Understanding molecular alphabets of the plant abiotic stress responses

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Abiotic stresses elicit complex cellular responses. This complexity made many believe for a long time that tolerance to abiotic stresses cannot be experimentally manipulated. Fortunately, this contention has proven wrong in recent years. Considered once to be an arduous task, genetic engineering for increasing tolerance to abiotic stresses has been achieved to an extent. This change has come due to the progress made in exploring and understanding plant abiotic stress responses at whole-plant, physiological, biochemical, cellular and molecular levels. Plant molecular biology is a fast-expanding research frontier of our times. This important branch of science has given several clues in understanding how plants respond under stressful regimes. A great deal of success has been achieved in unveiling gene/protein alterations associated with preparation of plants against the abiotic stresses. In parallel, major progress has been made in the characterization of stress-related promoters and transcription factors as well as stress signalling components. This article takes a broad look at molecular responses of plants to different abiotic stresses.

ALL plants have an in-built ability to adjust to circadian and seasonal environmental variables. In fact, these variables are often decisive factors in controlling certain physiological attributes (such as length of the vegetative phase, onset of the reproductive cycle, flowering intensity, timing of fruit set or of induction of whole plant senescence). Apart from the regular circadian and seasonal perturbations, there may be certain other rapid and unpredicted disturbances in the environment resulting in stressful conditions. For instance, paucity of water for long periods due to lack of irrigation, infrequent rains or lowering of water table causes drought stress, whereas excess water through rain, cyclones or frequent irrigation results in flooding or submergence or anaerobic stress. Similarly, cultivation of plants on saline soils or frequent irrigation with ground water leads to salinity stress and sudden atmospheric heating or cooling due to transient changes in wind patterns, cloud formation or excessive sunlight causes temperature stress. Since most crop plants have not been selected for meeting exigencies caused by such abiotic stress factors, the capacity of these to adjust to such conditions is usually limited.

Abiotic stress negatively influences survival, biomass production and accumulation, and grain yield of most crops[1–3]. Different crop ecosystems are affected by different abiotic stress factors, and to a differential extent[2,3]. Importantly, the degree of susceptibility of different plant species is often varied. There is also some level of variation associated with specific developmental stages of the plant. What adaptations (biochemical, physiological, whole-plant level) will allow survival of plants in response to stress regimes? At the heart of all metabolic adaptations are molecular events, and it is the molecular events that we mean when we aim at altering genetics of crops. But do we understand how plants face stress in terms of molecular alterations? This is the key issue in plant stress molecular biology studies today.

Light is the best studied environmental factor in plant research with respect to molecular details. Both the quality and quantity of light affect photosynthesis and growth of plants. Light is perceived through several different photoreceptors. A battery of molecular components is involved in transduction of light signal to the nuclei where it has been shown to regulate transcription of selective genes, in both positive as well as negative manner. It has been further shown that light-responsive elements (LREs) present in the promoters of the light-regulated genes and the transcription factors associated with light-regulated promoters interact to bring about regulated gene expression (details on the molecular biology of light perception and light-triggered gene expression events can be seen in ref. 4). However as against light, there is scarcity of information on how changes in temperature, water and salt levels are perceived and translated into cellular events.

Genetic engineering is the most revolutionary tool to impact agricultural research in recent years. The period of 1980s onwards has been the 'Phase of Recombinant DNA Technology'. In the recent past, techniques of protein analysis; identification, isolation, cloning and

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characterization of genes, promoter analysis, genetic transformation and new research on genomics and proteomics have made a significant contribution in plant molecular biology. This progress has culminated in production of a range of transgenics for varied traits[5–7]. The issue of genetically engineered abiotic stress-tolerant crops for high level stress tolerance has attracted a great deal of attention too[1,6,8,9]. A wealth of information has been generated on stress proteins that are specifically induced in response to abiotic stresses. Gene libraries enriched for stress-related cDNA clones have been constructed for several plant species. Availability of the genomic clones of stress genes has helped in the identification of the stress-related promoter sequences. Owing to a higher level of sophistication achieved in the isolation of cDNA clones which are present in minute amounts in the gene libraries, identification of genes encoding transcription factors as well as for proteins which mediate stress signalling has become possible in selected instances (refer refs 10–15 for details on these and other related aspects).

The need to further unravel the fundamentals of the plant stress responses is being constantly felt by both plant molecular biologists (for extending the understanding of the stress responses) and plant biotechnologists (for continued progress on raising stress-tolerant transgenic plants). We have presented details of the issues related to the science of raising stress-tolerant transgenics in several recent papers[2,6,7,16–24]. The present article gives a bird's-eye view of molecular changes elicited in plants in response to abiotic stresses (particularly due to lack of water, excess salt, low and high temperature and flooding). For want of space the reference list has been kept to minimal in this article (for more specific references the reader may refer to our earlier publications or e-mail us).

Macro- and micro-level stress effects

It is a general understanding that response of plants to stress agents is particularly of adaptive nature when the stress is sublethal[18,25]. On the other hand, response shown may be biased towards senescence or cell death if the stress is lethal (Figure 1). Application of sublethal stress regimes in experimentation has thus emerged as an effective approach for unveiling the fundamentals of stress responses[17,18]. Employing tools of physiological, biochemical and molecular relevance, intensive efforts have been made to unveil both the constitutive as well as inducible mechanisms associated with the survival of plant cells under stress conditions. It has been shown that while some of the stress effects are common amongst different abiotic stresses, others are unique to a particular stress type[26,27]. At the whole plant level, common effects caused by the above stress factors include reduced seed germination and seedling establishment, poor seedling vigour, decrease in the root length, leaf rolling, reduced pollen viability, leaf senescence, incomplete grain filling and reduction in grain yield[17,18]. On the other hand, at the cellular and sub-cellular level, stress-induced unique changes include increased unsaturation of the membrane lipids in response to low temperature stress, increased levels of different osmolytes in response to osmotic factors (such as dehydration, salinity and low temperature stresses), general repression of protein biosynthesis in response to water and high temperature stresses, selective changes in K^+/Na^+ levels in response to salt stress and finally, up-regulation of glycolytic and enzymes required for alcoholic fermentation by anaerobic stress[2,6].

While the above cellular changes have been dissected with mostly specific probes, a parallel development in stress studies is the application of shot-gun approach which aims at analysing what happens in the system under sublethal stress regimes. This approach banks on

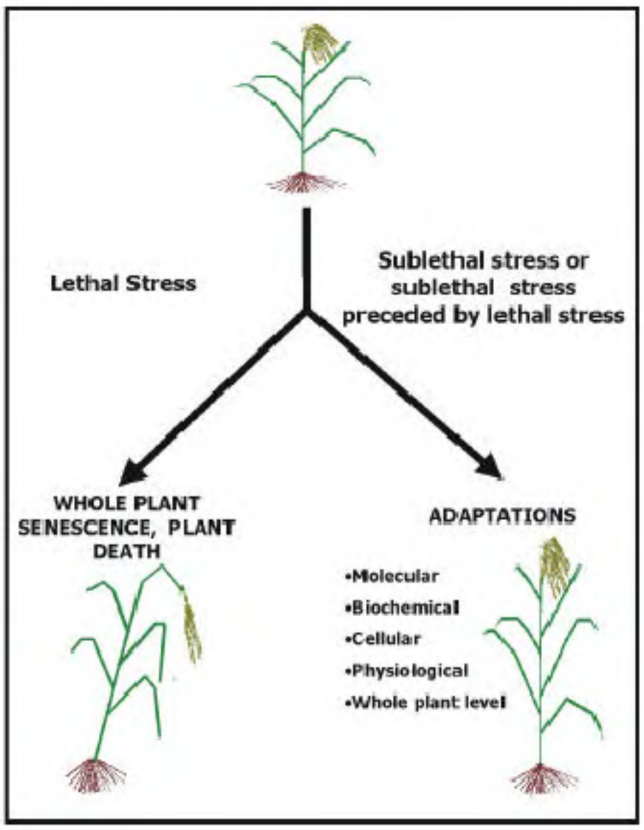

Figure 1. Response of plants to lethal and sublethal level of stresses. The plant in an unfavourable environment could face the following two situations: (i) lethal stress where the plant may ultimately die due to increased senescent activities and (ii) sublethal stress or lethal stress preceded by sublethal stress where certain adaptive changes may occur, leading to survival of the plant. These adaptations could be at the molecular level involving changes in gene expression, synthesis of stress proteins, etc. and at the biochemical level. The latter changes ultimately may bring about the physiological response and finally the whole plant response.

more sophisticated molecular tools and techniques such as examining stress-related changes in protein profiles and differential screening of gene libraries[16,17,26–28]. This development has paved the way to an unprecedented set of discoveries of stress proteins and stress genes, which we take up next.

Stress proteins and stress genes

Studies on the molecular basis of the stress responses began in earnest when it was found that even one minute of increased temperature brings about an altered puffing pattern of the polytene chromosome in *Drosophila*. Subsequently, it was shown that the heat shock (HS) conditions result in an altered protein profile in *Drosophila* cells. Soon thereafter, it was reported that HS induces comparable alterations in the protein profiles of plants too[29]. In the subsequent period, heat shock proteins (HSPs) have been detected and characterized in a number of plant species. Detailed information on biochemical and molecular aspects of the HSPs has been provided in several chapters/reviews[18,30–37]. Selected details on the plant HSPs are presented in Table 1 (refs 21, 24, 32–47). Further, stress proteins akin to HSPs have been identified in response to low temperature stress, salt stress, water stress and anaerobic stress. Selective details on these stress proteins are also presented in Table 1.

The most extensively characterized of the stress genes so far are the heat shock protein (*hsp*) genes, particularly in *Drosophila*, *Saccharomyces cerevisiae* and *E. coli*. HSPs are mostly encoded by nuclear genes, but these proteins are localized in different cell compartments, including cytoplasm, mitochondria, chloroplast and endoplasmic reticulum[18,32]. Broadly, *hsp* genes fall into following two categories based upon their mode of expression: (i) those that are constitutively expressed, often referred to as heat shock cognates (*hsc*), and (ii) those that are strongly induced under heat stress (*hsp*)[18,32]. Several plant *hsp* genes have been cloned and sequenced since the beginning of 1980s. The most thoroughly characterized plant *hsp* genes are those encoding low molecular weight HSPs and HSP70 (ref. 32, Table 1). The nucleotide sequence of the *hsp* genes is remarkably conserved[18,32]. The structural features of the *hsp* are also conserved[18,32]. Apart from *hsp* genes, a large number of genes induced in response to low temperature, water, salt and anaerobic stress have been cloned and characterized in recent years (Table 1).

Identification of the precise physiological roles of most stress genes/proteins has proven a challenging task. The best relevant details are perhaps available for 'Anaerobic Proteins' (ANPs; these are synthesized in response to hypoxia/anoxia stress) as most of these represent enzymes of the glycolytic and alcohol fermentation pathways[21]. From this analysis of the ANPs, it has emerged that respiratory pathway is affected in a major way in response to anaerobic stress[21]. Turning to high temperature stress, a major breakthrough was made in late eighties when biochemical and genetic tools enabled two groups to suggest that HSP70 is involved in the role of chaperoning[48,49]. In subsequent years, HSP60 and HSP90 have also been found important for chaperoning activities. HSP100 is shown to be critically required for resolubilizing protein aggregates formed due to action of stress[50,51].

Turning to other stress responses, 'Water Stress Proteins' (WSPs) have been implicated in several important metabolic functions (e.g. water channel proteins have a role in movement of water through membranes, whereas certain enzymes such as pyrroline 5-carboxylate synthase and choline oxidase are required for the biosynthesis of various osmoprotectants). The 'Late Embryogenesis Abundant' or LEA proteins and osmotin may protect macromolecules and membranes and chaperones and proteases are implicated in protein turnover and protein translocation. The detoxification enzymes such as glutathione S-transferase, catalases, superoxide dismutase and ascorbate peroxidases are involved in protection from reactive singlet oxygen species and finally proteins involved in regulatory functions and in signal transduction, including various protein kinases and transcriptional factors have a broader role in governing stress responses[24,45–47,52]. While selective details are available for the characteristics of several salt-regulated proteins including for osmotin, LEA/'Responsive to ABA' or RAB proteins/dehydrins, salT, NP24 and 'Responsive to Dehydration' or RD29, cellular functioning of these are by and large unknown[17,53]. When precise information on the biochemical role(s) of stress genes/proteins is gained, it should be possible to replace the operational stress gene/protein terms (such as HSPs, WSPs, LEA, RAB, etc.) with more specific functional nomenclature. We hope that studies in the near future will meet this end too!

In spite of the lacunae that exist with respect to the lack of the information on functioning of most stress proteins/genes, work employing stress proteins of known functions (such as choline oxidase, pyrroline 5-carboxylate synthase, mannitol-1 phosphate dehydrogenase, betaine aldehyde dehydrogenase, levan sucrase, trehalose 6-phosphate synthase, myo-inositol-*o*-methyl transferases, glycerol 3-phosphate acyltransferase or superoxide dismutase) as well as on those with relatively unknown functions (such as LEA proteins, HSP100 or osmotin) has made a major contribution in planning strategies for improving stress tolerance through transgenic technology[2,6]. This fact emphasizes that there is a constant need for identification, isolation and characterization of increasing number of stress pro-

Table 1. Stress-related proteins and genes. Selected abbreviations have been expanded in the footnote

Stress proteins and genes	Plant species examined	Inducing agents	Characteristic features	References
Heat shock proteins and genes				
HSP17.6, HSP17.9, HSP18, HSP18.1, HSP21, HSP70, HSP80, HSP81, HSP 83, HSP25, HSP104 *hsp16.9, hsp17.4, hsp17.5E, hsp17.6, hsp17.6L, hsp17.9, hsp18.2, hsp21, hsp22, hsp23.5, hsp70, hsp80, hsp81-2, hsp82B, hsp101*	*Arabidopsis thaliana, Brassica oleracea, Daucus carota, Glycine max, Helianthus annuus, Hordeum vulgare, Nicotiana tabacum, Oryza sativa, Phaseolus aureus, Pisum sativum, Triticum aestivum, Zea mays*	Mostly by high temperature stress; also by water stress, salt stress, low temperature stress, in some cases, also by abscisic acid	Classified as low molecular weight HSPs and high molecular weight HSPs, highly conserved amino acid sequence, nucleotide sequence of the corresponding genes is also conserved, selected HSPs are shown to act as chaperones	30–37
Cold stress proteins and genes				
BN115, BN26, BN19, COR6.6, COR15, COR19, COR11, COR39, CAS15, CAP85, CAP160, CAP85, HVA1, WCS120, WCS19 *blt101, cas17, cas18, cor6.6, cor11, cor15, cor19, cor39, cor47, cor85, cor140, dhn5, wcs120, wcs19, kin1, kin2, lti30, lti-40, lti45, lti65, ltn78, rab18, rab164*	*A. thaliana, H. vulgare, Medicago sativa, O. sativa, Spinacea oleracea, T. aestivum, Z. mays*	Mostly by low temperature stress; also by osmotic, oxidative stress and ABA	Conserved proteins of varied sizes and functions, synthesis of these proteins is mostly transcriptionally regulated, several *cor* genes are cloned and sequenced	38–40
Anaerobic stress proteins and genes				
LDH, ADH, PFK, PGM, ALDOLASE, PDC, MDH, PEPC, GPC, SUS, GPI, TPI, PK, SS, PPDK, XET, HGB *adh, pdc, mdh, pfk, gpc, xet*	*A. thaliana, H. vulgare, Helianthus tuberosum, O. sativa, S. tuberosum, Z. mays*	Mostly by anaerobic stress (caused by flooding or submergence stress)	Discovered initially in maize and latter shown to be universally present, most of the ANPs have been shown to be the enzymes of the fermentative or the glycolytic pathway	21, 41–44
Water stress and salt stress proteins and genes				
ARSK, AB1/2, BND22, CDPK1/2, ERA1, TMK1, MPK3, MYB2, PLC1, PROT, LEA5, LEA14, TAS14, TSW12 *athb-7, cDeT27-45, cyp15a, erd5, edr1, hva1, kin1, lti78, lti65, p5Cs, rab16, rab18, rab-25, rab21, rab17, rab28, rd17, rd19, rd21, rd22, rd29A*	*A. thaliana, B. napus, Craterostigma plantagineum, D. carota, O. sativa, P. sativum, T. aestivum, Z. mays*	Mostly by low water availability and salt stress, also induced by ABA	Varied molecular weights and cellular locations, these proteins are mostly the enzymes involved in diverse functions such as production of different osmolytes, protein degradation, signal transduction events, gene regulation and transport. Roles of some WSPs are not well defined (i.e. such as for dehydrins, late embryogenesis abundant proteins, etc.)	24, 45–47

ADH, alcohol dehydrogenase; ARSK, *Arabidopsis* root specific kinase; *athb-7* encodes *Arabidopsis* homeobox gene; *blt* encodes barley low temperature gene; CAP, cold acclimation protein; CAS, cold acclimation specific protein; COR, cold regulated proteins; CDPK, calcium-dependent protein kinase; LDH, lactate dehydrogenase; MDH, malate dehydrogenase; PDC, pyruvate decarboxylase; PEPC, phosphoenol pyruvate carboxylase; PFK-2, phospho-fructokinase 2; PGM, phospho glyceromutase; PROT, proline transporter; SS, sucrose synthase; TAS, tomato ABA-specific protein; WCS, wheat cold specific protein; XET, xyloglucan endotransglycosylase.

teins as well as for unravelling the functions of those stress proteins which have not been assigned any biochemical role thus far.

Stress-induced promoters

With an increasing number of stress genes becoming available in the cloned form and genetic transformation becoming a routine procedure, characterization of stress-induced promoters (particularly those induced by anaerobic, low or high temperature and salt stresses) has taken a firm footing[13,22,54]. Most of the stress promoters contain an array of stress-specific *cis*-acting elements that are recognized by the requisite transcription factors. Table 2 (refs 55–67) shows selected examples of *cis*-acting sequences which appear important in regulating expression of different genes in response to various abiotic stresses. Most of the work related to functionality of stress promoters has been carried out on *hsp* promoters. The transcriptional regulation of the *hsp* genes is mediated by the core 'Heat Shock Element' (HSE) located in the promoter region of the *hsp* genes, towards 5' of the TATA box. These elements were first identified in *Drosophila hsp70* gene. Comparison of the various *hsp* genes has indicated the presence of at least three 5-bp modules (NGAAN) arranged as contiguous inverted repeats (NGAANNTTCNNGAAN, Table 2). Further, it has been shown that 5′-NGAAN-3′ motif is conserved from yeast, slime mould and nematodes to mammals. The plant *hsp* genes sequenced to date have shown to contain partly overlapping multiple HSEs proximal to the TATA motif. Apart from *hsp* promoters, *rd29* (induced by osmotic stress) and *adh* (induced by anaerobic stress) gene promoters have been the subject of intensive research. The levels of *rd29* mRNA changes differentially in response to dehydration, low temperature, salt stress or exposure to ABA[68]. Corresponding to *rd29* cDNA, two genes, namely *rd29a* and *rd29b* have been isolated and cloned from *Arabidopsis thaliana*. It is reported that *rd29a* has at least two *cis*-acting elements, one involved in ABA-associated response to dehydration and the other induced by changes in the osmotic potential, and *rd29b* contains at least one *cis*-acting element which is involved in ABA responsive slow induction. A novel *cis*-acting 'Dehydration-Responsive Element' (DRE) containing 9 bp 5′-TACCGACAT-3′, involved in the first rapid response of *rd29a* to conditions of dehydration or high salt, has been identified[69]. Detailed studies have shown that the TACCGACAT element is essential for the regulation of dehydration-responsive gene expression of several stress responsive genes and is found in the promoter regions of the several dehydration and other cold inducible genes[70]. The regions of *adh1* gene promoter that are required for anaerobic induction include a string of bases called 'Anoxia Response Element' (ARE) with a consensus sequence of its core element as TGGTTT[44,71]. In fact, this sequence is the underlying basis for inducibility of several anoxia-induced genes[44]. Table 2 provides further information on 'Low Temperature Responsive Element' (LTRE), 'ABA Responsive Element' (ABRE) and a host of other stress-responsive *cis*-acting promoter sequences.

The basic findings on stress promoters have led to a major shift in the paradigm for genetically engineering stress-tolerant crops in recent years[22]. Shinozaki and coworkers[72] over-expressed the *DREB1A* cDNA in *A. thaliana* under the control of CaMV 35S (constitutive) and *rd29a* (stress-induced) promoters. The use of the strong CaMV 35S promoter to drive the expression of DREB1A also resulted in severe growth retardation under normal growing conditions. In contrast, expression of DREB1A under the control of *rd29a* gene promoter caused minimal negative effects on plant growth, while providing even greater tolerance compared to the CaMV promoter. It will become feasible to employ

Table 2. Selective reports on the *cis*-acting elements involved in up-regulation of stress-related genes. Selected abbreviations have been expanded in the footnote

Cis-acting elements	Genes analysed	References
Heat shock element or HSE (consensus sequence NGAANNTTCNNGAAN)	*gm hsp17.3B, gm hsp17.5E, gm hsp17.6L, ha hsp17.6G1*	55–59
Low temperature responsive element or LTRE (consensus sequence A/GCCGAC)	*bn115, cor6.6, cor15, cor78, kin1, wcs120*	60-63
Anaerobic responsive element or ARE (consensus sequence TGGTTT)	*adh1, gpc, aldolase*	64–66
Drought and ABA responsive element (ABRE has PyACGTGGC sequence; ASCE has CATGCATG sequence; MYBRS has PyAACPyPu sequence; MYCRS has CANNTG sequence; DRE1 has CGAGAAGAACCGAGA sequence; DRE2 has CCGGGCCACCGACGCACG sequence; GRA has CACTGGCCGCCC sequence; TT-MOTIF has TTTCGTGT sequence)	*cdeT27-45, em, hva1, hva22, rd29A, wcs120, rab17, rab21, rab28 rd22, dc3*	67

ABRE, ABA responsive element; ASCE, ABA-inducible sph-containing element; MYBRS, MYB recognition sequence; MYCRS, MYC recognition sequence; DRE, drought responsive element; GRA, GC-rich rab activator.

stress-induced promoters for raising transgenics against low or high temperatures, drought or salinity stresses when promoters responding to these are fully characterized and then cloned in suitable plant transformation vector systems.

Stress-related transcription factors

How do stress-associated *cis*-acting promoter elements come in action only when there is a stress? On a bigger canvas, it amounts to asking what is the mechanism(s) behind turning on and off of genes. In this context, the current focus is on transcription factors, which are also called gene switches[73–76]. For the regulation of HS promoter, heat shock transcription factor (HSF) genes have been identified, cloned and characterized from *E. coli*, *S. cerevisiae* and HeLa cells[77]. From tomato cell cultures, three *hsf* clones have been isolated – one of these has been shown to be constitutively expressed, while the other two are induced[78]. *Hsf* genes have also been cloned from plants like *A. thaliana*, *Zea mays* and *Glycine max* (Table 3, refs 54, 70, 78–87). From studies on yeast, *Drosophila* and human systems, it has emerged that HSF is present both in the cytoplasm and in the nucleus in a monomeric form and has no DNA binding activity in the unstressed cells[88]. In response to HS, however HSF assembles into a trimer in which form it can bind to DNA[88]. This response is rapid as activation and binding of HSF to HSE takes place within minutes of temperature elevation. The ability of HSF to promote transcription in yeast is modulated by a heat-induced change in its phosphorylation state[89,90]. Nucleotide sequences of the *hsf* gene, which lead to its oligomerization, have been finely mapped[91]. For the regulation of the *cor* (cold-regulated) genes, CBF1 (CRT/DRE binding factor) has been implicated to be the gene switch. The gene encoding CBF1 has been cloned from *A. thaliana*[92]. It encodes a protein which binds to the CRT/DRE sequences of a number of different *cor* genes (Table 3). *In vivo* foot-printing experiments have suggested that several different DNA binding proteins interact with the *adh1* gene promoter of maize. When grouped together according to the sequence of their binding sites, the *adh1* gene promoter interacting proteins are of the following types: (a) those that have a 5′GTGG 3′ core within their binding site and (b) those that have 5′GCCCC 3′ sequence in the same[93]. It is speculated that the GTGG binding protein may represent a group of general transcription factors, while the proteins that interact with the GCCCC sequence are uniquely a part of the ARE[94]. It is shown that a protein complex (termed ARF-B2) specifically binds to part of the ARE of maize *adh1*. Ferl and coworkers[93] have cloned some of the protein factors involved in regulating the expression of *adh* genes with the aim of understanding the possible associations that exist among the regulatory proteins and diverse cell signalling pathways.

Induction of stress tolerance through engineering for over-expression of transcription factor genes is emerging as an attractive proposition in recent years. The novelty as well as importance of this approach stems from the fact that the *cis*-acting promoter sequences of different stress responsive genes induced in response to the same stress are similar to an extent (discussed above) and thus can be possibly governed at the same time by modulating the transcriptional factor genes. Thomashow and coworkers[92] produced transgenic plants that over-expressed CBF1 in *A. thaliana*. Specific transformed lines exhibited transcripts in greater than the normal amounts of *cor6.6, cor15a, cor47* and *cor78* without the low temperature stimulus. Importantly, it was found that CBF1 over-expression increased tolerance to the freezing stress. Almost a similar approach was successfully used by Schoffl's group[95] to engineer for increased thermotolerance in *A. thaliana*. In this work, constitutive expression of HSF was obtained resulting in constitutive expression of certain HSPs. Table 3 gives a list of selected transcription factors which have been shown to be involved in stress responses.

Table 3. Selective reports on stress-related transcription factor genes/proteins. Selected abbreviations have been expanded in the footnote

Transcription factor	Plant species examined	References
High temperature stress		
At-Hsf1, At-HsfA2, At-HsfB1, AtHsf3, Lp-HsfA1, LpHsfA2, LpHsfB1, Zm-Hsfa, Zm-Hsfb, Zm-Hsfc	*A. thaliana, Lycoperiscon peruvianum, G. max, Z. mays*	54, 78
Cold temperature stress		
CBF1, CBF2, CBF3	*A. thaliana*	79, 80
Anaerobic stress		
AtMYB2	*A. thaliana*	81
Salinity stress		
Alfin1	*M. sativa*	82
Drought and ABA stress		
Athb-7, Athb-12, AtMYB2, DREB1A and 2A, *EmBP-1* and *Rd22BP1*	*A. thaliana, T. aestivum*	70, 83–87

CBF, CRT/DRE binding factor; DREB, DRE binding proteins; EmBP encodes for *Em* binding protein, and Rd22BP1 encodes *Rd22* binding protein.

Stress-related signal transduction components

In systems such as *Drosophila, Xenopus* and mammalian cells, diverse stimuli (such as small molecules,

gases and physical factors such as light and temperature) interact through remarkably conserved signalling mechanisms involving membrane receptors, GTP-binding proteins, G-protein effectors and protein kinases. The generalized scheme of signal transduction implies that the extracellular signal binds to a transmembrane receptor, which in turn activates GTP-binding proteins. The GTP-binding protein either regulates a cascade of kinases (MAPKKK, MAPKK, and MAPK; MAPK stands for mitogen-activated protein kinase) or a G-protein effector (such as adenylate cyclase, cAMP phosphodiesterase, guanylate cyclase, cGMP phosphodiesterase, phospholipase C, ion channels, etc.), leading to a change in the level of intracellular signals called second messengers (such as cAMP, cGMP, protein kinase C, Ca^{2+}-dependent kinases and calmodulin-dependent kinases). It is now clear that plant signal transduction mechanisms have a striking similarity to those in the animal systems in involving receptor-like protein kinases, heteromeric G-proteins, small GTP-binding proteins, cyclic nucleotides, Ca^{2+}/calmodulin, phospholipases, etc[96–99]. Ca^{2+} functions as a second messenger in several plant responses and the basic components of Ca^{2+} signalling such as Ca^{2+} transporters, calmodulin (CaM), CaM-dependent protein kinases, etc. have also been identified in plants[100] (see also other articles in this issue). The involvement of Ca^{2+} in low temperature signalling during cold acclimation has been inferred from the observed transient changes in its cytosolic levels[101]. Both MAPK-like kinase activity and mRNA levels of the components of MAPK cascades reportedly increase in response to environmental stress in plants, suggesting that some of the plant MAPK cascades have an important role in environmental stress signal transduction[102]. Table 4 (refs 103–114) highlights selective examples of signalling components which are associated with the response of the plants to high temperature stress, low temperature stress, osmotic stress, drought stress and anaerobic stress.

It is tempting to speculate that if the signal transduction components are altered such that the sensitivity of cells to stress is reduced or such that a low level of constitutive expression of stress genes is induced, it should be possible to bring about enhanced stress tolerance[6]. This approach worked successfully in *A. thaliana* for raising salt-tolerant transgenic plants by altering stress signalling through the Ca^{2+}/calmodulin-dependent protein phosphatase, calcineurin[20,115]. Yang and coworkers[115] over-expressed the catalytic and the regulatory subunits in transgenic tobacco plants and reconstituted a constitutively active phosphatase *in vivo*. Importantly, several different transgenics exhibiting substantial NaCl tolerance were made and this trait was linked to the genetic inheritance of the *CNB* genes.

Table 4. Selective reports on the signal transduction components involved in expression of stress-related genes. Selected abbreviations have been expanded in the footnote

Gene/protein	Plant species examined	References
Protein kinases		
AtDBF2 (induced by heat, salt, osmotic and cold stress), PKABA1 (induced by ABA), RPK1 (induced by dehydration, low temperature, salt and osmotic stress), AtPIP5K1 (induced by dehydration, ABA or salinity), AtMEKK1 (induced under high salt conditions), 45 kDa protein (induced in water stress, ARSK1 (stimulated by water deficit, NaCl and ABA), OSCPK2 and 11 (induced by anoxia)	*A. thaliana*, *O. sativa*, *P. sativum*, *T. aestivum*, *Z. mays*	103–109
Phosphatases		
AtPTP1 (induced by high salt and negatively regulated by cold stress), TAP42 (induced in response to chilling), AtPP2C (induced by ABA)	*A. thaliana*	110–112
Ca^{2+} binding proteins		
AtCBL1,2,3 (induced by wounding, cold and drought and salt stress), AtCP1 (induced in response to high salt concentration)	*A. thaliana*	113–114

PkABA1, ABA responsive protein kinase 1; RPK1, receptor like protein kinase1; AtPIP5K1, phosphatidylinositol-4-phosphate-5-kinase1; AtMEKK1, MAP kinase kinase kinase1; ARSK1, root specific kinase1; OSCPK, *O. sativa* calcium-dependent protein kinase; AtPTP1, protein tyrosine phosphatase1; TAP46, 2A phosphatase associated protein of 46 kDa; AtPP2C, protein phosphatase 2C; AtCBL, calcineurin B-like Ca^{2+}-binding protein; AtCP1, Ca^{2+} binding protein 1.

Future research avenues

The early work on the possible importance of stress proteins/genes in imparting stress tolerance was based on the following two simple observations: (a) stress proteins are synthesized at the time when there is a general repression of the protein biosynthesis machinery and (b) there is a remarkable level of conservation in structure and other features of stress proteins/genes. As discussed above, the role of stress genes/proteins in stress tolerance has been experimentally verified in selective instances in recent years. To obtain further gains, research in the following areas appears to be worth-pursuing in the future.

1. Our knowledge of stress proteins/genes is still far from complete. While it is reported that more than

100 transcripts are affected in plant cells when subjected to salt stress, only a handful of salt stress genes have been characterized. Abiotic stresses elicit multiple and complex alterations in the profile of stress proteins[26,27]. Further, a large number of stress-related expressed sequence tags (ESTs) have been identified[116]. Clearly there is a need to examine and analyse a much larger number of stress proteins/genes in order to understand the molecular complexity involved in plant abiotic stress responses. This is important for obtaining a complete picture of how plants respond to stresses. Modern methods of gene expression, including those coming through genomics and proteomics research, need to be intensively employed in this venture. The functional genomic approach which aims at better understanding and utilizing the large amount of DNA sequence information, accumulated from sequencing of complete genomes and ESTs, will provide a critical input. The genome of rice is being completely sequenced and complete or partial sequencing of the genomes of other major cereals is also underway. Completion of genome sequences will lead to the availability of a large number of genes with unknown functions. It is possible that some of these genes turn out to be crucial in response of plants to stress conditions. Work with techniques like the DNA microarray chips may allow identification of stress-related genes and thus must be taken up vigorously.

2. An urgent need has been felt for the recruitment of stress-induced promoters for driving expression of stress tolerance genes in genetic engineering experiments[22]. Lack of an in-depth analysis on identification, isolation and cloning of abiotic stress promoters, is the limiting factor in this aspect. As a large number of stress responsive genes have been identified in recent years, there is an urgent need that parallel efforts are made for characterization of the promoter regions, particularly the *cis*-acting regulatory sequences from the available genomic clones. There are other rapid-fire methods (such as the one based on PCR technique) developed in recent years for the isolation of the promoters if the gene sequences are known[117]. Application of such methods would make rapid changes in this respect. However, it is a general feeling that most of the stress-responsive promoters have poor strength of driving gene expression and thus, the expression level of the concerned gene is low compared to those with constitutive promoters[22,118]. It is therefore important that fine manipulation of these promoters is carried out so that their strength is increased without any negative effect with respect to their inducibility pattern. There is also a need that stress-induced promoters are cloned in vectors, which have other desired advantages for plant genetic transformation work. There is some recent work in optimizing vectors with HSE, ARE and ABRE in this respect, but this is yet to come to the level of routine use[22].
3. Isolation and cloning of transcription factor genes is a hot area of research in the present-day plant stress

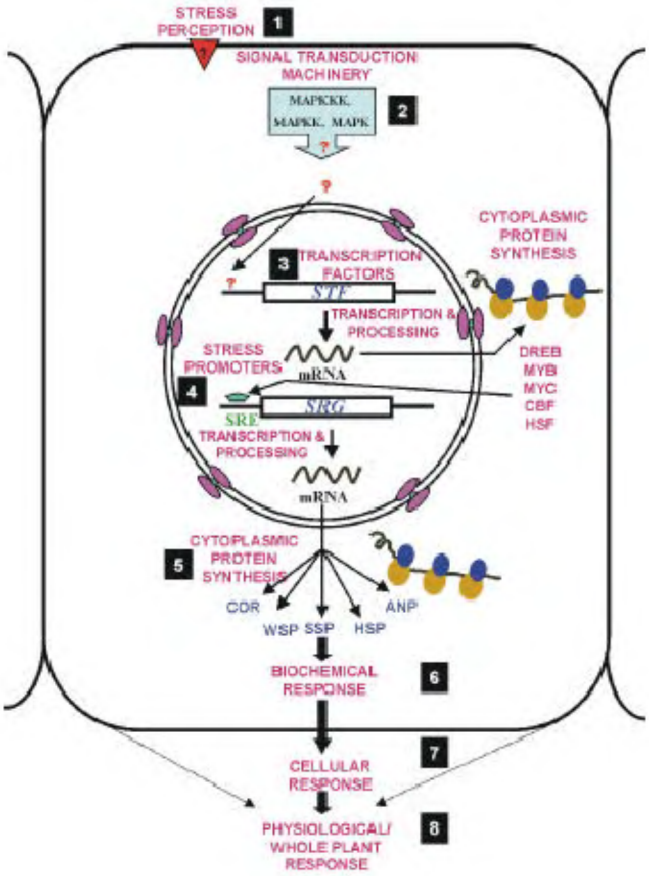

Figure 2. Model depicting various possible events involved in response of plant cells to different abiotic stresses. All these events have not been experimentally shown as yet, but are included in this hypothetical model to depict how the physical stress factors might elicit gene expression changes inside the nucleus of the cells. (**1**), Stress perception may involve specific components, about which not much is known. (**2**), Following the 'sensing' of the stress, stress signal is possibly amplified and transduced through the signal transduction machinery, which may involve protein kinases, phosphatases, and Ca^{2+}-binding proteins. (**3**), Through activated signalling intermediates, the stress signal is transduced inside the nucleus where the genes encoding the stress transcription factors (STF; e.g. *dreb, myc, myb, cbf* and *hsf*) are possibly synthesized/activated. The synthesis of transcription factors must involve cytoplasmic ribosomes, which means that nucleus–cytoplasmic crosstalk is an important feature in this respect. After the synthesis, the *trans*-acting factors re-enter the nucleus where (**4**), they bring about the transcriptional activation of stress responsive promoters. These have stress-responsive elements (SRE; e.g. ABRE, LTRE, DRE, HSE and ARE) to which possibly these transcriptional factors bind. (**5**), Stress responsive genes (*SRG*) are transcribed and translated on the cytoplasmic ribosomes, leading to the synthesis of the stress proteins. (**6**), These stress proteins initiate a biochemical response and subsequently the (**7**), cellular response, which would then bring about the (**8**), physiological and finally the whole plant response. (See text for details on the components shown in the figure).

molecular biology[119]. These genes are normally expressed at low levels. Techniques such as subtractive hybridization, cold-plaque screening, south-western blotting, electrophoretic mobility shift assays (EMSAs), foot-printing or random binding site selection (RBSS) have shown a great deal of potential in the analysis of transcription factor genes in model systems like yeast and HeLa cells[120,121]. There is a need to bring a higher level of sophistication in the analysis of transcription factor genes in plant systems too, but only few laboratories are actively carrying out this programme at present.

4. Research on plant stress signalling mechanism is fast emerging as an important area[122]. However, there is lack of clarity on the cascade of events involved in transduction of abiotic stress signals and it remains a challenge to define these cascades fully. The study of the precise mechanisms underlying stress perceival has been more or less ignored in plants. In yeast, which has been extensively studied in recent years, osmo-sensing mechanism involves specific members of MAP kinase and MAPKK gene families which are implicated for restoring the osmotic gradient across the cell membrane in response to increased external osmolarity[123–125]. In *E. coli*, it has been found that histidine kinases function as sensor molecules that transduce extracellular signals to the cytoplasm where they are received by response regulators[77]. Recently, it is shown that a transmembrane hybrid-type histidine kinase in *Arabidopsis* functions as an osmosensor[126]. However, detailed analysis of such mechanism(s) is awaited in plant systems.

The foregoing account has dealt with the overall progress made in molecular biology of abiotic stress responses. From this presentation, it is clear that the plant response to stress conditions consists of several events, including (1) stress perceival, (2) stress signal transduction, (3) transcriptional activation of stress genes, (4) synthesis and accumulation of stress proteins, resulting finally in (5) biochemical, (6) cellular and (7) physiological manifestations (see Figure 2). This presentation has also highlighted the areas that need to be given further attention. It is beyond any doubt that basic stress molecular biology science has played the important background role in production of stress-tolerant transgenics. There is need to not only continue but to encourage work with added zeal on this important area of plant molecular biology in the days when the new areas of genomics and proteomics are expected to lead to major discoveries.

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