State of the Field

Abiotic Stress Tolerance in Grasses. From Model Plants to Crop Plants

Mark Tester* and Antony Bacic

Australian Centre for Plant Functional Genomics, Glen Osmond, South Australia, 5064, Australia (M.T., A.B.); School of Agriculture and Wine, University of Adelaide, Adelaide, South Australia, 5005, Australia (M.T.); and School of Botany, University of Melbourne, Victoria, 3010, Australia (A.B.)

Abiotic stresses, notably extremes in temperature, photon irradiance, and supplies of water and inorganic solutes, frequently limit growth and productivity of major crop species such as wheat (*Triticum aestivum*; http://www.cimmyt.org/Research/Wheat/map/research_results/wphysio/wphysio.html). In addition, more than one abiotic stress can occur at one time. For example, high temperature and high photon irradiance often accompany low water supply, which can in turn be exacerbated by subsoil mineral toxicities that constrain root growth. Furthermore, one abiotic stress can decrease a plant's ability to resist a second stress. For example, low water supply can make a plant more susceptible to damage from high irradiance due to the plant's reduced ability to reoxidize NADPH and thus maintain an ability to dissipate energy delivered to the photosynthetic light-harvesting reaction centers.

If a single abiotic stress is to be identified as the most common in limiting the growth of crops worldwide, it most probably is low water supply (Boyer, 1982; Araus et al., 2002). However, other abiotic stresses, notably salinity and acidity, are becoming increasingly significant in limiting growth of both forage grasses and the cereals. Globally, low temperature also is a major limitation of plant growth, and this has a major impact on grasses via, for example, vernalization and low temperature damage at anthesis. In this focus issue, there are articles addressing three aspects of these abiotic stresses.

Traditional approaches to breeding crop plants with improved abiotic stress tolerances have so far met limited success (Richards, 1996). This is due to a number of contributing factors, including: (1) the focus has been on yield rather than on specific traits; (2) the difficulties in breeding for tolerance traits, which include complexities introduced by genotype by environment, or G × E, interactions and the relatively infrequent use of simple physiological traits as measures of tolerance, have been potentially less subject to G × E interferences; and (3) desired traits can only be introduced from closely related species.

Most cereals are moderately sensitive to a wide range of abiotic stresses, and variability in the gene pool generally appears to be relatively small and may provide few opportunities for major step changes in tolerance. Of potentially larger impact on abiotic stress tolerance is the use of genetic manipulation technologies to generate such step changes. Having said this, more immediately achievable, if modest, increases in tolerance may be introgressed into commercial lines from tolerant landraces using marker-assisted breeding approaches (Dubcovsky, 2004), facilitated by recent breakthroughs with positional cloning (e.g. Yan et al., 2003, 2004) that are likely to enable identification of extant tolerance genes within cereal germplasms (see www.acpfg.com.au). Of course, the sequencing of the rice (*Oryza sativa*) genome provides an invaluable resource for work on rice and, by exploiting syntenic alignment with many other grasses (Devos and Gale, 2000), facilitates fine mapping in the unsequenced genomes of many other grasses.

It is exploitation of this latest resource that, combined with steadily increasing transformation frequencies for many grasses, is making the functional genomics approach to the study and manipulation of abiotic stresses in grasses increasingly tractable. The need to use a model plant such as Arabidopsis (*Arabidopsis thaliana*) for such work is steadily decreasing, and will continue to do so, as the principles uncovered in this model organism are refined (or even supplanted) by knowledge gained in the plants that are the ones in which this knowledge needs to be applied (this means, of course, primarily the grasses, both cereals and forage species). Furthermore, in addition to the obvious fundamental differences in development and anatomy between monocotyledons and dicotyledons, many of the mechanisms of tolerance to abiotic stresses can have fundamentally different characteristics between these two major plant groups, so transferring knowledge from Arabidopsis to the major crops often is not possible. For example, when grown in saline soils, many dicotyledonous halophytes accumulate much higher concentrations of Na^+ in their shoots than monocotyledonous halophytes, a feature that may be related to the observation that succulence is observed more commonly in dicotyledons than monocotyledons, particularly the grasses.

The possibilities for increasing tolerance to abiotic stresses are enormous, although it is notable that the actual production of transgenic plants with demonstrably improved abiotic stress tolerance has been slow. There have been dozens of reviews published outlining the possibilities for inducing stress tolerance, but, to date, only a handful of papers have been published presenting results from transgenic plants. It is also notable that in a critique of all the papers claiming to have generated plants with increased salinity tolerance, Flowers (2004) found the critical evidence substantiating such claims to be rather wanting due to incomplete implementation of experiments and/or fundamental errors in the design of experiments arising from incomplete knowledge of the basic physiology of salinity tolerance. Such calls for increased rigor should be heeded.

The possibilities for increasing tolerance to abiotic stresses are reviewed in textbooks (e.g. Taiz and Zeiger, 2002), in a range of review articles (e.g. high Al, Samac and Tesfaye, 2003; high Na^+, Tester and Davenport, 2003; low N, Masclaux et al., 2001; low P, Raghothama, 1999; low temperature, Thomashow, 1999; low water, Shinozaki et al., 2003; ozone, UV-B, etc., Blokhina et al., 2003), in research papers correlating particular traits with increased tolerance, and in ongoing reductionist/reverse genetic work probing the molecular and cellular processes likely to be involved in adaptive plant responses to abiotic stresses.

Examples include the articles in this focus issue, in which work ranges from protein structure (Délye et al., 2005) through cell biology (Gilliham and Tester, 2005) to whole plant processes (Davenport et al., 2005).

Knowledge from such work in grasses can be applied for increasing abiotic stress tolerance in commercial lines. The use of $\delta^{13}C$ assays for screening wheat lines to generate the new drought tolerant line Drysdale is an excellent, if rare, example of the use of new technologies developed from pure plant physiology in otherwise traditional breeding programs to increase abiotic stress tolerance (Rebetzke et al., 2002). There is also promise in the potential application of work in the articles published in this issue—such applications are highlighted in each article.

Physiological knowledge, such as that presented in this focus issue, may also underpin some early breakthroughs using genetic manipulation technologies to increase abiotic stress tolerance in grasses. Again, such promise is discussed by articles in this issue. Examples where such knowledge has led to the development of increased tolerance to abiotic stresses in grasses are discussed below.

Step changes in tolerance may arise from the introduction of de novo characteristics that are apparently completely absent from a particular gene pool. For example, no wheat variety apparently has the ability to synthesize the sugar alcohol mannitol (Abebe et al., 2003). This can be accumulated to large concentrations in some plants, where it can act as a compatible solute, decreasing the osmotic potential in the cyto-

plasm and thus increasing the ability of the cytoplasm to retain water in the face of reduced water supply. However, when the bacterial gene encoding the key enzyme to allow mannitol synthesis was inserted into wheat plants, with expression driven constitutively, it usually caused severe stunting of plants and sterility (Abebe et al., 2003). Interestingly, in the lines that synthesized very low concentrations of mannitol (less than 0.7 μmol g^{-1} fresh weight, a level that is osmotically irrelevant), vegetative growth was inhibited less by either the addition of 150 mM NaCl to the liquid medium in which they were grown or by reduced watering of soil-grown plants. There appeared to be no reduction in growth of these plants in unstressed conditions. It will be interesting to know the effects of this manipulation on seed yield.

The above example involves manipulation of processes involved in some of the later responses to stress, related more to processes of damage limitation, rather than damage prevention. A more preemptive defense against abiotic stress could involve processes involved in the early detection of and response to stress. This approach also has the benefit of potentially facilitating the coordinated response to a stress since many stresses require more than one response for tolerance to occur. In addition, the observation that many abiotic stresses can impinge upon a plant simultaneously has created much interest in investigating the possibility of generating plants with catch-all alterations involving the signaling pathways and their early responses that are common to several abiotic stresses (Seki et al., 2003). This could be done by altering levels or patterns of expression of higher level transcription factors involved in the early responses to stresses, as has been described to some extent in Arabidopsis (Dubouzet et al., 2003). Although increased tolerance was associated with reduced growth in unstressed conditions, placing such genes behind a strong stress-inducible promoter reduced this growth inhibition in unstressed conditions (Kasuga et al., 1999). However, such an approach has not been extensively tested in grasses, although field trials of wheat expressing DREB1A have commenced in Mexico (http://www.cimmyt.org/english/webp/support/news/dreb.htm).

Using the same rationale of trying to prevent damage from occurring in the first place, increased tolerance to NaCl was observed in plants in which expression of a Na^+ transporter, HKT1, was reduced by antisense (Laurie et al., 2002). This was associated with a decrease in net Na^+ influx and root Na^+ concentration. The potential of this manipulation to increase Na^+ tolerance in wheat is significant, especially given the clear empirical observations, such as by Munns et al. (2003), of Na^+ exclusion being proportional to Na^+ tolerance. Related to this concept are the articles by Davenport et al. (2005) and Gilliham and Tester (2005) in this focus issue.

Unfortunately, some of the alterations described above appear to come with a growth and/or yield

penalty in conditions of reduced stress. A strategy to avoid this outcome would be to drive expression of these genes in response to stress by an inducible promoter, as has been done for controlling expression of a gene encoding a key Pro-synthesizing enzyme in rice (Su and Wu, 2004). This has successfully removed inhibition of growth in low-stress conditions. Such an approach should now be attempted for manipulations in wheat.

To conclude, physiological knowledge of the processes of abiotic stress tolerance in grasses is still developing, and it is clear that significantly more effort needs to be invested to both complement and guide both breeding and genetic manipulation programs. It would be desirable that future work exploit further the synergies to be gained by the interfacing of physiological and molecular/genetic research.

LITERATURE CITED

Abebe T, Guenzi AC, Martin B, Cushman JC (2003) Tolerance of mannitol-accumulating transgenic wheat to water stress and salinity. Plant Physiol **131:** 1748–1755

Araus JL, Slafer GA, Reynolds MP, Royo C (2002) Plant breeding and drought in C$_3$ cereals: What should we breed for? Ann Bot (Lond) **89:** 925–940

Blokhina O, Virolainen E, Fagerstedt KV (2003) Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann Bot (Lond) **91:** 179–194

Boyer JS (1982) Plant productivity and environment. Science **218:** 443–448

Davenport R, James RA, Zakrisson-Plogander A, Tester M, Munns R (2005) Control of sodium transport in durum wheat. Plant Physiol **137:** 807–818

Délye C, Zhang X-Q, Michel S, Matéjicek A, Powles SB (2005) Molecular bases for sensitivity to acetyl-coenzyme A carboxylase inhibitors in black-grass. Plant Physiol **137:** 794–806

Devos KM, Gale MD (2000) Genome relationships: the grass model in current research. Plant Cell **12:** 637–646

Dubcovsky J (2004) Marker-assisted selection in public breeding programs: the wheat experience. Crop Sci **44:** 1895–1898

Dubouzet JG, Sakuma Y, Ito Y, Kasuga M, Dubouzet EG, Miura S, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2003) OsDREB genes in rice, *Oryza sativa* L., encode transcription activators that function in drought-, high-salt- and cold-responsive gene expression. Plant J **33:** 751–763

Flowers TJ (2004) Improving crop salt tolerance. J Exp Bot **55:** 307–319

Gilliham M, Tester M (2005) The regulation of anion loading to the maize root xylem. Plant Physiol **137:** 819–828

Kasuga M, Liu Q, Miura S, Yamaguchi-Shinozaki K, Shinozaki K (1999) Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. Nat Biotechnol **17:** 287–291

Laurie S, Feeney KA, Maathuis FJM, Heard PJ, Brown SJ, Leigh RA (2002) A role for HKT1 in sodium uptake by wheat roots. Plant J **32:** 139–149

Masclaux C, Quillere I, Gallais A, Hirel B (2001) The challenge of remobilisation in plant nitrogen economy. A survey of physio-agronomic and molecular approaches. Ann Appl Biol **138:** 69–81

Munns R, Rebetzke GJ, Husain S, James RA, Hare RA (2003) Genetic control of sodium exclusion in durum wheat. Aust J Agric Res **54:** 627–635

Raghothama KG (1999) Phosphate acquisition. Annu Rev Plant Physiol Plant Mol Biol **50:** 665–693

Rebetzke GJ, Condon AG, Richards RA, Farquhar GD (2002) Selection for reduced carbon isotope discrimination increases aerial biomass and grain yield of rainfed bread wheat. Crop Sci **42:** 739–745

Richards RA (1996) Defining selection criteria to improve yield under drought. Plant Growth Regul **20:** 57–166

Samac DA, Tesfaye M (2003) Plant improvement for tolerance to aluminium in acid soils—a review. Plant Cell Tissue Organ Cult **75:** 189–207

Seki M, Kamei A, Yamaguchi-Shinozaki K, Shinozaki K (2003) Molecular responses to drought, salinity and frost: common and different paths for plant protection. Curr Opin Biotechnol **14:** 194–199

Shinozaki K, Yamaguchi-Shinozaki K, Seki M (2003) Regulatory network of gene expression in the drought and cold stress responses. Curr Opin Plant Biol **6:** 410–417

Su J, Wu R (2004) Stress-inducible synthesis of proline in transgenic rice confers faster growth under stress conditions than that with constitutive synthesis. Plant Sci **166:** 941–948

Taiz L, Zeiger E (2002) Plant Physiology, Ed 3. Sinauer, Sunderland, MA

Tester M, Davenport RJ (2003) Na$^+$ transport and Na$^+$ tolerance in higher plants. Ann Bot (Lond) **91:** 503–527

Thomashow MF (1999) Plant cold acclimation: freezing tolerance genes and regulatory mechanisms. Annu Rev Plant Physiol **50:** 571–599

Yan L, Loukoianov A, Tranquilli G, Helguera M, Fahima T, Dubcovsky J (2003) Positional cloning of the wheat vernalization gene. Proc Natl Acad Sci USA **100:** 6263–6268

Yan LL, Loukoianov A, Blechl A, Tranquilli G, Ramakrishna W, SanMiguel P, Bennetzen JL, Echenique V, Dubcovsky J (2004) The wheat VRN2 gene is a flowering repressor down-regulated by vernalization. Science **303:** 1640–1644