another gene that encodes a vacuolar Na^+/H^+ antiporter was isolated from *Atriplex gmelini* (AgNHX1) and transferred to *O. sativa.* The transgenic rice plants survived up to 300 mM NaCl for 3 days and conferred significant improvement in salt stress tolerance (Ohta *et al.*, 2002). According to Horie *et al.* (2001) plant growth under salt stress conditions requires the maintenance of a high cytosolic K^+/Na^+ concentration ratio. Therefore, relevant ion transporters are the likely candidates to be tested in transgenic plants. Rus *et al.* (2001) found that a high affinity potassium transporter (HKT1) from *A. thaliana* functions as a selective Na^+ transporter and also mediates K^+ transport. A HKT gene was introduced into wheat in sense and antinsense orientation and the transgenic lines showed enhanced growth in the presence of 200 mM NaCl. Na^+: K^+ ratios were reduced in salt-stressed transgenic tissue when compared to control (Laurie *et al.*, 2002).

The regulation of ion homeostasis under salt stress has been extensively studied by using salt overly sensitive (SOS) mutants of Arabidopsis (for review see Zhu, 2003; Gong *et al.*, 2004). There is substantial evidence that SOS pathway involving several SOS genes plays a key role in regulation of ion transporter expression. For instance, one of these genes, SOS3 encodes a novel EF-hand Ca^{2+} sensor (Liu and Zhu, 1998) and their associated SOS2 gene (Ser/ Thr protein kinase) interacts physically. The SOS3-SOS2 complex mediates expression of Na^+/ H^+ antiporter (SOS1) gene. The transporter in turn, maintain low Na^+ and high K^+ levels in the cytoplasm during salt stress. Ion homeostasis during salt stress is also dependent on signaling via the calcium- and calmodulin-dependent protein phosphatase calcineurin (Liu and Zhu, 1998; Pardo *et al.*, 1998). A truncated form of the catalytic subunit, and the regulatory subunit of yeast calmodulin-dependent protein phosphatase calcineurin (CaN) were coexpressed in transgenic tobacco plants to activate the phosphatase *in vivo.* Like in yeast, transgenic tobacco plants expressing activated CaN exhibited substantial NaCl tolerance by regulating the calmodulin-dependent CaN signal pathway (Pardo *et al.,* 1998). A rice gene encoding Ca^{2+}-dependent protein kinase (CDPK) was overexpressed in transgenic rice plants and shown to enhance induction of stress-responsive genes in response to salt and drought stress (Saijo *et al.*, 2000). The authors concluded that CDPK is a positive regulator commonly involved in tolerance to both salt and drought stress in transgenic rice plants overexpressing CDPK.

2.2.5. Engineering of Regulatory Genes

Transcription factors that control gene expression under stress conditions play an important role during stress adaptation. Here, we describe mainly two major families, ERF and bZIP proteins. The ERF (ethylene-responsive-element-binding factor) family is a large group of transcription factors containing a C-repeat dehydration-responsive element (DRE), which is unique to plant systems. DRE elements play an important role in the regulation of gene expression in response to various stresses (Yamaguchi-Shinozaki and Shinozaki, 1994). It was found that the transcription factor DREB1A specifically interacts with the DRE motif and induces the expression of stress tolerance genes. Overexpression of the DREB1A gene under control of the stress inducible rd29A promoter resulted in a better growth of the transgenic plants in comparison to those transformed with a CaMV35S promoter-DREB1A construct (Kasuga *et al.*, 1999). This work indicates the importance of stress-inducible promoters for generation of transgenic plants. When a DREB1 gene was introduced into wheat, transformants survived a short but intensive water stress (Pellegrineschi *et al.*, 2002).

Another large family of transcription factors in plants are the bZIPs, among which one subclass ABRE/ABF (ABA-responsive-element-binding protein/ ABRE binding factor) is a well-studied example, which is linked to stress signaling, including salt, drought and UV light stresses. Different abiotic stresses and ABA induce ABRE/ABF expression and ABA triggers ABRE phosphorylation. This phosphorylation is necessary to induce downstream genes, which could occur on the casein kinase II phosphorylation sites. Therefore, ABA and different abiotic stresses induce both transcriptional and post-translational regulation of several bZIP transcription factors (Jakoby *et al.*, 2002). In rice, a cDNA encoded bZIP protein (OSBZ8) was shown to bind G-box-like elements including ABREs (Nakagawa *et al.*, 1996). Constitutive overexpression of ABRE binding factors (ABF3 or ABF4) led to altered expression of ABA/stress-regulated genes and in turn reduced transpiration and enhanced drought tolerance (Kang *et al.*, 2002).

2.2.6. The Future of Developing Stress-Tolerant Transgenic Cereals

So far, relatively few transgenic cereals (mostly rice and wheat) have been developed, each containing usually only one stress response gene (Table 4). However, this may not be enough to serve the purpose since many genes and components control stress tolerance. Furthermore, plants have to be tolerant and at the same time have to produce high yields (Pental, 2003).

Thus, only introduction of multiple genes into a single plant might yield higher tolerance without negative effects on other important agronomic parameters. Further, the expression levels of the transgenes have to be increased and expression must be controlled preferentially by using stress-induced promoters. Moreover, no scientific reports have been published yet on extensive field tests, which can give clear results about tolerance levels. Positive transgenic lines tested in this way, could be used for further development of stress tolerant varieties through breeding.

2.3. AB-QTL Analysis and Genetical Genomics

Dense molecular marker maps are now available for a number of cereals like wheat (reviewed in Gupta *et al.*, 1999), barley (reviewed in Varshney *et al.*, 2004; Forster *et al.,* 1997), maize (http://www.agron.missouri. edu/maps.html), rice (http://rgp.dna.affrc.go.jp/ Publicdata.html; Kikuchi *et al.*, 2003) and sorghum (http://sorghumgenome. tamu.edu). In all major crops including cereals, these molecular genetic maps and the available molecular markers were extensively used for identification of genes or QTLs for a variety of traits. In particular, the molecular markers linked with QTLs that confer tolerance to abiotic stresses have a great potential for their use in marker-assisted selection (MAS) in breeding programmes aimed at crop improvement. This aspect has been discussed in detail in Chapter 9 by Tuberosa and Salvi, and in Chapter 10 by Koebner.

Advanced-backcross QTL analysis (ABQA) for simultaneous discovery and transfer of QTLs from a wild species to a crop variety, proposed earlier by Tanksley and Nelson (1996), may also be useful for the development of tolerance to abiotic stresses in cereals. In this approach, a wild species is backcrossed to a superior cultivar, and during backcrosses, the transfer of desirable gene/QTL is monitored by employing molecular markers. The segregating BC2F2 or BC2F3 population is then used not only for recording data on the trait of interest, but also for genotyping it using polymorphic molecular markers. This data is then used for QTL analysis, leading to simultaneous discovery of QTLs, while transferring these QTLs by conventional backcrossing. However, for transfer of tolerance to abiotic stresses, this ABQA approach has yet to be utilized in cereals, although for other traits like yield and yield components it has already been successfully used in tomato (Tanksley *et al.*, 1996), rice (Xiao *et al.*, 1998; Moncada *et al.,* 2001), wheat (Huang *et al.*, 2003) and barley (Pillen *et al.*, 2003; Talamè *et al.*, 2003).

Recently, a new approach, called 'genetical genomics' has also been proposed, where QTL mapping is combined with expression profiling of individual genes in a segregating (mapping) population (Jansen and Nap, 2001). In this approach, total mRNA or cDNA of the organ/tissue from each individual of a mapping population is hybridized onto a microarray carrying a high number of cDNA fragments representing the species/tissue of interest and quantitative data are recorded reflecting the level of expression of each gene on the filter. Under the presumption, that every gene showing transcriptional regulation is mapped within the genome of the species of interest, the expression data can be subjected to QTL analysis, thus making it possible to identify the so-called 'ExpressQTLs' (eQTLs). The recently developed software tool 'Expressionview' for combined visualization of gene expression data and QTL mapping (Fischer *et al.*, 2003) will be very useful in this connection. Based on segregating populations, eQTL analysis identifies gene products influencing the quantitative trait (level of mRNA expression) in *cis* (mapping of the regulatled gene within the QTL) or *trans* (the gene is located outside the QTL). The latter gene product (second order effect) is of specific interest because more than one QTL can be connected to such a *trans*-acting factor (genes acting on the transcription of other genes) (Schadt *et al.*, 2003). The mapping of eQTLs allows multifactorial dissection of the expression profile of a given mRNA/cDNA, protein or metabolite into its underlying genetic components, and also allows locating these components on the genetic map (see Jansen and Nap, 2001; Jansen, 2003). Eventually, for each gene or gene product analyzed in the segregating population (by using expression profiling methodology), eQTL analysis will underline the regions of the genome influencing its expression. For crops like rice, where sequence of the whole genome is available, the annotation of those genomic regions will be helpful for the identification of the genes and their regulatory sequences involved in the expression of an individual trait.

Recently, in mouse, humans and maize, 'genetical genomics' approach has been used for a genome-wide study of the genetics of variation in expression of individual genes/QTLs for specific triats (Schadt *et al.* 2003). For instance, eQTLs were identified that influenced expression of about 10% of genes, differentially expressed in two typical inbred lines of maize- a stiff salk syntethic type and a Lancaster type. Gene-gene interactions similar to epistatsis were also noticed, and the interacting eQTLs were sometimes found on different chromosomes. This approach provides a powerful source to implicate genes as being involved either in the same or related transduction pathways involved in the expression of individual genes. Although the 'genetical genomics' approach is still in its infancy, efforts are underway in this direction in some plant species like tomato (Bai

et al. 2003), eucalyptus (Kirst *et al.* 2003) and barley (Potokina *et al.* 2003). We believe that availability of large EST collections for genome-wide expression profiling (see section 2.1) and analytical tools for molecular marker analysis in different cereals will accelerate the use of this approach in cereals for different agronomic traits including abiotic stress tolerance.

3. SUMMARY AND OUTLOOK

In the last two decades, biochemical pathways that are involved in conferring tolerance against abiotic stresses were studied in great detail, and genes involved in different steps of these biosynthetic pathways were isolated and characterized. Based on these studies, it is now known that accumulation of osmolytes, scavenging of reactive oxygen species, higher expression of chaperones and a control over sodium uptake might bring about at least partial tolerance against drought, salinity and cold. Nevertheless, we are still far from having a complete understanding of the molecular basis and regulatory mechanisms involved in conferring abiotic stress tolerance/susceptibility. A dissection of the complexity of tolerance against salinity, drought and temperature stress in (tolerant) crop plants will be possible in future through a variety of approaches. These approaches include whole genomic sequencing, high-throughput transcript profiling and discovery of gene functions. This will facilitate identification of candidate genes conferring tolerance, development of transgenic crops with higher tolerance, and selection of markers for marker-assisted selection/breeding. New approaches like 'genetical genomics' offer great promise to identify genes or genomic regions (QTLs) that are involved in conferring tolerance to abiotic stresses.

4. ACKNOWLEDGEMENTS

We are gratefully indebted to Prof. U. Wobus, Director, IPK, Gatersleben (Germany) and Prof. P.K. Gupta, Ch Charan Singh University, Meerut (India) for helpful comments and improving the quality of manuscript. This work was supported by IPK internal funds.

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