# Enhancing Abiotic Stress Tolerance in Cereals Through Breeding and Transgenic Interventions

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## ABBREVIATIONS

| | |
|---|---|
| ABA | Abscisic acid |
| AFLP | Amplified fragment length polymorphism |
| Al | Aluminum |
| ASI | Anthesis silking interval |
| BA | Betaine aldehyde |
| BADH | Betaine aldehyde dehydrogenase |
| cDNA | Complementary deoxyribonucleic acid |
| CGIAR | Consultative Group on International Agricultural Research |
| CIMMYT | International Maize and Wheat Improvement Center |
| CMO | Choline monooxygenase |
| CSIRO | Commonwealth Scientific and Industrial Research Organization (Australia) |
| DMB | Dragon Motif Builder |
| DNA | Deoxyribonucleic acid |
| DT | Drought tolerance |
| ECe | Electrical conductivity |
| EST | Expressed sequenced tags |
| eQTL | Expression quantitative trait loci |
| GB | Glycinebetaine |
| GCP | Generation Challenge Program |
| GO | Gene oncology |
| HI | Harvest index |

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|---|---|
| ICRISAT | International Crops Research Institutue for the Semi-Arid Tropics |
| LEA | Late embryogenesis abundant |
| MABC | Marker-assisted backcross |
| MAS | Marker assisted selection |
| MATE | Multidrug and toxic compound extrusion |
| NIL | Near-isogenic line |
| NSF | National Science Foundation |
| NUE | Nitrogen use efficiency |
| OA | Osmotic adjustment |
| PA | Polyamine |
| PEG | Polyethylene glycol-6000 |
| PNHI | Panicle harvest index |
| QTL | Quantitative trait loci |
| RED | Rice expression database |
| RFLP | Restriction fragment length polymorphism |
| ROS | Reactive oxygen species |
| RT-PCR | Reverse transcriptase-polymerase chain reaction |
| RWC | Relative water content |
| SAGE | Serial analysis of gene expression |
| SSR | Simple sequence repeat |
| SOD | Superoxide dismutases |
| SPAD | Soil-plant analyses development |
| TE | Transpiration efficiency |
| WSC | Water-soluble carbohydrate |
| WUE | Water use efficiency |

## I. INTRODUCTION

The success of plant breeding in the 20th century led to new cultivars that, to date, have provided enough food for an increasing world population (Conway and Toenniessen 1999; Mifflin 2000). The results of the Green Revolution—led in the 1960s by Henry M. Beachell and Norman E. Borlaug—resulted in a dramatic increase in rice and wheat grain yields (Milford and Runge 2007; Ortiz et al. 2007). However, abiotic stresses and climate change are becoming increasingly serious threats to crop production worldwide at a time when food staple supply will need to be significantly higher to meet the demand of the growing human population. Water scarcity (Rockström et al. 2007), salinity (Rengasamy 2006), and low soil fertility (Sanchez and Swaminathan 2005) rank among the most important abiotic stresses worldwide. Similarly, increased climatic disturbances due to global warming are causing the major stresses that

necessitate crop improvements to safeguard grain supply, particularly in the developing world (Kumar 2006). Hence, genetic enhancement of cereal crops with respect to abiotic stress tolerance will be essential for ensuring grain yields in water-limited, increasingly hotter agricultural zones, particularly if these conditions combine with poor and saline soils, conditions that prevail in parts of the developing world. Crop breeding for adaptation to abiotic stress–prone environments remains a challenging task, not least because of the complexity of the stress-adaptive mechanisms in plants and particularly cereal crops, which are the staple of most of the world's population (Reynolds et al. 2005).

Limited available water is the single most important factor that reduces global crop yields. Agriculture currently accounts for 70% of the fresh water used by humans, an amount that is expected to increase 17% by 2025 (Molden 2007). The underground aquifers are rapidly being depleted due to excessive water use (Morison et al. 2008). Although the perception of "drought" may be distinctly different among people, farmers and scientists agree that water shortage is a key limiting factor for crop yields (Passioura 2007). For a cereal agronomist, drought is the coefficient of variation in grain yield across environments differing in rainfall. Water deficiency significantly impedes sexual plant reproduction, particularly at the early stages, when development may irreversibly cease although the plant may remain alive (Boyer and Westgate 2004). Ovule abortion or pollen sterility occurs in cereal crops under water stress, and this in turn increases abscisic acid in the plant that may lead to inhibited photosynthesis. Water-use efficiency or water productivity are the distinct terms used by agronomists, economists, plant physiologists, and crop breeders to refer to crop performance in drought-prone environments (Bennet 2003). Irrespective of their understanding, any farmer measures the success of a new cultivar crop by a known (often >10%) yield advantage in challenging water stress–prone environments. While some progress made in the private or public sector in the genetic enhancement of cereal crops for drought-prone environments, both through conventional or genomics-led approaches (Campos et al. 2004; Bänziger et al. 2006), farmers worldwide continue to struggle to obtain satisfactory grain yields under conditions of water scarcity.

Salinity affects crop yields and pollutes the environment, thereby negatively impacting the economic welfare of nations. There are about 100 countries directly affected by salinity (Rengasamy 2006) with the major impact in those where soil degradation, aridity, and salinization, in part caused by deforesation, prevail. Most of the commonly used food staples are sensitive to salinity (Flowers and Colmer 2008). Researchers are assessing halophytes (i.e., salt-tolerant plants), which constitute about 1% of the world's flora, as potential gene sources to improve

salinity tolerance in field crops. Halophytes have evolved a range of adaptation mechanisms to tolerate high salinity. These include adjustment of their internal water relations through ion compartmentation in vacuoles, the accumulation of compatible organic solutes, succulence, and salt-secreting glands and bladders (Flowers et al. 1986).

In addition, acid soils constitute about 38% of farmland in Southeast Asia; 31% of Latin America; and 20% in East Asia, sub-Saharan Africa, and North America. Aluminum (Al) toxicity in these soils is the major constraint to crop production (Wood et al. 2000). It is the second most important abiotic constaint to world food production after drought (von Uexküll et al. 1995). At low pH (<5), Al is released as $Al^{3+}$ into the soil, reducing root growth and development due to poor uptake of water and nutrient and resulting in reduced crop yields (Kochian 1995). The use of lime in acidic soils is a common agricultural practice to raise soil pH and minimize adverse effects of Al toxicity on crop plants. However, the energy costs for application or actual cost of lime often prohibits widespread adoption of this practice. Natural genetic variation for Al tolerance has been identified in many crop species, and Al-tolerant cultivars in some crops are commercially grown (Samac and Tesfaye 2003).

Climate change caused by global warming is expected to significantly affect grain yields of the most important cereals (Jones and Thornton 2003; Peng et al. 2004; Ortiz et al. 2008), although in cooler regions we may expect grain yield increases. For example, rice grain yield declined by 10% for each 1 °C increase in growing-season minimum temperature in the dry season in the Philippines (Peng et al. 2004). A projected 10% yield reduction in maize will bring losses equal to U.S. $2 billion in Africa and Latin America (Jones and Thornton 2003). The wheat yield is expected to decline 20% in China by 2070 (Hui et al. 2005) and 13% to 32% in south Australia (Luo et al. 2005). Breeding for drought and heat tolerance will therefore be the main task for adapting cereal crops, particularly in the most affected areas of the developing world (Long et al. 2006; Barnabás et al. 2008).

Furthermore, nitrous oxide ($N_2O$) is a potent greenhouse gas generated through use of the manure or nitrogen fertilizer and susceptible to denitrification, thus often unavailable for crop uptake and utilization. Consequently, the transfer of nitrogen use efficiency (NUE) gene(s) to main cereal staples will also be an important crop improvement goal to react to climate change in an integrated and sustainable fashion.

It is not within the scope of this section to provide a comprehensive review of drought, salinity, and aluminum toxicity. More extensive coverage of the problems and possible strategies to overcome them has been presented elsewhere (Araus et al. 2002; Chaves et al. 2003; Samac

and Tesfaye 2003; Condon et al. 2004; Bartels and Sunkar 2005; Munns 2005; Parry et al. 2005; Reynolds et al. 2005; Slafer et al. 2005; Tuberosa and Salvi 2006; Mathur et al. 2007; Barnabás et al. 2008; Pennisi 2008; Takeda and Matsuoka 2008). The aim of this review is to provide updates on novel sources of variation and genomic regions (quantitative trait loci; QTL) associated with abiotic stresses and trait-based breeding for abiotic stress tolerance, and to assess the usefulness of novel genes and regulatory pathways to increase abiotic stresses tolerance (drought, heat, salinity, and aluminum toxicity) in cereal crops. Transgenic approaches for improving crops in high-stress environments will need to address new and vigorous biosafety assessments before obtaining regulatory approval. New crop varieties with enhanced performance in abiotic stress-prone environments are likely to be developed, and this in turn will increase competitiveness and have socioeconomic downstream effects. However, new stress-tolerant crops obtained as a result of transgenic interventions may pose serious questions regarding their safety and impact on the environment. Although these are important aspects, they do not fall within the scope of this chapter.

## II. NATURAL GENETIC VARIATIONS AND TRAIT-BASED BREEDING

Natural variation is the foundation of genetic improvement in plants. Identification and utilization of undiscovered variation for abiotic stress tolerance could enhance the adaptation of cereal crops. The Consultative Group on International Agricultural Research (CGIAR) institutions house 336,325 accessions of cultivated and wild relatives of the major cereal crops (http://singer.grinfo.net) that provide a unique resource for the discovery of variation that eventually can be harnessed in crop improvement programs. Precise phenotyping is the key to finding and introducing new genes for abiotic stress tolerance. Phenotypic screens for drought, salinity, and aluminum tolerance have been developed for identifying tolerant germplasm and for dissecting the genetic, physiological, and molecular basis of tolerance. Physiological and molecular understanding of tolerance mechanisms will contribute toward developing more tolerant crops. In this section we discuss the progress made in identifying germplasm resources tolerant to abiotic stresses, the physiological traits associated with these stresses, genomic information for dissecting the genetic structure and diversity in germplasm resources, and use of trait-based selection to improve abiotic stress tolerance in cereal crops.

Natural genetic variations for tolerance to drought (Table 2.1), salinity (Table 2.2), and Al toxicity (Table 2.3) have been reported in cereal crops.

**Table 2.1.** Sources of drought tolerance in barley, maize, pearl millet, rice, sorghum, and wheat from 1996 to 2007.

| Source | Reference |
|---|---|
| Barley (*Hordum vulgare*)<br>Gharamalek, Giza 2000, IB78-26403, JR1 and JR2 of *Hordeum spontaneum*, Tadmor, Arta | Guo et al. 2008; Liu and Song 2007; Noaman et al. 2007; Suprunova et al. 2007; Baheri et al. 2003; Teulat et al. 1998 |
| Maize (*Zea mays*)<br>95TZEE-W, 95TZEE-Y, Ac7643, Ac7643S5, Chang 3, CML 269/CML343, CML 449/CML 343, D 978, Danhuang 02, HI 209, HI 295, HI 536, HI1040, K 10, K 22, TSC 8, TZE-COMP 3 DT, X178, Xi 502, Ye 8001, Yedan-13, Zheng 22 | Monneveux et al. 2007; Ribaut and Ragot 2007; Kamara et al. 2006; Vargas et al. 2006; Lu et al. 2005; Sánchez-Urdaneta et al. 2005; Xiao et al. 2005; Kumari et al. 2004 |
| Pearl millet (*Pennisetum glaucum*)<br>863B, ICMP-83720, ICMV-9413, ICMV-94472, and PRLT 2/89-33 | Ashraf et al. 2003; Yadav et al. 2002; Andrew and Kumar 1996 |
| Rice (*Oryza sativa*)<br>ASD 16, ASD 18, Azucena, Bala, Bg 300, Bg 400, CAR 3, CS 94, CT9993, Daitun 502, Doddi, Gayabyeo, Han-501, Han-A03, Hu-han 3, Hu-han 7, IR55419-04, IR57514-PMI-5-B-1-2, IR58821-23-B-1-2-1, IR62266-42-6-2, IRAT 109, IRAT 216, JC 81, Jhona 349, Kantomochi 168, Khumal 4, Lemont, Nagina 22, Norinmochi 4, OM 997, OM 1723, Q 5, Safri 17, Shwe Thwe Yin Hyv, Tarom molaii, TKM 9, Wuyujing, Y134, Yumenohatamochi, Yunhui 290, Zale, Zhong-han 3, drought-tolerant CMS and maintainer line Hu-han 1A and Hu-han 1B, drought-tolerant core collection of 86 accessions | Kumar et al. 2008; Yang et al. 2007; Hirayama et al. 2006; Lafitte et al. 2006; Liu et al. 2006; Ouk et al. 2006; Yue et al. 2005; Hirasawa et al. 2004; Jiang et al. 2004; Nguyen et al. 2004; Robin et al. 2003; Ali et al. 2000; Price et al. 2000; Zheng et al. 2000 |

(continued)

Table 2.1. (Continued)

| Source | Reference |
|---|---|
| **Sorghum (*Sorghum bicolor*)** | |
| Ajabsido, B35, BTx642, BTx3197, CSM-63, El Mota, E36xIr16 8/1, Gadambalia, IS12568, IS22380, IS12543C, IS2403C, IS11549C, IS12553C, IS12555C, IS12558C, IS17459C, IS3071C, IS6705C, IS8263C, ICSV-272, Koro Kollo, KS19, P898012, P954035, QL10, QL27, QL36, QL41, SC414-12E, Segaolane, TAM422, Tx430, Tx432, Tx2536, Tx2737, Tx2908, Tx7000, Tx7078 | Harris et al. 2007; Tsuji et al. 2005; Rooney 2004; Sharma and Singh 2003; Mahalakshmi and Bidinger 2002; Xu et al. 2000 |
| **Wheat (*Triticum aestivum*)** | |
| Baviacora, C306, Cham 1, CROC_1/AE.SQUARROSA (224)//OPATA (SYN), Cunningham, Dharwar Dry, Dingxi 24; Excalibur, Hanfeng 9703, Jennah Khetifa, MES, MEX94.2.19, MEX94.15.34, MEX94.12.2.39, MEX94.27.1.20, Njoro BW1, Oued Zenati, Pastor, PUB94.16.24, PUB94.15.1.12, Quarrion, Sokoll, Weebil 1, ZAM-04, OAX93.24.35, several substitution lines in Sheynne background having 7A, 4D, 6D, and 2D chromosomes | Chopra and Selote 2007; Kirigwi et al. 2007; Reynolds et al. 2007; Monneveux et al. 2006a; Xue et al. 2006c; Farshadfar and Mohammadi 2005; Nasir et al. 2005; Njau et al. 2005; Zhu et al. 2005; Li et al. 2003 |
| Several accessions of wild emmer wheat (*Triticum turgidum* spp. *dicoccoides*); wild emmer wheat exhibiting greater carbon isotope ratio ($\delta^{13}$C) and higher plasticity of $\delta^{13}$C relative to cultivated controls under hot dry locations; several *Aegilops geniculata* accessions with high temperature and drought tolerance; *T. tauschii*—a source of genes for increased yield under terminal drought (higher grain filling and larger grains) and heat stress during grain filling | Peleg et al. 2005; Gororo et al. 2002; Zaharieva et al. 2001 |

Table 2.2. Sources of salinity tolerance in barley, maize, pearl millet, rice, sorghum, and wheat from 1981 to 2007.

| Source | Reference |
|---|---|
| **Barley** | |
| AC Burman, Accsad 176, CM 72, Gerbel, Golden Promise, HS 26/55C, Line 5, Numar, Rum, ZUG293, ZUG 797. | Chen et al. 2007; Thalji and Shalaldeh 2007; Forster et al. 2002; Flowers and Hajibagheri 2001 |
| Seven wild *Hordeum* species with better Na $^+$ and Cl$^-$ exclusion and high leaf K$^+$, *H. agriocrithon* > *H. spontaneum* > other species with high NaCl tolerance at seedling stage | Garthwaite et al. 2005; Mano and Takeda 1998 |
| **Maize** | |
| SR 03, SR 12, and several landraces and commercial cultivars/hybrids | de Costa et al. 2007 |
| C 12338, Champ, Conquest, Lg 20.80, PI 213714, PI 503567, PI 503568, PI 508270, Sundance, Zea 670 | Khan et al. 2003 |
| **Pearl millet** | |
| 863-B, CZI 98-11, CZI 9621, HTP 94/54, ICMB 02111, ICMB 94555, ICMB 95333, ICMB 00888, ICMB 01222, ICMP 451, IP 3732, IP 3757, IP8210, PRLT 2/89-33 | Krishnamurthy et al. 2007b; Kusaka et al. 2005 |
| **Rice** | |
| AT69-5, Azucena, BR 28, Cheriviruppu, CSR 11, Damodar, DH, Getu, FL 378, FL 478, IAEA-2, IR 64, IR 4595-4-1-13, IR 4630-22-2-5-1-3, IR 51491-AC10, IR 5273-2B-8-2B-1-2, IR 51500-AC11-1 (Bicol in Philippines), IR 51500-AC17 (CSR 21 in India), IR 51485-AC6534-4 (CSR 28 in India), IR 58443-6B-10-3, IR 60494-2B-18-2-3, IR 61920-3B-22-2-1, IR 63291-B-3R-8-3, IR 63307-4B-4-3, IR 65185-3B-8-3-2, IR 65192-4B-10-3, IR 65195-3B-2-3, IR 69997-AC1, IR 69997-AC2, IR 69997-AC3, IR 69997-AC4, IR 69997-AC1-2-3-4, IR 72132-AC 6-1, IR 9884-54-3, IR-10167-129-3-4, IR 10198-66-2, IR 10206-29-2-1, Kararata, Ketumbar, Khao Seetha, Nona Bokra, NV, Pat, Pokkali, PSBRc 50, ROK 5, Shorawaki, Soc Nau, Solla, SR26B, TCCP 266-2-49-2B-3, VTL 1, VTL 2, VTL 3, VTL 4, VTL 5, VTL 6, Wagwag | Ismail et al. 2007; Lee et al. 2007; Shylaraj et al. 2006; Natrajan et al. 2005; Shylaraj and Sashidharan 2005; Díaz De León et al. 2000; Gregorio et al. 2002; Shylaraj et al. 1998; WARDA 1994 |

**Table 2.2.** (Continued)

| Source | Reference |
| --- | --- |
| **Sorghum** CSF 20, CSV 15, Dorado, G 114, Giza 113, Hagen Shandawil, ICSB 589, ICSB 676, ICSB 766, ICSV 95030, NTJ 2, S 35 | Krishnamurthy et al. 2007a; Alves da Costa et al. 2005; Azooz et al. 2004; Hassnian and Azab 1993 |
| **Wheat** Berkut, Bin-bashair, Chadianhong, Cham 3, Cham 6, Drysdale, Janz, Jumaizah, Kharchia 65, KRL1-4, KRL 19, KTDH 19, Line 149, LU 26S, Pitic 62, Punjab 85, RH 8706-49, S 24, SARC-1, Sakha 8, Sakha 93, Snap, TW 161, W 4909, W 4910, Wentworth, Yecora Rojo, 34 durum and bread wheat accessions, several hexaploid-based resynthesized wheat and translocation lines | Genc et al. 2007; El-Hendawy et al. 2007; Ma et al. 2007; Mullan et al. 2007; Thalji and Shalaldeh 2007; Munns et al. 2006; Wang et al. 2003a; Pritchard et al. 2002; Suo et al. 2001 |
| Wild relatives: *Aegilops, Thinopyrum bessarabicum, T. elongatum, T. junceiforme, T. junceum, T. intermedium, T. runemarkii, T. ponticum, Elytrigia elongata, E. scirpea, E. pontica, E. junceiformis, E. diae, E. Leymus, Lophopyrum elongatum, T. tauschii* (CPI 110791 and AUS 18905) | Colmer et al. 2006; Wang et al. 2003b; Schachtman et al. 1991; McGuire and Dvořák 1981 |

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**Table 2.3.** Sources of aluminum (Al) tolerance in barley, maize, rice, sorghum, and wheat from 1965 to 2007

| Source | Reference |
| --- | --- |
| **Barley** CI 8411, Colonia 2, Dayton, Honen, Kaniere, L 1327, Murassaki Mochi, Nakano Wase, RL 819/2, RL 820/6, Smooth Awn 86, Smooth Awn 203, Sunrise, WB 229 | Wang et al. 2006; Ninamango-Cárdenas et al. 2003; Raman et al. 2002; Nawrot et al. 2001; Minella and Sorrells 1992 |
| **Maize** Adour 250, ATP-Y, Bozm 1335, Bozm 1337, Bozm 1536, BR 201 F, Cateto-Colombia, C 525 M, CMS 36, DK 789, IAC-TAIUBA, SA 3, Sikuani, Zea 769, several Madeiran germplasm accessions | Kochian et al. 2005; Carvalho et al. 2004; Mariano and Keltjens 2003; Khan and McNeilly 1998; Llugany et al. 1995 |
| **Rice** 63-83, Asominori, Azucena, CT9993, Chiembu, IRAT 13, IRAT 20, IRAT 121, IRAT 216, Koshihikari, Moroberekn, Nipponbare, Xiangnuo 1 | Xue et al. 2006a,b; Xu et al. 2004b; Ma et al. 2002; Nguyen et al. 2002; Wu et al. 2000 |
| **Sorghum** 3DX, 9DX, 5DX, 9929034, ATF 14, Brandes, CMS 153J, CMS 225, CMS 226, CMS 227, IS 8577, SC 283, SC 566-14, SC 112, SC 549, SC 175, V20 | Caniato et al. 2007; Magalhaes et al. 2004 |
| **Wheat** 1BL.1RS, 1AL.1RS, AMC15, AMC19, Atlas 66, BH 1146, Carrazinho, Chinese Spring, CROAT1A 7, Currawong, DES 1032, Diamondbird, Dollarbird, Drysdale, ET 3, ET 8, Embrapa, Frontana, Goldmark, Halberd, Hartog, Kitakami B, LEON 15, Maringa, OK91G 103, OK91G 104, OK91G 105, OK91G 106, Inia 66, Thorne, Seneca, ONW 21A, ONW 25A, ONW 26A, ONW 32A, ONW 40A, ONW 65A, ONW 67A, ONW 77A, ONW 90A, ONW 91A, ONW 92A, ONW 93A, ONW 95A, ONW 96A, ONW 98A, ONW 99A, ONW106A, ONW 108A, OW146, Polyssu, VARNA 25, TUNIS 18, SALAMANCA, SEVILLE1, Tasman, Trintecino, WW 11324, 88 accessions with moderate resistance to Al toxicity | Stodart et al. 2007; Zhou et al. 2007a,b; Ping et al. 2006; Raman et al. 2005, 2006; Hossain et al. 2005; Kochian et al. 2005; Riede and Anderson 1996; McKendry et al. 1996; Carver et al. 1993; Foy et al. 1965 |
| *Aegilops uniaristata* (2n = 2x = 14, NN genome) and chromosome 3n addition lines of *A. uniaristata* into wheat | Miller et al. 1997 |

From the published reports, it appears that the landraces and/or traditional cultivars provided a rich source of diversity for tolerance to these stresses in maize, rice, wheat, barley, pearl millet, and sorghum. *Landrace* is defined as an autochthonous (primitive) cultivar with a high capacity to tolerate biotic and abiotic stresses, resulting in high yield stability and an intermediate yield level in a low-input agricultural system (Zeven 1998). For enhancing the use of plant genetic resources in crop breeding, researchers have developed core collections in barley, maize, pearl millet, rice, sorghum, and wheat (reviewed in Dwivedi et al. 2007), thus providing crop breeding programs with a systematic yet manageable entry point into global germplasm resources. Trait-based core collection for drought tolerance, consisting of 86 accessions (selected after evaluating more than 2,000 rice accessions under rainout shelter with a powerful water management system [www.plantstress.com/methods/RAINOUT/Rainouts.htm]) has been reported for diverse uses in rice breeding and genomics (Liu et al. 2006). More recently, the Generation Challenge Programme (GCP) provided support for the development of composite collections (3,000 accessions, or no more than 10% of the total number of available accessions for inbreeding crops and 1,500 accessions for outbreeding species where each accession must be treated as a population) to cover global diversity for most of the CGIAR-mandated crops including cereals, that have been molecularly profiled using polymerase chain reaction (PCR)–based markers to study population structure and form a genotype-based reference set (about 200–300 accessions) (www.generationcp.org) for diverse uses in breeding and genomics studies.

Malysheva-Otto et al. (2006) studied the molecular diversity in 953 cultivated barley germplasm (originating from various geographic areas worldwide, end use qualities, and form of spike) using 48 simple sequence repeat (SSR) loci (distributed over the whole barley genome) and detected rich allelic diversity (799 alleles) with most of the molecular diversity associated with geographic origin or agronomic traits. The findings thus provide opportunities to identify germplasm with beneficial traits for use in barley genetic improvement. Analyzing 3,942 accessions of the bread wheat originating from 73 countries with 38 SSR loci, Balfourier et al. (2007) detected 908 alleles and together with passport data selected a subset of 372 accessions capturing most of the geographic regions and 98% of the allelic diversity. There are large numbers of landraces in rice, most of them untapped for rice breeding. To better understand the allelic diversity, Thomson et al. (2007) studied the genetic diversity in 330 rice accessions, including 246 Indonesian landraces, using 30 fluorescently leveled SSR markers. They detected

394 alleles and characterized the landraces as 68% *indica* and 32% *japonica*, with an *indica* gene diversity of 0.53 and a *japonica* gene diversity of 0.56, and an $F_{st}$ (fixation index, a measure of population differentiation based on genetic polymorphism) of 0.38 between the two groups. This turns out to be a valuable resource for improving Indonesian rice. In an another example of the use of marker-based approach to broadening the genetic base of rice in the United States, Xu et al. (2004a) analyzed the genetic structure of 236 rice accessions using 113 restriction fragment length polymorphism (RFLP) and 60 SSR loci. They detected 274 RFLP and 714 SSR alleles and identified a subset of 31 rice accessions embodying 95% RFLP and 74% of SSR alleles offering a valuable resource for future crop improvement. Using 206 maize inbred lines (representing temperate, tropical, and subtropical groups) and 94 SSR loci, Liu et al. (2003) detected 2309 alleles that grouped the inbred lines into five clusters corresponding to major breeding groups and a set of lines showing evidence of mixed origins. Further, comparison of diversity revealed that maize inbred lines capture about 80% of the alleles in the landraces and identified a core subset of inbreds that capture maximum allelic richness that can be used for a variety of genetic applications in maize. The sorghum reference set of 384 accessions, selected from a composite collection (3,367 accessions and 41 SSR loci data) captured 78% (615 of the 789 alleles) allelic diversity of the collection (www.generationcp.org).

Wild relatives are the potential reservoirs of novel genes and alleles to improve abiotic stress tolerance. Limited screening of the wild relatives identified a number of accessions better adapted to drought and salinity in barley and wheat (Tables 2.1 and 2.2) and to Al toxicity in wheat (Table 2.3). In 110 wild emmer wheat accessions studied under two moisture regimes (well-watered and water-limited conditions), Peleg et al. (2005) detected wide genetic diversity between and within the wild emmer populations and greater productivity (spike and total dry matter) over controls under water-limited conditions. Most wild emmer wheat accessions exhibited a greater carbon isotope discrimination ($\delta^{13}C$) and higher plasticity of $\delta^{13}C$ relative to controls, and populations originating from hot dry locations showing outstanding drought-tolerance capacity. Nevo (2001) also reported wild emmer wheat germplasm as useful genetic resources to improve drought tolerance in cultivated wheat.

## A. Drought Tolerance

Breeding for drought tolerance is extremely challenging due to the complexity associated with various stress-adaptive mechanisms, uncertainty in onset of stress, and large genotype × environment interaction.

Traditional approaches to improve drought tolerance in field crops have so far met with limited success. A number of factors contributed to low genetic gain in drought-tolerance breeding: the focus on yield, lack of simple physiological traits as a measure of tolerance, and complex inheritance. Understanding the physiological basis of crop performance under drought conditions will contribute to the identification and manipulation of traits associated with improved water use efficiency and yield under rainfed conditions. The most effective drought-adaptive mechanism is drought avoidance through phenological escape, such as early maturity to escape terminal drought (Reynolds et al. 2005; Slafer et al. 2005). Using conventional crossing and selection, breeding for early maturity has been successful in many cereal crops with potential to escape for terminal drought stress. A review of breeding progress on drought tolerance revealed that selection for high yield in stress-free conditions has, to certain extent, indirectly improved yield under different water-limiting conditions. Further progress will require the introduction of traits that reduce the gap between yield potential and actual yield in drought-prone environments. Plant physiology has provided new insights and new tools for understanding the complex network of drought-related traits (Tuberosa and Salvi 2006; de Dorlot et al. 2007). Many QTL affecting yield under drought or the expression of drought tolerance-related traits have been discovered, and these QTL need further dissection to identify candidate genes to improve abiotic stress tolerance through genetic engineering. Plant breeders should integrate both physiological and genetic information by accumulating the most effective QTL and/or transgenes to improve important physiological attributes into new elite lines without incurring negative effects on yield potential. This strategy is likely to lead to new cultivars with high yield potential and stability that in turn will result in superior performance in dry environments (Cattivelli et al. 2008).

**1. Rice.** Selection for high relative water content, panicle length, grains per panicle, harvest index, biomass yield, root/shoot ratio, root length, and low scores for leaf rolling, leaf drying, and drought recovery rate has enabled researchers to improve drought tolerance and yield in rice (Manickavelu et al. 2006). Donor lines with high drought tolerance, identified through use of drought response index, are utilized in breeding better-adapted cultivars for the rainfed lowlands (Ouk et al. 2006). Lafitte et al. (2006) initiated large-scale backcross breeding by including over 160 donor cultivars from 25 countries to improve drought tolerance. By evaluating these lines under drought conditions, they identified several lines with significant variation in response to drought that they

crossed to one to three recurrent parents and subsequently evaluated $BC_2F_2$ (second backcross generation after F2) bulk populations under drought in lowland or upland nurseries. While severe stress eliminated almost all seed set in recurrent parents, many putative drought-tolerant lines produced grains under such stress conditions, thus representing a useful resource to develop improved cultivars in rainfed or water-scarce rice-growing regions. More recently, direct selection for grain yield under reproductive-stage drought stress showed 25% to 34% yield advantage over random lines, when evaluated at stress levels similar to those in which they were selected, and it was noted that the choice of donor is of particular importance in breeding for drought tolerance (Venuprasad et al. 2007). Furthermore, Kumar et al. (2008) also reported that selection under severe drought stress (under both lowland and upland conditions) at the reproductive stage resulted in greater gains under similar stress levels (yield reduction of 65% or greater under stress) than did selection under non-stress conditions with no yield reduction under nonstress conditions (Kumar et al. 2008). The major QTL with large effects (32–33% variation) for grain yield under drought conditions in upland and lowland rice have recently been reported (Bernier et al. 2007; Kumar et al. 2007).

The ability of root systems to provide for evapotranspirational demand from deep soil moisture and capacity for osmotic adjustment (OA) are now considered major drought-tolerance traits in rice. Azucena, a *japonica* cultivar, is a good source of drought tolerance because of its thick and deep root system. Four QTL on chromosomes 2, 7, 9, and 11 contribute between 5% to 30% phenotypic variance for these traits. Using marker-assisted backcross, Steele et al. (2006) successfully transferred QTL on chromosome 9 in near-isogenic lines (NIL) in Kalinga III genetic background that consequently showed significantly increased root length under both irrigated and drought-stress environments. Shen et al. (2001) also showed the effectiveness of QTL-based transfer of root traits from Azucena to NIL in IR64 genetic background. Several shoot-related traits (rapid leaf rolling, rapid stomatal closure, high water use efficiency, thick epicuticular wax, osmotic adjustment, dehydration tolerance, membrane stability, and photoinhibition resistance) also confer drought tolerance (Price et al. 2002c and references therein).

NERICA, new rice for Africa, was developed from a cross between African rice (*Oryza glaberrima* Steud.) and Asian rice (*O. sativa* L.), combining the local-stress adaptation of African rice with high yield potential of the Asian rice, revolutionizing rice farming in sub-Saharan Africa. The characteristics of NERICA are profuse early vegetative growth giving rapid ground cover and upright growth at reproductive stage, enabling plants to support heavy seed heads from maturity to

harvest. In addition, NERICA matures early (90–100 days), is tolerant to drought, and resistant to pest and diseases. High growth and grain yield of NERICA under drought conditions is associated with high stomata conductance and low water uptake (Fujii et al. 2004).

**2. Wheat.** Early flowering has the potential to avoid terminal drought. The relative contribution of stem reserves and ear photosynthesis to grain yield varies depend on genotypes and environments, and reduction in photosynthetic assimilation under postanthesis moisture stress induces greater stem reserve mobilization and utilization by the grains. Thus, a significant proportion of reserves usually are retranslocated to grains under drought to buffer effects of accelerated leaf senescence (Bidinger et al. 1977; Schnyder, 1993; Blum et al. 1994; Blum 1985; Yang et al. 2000). Foulkes et al. (2007) studied the association of specific target traits (early flowering, high accumulation of stem water-soluble carbohydrate [WSC] reserves, presence of awns, and high green flag-leaf area persistence) under late-season drought to quantify associations between target traits and yield response to drought to prioritize traits. Their study revealed that flowering time variation had neutral effects on absolute yield loss under drought, suggesting that there may be a trade-off between water-saving behavior in the shorter preflowering period with early flowering and a reduced capacity to access water associated with a smaller rooting system. The potential advantage of awns for increased water use efficiency (WUE) and sensible heat transfer responsible for a cooler canopy is of less significance under moderate drought than under severe drought. Stem WSC is positively associated with grain yield under both irrigated and drought conditions while green flag-leaf area persistence is clearly associated with the ability to maintain yield under drought, demonstrating its potential usefulness as a selection criterion for yield under drought. A combination of flowering time, stem WSC, and green flag-leaf persistence may therefore be useful as indirect criteria to select for drought tolerance. More recently, Zhang et al. (2008) mapped three 1-fructan exohydrolases genes (*1-FEH*) to Chinese Spring chromosome 6A, 6B, and 6D that corresponded to published *1-FEH w1 (6A)*, *1-FEH w2 (6D)*, and *1-FEH w3 (6B)* cDNA sequences. The overall correlation of the mRNA accumulation profile for the *1-FEH* genes in stem and sheath leaf tissue in relation to the profile of soluble carbohydrate accumulation was consistent with their postulated role in stem soluble carbohydrate accumulation. The accumulation of the *1-FEH-6B (1-FEH w3)* mRNA was 300-fold greater than that of *1-FEH-6A* and *1FEH-6D*. Thus, *1-FEH-6B* can serve as new class of molecular marker to select for stem carbohydrates and grain filling in wheat breeding.

High WUE or transpiration efficiency (TE) in wheat is a desirable physiological trait for increasing grain yield under water-limited environment. Stable carbon isotope composition ($\delta^{13}C$) of dry matter has been widely investigated as a selection tool in cereal breeding programs. However, reports on the possibilities of using stable oxygen isotope composition ($\delta^{18}O$) as yield predictor are very scarce and only cover stresses other than water stress. Ferrio et al. (2007) studied the relationships of grain $\delta^{13}C$ and $\delta^{18}O$ with wheat phenology and yield under water-limited conditions. Both $\delta^{13}C$ and $\delta^{18}O$ showed higher broad-sense heritability than grain yield. Grain yield was negatively correlated with $\delta^{13}C$ but not with $\delta^{18}O$. Both isotopes were correlated with grain filling duration, whereas $\delta^{18}O$ was also affected by crop duration from planting to anthesis. $\delta^{13}C$ in grains is therefore a more robust tool as its relationship with grain yield is independent of phenology under drought conditions while $\delta^{18}O$ under such conditions is not independent of phenology. Selection for low $\delta^{13}C$ in the drier environments showed increased grain yield, greater biomass, harvest index, and grain weight in wheat, which resulted the release of the drought-tolerant wheat cultivars Drysdale and Rees in Australia, with no yield penalty in absence of soil water deficit (Rebetzke et al. 2002; Richards et al. 2002; Condon et al. 2004). Molnár et al. (2007) used wheat-barley 4H(4D) disomic substitution lines to study the ability of barley 4H chromosome to compensate for wheat 4D in response to mild drought stress. Their study revealed that genes localized on the 4H chromosome of barley are able to increase the WUE of the wheat substitution line and are thus suitable for improving wheat through intergeneric crosses. More recently, Tambussi et al. (2007) reported that ears of barley and durum wheat have a higher $WUE_{instantaneous}$ than flag leaf, both under well-watered and drought conditions, probably because of higher refixation capacity (i.e., the capacity to reassimilate respired carbon dioxide [$CO_2$]). At the crop level, decreasing soil evaporation is a crucial factor to improve $WUE_{yield}$ in Mediterranean conditions for which the early vigor (fast initial growth of the crop) seems to be the relevant trait. Modern wheat cultivars with gibberellic acid-insensitive dwarfing genes have higher yields but, concomitantly, have lower initial growth performance. However, some newly developed giberellic acid-sensitive semidwarf cultivars with high grain yield and high early vigor have been bred, thus opening new avenues to increase $WUE_{yield}$ in wheat.

Wheat productivity under drought conditions is strongly related to photosynthetic activity and TE. TE can be evaluated at the leaf level as the ratio of leaf $CO_2$ exchange rate (CER) to stomatal conductance ($g_s$) (Xue et al. 2002). Photosynthesis-related traits at anthesis and carbon

isotope discrimination ($\Delta$) in flag leaf at anthesis ($\Delta L_a$) and in grains at maturity ($\Delta G_m$) under rainfed and irrigated conditions in five durum wheat cultivars grown under Mediterranean environmental conditions show highly significant correlation between both $\Delta L_a$ and $\Delta G_m$ under rainfed conditions, leaf CER, $g_s$, and internal to ambient $CO_2$ concentration ratios (Monneveux et al. 2006b). $\Delta G_m$ was correlated negatively with TE under rainfed conditions, and positively under irrigated conditions. At both conditions, $\Delta G_m$ was positively associated with grain yield, while $\Delta L_a$ and grain yield were not related. Breeding for high $\Delta G_m$ would therefore lead, under Mediterranean drought conditions, to higher stomatal conductance, lower TE, and higher grain yield.

Senescence is an important stage of plant development, characterized by the loss of the green pigments regulated by specific genes (Rampino et al. 2006 and references therein). Elucidation of the molecular basis of stay-green is not only relevant to understanding the senescence phenomenon but also has potential significance for yield improvement. Rampino et al. (2006) characterized a mutant of durum wheat cultivar Trinakria as a delayed leaf senescence type, and analysis of photosynthetic parameters showed that this mutant is functionally stay-green. The differentially expressed cDNAs in the mutant showed homology with photosynthesis-related genes. Further analysis using reverse transcriptase-polymerase chain reaction (RT-PCR) revealed altered expression profiles of these genes in the stay-green mutant. This mutant therefore has potential to increase yields by extending the period of active photosynthesis during grain filling, especially in water- and nitrogen-limited environments where postanthesis photoassimilation is a likely limit to yield.

The International Maize and Wheat Improvement Center (CIMMYT) is using resynthesized wheat derivatives (obtained from interspecific hybridization of the ancestral genomes) and landraces originating in abiotic stress environments to increase the allelic diversity for drought tolerance in wheat. Reynolds et al. (2007) recently reported that some of the drought-adapted landraces were not only distant from the control cultivars but also showed significant allelic diversity among themselves. Further, they demonstrated that improved performance of resynthesized wheat-derived lines compared with their recurrent parents was not associated with a larger overall increase in root dry weight but with increased partitioning of root mass to deeper soil profiles (between 60 cm and 120 cm) and increased ability to extract moisture. Under drought conditions, the best Mexican landraces showed superior ability to extract water from soil depth as well as increased concentration of soluble carbohydrates in stem shortly after anthesis. CIMMYT is making strategic

crosses based on theoretical combination of useful stress-adaptive traits (high biomass under drought combined with favorable expression of physiological traits such as stem carbohydrates, water extraction characteristics, and transpiration efficiency) with a view to realizing additive gene action in selected progeny. It is yet to be seen whether such strategic crosses based on theoretical combination of useful stress-adaptive traits have any merit to exploit additive gene action with desired combinations of traits into progenies since compensatory or adversary mechanisms may be in operation to offset the gains.

**3. Maize.** In maize too, drought at flowering reduces grain yield substantially. Drought causes a delay in silking, an increase in anthesis silking interval (ASI) and therefore a decrease in grain yield. ASI is an important secondary trait with relatively high heritability and more stability than grain yield. Zaidi et al. (2004) evaluated the performance of hybrid progenies of drought-tolerant populations in stressed (drought and low-N) and unstressed environments and concluded that recurrent selection for midseason drought tolerance in maize resulted in morpho-physiological changes (reduced ASI, increased ears per plant, delayed senescence, and relatively high leaf chlorophyll content during the late grain filling) that proved advantageous under both drought and low-N stress, without incurring significant yield penalties. Selection for tolerance to midseason drought stress consistently reduced ASI and increased ears per plant under both drought and low-N stress, and these constitutive changes facilitated not only synchronization but also decreased ears and kernel abortion under stress conditions. Using recurrent selection, Monneveux et al. (2006a) demonstrated significant yield gains as a means of improving tropical maize source populations under water deficit conditions associated with a significant increase in numbers of ears per plant and grains per ear concomitant with significant reductions in ASI, ovule number, and abortion rate during grain filling. The mechanism underlying these changes also caused improved partitioning of assimilates to the ear at flowering at the expense of tassel and stem growth.

Osmotic adjustment (OA) contributes effectively to yield maintenance under drought conditions. Chimenti et al. (2006) demonstrated that OA contributes to drought tolerance in maize crops exposed to water deficit both before and during flowering and that the trait carries no yield penalty under irrigation. High-OA maize population extracted significantly more water from deeper in the soil, exhibited higher leaf area and harvest index and higher grain yields, in drought-stressed crop at flowering than the low-OA population.

In 1997, CIMMYT initiated a product-oriented breeding program targeted at improving maize for drought-prone mid altitudes of southern Africa. When compared a number of CIMMYT-bred hybrids with released and prereleased hybrids produced by private seed companies in 36 to 65 trials across eastern and southern Africa, the hybrids from CIMMYT's stress breeding program showed a consistent advantage over private company hybrids at all yield levels (average yield ranging from less than $1\,t\,ha^{-1}$ to above $10\,t\,ha^{-1}$), with largest selection differentials between 2 and $5\,t\,ha^{-1}$ yield levels. The 40% yield advantages at the 1 t yield level were decreased to 2.5% at the 10 t yield level. It appears that selection under high-priority abiotic stress conditions, including drought, by a breeding program with adequate weighing can significantly increase maize yields in a highly variable drought-prone environment and particularly at lower yield levels (Bänziger et al. 2006).

A number of QTL associated with drought tolerance traits have been reported in maize (Section III.A, Table 2.4). The introgression of favorable alleles at five target regions controlling yield components and flowering traits increased grain yield and reduced the asynchrony between male and female flowering under water-limited conditions. More recently, Ribaut and Ragot (2007) recovered 85% of the recurrent parent genome at nontarget loci by using marker-assisted backcross (MABC) selection in four generations by screening large segregating populations for three of the four generations. They crossed selected MABC-derived $BC_2F_3$ families with two testers that they evaluated under different water regimes. Mean grain yield of MABC-derived hybrids was consistently higher than that of control hybrids under severe water conditions. The best five MABC-derived hybrids under those conditions yielded, on average, at least 50% more than control hybrids. However, under mild moisture stress, defined as resulting in <50% yield reduction, no difference was observed between MABC-derived hybrids and the control plants, thus confirming that genetic and transcriptional responses to drought are dependent on stress intensity.

More recently, Monneveux et al. (2007) used carbon isotope discrimination ($\Delta$) as a selection criterion for yield under drought and well-watered conditions in different organs of maize inbred lines differing for drought tolerance. Drought stress significantly increased $\Delta$ in all organs and was higher in leafs than ears and silk. Under drought, tolerant inbred lines showed significantly higher $\Delta$ than susceptible ones. There was a significant positive correlation among leaf, ear, silk $\Delta$, and ear dry weight at flowering, a trait closely associated to grain yield. Further, drought-tolerant hybrids have significantly higher grain yield and $\Delta$ than the

**Table 2.4.** Quantitative trait loci (QTL)/markers associated with drought tolerance in barley, maize, pearl millet, rice, sorghum, and wheat from 1996 to 2008.

| Summary of QTL/marker information | Reference |
|---|---|
| Barley | |
| 2 major loci, located on chromosome 2H, involved in regulation of fluorescence parameter (maximum quantum efficiency of PSII, $F_v/F_m$) contribute 9% to 15% variation under drought stress conditions | Guo et al. 2008 |
| 4 of the 12 drought tolerance QTL of consensus map associated with regulatory candidate genes, encoding transcription factors for abiotic stresses, on chromosome 2H, 5H, and 7H, and 2 QTL with effector genes on chromosome 5H and 6H | Tondelli et al. 2006 |
| 2 candidate genes and 10 differentially expressed sequences linked with QTL for 6 drought-tolerance traits | Diab et al. 2004 |
| 8 of the 10 QTL for grain $\delta^{13}C$ colocated with QTL for physiological traits related to plant water status and/or osmotic adjustment, and/or agronomic traits | Teulat et al. 2002 |
| 3 QTL for relative water content, 1 to 4 QTL for osmotic adjustment related traits mapped on chromosome 1 (7H) and 6 (6H) | Teulat et al. 1998 |
| Maize | |
| Colocalization of 4 QTL for ASI and three QTL for ears per plant under low N and water-limited conditions | Ribaut et al. 2007 |
| Leaf growth and ASI the main determinants of source and sink strengths; 3 of the 5 QTL for leaf growth (maximum elongation rate per unit thermal time) colocalized with QTL for ASI; the alleles conferring high leaf elongation rate conferred a low ASI (high silk elongation rate) | Welcker et al. 2007 |
| Under differing moisture stress regimes, main effect QTL for grain yield on chromosomes 3 and 8 and QEI effect on 1 and 10; main effect QTL for ASI on 1, 2, 6, 8, and 10 and QEI effect on 8, with contribution varying from 36% to 64%; min. and max. temperature during flowering explained 78% and 24% variation, respectively | Vargas et al. 2006 |
| 18 QTL on well-watered and 20 QTL on water-stressed conditions detected. Few QTL for grain yield, kernel weight, and ear weight under irrigated; grain yield and cob weight under water-stressed; and ear weight, kernel weight, and ear number under both regimes overlapped either on chromosome 1.03 near bnlg176 or on chromosome 9.05 and 9.07 near umc1657 and bnlg1525 | Xiao et al. 2005 |
| 181 QTL associated with 9 morphophysiological traits in 10 mapping populations under drought stress conditions; 15 universal drought tolerance QTL identified | Li et al. 2005a |
| A major QTL for leaf-abscisic acid concentration (L-ABA), reported on chromosome 2 (bin 2.04) [Tuberosa et al. 1998], validated on bin 2.04 near csu133 that affects root traits and relative water content | Landi et al. 2005 |

(continued)

Table 2.4. *(Continued)*

| Summary of QTL/marker information | Reference |
| --- | --- |
| Near-isogenic hybrids, by crossing BDLs differing for the parental alleles at a major QTL for L-ABA, confirmed QTL effect on L-ABA (reduced stomatal conductance) and root lodging through a constitutive effect on root architecture, probably due to pleiotropic effects | Giuliani et al. 2005 |
| Several chromosome regions affecting root traits and grain yield under well-watered and/or drought-stressed conditions in four populations, with most important QTL effects on chromosome bins 1.03, 1.06, 1.08, 2.03, 2.04, 7.02, 8.06, and 10.04 | Tuberosa et al. 2003 |
| 7–9 QTL for grain yield (GY) and drought tolerance index (DTI) under well-watered and water-stressed field conditions; QTL for the seminal root weight overlapped with QTL for GY and DTI under both the water regimes | Tuberosa et al. 2002 |
| Several QTL associated with drought tolerance for grain yield components; with more than 50% QTL common in well-watered and water-stressed conditions; also several QTL common to 2 or more traits | Frova et al. 1999 |
| 13 genomic regions associated with 1 or more measures of postflowering drought tolerance; 2 QTL with major effects on yield and stay-green under postflowering drought had pleiotropic effects on yield under nonstress conditions | Sari-Gorla et al. 1999 |
| 16 of the 17 QTL regions influencing L-ABA also harbored QTL for 1 or more of these traits: stomatal conductance, a drought sensitivity index, leaf temperature, leaf relative water content, ASI, and grain yield | Sanguineti et al. 1999 |
| 4 of the 16 QTL significantly influence L-ABA, accounting for 66% phenotypic variation, with 2 QTL mapped on chromosome 2 near csu133 and csu109α | Tuberosa et al. 1998 |
| Reduced ASI correlated with improved yield under drought stress; 6 QTL for ASI mapped on 6 chromosomes explaining 47% variation; ASI alleles from resistant line contributed 7-day reduction in ASI that were stable over years/stress levels | Ribaut et al. 1996 |
| **Pearl millet** | |
| 3 QTL on LG2, LG3, and LG4 for improved grain yield across variable postflowering moisture stress environments; QTL on LG2 and LG3 explained 13% to 25% variation and comapped with QTL for HI across environment and with QTL for both grain number and individual grain mass under severe terminal drought stress | Bidinger et al. 2007 |
| A significant genotype × drought interaction for grain and stover yield; some genomic regions more affected while others detected across drought stress environments and tester background; HI and PNHI comapped with grain yield, thus possible to select for increased drought tolerance and yield | Yadav et al. 2004 |
| Major G × S QTL for flowering time (LG 4 and LG 6) mapped to the same intervals as G × S QTL for stover yield, HI, biomass yield, and panicle number; the 3 QTL for grain yield unaffected by G × S interaction; QTL × E (S) had no effect on stover yield QTL (LG 2, LG 4, and LG 6) and a QTL for grain yield (LG 4) | Yadav et al. 2003a |
| A QTL associated with grain yield per se and for the drought tolerance mapped on LG 2 explained up to 23% variation; with some common QTL across stress environments; increased drought tolerance either through variation; with some common QTL across stress environments; increased drought tolerance either through better than average maintenance of HI or HI and biomass productivity | Yadav et al. 2002 |
| **Rice** | |
| *qtl12.1* with a large effect on grain yield under drought stress (172 kg ha⁻¹ per year; representing 47% of the average yield under stress and explaining 51% variation), mapped between RM28048 and RM511 on chromosome 12, under severe drought stress in the field but this QTL had no effect on any trait under nonstress conditions | Bernier et al. 2007 |
| *dis4.1, Ir8.1, Ir10.1,* and *Ir12.1* on chromosomes 4, 8, 10, and 12, respectively, linked with drought tolerance while 5 QTL RM136 *(y6.1,* chromosome 6); RM537 *(y4.1,* chromosome 4), RM5443 *(y1.1,* chromosome 1), RM3231 *(y8.1,* chromosome 8), and RM3 *(y6.2,* chromosome 6) associated with yield | Lin et al. 2007 |
| 2 candidate genes, *EXP15* and *EXP13,* associated with root number and silicon content in the stem, respectively, under different moisture regimes | Vinod et al. 2006 |
| 27 QTL for 7 fitness- and yield-related traits; 36 QTL for 5 root traits under control and 38 QTL for 7 root traits under drought stress conditions, only a small portion of QTL for fitness-and yield-related traits overlapped with QTL for root traits | Yue et al. 2006 |
| 38 regions associated with both drought tolerance and yield traits, and 18 of these closely linked with DNA markers | Manickavelu et al. 2006 |
| 13 main effect QTL associated with chlorophyll content on chromosomes 1, 2, 3, 4, 5, 6, and 10, of which 6 under drought stress explaining 47% variation, the remaining 7 QTL under irrigated conditions contributing 56% phenotypic variation | Hu et al. 2006 |
| 32 QTL for grain yield and its components under well-watered and drought stress conditions, with several main effect QTL affecting spikelet fertility, 1,000-grain weight, and panicle and spikelet number consistently across 2 years under both water levels and mapped to the same regions on chromosome 4 and 8 | Zou et al. 2005 |

*(continued)*

Table 2.4. (Continued)

| Summary of QTL/marker information | Reference |
|---|---|
| 36 QTL affecting flowering, plant height, grain yield, and related traits under irrigated and water-stressed conditions. Of these, 12 expressed under both stress and nonstress conditions; 17 and 7 under irrigated and water-stressed conditions, respectively | Xu et al. 2005 |
| QTL for drought response index, spikelet fertility, plant water status, and root traits, explaining 5% to 32% variation; with 2 QTL for plant water status detected in 2 environments; colocation of QTL for canopy temperature and flowering; correlation and QTL congruence between root traits and putative drought tolerance traits | Yue et al. 2005 |
| 7 QTL and 15 epistatic QTL for basal root thickness (BRT) and root numbers (RN); 12 QTL and 17 epistatic QTL for BRT, RN, maximum root length (MRL), root fresh weight (RFW), ratio of root fresh weight to shoot fresh weight (RFW/SFW), and for root dry weight (RDW)/shoot dry weight (SDW); 4 QTL and 1 epistatic QTL for index of drought resistance (IDR). 5 colocalized QTL controlling RFW, RDW, RFW/SFW, RDW/SDW, IDR, BRT, RN, MRL, and IDR; few common QTL across different genetic background | Li et al. 2005 |
| 77 QTL for grain yield and its components under varying water stress, with 7% to 56% phenotypic variance. The aggregate effects of QTL on chromosomes 3, 4, and 8 associated with grain yield traits resulted in higher grain yield under drought stress | Lanceras et al. 2004 |
| 46 main effect QTL for 6 stay-green traits localized to 25 chromosomal regions, and 56 digenic interactions involving 66 loci distributed on all 12 chromosomes | Jiang et al. 2004 |
| 42 QTL for osmotic adjustment (OA) and root traits; mapped 11 drought-inducible genes, of which 7 novel cDNA clones to the vicinity of OA, root thickness, total root dry weight, and root pulling force | Nguyen et al. 2004 |
| 47 QTL associated with drought tolerance, 5% to 59% variation; chromosome 4 harbored major QTL for plant height, grain yield, and grains/panicle; root trait QTL on chromosome 4 had pleiotropic effects on yield traits under drought | Babu et al. 2003 |
| 14 QTL for osmotic adjustment on chromosomes 1, 2, 3, 4, 5, 7, 8, and 10, together explained 58% variation; some QTL on same position as in previous studies | Robin et al. 2003 |
| 18 QTL for seminal root length (SRL), adventitious root numbers (ARN), lateral root length (LRL), and lateral root number (LRN) on the seminal root under flooding and upland conditions; several identical QTL for root elongation across 3 populations; 2 candidate genes for cell expansions (OsEXP2 and endo-1,4-β-D-glucanase EGase) and 4 cDNA-AFLP clones mapped on the intervals carrying the QTL for SRL and LRL under upland conditions | Zheng et al. 2003 |
| 24 genomic regions containing several QTL for root traits, 7 QTL with potential value in breeding for root traits | Price et al. 2002b |
| 17 QTL for leafrolling, leaf drying, and leaf relative water content (RWC), QTL for RWC mapped on chromosome 8 and congruent with an osmotic adjustment QTL | Price et al. 2002c |
| 2 QTL for shoot biomass, 12 for deep root morphology, and 8 for root thickness, contributing 6% to 30% variation; 14 and 9 QTL overlapped across different populations for deep root morphology and root thickness | Kamoshita et al. 2002 |
| 41 QTL for OA and root traits, explaining 8–38% variation; chromosome 4 harbored major QTL for root traits; consistent QTL for drought responses across genetic backgrounds; comparative mapping identified 3 genomic regions associated with physiological responses to drought in several grass species | Zhang et al. 2001 |
| 4 QTL each for root-penetration ability and root thickness, 2 QTL each for number of penetrated root and for total root number; 8% to 16% variation; a QTL between RG104 and RG348 influences both root-penetration ability and root thickness | Zheng et al. 2000 |
| 28 QTL for 5 root traits on chromosomes 1, 2, 3, 4, 6, 7, 10, and 11, with individual QTL accounting 6% to 27% variation; many common QTL consistent across genetic backgrounds and environments | Ali et al. 2000 |
| 7 QTL each for number of roots penetrating the wax layer and for the ratio of penetrated to the total number of roots | Price et al. 2000 |
| **Sorghum** | |
| Physiological analysis of NILs containing stay-green QTL, completely or partially spanning Stg1, Stg2, Stg3, or Stg4 from the donor parent (BT × 642), revealed that BT × 642 alleles in each of these loci contribute to the stay-green phenotype; with Stg2 alleles retaining more green leaf area at maturity under terminal drought | Harris et al. 2007 |
| 9 QTL for stay-green on 7 LGs across environments; 3 QTL on LGs A, G, and J corresponded to stay-green QTL in maize and congruent with other drought-related traits in maize and rice; 4 QTL for preflowering drought tolerance | Kebede et al. 2001 |
| 4 stay-green QTL (Stg1 and Stg2 on A, Stg2 on D, and Stg4 on J), Stg1 and Stg2 13% to 30% variation under postflowering drought stress across locations; 3 QTL for chlorophyll content (Chl1, Chl2, and Chl3), 25% to 30% variation, coincided with Stg1, Stg2, and Stg3 and together contributed 46% variation | Xu et al. 2000 |
| Consistency of 4 stay-green QTL across different environments and different genetic backgrounds | Subudhi et al. 2000 |
| 13 QTL associated with 1 or more measures of postflowering drought tolerance, with 2 major QTL showing pleiotropic effects on yield under irrigated conditions | Tuinstra et al. 1997 |

*(continued)*

Table 2.4. *(Continued)*

| Summary of QTL/marker information | Reference |
|---|---|
| **Wheat** | |
| 6 QTL for 1,000-grain weight, 1 for grain yield, 3 for peduncle length, and 5 for grains per panicle under drought stress, with QTL effects ranging from 13% to 34%; several QTL for drought tolerance indicators with effects ranging from 13% to 36% | Dashti et al. 2007 |
| Genes on homologous group 2 chromosomes associated with stay-green variation under drought; a stable QTL on chromosome 6A consistent in different populations, seasons, and environments (drought and irrigated) | Snape et al. 2007 |
| A grain yield QTL on chromosome 4AL associated with improved performance under reduced moisture; several QTL on chromosome 4A associated with yield and yield components 15% to 41% variation; Xwmc89 linked with all QTL (7.7 cM) | Kirigwi et al. 2007 |
| Xgwm617a and Xgwm427b on long arm of chromosome 6A associated with number of roots penetrating the PV disc, root penetration index; a QTL for root dry weight linked to Xgwn11 on chromosome 1B | Kubo et al. 2007 |
| 15 chromosome regions significantly associated with variability in root architecture, major effects on chromosome 2AL (at Xgwm294), 7AL (at Xcfa2257 and Xgwn332), and 7BL (at Xgwm577 and Xcfa2040) | Sanguineti et al. 2007 |
| Several drought-tolerance QTL on chromosomes 7A, 4D, 6D, and 2D in Sheynne background | Farshadfar and Mohammadi 2005 |
| Multiple genetic loci for the leaf carbon isotope discrimination ($\delta^{13}C$) | Shahram-Moammady et al. 2005 |
| QTL for senescence on chromosomes 2B and 2D under drought-stressed and optimal environments | Verma et al. 2004 |

controls; however, no correlation between $\Delta$ and grain yield within tolerant hybrids was detected. It was concluded that use of $\Delta$ appears an accurate measure for a first screening of lines or hybrids highly contrasting for drought tolerance but not for a more advanced selection among tolerant hybrids.

**4. Barley.** Using participatory plant breeding in water-limited environments in Syria, Ceccarelli et al. (2007) developed two breeding lines with grain yield potential of about $600\,kg\,ha^{-1}$ in situations where the farmers did not consider it worth harvesting the local landrace. On average, the new lines outyielded the local landrace by 89% to 108%. Forster et al. (2004) tested a wide range of barley germplasm (cultivars, landraces, and wild relatives) and identified positive traits associated with drought tolerance in each gene pool that they crossed. When this material was evaluated under varying moisture regimes in contrasting environments, some of the progenies outperformed the best local cultivar. Several QTL associated with drought-responsive traits cluster around major genes controlling flowering time (*sgh1*), plant stature (*sdw1* and *arieGP*), and ear type (*vrs1*). These associations probably represent pleiotropic effects of the developmental genes on drought responsive traits. *Hordeum spontaneum* 41–1 when crossed with Arta contributed drought and cold tolerance in its progenies in addition to contributing positive alleles for agronomic traits (Baum et al. 2003). Barley primitive landraces and related wild gene pools show greater variation to abiotic stresses. The fingerprinting of these gene pools revealed DNA markers associated with site-of-origin eco-geographic factors as well with the experimentally imposed stresses. Genotypes and collection sites show the desired variation for particular stresses. They have been genetically mapped, and the marker/QTL association can be used to select for drought tolerance (reviewed in Forster et al. 2002). As in wheat, several QTL associated with grain $\delta^{13}C$ in barley have been reported from drought-prone adapted cultivars and many colocated with QTL for physiological traits related to plant water status, osmotic adjustment, and/or for agronomic traits (Teulat et al. 2002).

Guo et al. (2008) identified two major loci on chromosome 2H at 116 cM and 135.7 cM, contributing 9% and 15% phenotypic variation associated with chlorophyll fluorescence parameters under postflowering drought conditions in barley. This observation, which is in agreement with the results from wheat by Araus et al. (1998), suggests that chlorophyll fluorescence could be used as a selection criterion for grain yield under drought conditions.

**5. Pearl Millet.** Genotypes that flower early, possess few but effective basal tillers, are low in biomass, and have high harvest index (including panicle harvest index) perform better under terminal drought stress (Yadav et al. 2003b; Bidinger et al. 2005). Trait-specific QTL (grain yield, grain number, individual grain mass, and harvest index) associated with terminal drought tolerance have been reported in pearl millet (Table 2.4). Recently, Bidinger et al. (2007) identified three QTL (on LG 2, LG3, and LG4) as primary candidates for marker-assisted selection for improved grain yield across variable postflowering moisture stress environments. The QTL on LG2 and LG3 explained 13% to 25% of variation for grain yield across environments. These QTL comapped with QTL for harvest index across environments and with QTL for both grain number and individual grain mass under severe terminal stress. Neither QTL had a significant QTL × environment interaction. Both QTL are linked to SSR markers and are therefore amenable to efficient marker-assisted selection (MAS). The predicted response for each of the identified grain yield QTL into other genetic backgrounds by MAS ranged from 7 to $10\,g\,m^{-2}$ ($70–100\,kg\,ha^{-1}$) across environments and as much $16\,g\,m^{-2}$ in individual moisture environments.

**6. Sorghum.** Both preflowering and postflowering drought stress are reported to significantly reduce the grain yields in sorghum. Postflowering drought is associated with stay-green, and such genotypes retain greater green leaf area, higher levels of stem carbohydrates, and higher transpiration efficiency than non–stay-green genotypes (Borrell et al. 2000a,b,c; Mahalakshmi and Bidinger 2002). Several chromosome regions with major effects on stay-green phenotype have been reported in sorghum, and some of these regions are syntenic with regions of the maize genome associated with stay-green (see Section III.A). Other traits associated with drought resistance include heat tolerance, transpiration efficiency, rooting depth and patterns, and epicuticular wax (reviewed in Rooney 2004). Limited studies at the International Crops Research Institutue for the Semi-Arid Tropics (ICRISAT) revealed that stay-green genotypes had a larger proportion of roots (15% to 35%) in the deeper layers of soil (deeper than 150 cm) than senescent genotypes (5% to 10%) under drought stress conditions. Interestingly, stay-green and senescent genotypes did not vary for root length under well-watered conditions (ICRISAT 2006). Selection for stay-green trait has been the most effective approach to enhance postflowering drought tolerance in sorghum (Rooney 2004). Harris et al. (2007) constructed near-isogenic lines containing 1 to 4 stay-green QTL that showed delayed onset of leaf senescence under postanthesis water deficit

conditions. ICRISAT has also initiated marker-aided introgession of stay-green QTL into elite parental background for enhanced postflowering drought tolerance in sorghum. Some of the marker-based introgressed lines containing stay-green QTL, when evaluated for grain yield under water-stressed and well-watered conditions under vertisol at Patancheru (India), showed significantly higher grain yield under moderate water-stressed than under well-watered conditions (ICRISAT 2007).

## B. Salinity Tolerance

Salinization is the accumulation of water-soluble salts in the soil to a level that impacts on agricultural production, environmental health, and economic welfare. A soil is considered saline if the electrical conductivity of its saturation extract ($EC_e$) is above 4 dS $m^{-1}$. Salinity stress inhibits plant growth due to reduced water uptake and ion toxicity. Soil salinity can also induce nutritional imbalances in plants. Seepage salinity and transient salinity are the two major forms of the dryland salinity. The former is caused by rising water tables that mobilize salts in the soil and brings them into the root zone while the latter is associated with accumulation of natural occurring high salt loads in sodic soils (reviewed in Rengasamy 2006). Salt tolerance is a complex trait, controlled by many genes encoding specific channel proteins, structural components, and regulatory protein such as kinases. Inadequate screening techniques, low selection efficiency, and limited knowledge of the stress and environmental interactions have contributed to slow progress in breeding salinity-tolerant crops (Gregorio et al. 2002; Flowers and Flowers 2005; Ismail et al. 2007).

Barley, rice, and wheat are the most extensively studied cereal crops for understanding the physiological basis of salt tolerance. The key traits that govern salt tolerance include salt exclusion or low uptake, enhanced potassium/sodium ($K^+/Na^+$) discrimination, retention of toxic ions in structural and older tissues, tissue tolerance, responsive stomata that close faster upon exposure to salt stress, up-regulation of antioxidant systems for protection against reactive oxygen species, osmotic adjustment, enhanced vigor, water use efficiency, early flowering, and vigorous growth to dilute salt concentration in plant tissue (Colmer et al. 2005; Ismail et al. 2007).

**1. Rice.** Several sources of salt tolerance have been reported in rice (Table 2.2). Many are already used as donor parents in breeding programs. A few salt-tolerant lines have been released as cultivars for

Table 2.5. Quantitative trait loci (QTL)/markers associated with salt tolerance in barley, rice, and wheat from 1997 to 2007.

| Summary of QTL/marker information | Reference |
|---|---|
| **Barley** | |
| QTL for salt tolerance at germination on chromosomes 4(4H), 5(1H), 6(6H), 7(5H) while at seedling stage on 2(2H), 5(1H), 6(6H), and 7(5H) | Mano and Takeda 1997 |
| **Rice** | |
| 8 QTL detected in more than 1 test condition, markers explaining between 37% and 48% variation in yield | Manneh et al. 2007 |
| $qST1$ and $qST3$ confer salt tolerance, 35% to 37% variation at young seedling stage, mapped on chromosome 1 and 3, respectively | Lee et al. 2007 |
| 8 QTL for salt tolerance traits on 5 chromosomal regions, with qSNC-7 for shoot $Na^+$ and qSKC-1 for shoot $K^+$ concentration explained 40% to 48% variation | Lin et al. 2004 |
| 1 QTL for $Na^+$ uptake on chromosome 1 (9% variation); 3 QTL for $K^+$ uptake on chromosomes 4, 6, and 9 (34% variation); 2 QTL for $Na^+$ concentration on chromosomes 4 and 6; 2 QTL for $K^+$ concentration on chromosomes 1 and 4; and 2 QTL for $Na^+$ : $K^+$ ion ratio on chromosomes 1 and 4 (18.7%) | Koyama et al. 2001 |
| **Wheat** | |
| 47 QTL for salt tolerance: 10 at the germination stage while 37 at the seedling stage; QTL on chromosome 4DL effective during germination stage and QTL on chromosome 6DL to the seedling stage while QTL on chromosome 3AS and 3BL effective at both the germination and seedling stages | Ma et al. 2007 |
| Nax1, a salt tolerance locus mapped on chromosome 2AL, accounted for 38% variation for low $Na^+$ concentration in durum wheat, and markers linked to Nax1 locus also associated with low $Na^+$ progeny in a genetically unrelated population | Lindsay et al. 2004 |
| Higher $K^+/Na^+$ ratio in leaves of bread wheat correlates with its higher salt tolerance, controlled by single locus, Kna1, and completely linked with Xwg199, Xabc305, Xbcd402, Xpsr567, Xpsr375 | Dubcovsky et al. 1996 |

salt-affected areas in India, Indonesia, Philippines, Thailand, and Vietnam (Gregorio et al. 2002; Ismail et al. 2007). In a large-scale backcross breeding program using 3 recurrent parents and 203 donors representing a significant portion of the genetic diversity in the primary gene pool of rice, Ali et al. (2006) demonstrated significant progress toward developing large numbers of introgression lines with improved tolerance. Introgression of genes from a diverse source of donors into elite genetic backgrounds through backcross breeding and efficient selection under severe stress conditions is a powerful way to exploit hidden diversity for improving abiotic stress tolerance.

Several QTL associated with salt tolerance have been reported (see Section III.A; Table 2.5). A microsatellite marker, RM223 on chromosome 8, associated with salt tolerance at both vegetative and reproductive stages, predicted the performance of $F_3$ plants under salt stress with greater than 80% accuracy (Lang et al. 2001). A major QTL, *Saltol*, fine mapped (within 1.2 Mb), is currently being introgressed into several popular rice cultivars using MABC breeding (Ismail et al. 2007). Using stepwise multiple regression analysis, Manneh et al. (2007) identified superior genotypes based on their marker signatures, thus overcoming the problem of low selection efficiency encountered during phenotypic selection for yield under saline environment. A candidate gene for salt tolerance, *OsHKT8*, has been identified (Ren et al. 2005), and this might be a good source to introduce salt tolerance into other rice cultivars using a transgenic approach.

2. **Wheat and Barley.** Genetic variation in leaf $Na^+$ and $K^+/Na^+$ discrimination ratio have been reported in barley and wheat (Table 2.2). Bread wheat maintains a lower shoot $Na^+$ concentration than durum wheat. Barley uses a different mechanism to cope with salt than wheat does. Rather than excluding salt, barley absorbs, compartmentalizes and tolerates it within the plant leaves—a mechanism known as tissue tolerance. The plant uses the segregated salt to balance the pressure from the salt in the soil (Colmer et al. 2005). The potential sources of tissue tolerance have also been reported in *Triticum* species (Munns and James 2003). A novel source of $Na^+$ exclusion conferring salt tolerance has been found in durum wheat, Line 149, which has low $Na^+$ concentrations and high $K^+/Na^+$ ratios in the leaf blade similar to bread wheat (Munns et al. 2000). It contains two genes, *Nax1* and *Nax2*, that exclude salts from different part of the plant; *Nax1* is expressed in leaves while *Nax2* is expressed in the roots (Munns et al. 2003). The physiological traits associated with $Na^+$ accumulation include the rate of transfer from the root to the shoot (xylem loading)—this was much

lower in Line 149—and the capacity of the leaf sheath to extract and sequester Na$^+$ as it enters the leaf (Davenport et al. 2005).

Screening for salinity at three-leaf stage has been more likely to reveal salinity tolerance in both wheat and barley genotypes than in advanced growth stages, and the K$^+$/Na$^+$ ratio could be used as selection criteria for salinity tolerance as it is highly correlated with seed and straw yields (Tajbakhsh et al. 2006; Thalji and Shalaldeh 2007). Salinity causes not only ion toxicity and imbalance, but also low photosynthesis in plants. Stomatal conductance and chlorophyll content in leaves can be measured by a nondestructive, rapid, and easy technique using a porometer and soil-plant analyses development (SPAD) meter, respectively. El-Hendawy et al. (2007) reported genetic variation in net photosynthetic rate, stomatal conductance, and SPAD values while screening for salinity tolerance in wheat, thus demonstrating the potential of SPAD reading as a tool for large-scale screening for salt tolerance.

Large genetic variations for Na$^+$ and chlorine (Cl$^-$) exclusion have also been reported in wild relatives of wheat and barley (Table 2.2). Several wild *Hordeum* species including *H. vulgare* subsp. *spontaneum* had better Na$^+$ and Cl$^-$ exclusion and high leaf K$^+$ concentration while bread wheat progenitors [*Aegilops tauschii*, DD genome and *Triticum urartu*, AA genome] and resynthesized wheat hexaploids involving *T. urartu* showed enhanced Na$^+$ exclusion or K$^+$/Na$^+$ discrimination (Colmer et al. 2005). Interestingly, wild relatives of wheat have shown higher salt tolerance than bread wheat, with wheat-tall wheatgrass (*Agropyron elongatum*) amphiploids showing better salt tolerance and less reduction in grain yield in comparison with control cultivars under high saline conditions. *Lophopyrum elongatum* is a wild relative of wheat that provides a source of novel genes for improvement of the salt tolerance of bread wheat because of its improved Na$^+$ exclusion associated with salt tolerance. Chromosome 3E largely (ca. 50%) controls the regulation of leaf Na$^+$ concentrations in wheat–*L. elongatum* amphiploids. Mullan et al. (2007) used comparative genomics to identify wheat orthologs of Arabidopsis genes involved in Na$^+$ regulation during salt stress. The majority of exons for Arabidopsis, rice, and wheat orthologs of *NHX1*, *NHX5* and *SOS1* were conserved except for those at the amino and carboxy terminal ends. However, additional exons were identified in the predicted rice and wheat *NHX1* and *SOS1* genes as compared with Arabidopsis, revealing gene rearrangement during evolution from a common ancestor. The identification of wheat orthologs of Arabidopsis genes, map locations, and gene expression profiles will increase our knowledge of the complex mechanisms regulating Na$^+$ transport in

wheat and wheat–*L. elongatum* lines under salt stress. In conclusion, wheat–*L. elongatum* substitution lines will be suitable for developing wheat germplasm with reduced Na$^+$ accumulation. *Thinopyrum bessarabicum* Savul is tolerant of high levels of salt (upto 350 mol m$^{-3}$ of NaCl) because it can limit the accumulation of sodium and chloride ions in its leaves (Gorham et al. 1985). A new salt-tolerant cereal, Tritipyrum, an amphiploid involving wheat and *Th. bessarabicum*, has been developed and is the most salt tolerant at 150 mol m$^{-3}$; hence genes from *Th. bessarabicum* have the potential for breeding salt-tolerant wheat (King et al. 1997a,b).

Targeted breeding for salt tolerance in wheat resulted in the successful release of a few salt-tolerant cultivars in India, Pakistan, Egypt, and the United Kingdom (Munns et al. 2006). Using highly accurate selection methods, the newly identified source of salinity tolerance, and molecular markers associated with salt tolerance (Lindsay et al. 2004), CSIRO (Commonwealth Scientific and Industrial Research Organization, Australia) researchers developed durum wheat lines, which are now in advanced field trials in Australia, with same level of salt tolerance as bread wheat (www.kondinin.com.au).

**3. Maize.** Genetic variation for salt tolerance has been reported in few commercial cultivars, hybrids, and landraces (Table 2.2). Using relative root length at salinity threshold (NaCl concentration at which root growth start to decrease) and salt concentrations at which roots stop growing, Khan et al. (2003) detected considerable differences between accessions. The expression of root growth as a function of NaCl concentrations provides a useful guideline for salt tolerance in maize. More recently, de Costa et al. (2007) reported that the combination of partial osmotic adjustment, possible reduction of the sensitivity of leaf growth under salt stress to increased abscisic acid (ABA) concentrations, and growth-promoting function regulated by ABA is responsible for resistance in the first stage of salt stress in maize and that the genotypic variation in these mechanisms can be utilized to breed salt-resistant genotypes in maize.

**4. Sorghum and Pearl Millet.** Whole-plant tolerance to salinity in sorghum and pearl millet is associated with reduced shoot Na$^+$ concentration while K$^+$/Na$^+$ and Ca$^{2+}$/Na$^+$ ratios are of lesser importance. Genetic variation exists for salt tolerance biomass production (Table 2.2) and that Na$^+$ exclusion from the shoot may be a major mechanism involved in that tolerance in sorghum and pearl millet (Krishnamurthy et al. 2007a,b).

## C. Aluminum Tolerance

Aluminum (Al) resistance can be achieved by the mechanisms that facilitate Al exclusion from the root apex (Al exclusion) or by mechanisms that confer the ability of plants to tolerate Al (Al tolerance). Exclusion mechanism is based on Al-induced carboxylate (malate, citrate, and oxalate) release from the growing root tip under acidic soils while tolerance is based on the internal detoxification of Al with carboxylate ligands and the sequestration of the Al-carboxylate complexes in the vacuole (Kochian et al. 2005). Eticha et al. (2005) studied the maize cultivars differing in Al resistance for variation in cell-wall (CW) pectin content and its degree of methylation in root apices and concluded that in addition to the release of organic anions and Al-inducible changes in organic acid synthesis and compartmentalization (Piñeros et al. 2002; Mariano and Keltjens 2003), differences in CW pectin and its degree of methylation contribute to genotypic differences in Al resistance in maize (Piñeros et al. 2002; Mariano and Keltjens 2003). Hematoxylin staining of root tips (Polle et al. 1978) and root growth evaluations (Baier et al. 1995) are the most widely used methods to screen for Al tolerance. The former is a qualitative measure, but the latter provides a quantitative measure of root health.

Novel genetic variations for Al tolerance associated with increased root length following exposure to Al have been reported in cereal crops (Table 2.3), representing a valuable resource for expanding the diversity of the gene pool from which Al-tolerant cultivars can be developed. For example, AFLP analysis of 35 Al-tolerant landraces in wheat revealed that the germplasm are genetically diverse, which may offer unique combinations of alleles for improvement of cultivated wheat (Stodart et al. 2007). In another study involving 590 wheat accessions from diverse geographic regions or breeding programs, Zhou et al. (2007a) identified 88 accessions tolerant to Al toxicity. When molecularly profiled using SSR markers, they detected two major clusters, an Asian and an American-European cluster, and hence new germplasm for Al resistance different from those derived from Brazil. Similarly, a global SSR-based genetic diversity analysis using a broader sorghum set revealed the presence of both multiple $Alt_{SB}$ alleles and different Al tolerance genes within highly related accessions (Caniato et al. 2007). These results suggest that efforts toward broadening the genetic basis of Al tolerance may benefit from a detailed analysis of Al-tolerance gene diversity in crop plants.

Several key genes associated with resistance to Al have been reported in barley, sorghum, and wheat, but it appears that more complex inher-

itance governs Al resistance in maize and rice (Samac and Tesfaye 2003; Kochian et al. 2005). Targeted breeding for Al resistance in wheat has led to the development and release of Al-resistant cultivars in Brazil, Australia, and the United States. Several resynthesized wheat and advanced backcross lines of barley and wheat, all possessing resistance to Al toxicity, have been developed (Samac and Tesfaye 2003; reviewed in Dwivedi et al. 2007). Further, database searches revealed that Al tolerance derived from Brazilian sources has been utilized in over 100 cultivars released internationally (www.genbank.vurv.cz/wheat/pedigree/).

A wild relative of wheat, goatgrass (*Aegilops uniaristata*), has been reported as a useful source of Al tolerance. The 3n chromosome carrying the Al-tolerant gene has been transferred into a wheat cultivar, Chinese Spring. However, this introgression also led to the transfer of the undesirable "split neck break" trait. Subsequently, the undesirable effect was reduced either by inducing recombination between wheat and 3n or by irradiation with fast neutrons and highly aluminum-tolerant lines, which the CIMMYT breeders are using to transfer into a range of popular wheat cultivars from Brazil, Ethiopia, Kenya, and Nepal (www.dfid-psp.org/ccstudio/publications/Annual Report/2004_aluminum.pdf). Further, an SSR marker, *barc 1040*, has been found to be associated with Al tolerance in goatgrass.

## III. ENHANCING ADAPTATION TO ABIOTIC STRESSES THROUGH BREEDING AND TRANSGENIC INTERVENTION

### A. Mapping and Cloning Quantitative Trait Loci Associated with Tolerance to Drought, Salinity, and Aluminum (Al) Stresses

High-density genetic linkage maps are a useful resource for identifying markers tightly linked to agronomically important QTL for marker-assisted selection and for cloning gene/QTL. Genetic maps of varying marker density and genome coverage are available in cereal crops. High-density maps have been developed in barley, maize, rice, sorghum, and wheat while a less saturated map is available in pearl millet (reviewed in Dwivedi et al. 2007). In this section we review aspects of the progress made in identifying and fine-mapping of QTL for traits associated with better adaptation to drought, salinity, and aluminum stresses; QTL validation across different genetic backgrounds and environments, which are essential step for marker-aided genetic enhancement; and determination of the genetic function of individual QTL.

**1. Drought Tolerance.** Variation in root traits in rice, maize, wheat, and sorghum; osmotic adjustment in barley and rice; leaf-abscisic acid (L-ABA), leaf relative water content (RWC), leaf (or canopy) temperature, and anthesis silking interval (ASI) in maize; harvest index (HI), panicle harvest index (PNHI), grain number and individual grain mass in pearl millet; stay-green and chlorophyll content and fluorescence parameters in barley, sorghum, and wheat are diagnostic traits that confer better adaptation to drought stress (see Section II). There is a large body of literature covering the identification of putative QTL for drought tolerance in barley, maize, pearl millet, rice, sorghum, and wheat (Table 2.4). Colocalization of QTL have also been reported, for example, QTL for ASI and ears per plant, leaf growth (maximum elongation rate per unit thermal time) and ASI, L-ABA and leaf temperature, RWC, ASI, and grain yield in maize; QTL for grain yield, HI, PNHI, grain number, and individual grain mass in pearl millet; QTL for grain yield and root traits, canopy temperature, and flowering and RWC and OA in rice; QTL for stay-green and chlorophyll content in sorghum; and QTL for chlorophyll and chlorophyll fluorescence parameters in barley. Further, QTL for stay-green in sorghum corresponded to stay-green QTL in maize and was congruent with other drought-related traits in maize and rice. Several QTL with major effects have been reported under varying moisture stress regimes; for example, QTL for grain yield, ASI, L-ABA, root lodging, and stay-green in maize; QTL for chlorophyll fluorescence in barley; QTL for grain yield in pearl millet and rice. QTL validation studies revealed only a few QTL common across different genetic background or environments (varying moisture stress regimes); for example, a major QTL for L-ABA concentration and root lodging, several QTL for morphophysiological, root-, and grain yield-related traits in maize; QTL for spikelet fertility, 1,000-grain weight, panicle and spikelet numbers, OA and root traits in rice; and a QTL for stay-green in wheat. Further work is in progress to clone major QTL for drought tolerance; for example, *root-ABA1* (influences root architecture), L-ABA concentration, and grain yield in maize, thus providing a more direct way to access and manipulate genetic diversity for traits enhancing the adaptation to drought stress (Tuberosa and Salvi 2006).

Candidate genes may encode structural or regulatory proteins or proteins involved in biochemical pathway affecting the trait expression (Byrne and McMullen 1996). Four candidate genes have been mapped in rice. *OxEXP2* and *EGase*, involved in cell expansion, mapped on the intervals carrying the QTL for root traits common to the three populations (Table 2.4). *EXP15* and *EXP13* are associated with root number and silicon content in the stem respectively and under varying moisture

regimes. Using differential gene expression in response to polyethylene glycol (PEG) simulated drought in three unique rice genetic materials (a lowland rice, IR64 and its derived line PD86 introgressed with 11 drought-tolerant (DT) QTL, and an upland rice IRAT109), Fu et al. (2007) identified more than 300 unique cDNA sequences covering genes of diverse cellular activities and functions. They found that levels and mechanisms of DT of these lines was associated with the number and types of differentially expressed genes, suggesting different DT mechanisms in rice are controlled by different sets of genes and different metabolic pathways. In addition, most differentially expressed genes under drought were able to contribute to DT. Second, there appears to be a high correspondence in genomic location between DT QTL and clusters of differentially expressed genes in rice, suggesting that some DT QTL may represent clusters of coregulated and functionally related genes. Four to six QTL for several drought-tolerance traits were found related to candidate genes in barley (Table 2.4). Gene expression profiles of water-stress–tolerant versus water-stress–sensitive wild barley using cDNA-AFLP analysis of dehydrated samples at the seedling stage, Suprunova et al. (2007) detected 70 transcript-derived fragments (TDFs) that displayed differential expression between control and stress conditions, of which 11 showed distinct differences (up- or down-regulation) between tolerant and susceptible genotypes. They selected TDF-4 as a promising candidate gene for stress tolerance and sequenced the corresponding gene, designated *Hsdr4*, and determined the transcribed and flanking regions. Further analysis of the *Hsdr4* promoter region revealed a new putative miniature inverted-repeat transposable element (MITE) and several stress-related transcription factors (MYC, MYB, LTRE, and GT-1), suggesting a role of the *Hsdr4* gene in plant tolerance to dehydration stress. *Hsdr4* gene mapped to the long arm of chromosome 3H between markers *EBmac541* and *EBmag 705*, within a region that previously shown to affect osmotic adaptation in barley.

By using a microarray of approximately 16,000 unique wheat ESTs and progenies, derived from Quarrion (high-transpiration efficiency, TE) and Genaro 81 (low TE), with contrasting TE, Xue et al. (2006c) identified 93 genes that differentially expressed between high- and low-TE progeny lines. One-fifth of these genes were markedly responsive to drought stress. Several growth-related regulatory genes that were downregulated by drought were expressed at a higher level in the high-TE lines than the low-TE lines and were associated with a biomass production component of the Quarrion-derived high-TE trait. Further, the expression of 11 genes was positively associated with the high-TE trait when measured as $\delta^{13}C$, thus these genes are candidates for investigating processes that underlie the high-TE trait.

**2. Salinity Tolerance.** Salinity tolerance comes from genes that limit the rate of salt uptake from the soil and the transport of the salt throughout the plant, adjust the ionic and osmotic balance of cells in roots and shoots, and regulate leaf development and the onset of senescence (Munns 2005). Salt tolerance in cereals is associated with sodium exclusion, which limits the entry of sodium into the plant and its transport to leaves, and results in high $K^+$/$Na^+$ ratio in leaves. For example, bread wheat restricts $Na^+$ transport to leaf tissues (i.e., through $Na^+$ exclusion) and maintains high selectivity of $K^+$ over $Na^+$ while barley is not so efficient with respect to these traits. However, the adverse affects of $Na^+$ within leaves of barley are minimized by its compartmentalization into vacuoles (with $Cl^-$) and the production of organic solutes to osmotically balance the cytosol (Garthwaite et al. 2005 and reference therein). Genetic variations for salt tolerance has been reported in cereal crops (see Section II, Table 2.2); however, genomic regions associated with salt tolerance have been reported only in barley, rice, and wheat (Table 2.5)—for example, QTL for $Na^+$ and $K^+$ uptake, $Na^+$ and $K^+$ concentration, and $Na^+$/$K^+$ ratio in rice and *Kna1* in bread wheat and *Nax1* in durum wheat, both regulating low $Na^+$ uptake. Additionally, *qST1* and *qST3* confer salt tolerance at seedling stage in rice and several QTL conferring salt tolerance at germination and at seedling stage in wheat and barley. It is not yet known whether any of these QTL is common across these species; however, a few common QTL associated with salt tolerance at germination and seedling stage in rice or wheat were detected. Lindsay et al. (2004) validated SSR markers, gwm312 and wmc170, closely linked to the *Nax1* locus that has proven useful for the selection of the $Na^+$ exclusion in durum wheat populations from a range of genetic backgrounds.

A durum wheat, Line 149, contains two major genes for excluding $Na^+$ from leaf blades, *Nax1* and *Nax2* that James et al. (2006) separated into families containing a single gene and later developed homozygous NIL containing a single gene. Subsequently lines containing either *Nax1* or *Nax2* had lower rates of $Na^+$ transport from roots to shoots than their NIL pairs due to lower rates of net loading of the xylem, not to lower rates of net uptake from the soil or higher rates of retranslocation in the phloem. *Nax1* and *Nax2* lines also had higher rates of $K^+$ transport from root to shoot, resulting in an enhanced discrimination of $K^+$ over $Na^+$. Further, lines containing *Nax1* differed from those containing *Nax2* by unloading $Na^+$ from the xylem as it entered the shoot so that $Na^+$ was retained at the leaf base leading to a high sheath-to-blade ratio of $Na^+$ concentration. *Nax2* line did not retain $Na^+$ at the leaf base, suggesting that it functioned only in the root. It appears that *Nax2* has a similar function to

*Kna1* in bread wheat. More recently, two putative sodium transporter genes (*TmHKT7-A1* and *TmHKT7-A2*, with 83% amino acid identity) related to rice *OsHKT7* mapped to wheat chromosome 2AL. *TmHKT7-A1* cosegregated with *Nax1*. *TmHKT7-A2* expressed in the root and leaf sheaths of the salt-tolerant durum wheat Line 149 and its expression pattern matches the physiological role of *Nax1* in reducing $Na^+$ concentration in leaf blades by retaining $Na^+$ in the sheaths and the control of $Na^+$ unloading from xylem in roots and sheaths (Huang et al. 2006). Furthermore, the *Nax2* locus on 5AL coincides with the locus for a putative $Na^+$ transporter, *HKT1;5* (*HKT8*). The *Nax2* region on 5AL is homoeologous to the region on chromosome 4DL containing the major $Na^+$ exclusion locus in bread wheat, *Kna1*. A gene member of the *HKT1;5* family colocates to the deletion bin containing *Kna1* on chromosome 4DL providing evidence that *Nax2* and *Kna1* are strongly associated with *HKT1;5* (Byrt et al. 2007).

QTL for salt tolerance (*SKC1*) in rice have been cloned and encode a sodium transporter involved in regulating $K^+$/$Na^+$ homeostasis under salt stress (Ren et al. 2005); *SKC1* is preferentially expressed in the parenchyma cells surrounding the xylem vessels.

**3. Al Tolerance.** Genetic variation (see Section II.C, Table 2.3) and QTL with major effects associated with Al tolerance have been reported (Table 2.6), for example, eight QTL associated with relative root elongation under Al-stress conditions in rice, of which *qRRE-9* reduced to the single Mendelian factor, *Alt-9*, present in different genetic backgrounds while *qRRE-5* and *qRRE-11*, respectively, syntenic to the major Al resistance gene on chromosome 6 and 10 of maize; *Alt_{SB}*, a major Al tolerance locus in sorghum is an orthologue of a major Al tolerance QTL on rice chromosome 1. One of the two major QTL associated with variation in net root growth in Al-tolerant wheat cv. Atlas on chromosome 4DL cosegregated with Al-activated malate transporter gene, *ALMT1*. A candidate gene, *HvMATE*, has been associated with Al tolerance and Al-activated citrate efflux in barley cv. Dayton.

Sasaki et al. (2004) were the first to clone a wheat gene, *ALMT1* (Al-activated malate transporter), cosegregating with Al tolerance, that encodes a membrane protein constitutively expressed in the root apices of the Al-tolerant line at greater levels than in the near-isogenic but Al-sensitive line. Transgenic barley overexpressing the malate transporter *ALMT1* showed higher levels of Al resistance than wild type (Delhaize et al. 2004). Homologs of wheat *ALMT1* have been cloned in *Arabidopsis thaliana*, rape, and rye; although the expression patterns of these genes differ among these plant species (Hoekenga et al. 2006; Ligaba et al. 2006;

**Table 2.6.** Quantitative trait loci (QTL)/markers associated with aluminum (Al) tolerance in barley, maize, rice, sorghum, and wheat from 1996 to 2007.

| Summary of QTL/marker information | Reference |
|---|---|
| **Barley** | |
| ABG715, Bmag353, GBM1071, GWM165, HvMATE, and HvGABP exhibited complete linkage with the *Alp* locus accounting for 72% variation for Al tolerance. HvGABP and ABG715 delineated *Alp* locus to a 0.2 cM interval. | Wang et al. 2007 |
| A candidate gene, *HvMATE*, expressed 30-fold greater RWC in Dayton and its expression in $F_{2:3}$ families significantly associated with Al tolerance and Al-activated citrate efflux | Wang et al. 2006 |
| A single Al tolerance locus *Alt* validated on long arm of chromosome 4H in Honen, Bmag353 and HVM68 flanked *Alt* locus at $1.6 \pm 0.9$ and $5.1 \pm 1.7$ cM, respectively | Ma et al. 2004 |
| A major QTL associated with Al tolerance on chromosome 4H explained 50% variation in Al-activated citrate secretion; Bmag353 a tightly linked marker | Raman et al. 2002 |
| *Alt* gene mapped on chromosome 4H, large allelic variation for Al tolerance detected with marker Bmag353 | Tang et al. 2000 |
| *Alp* in Dayton confers a high level of Al tolerance; mapped on 4HL, 2.1 cM proximal to *Xbcd1117* and 2.1 cM distal to *Xwg464* and *Xcdo1395*; *Xcdo1395* also linked to wheat Al-tolerance gene *Alt_BH* located on 4DL | |
| **Maize** | |
| 5 QTL on chromosomes 2, 6, and 8 explained 60% variation; $QTL_4$ close to genes encoding for enzymes involved in the organic acids synthesis pathways; $QTL_2$ mapped in the same region as *Alm2*, also associated with Al tolerance in maize | Ninamango-Cárdenas et al. 2003 |
| 6 RFLP markers on chromosomes 6 and 4 RFLP on 10 cosegregated with Al tolerance in Cat-100-6; chromosome 10 locus with strongest effect on Al tolerance | Sibov et al. 1999 |
| **Rice** | |
| 3 QTL on chromosomes 1, 9, and 11, explaining 13% to18% variation; *qRRE-9* reduced to the single recessive Mendelian factor, *Alt-9*, that mapped between *RM24702* and *ID47-2* on chromosome 9 and cosegregated with RM5765 | Xue et al. 2007 |
| *qRRE-1*, *qRRE-9*, and *qRRE-11* detected on chromosomes 1, 9, and 11, respectively, explaining 13.5% to 17.7% variation for relative root elongation. *qRRE-1* and *qRRE-9* consistent among different populations. *qRRE-11* a newly detected QTL and syntenic with a major Al-resistance gene on chromosome 10 of maize | Xue et al. 2006 |
| *qCRE-6*, *qCRE-8*, and *qCRE-9* for root elongation under nonstress conditions; *qSRE-4* for root elongation under Al stress; *qRRE-5*, *qRRE-9*, and *qRRE-10* for relative root elongation; *qRRE-5* syntenic to a major Al-tolerance gene on chromosome 6 of maize; *qRRE-9* similar in different rice populations | Xue et al. 2006c |
| 9 QTL including 1 for root length under nonstress conditions, 3 for root length under Al stress and 5 for relative root length (RRL), explained 9% to 25% variation; QTL for RRL, mapped on chromosomes 1 and 9, consistent across populations; a major QTL for RRL on chromosome 3 conserved across cereal species | Nguyen et al. 2003 |
| 3 QTL controlling Al tolerance on chromosomes 1, 2, and 6 together explained 27% phenotypic variation | Ma et al. 2002 |
| 20 QTL for root growth under Al stress and control conditions on 10 rice chromosomes; *qALRR-1-1* and *qARR8*, major QTL, for root length ratio on chromosomes 1 and 8, respectively; conserved genomic region on chromosome 1 associated with Al tolerance across 3 rice genetic backgrounds | Nguyen et al. 2002 |
| 9 genomic regions on 8 chromosomes linked with root and shoot growth under Al stress; region near WG110 on chromosome 1 showed greatest effect on Al tolerance that does not correspond to Al-tolerance genes mapped in other species | Nguyen et al. 2001 |
| **Sorghum** | |
| A single locus, *Alt_SB* mapped near the end of sorghum chromosome 3, controls Al tolerance in 2 highly Al-tolerant sorghum cultivars; a major Al-tolerance QTL on rice chromosome 1 orthologous to *Alt_SB* whereas another rice QTL on chromosome 3 corresponds to the Triticeae group 4 Al-tolerance locus | Magalhaes et al. 2004 |
| **Wheat** | |
| 2 QTL for Al resistance in Atlas 66 together contributed 57% variation in hematoxyline staining score and 50% variation in net root growth. Of these, a major QTL on chromosome 4DL cosegregated with Al-activated malate transporter gene (*ALMT1*) while expression of a minor QTL on chromosome 3BL was suppressed by the major QTL; the expression of these QTL also verified in another population | Zhou et al. 2007 |
| *Qualt.pser-4D*, *Qualt.pser-5A*, and *Qualt.pser-2D* associated with enhanced root growth under Al stress in Chinese Spring; 2 minor QTL, *Qualt.pser-5A* and *Qualt.pser-2D* on chromosome 5AS and 2DL, respectively | Ma et al. 2006 |
| A major Al-tolerance gene, *ALMT1*, mapped on chromosome 4DL, and loss of *ALMT1* coincide with the loss of both Al-tolerance and Al-activated malate efflux | Raman et al. 2005 |
| A major QTL on distal region of chromosome 4DL, where a malate transporter gene mapped, accounted for nearly 50% variation for Al tolerance, and SSRs *Xgdm125* and *Xwmc331* flanking the QTL identified | Ma et al. 2005 |
| An RFLP marker, bcd1230, on chromosome 4DL (1.1 cM), explained 85% variation in Al tolerance | Riede and Anderson 1996 |

Fontecha et al. 2007). Recently, two Al-resistant genes, *HvACCT1* in barley and *SbMATE* in sorghum, belonging to the multidrug and toxic compound extrusion (MATE) family, have been cloned (Furukawa et al. 2007; Magalhaes et al. 2007). MATE proteins are members of a large and complex family of transporters involved in the efflux of small organic molecules that confer resistance to toxins (Diener et al. 2001; Li et al. 2002). *HvACCT1* is an Al-activated citrate transporter that constitutively expresses mainly in the roots of the Al-resistant barley cultivar. Overexpression of this gene in tobacco-enhanced citrate secretion and Al resistance compared with the wild-type plants. *HvACCT1* is localized in the epidermal cells of the barley root tips. $Alt_{SB}$ is the major Al-tolerance locus in sorghum mapped to the terminal region of chromosome 3 (Table 2.6). *SbMATE* is also an Al-activated citrate transporter and expressed only in the root of the Al-tolerant sorghum NIL that correlates closely with the observed increase in the root citrate exudation. Furthermore, when examined in a genetically diverse sorghum panel, the differences in *SbMATE* expression explained over 95% of the phenotypic variation for Al tolerance, providing strong evidence that *SbMATE* underlies $Alt_{SB}$ and that those differences in gene expression constitute the basis for allelic variation at this locus (Magalhaes et al. 2007). The major Al-tolerance loci in sorghum and wheat, $Alt_{SB}$ and $Alt_{BH}$, are distinctly different genes and presumably evolved independently to encode similar physiological mechanisms of Al tolerance involving Al exclusion from root apices based on organic acid release (Magalhaes et al. 2007), whereas a major Al tolerance QTL on rice chromosome 1 is possibly orthologous to $Alt_{SB}$ (Nguyen et al. 2001; Magalhaes et al. 2004).

Recent progress in detecting and isolating salinity and Al-tolerance genes in cereals clearly opens the possibility of introgressing these genes into locally adapted sensitive cultivars by using transgenic approach. Obviously, more efforts are needed to clone QTL associated with traits enhancing adaptation to drought stress in cereals before we see the development of agronomically viable cultivars in the field.

## B. Genes and Approaches to Engineering Increased Abiotic Stress Tolerance

Plant adaptation to abiotic stress is achieved by cumulative action of a number of genes involved in different stress tolerance mechanisms in a coordinated fashion. The coordinated involvement of these genes and their interaction with timing and intensity of stress has made it difficult

for plant breeders to develop stress-tolerant plants by conventional plant breeding. Advances in genetics and genomics are beginning to enable researchers to identify a large numbers of genes that express specifically under abiotic stress conditions in different tissues with particular temporal signatures (Denby and Gehring, 2006). Some of these genes will be candidates for transgenic interventions as stress tolerance enhancers. Transfer and expression of such candidate genes into crop species through genetic engineering offers considerable promise. In this section, we provide evidence that candidate transgenes in barley, maize, rice, and wheat can confer improved tolerance to abiotic stresses, mainly drought, salinity, and extreme variation in temperature (Table 2.7).

Trehalose, a nonreducing disaccharide of glucose, is one of the most effective osmoprotectants, and significant levels of trehalose accumulation have been shown to result in abiotic stress tolerance in transgenic plants. Garg et al. (2002) overexpressed *E. coli* trehalose bisynthetic genes (*otsA* and *otsB*) as a fusion gene under the control of either tissue-specific or stress-responsive promoter to increase accumulation of trehalose to enhance abiotic stress tolerance in rice. The transgenic lines accumulated 3 to 10 times more trehalose and showed better growth, less photo-oxidative damage, and favorable mineral balance under salt, drought, and low-temperature stress conditions. Recently, engineering trehalose metabolism with a yeast trehalose-6-phosphate synthase (TPS)- trehalose-6-phosphate phosphatase (TPP) bifunctional enzyme, using either *35S* or *rd29A* promoter, confered multiple stress protection (drought, extreme variation in temperature, and salinity) with no adverse effect in *Arabidopsis thaliana*, making it a potential tool to improve stress tolerance in crops (Miranda et al. 2007). Naurally, the agronomical success of such future interventions is also dependent on the metabolic cost incurred by expressing a transgene and producing new proteins and/or metabolites *in planta*.

Majee et al. (2004) isolated and characterized a novel salt-tolerant gene *L-myo-inositol 1-phosphate synthase* from halophytic rice, *Porteresia coarctata*. Incorporating this gene into rice and other plant species increased synthesis of inositol under salinity stress and enhanced tolerance to salinity stress (Das-Chatterjee et al. 2006). Superoxide dismutases (SOD) play an important role in scavenging the reactive oxygen species (ROS). Jithesh et al. (2006) isolated a cDNA (*Sod1*) encoding a cytosolic Cu/ZnSOD that accumulated during oxidative stress in the mangrove species *A. marina*. Prashanth et al. (2008) transformed Pusa Basmati-1 with *Sod1*, and the resulting transgenic plants were more tolerant to oxidative, salinitiy (150 mM NaCl), and drought

Table 2.7. Examples of transgenic barley, maize, rice, and wheat with tolerance to drought, salinity, and cold tolerance.

| Gene | Source | Gene product | Cellular role | Effect of the transgene | Reference |
|---|---|---|---|---|---|
| Barley HVA1 | Barley | LEA protein | Possibly membrane protection | Enhanced growth under water deficit | Sivamani et al. 2000 |
| Maize ZmPLC1 | Maize | Phosphatidylinositol-specific phospholipase C (PI-PLC) | — | Improved drought tolerance and higher grain yield under drought stress conditions | Wang et al. 2008b |
| ZmDREB2A | Maize | DRE-binding protein | — | Improved drought and heat stress tolerance | Qin et al. 2007 |
| ZmNF-YB1 | Maize | — | — | Enhanced drought tolerance | Nelson et al. 2007 |
| NPK1 | Tobacco | Mitogen-activated protein kinase kinase kinase (MAPKKK) | — | Enhanced drought tolerance | Shou et al. 2004 |
| betA | E. coli | Choline dehydrogenase | Glycinebetaine synthesis | Increased tolerance to drought stress | Quan et al. 2004 |
| Rice Sod1 | Avicennia marina | Sod1 protein | Defence against free oxygen radicals | Tolerant to salinity and drought | Prashanth et al. 2008 |
| HARDY | Arabidopsis | AP2/ERF-like transcription factor | — | Improved water use efficiency and photosynthetic assimilation; reduced transpiration; enhanced drought tolerance | Karaba et al. 2007 |
| OsNAC6 | Rice | NAC transcription factor | — | Improved tolerance to drought, salinity, and blast disease | Nakashima et al. 2007 |
| OsCIPK03, OsCIPK12, OSCIPK15 | Rice | Calcineurin B-like protein-interacting protein kinase | — | Enhanced cold and drought stress tolerance | Xiang et al. 2007 |
| CMO | Spinach | Choline monoxygenase | Glycinebetaine synthesis | Enhanced tolerance to salt and temperature stress | Shirasawa et al. 2006 |
| SsNHX1 | Suaeda salsa | Na$^+$/H$^+$ antiporter | Vacuolar Na$^+$/H$^+$ antiporter | Enhanced tolerance to salt and water stress | Zhao et al. 2006b |
| SsNHX1 and AtAVP1 | Suaeda salsa and Arabidopsis | Na$^+$/H$^+$ antiporter H$^+$-pyrophosphatase | Na$^+$/H$^+$ antiporter and H$^+$ pyrophosphatase | Enhanced salt tolerance | Zhao et al. 2006c |
| SOD2 | Yeast | Na$^+$/H$^+$ antiporter | Plasma membrane Na$^+$/H$^+$ antiporter | Enhanced salt tolerance | Zhao et al. 2006a |
| PcINO1 | Porteresia coarctata | L-myoinositol-1-phosphate synthase | Inositol synthesis | Enhanced salt tolerance | Das-Chatterjee et al. 2006 |
| OsDREB1 | Rice | DRE-binding protein | — | Enhanced drought, salt, and low temperature stress | Ito et al. 2006 |
| SNAC1 | Rice | NAM, ATAF, and CUC (NAC) transcription factor | — | Enhanced drought tolerance | Hu et al. 2006a |
| nhaA | E. coli | Na$^+$/H$^+$ antiporter | Na$^+$/H$^+$ antiporter | Enhanced salt and drought tolerance | Wu et al. 2005 |
| Mn-SOD | Pea | Mn-Superoxide dismutase | Reduction of ROS | Improved drought tolerance | Wang et al. 2005 |
| ABF3 | Arabidopsis | ABRE binding protein | — | Improved drought tolerance | Oh et al. 2005 |
| Calcineurin | Mouse | Ca2$^+$- and calmodulin-dependent serine/threonine phosphatase | Regulation of ion homeostasis | Enhanced salt tolerance | Ma et al. 2005 |
| Adc | Datura stramonium | Arginine decarboxylase | Polyamine synthesis | Enhanced drought tolerance | Capell et al. 2004 |

(continued)

**Table 2.7.** (Continued)

| Gene | Source | Gene product | Cellular role | Effect of the transgene | Reference |
|---|---|---|---|---|---|
| *OsNHX1* | Rice | (Na$^+$, K$^+$)/H$^+$ antiporter | Vacuolar (Na$^+$, K$^+$)/H$^+$ antiporter | Enhanced salt tolerance | Fukuda et al. 2004 |
| *HVA1* | Barley | LEA protein | Possibly membrane protection | Increased tolerance to drought and salt stress | Chandrababu et al. 2004 |
| *otsA and otsB* | *E. coli* | Trehalose 6-P synthase and Trehalose-6-P phosphatase | Trehalose | Increased photosynthesis under salt, cold, and drought stress | Garg et al. 2002 |
| *SAMDC* | Tritordeum | S-adenosylmethionine decarboxylase | Polyamine synthesis | Increased seedling growth under salt stress | Roy and Wu 2002 |
| *AgNHX1* | *Atriplex gmelini* | Na$^+$/H$^+$ antiporter | Vacuolar Na$^+$/H$^+$ antiporter | Enhanced salt tolerance | Ohta et al. 2002 |
| *codA* | *Arthobacter globiformis* | Choline oxidase A | Glycinebetaine synthesis | Enhanced tolerance to salt stress | Mohanty et al. 2002 |
| *Adc* | Oat | Arginine decarboxylase | Polyamine synthesis | Increased biomass under salinity stress | Roy and Wu 2001 |
| *Choloro-plastic-GS2* | Rice | Glutamine synthetase | Enhances photorespiration | Enhanced salt tolerance | Hoshida et al. 2000 |
| *BADH* | Barley | Betaine aldehyde dehydrogenase | Glycinebetaine synthesis | Enhanced tolerance to salt, cold, and heat stress | Kishitani et al. 2000 |
| *OsCDPK7* | Rice | Calcium dependant protein kinase | — | Enhanced cold and salt/drought tolerance | Saijo et al. 2000 |
| *Mn-SOD* | *S. cerevisiae* | Mn-Superoxide dismutase | Reduction of ROS | Enhanced salt tolerance | Tanaka et al. 1999 |
| *P5CS* | Moth bean | Δ-pyrroline-5-carboxylate synthetase | Proline synthesis | Increased biomass under salt and moisture stress | Zhu et al. 1998 |
| Wheat *AtNHX1* | *Arabidopsis* | Na$^+$/H$^+$ antiporter | Vacuolar Na$^+$/H$^+$ antiporter | Enhanced salt tolerance | Xue et al. 2004 |
| *DREB1A* | *Arabidopsis* | DRE-binding protein | — | Enhanced drought tolerance | Pellegrineschi et al. 2004 |

stresses. To the best of our knowledge, this is the first report of the transfer of a mangrove gene to rice and may make mangrove potential sources for other abiotic stress-tolerance candidate genes.

In addition, the transgenic wheat constitutively expressing barley *HVA1* gene outyielded the control in yield attributes and relative water content (Bahieldin et al. 2005) while rice overexpressing *OsLEA3-A* showed enhanced drought tolerance with no yield penalty under nonstress conditions (Xiao et al. 2007).

The Na$^+$/H$^+$ antiporters play an important role in the maintenance of cellular ion homoeostasis, cytoplasmic pH regulation, and cell turgor leading to salt tolerance in plants (Horie and Schroeder 2004). Overexpression of vacuolar Na$^+$/H$^+$ antiporter gene *OsNHX1* enhanced salt tolerance in rice (Fukuda et al. 2004) whereas overexpression of the same gene from wheat together with H$^+$-pyrophosphatase (*TVP1*) improved both salt and drought tolerance in *Arabidopsis* (Brini et al. 2007). Although transgenic plants accumulated more Na$^+$ and K$^+$ in the leaf tissue, toxic effects of Na$^+$ in cytosol were reduced by the sequestration of the Na$^+$ in vacuole. The *V-NHX1* gene from *Suaeda salsa* increased salt tolerance, photosynthetic activity, and activity of shoot *V-ATPase* in rice (Zhao et al. 2006a). When the *SsNHX1* was combined with the *Arabidopsis AVP1* (vacuolar H$^+$-PPase), the transgenic lines have higher K$^+$/Na$^+$ ratio in their shoots and showed higher level of salt tolerance than the *SsNHX1*-transformed lines upon salt treatment (Zhao et al. 2006b). Ohta et al. (2002) used a *NHX1* gene from a halophyte *Atriplex gmelini* to generate transgenic rice lines that showed eightfold higher activity of this gene compared to the wild rice. These transgenic lines survived in 300 mM NaCl for 3 days, but the wild types failed to survive. A field trial of wheat transgenic lines expressing *AtNHX1* gene exhibited higher grain yield and heavier and larger grains in saline conditions (Xue et al. 2004). There was less Na$^+$ and more K$^+$ accumulation in leaves in transgenic lines compared with nontransgenic lines.

It is widely recognized that multiple mechanisms cooperate to confer stress tolerance in plants. Introduction of a single downstream gene may not significantly increase stress tolerance. Therefore, regulatory genes (e.g. encoding transcription factors) are likely to cause a cascade leading to the transcription of many stress-responsive genes that ultimately confer abiotic stress tolerance. Indeed, transcription factor that upregulate stress responsive genes have been utilized to enhance abiotic stress tolerance. Kasuga et al. (1999) showed in transgenic *Arabidopsis* that overexpression of *DREB1A* under *35S* promoter improved tolerance to drought, salinity, and freezing stress but incurred severe retardation of growth. When a stress-inducible promoter *rd29A* was used to drive this

*DREB1A* gene, growth was normal but so was the stress tolerance. In contrast, Oh et al. (2005) reported that *DREB1A* and *ABF3* rice transgenic lines did not show any stunted growth despite constitutive expression, However, transgenic plants exhibited drought and salinity stress tolerance but low level of freezing stress tolerance. DREB homologs have been isolated from rice: *OsDREB1A* and *OsDREB1B* are cold inducible, *OsDREB2A* is induced by dehydration and salinity stress (Dubouzet et al. 2003). Overexpression of the *OsDREB1* gene in rice and *Arabidopsis* correlate with improved salt, drought, and low temperature tolerance (Ito et al. 2006). *OsDREB1F* is one of the most upregulated genes isolated from upland rice (Wu et al. 2005), The transgenic plants harboring *OsDREB1F* gene led to enhanced tolerance to salt, drought, and low temperature in both rice and *Arabidopsis* (Wang et al. 2008a). Transgenic wheat with *DREB1A* gene under the control of stress-inducible promoter *rd29A* exhibit improved drought tolerance under greenhouse conditions (Pellegrineschi et al. 2004). A *DREB2* homolog from maize, *ZmDREB2A*, is induced in response to cold, drought, salt, and heat stresses in seedlings (Qin et al. 2007). Maize transgenic lines with *ZmDREB2A* improved drought and heat stress tolerance, which might be due to the induction of LEA (late embryogenesis abundant), heat shock, and detoxification genes.

*OsNAC6* is a member of the NAC transcription factor gene family in rice that is induced by abiotic stresses, including cold, drought, and high salinity. Transgenic rice plants constitutively overexpressing *OsNAC6* showed tolerance to dehydration, salinity, and blast disease (Nakashima et al. 2007); however, the plants exhibited growth retardation and low reproductive yields. By utilizing stress-inducible promoters, such as the native *OsNAC6* promoter, it is hoped that specific stress-inducible overexpression of *OsNAC6* in rice can improve stress tolerance by eliminating effects of indiscriminate overexpression.

*SNAC1* (stress-responsive NAC1) when overexpressed in rice improved both salt and drought tolerance (Hu et al. 2006a). *SNAC1* encodes a NAM, ATAF, and CUC (*NAC*) transcription factor and is expressed in guard cells under drought stress. Many stress responsive genes were upregulated in transgenic rice, and seed set was higher than control under severe moisture stress under field conditions with no morphological abnormality or yield reduction.

The *HARDY* (*HRD*) gene, an AP2/ERF-like transcription factor identified by a gain-of-function Arabidopsis mutant *hrd-D* having roots with enhanced strength, branching, and cortical cells, exhibits drought resistance and salt tolerance accompanied by an enhancement in the expression of abiotic stress associated genes. Overexpression of *HRD*

in rice improves water use efficiency by enhancing photosynthetic assimilation and reducing transpiration. The transgenics are drought-tolerant, low-water-consuming rice plants that exhibit increased shoot biomass under well-irrigated conditions and an adaptive increase in root biomass under drought stress. Further, overexpression of *HRD* in Arabidopsis produces thicker leaves with more chloroplast-bearing mesophyll cells, and in rice, there is an increase in leaf biomass and bundle sheath cells that probably contributes to the enhanced photosynthesis assimilation and efficiency. Thus, a gene from the model plant Arabidopsis improves water use efficiency coincident with drought resistance in rice (Karaba et al. 2007).

AtNF-YB1, a transcription factor from nuclear factor Y (NF-Y) family, also confers improved performance in Arabidopsis under drought conditions. Nelson et al. (2007) identified maize homologs (ZmNF–YB2) that they used to transform elite maize inbred plants. The transgenic maize plants constitutively expressing ZmNF–YB2 showed less leaf rolling, cooler leaf temperature, and a higher chlorophyll index, photosynthetic rate, and stomotal conductance. All these stress adaptive traits contributed to grain yield advantage under water-limited environments. The best-performing transgenic lines produced about 50% increase in grain yield relative to the irrigated control.

Phospatidylinositol-specific phospholipase C (PI-PLC) plays an important role in a variety of physiological processes in plants, including drought tolerance (reviewed in Wang et al. 2008b), and the maize *ZmPLC1* gene is upregulated in roots under dehydration conditions (Zhai et al. 2005). Using *Agrobacterium*-mediated transformation, Wang et al. (2008b) produced transgenic maize expressing a *ZmPLC1* transgene in sense or antisense orientation. The sense transgenic plants under drought stress conditions showed higer relative water content, better osmotic adjustment, increased photosynthetic rates, lower percentage of ion leakage, and less lipid membrane peroxidation and higher grain yield than the wild type, whereas the antisense transgene exhibited inferior characters compared with the wild type. Thus, drought tolerance could be improved substantially by the manipulation of *ZmPLC1* gene expression in maize.

Cold shock proteins (CSPs) from bacteria, CspA from *Escherichia coli* and CspB from *Bacillus subtilis*, promote stress adaptation in many plant species. Castiglioni et al. (2008) demonstrated that expression of CSP proteins in maize is not associated with negative pleiotropic effects, indicating that stress tolerance does not have to come at a cost to crop productivity under well-watered conditions. The CspA transgenic entries provided yield increase of 4.6% under water stress, with the two

best-performing events demonstrating superiority of 30.8% and 18.3%. Yield advanatages of CspB-positive plants as a group were significantly greater than control by 7.5%. The best-performing events, CspB-Zm event 1 and 2, demonstrated yield improvement of 20.4% and 10.9%, respectively. These are the same events that demonstrated significant improvement in leaf growth, chlorophyll content, and photosynthetic rates, providing evidence that these improvement in vegetative productivity will translate into improvements in reproductive performance and grain yield. Furthermore, CspB-Zm positive entries exhibited improvements in end-of-season grain yield across the different hybrid background under both water-stress regimes when compared to a conventional wild type control of the same genetic background. The yield benefit ranged from 11% to as much as 21% across yield values that averaged 6.4 to 8.5 t ha$^{-1}$. The transgenic CspB plants consistently outyielded the nontransgenic controls by at least 0.5 t ha$^{-1}$ across most of the reproductive and vegetative stress treatments, highlighting the potential agricultural benefits that this technology can deliver. This research demonstrate that as water resources become increasingly scarce and the global demands for grain continue to increase, the ability to bring yield stability across water-limiting environments presents an important advancement in the area of stress tolerance research.

Drought accelerates leaf senescence leading to a decrease in canopy size, loss in photosynthesis, and reduced yields in plants. Senescence is a type of cell death program that could be activated inappropriately in different plants during drought; however, it is possible to enhance drought tolerance by delaying drought-induced leaf senescence. The first proof of concept came when stay-green QTL were detected in maize, rice, sorghum, and wheat tolerant to postflowering drought (see Section III.A). Leaf senescence could also be delayed in plants by expressing isopentenyltransferase (*IPT*), an enzyme that catalyzes the rate-limiting step in cytokinin (CK) synthesis (Gan and Amasino 1995). Rivero et al. (2007) recently introduced *IPT* gene driven by a stress- and maturation-induced promoter in tobacco that resulted in outstanding drought tolerance as shown by, among other responses, vigorous growth after a long drought period that killed the control plants. The transgenic plants maintained high water contents and retained photosynthetic activity (albeit at a reduced level) during the drought. Moreover, the transgenic plants displayed minimal yield loss when watered with only 30% of the amount of water used under controlled conditions. The expression of *IPT* gene induced the synthesis of CKs in the plant, which contributed to the enhanced drought tolerance of the transgenic plants. The introgression of *IPT* gene may facilitate the

development of transgenic crops able to grow with reduced irrigation without significant yield penalties.

The production of transgenics with conventional techniques (using *Agrobacterium tumefaciens* T-DNA plasmid or biolistic delivery of DNA-coated particles) has its limitations, such as intereference with plant genes, poor stability and expression of transgene, difficulty to add multiple genes at one time, and introduction of only limited amount of DNA. Integration at random sites results in unpredictable transgene expression due to positional effect variation, variable copy numbers from tandem integrations, and frequent loss of gene integrity. Moreover, it takes large numbers of transgenic events to identify insertions with the desired expression level, copy number, structure, and genomic location in addition to the long time frame required to produce varieties with multiple genes (Birch 1997; Lorence and Verpoorte 2004). Autonomous minichromosomes are convenient for manipulating and delivering DNA segments containing multiple genes. Carlson et al. (2007) constructed maize minichromosomes, using DNA sequences naturally occuring in maize centromeres. When minichromosome 1 (MMC1) is introduced into maize cells, it behaves like an ordinary chromosome, it remains distinct from the other chromosomes, the gene cassette remains structurally stable from generation to generation (at least for the four generations they tested), and the genes are expressed and transmitted through mitosis and meiosis. This novel approach for plant transformation can facilitate crop biotechnology by combining several trait genes on a single DNA fragment, arranging genes in a defined sequence context for more consistent gene expression, and providing an independent linkage group that can be introgressed rapidly into various germplasms. This technology could be used to all traits including those that increase hardiness, yield, and nutritional content of plants. The United States Patent and Trademark Office issued Chromatin patent No. 7,119,250, which extends the exclusive right to use these minichromosomes to all plants.

While recent advances in biological sciences and in particular plant genetics, plant physiology, plant biochemistry, and plant molecular biology have led to an impressive body of work aimed, directly or indirectly, at understanding abiotic stresses with the ultimate goal to engineer stress-tolerant crop plants (Xiong et al. 2002; Seki et al. 2003; Ashraf and Harris 2004; Horie and Schroeder 2004; Denby and Gehring 2005; Valliyodan and Nguyen 2006), it is perhaps surprising that, to date, there are no transgenic crop plants with increased abiotic, and in particular drought and salinity, stress tolerance in the field. What are the main reasons for this? First, strategies learned from highly drought-stress-tolerant plant families such as Mesembryanthemacae cannot be

transferred to crop plants, because the latter lack the specialized organs such as salt glands that confer the high degree of tolerance. Second, it becomes increasingly clear that single-gene modifications in transgenic plants are not a viable long-term strategy. While single-gene modifications can confer significant salt tolerance in the model plant Arabidopsis (Kasuga et al. 1999; Shi et al. 2003), such interventions, if stable, will almost certainly disturb development and physiology of the plant, thus having a significant fitness cost. The use of specific stress-inducible promoters can help reduce gross growth effects (Kasuga et al. 1999), but such transgenic lines have not been evaluated for fitness parameters such as seed yield let alone agronomic performance under field conditions. Third, it becomes increasingly clear that the knowledge gained from experimental model plants such as Arabidopsis cannot be directly applied to crops and especially cereals, hence the minimal success in the field. In summary, it is becoming increasingly obvious that systems-based research strategy with relevant plant species can yield insights that will eventually translate into biotechnologically successful applications. One important step to achieve this goal is the development of appropriate experimental model systems.

## C. Experimental Model Systems and Genomic Tools for Cereal Improvement

What makes an ideal experimental model system and in this case model plant?

1. The model plant should be easy to grow, both under sterile conditions and in a glasshouse.
2. Since classical genetics remains a most valuable tool and phenotypes need assessment at all stages *in planta*, generation times should be short and ploidy ideally should not exceed diploid level.
3. Model plants should easily transform and regenerate.
4. The complete genome should be available in the public domain.

All conditions are fulfilled by *Arabidopsis thaliana,* and it is no surprise that it has served, and continues to serve, as the first and foremost model system in higher plants (Rothan and Causse 2007). Concomitant with its use as model plant is an ever-increasing amount of data and resources. They include a large number of well-described mutants, the availability of the complete genomic sequence, large repositories of expression data both in the wild type and mutants easily accessible through "Genevestigator" (www.genevestigator.ethz.ac.ch; Zimmermann et al. 2004) and gene ontology servers (Al Shahrour

et al. 2007) to name just a few. These are invaluable tools for a systems-oriented biology where the focus is not on the response of a single gene but on the transcriptome as a whole: the proteome and ultimately the metabolome. In fact, a recent example demonstrates how the sequential applications of Web-based tools can assist in functional annotation of a novel gene and lead researchers to infer the function of previously unknown genes in Arabidopsis at the systems level (Meier et al. 2008). In essence, this systems approach to study stress responses, for example, requires access to the complete genome and a sizable repository of publicly available microarray data, two conditions that will be fulfilled by an ever-increasing number of crop and in particular cereal species.

Furthermore, a detailed analysis of the heat shock response and the transcription regulatory network required to convey temporal, spatial, and stimulus specificity has been published (Swindell et al. 2007). A genome-wide functional analysis of the responses to salt and osmotic stress revealed highly differentiated expression patterns, both temporally and spatially, and the fact that the responses to osmotic stress are much more complex than the responses to salt stress. Perhaps surprisingly, there is little overlap between the two response types. This may reflect different adaptation strategies of Arabidopsis to either stress (Denby and Gehring 2005), and it is reasonable to expect that rather different response patterns may have evolved in other species.

It has also been pointed out that our understanding of drought-tolerance mechanisms is focused mainly on plant developmental stages other than those immediately before and after flowering, while in fact in most cereals, the agronomical interest is the reproductive parts since they constitute the harvest (Valliyodan and Nguyen 2006). Consequently, success in producing crops with increased drought tolerance may rely on improving reproductive success under specific abiotic stress conditions, and the value of individual transgenes or pathways for drought tolerance ultimately will be judged by agronomic performance rather than biological extremities achieved in model plants.

One of several advantages of Arabidopsis over cereals as a system is the relative ease with which reasonably stable homozygous transgenics can be obtained. This is mainly due to the application of the *Agrobacterium tumefaciens* transformation system and ease of regeneration. However, more recently, significant success has been achieved with *Agrobacterium*-mediated transformation in cereals, notably in maize (Ishida et al. 2007), but also rice (Datta and Datta 2006), wheat (Wu et al. 2008), and other cereal crops such as barley, sorghum, and sugarcane (Shrawat and Lorz 2006). Given that Arabidopsis, despite the experimental convenience it offers and the wealth of available data, is of only limited use

in cereal research, we shall briefly evaluate some aspects of cereal-based experimental systems and tools.

The genome sequencing status of different cereals is at different stages and progressing rapidly. Most advanced is rice with a virtually complete genome of 389 Mb in the public domain (IRGSP 2005). Rice is diploid, and of its 37,544 nontransposable-element-related protein-coding genes, 71% have a putative homolog in Arabidopsis, while 90% of the Arabidopsis proteins have a putative homolog in the predicted rice proteome (IRGSP 2005). The fact that in excess of 20% of rice genes have no homolog in Arabidopsis is a strong indication of significant structural and functional divergence.

An additional level of complexity when working with rice stems from the asymmetry of the genome, meaning the presence of orthologous genes only in a small subset of the species. Genomic studies suggest that structural rearrangements are frequent between genomes in the same species. Two recently sequenced rice genomes, var. Nipponbare (*japonica* rice) and var. 93–11 (*indica* rice), have provided an opportunity to investigate the extent of this polymorphism systematically. When gene contents and the genomic locations between two rice genomes were compared (Ding et al. 2007), it was concluded that at least 10% of the genes in the genomes were either under presence/absence polymorphism (5.2%) or asymmetrically located between genomes (4.7%). The proportion of these "asymmetric genes" varied largely among gene groups in which disease-resistance genes (*R*) and the receptor-like kinase (RLK) gene group had 11.6 and 7.8 times higher proportion of asymmetric genes than housekeeping genes (*Myb* and *MADS*). The significant difference in the proportion of asymmetric genes among gene groups suggests that natural selection is responsible for maintaining genomic asymmetry. However, the nucleotide diversity in 17 *R* genes under presence/absence polymorphism was generally low. It was concluded that the genomic symmetry was disrupted by 10% of asymmetric genes, thus causing genetic variation through more unequal crossing over, since these genes have no allelic counterparts and therefore are free to pair with homologs at nonallelic loci during meiosis in heterozygotes (Ding et al. 2007).

With regard to functional analyses in rice, there are several tools available for expression analyses. They include the "Rice Expression Database" (RED) (http://red.dna.affrc.go.jp/RED/) and notably the NSF Rice Oligonucleotide Array Project (www.ricearray.org/), where the whole genome can be queried for multiple expression conditions.

While rice is representative of temperate grasses using "C3" photosynthesis, sorghum (*Sorghum bicolour*), like sugarcane, is representative of the tropical grasses using "C4" photosynthesis, which requires a complex

combination of adaptations, both structural and functional, resulting in more efficient carbon assimilation at higher temperatures. For these reasons, sorghum is a preferred candidate for biofuel production.

Sorghum has a genome size of approximately 770 Mb arranged in $2n = 20$ chromosomes encoding 54,067 proteins. More recently, initial assembly, annotation, and preliminary analysis of the sorghum genome was announced (Paterson et al. 2009). Considering that sorghum shows comparatively high abiotic stress tolerance, it is not surprising that transcriptomic responses to dehydration, high salinity, ABA (Buchanan et al. 2005), methyl jasmonate, salicylic acid, and aminocyclopropane carboxylic acid (Salzman et al. 2005) have been investigated with a view to improving our basic understanding of how plants respond to stresses and thus serve as a source of genes of potential value to agriculture.

Sorghum and sugarcane are thought to have branched from a common ancestor about 5 million years ago. The two species have largely retained a common gene order, and some genotypes can still be intercrossed. However, sugarcane has undergone at least two cycles of whole-genome duplication, resulting in a genome larger than that of *Homo sapiens* and with fourfold or higher redundancy of most genes. By contrast, sorghum is diploid with a genome about 25% the size of maize (*Zea mays*) or sugarcane (*Saccharum officinarum*), the latter having arguably the most complex genome of any crop plant (Cuadrado et al. 2004).

The *Sorghum* genus is also noteworthy in that it includes "Johnson grass" (*S. halepense*), one of the most noxious weeds. The same features that make it so persistent are actually desirable in many forage, turf, and biomass crops that are both genetically complex and much less studied. Therefore, sorghum may inform novel approaches and tools relevant to weed biology and thus inform strategies toward the improvement of a wide range of other crops.

While maize is and has been a classical model for genetic and physiological studies as well as being a crop of great economic importance, its genome is just becoming available (CINVESTAV, Irapuato, Mexico, 2007, unpublished results). The reasons for this are the large size of the genome and the expectation that upward of 80% of the genome consists of repetitive elements that are unsuitable for conventional sequencing and genome assembly methods. More promising approaches are based on targeting gene-rich regions rather than concentrating on whole-genome sequencing.

Positional cloning remains a powerful method for gene identification in Arabidopsis. With the completion of the rice genome, positional cloning in rice has also taken off, leading to the cloning of several quantitative trait loci (see Section III.A; also reviewed in Dwivedi

et al. 2007). In maize, with its large genome, this method was considered nearly impossible because of the vast amounts of repetitive DNA. Nevertheless, conserved synteny across the cereal genomes together with new maize resources made this approach feasible (Bortiri et al. 2006). The consequently enabled "chromosome walking" is much more efficient than gene isolation by transposon tagging. In addition, there are a number of Web sites dedicated to genome resources (e.g., www.maizegenome.org/, www.maizegdb.org/genome/ and www.maizecdna.org/outreach/resources.html). Expression data can be viewed and analyzed for more than 55,000 genes (www.maizearray.org). The studies in the array database include aluminum treatment and tolerance, response of maize to far-red light and UV-B, low or high nitrogen, as well as tissue specific expression.

In wheat, the situation is somewhat similar to maize in that its hexaploid genome has not been completely characterized due to its complexity, large size of about 17,000 Mb, and the high content of repetitive elements within the genome. However, a number of genomic resources have been developed or are being developed, including a collection of more than 500,000 wheat-expressed sequence tags (EST), bacterial artificial chromosome clones, and more than 5,000 bin-mapped EST markers (www.tigr.org/tdb/e2k1/tae1/). A wheat genome microarray offers comprehensive coverage of the wheat genome containing, for example, 61,127 probe sets representing 55,052 transcripts for all 42 chromosomes (GeneChip® Wheat Genome Array) in the genome for mapping translocation breakpoints (Bhat et al. 2007) or studying expression profiles (Gregersen and Holm 2007). Recently aspects of genetic engineering in wheat have been reviewed (Bhalla 2006) and, much like in other cereals, the emphasis is on increasing abiotic stress tolerance (e.g. drought, salt, oxidative stress, aluminum, boron, and temperature) and improving responses to fungal, bacterial, and viral pathogens as well as insect and nematode pests. Other targets for biotechnological interventions are agronomic traits such as improved grain quality, including improved micronutrient content.

Barley is the fifth most produced cereal, its use being mostly in animal feeds and production for malting. While its genome (5,000 Mb) is more than three times smaller than that of wheat, we are not likely to see its genome assembled before that of wheat. Again, in barley much like in wheat, several generations of selectable molecular markers have been included in the genetic maps, and large numbers of qualitative and quantitative traits have been located, some of which are being selected in marker-assisted breeding. In addition, the number of EST markers generated is steadily increasing, and these have been used for the

development of functional molecular markers, preparation of transcript maps, and construction of cDNA arrays. These functional genomic resources combined with new approaches, such as expression genetics, association mapping, allele mining, and bioinformatics tools and their applications, will support the identification of genes responsible for desirable traits and their deployment in breeding programs (Varshney et al. 2007).

## D. From Target Gene Selection to Increased Abiotic Stress Tolerance

Since all single-gene (and/or single-promoter) modifications have profound systemic implications that will affect the whole organism and ultimately agronomical traits, a systems-based approach to target gene identification and modification is called for (Fig. 2.1). This requires the availability of new tools that can extract and analyze information from
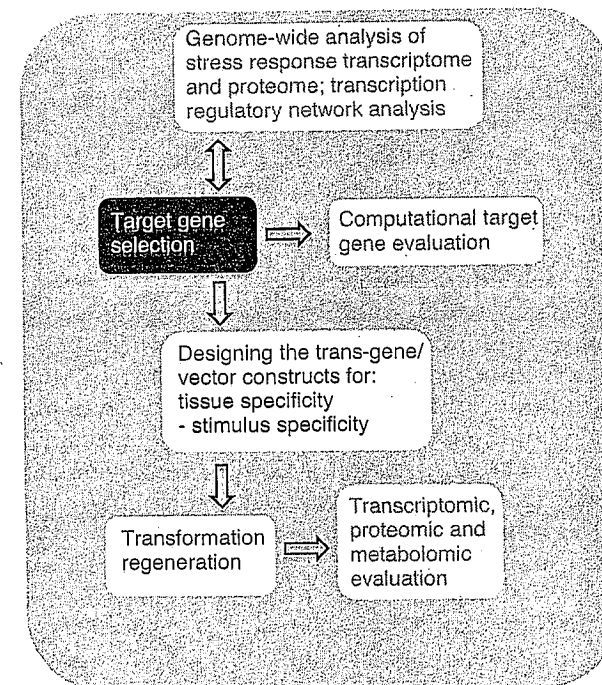


Fig. 2.1. Overview of the key steps in the development of suitable transgenics with increased abiotic stress tolerance.

the ever-increasing quantity of data. One such tool is transcription regulatory network builders.

A transcription regulatory network is a representation of relatedness (presented as a link/edge in the network) based on significant promoter similarities, such as shared transcriptional regulatory potential as represented by the identified common motif families. The rationale behind finding other genes in a genome that share a high number of common family motifs with a particular target gene is that these genes are likely to be coregulated with the original gene and their functions would be complementary to each other in producing common cellular responses. In other words, a promoter-based network represents dynamic functional similarities of coregulated gene families in pathways or in response types. Typically, one would start with a target gene and either its functional or structural relatives (say in a pathway) or one would look for expression of correlated genes. In order to build the actual network, promoter sequences [−2000, +200] of these genes relative to the transcription start site (TSS) need to be extracted. Using the "Dragon Motif Builder" system (DMB; Huang et al. 2005), an *ab-initio* methodology is applied to determine most common motif families present in the analyzed promoter sequences. For each of these motifs or motif families (cis-elements), one determines the consensus sequence and then matches this sequence to the known binding sites of plant transcription factors (TFs). For this task, the PATCH program of the Transfac Professional version 10.4 database is used. In summary, the output is twofold: (1) a network that can be viewed and displayed in different formats (using, e.g., "Cytoscape," a program in the public domain) and (2) a list of specific TFs that are annotated as being involved in specific responses can be obtained. The interpretation of the data, including the expression data from microarray or serial analysis of gene expression (SAGE) databases—often referred to as expression profiling—will then allow one to interpret responses of individual genes or groups of genes in a systemic transcriptional context. This approach, together with gene expression correlation and gene ontology category studies, will yield invaluable new information about and insights into gene function(s), thereby greatly enhancing target gene selection and evaluation (Fig. 2.1).

An important suite of tools for target gene identification are controlled and structured vocabularies, such as Gene Ontology (www.geneontology.org), that can significantly contribute to the analysis of genome-wide response patterns. The Gene Ontologies (GO) addresses the need for uniform and consistent descriptions of gene products in terms of their associated biological processes, cellular components, and molecular functions in different databases and thus provides a link between

different (model) species. Simplified vocabularies, such as the Plant GO slim (www.geneontology.org/GO.slims.shtml), are particularly useful in that they provide a summary of the results of GO annotation of a genome, microarray, or cDNA collection when broad classification of gene product function is required.

Since the response to abiotic stress is highly complex, it is unlikely that modification of a single structural or metabolic component will be suitable for engineering drought and salt tolerance. It is more likely that the molecular modulation of signaling molecules can mediate stress response pathways and consequently may enable successful increases in biotic stress resistance. However, the danger of such a top-down approach is that it could cause undesirably broad responses. Still, a particularly encouraging recent example illustrates potential benefits of using signaling components that critically depend on posttranslational activation. The SRK2C kinase in Arabidopsis is activated by osmotic stress, and overexpression of this kinase confers increased drought tolerance (Umezawa et al. 2004); conversely, knockout mutants of SRK2C exhibited drought hypersensitivity in their roots, suggesting that SRK2C is indeed a positive regulator of drought tolerance in Arabidopsis roots. Despite accumulation of SRK2C mRNA, there was no detectable SRK2C kinase activity in untreated overexpressing transgenic lines. However, after drought, SRK2C kinase activity increased much more in the overexpressors compared to the wild type. Microarray analysis of untreated transgenic lines identified only a small number of genes upregulated compared to the wild type suggesting that (1) the majority of SRK2C effects happen after activation of the kinase by drought and (2) the extra cost of transcription due to the upregulation of coregulated genes is kept low. The use of signaling components that require stress activation despite overexpression of the gene may thus prove to be a promising approach, since the functional response ultimately is triggered by the abiotic stress itself. In this sense, we are looking at a modified stimulus specific response rather than a preventive and almost certainly more costly adaptation strategy. While it is by no means sure that homologous genes in cereals would be showing equally promising results, we have reasons to believe that such a top-down strategy can yield promising results in systems other than Arabidopsis.

Furthermore, it may turn out that microRNAs (miRNAs) may have a role in stress tolerance bioengineering. MiRNAs are single-stranded RNA molecules of about 21 to 23 nucleotides in length that regulate gene expression. They are not translated; instead, they are processed from primary transcripts known as pri-miRNA to short-stem loop structures called pre-miRNA and finally to functional miRNA. Mature

miRNA molecules are partially complementary to one or more mRNA molecules, and their main function is to downregulate gene expression. A recent report suggested that miRNAs are responsive to high salt and water stress (Sunkar and Zhu 2004). These small regulatory RNAs affect gene expression through either degradation of transcripts or inhibition of translation (Dugas and Bartel 2004). Genome-wide expression profiling of plants with altered miRNA expression has already been used to identify downstream target genes of miRNAs (Palatnik et al. 2003) and may prove to be a valuable tool for understanding the role that this class of transcript has in the adaptation of a plant to abiotic stress. It is conceivable that the design of specific miRNAs could be yet another emerging approach to genetic engineering. The limitation of this method is that the expression of target genes can be modulated downward only, but it might be useful to target transcriptional repressors of abiotic stress responses identified via analysis of whole-genome expression profiles.

Engineering cereals with increased abiotic stress tolerance has become more and more reliant on novel systems approaches, including genomics, proteomics, and metabolomics. These approaches have led to new computational and predicting tools as well as to large and growing data repositories that are invaluable for all steps, from the identification of target genes through to the evaluation of target genes and performance testing of engineered species. Naturally, the full benefit of this approach requires access to the complete genomes.

At the center is the identification of target genes (black box). Computational support tools include transcriptomics, proteomics (including the analysis of the phospho-proteome), and metabolomics (in red) as well as the promoter content analysis that will identify coregulated genes and putative transcription factors that play a critical role in the target gene regulation. In addition, in the model organism like *Arabidopsis*, extended computational target gene evaluation is made possible by co-expression analysis of candidate target genes and expression studies in mutants. While these investigations are not directly applicable to cereals, they still yield considerable insight into abiotic stress response biology. The step following regeneration includes the use of "omics" technologies to assess transgenic interventions *in planta*.

## IV. OUTLOOK

In the last two decades, crop genetic enhancement has made significant progress toward developing phenotypic screens, identifying novel sources of genetic variation, dissecting physiological and molecular

basis of the response and adaptation to abiotic stresses, and detecting multiple genes or quantitative trait loci associated with tolerance to abiotic stress in cereals. While resistance to aluminum is associated with major gene(s), drought and salinity tolerance are multigenic. Traditional approaches to breeding for drought and salinity tolerance had limited successes; however, when supported by applied genomics tools, the QTL associated with drought tolerance in maize, rice, and pearl millet; salinity tolerance QTL in rice, wheat, and barley; and aluminum tolerance QTL in wheat and barley were introduced into improved genetic background. Wild relatives in barley, rice, and wheat have contributed genes for better adaptation to abiotic stresses; the need to identify new sources of genetic variations from tertiary gene pools and the use of biotechnological tools to transfer these novel genes into genetic resources of cereal crops remains. Candidate genes associated with tolerance to drought, salinity, and aluminum has been proposed. Our understanding of physiological traits associated with adaptation to abiotic stresses has been enhanced, and this knowledge assists in cereal breeding. A major QTL for salinity tolerance in rice and aluminum tolerance in sorghum, wheat, and barley have been cloned, and ongoing research includes cloning major QTL associated with better adaptation to drought in maize.

A large number of candidate genes conferring tolerance to abiotic stresses have been reported in a number of organisms, and some were introduced through genetic transformation. The resulting transgenic rice, barley, maize, and wheat showed improved tolerance to drought, salinity, and extreme variation in temperature. However, in some cases, this tolerance was associated with yield penalty in transgenic plants. Use of stress-inducible promoter rather than constitutive promoter has been suggested to minimize yield penalty in transgenic plants. Moving from single gene intervention to the introduction of multiple genes may enhance the outcome. New approaches are being discovered that avoid disrupting the native genes and have the ability to introduce multiple genes at one time. The introduction of DNA segments containing multiple genes using autonomous mini-chromosomes may be a way forward.

We are currently experiencing a major paradigm shift where the approach of searching for "the one-gene" solution to increased abiotic stress tolerance is superseded by a system-based approach. The system-based approach makes increasing use of the "omics technologies"—genomics, transcriptomics, proteomics, and metabolomics—and uses computer-based tools to predict and monitor transgenic intervention strategies. We have also become aware that the mechanisms that govern responses to stresses such as drought have highly complex temporal

signatures, ranging from seconds in the case of signaling molecules, and second messengers in particular (Donaldson et al. 2004), to long-term adaptive changes that include functional, developmental, and structural changes. In consequence, the biology of abiotic stresses will have to cover multiple aspects from the sensing of stress through to the long-term biological adaptation. It is likely that the latter will most profoundly affect agronomical performance. Clearly, there is no point in engineering abiotic stress resistance into such crops as rice and wheat if the price to pay is inferior yield or quality.

While the system-based approach to genetic engineering or molecular marker assisted breeding may likely lead to positive results, there may be alternative strategies based on hitherto unexplored plants that could be exploited for agricultural use. At the level of experimental model, this may be the case with *Brachypodium distachyon*—purple false brome—which has several advantages since it is a temperate grass with a relatively small genome size (300–320 million base pairs on 5 chromosomes), thus making it useful for genetic mapping and sequencing. *Brachypodium distachyon* is also a small plant with a short life cycle essential for genetic studies.

Finally, there is the important question of public acceptance of genetically modified plants. While "marker-free" genetic engineering is now becoming the norm, it is by no means certain that genetically modified (GM) crops will be acceptable to the majority of consumers. Arguments used on both sides often are not governed by reason. To ask the genetic engineers to affirm, let alone prove, that an intervention is not in all imaginable circumstances without side effects is not possible and never will be. What we may see is the development of novel transgenic strategies that guarantee containment to a large extent of engineered traits. One such strategy is built around chloroplast-based maternally transferred gene technologies. Interestingly, chloroplast transformations recently have been shown to be less stable (in the sense of containing) than initially believed; it was demonstrated that a surprisingly high proportion of genes move from the chloroplast genome to the nucleus (Huang et al. 2003). Finally, when it becomes clear that GM crops can deliver obvious benefits to the consumer, acceptance of transgenic interventions will almost certainly grow.

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# Verticillium Wilt in Solanaceous Crops

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