

Engineering drought and salinity tolerance in plants: lessons from genome-wide expression profiling in *Arabidopsis*

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World food security is increasingly dependent on continuous crop improvement and, in particular, the development of crops with increased drought and salinity tolerance. The completed genomic sequence of the model plant *Arabidopsis thaliana* and the development of whole-genome microarrays, together with increasing repositories of publicly available data and data analysis tools, have opened new avenues to genome-wide systemic analysis of plant stress responses. Here we outline examples of how this full-genome expression profiling can contribute to our understanding of complex stress responses and the identification and evaluation of novel transgenes that could hold the key to the development of commercially viable and sustainable crop plants.

The salinity and drought problem

There is broad consensus that climate change continues to occur and that stresses from climatic extremes will continue, and possibly increase, and thus impose significant difficulties to plant and crop growth in many parts of the world [1,2]. These difficulties will be particularly pronounced in currently semi-arid agricultural zones and/or under conditions of irrigation that often exacerbate soil salinization [3]. Sustainable and equitable global food security is therefore, at least in part, dependent on the development of crop plants with increased resistance to abiotic stresses, such as drought and salinity. The realization of the urgent need to use rational approaches to develop crop plants with increased abiotic stress tolerance has led to an impressive body of work in the area of plant genetics, plant physiology, plant biochemistry and plant molecular biology [4–7]. However, despite the significant progress in these fields, to date there are no reports of agriculturally successful applications of biotechnology to increasing drought and salinity tolerance. Single-gene modification approaches have been used to confer significant salt tolerance [8–10] in transgenic plants; however, such interventions are likely to unbalance the development and physiology of the plant, thus having a significant

fitness cost. Indeed, salt-tolerant *DREB1A* (GenBank accession no. NM_118680) overexpressors and *ADR1* (GenBank accession no. ATH581996) overexpressors are both severely stunted [10] and recent gene expression profiling of plants lacking the *AtNHX1* vacuolar antiporter (GenBank accession no. AF106324), a gene used to successfully increase salt tolerance [8], indicated that levels of this protein influence a wide range of cellular processes, including development, protein modification and trafficking [11]. The use of stress-inducible promoters can help reduce gross growth effects [10] but such transgenic lines have not been evaluated for fitness parameters such as seed yield. It is becoming increasingly clear that only a systems-based approach can yield insights that will eventually translate into biotechnologically successful applications.

In this article, we argue that new and rapidly expanding resources of the *Arabidopsis thaliana* model system and novel tools available in the post-genomic era can inform integrated strategies towards a sustainable improvement of abiotic stress tolerance in plants. It is not our aim to review the response mechanisms that can be used to increase tolerance to abiotic stress (this has been done recently [12]) but to highlight the potential value of full-genome expression profiling in such engineering.

Target gene discovery

To date, there is no satisfactory experimental crop plant model system available to study water and salt stress at a molecular and systems level. The requirements for a system for this kind of investigation are the complete genomic sequence in the public domain, easy transformation protocols, short generation times, expressed sequence tags (EST), microarray and proteomics data, and ideally a large set of well-characterized mutants. These conditions are true for *Arabidopsis* and with the considerable body of physiological data on ion homeostasis [13] have made *Arabidopsis* the system of choice for molecular and system-wide plant studies of abiotic stress. There are some limitations to using *Arabidopsis* for the study of salt tolerance. For example, plant biomass does not appear to be negatively correlated with shoot sodium

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concentrations [14]. However, it could be argued that the successful strategies of the plant to cope with an increased sodium content could contain important lessons for improvement strategies.

Experiments using the *Arabidopsis* model must be designed in a rational way to increase the likelihood of identifying target genes with the potential for engineering drought and salt tolerance. Water stress (dehydration or osmotic stress), salinity and, to some extent, cold have been treated as a group (e.g. [12]) and overlap; for example, salinity also leads to osmotic stress. Physiological studies have indicated that during salt stress early effects (minutes to hours) on plant growth are due to water stress, whereas salt-specific effects only appear much later (days to weeks) [4]. Many molecular experiments are conducted over short time periods and might not distinguish between these stresses. However, differential calcium and cGMP signatures in response to salt and osmotic stress [15], as well as salt-specific activation of the SOS pathway [16], indicate that differential sensing and signaling networks are operating and are part of early responses. Although the age of the experimental plants and the method used to induce abiotic stress should be compatible with agriculturally plausible biological conditions to gain the full benefit of the model system, it must be stressed that an eventual application to crop plants might not prove to be straightforward. For example, signal transduction and gene regulatory networks could differ between *Arabidopsis* and crop plants, particularly those that are more distantly related.

Microarray gene-expression profiling is one of the major techniques emerging as a result of genomic research that is widely used for target gene selection, the first step towards biotechnological applications. Recently, this approach was used to address the following key issue: does the model plant, in this case the glycophyte *Arabidopsis*, contain genes that can be used to increase salt tolerance, or do halophytes have novel genes absent in glycophytes that confer the trait of interest? Microarray profiling of the halophyte *Thellungiella halophila* (salt cress) with *Arabidopsis* microarray slides found that a relatively small number of genes were upregulated by salt in *T. halophila* compared with *Arabidopsis* (6 versus 40) [17]. However, many genes that were highly expressed in untreated *T. halophila* compared with untreated *Arabidopsis* were genes that are stress-inducible in *Arabidopsis*. This suggests that *T. halophila* constitutively expresses genes that are part of a stress response in *Arabidopsis* and that genes underlying mechanisms of salt tolerance are present in glycophytes. Hence, it is presumably their differential regulation in halophytes that confers salt tolerance. This finding was crucially important to the current assumptions that modifying expression patterns of stress-inducible genes in *Arabidopsis* could increase salt and drought tolerance. However, transcripts in *T. halophila* with low sequence identity to *Arabidopsis*, or transcripts that are unique to *T. halophila*, would not be identified in these experiments. Several paralogs of *Arabidopsis* stress-inducible genes are present in *T. halophila* and absent in *Arabidopsis*, some of which are over-represented in a *T. halophila* EST library generated from salt-stressed plants [18]. The role of such

genes can be tested via forward and reverse genetic studies.

Two microarray studies afforded some insight into the complexity of salt and water stress responses in *Arabidopsis* and indicated that there are large-scale changes in the transcriptome in response to specific abiotic stresses [19]. In the first, microarray slides of 8000 *Arabidopsis* cDNAs were used and highlighted >1000 genes with at least twofold up- or downregulation in response to salt and osmotic stress in seedling roots and shoots [19]. Three hours after treatment, ~20% of the genes whose expression changed after salt or osmotic stress were common to both responses, whereas after 27 h the overlap was <10%. In the second study using 7000 cDNAs, 356 genes whose expression changed by more than fivefold in response to dehydration, and 283 in response to salt, were identified [20]. In this experiment, early and late time points and tissue types were combined and it was reported that ~40% of genes were common to both stress conditions. These experiments were a first indication that the response to salt and dehydration leads to a significant transcriptional change with a complex temporal induction pattern. Only a small percentage of genes showed a consistent change in expression over time [19], whereas the majority showed variable and transient expression patterns [20] consisting mainly of two groups of genes that were upregulated in response to dehydration. One group was induced rapidly and peaked within 2 h of treatment and a second group reached a maximum 10 h after treatment. It is conceivable that the first group includes many regulatory genes whose protein products mediate downstream signal transduction and gene expression of the second group. Consistent with this, functional annotation indicated that several genes encoding transcription factors were present in the early dehydration-inducible class [20].

These studies are not representative of the complete transcriptome and the next generation of microarray experiments have been enabled by the availability of oligonucleotides representing every known and predicted gene in the genome. This advance also resulted in curated, publicly available full-genome microarray data. A particularly valuable resource is the data generated by AtGenExpress, a multinational effort to profile the transcriptome of *Arabidopsis* (<http://web.uni-frankfurt.de/fb15/botanik/mcb/AFGN/atgenex.htm>). The full AtGenExpress data can be accessed via the TAIR (<http://www.Arabidopsis.org>) and NASC (<http://affymetrix.Arabidopsis.info/narrays/experimentbrowse.pl>) databases. In addition, Genevestigator is a user-friendly web-based tool that enables researchers to visualize the expression of relatively small sets of genes from a variety of microarray experiments, including AtGenExpress [21]. Even if we consider the limitations of quantification of microarray data, as well as the fact that analyzed plant organs are composed of tissue and cell types that do not react in a physiologically homogenous way to environmental or hormonal stimuli [22,23], such microarray analyses are still a powerful step towards a comprehensive systemic characterization of stress responses.

As an example of a genome-wide transcriptional investigation, we present the responses to both salt

(150 mM) and osmotic stress (300 mM mannitol) in seedlings corresponding to the publicly available AtGen-Express: stress treatments control samples, osmoticity and salinity (Experiments 120, 122 and 123 in Genevestigator). Signal intensities of replicate arrays were averaged and the time course condensed into an early time point (30 min to 3 h after stress treatment) and late time point (6 h to 24 h after stress treatment) by taking the highest average value within these time frames as representative. Differentially expressed genes were defined as those with a signal intensity at least fivefold higher than the corresponding control value.

This genome wide display (Figure 1) tells us that the responses to salt and osmotic stress are highly differen-

tiated, both temporally and spatially, that the response to osmotic stress is much larger than to salt stress and that there is surprisingly little overlap between the responses (Figure 2a,b), possibly reflecting different adaptation strategies of the plant. It is noteworthy that the distinction between the transcriptional response to salt and osmotic stress is more pronounced in the full genome dataset than in previously published data [19,20] and, strikingly, the difference is most pronounced during the early response in root and late response in shoot (Figure 2a,b). Although salinity leads to both ionic and osmotic stress, the transcriptional response to osmotic

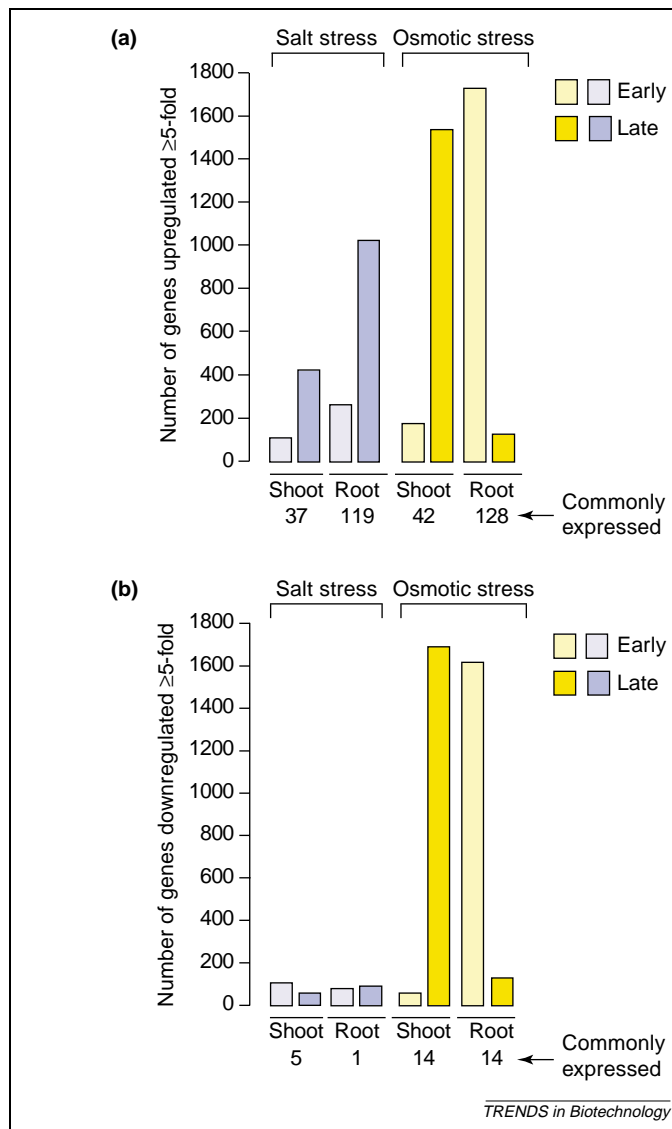


Figure 1. Representation of the genome-wide transcriptional response to salt (150 mM NaCl) and osmotic (300 mM mannitol) stress in *Arabidopsis*. The data are derived from Genevestigator (<http://www.genevestigator.ethz.ch>) experiments 120, 122 and 123, and the values represent the numbers of genes that have (a) increased or (b) decreased expression by a factor of 5 or more after salt (blue) or osmotic (yellow) stress, compared with the control levels. The two organs compared are the shoot and the root and the two stages compared are 'early' (30 min to 3 h after stress treatment; pale bars) and 'late' (6 h to 24 h after stress treatment; dark bars). Commonly expressed values indicate the number of genes that are (a) upregulated or (b) downregulated at least fivefold in both early and late time points in the same tissue.

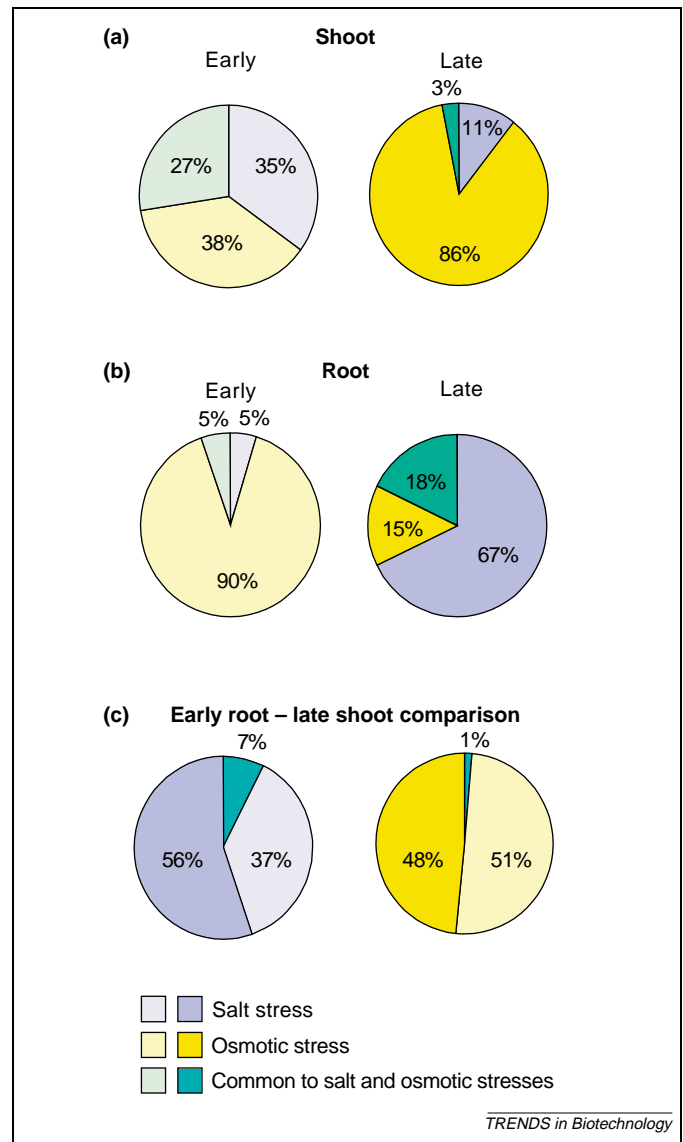


Figure 2. Display of unique and commonly expressed genes in response to salt (150 mM NaCl) and osmotic (300 mM mannitol) stress in *Arabidopsis*. The data are derived from Genevestigator (<http://www.genevestigator.ethz.ch>) experiments 120, 122 and 123. Segments of pie charts indicate the relative percentage of genes differentially expressed (up- or downregulated) after salt stress only (blue), osmotic stress only (yellow) or both (green). This is shown for (a) shoot and (b) root. The two stages compared are 'early' (30 min to 3 h after stress treatment; pale segments) and 'late' (6 h to 24 h after stress treatment; dark segments). (c) Comparison of transcriptional response between early root and late shoot after salt (blue) and osmotic stress (yellow). Segments are the percentage of genes differentially expressed (up- or downregulated) in early root only (pale segments), late shoot only (dark segments) or both (green). 100% represents the total number of at least fivefold transcriptionally changed genes in a given set of samples.

stress is clearly a distinct transcriptional change and not merely a subset of the response to salt stress. In addition, the low number of commonly up- or downregulated genes during early and late responses in both the shoot and the root (Figure 1) suggests a complex transcriptional response pattern with pronounced transient profiles (i.e. that the overall transcriptional profile undergoes significant reprogramming over time). Both salt and osmotic stress are likely to be sensed by roots with subsequent adaptive responses occurring throughout the plant and, certainly in the case of osmotic stress, this can be observed by the magnitude and timing of the transcriptional response in these organs. Furthermore, it is not simply that the shoot has delayed sensing of, and hence response to, osmotic stress compared with the root because the overlap in gene expression changes between early root and late shoot are almost negligible (Figure 2c). In fact, they appear to be two distinct profiles. This type of full genome expression profiling incorporating both spatial and temporal specificity enables researchers a significantly more comprehensive picture of the response of the plant to stress.

Target gene selection

Whole-genome expression profiling can identify a vast number of target genes but the real challenge lies in selecting target genes with an important function in abiotic stress tolerance and biotechnological potential. The use of controlled vocabularies, such as Gene Ontology (<http://www.geneontology.org>), can contribute significantly to the analysis of genome-wide response patterns. Simplified vocabularies, such as the Plant GO slim (<http://www.geneontology.org/GO.slims.shtml>), can help in identifying broad functional categories of potential target genes, in terms of either molecular function or biological process. The response to abiotic stress is complex and it is unlikely that modification of a single component will be suitable for engineering drought and salt tolerance. The manipulation of signaling molecules mediating stress response pathways could enable successful modular intervention; however, such an approach could also lead to an undesirably broad response. A particularly encouraging recent example highlights a potential benefit of using signaling components that must be post-translationally activated. The SRK2C kinase in *Arabidopsis* (GenBank accession no. NM_106478) is activated by osmotic stress and overexpression of this kinase leads to increased drought tolerance [24]. Despite accumulation of SRK2C mRNA, there was no detectable SRK2C kinase activity in untreated overexpressing transgenic lines. By contrast, after dehydration SRK2C kinase activity increased much more in the overexpressors than in wild-type controls. Microarray analysis of untreated transgenic lines identified only a small number of genes that were upregulated compared with wild-type, again suggesting that the majority of the effects of SRK2C occur after activation of the kinase by dehydration. The use of signaling components that require stress-activation rather than just overexpression of the gene might thus prove to be a promising approach by reducing the fitness costs of large-scale constitutive gene expression but priming the plant for a fast and effective adaptation to the stress.

Analysis of potential target genes in additional stress response expression profiling experiments could indicate whether particular target genes are likely to confer cross-tolerance to abiotic stress. Given the different nature of the transcriptional responses to salt and osmotic stress, it is not to be expected that cross-tolerance will always occur; however, within the signaling pathways mediating abiotic stress responses there are numerous points of potential cross-talk [25]. Overexpression of the transcription factor DREB1A has been shown to result in increased drought and salt tolerance [10], whereas enhanced expression of the *ADR1* gene confers significant drought tolerance but increased sensitivity to salinity [26]. A comparative microarray analysis of *ADR1* overexpressing mutants could provide clues as to why it is that salt sensitivity is increased.

Selection of target genes can also be informed through the understanding of networks of signal transduction and gene interaction. Another key outcome of microarray experiments is the identification of groups of co-regulated genes that can be used to help piece together regulatory networks. Given the *Arabidopsis* genome sequence, it is straightforward to obtain upstream promoter sequence of co-regulated genes for analysis of *cis*-elements and potential transcription factors if known. There are several databases that enable *cis*-element or transcription factor searches and the ever-increasing number of *cis*-elements and characterized transcription factors will contribute to building maps of regulatory networks. In our experience, the *Arabidopsis* gene regulatory information server [27] is particularly user-friendly, providing access to >25 000 promoter sequences of annotated *Arabidopsis* genes with a description of putative *cis*-regulatory elements and information on >1550 transcription factors grouped based on conserved DNA-binding domains. Although the database is not suitable for bulk analysis, it will support detailed analysis of individual target genes. Information from expression profiling of transgenic and/or mutant lines with altered expression of transcription factors and/or altered abiotic stress tolerance is also valuable for constructing networks of regulatory interactions.

Transcriptional profiles differ dramatically between root and shoot and hence crude overexpression of a target gene throughout the whole plant is unlikely to be a useful approach. In addition, salt-specific and osmotic stress responses are distinct and complex in terms of timing and tissue specificity [4] and, therefore, understanding the precise nature of transcriptional changes after salt and osmotic stress will be key in target gene selection and in designing downstream transgenic plants. Where a transgene is expressed and how it is expressed are likely to be vital to the goal of identifying genes that can be used in a viable biotechnology strategy to improve salt and drought tolerance, and tissue-specific expression profiling can inform such use of a transgene.

It must be noted that the majority of microarray experiments merely show a correlation between the expression of a gene and a stress response and do not demonstrate function. A genome-wide deletion analysis in yeast indicated that a surprisingly small fraction of genes with significant increases in expression after salt stress

actually altered the ability of the yeast to grow under salinity conditions [28]. There could be a similar scenario in *Arabidopsis*; changes in mRNA accumulation might not be reflected as changes in protein levels and key components of adaptation to abiotic stress might be regulated post-translationally without a change in expression level. Future proteome studies of drought and salt stress will contribute to the selection of target genes with promise but the real test is the phenotype of individual transgenic knockout plants.

Target gene evaluation

After the selection of target genes and design of transgenic lines, post-genomic tools can inform the evaluation of the transgenic constructs for suitability in generating drought and salt-tolerant crop plants. One possibility is to use transcriptional profiling to identify transgenic lines with an increased chance of improving drought or salt tolerance without the heavy penalty of reduced fitness and unsatisfactory agricultural performance. In drug discovery, a similar approach – termed toxicogenomics – uses microarray analysis to predict drugs with an increased probability of safety in a relatively quick and cost-effective manner [29]. Expression profiling of transgenic lines with altered drought and/or salt tolerance could enable early screening to identify lines with focused changes in the transcriptome. The use of public microarray data from stress treatments and information on the biological process associated with particular genes via controlled vocabularies will help in determining specific transcriptional responses. Ultimately, the use of microarray analysis in the evaluation of transgenic lines will depend on the development of a reference database containing expression profiles of transgenic lines with associated data on the degree of drought and/or salt tolerance and fitness measurements, such as transpiration, growth rate and seed set.

A recent exciting development that could prove to be useful in engineering abiotic stress tolerance is the discovery of microRNAs (miRNAs) that are responsive to high salt and water stress [30]. These small regulatory RNAs affect gene expression through either degradation of transcripts or inhibition of translation [31]. Genome-wide expression profiling of plants with altered miRNA expression has already been used to identify downstream target genes of miRNAs [32] and would be a valuable tool for understanding the role that miRNAs have in the adaptation of a plant to abiotic stress. It is conceivable that the design of specific miRNAs could be an emerging approach to genetic engineering. The limitation of this method is that the expression of target genes can only be reduced but this could be used to target transcriptional repressors of abiotic stress responses identified via analysis of whole-genome expression profiles.

Concluding remarks and outlook

The amount of *Arabidopsis* gene expression data, and in particular microarray data, available in the public domain is growing rapidly and is a valuable resource for research on salt and drought tolerance. User-friendly tools provide non-specialists in the field of computational biology with

access to this large repository of under-analyzed data. Genome-wide expression profiling data can be of particular use at three stages of strategies to improve salt and drought tolerance through genetic engineering: (i) during target gene discovery; (ii) aiding in target gene selection; and (iii) at the level of target gene evaluation via profiling of transgenic plants. The creative use of microarray expression profiling extends our understanding of how a plant adapts to abiotic stress as a whole system, what responses occur when and where, and what is the nature of interactions between different components of the response. Deposition of such data into public databases will provide a strong background for improved throughput of candidate genes in biotechnology from the initial identification to an eventual agriculturally successful use in crop plants.

On a more general level, a system-wide approach to understanding plant function will integrate expression data with ecophysiological, physiological, biochemical and metabolic information from wild-type, mutant and knockout lines and will contribute to the construction of a virtual plant simulating complex reaction patterns *in silico*. Such a resource will inform experimental strategies towards improving abiotic stress tolerance in the *Arabidopsis* model system and contribute to engineering improved crops with both desirable and sustainable traits.

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References

- 1 Wang, W. *et al.* (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta* 218, 1–14
- 2 White, J.W. *et al.* (2004) Genomics and crop response to global change: what have we learned? *Field Crops Res.* 90, 165–169
- 3 Boyer, J.S. (1982) Plant productivity and environment. *Science* 218, 443–448
- 4 Munns, R. (2002) Comparative physiology of salt and water stress. *Plant Cell Environ.* 25, 239–250
- 5 Ashraf, M. and Harris, P.J.C. (2004) Potential biochemical indicators of salinity tolerance in plants. *Plant Sci.* 166, 3–16
- 6 Xiong, L. *et al.* (2002) Cell signaling during cold, drought, and salt stress. *Plant Cell* 14, S165–S183
- 7 Flowers, T. (2004) Improving crop salt tolerance. *J. Exp. Bot.* 55, 307–319
- 8 Apse, M.P. *et al.* (1999) Salt tolerance conferred by overexpression of a vacuolar Na⁺/H⁺ antiporter in *Arabidopsis*. *Science* 285, 1256–1258
- 9 Shi, H. *et al.* (2003) Overexpression of a plasma membrane Na⁺/H⁺ antiporter gene improves salt tolerance in *Arabidopsis thaliana*. *Nat. Biotechnol.* 21, 81–85
- 10 Kasuga, M. *et al.* (1999) Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. *Nat. Biotechnol.* 17, 287–291
- 11 Sottosanto, J.B. *et al.* (2004) DNA array analyses of *Arabidopsis thaliana* lacking a vacuolar Na⁺/H⁺ antiporter: impact of AtNHX1 on gene expression. *Plant J.* 40, 752–771
- 12 Vinocur, B. and Altman, A. (2005) Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. *Curr. Opin. Biotechnol.* 16, 123–132
- 13 Salt, D. (2004) Update on plant ionomics. *Plant Physiol.* 136, 2451–2456
- 14 Essah, P.A. *et al.* (2003) Sodium influx and accumulation in *Arabidopsis*. *Plant Physiol.* 133, 307–318

- 15 Donaldson, L. *et al.* (2004) Salt and osmotic stress cause rapid increases in *Arabidopsis thaliana* cGMP levels. *FEBS Lett.* 569, 317–320
- 16 Zhu, J.K. (2002) Salt and drought stress signal transduction in plants. *Annu. Rev. Plant Biol.* 53, 247–273
- 17 Taji, T. *et al.* (2004) Comparative genomics in salt tolerance between *Arabidopsis* and *Arabidopsis*-related halophyte salt cress using *Arabidopsis* microarray. *Plant Physiol.* 135, 1697–1709
- 18 Inan, G. *et al.* (2004) Salt cress. A halophyte and cryophyte *Arabidopsis* relative model system and its applicability to molecular genetic analyses of growth and development of extremophiles. *Plant Physiol.* 135, 1718–1737
- 19 Kreps, J.A. *et al.* (2002) Transcriptome changes for *Arabidopsis* in response to salt, osmotic, and cold stress. *Plant Physiol.* 130, 2129–2141
- 20 Seki, M. *et al.* (2002) Monitoring the expression profiles of 7000 *Arabidopsis* genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. *Plant J.* 31, 279–292
- 21 Zimmermann, P. *et al.* (2004) GENEVESTIGATOR. *Arabidopsis* microarray database and analysis toolbox. *Plant Physiol.* 136, 2621–2632
- 22 Kiegle, E. *et al.* (2000) Cell-type-specific calcium responses to drought, salt and cold in the *Arabidopsis* root. *Plant J.* 23, 267–278
- 23 Ludidi, N. *et al.* (2004) A recombinant plant natriuretic peptide causes rapid and spatially differentiated K⁺, Na⁺ and H⁺ flux changes in *Arabidopsis thaliana* roots. *Plant Cell Physiol.* 45, 1093–1098
- 24 Umezawa, T. *et al.* (2004) SRK2C, a SNF1-related protein kinase 2, improves drought tolerance by controlling stress-responsive gene expression in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. U. S. A.* 101, 17306–17311
- 25 Chinnusamy, V. *et al.* (2004) Molecular genetic perspectives on cross-talk and specificity in abiotic stress signalling in plants. *J. Exp. Bot.* 55, 225–236
- 26 Chini, A. *et al.* (2004) Drought tolerance established by enhanced expression of the CC-NBS-LRR gene, ADR1, requires salicylic acid, EDS1 and ABI1. *Plant J.* 38, 810–822
- 27 Davuluri, R. *et al.* (2003) AGRIS: *Arabidopsis* Gene Regulatory Information Server, an information resource of *Arabidopsis* cis-regulatory elements and transcription factors. *BMC Bioinformatics.* 4, 25
- 28 Giaever, G. *et al.* (2002) Functional profiling of the *Saccharomyces cerevisiae* genome. *Nature* 418, 387–391
- 29 Yang, Y. *et al.* (2004) Toxicogenomics in drug discovery: from preclinical studies to clinical trials. *Chemico-Biol. Inter.* 150, 71–85
- 30 Sunkar, R. and Zhu, J.K. (2004) Novel and stress-regulated microRNAs and other small RNAs from *Arabidopsis*. *Plant Cell* 16, 2001–2019
- 31 Dugas, D.V. and Bartel, B. (2004) MicroRNA regulation of gene expression in plants. *Curr. Opin. Plant Biol.* 7, 512–520
- 32 Palatnik, J. *et al.* (2003) Control of leaf morphogenesis by microRNAs. *Nature* 425, 257–263

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