

Sustaining and enhancing crop productivity in an era of climate change

Ajay Parida* and Suja George

M.S. Swaminathan Research Foundation, Taramani, Chennai 600 113, India

The earth is experiencing a faster change in climate in the 21st century than it had in the past. Abiotic stresses such as drought and salinity, exacerbated by the fast changing climatic conditions pose a major hurdle in sustaining crop productivity. Developing crop plants able to yield better under abiotic stresses offer hope in this situation. Understanding abiotic stress-tolerance mechanisms in a plant system is crucial to improve the stress tolerance. The present review discusses broad molecular mechanisms of plant abiotic stress tolerance and outlines the latest biotechnological advances aiding plant abiotic stress research, with particular reference to the work carried out at the M.S. Swaminathan Research Foundation. The advantages of using extremophiles as model organisms (as conceptualized by M. S. Swaminathan) for identification of novel genetic combinations and understanding stress tolerance are discussed here.

Keywords: Abiotic stress, crop productivity, extremophiles, omics, transgenic.

Introduction

THE earth's climate is not static and has been changing over the centuries. Since the beginning of Industrial Revolution, anthropogenic activities have contributed substantially to accelerating the climate change by adding CO₂ and other greenhouse gases (GHG) to the atmosphere. These GHG emissions have increased the greenhouse effect and caused earth's surface temperature to rise. Climate change predictions indicate that temperatures are likely to increase by 1.4°C to 3.8°C by 2100, relative to 1980–1990 temperatures. Rising sea level is consistent with warming. Sea levels have been estimated to rise between 0.18 and 0.59 m by 2099 as warming sea water expands, and mountain and polar glaciers melt¹. Increasing temperatures and sea level rise negatively impact the existing ecosystem. Warmer temperatures extend the growing season of many plant species leading to more water requirement, while at the same time increasing the drought conditions globally, as less summer rainfall and increased evaporation combine to reduce surface water availability. In some ecosystems, maximum daily

temperatures might exceed the tolerance of indigenous plant or animal and those species/ecosystems, that cannot quickly migrate or adapt, face extinction. Rising sea levels, among other factors contribute to increase in soil salinity. One-fifth of irrigated arable lands in the world has been reported to be adversely influenced by high soil salinity².

Impacts of climate change on agriculture

Increasing events of drought and soil salinity brought out by changing climates is one of the major causes of crop yield losses in agriculture. Several studies have revealed correlations between reduction in crop yield and changing climate^{3,4}. The International Food Policy Research Institute (IFPRI) estimated the climate change induced changes in yield under the projected 2050 climate and compared with 2000 climate for major cereal crops. According to their results, the Asia-Pacific region will experience the worst effect on rice and wheat yields worldwide, and decreased yields could threaten the food security of 1.6 billion people in South Asia. The crop models indicate that in South Asia, average yields in 2050 for crops will decline from 2000 levels by about 50% for wheat, 17% for rice and about 6% for maize because of climate change. In East Asia and the Pacific, yields in 2050 for crops will decline from 2000 levels by up to 20% for rice, 13% for soybean, 16% for wheat and 4% for maize because of climate change⁵. India among the Asian countries is most vulnerable to climate change along with Afghanistan, Bangladesh, Cambodia, Myanmar and Nepal.

To meet the food and nutritional needs of a growing population, increasing crop yield under the changing climatic conditions is crucial. Developing crop plants able to yield better under abiotic stresses such as drought, high temperature, submergence and salinity offer hope in this situation.

Understanding plant abiotic stress tolerance

Understanding abiotic stress-tolerance mechanisms in a plant system is crucial to improve the stress tolerance. Abiotic stress leads to a series of morphological, physiological, biochemical and molecular changes that adversely

*For correspondence. (e-mail: ajay@mssrf.res.in)

affect plant growth and productivity⁶. Drought, salinity, extreme temperatures and oxidative stress are often interconnected and may induce similar cellular damage. For example, drought and/or salinization are manifested primarily as osmotic stress, resulting in the disruption of homeostasis and ion distribution in the cell^{7,8}. Oxidative stress, which frequently accompanies high temperature, salinity, or drought stress, may cause denaturation of functional and structural proteins⁹. As a consequence, these diverse environmental stresses often activate similar cell signalling pathways¹⁰⁻¹³ and cellular responses, such as the production of stress proteins, up-regulation of antioxidants and accumulation of compatible solutes¹⁴⁻¹⁶. The negative effects of abiotic stress can be divided into three broad categories: stress-imposed homeostatic imbalance, disruption of growth and metabolic activities, and generation of reactive oxygen species (ROS).

At the molecular level, plants acclimatize to abiotic stresses by triggering a cascade or network of events that start with stress perception and ends with the expression of a battery of target genes. The key components of the stress response are stress stimuli, transducers, transcription regulators and target genes, the activity of which results in stress responses including morphological, biochemical and physiological changes. Plants make use of unique and common pathways and components in the stress response. This phenomenon, known as cross-tolerance, allows plants to adapt/acclimate to a range of different stresses that might occur simultaneously¹⁷. The molecular adaptive responses could be grouped into three aspects: (i) restoration of homeostasis, which include ion and osmotic homeostasis which is required to re-establish the ion and osmotic gradient; (ii) stress damage control and repair or detoxification, which include scavenging of ROS, protecting nucleic acids and proteins from further damage and removal of damaged proteins, and (iii) growth control, which involves controlling cell division and expansion during stress¹³. Several studies have focused on these three broad aspects for better understanding of plant stress response.

Genes involved in restoration and maintenance of ion and osmotic homeostasis

Maintenance and re-establishment of cellular ion homeostasis during stress and/or following stress is extremely important for plant survival and growth. Ion transporters selectively transport ions and maintain them at physiologically relevant concentrations. Na⁺/H⁺ antiporters play a crucial role in maintaining cellular ion homeostasis, thus permitting plant survival and growth under saline conditions⁷. Plant Na⁺/H⁺ antiporters have been isolated from various species including *Arabidopsis*^{18,19}, rice²⁰ and from the halophytic plants such as *Atriplex gmelini*²¹, *Porteresia coarctata*²² and *Mesembryanthemum crystal-*

*linum*²³. Overexpression of the vacuolar Na⁺/H⁺ antiporter AtNHX1 in *Arabidopsis* plants²⁴ promoted growth and development under salt stress²⁴. The vacuolar H⁺-PPase pumps H⁺ from the cytoplasm into vacuoles with PPI dependent H⁺ transport. Over expression of H⁺-PPase genes from *Thellungiella halophila* (TsVP) enhanced the salt tolerance of tobacco²⁵ and cotton²⁶. Potassium transport in plants is mediated by K⁺ channels as well as high affinity K⁺ transporters both in the plasmalemma and in the tonoplast of plant cells²⁷. *McHKT1*, a potassium transporter isolated from *M. crystallinum*, is up-regulated after a sudden increase in external salinity²⁸. The decreased storage of Na⁺ in the root and enhanced transport to the shoot, with the up-regulation of *McHKT1* suggested to contribute to storage of Na⁺ in the leaves in *M. crystallinum*²⁸. In *Suaeda salsa*, *SsHKT1* transcript was developmentally controlled and significantly up-regulated by K⁺ deprivation and NaCl treatment, suggesting its role in ion homeostasis and salt tolerance²⁹. An AKT1-type K⁺ channel gene from *Puccinellia tenuiflora* (PutAKT1), a salt-tolerant plant was induced by K⁺-starvation stress in the roots and was not down-regulated by the presence of excess Na⁺. *Arabidopsis* plants over-expressing PutAKT1 showed enhanced salt tolerance compared to wild-type plants and a decrease in Na⁺ accumulation in shoot and root³⁰.

One way many plants and other organisms cope with disturbances in osmotic balance is to synthesize and accumulate compounds termed osmoprotectants (or compatible solutes). Osmoprotectants are small neutral molecules that are non-toxic to the cell at molar concentrations and stabilize proteins and cell membranes against the denaturing effect of stress conditions on cellular functions³¹. There are four main classes of solutes that could have an osmotic or protective role: N-containing solutes such as proline and glycine betaine, sugars such as sucrose and raffinose, straight-chain polyhydric alcohols (polyols) such as mannitol and sorbitol, and cyclic polyhydric alcohols (cyclic polyols)³².

Genes involved in glycinebetaine (GB) biosynthetic pathway such as choline monooxygenase (CMO), betaine-aldehyde dehydrogenase (BADH), etc. have been isolated and characterized in detail from many plant systems including *Beta vulgaris* (*BvCMO*)³³, *Atriplex hortensis* (*AhCMO*, *AhBADH*)³⁴, *Atriplex nummularia* (*AmCMO*)³⁵, *Avicennia marina* (*AmBADH*)³⁶ and *Suaeda liaotungensis* (*SIBADH*)³⁷. Over-expression of *AhCMO* improved drought tolerance in transgenic tobacco and the transgenic plants also performed better under salt stress³⁸. Transplastomic tobacco plants over-expressing *BvCMO* gene exhibited increased tolerance to salt and drought stress. Overexpression of *AhBADH* gene into rice³⁹, wheat⁴⁰ and white clover⁴¹ and *SIBADH* into tobacco plants³⁷ improved salt tolerance. Proline is another compatible osmolyte that accumulates in many plants in response to abiotic stresses⁴². Increased levels of proline accumulation have

been observed in salt-stressed calli of *Suaeda nudiflora* suggesting that proline protects the callus cells from membrane damage caused by free radicals formed during salt stress⁴³. The role of genes functioning in proline biosynthesis such as Δ 1-pyrroline-5-carboxylate synthetase (P5CS) and catabolism such as proline dehydrogenases (PDH) in stress tolerance have been demonstrated in many plant systems⁴⁴⁻⁴⁶. Studies have reported correlation between accumulation of polyols, either straight-chain metabolites such as mannitol and sorbitol, or cyclic polyols such as *myo*-inositol or its methylated derivatives such as pinitol with tolerance to drought and/or salinity⁴⁷.

Genes involved in stress damage control, repair and detoxification

Salt, drought, heat and oxidative stress are accompanied by the formation of ROS such as O₂, H₂O₂ and OH[•], which damage membranes and macromolecules. Plants have developed several antioxidation strategies to scavenge these toxic compounds. Enhancement of antioxidant defense in plants can thus increase tolerance to different stress factors. Antioxidants (ROS scavengers) include enzymes such as catalase, superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase, as well as non-enzyme molecules such as ascorbate, glutathione, carotenoids and anthocyanins. Additional compounds, such as osmolytes, proteins (e.g. peroxiredoxin) and amphiphilic molecules (e.g. tocopherol) can also function as ROS scavengers^{49,50}. The expression of a cytosolic copper/zinc superoxide dismutase from mangrove plant *Avicennia marina* (AmSOD1) was found to be induced under drought, salinity and oxidative stress. Transgenic rice plants overexpressing AmSOD1 showed increased tolerance to salinity stress⁵¹. A chloroplast localized glutathione S-transferase from drought tolerant plant *Prosopis juliflora* exhibited glutathione peroxidase activity in *in vitro* assays and conferred drought stress tolerance to transgenic tobacco plants⁵². Monodehydroascorbate reductase (MDAR) plays a key role in regeneration of ascorbate from monodehydroascorbate for ROS scavenging. Transgenic tobacco plants overexpressing a salt inducible chloroplastic MDAR gene from *A. marina* (Am-MDAR) survived better under salt stress compared to untransformed control plants. The transgenic lines showed an enhanced redox state of ascorbate and reduced levels of malondialdehyde indicating its enhanced tolerance to oxidative stress⁵³. Upregulation of a catalase gene (*AmCat1*) and ferritin gene (*AmFer1*) under salt, iron, light stress and direct H₂O₂ stress treatment was observed in *A. marina* confirming their role in oxidative stress response⁵⁴. In Arabidopsis, overexpression of the chyB gene that encodes beta-carotene hydroxylase (an enzyme active in the zeaxanthin biosynthetic pathway) resulted in

a 2-fold increase in the pool of the xanthophyll cycle⁵⁵. These transgenic plants showed greater tolerance to high light and increased temperatures, and it was suggested that the stress protection was most likely due to the action of zeaxanthin in preventing oxidative damage to membranes.

During stress, many enzymes and structural proteins undergo deleterious structural and functional changes. Maintaining proteins in their functional conformation, preventing aggregation of non-native proteins, refolding of denatured proteins to regain their functional conformation and removal of non-functional but potentially harmful polypeptides are particularly important for cell survival under stress. Molecular chaperones are proteins responsible for protein folding, assembly, translocation and degradation in a broad array of normal cellular processes; they also function in the stabilization of proteins and membranes, and can assist in protein refolding under stress conditions.

Many stress-responsive proteins, especially heat-shock proteins (HSPs), have been shown to act as molecular chaperones⁵⁶. Various studies have shown that plant HSPs are not only expressed in response to heat shock but also under water, salt, oxidative stress, and at low temperature and are able to confer stress tolerance in transgenic plants⁵⁷⁻⁶².

Late embryogenesis abundant (LEA) proteins have been reported in a wide range of plant species and fall into a number of families, with diverse structures and functions⁶³. It has been suggested that LEA-type proteins act as water-binding molecules, in ion sequestration and in macromolecule and membrane stabilization⁶⁴. An atypical LEA protein (Pj LEA3) and its promoter were isolated and characterized from *Prosopis juliflora*, a hardy plant reported to be tolerant to drought, salinity, extremes of soil pH, and heavy metal stress. PjLEA3 showed upregulation under H₂O₂ stress in *P. juliflora* leaves⁶⁵. LEA proteins from different families have been shown to be upregulated under various stress conditions and confer stress tolerance in transgenic plants⁶⁶⁻⁶⁹.

Genes involved in growth control

Stress negatively affects plant growth. Plants grown under stressful conditions are almost always smaller than they would be under favourable conditions. One of common cellular responses upon stress application is transient inhibition of proliferation and cell growth. While this response can lead to reduced yield in crops, it has a more general adaptive significance in that the resultant plant is more likely to survive.

Members of the wheat sucrose nonfermentation 1 (SNF1)-related kinases (SnRKs) family were implicated in the regulation of enhanced abiotic stress tolerance and growth under normal and stress conditions. Overexpression

of these genes favoured root development, benefited water and nutrient uptake and did not retard the growth of transgenic plants, indicating their strong potential in transgenic breeding assays for the improvement of abiotic stress tolerance in crops⁷⁰. Members of the *Arabidopsis* SnRK2 family are reported to be strongly activated by ABA and regulate growth and reproduction⁷¹ member of the NIMA (never in mitosis A)-related kinases (NEKs) was implicated to osmotic stress response and regulation of plant growth. Animal and fungi NEKs were reported to regulate cell cycle progression⁷⁰.

Cyclin-dependent protein kinases (CDKs) are a large family of serine/threonine protein kinases with an important role in ensuring that cells progress in an orderly fashion over the different stages of cell division^{72,73}. Almost all CDKs require binding with a cyclin for their activity. In parsley cell suspensions, the expression of mitotic CDK and cyclin genes was suppressed following treatment with both UV irradiation and fungal elicitors⁷⁴. Mild heat stress (30°C) induced a transient cell cycle arrest at G1/S or G2/M in BY2 cell suspensions, depending on the stage at which the stress was applied⁷⁵. A rice gene (*Oryza*; *E2L*) was transcriptionally induced by low temperatures and was proposed to couple cell cycle progression to cold stress response pathway⁷⁰. Similarly, cold stress was reported to induce the rice transcription factor OsMYB3R-2 that resulted to higher transcript levels of several G2/M phase specific genes, including OsCycB1;1. Additionally, plants overexpressing OsCycB1;1 displayed enhanced resistance to cold stress, suggesting that this gene is involved in the regulation of cell cycle progression under chilling conditions⁷⁰. Mild water deficit reduced both the cell division rate and CDKA1 activity almost by half in maize leaves⁷⁶. Decreased transcript levels of CDKA/CDKB and CycA/CycB resulting in transient downregulation of mitotic activity in the shoot and root apex of *Arabidopsis* plants and cell cycle arrest were reported in response to salt stress resulting in a smaller meristem and limited growth⁷⁰. As one of the largest gene families, F-box domain proteins have important roles in regulating various developmental processes and stress responses. Expression of a rice F-box domain gene, MAIF1 was induced rapidly and strongly by ABA and abiotic stresses. Overexpression of MAIF1 reduced ABA sensitivity and abiotic stress tolerance and promoted root growth in rice⁷⁷.

Although plant growth is controlled by a multitude of physiological, biochemical and molecular processes, photosynthesis is a key phenomenon, which contributes substantially to plant growth and development. Abiotic stresses such as drought, salinity and unfavourable temperatures considerably hamper the process of photosynthesis in most plants by altering the ultra structure of the organelles and concentration of various pigments and metabolites including enzymes involved in this process as well as stomatal regulation. Drought, salinity and cold

are well known to induce stomatal closure^{13,78,79}, slowing CO₂ assimilation and consequently reducing the photosynthetic rate. Downregulation of genes involved in photosynthesis was reported in *Arabidopsis* and rice under drought and bacterial stress⁸⁰. Genes functioning in photosynthesis were found to be repressed in rice plants subjected to salt stress⁸¹.

Biotechnological advances aiding plant abiotic stress research

Since 2000, plant science has moved forward into an era of post-genomics. With the next generation sequencing technologies from companies such as Roche, Illumina and Applied Biosystems sequencing of the whole genome of higher organisms is being done with unprecedented speed. 'Omic' technologies adopt a holistic view of the molecules that make up a cell, tissue or organism. They are aimed primarily at the universal detection of genes (genomics), mRNA (transcriptomics), proteins (proteomics) and metabolites (metabolomics) in a specific biological sample in a non-targeted and non-biased manner. The basic aspect of these approaches is that a complex system can be understood more thoroughly if considered as a whole. Researchers all over the world have been enthusiastically exploiting the latest technologies and bioinformatics tools to throw better insights into various facets of abiotic stress tolerance and a great deal of research has been carried out in the recent past in the field of plant abiotic stress tolerance encompassing genomics, transcriptomics, proteomics and metabolomics.

Genomics

Major genomics initiatives have generated valuable data for the elucidation of the expressed portion of the genomes of higher plants. The genome sequencing of *Arabidopsis thaliana* was completed in 2000 (The Arabidopsis Initiative), whereas the finished sequence for rice was published in 2005 (project IRGS). Since then, many higher plant genomes have been completely sequenced. Many of these sequencing projects have been instrumental in comparative analysis of plant genomes, understanding evolutionary relationships between related species and in analysis of stress-tolerance mechanisms. For example, genome sequencing of the model lycophyte *Selaginella moellendorffii* revealed that the transition from a gametophyte- to a sporophyte-dominated life cycle required far fewer new genes than the transition from a nonseed vascular to a flowering plant⁸². Sequencing of genomes from three Brassicaceae species (*Leavenworthia alabamica*, *Sisymbrium irio* and *Aethionema arabicum*) and their joint analysis with six previously sequenced crucifer genomes revealed the central importance of noncoding DNA in gene regulation and evolution⁸³.

Eutrema salsugineum is a halophytic species in the Brassicaceae that can naturally tolerate multiple types of abiotic stresses that typically limit crop productivity, including extreme salinity and cold. It has been widely used as a laboratory model for stress biology research in plants. The reference genome of *E. salsugineum* was sequenced and compared it to its close relative *A. thaliana*⁸⁴. The comparative analyses of the genome structures, protein-coding genes, microRNAs, stress-related pathways, and estimated translation efficiency of proteins between *E. salsugineum* and *A. thaliana* suggested that halophyte adaptation to environmental stresses may occur via a global network adjustment of multiple regulatory mechanisms. Genome resources such as that of *E. salsugineum* help to identify naturally occurring genetic alterations contributing to the adaptation of stress-tolerant plants to abiotic stress. *Thellungiella parvula* is related to *A. thaliana* and is endemic to saline, resource-poor habitats, making it a model for the evolution of plant adaptation to extreme environments. The draft genome of the extremophile *T. parvula* was published in 2011 (ref. 85). The sequencing identified a number of tandem duplications that suggested a possible basis for *T. parvula*'s extremophile lifestyle.

The sequencing of plant genomes enables the first generation of functional genomics that help to define the roles of hundreds of genes, provides unprecedented access to sequence-based markers for breeding, and provides glimpses into plant evolutionary history. Genome resources of extremophiles such as that of *E. salsugineum* and *T. parvula* help to identify naturally occurring genetic alterations contributing to the adaptation of stress-tolerant plants to abiotic stress.

Transcriptomics

Before the advent of cheaper sequencing technologies, transcriptomics was largely limited to model species using oligo arrays hybridized to transcript libraries. However, studies involving large scale sequencing of expressed sequence tags (ESTs) resulted in the identification of expressed genes from various non-model plant species^{86,87}. Many researchers have used EST-based approaches to identify genes upregulated under abiotic stress conditions⁸⁸⁻⁹⁰ resulting in better understanding of stress-tolerance mechanisms in the selected plant species. With the arrival of NGS technologies, transcriptomic studies leading to gene discovery, SNP detection, simple sequence repeat discovery, and gene pathway description were made possible even in non-model organisms through generation of reference transcriptomes *de novo*. Comparative transcriptome profiling of plant tissues between control no-stress and stressed conditions leads to the discovery of genes differentially expressed under various abiotic stresses.

Ammopiptanthus mongolicus is an evergreen broadleaf shrub grown in the central Asian desert reported to be highly tolerant to cold and drought stresses. A comparative transcriptome profiling of drought- and cold-treated *A. mongolicus* seedlings showed that 971 genes were co-regulated by both stresses. Functional enrichment analyses indicated that flavonoid biosynthesis genes were enriched in the differentially expressed genes (DEGs) co-up-regulated by both stresses, while membrane protein genes and genes related to chloroplasts were abundant in the DEGs specifically up-regulated by drought or cold respectively⁹¹. The molecular mechanisms of salt stress tolerance in the halophytic plant *Salicornia europaea* were analysed by whole transcriptome profiling under salt stress. Differentially expressed genes indicated that cell wall metabolism and lignin biosynthetic pathways were significantly enriched in *S. europaea* to promote the development of xylem under saline conditions⁹². Genome-wide gene expression profiling and detailed physiological investigations were carried out in *Gossypium herbaceum* for understanding the molecular mechanism and physiological response to drought conditions⁹³. Comparative transcriptome profiling of osmotic stress tolerant and sensitive *G. herbaceum* accessions indicated that the tolerant accession has the inherent ability to sense drought at a much earlier stage and to respond to it in a much more efficient manner than the sensitive accession.

Transcriptomics have been highly successful in deepening our understanding of plant tolerance to various abiotic stresses. To exploit the maximum potential of these studies, gene resources made available through these studies should be functionally characterized in detail.

Proteomics

The transcriptome analyses of gene expression at the mRNA level have contributed greatly to our understanding of abiotic stress tolerance in plants. However, the level of mRNA does not always correlate well with the level of protein, the key player in the cell⁹⁴. Therefore, it is insufficient to predict protein expression level from quantitative mRNA data. This is mainly due to post-transcriptional regulation mechanisms such as nuclear export and mRNA localization, transcript stability, translational regulation, and protein degradation. Proteome studies aim at the complete set of proteins encoded by the genome and thus complement the transcriptome studies.

Several researchers have used the proteomics approach to identify specific proteins involved in stress response. In rice, a sub-cellular proteomics approach was applied to understand the plasma membrane (PM) proteome response to salt stress⁹⁵. Comparative two-dimensional electrophoresis revealed 24 proteins being differentially expressed in response to salt stress. Most of the proteins identified were involved in several important mechanisms of plant

adaptation to salt stress including regulation of PM pumps and channels, membrane structure, oxidative stress defense, signal transduction, protein folding, and the methyl cycle. These results point out the suitability of proteomics approach in identification of stress-regulated proteins. Using a novel mass spectrometry-based label-free quantitation method, global phosphorylation changes during osmotic stress in *Arabidopsis* have been analysed⁹⁶. The results revealed several known and novel components in the osmotic stress pathway. Recent research on proteomics in wheat⁹⁷ and rice⁹⁸ using the techniques of 2-DE and mass spectrometry, MALDI-TOF, shows changes in protein expression under water stress conditions. Several studies related to the comparative analysis of proteome between plants subjected to salt stress and control treatments have been performed in species like rice^{94,99}, wheat¹⁰⁰, sorghum¹⁰¹ and *Arabidopsis*¹⁰², using the techniques of 2-DE and mass spectrometry, MALDI-TOF/TOF-MS. Proteomic approach has been adopted to study the low-abundant proteins in rice leaf in response to cold stress¹⁰³. Another group of researchers also used proteomics approaches to get new insights into chilling stress responses in rice¹⁰⁴. Interestingly, gene expression analysis of 44 different proteins by quantitative real time PCR showed that the mRNA level was not correlated well with the protein level. This underlines the importance of proteomics in identification of key components in stress tolerance. The same group previously used proteomic successfully to study the salt stress-responsive proteins in rice cv. Nipponbare roots⁹⁴. Analysis of differential expression of rice phosphoproteome under salt stress revealed the critical role of protein phosphorylation in response in plants¹⁰⁵. Gel-based proteomic and LC-MS/MS-based proteomic techniques were used to determine differentially expressed cell wall proteins in wheat under flooding stress¹⁰⁶. The implications of different drought treatments on the protein fractions in grains of winter wheat were examined using (1)H nuclear magnetic resonance spectroscopy followed by chemometric analysis¹⁰⁷. The results showed that a single drought event during the generative stage had as strong influence on protein metabolism as two consecutive events of drought.

Proteomics studies can substantially contribute to revealing virtually every aspect of cellular function in plant stress responses, unravelling possible relationships between protein abundance and/or modification and plant stress tolerance. This would enable the development of novel breeding strategies resulting in an increase in crop productivity and environmental performance.

Metabolomics

Even after the completion of the whole genome sequencing in many plants, networks of gene-to-metabolite

remain largely unknown. To reveal the function of genes involved in metabolic processes and gene-to-metabolite networks, the metabolomics-based approach is regarded as a direct way. In particular, integration of comprehensive gene expression profile with targeted metabolite analysis is shown to be an innovative way for identification of gene function for specific product accumulation in plants¹⁰⁸.

Metabolomics represents the exhaustive profiling of metabolites contained in organism. Proteomics and transcriptomics are both considered to be a flow of media concerning genetic information. In contrast, metabolomic should be thought as being concerned with phenotype¹⁰⁹. Perturbations including environmental change, physical stress, abiotic stress, nutritional stress, mutation, etc. lead to changes in the metabolome. Analysis of these changes serves to fine tune our knowledge on plant response to environmental changes, physical stress, abiotic stress, nutritional stress, mutation, etc.

Metabolomics has been successfully applied to the study of molecular phenotypes of plants in response to abiotic stress in order to find particular patterns associated to stress tolerance¹¹⁰. Capillary electrophoresis mass spectrometry (CE-MS) and capillary electrophoresis diode-array detection (CE-DAD) were used to analyse the dynamic changes in the level of 56 basic metabolites in rice foliage at hourly intervals over a 24 h period¹¹¹. The results showed that in response to environmental stress, glutathione and spermidine fluctuated synchronously with their regulatory targets.

Overexpressing *YK1* gene, the homologue of the HC-toxin reductase (HCTR) gene, in transgenic rice was found to be accompanied with an increase in the amounts of NAD(P)(H). Besides HCTR activity, *YK1* also possessed dihydroflavonol-4-reductase (DFR) activity¹¹². The overexpression of *YK1* was found to induce the activation of enzymes in the NAD synthetic pathway, which resulted in increase in the amount of NAD(P)(H). These results implied that the coupled increase of DFR activity and amounts of NAD(P)(H) may contribute to biotic and abiotic stress tolerance. Profiling several metabolites of glycolysis, the tricarboxylic acid (TCA) cycle and the pentose phosphate pathway in *YK1* transgenic rice by capillary electrophoresis mass spectrometry analysis (CE/MS) was carried out¹¹³. In addition, the concentrations of sugars and ion were quantified. The results indicated that in *YK1* overexpressing plants, the concentrations of cis-aconitate, isocitrate and 2-oxoglutarate were higher in leaves, whereas those of fructose-1,6-bisphosphate and glyceraldehyde-3-phosphate were lower in roots. In seeds, the amounts of free amino acids and metals were altered, whereas sugars in seeds were kept constant. While the overexpression of *YK1* was associated with only slight changes in the amounts of several metabolites analysed, glutathione derivatives were substantially increased in suspension cultured cells.

Transcriptomic and metabolomic approaches were used to study the impact of nitrogen (N) and sulphur (S) deficiency on N and S remobilization from senescing canopy tissues during grain filling in winter wheat¹¹⁴. Nuclear magnetic resonance (NMR) metabolite profiling revealed significant effects of suboptimal N or S supply in leaves but not in developing grain. Analysis of amino acid pools in the grain and leaves revealed a strategy whereby amino acid biosynthesis switches to the production of glutamine during grain filling. Glutamine was found to accumulate in the first 7 days of grain development, prior to conversion to other amino acids and protein in the subsequent 21 days. Transcriptome analysis indicated down-regulation of the terminal steps in many amino acid biosynthetic pathways. These results indicated that vegetative tissue N has a greater control of the timing and extent of nutrient remobilization than S.

Metabolites reflect the integration of gene expression, protein interaction and other different regulatory processes and are therefore closer to the phenotype than mRNA transcripts or proteins alone. Amongst all *-omics* technologies, metabolomics is the most transversal and can be applied to different organisms with little or no modifications. Metabolomics studies have highlighted the essential involvement of many primary and secondary metabolites as direct markers of stress response. The presence/absence and relative accumulation of certain metabolites along with gene expression data provides accurate markers for tolerant crop selection in breeding programmes.

Extremophiles as model systems for understanding plant abiotic stress tolerance

Despite the wealth of information on abiotic stress and stress tolerance in plants, many aspects still remain unclear. One of the effective ways of analysing a stress response is to use model organisms, chosen either for their relative amenability to study or for tolerance to the stress in question. Detailed analysis of physiological and molecular mechanisms underlying stress tolerance in model species will enable future molecular dissection of tolerance mechanisms in important crop plants.

A. thaliana has been a long time favourite of plant biologists due to its small genome, availability of extensive genetic and physical map of all chromosomes, rapid life cycle, prolific seed production, easy cultivation in restricted space, efficient transformation methods utilizing *Agrobacterium tumefaciens* and the availability of a large number of mutant lines and genomic resources. Many of the abiotic stress tolerance pathways were first deduced in *Arabidopsis* (SOS, pathway of salt tolerance)⁸, with the advent of modern biotechnological tools, more researchers have begun to choose stress-tolerant plants as model systems for understanding plant abiotic stress responses. It is possible that these tolerant plants might have evolved

unique pathways of stress tolerance, the elucidation of which would greatly improve our understanding of stress tolerance and would help in engineering similar pathways in susceptible economically important plant species.

Regular drought-tolerant plants can withstand moderate dehydration conditions of about 30% water loss. In contrast, desiccation-tolerant plants (generally referred to as resurrection plants) can further tolerate cell dehydration (around 90% water loss) and also have the ability to rehydrate successfully. Resurrection plants have been widely used as model plants for dehydration studies¹¹⁵. Transcriptome profiling of the resurrection plant *Craterostigma plantagineum* leaves at four stages of dehydration and rehydration revealed mechanisms used to survive environments with extreme dehydration and restricted seasonal water¹¹⁶. *P. juliflora* is an extremely drought-tolerant tree species of Fabaceae, which in addition to being drought tolerant, is found to be tolerant to salinity, heavy metals and extremes of pH. Large scale EST sequencing of a drought enriched leaf tissue library of *P. juliflora* resulted in the identification of several genes functioning in stress tolerance in this species⁸⁸. Several stress-responsive genes and their promoters were characterized from this plant^{52,65,117–119}.

Similarly, the nature of salinity tolerance was studied in several halophytes. Salt stress response mechanisms in the ice plant *M. crystallinum* were studied with respect to both the salt-stress-induced C3/CAM shift and the consequent oxidative stress¹²⁰, and through the characterization of Na⁺/K⁺ transporters¹²¹ and aquaporins¹²². *Thellungiella salsuginea* (*T. halophila*) is a halophytic close relative of *Arabidopsis* that tolerates extreme cold, drought, and salinity¹²³. *T. salsuginea*'s short life cycle, self-fertility, amenability to transformation, relatively small genome size and the availability of ecotypes that show a range of stress responses make the species an excellent model for unravelling the factors that constitute abiotic stress tolerance¹²⁴. Specific root ion-channel features leading to superior K⁺/Na⁺ homeostasis under salinity stress compared to *Arabidopsis* have been discovered in this plant¹²⁵. Genome sequencing of *T. halophila* provides insights into its salt tolerance¹²⁴.

A. marina is a pantropical mangrove species that can survive in highly saline conditions. Large scale EST sequencing from salt-stressed leaf tissue library of *A. marina* resulted in the identification of several salt stress responsive genes⁸⁹. Full length cDNAs and promoters of several of these genes were isolated and characterized in detail^{53,126–129}. Different efforts in large-scale EST sequencing and analysis have been performed in a few other halophytes, such as *Suaeda salsa*¹³⁰, *Tamarix hispida*¹³¹, *Limonium sinense*¹³², etc.

The elucidation of molecular mechanisms of stress tolerance in extremophiles will expand the current knowledge on plant abiotic stress response and lead to

extensive applications in generation of economically important abiotic stress-tolerant crop plants.

Molecular strategies for generating stress-tolerant plants

Improving abiotic stress tolerance of sensitive crop varieties with suitable agronomic characteristics through conventional breeding requires identification of genetic variability to different abiotic stresses among existing crop varieties, or among sexually compatible species, and introduction of the tolerance trait into the sensitive variety. Conventional breeding for abiotic stress tolerance has been successful in many crop species including rice, wheat, Indian mustard, maize, etc.¹³³. Ideotype breeding also has been successfully exploited for increasing yield potential in cereals such as wheat, rice and sorghum¹³⁴. Many researchers have conducted wide hybridization with drought, saline tolerant wild species, weedy races, as well as intra-specific groups, to widen gene pools¹³⁵. Breeding strategies that result in accumulation of many tolerance traits in a single variety will achieve higher levels of tolerance¹³⁶. The recent developments in molecular marker analysis have made it feasible to analyse both simply inherited as well as the quantitative traits, and identify individual gene controlling the trait of interest. Molecular markers could be used to tag quantitative trait loci (QTL) and to evaluate their contributions to the phenotype by selecting for favourable alleles at these loci in a marker-aided selection scheme aiming to accelerate genetic advance. QTL mapping followed by marker-assisted selection (MAS) allows to genetically dissect tolerance into discrete QTLs for different physiological traits, and then pyramid them for multiple stress tolerances. Marker-assisted backcrossing (MABC) combines 'foreground' selection of donor alleles linked to QTLs and 'background' selection of recurrent parent alleles in the BC₂ and later generations. Map based cloning of QTLs has led to the identification of important genes in stress tolerance¹³³.

Progress in breeding and the adoption of new varieties has been slow due to the large number of mechanisms involved, the complexity of these mechanisms, and the diversity of target environments where multiple abiotic stresses often coexist. Breeding for abiotic tolerance also requires reliable screening techniques that are rapid enough to keep pace with the large amount of breeding materials generated. The introgression of multiple QTLs into elite varieties is an expensive and laborious process. In the case of stress-related QTLs, the results of MAS are limited^{137,138} owing to difficulties such as QTLs that have epistatic interactions and do not contribute significantly in a novel genetic background. For effective MAS programmes, QTLs must be expressed and beneficial in new genetic backgrounds and need to be stable across different mapping populations and environments. Several mod-

ern plant types developed through breeding do not possess the level of tolerance found in the donors. Difficulty in transferring the tolerance trait arise because donors have too many undesirable traits which are linked to stress tolerance, preventing transfer to an improved plant type. Development of a promising stress-tolerant line through hybridization and selection can take about 8–10 years. This is aggravated by the fact that in areas where abiotic stresses are found, only one cropping season per year is possible. Therefore, conventional breeding methods need to be supplemented with recent advances biotechnology to meet the needs of the growing world population¹³⁹.

During the last 15 years, many independent transgenic experiments have resulted in improved crop varieties with respect to a number of agronomically important traits, including quality improvement and increased abiotic stress tolerance. These improvements could not have been achieved through the application of conventional breeding technologies. Model organisms such as *Arabidopsis*, resurrection plants and halophytes have been used as sources for mining genes for engineering tolerance in sensitive plants^{51,140–143}. Over expression of genes from divergent sources functioning in various aspects of stress tolerance such as efflux of Na⁺ from the cell, compartmentalization of Na⁺ ions inside the cell, oxidative stress tolerance, ion transport, osmotic stress tolerance, transcription factors controlling the expression of genes involved in stress tolerance, etc. in transgenic crop systems have resulted in improved tolerance to abiotic stress without compromising on phenotypic characters and yield levels¹³³. Although abiotic stress tolerance is known to be governed by multiple genes, significant increases in stress tolerance can be achieved by single gene manipulations as revealed by *SOS1*¹⁴⁴, *GST/GPX*¹⁴⁵ and *NHX1*²⁴ over-expression. Due to the similar cellular response to various stresses such as drought, low temperature and salinity, many of these experiments have achieved tolerance to multiple stresses with single gene transformations. These transgenics are capable of growing and surviving better under respective stress conditions as compared to wild type plants without any growth abnormalities. The evaluation of success of transgenic experiments should be based on proper experimentation methods under field conditions at the whole plant level including quantitative estimates of plant growth and taking the transpiration rate into account¹⁴⁶.

Conclusions

Researchers all over the world have been exploring the latest innovations in biotechnology to better understand plant abiotic stress tolerance and to develop crop plants tolerant to these stresses. Genomics, transcriptomics, proteomics and metabolomics investigate different facets of

a given scientific issue like abiotic stress tolerance, but complement each other. Integration of phenotypic, genetic, transcriptomic, proteomic and metabolomic data will enable accurate and detailed gene network reconstruction. This will ultimately result in the elucidation of the molecular pathways involved in complex phenotypic traits. Using extremophiles as model organisms for understanding abiotic stress tolerance would expedite unravelling of stress-tolerance mechanisms. A better understanding of genetic and cellular mechanisms behind abiotic stress tolerance would facilitate generation of tolerant crop plants though conventional as well as modern molecular tools.

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