Review Article

# Genetic engineering for heat tolerance in plants

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**ABSTRACT**

High temperature tolerance has been genetically engineered in plants mainly by over-expressing the heat shock protein genes or indirectly by altering levels of heat shock transcription factor proteins. Apart from heat shock proteins, thermotolerance has also been altered by elevating levels of osmolytes, increasing levels of cell detoxification enzymes and through altering membrane fluidity. It is suggested that Hsps may be directly implicated in thermotolerance as agents that minimize damage to cell proteins. The other three above approaches leading to thermotolerance in transgenic experiments though operate in their own specific ways but indirectly might be aiding in creation of more reductive and energy-rich cellular environment, thereby minimizing the accumulation of damaged proteins. Intervention in protein metabolism such that accumulation of damaged proteins is minimized thus appears to be the main target for genetically-engineering crops against high temperature stress. **[Physiol. Mol. Biol. Plants 2008; 14(1&2) : 155-166]** *E-mail : anil.anilgrover@gmail.com*

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High temperature-induced gene expression system is one of the best-studied model systems for analyzing induced gene expression. The molecular basis of heat shock (HS) response was revealed for the first time when Ritossa (1962) reported that temperature elevation brings about altered puffing pattern of polytene chromosomes in *Drosophila*. Tissieres *et al.* (1974) for the first time showed that the HS condition results in altered protein profile in the *Drosophila* cells. Further studies established that nearly all organisms, ranging from bacteria to man, respond to HS by synthesizing a new set of proteins called heat shock proteins (Hsp). The HS system has been investigated in great depth using diverse biological systems including microbes (e.g. *Escherichia coli*, *Saccharomyces cerevisiae*), animals (e.g. *Drosophila melanogaster*, *Homo sapiens*) and plants (e.g. *Arabidopsis thaliana*, *Oryza sativa, Solanum lycopersicon*) species. In recent years, detailed understanding has been gained on various components of the heat shock response (HSR) in living organisms including features like heat shock genes/proteins, heat shock promoters and heat shock elements (HSEs), heat shock factors (HSFs), possible receptors of the heat shock response, signaling components and chromatin re-modeling aspects (Grover, 2002, Wahid *et al.,* 2007).

Plant scientists have a concern in altering the HS response as high ambient temperature is one of the major constraints in obtaining maximum output from the crop plants. Most crops are affected by daily fluctuations in high day and/or night temperatures. While some stages of plant growth may be more sensitive to high temperature than others, there is an overall reduction in plant performance when temperature is higher than the optimal temperature at specific growth stages. Conventional breeding for high temperature stress tolerance has not been much successful due to several reasons like lack of suitable source of genes in sexually-compatible gene pools, complex nature of the HS trait, lack of understanding on the genetic mechanisms of the high temperature tolerance response etc. Advent of recombinant DNA (rDNA) technology methods has opened avenues for tackling issues relating to complex genetic traits. In early 1990s, low temperature tolerant transgenic tobacco plants were raised by over-expressing desaturase gene isolated from *Arabidopsis* or cucurbits, heralding the beginning of transgenic solution to the problems of abiotic stresses (Murata *et al.,* 1992). During the past 15 years of research (1992-2007), there have umpteen reports on the production of abiotic stress tolerant transgenic plants (Grover *et al.,* 1998; Grover *et al.,* 1999; Grover *et al.,* 2001a; Grover *et al.,* 2001b;

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Grover *et al.,* 2003; Kotak *et al.,* 2007; Wahid *et al.,* 2007; see detailed list of other recently published abiotic stress-related reviews at *http:://plantstress.com/FilesPresentations*), providing testimony that rDNA approach has great potential in alleviating abiotic stress-induced injuries. The need for raising high temperature tolerant crops using rDNA methods was felt since the early days of rDNA science but however, not much could be achieved as the underlying physiological processes, biochemical enzymes and molecular mechanisms,that impart high temperature tolerance were not precisely understood. In recent times, several reports have appeared wherein it has been possible to address the issue of raising high temperature stress tolerant plants by manipulating the HSR components. It has been shown that plants for high temperature tolerance can be genetically-engineered by altering Hsps either directly or through regulatory circuits that govern Hsp levels, levels of osmolytes, components of the cell detoxification mechanisms and components that regulate membrane fluidity (Grover *et al.,* 2000; Burke and Chen, 2006). In this article, we first provide specific examples for each of the above four routes through which high temperature tolerance of plants has been altered genetically. Subsequently, we move on to synthesize a common model showing that these four routes may be acting through a common mechanism dealing with the metabolism of denatured proteins under stress conditions.

## High temperature tolerant transgenics raised through altering HSPs

High temperature stress is, in general, simulated in laboratory experiments by subjecting biological systems to HS treatment. Plants mount resistance to HS by eliciting specific metabolic adjustments. The temperature for the induction of plant HSR varies amongst different plant species but an increase of 5-10ºC over and above the ambient temperature is generally sufficient to elicit the HSR. Reports on HS-induced alterations in protein profile of plants started appearing 1980s onwards (Barnett *et al.,* 1980). While Hsps have been reported from a large number of plants, those plant species which are extensively analyzed for Hsps include *Arabidopsis*, maize, tomato, rice and wheat. Hsps are broadly classified on the basis of their molecular weights as high molecular weight (HMW-Hsps; 70-100 kDa) and low molecular weight (sHsps; 15-20 kDa) Hsps. The level of accumulation of different Hsps is often variable. Under stress conditions, sHsps may comprise up to 1% of the cellular proteins (Agarwal *et al.,* 2003). During the past nearly thirty years of research, Hsps have been extensively-analyzed for their physiological, biochemical, cellular and molecular properties (Vierling, 1991; Agarwal *et al.,* 2001; Katiyar-Agarwal *et al.,* 2001; Scharf *et al.,* 2001; Agarwal *et al.,* 2003). It has been shown that Hsp are highly-conserved proteins across different species (Katiyar-Agarwal *et al.,* 2001). Detailed characterization of Hsps in terms of their (a) molecular weights, (b) inducers that trigger Hsps synthesis, (c) cellular localizations, (d) expression patterns, (e) synthesis under field-conditions, (f) cellular levels and (g) conservation of amino acid sequence have been discussed at length elsewhere (Agarwal *et al.,* 2003; Wang *et al.,* 2003; Wang *et al.,* 2004; Kotak *et al.,* 2007). Besides HS, selective plant Hsps are induced in response to several different abiotic stresses as well such as heavy metal stress, water stress, wounding stress, salt stress, cold shock stress and anoxia stress. Plants, in general, survive lethal high temperature stress more efficiently after prior exposure to a mild high temperature stress as against direct response to lethal high temperature stress (Singla-Pareek *et al.,* 1997). This phenomenon is termed as acquired thermotolerance (Sanchez and Lindquist, 1990; Kumar *et al.,* 2007). Hsps are believed to be important for the protection of cells against heat injury both in basal thermotolerance (i.e. thermotolerance achieved without prior HS) as well as in acquired thermotolerance responses. Over the years, large number of *hsp* genes has been isolated, sequenced and cloned (Agarwal *et al* 2003; Grover *et al*., 2003). This has been achieved from varied plant species representing diverse taxonomic classes. Availability of complete genomic sequence data of *Arabidopsis thaliana* and *Oryza sativa* has provided vast information on different families of the *hsp* (Agarwal *et al.,* 2001; Krishna and Gloor, 2001; Scharf *et al.,* 2001; Batra *et al.,* 2007).

The question how important Hsps are in controlling thermotolerance has been repeatedly addressed to. Several groups have altered levels of sHsps in bacterial systems and shown that when over-expressed in bacterial cells, sHsps have a role in conferring thermotolerance. We herewith describe three specific reports to illustrate this observation. Yeh *et al.* (2002) showed that over-expression of *Oshsp16.9* in *E. coli* confers thermotolerance to the bacterial cells. To define the regions for this intriguing property, deletion mutants of this sHsp were constructed and over-expressed in *E. coli*. The deletion of amino acid residues 30 through 36 (PATSDND) in the N-terminal domain or 73 through 78 (EEGNVL) in the consensus-II domain of OsHsp16.9 caused the loss of chaperone activities and also rendered the *E. coli* incapable of surviving at 47.5°C. Light scattering changes of OsHsp16.9 mutant proteins under

heat treatment (either by themselves or in the presence of a thermo-sensitive enzyme, citrate synthase) reflected that regions of amino acid residues 30 through 36 and 73 through 78 were responsible for stability of OsHsp16.9 and its interactions with other unfolded protein substrates (such as citrate synthase). Studies of two point mutations of OsHsp16.9, GST-N74E73K and GST-N74E74K, indicated that amino acid residues 73 and 74 are an important part of the substrate-binding site of OsHsp16.9. Yeh *et al.* (1997) introduced *Oshsp16.9* in *E. coli* using the pGEX-2T expression vector to analyze the possible function of this sHsp under heat stress. The survivability of *E. coli* XL1-blue cells transformed with a recombinant plasmid containing glutathione S-transferase (GST)-OsHsp16.9 fusion protein (pGST-FL cells) was compared with the control *E. coli* cells (transformed with the pGEX-2T vector; pGST cells) under HS. The pGST-FL cells demonstrated thermotolerance at 47.5°C, a treatment that was lethal to the pGST cells. When the cell lysates from pGST and pGST-FL transformants were heated at 55°C, the amount of protein denatured in the pGST-FL cells was 50% less than that of the pGST cells. Results similar to pGST-FL cells were obtained in pGST-N78 cells (cells producing a fusion protein with only the N-terminal 78 amino acids in the OsHsp16.9 portion) but not in pGST-C108 cells (cells produced a fusion protein with C-terminal 108 amino acids in the OsHsp16.9 portion). Pike *et al.* (2001) addressed to the role of sHSPs in the acquired and transgenic thermotolerance of *Synechococcus* sp. PCC7942. The genes for three-minimally related sHSPs namely OsHsp from *Oryza sativa* cytoplasm, tom111 from *S. lycopersicon* chloroplasts and 6803 Hsp from *Synechocystis* sp. PCC6803 were transformed into *E. coli* and over-expressed. All three proteins were able to protect malate dehydrogenase (MDH) from *in vitro* thermal aggregation. sHsps could also protect several soluble proteins in *E. coli* extracts from thermal aggregation *in vitro*, as well as protecting phycocyanin in extracts from *Synechococcus* sp. PCC7942.

The involvement of Hsps in regulating thermotolerance in plants has been indicated by down-regulating their levels through antisense and RNAi approach. Mutants of *Zea mays* and *A. thaliana* plants under-expressing their respective Hsp100 proteins are observed to lack both basal as well as induced thermotolerance (Hong and Vierling 2000; Hong and Vierling 2001; Nieto-Sotelo *et al.,* 2002). Yang *et al.* (2006) showed that the tomato plants silenced for Hsp100/ClpB protein were impaired in thermotolerance. Acquisition of thermotolerance has been found to be negatively affected in Hsp70 antisense *A. thaliana* plants (Lee and Schoffl, 1996). Hsa32 in *A. thaliana* is heat-inducible protein (Charng *et al.,* 2006). Mutant/ RNAi plants lacking Hsa32 do not survive a severe HS treatment even after a pre-treatment at a sublethal temperature (Charng *et al.,* 2006). Very recently, Charng *et al.* (2007) have shown that HsfA2 (heat-inducible trans-activator protein which sustains the expression of Hsp genes and prolongs the acquired thermotolerance in *A. thaliana*) under-expression results in an increased sensitivity of the mutant plants to HS. The dehydration response element binding proteins/ C-repeat binding proteins (DREB/CBF family) are transcriptional regulators involved in plant response to draught, salt and cold stress. It was recently demonstrated that DREB2A is heat stress-induced and a regulator of the heat stress response of *Arabidopsis.* Schramm *et al.* (2007) provided evidence that HsfA3 is transcriptionally induced during HS by DREB2A.

Conversely, up-regulation of Hsps has been achieved in a large number of studies. Malik *et al.* (1999) produced transgenic carrot cell lines and plants in which carrot sHsp17.7 was over-expressed. Modified expression of sHsp17.7 in this experiment resulted in enhanced survival of transgenic cell lines and plants at high temperature (thermotolerance assessed in terms of cell viability and growth, as well as electrolyte leakage in plants after severe stress). Park and Hong (2002) raised transgenic tobacco plants over-expressing tobacco class I sHsp. The transformed seedlings showed higher cotyledon opening rate (on the other hand, seedlings raised with the antisense construct in this experiment showed increased sensitivity to HS). Transgenic rice plants over-expressing *Oshsp17.7* gene showed increased thermotolerance as well as increased resistance to UV-B irradiation (Murakami *et al.,* 2004). Tomato *Lehsp* (mitochondrial) gene over-expressed in tobacco showed that transgenics were more thermotolerant at 48ºC than the transgenics produced with the antisense construct of the same gene (Sanmiya *et al.,* 2004). In selective instances, levels of HMW-Hsp have also been over-expressed in plants. Queitsch *et al.* (2000) produced transgenic *A. thaliana* plants by modifying level of AtHsp100 protein. Transgenic plants in this study survived as high as 45ºC (1 h) temperature stress as they showed vigorous growth after the removal of stress. The vector-transformed control plants could not regain growth during the post stress recovery period. Katiyar-Agarwal *et al.* (2003) over-expressed AtHsp100 protein in rice plants. The transgenic rice lines showed re-growth in the post-high temperature stress recovery phase while the untransformed plants could not recover to the similar extents (Katiyar-Agarwal *et al.,* 2003).

There are clear evidence showing that HSEs interact with positively-acting regulatory HSF proteins to bring about increased transcription of *hsp* genes (Wu, 1995). In recent years, *hsf* gene induction system has emerged as a powerful target for manipulating levels of Hsps in transgenic experiments. Lee *et al.* (1995) showed that AtHSF1 is constitutively expressed but its activity for DNA binding, trimer formation and transcriptional activation of the *hsp* genes is repressed at normal growth temperatures. They were able to de-repress the HSF function which led to constitutive expression of Hsps. The level of basal thermotolerance of transgenic *A. thaliana* plants producing constitutively higher levels of Hsps was found to be significantly enhanced. Prandl *et al.* (1998) over-expressed *Athsf3* in *A. thaliana* using CaMV35 promoter and showed a clearly enhanced thermotolerance in transgenic plants. In another independent study, *hsf3* gene over-expressed in *A. thaliana* resulted in increased thermotolerance (Panchuk *et al.,* 2002). Mishra *et al.* (2002) over-expressed tomato *hsfA1* gene in tomato plants. In this study, over-expressing lines showed increased thermotolerance while transgenic lines in which transgene was silenced due to cosuppression were thermosensitive. Sakuma et al., (2006) provided evidence that DREB2A has a direct role in heat stress responsive gene expression in *Arabidopsis*: thermotolerance was significantly induced in plants over-expressing DREB2ACA (mutant version which is constitutive active form) and decreased in DREB2A knockout plants. Table 1 provides comprehensive details on plant transgenics raised for high temperature tolerance.

**High temperature tolerant transgenics raised through altering osmolytes**

Certain low molecular weight compounds such as amino acids (e.g. proline), polyamines (e.g. putrescine), quaternary ammonium compounds (e.g. glycinebetaine), sugars (e.g. mannitol, fructans, sorbitol, trehalose) and sugar alcohols (e.g. polyols) help plants to acclimatize against largely the osmotic stresses (Gepstein *et al.,* 2005). Such compounds are usually referred to as osmolytes. Glycinebetaine is proposed to protect the photosynthesis machinery by stabilizing $O_2$ evolving photosystem II complex (Papageorgiou and Murata, 1995). Alia *et al.* (1998) achieved increased biosynthesis of glycinebetaine in *Arabidopsis* plants by introduction of bacterial *codA* gene (that encodes for choline oxidase protein). The seeds of transgenic plants were more tolerant to heat stress as compared to the wild type, at the imbibition and germination stage. Overproduction of glycinebetaine also provided significant advantage to the growth of young transgenic seedlings at supra-optimal temperature in this study. Yang *et al.* (2005) overexpressed betaine aldehyde dehydrogenase protein from spinach into tobacco plants, to increase glycinebetaine levels. Tobacco transformants in this study showed increased thermotolerance in terms of growth of young seedlings as well as $CO_2$ assimilation rates.

However, data on heat tolerance of large number of plants raised for increased osmotic stress tolerance through overproducing levels of proline, mannitol, trehalose etc are largely not reported (Grover *et al.,* 2003).

**High temperature tolerant transgenics raised through altering Membrane fluidity**

Living cells adapt to the extracellular low temperature stress through alteration in the composition of membrane lipids. Murata (1983) showed that the membrane fluidity is increased in response to low temperature stress due to elevated activity of desaturase enzymes (responsible for bringing out increased unsaturation of membrane lipids). Thomas *et al.* (1986) noted that saturation of membrane lipids is increased when cells are subjected to supra-optimal temperatures, resulting in increased membrane rigidity. Murakami *et al.* (2000) produced transgenic tobacco plants in which the gene encoding the chloroplast- localized fatty acid desaturase was silenced. In this study, significant reduction was observed in the amount of trienoic fatty acids in homozygous transgenic lines in comparison to wild type plants. Thermotolerance assays revealed that transgenic tobacco plants were resistant to high temperature stress (41ºC, 2 h), whereas wild type plants could not survive in response to such extreme temperature treatment. Zhang *et al.* (2005) over-expressed *Brassica napus* cytosolic Fad8 protein in tobacco and showed that over-expression of Fad8 imposed much greater heat sensitivity than that observed with other desaturases (such as Fad3).

**High temperature tolerant transgenics raised through altering cell detoxification components**

Reactive oxygen species (ROS) are produced in cells under normal as well as under stress conditions. Levels of ROS can be immensely high under stressed conditions (Alscher *et al.,* 2002). Electron transport systems in chloroplasts and mitochondria are the major source for the production of the $O_2$ radicals: free radicals are by-products of the electron transport chain during the generation of cellular energy. Biological systems have evolved defense systems against ROS, involving both

| Gene | Protein | Source | Cellular Function | Trans host | Promoter | Comments | Reference |
|---|---|---|---|---|---|---|---|
| **Using HSFs and Hsps** | | | | | | | |
| *Athsf1* | HSF | *A. thaliana* | TF | *A. thaliana* | CaMV35S | Transformants exhibited thermotolerance and also constitutive expression of the *hsp* genes at normal temperature. | Lee *et al.* (1995) |
| *Athsf3* | HSF | *A. thaliana* | TF | *A. thaliana* | CaMV35S | *Arabidopsis* plants showed an increase in basal thermotolerance, indicating the importance of HSFs and HSF-regulated genes as determinants of thermo-protective processes. | Prandl *et al.* (1998) |
| *AtHsfA2* | HSF | *A. thaliana* | TF | *A. thaliana* | CaMV35S | The mutants displayed reduced basal and acquired thermotolerance as well as oxidative stress tolerance while the over-expression lines displayed increased tolerance. | Li *et al.* (2005) |
| *OsHSFA2e* | HSF | *O. sativa* | TF | *A. thaliana* | Maize Ubi1 | *Arabidopsis* plants showed enhanced thermotolerance. | Yokotani *et al.* (2007) |
| *HsfA1* | HSF | *S. lyco-persicon* | TF | *S. lyco-persicon* | CaMV35S | HsfA1 over-expression in plants provided distinct advantage to growth and fruit ripening processes under high temperature stress conditions; in case of HsfA1 cosupression, plants and fruits were sensitive to elevated temperatures. | Mishra *et al.* (2002) |
| *HSF3* | HSF | *A. thaliana* | TF | *A. thaliana* | CaMV35S | HSF3 over-expressing plants showed a lower threshold temperature for the expression of HSPs than wild type plants along with the presence of a novel heat stress induced thermostable isoform of ascorbate peroxidase which was absent in the wild type. | Panchuk *et al.* (2002) |
| *TLHS1* | TLHS1 | *N. tabacum* | Chaperone | *N. tabacum* | CaMV35S | Over-expressing plants showed significant increase in thermotolerance as was evident by the rate of cotyledon opening after heat stress treatments. Antisense plants showed severe defect in withstanding stress. | Park and Hong (2002) |
| *hsp17.7* | HSP 17.7 | *Daucus carota* | Chaperone | *D. carota* | CaMV35S | Transformants expressed *hsp17.7* gene in the absence of heat shock and showed increased thermotolerance. | Malik *et al.* (1999) |

| Gene | Protein | Source | Cellular Function | Trans host | Promoter | Comments | Reference |
|---|---|---|---|---|---|---|---|
| *sHSP* | Mt-sHSP | *S. lycopersicon* | Chaperone | *N. tabacum* | CaMV35S | Plants which over-express the MT-sHSP gene exhibited thermotolerance, while the antisense plants in which the expression of the gene was suppressed exhibited susceptibility. | Sanmiya *et al.* (2004) |
| *hsp21* | HSP21 | *S. lycopersicon* | Chaperone | *S. lycopersicon* | CaMV35S | Protects PSII from temperature-dependent oxidative stress; also plays role in accumulation of carotenoids during fruit ripening. | Neta-Sharir *et al.* (2005) |
| *hsp101* | HSP 100 | *A. thaliana* | Chaperone | *A. thaliana* | CaMV35S | Transformants constitutively expressing hsp101 tolerated sudden shifts to extreme temperature better than the controls. | Quietsch *et al.* (2000) |
| *hsp101* | HSP 100 | *A. thaliana* | Chaperone | *O. sativa* | Maize Ubi1 | Transformants expressing hsp101 showed enhanced tolerance to high temperature. | Katiyar-Agarwal *et al.* (2003) |
| **Using proteins involved in ROS scavenging system** | | | | | | | |
| *Cu/Zn SOD*<br>*APX* | Cu/Zn superoxide dismutase;<br>ascorbate peroxidase | *Manihot esculenta*<br>*Pisum sativum* | ROS-scavenging enzymes | *Solanum tuberosum* | SWPA2 (oxidative stress inducible) | Transgenic plants showed enhanced tolerance to 250 μM methyl viologen, and visible damage in these transgenic plants was one-fourth that of non-transgenic plants that were almost destroyed. | Tang *et al.* (2006) |
| *HvAPX1* | Ascorbate peroxidase | *Hordeum vulgare* | $H_2O_2$ detoxification | *A. thaliana* | CaMV35S | Ascorbate peroxidase is involved in detoxification of photo-produced $H_2O_2$. Transgenic plants were significantly more tolerant to heat stress. | Shi *et al.* (2001) |
| **Using protein involved in osmolyte synthesis** | | | | | | | |
| *badh* | Betaine aldehyde dehydrogenase | *Spinacia oleracea* | Glycine-betaine synthesis | *N. tabacum* | CaMV35S | Transgenic plant accumulated glycinebetaine mainly in chloroplasts, which resulted in enhanced tolerance to high temperature stress during growth of young seedlings. | Yang *et al.* (2005) |
| *cod A* | Choline oxidase A | *A. globiformis* | Glycine-betaine synthesis | *A. thaliana* | CaMV35S | Transformants showed tolerance to high temperature during imbibition and germination of the seeds. | Alia *et al.* (1998) |

| Gene | Protein | Source | Cellular Function | Trans host | Promoter | Comments | Reference |
|---|---|---|---|---|---|---|---|
| **Using proteins involved in lipid metabolism associated with membranes** | | | | | | | |
| *fad 7* | ω-3-fatty acid desaturase | *A. thaliana* | Causes reduction of trienoic fatty acids and hexadecatrienoic acid | *N. tabacum* | CaMV35S | Transformants showing silencing of the gene were better able to acclimate to higher temperature. | Murakami *et al.* (2000) |
| *fad 7* | ω-3-fatty acid desaturase | *A. thaliana* | Causes reduction of trienoic fatty acids and hexadecatrienoic acid | *O. sativa* | Maize Ubi1 | Transformants showing silencing of the gene showed better growth, higher chlorophyll content and photochemical efficiency. | Sohn and Bach (2007) |
| *fad8* | Desaturase | *Brassica napus* | Oxidoreductase acting on paired donors, with incorporation or reduction of molecular oxygen. | *N. tabacum* | CaMV35S | Over-expression of FAD8 imposes much greater heat sensitivity than does FAD3 over-expression. | Zhang *et al.* (2005) |
| **Others** | | | | | | | |
| *mbf1c* | Multiprotein bridging factor 1c | A. *thaliana* | Transcription regulation | A. *thaliana* | CaMV35S | Enhances the tolerance of transgenic plants to bacterial infection, heat and osmotic stress. The enhanced tolerance of transgenic plants to osmotic and heat stress was maintained even when these two stresses were combined. | Suzuki *et al.* (2005) |
| *fld* | Flavodoxin | *Anabaena* | Electron carrier | *N. tabacum* | CaMV35S | Tobacco plants expressing Fld in chloroplasts displayed increased tolerance to multiple sources of stress, including redox-cycling herbicides, extreme temperatures, high irradiation, water deficit and UV radiation. | Tognetti *et al.* (2006) |
| *SBPase* | Sedoheptulose-1,7-bisphosphatase gene | *O. sativa* | Calvin cycle | *O. sativa* | Maize Ubil | Transgenic plants were more tolerant to high temperature stress during seed development. | Feng *et al.* (2007) |
| *rolB* | β-glucosidase | *Agrobacterium rhizogenes* | Root formation | *S. lycopersicon* | TPRP-F1 (early fruit specific promoter) | Transgenic line expressing rolB specifically during early stages of fruit development performed significantly better than the parental line at both high and low temperatures. | Shabtai *et al.* (2007) |

limiting the formation as well as removing the excess levels. Component of cell detoxification mechanisms have been employed in specific experiments to alter thermotolerance response in transgenic plants. Over-expression of barley *hvapx1* gene (encoding for peroxisomal ascorbate peroxidase) in *Arabidopsis* caused increased thermotolerance of transgenic plants as compared to wild type plants (Shi *et al.,* 2001). Chen *et al.* (2004) overexpressed tomato gene encoding for glutathione peroxidase in tobacco. Transient expression of transgene protected transgenic leaves from salt and heat stress. Overexpression of Cu/Zn superoxide dismutase is also noted to protect plants from high temperature stress (Tang *et al.,* 2006).

**Synthesis to propose a model**

Cellular proteins lose their biological activity upon heat shock due to aggregation and/or misfolding (Georgopoulos and Welch, 1993). There are evidence suggesting that the stress-induced accumulation of aggregated/misfolded proteins proves deleterious to cells and the abnormal state of proteins triggers the HSR in living organisms (Vierling 1991). HS is known to enhance the synthesis of specific proteases involved in the degradation of abnormal proteins (Katiyar-Agarwal *et al.,* 2001). Hsps reportedly function as molecular chaperones that co-operate as a functional-network in protecting cells against damage due to HS. Hsp16.9, Hsp17.1, Hsp17.3 and Hsp18.1 are shown to prevent the aggregation or denaturation of proteins during HS (Lee *et al.,* 1995; Yeh *et al.,* 1997; Young *et al.,* 1999; Low *et al.,* 2000). Hsp100 is shown to rescue the heat-induced protein aggregates by their re-solubilization during the recovery phase (Glover and Lindquist, 1998). Certain other Hsps such as Hsp40, Hsp60, Hsp70 and Hsp90 (alone or in co-operation) stabilize the heat-denatured proteins (Hartl *et al.,* 1994; Glover and Lindquist, 1998; Buchner, 1999). Detailed studies in model systems like *Arabidopsis*, yeast and human cell lines show that several different Hsps act in synchronous manner to prevent aggregation or re-fold the aggregated proteins. There is definite evidence showing that Hsp100, Hsp70 and sHsps act in a coordinated manner to prevent toxicity due to denatured proteins (Lee *et al.,* 2005; Zhang and Guy, 2005; Dragovic *et al.,* 2006; Raviol *et al.,* 2006; Doyle *et al.,* 2007).

In the account presented above, large number of reports are discussed which highlight that the increased synthesis of Hsps, elevation in the levels of osmolytes, specific alterations in membrane fluidity and increased expression of cell detoxification enzymes result in thermotolerant phenotype in transgenic plants. As suggested above, increased Hsps may have a direct bearing on levels of denatured / aggregated proteins through their chaperoning action. But then, how the other three agents are implicated in thermotolerant phenotype remains to be debated. It is possible that increased osmolytes make cell environment more reductive due to higher energy status and thus reduce damage to proteins. There are indications that osmolytes bind with the cellular proteins to protect them from denaturation / aggregation (Ou *et al.,* 2001; Ignatova and Gierasch, 2006). Cell detoxification components may likewise be important in making reductive cell environment and minimizing the loss of active proteins. It is possible that membrane fluidity has a role in maintaining cell volumes and stress levels such that cellular proteins are most protected. The physical state of the membrane has been shown to influence gene expression (Vigh *et al.,* 1998). Alterations in lipids can affect selective stress signaling either through global effects on the physical state of membrane, or via specific interactions of lipids with proteins (Vigh *et al.,* 2007). We hereby present a model (Fig. 1) showing that all the four mechanisms through which thermotolerance has been achieved in transgenic plants may basically be acting at the level of reducing damage to cell proteins.

There is a definite need to work out the detailed aspects of the mechanisms that play role in preventing protein denaturation, removal of denatured proteins and dissolving protein aggregates in post-stress period to reduce toxicity. It should also be important to delineate various cellular events that may be affected by the aberrations caused in protein metabolism. Salvucci *et al.* (2006) recently showed that thermal denaturation of rubisco activase is one of the key factors responsible for loss of rubisco activation under heat stress. According to Sage and Kubien (2007), limitations in electron transport and rubisco activase capacity should be more common, in the warmer, high $CO_2$ conditions expected by the end of the century. How Hsps (or other methods of eliciting thermotolerance discussed in this chapter) can play a role in protecting such important cellular reactions need to be taken up for further research. Chen *et al.* (2006) recently isolated series of *Arabidopsis* mutants that are defective in the acquisition of thermotolerance in response to sublethal high temperature treatment. In the specific mutant type named *dgd1-2* which has lesion with respect to loss of acquired as well as basal thermotolerance, affected gene was shown to encode for digalactosyldiacylglycerol synthase 1 (DGD1). Importantly, this group showed that expression patterns of Hsps in heat-treated *dgd1-2* plants were similar to those from identically-treated wild type plants,

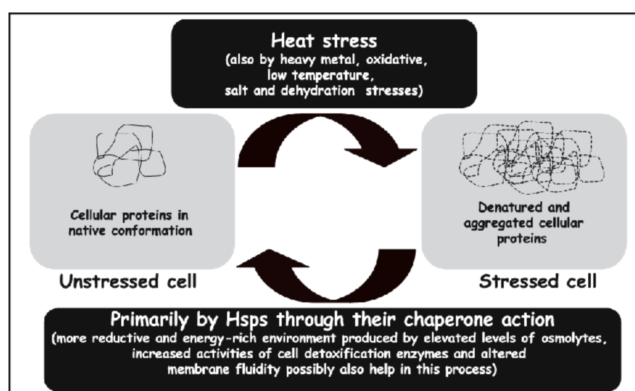


**Fig. 1.** Model showing how protein metabolism is affected under stress in cells. Stressed cells tend to have higher levels of denatured / aggregated proteins caused by the stress treatment (primarily by heat shock; also by other stresses). Stressed cells tend to return to their unstressed state by minimizing damage to protein, primarily through chaperoning activity of the Hsps. We propose that agents that can help in reducing damage to proteins may have positive role in high temperature tolerance.

suggesting that the thermosensitivity in the *dgd1-2* mutant was not caused by defect in Hsps induction. On the other hand, lipid profiles in this case appeared extremely relavant to thermotolerance capacity. These aspects are important for future research, in order to make further in-roads for engineering higher level stress tolerance in crops.

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