Heat shock proteins – Role in thermotolerance of crop plants

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Thermotolerance is required in crop plants in order to maintain productivity under heat stress. At the cellular level, thermotolerance is linked with the induction of heat shock proteins (HSPs), a response conserved from prokaryotes to eukaryotes. HSPs belong to six families and each family has several members, of which only a few may be involved in acquired thermotolerance. Molecular approaches may help to assign specific role to HSPs involved in thermotolerance. Thermotolerant genotypes show adaptations at various levels of organization besides showing qualitative and quantitative differences in HSPs as compared to the thermosensitive genotypes. In future, HSPs and enzymes with broader thermal kinetic windows may be the desired selection criteria at molecular level for breeding thermotolerant crop plants.

AGRICULTURE production must continue to meet the demands of the growing population. In the past decades agricultural production increased due to higher yields and by bringing more land under agricultural production. The scarcity of productive agricultural land may force us to grow agricultural crops in harsher environments. Temperature is an important environmental factor affecting crop productivity. Crops have high productivity when grown in temperatures optimum for various growth and metabolic processes. Temperatures higher than the optimum decrease both the rate and duration of metabolic processes and thus decrease the yield.

Being poikilothermic, plants have to keep their temperatures below ambient through transpiration. In water-limited environments, transpiration is reduced, thus plants experience both water and high temperature stress. Howard[1] remarked that, 'Wheat production in India is a gamble in temperature'. Even today, the wheat season in North India is limited by temperature at both ends of the crop growth. It has been observed that for every one degree rise in mean temperature over the range of 12.2–27.5°C the wheat yield is reduced by 4%. Therefore, to sustain the agricultural production it is necessary to breed varieties which are tolerant to high temperature stress. Further, the current estimates of global warming predict an increase of 0.5°C annual mean temperature by 1995–2005, 1.5°C by 2050 and 3°C by 2050–2100 AD. These changes in mean values of temperature may be accompanied by high frequencies of extreme levels of heat and moisture.

Thus, it is necessary to understand the physiological and molecular basis of high temperature stress tolerance. In this review, we focus on role of heat shock proteins in high temperature stress tolerance and productivity of crop plants.

Heat shock proteins

Heat stress (5–10°C above the normal growing temperature of organism) induces expression of specific gene families called heat shock genes (*hsps*), which lead to the synthesis of a new set of proteins called heat shock proteins (HSPs). After Tissieres *et al.*[2] demonstrated for the first time that heat-induced chromosomal puffing of *Drosophila melanogaster* was accompanied by the high level expression of an unique set of proteins called heat shock proteins, HSPs have been found in every organism in which it has been sought from unicellar prokaryotes to highly evolved complex multicellular organisms including *Homo sapiens*. In fact, HSP induction upon heat shock is a highly conserved universal genetic response among organisms from Antarctic algae to archaeabacteria[3–5].

The heat shock response of all organisms shares the following common features:

1. Immediately following the heat shock, a new set of unique HSPs are synthesized from newly-transcribed mRNAs.
2. Heat treatments, which induce HSP synthesis, also lead to acquired thermotolerance, i.e. the ability of an organism to withstand a normally lethal temperature if it is first given a heat shock at non-lethal temperature.

Heat shock proteins are classified into two broad categories based on their expression. The heat shock-inducible proteins are called HSPs while the HSP homologues, which are expressed in the cell during normal cell growth and differentiation, are called Heat Shock Cognates (HSCs). Based on their molecular

Table 1. HSP families

Family	Expression	Location	Functions
HSP110			
HSP104-yeast	Heat inducible	Cytosol	Thermotolerance,
HSP101-soybean		Nucleus	EtOH tolerance
-*Arabidopsis*			
-rice	ABA		
Casinolytic protease (CLP)-pea, tomato			
-*Arabidopsis*			
ERD 1-*Arabidopsis*	Dehydration		
HSP90			
80-90 kDa	Constitutive	Cytosol	
HSP81,82-*Arabidopsis*	Heat inducible	ER	Chaperone
HSP90 -*Brassica*		Nucleus	
HSP82-yeast			Thermotolerance essential for viability
HSP70			
Dnak (63-79kDa)	Constitutive	Nucleus	Thermotoleranace, negatively regulated hsp expression
HSP70-soybean, maize	Heat inducible	Cytosol	molecular chaperone
HSC70-tomato, pea		Mitochondria, Chloroplast	
SSA, SSB, KAR2-yeast			
HSP60			
53-62 kDa	Constitutive	Nucleus	Molecular chaperone
GroEL homolog	Heat inducible	Chloroplast	
HSP60-maize	Developmental		
HSP20			
10–30 kDa	Heat inducible	Nucleus	Protection of organelle macromolecules
HSP20-soybean	Constitutive	Cytosol	
wheat, carrot	Developmental	Mitochondria	
petunia		Chloroplast	
Vigna sinensis	Heat inducible	Chloroplast	
Ubiquitin			
8.5 kDa-maize	Constitutive	Cytosol	Proteolysis
-wheat	Heat inducible		
-*Arabidopsis*			

weight, HSPs (and HSCs) are grouped into six families (Table 1). The high molecular weight HSPs predominate in prokaryotes, yeast and animals. In higher plants the low molecular weight HSPs predominate and may play crucial role in heat stress tolerance[6]. All the HSPs are coded by nuclear genome except a HSP20 family member of *Vigna sinensis*, which is coded by chloroplast genome.[7]

Mechanism of protection

The role of HSPs during normal cell growth and development in the proper folding of polypeptides, formation of multimeric enzyme complexes and transport of proteins to the proper site/organelle has been well studied[5]. Historically, HSPs are believed to prevent the accumulation of aberrant proteins generated as a result of exposure to high temperature or other forms of stresses. Evidences available so far indicate that HSPs protect proteins from denaturation, salvage the denatured proteins and target the aberrant proteins for proteolysis. In soybean, both high and low molecular weight HSPs protected soluble proteins from heat denaturation[8] and the degree of protection showed dose-dependent character and did not require any additional energy[9]. Repair of heat damaged/denatured proteins is essential for both

survival and recovery from heat stress. HSP104 of yeast, which is essential for survival under heat stress, functions by reactivation of heat-damaged proteins. Thus HSP104 repaired the denatured luciferase to the control level *in vivo* within two hours[10]. This function of HSP has been confirmed *in vitro*, but needs to be confirmed *in vivo* in higher plants. Similarly, HSP60 from etiolated *Avena sativa* seedlings stimulated the refolding of chemically-denatured phytochrome to a photoactive form within an hour. The reactivation required additional energy as ATP[11]. Proteolysis of denatured proteins is another strategy, used by cells to prevent accumulation of denatured proteins. Ubiquitins which target the damaged proteins for proteolysis are coded by multigene family[12] and show heat induction[13] in higher plants. (In wheat (*T. aestivum* L. cv. Len), heat stress elevated the levels of ubiquitin–protein conjugates, induced HSP synthesis and elevated degradation of soluble proteins.) These results indicate that ubiquitin targets the denatured proteins for proteolysis under heat stress[13]. The protection of damaged proteins is less energy dependent while proteolysis is more energy dependent.

Regulation of HSP expression

The remarkable conservation of heat-stress response from prokaryotes to eukaryotes includes not only the structure and function of the HSPs but also the control of their stress-dependent expression. The environmental stimuli that induce *hsps* other than heat stress are toxic metals, inhibitors of energy metabolism, amino acid analogues, etc. Although HSP inducers are bewildering in their variety[14–17], many of them have in common the capacity to cause protein denaturation. Hightower[18] suggested that the accumulation of denatured or abnormally-folded proteins in cells initiated a stress response and the stress proteins might somehow facilitate the identification and removal of denatured proteins. This proposal was confirmed when Ananthan *et al.*[19] showed that injecting denatured proteins into living cells was sufficient to induce *hsp* genes in eukaryotes.

In eukaryotes, the induction of transcription of *hsps* is mediated by pre-existing transcription factors, the heat shock factors (HSF). HSFs are transacting factors, which upon activation bind to heat shock promoter elements (HSEs) at the 5′ upstream end of *hsps*, and induce the *hsp* expression[5,20]. The binding motif of HSE is composed of 5 bp (nGAAn) blocks in alternating orientation and atleast three units are required for stable binding[21]. In higher organisms, binding of HSF to HSE is heat stress inducible[22,23] and requires conversion from a latent monomer to an active trimer[20]. HSF genes have been isolated from the yeast (*Saccharomyces cerevisiae* K. lactis), higher plants (tomato and *Arabidopsis*), fruit fly, chicken, mouse and man. HSFs have two highly-conserved regions: an NH_2-terminal DNA binding domain of ~100 amino acids and an adjacent trimerization domain containing 3 hydrophobic heptad repeats, leu zippers. For the higher eukaryotes there is a fourth zipper domain near COOH-terminus that appears to interact directly with the more NH_2-terminal Leu zippers array to prevent trimerization under non-stress condition[5]. HSF is encoded by a single gene in yeast and *Drosophila* and by three genes in tomato[23]. Using tomato HSF genes (*hsf*s) as heterologous probes, *Arabidopsis hsf1* has been cloned[24]. Sequence comparison of *hsf* genes from different species demonstrates a strong conservation but only in their DNA binding and oligomerization domains. Out of three *hsf*s of tomato, *hsf8* is constitutively expressed, while *hsf24* and *hsf30* are induced by heat stress[25]. The *hsf1* is also constitutively expressed but the level is increased to two to threefold upon heat shock.

HSF activation may also involve cellular factors as intermediary sensors to regulate activity of HSFs under non-stress conditions. HSFs are maintained in monomeric form through transient interaction with HSP70 and/other HSPs which are constitutive. During heat shock, due to the availability of denatured/misfolded proteins, which are substrate for HSPs, they release HSFs and bind to denatured proteins. These free HSFs can then form trimers to bind HSEs[5,20]. Thus in cells, a homeostatic mechanism involving the free level of HSPs (=HSP70) provides a thermometer for reacting to temperature changes. HSFs which are expressed during normal temperature and heat stress are different and heat-induced phosphorylation of some HSFs suggests that other kinds of activation of HSFs also occur in cell[26]. Species-specific expression of *hsp*s may be regulated by the specific upstream sequences on the *hsp* genes, as in case of barley *hsp17* (ref. 27).

Role of HSPs in thermotolerance of higher plants

The induction of HSPs is characteristic of an emergency response, i.e. they are extremely rapid and very strong. For example, in *Glycine max* seedlings, hsp mRNAs are observed within 5 min of heat shock, and up to 20,000 fold induction of HSPs occurs[28]. Secondly, the induction temperature reflects stress conditions for the organism (Table 2).

The plant species adapted to temperate environment (soybean, maize, pea and wheat) begin to synthesize HSPs when tissue temperature exceeds 32–33°C (ref. 29). Thus the HSP-inducing temperature of these organisms reflects the thermal characteristic of the environment in which the organisms are growing. Under field conditions, soil water deficit enhanced midday canopy

Table 2. *hsp* induction temperature of organisms

Organism HSP maximum	Growth temperature optimum (°C)	Induction temperature (°C)
1. Archaebacteria		
Extreme thermophile *Pyrodichim occultum*	102	108
11. Prokaryote: *E. coli*	38	42
III. Eukaryotes		
1. Arctic fishes	0	5–10
2. *Drosophila*	25	33–38
3. Birds	25–35	43–45
4. Mammals		45
5. Snow fungus *Fusarium nivale*	12	25
6. Antarctic algae *Plocamium cartilagineum*	0	5
7. Yeast *Saccharomyces cervisiae*	25–28	37
8. Higher plants		
a) *Lolium temulentum*	25–30	35
b) *Triticum* sp.	20–25	32–40
c) *Glycine max*	28	40
d) *Sorghum bicolor*	35	43–45
e) Maize	35	43–45
f) *Pennisetum glaucum*	35	45
g) Tomato	25	35–37
h) Cotton	30	40
i) *Arabidopsis thaliana*	22	35

temperature of 40°C induced HSPs in cotton[30]. Even in irrigated wheat, HSPs were expressed in the field condition when the flag leaf temperature reached 32–35°C and HSP expression was correlated with the thermotolerance[31]. Hence HSPs are expressed in these organisms, when their temperature increases above the normal, rather than at a universal temperature threshold. The kinetics of HSP induction and acquired thermotolerance are tightly coupled and highly conserved among the evolutionarily diverse organisms. Moreover, HSPs show high similarity in nucleotide and amino acid sequences among eukaryotes, and in some cases also with prokaryotes. These evolutionary conservations clearly suggest the importance of HSPs in heat stress tolerance of organisms.

Correlation between HSP expression and thermotolerance

Acquired thermotolerance is correlated with HSP synthesis in many organisms, including higher plants[3,29,32]. Using etiolated soybean seedlings Lin *et al.*[33] have shown that the rate of synthesis of low molecular weight HSPs is correlated with acquired thermotolerance. Soybean seedlings are able to acquire thermotolerance by a pre-treatment of 2 h at 40°C or 10 min at 45°C followed by 2 h at 25°C. Thus, the pre-heat shock temperature which induced HSP synthesis resulted in the tolerance of seedlings at 45°C, 2 h heat shock. The 40°C pre-heat shock not only induced the HSP synthesis in soybean seedlings, but also resulted in the localization and stable association of HSPs with cell organelle fractions (nuclei, mitochondria and ribosome). Similarly in other crops like wheat[34–37], sorghum, pearl millet[38,39], and maize[40], the seedling thermotolerance at otherwise lethal temperature is correlated with the kinetics of HSP synthesis. These studies strongly suggest that the accumulation of HSPs is important for protection from thermal killing. If so, the next question arises, whether the quantity of HSPs or the quality of HSPs is important in providing thermotolerance. *Triticum monococcum* L cultivars, M_3 and M_9, which differ in thermotolerance, did not differ in the quality of HSP induced at 37°C. But Northern analysis using HSP cDNAs as probes revealed that the tolerant genotype M_3 was able to accumulate higher steady state mRNA level of 16.9, 26 and 70 kDa HSPs than the heat susceptible M_9 during heat hardening period[41].

In *Triticum aestivum*, the heat tolerant variety Mustang maintained its cell viability up to 80% when pre-heat shocked (at 34°C) wheat leaves were exposed to 50°C for 1 h, while the susceptible genotype Sturdy could maintain only 40% cell viability. Mustang also maintained its capacity to synthesize a small subunit of Rubisco at 34°C, while Sturdy could not. Two-dimensional gel electrophoretic analysis revealed the presence of three unique HSPs (16, 17 and 26 kDa) in

Mustang[35]. In case of maize also, the drought and heat-tolerant genotype ZPBL1304 synthesized an unique 42 kDa HSP, which was absent in susceptible line ZPL389 (ref. 40). Thus, not only are the HSP synthesis and acquired thermotolerance tightly coupled, but also the intraspecific differences in quantity, quality and the rate of accumulation of HSPs are highly correlated with thermotolerance.

Cellular localization of HSPs: Heat shock dependent

The positive correlation between acquisition of thermotolerance and HSPs appears to depend not only upon synthesis of HSPs but also on their cellular localization[33,42,43]. In soybean, heat hardening, which induced seedling thermotolerance, also induced synthesis and selective localization of HSPs. Cell fractionation studies revealed that the low molecular weight 15–18 kDa HSPs selectively localized and associated with nuclei, mitochondria and ribosomes, a lesser amount of 68–70 and 90 kDa HSPs localized in these organelles. While some 22–24 kDa HSPs remained soluble in cytosol, they remained organelle associated during a chase at 40°C, but dissociated gradually during a chase at 28°C. If again 10 min heat shock at 45°C was given, the localization occurred within 15 min. Arsenite-induced HSPs did not localize at 28°C, but they became organelle associated during subsequent heat stress[33].

Similarly, pea HSP22 was strongly associated with chloroplast thylakoids when the temperature was raised above 38°C, at high light intensities[44–46]. Studies conducted in *Chlamydomonas* showed association of LMW HSP with PS II which protected the PS II from photoinhibition[47]. In higher plants too, the localization and association of HSPs with organelle demonstrated the protective role of HSPs during heat stress. In soybean seedlings, at 38°C, all HSPs were synthesized, but their organelle localization occurred at 42.5°C. The 15–18 kD HSP and 70 kDa HSP were associated with mitochondria at 42.5°C and dissociated during 4 h recovery period at 20°C. This association protected the mitochondrial phosphorylation at non-permissive temperatures[48].

Cross tolerance and HSPs

HSPs are induced by many other stresses such as ethanol, malonate, arsenite[33], amino acid analogues[49], abscisic acid[50–52], drought stress[51], wounding[50], τ-rays[53] and cold[54]. If thermotolerance is a direct result of HSP synthesis, then the other treatments which induce HSP synthesis should also induce thermotolerance. Arsenite induced HSP synthesis at 28°C in soybean and was able to provide thermotolerance to the seedlings[33]. Also, the arsenite-induced HSPs showed heat-induced localization and association with organelles. In 40-day-old seedlings of sorghum and pearlmillet, arsenite (100 µM) and malonate (25 mM) induced HSPs synthesis comparable to that of 45°C heat hardening induced HSPs, which also gave thermal protection to the protein synthesis and seedling growth at 50°C[38]. Ethanol (4%, 6%) which was able to induce HSP synthesis and provide cross tolerance in yeast[55], was unable to induce either HSPs or thermotolerance in sorghum and pearlmillet[38]. Cell free fractions (soluble proteins) isolated from control and ABA treated cells of *Bromus inermis* Leyss showed different temperature tolerance in temperature-induced protein coagulation assay. Addition of 50 µg (5%) of ABA induced heat stable proteins and decreased the rate of heat-induced coagulation of cell free fractions *in vitro*[50]. Thus, it seems logical to conclude that HSPs must be playing an important role in thermal protection of higher plants.

Genetic complementation

Not only the heat shock response, but also the HSPs themselves and their regulation of expression is highly conserved among evolutionarily diverse organisms[20,29]. HSP101 of *Arabidopsis* and soybean show 43% identity to *Saccharomyces cervisiae* HSP104 at the amino acid level. The conservation of structure and mode of expression suggests that the functionality must have also been conserved. To test this possibility, yeast HSP104 mutants, which did not acquire thermotolerance[56], were transformed with *Arabidopsis thaliana hsp101* and soybean HSP101 gene. Higher plant HSP101 is undetectable in yeast (transformed with *Arabidopsis thaliana hsp101/Glysine max hsp101*) in the absence of heat stress, but accumulated to high levels during exposure to high temperature. Both *Arabodopsis thaliana* HSP101 and *Glysine max* HSP101 are able to complement the thermotolerance defect caused by HSP104 gene mutation[57,58]. HSP104 in yeast functions in thermotolerance by promoting the reactivation of heat-damaged proteins after high temperature stress[10]. Since higher plant HSP101 was able to complement the thermotolerance deficiency of hsp104 mutant yeast strain, it seems plant HSP104 is able to functionally complement the HSP104 of yeast. Therefore, it appears that HSP104 provides thermotolerance to higher plants in a manner which is functionally similar to that of yeast HSP104.

Molecular biology approaches

Molecular biological approaches were used to prove the role of HSPs in thermal stress tolerance in several organisms. HSP mutations in yeast[56] and *E. coli*[59] resulted in temperature sensitivity. Complementation of the yeast

hsp104 mutatation by either yeast or higher plant HSP101 resulted in restoration of acquired thermotolerance[57,58]. Over-expression of HSP70 in *D. melanogaster* resulted in faster acquisition of thermotolerance[60]. Similarly selection of thermotolerant cell lines of Chinese hamster fibroblast cell showed high level expression of HSP70 (ref. 61). Competitive inhibition at transcriptional level of *hsp70* gene in Chinese hamster ovary (CHO) cells reduced the heat-induced expression of *hsp* by at least 90%, which resulted in elevated thermosensitivity[62]. Expression of *hsp27* gene from metallothionein-regulated promoter in CHO cells, conferred metal regulated thermotolerance[63]. Affinity purified monoclonal antibodies to HSP70 when introduced into human fibroblasts by microinjection impaired heat-induced translocation of HSP70 into nucleus after mild heat shock and rendered the cells thermosensitive[64]. *D. melanogaster* and mammalian cells transformed with *hsp70* and *hsp90* antisense genes respectively, accumulated HSP70 at a slower rate and showed reduced thermotolerance[60,65].

These studies conclusively prove that some or other kind of HSP is involved in the protection of cells under high temperature stress. These kinds of molecular approaches have been limiting in higher plants because of

1. Existence of *hsp* multigene families showing high homology among the members.
2. Polyploidy nature of several plant species.
3. Lack of knowledge of roles of each HSP family under normal/stress environment.

However, Schöffl[66] suggested two gene manipulation strategies for HSP analysis in higher plants which includes:

a) Selection of cells and plants with constitutively repressed gene for which antisense mRNA approach appears to be more promising because within the members of a family ~90% homology is present. Hence, it should be possible to repress the expression of all family members by a temperature dependently transcribed antisense mRNA of single gene.

b) Generation of plants that overexpress the desired HSPs, which will be useful to examine the biological effect of protein dosage, protein structure and changed specificity under thermal stress.

Already studies have been initiated to study the regulation of HSP expression in higher plant using 1) GUS gene fused with *hsp* promoter[67]. 2) Soybean *hsp70* fused with Drosophila *hsp70* promoter[68], which showed regulated expression of HSPs environmentally and developmentally. Efforts have also been made to over-express/to inhibit synthesis by antisense mRNA approach. The tobacco transgenic tobacco plants developed by soybean HSP17.6 fused with cauliflower mosaic virus 35S promoter expressed constitutively to the level comparable to that of heat induction[69]. However, upon heat shock in the transgenic tobacco plants Gm HSP 17.6 was inhibited which indicated that CaMV35S promoter was not transcriptionally competent under heat stress. In antisense transgenic tobacco (soybean hsp17.6 fused with cauliflower mosaic virus 35S promoter in the antisense orientation) the level of expression was very low, the reasons being the long distance of the inverted gene from its promoter site and lack of a suitable 3′.termination signal of transcription[69].

Developmental expression of HSPs

All the cells/tissues so far examined are capable of synthesizing HSPs in response to heat shocks, except germinating pollen[70,71] and pre-torpedo stage of very early embryo development[72]. So, in all other stages of plant development, HSPs are expressed in response to heat stress. The question then asked is, are HSPs developmentally regulated in the absence of heat stress? Studies from eukaryotes including plants clearly indicate that there is, in fact, a tissue and developmental specificity in the expression of HSPs. Expression of HSPs in optimal growth environments occurs in flowers, pods and seeds of pulses[73], sepals, filaments and styles of transgenic $P_{hsp18.2}$::Gus marker gene *Arabidopsis thaliana* plants[68] and during embryogenesis[74–77]. HSC70 is shown to express during vegetative and reproductive stages of tomato[75,76]. HSPs did not express in germinating pollen and early imbibing embryos although both were very thermotolerant, the preformed HSPs may be playing a potential role in providing thermotolerance. HSC are stored in mature pollen[74] and seeds, probably to ensure survival in anticipation of potential heat stress. The involvement of HSPs in temperature stress tolerance and normal development has to be further tested.

HSP and crop productivity under stress

Crop productivity or grain yield is the result of a series of processes involving growth and development spread over the entire life span of the crop. These processes are supported by and regulate the various metabolic processes at the cellular level. Grain yield represents the dry matter partitioned towards grains and hence is directly related to the total dry matter accumulation by the crop. Dry matter accumulated over a period of time is related to the net photosynthesis rate and the total leaf area. High grain yield is the culmination of complementary relationship between 'source' (photosynthate availability) and 'sink' (grain no. × grain weight)[78].

High temperature stress causes accelerated plant development and consequently reduces both vegetative growth and grain yield[79]. At the cellular level, heat stress results in metabolic disturbances, depletion of

respiratory substrates and reduction of photosynthetic activity. It may also cause denaturation of proteins, inactivation of enzymes and damage to cellular structures. Heat stress is especially deleterious during grain filling stage when it inhibits starch accumulation leading to grain weight decrease[80].

Breeding high temperature-tolerant varieties of crops is an important component of breeding programmes. Stability in grain yield in stress environment is an acceptable criterion for expressing the relative thermotolerance of varieties and species. Stability analysis helps in identifying contrasting genotypes and species which provide ideal material for analysing the basis of thermotolerance at various levels of organization. Infact the response of plants to high temperature has been identified as a two-tier response. For a temperate crop such as wheat, increasing temperatures in the range of 18–32°C constitute high temperature stress while temperatures above 32–40°C constitute the heat shock range. The two ranges of temperature evoke distinct responses which differ considerably.

Although the deleterious effects of heat stress on wheat productivity were known and emphasized in the beginning of the century[1,81], in India and Australia, emphasis on understanding the mechanism underlying heat tolerance is only recent. It is realized that a complex character like heat tolerance with respect to grain yield may not be linked to a single metabolic process. Plants have a multitude of mechanisms which help them to survive and propagate under high temperature stress. These include heat stress avoidance and heat tolerance mechanisms.

The heat avoidance mechanisms enable the plants to keep their temperature lower than ambient, through mechanisms such as

1) Transpirational cooling (in spring wheat genotypes, canopy temperature depression was significantly and positively correlated with yield stability under unirrigated conditions[82]).

2) Differences in reflection of solar radiation through increase in leaf hairiness and wax deposition.

3) Leaf shading of tissues that are sensitive to sun burn.

The heat tolerance mechanisms operate in situations when tissue temperature is higher and yet plant functions are maintained. These include

1) Biomembrane saturation[83,84].

2) Synthesis of enzymes and isozymes with broad thermal kinetic windows and protective enzymes such as glutathion reductase, peroxidase, catalase, super oxide dismutase, etc.

3) Protection of biomembrances, molecules, organelles and maintaining their function, where HSPs may play a very crucial role.

Over 7 million ha of wheat cultivated in the subtropics suffers from heat stress. In central and southern parts of India, wheat suffers from heat stress at both the ends of crop growth. Sorghum and pearl millet also suffer in Rajasthan where during seed germination the soil temperature ranges from 50 to 60°C. High temperature stress causes yield reduction through accelerated phasic development, accelerated senescence, reduction in photosynthesis, increase in respiration (maintenance respiration) and inhibition of metabolic process of grain development such as starch synthesis. Rice also suffers from high temperature stress in tropical areas. When temperature rises from 24°C to 28°C, quantum efficiency of photosynthesis is decreased by 5%, while the rate of respiration increased by 30%. Thus the dark respiration is a primary limiting factor for energy fixation by canopies in tropical rice cultivation.

The amount of solar energy harvested during crop growth depends on the leaf area, which depends on the proper germination, seedling establishment and tillering/branching. High temperature stress drastically reduced germination in wheat[34,85], sorghum, pearl millet[38] and maize[86]. In all these crops, thermotolerance of germination and seedling growth, and the kinetics of HSP synthesis were positively correlated[33,34,36,38,41]. In cereals, leaf and shoot growth occurs from meristems situated near the soil surface and thus high soil surface temperature may have adverse effect on tiller and leaf production. In Central India, where soil temperature reaches 35°C to 40°C at the time of sowing, wheat variety Hindi 62 performs better than high yielding varieties because of its ability to germinate under heat stress and maintenance of tiller production. Hindi 62 which has the capacity to germinate at high temperatures exhibits high amylase activity at 30°C compared to a susceptible variety[87]. Also, in seedling stage Hindi 62 showed higher and faster accumulation of hsp16.9, hsp17.3 and hsp26.4 transcripts at 35, 40 and 45°C compared to susceptible varieties (Viswanathan and Khanna-Chopra, unpublished). Thus, it seems logical to conclude that HSPs may be an important component of stress tolerance during germination and seedling establishment.

After seedling establishment the biomass accumulation depends on two important processes, i.e. i) photosynthesis and ii) respiration (growth and maintenance). Photosynthesis is highly susceptible to high temperature stress. Photosynthate availability decides sink size and in turn crop yield. In C_3 plants, quantum yield decreased by 22% when the temperature increased from 20°C to 35°C. Net photosynthesis in wheat started to decline beyond 28°C. In photosynthesis PS II is the most susceptible component to high temperature stress[88]. Heat shock induced integration of 22–25 kDa HSPs into thylakoid membranes in pea and localized onto the stroma of chloroplast[3,29]. In *Chlamydomonas* HSPs 22 and 29 kDa were localized into grana lamellae during heat

stress and protected Photosystem II from photoinhibition[47]. Rubisco, the most abundant protein on earth, is also susceptible to high temperatures. In wheat the synthesis of Rubisco SSU is inhibited at 34°C in the susceptible wheat cv. Sturdy, while cv. Mustang was able to maintain Rubisco SSU synthesis and was correlated with synthesis of unique HSPs[40]. Although in higher plants, existence of several chloroplast-specific HSPs has been demonstrated in *Pisum sativum, Phaseolus vulgaris, Arabidopsis thaliana, Vigna sinensis, Zea mays* and wheat, their correlation with protection of photosynthesis has yet to be demonstrated. Thus, HSPs may be an important component of thermotolerance of photochemical as well as biochemical components of photosynthesis. Under high temperature stress, cells need more energy to protect/repair the heat damaged macromolecules, biomembranes, organelles and for acclimation/adaptive reactions, i.e. the maintenance respiration need will be more under heat stress. Heat stress drastically reduces mitochondrial respiration. This may lead to metabolic aberrations, even if it happens for 2–3 hours in the midday, and to low crop productivity. *Phaseolus acutifolius* had maintained its mitochondrial efficiency at 32°C and thus plant growth, while *P. vulgaris* did not maintain its mitochrondrial efficiency and thus reduced plant growth[89]. The direct correlation between HSPs synthesis, its localization in mitochondria and maintenance of mitochondrial efficiency at 42.5°C had been demonstrated in soybean seedlings[48]. Thus, HSPs appear to play a vital role in protecting mitochondrial respiration of crop plants.

At molecular level by protecting and repairing the macromolecules (enzymes, carrier proteins, ion channels, etc.) and by targetting the denatured macromolecules for proteolysis, HSPs may play a vital role in thermotolerance of all the metabolic processes, throughout the crop growth period. Increased solute leakage is another detrimental effect caused by heat stress at organelle and cellular levels. Under heat stress the membrane thermostability is an important component of thermotolerance and is highly correlated with yield stability[82]. By selecting genotypes for high membrane thermostability, yield increase had been achieved under heat stress[90]. Increased solute leakage is attributable to loss of membrane integrity through lipid phase transitions and the effect on membrane proteins. Leakage of substances (amino acids, sugars, ions) into the incubation medium from soybean seedlings at 45°C was prevented if the seedlings were pretreated at 40°C, 2 h and during this process a 15 kDa HSP associated with the plasmamembrane, appeared to play a role in the protection of membrane proteins during heat stress[91]. The other component of membrane thermostability is through membrane lipid saturation, relatively a long-term adaptive process. Therefore, it appears logical to conclude that HSPs may be one of the essential components of thermotolerance mechanism of crop plants and crop productivity under heat stress. If HSP is to be used as a selection criterion in breeding for thermotolerance, its genetics and heritability must be known. Efforts to link HSP accumulation with QTLs have not given very promising results[92]. Hence more studies are needed in this direction.

Future prospects

The role of HSPs in thermotolerance has been questioned in higher plants[93,94], yeast[95] and *E. coli*[96]. Plants have at least six *hsp* families with several members in each family. These *hsps* show differential expression under stress and development, hence all the HSPs may not be required for stress tolerance in all the tissues. Can thermotolerance of crop plants be increased by altering the *hsp* expression? Studies to answer this question are stymied because the following questions also remain to be answered.

1. Identification and assignment of the role in stress tolerance of each HSP *in vivo*.
2. In stressed cells, what decides the damaged protein to choose the salvage or proteolysis pathway?
3. How is the expression/action of HSF controlled under stress and development?

To engineer plants with temperature stress tolerance, *hsp* expression may become an important approach along with alteration in thermal kinetic windows of key enzymes[97] and membrane lipid unsaturation. Further, HSPs can be used as a selection criterion in breeding programmes aimed at thermotolerance.

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Slower-chemical or faster-electrical signalling under stress in plants: Is it the hare and tortoise story of a slower signal winning the race?

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In stress physiology, one of the controversies related to root to shoot communication under stress, has been whether electrical signals from roots precede the chemical signal, represented by the predominant positive signal, abscisic acid (ABA) which accumulates up to 50 fold in the roots and xylem sap of stressed plants. Electric signals can be produced and transmitted to the shoots 18 cm away from the roots in 25 s when an osmotic stress is given to the roots. However a recent finding that ABA applied to the roots itself can generate electrical signals has only fuelled or exacerbated the controversy. In this paper we have attempted to analyse the relative merits of a faster but apparently short distance intense signal, with the slower chemical signals. We have critically assessed what appears to be a 'deliberate strategy' of the plants to spatially separate two diverse but equally effective signals. The question we pose in this paper is, can a chemical signal still precede an electrical signal? If true, the plant must devise a different way to release an already available sequestered chemical signal. This is akin to resolving the classical dilemma of what comes first the chicken or the egg.

MAN has been concerned with plant stress adaptations since the first pre-historic cave dweller selected seed for propagation from plants that performed better than their neighbours. Physical and biochemical responses of plants to environmental stresses have been studied for over a century and a great mass of data is available. These responses embrace a fascinating spectrum of adaptation, ranging from the survival of the unicellular algae Dunaliela in the harsh saline waters of the dead sea of Israel through a process called osmoregulation[1], to the survival of Opuntia, the common cactus, in the Californian desert when the temperature of its shoot reaches 65°C, i.e. 17° above the air temperature[2].

Although these two examples represent plant adaptations to a saline and high temperature stress respectively, the predominant abiotic stress affecting plant growth and development is by far drought or water deficits. This concern is reflected by the number of

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