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Plant heat-shock proteins: A mini review

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Abstract Plants as sessile organisms are exposed to persistently changing stress factors. The primary stresses such as drought, salinity, cold and hot temperatures and chemicals are interconnected in their effects on plants. These factors cause damage to the plant cell and lead to secondary stresses such as osmotic and oxidative stresses. Plants cannot avoid the exposure to these factors but adapt morphologically and physiologically by some other mechanisms. Almost all stresses induce the production of a group of proteins called heat-shock proteins (Hsps) or stress-induced proteins. The induction of transcription of these proteins is a common phenomenon in all living things. These proteins are grouped in plants into five classes according to their approximate molecular weight: (1) Hsp100, (2) Hsp90, (3) Hsp70, (4) Hsp60 and (5) small heat-shock proteins (sHsps). Higher plants have at least 20 sHsps and there might be 40 kinds of these sHsps in one plant species. It is believed that this diversification of these proteins reflects an adaptation to tolerate the heat stress. Transcription of heat-shock protein genes is controlled by regulatory proteins called heat stress transcription factors (Hsfs). Plants show at least 21 Hsfs with each one having its role in regulation, but they also cooperate in all phases of periodical heat stress responses (triggering, maintenance and recovery). There are more than 52 plant species (including crop ones) that have been genetically engineered for different traits such as yield, herbicide and insecticide resistance and some metabolic changes.

In conclusion, major heat-shock proteins have some kind of related roles in solving the problem of misfolding and aggregation, as well as their role as chaperones.

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1. Introduction

Plants interact with not only climatic factors (such as irradiation, temperature, and drought) but also soil factors (such as salinity) and biotic factors (such as herbivores and pathogens). All these factors put the plant under interrelated stresses (Levitt, 1980). Moreover, daily sudden changes in the temperature and the presence of heavy metals, toxins, and oxidants due to human activities could result in extra stresses on plants (Vierling, 1991).

Basic Stresses such as drought, salinity, temperature, and chemical pollutants are simultaneously acting on the plants causing cell injury and producing secondary stresses such as osmotic and oxidative ones (Wang et al., 2003). Plants could not change their sites to avoid such stresses, but have different ways and morphological adaptations to tolerate these stresses. Some of these are, the dominance of sporophyte that embraces the sensitive gametophyte, the presence of epidermis with stomata for gases exchange, the formation of dormant organs, and the presence of conducting tissues for long distant transport. Other ways of defense at the molecular level are very important for the survival and growth of plants. Plants show a series of molecular responses to these stresses. The physiological processing basis for these molecular responses will not be covered here as it has been reviewed in depth lately (Shao et al., 2007a).

Heat stress as well as other stresses can trigger some mechanisms of defense such as the obvious gene expression that was not expressed under "normal" conditions (Morimoto, 1993; Feder, 2006). In fact, this response to stresses on the molecular level is found in all living things, especially the sudden changes in genotypic expression resulting in an increase in the synthesis of protein groups. These groups are called "heat-shock proteins" (Hsps), "Stress-induced proteins" or "Stress proteins" (Lindquist and Crig, 1988; Morimoto et al., 1994; Gupta et al., 2010). Almost all kinds of stresses induce gene expression and synthesis of heat-shock proteins in cells that are subjected to stress (Feige et al., 1996; De Maio, 1999). In *Arabidopsis* and some other plant species low temperature, osmotic, salinity, oxidative, desiccation, high intensity irradiations, wounding, and heavy metals stresses were found to induce the synthesis of Hsps (Swindell et al., 2007). However, stressing agents lead to an immediate block of every important metabolic process, including DNA replication, transcription, mRNA export, and translation, until the cells recover (Biamonti and Caceres, 2009).

It was known a long time ago that the most damage to crop plants in fields occurs when two or more stresses are prevailing (Mittler, 2006). Hence, in order to study the plant tolerance, it is very necessary to mimic the natural conditions in a specific area. Most recent studies indicate that the plant responses to two or more factors are unique and differ from the response to one factor only. For example, subjecting the plants to drought only leads to high content of proline, but subjecting the same species to drought combined with high temperature leads to high content of sucrose and other sugars, but not proline (Rizhsky et al., 2004). Hence, Mittler (2006) studying all prevailing abiotic factor;[s has suggested to treat this situation as a new stress condition that he called "Stress combination". The mechanisms of plant tolerance to a combination of diverse stress conditions, particularly those that mimic the field environment, have gained interest particularly for the biotechnologists (Chen and Zhu, 2004; Al-Babili and Beyer, 2005; Luo et al., 2005; Munns, 2005; Shao et al., 2007b).

Heat stress – high temperature – affects the metabolism and structure of plants, especially cell membranes and many basic physiological processes such as photosynthesis, respiration, and water relations (Wahid et al., 2007). On the molecular level, this effect of heat stress reflects the temperature dependence of Michaelis–Menton constant (K_m) of every enzyme participating in the process (Mitra and Bhatia, 2008). Plants must cope with heat stress for survival, so they developed different mechanisms including the maintenance of cell membrane stability, capturing the reactive oxygen species (ROS), synthesis of antioxidants, accumulation and osmoregulation of osmoticum, induction of some kinases that respond to stress, Ca-dependent kinase proteins, and enhancing the transcription and signal transfer of chaperones (Wahid et al., 2007).

The induction and synthesis of heat-shock proteins due to high temperature exposure are common phenomena in all living organisms from bacteria to human beings (Parsell and Lindquist, 1993; Vierling, 1991; Gupta et al., 2010). It seems that the synthesis of these proteins is costly energy wise that is reflected on the yield of the organism.

2. Heat-shock proteins classification

Historically, the observation of the Italian Scientist R. Ritossa on gene expression of the puffing in the chromosomes of *Drosophila melanogaster* after exposure to heat was the start of discovering the heat-shock proteins. The result was an increase in protein synthesis that occurred also by the use of other stress factors such as azide, 2,4-dinitrophenol, and salicylate (Ritossa, 1962). After that report, these proteins were identified and named as heat-shock protein (Hsp) (Tissieres et al., 1974). Researchers started studying the relationship of the synthesis of these proteins with the tolerance of stresses. On the other hand, it was reported that the induction of Hsp synthesis in *Glycine max* var. Wayne seedlings is accompanied by the reduction of other proteins synthesis after the exposure of such seedlings to heat shock (from 28 to 45 °C) for 10 min (longer periods killed the seedlings). Moreover, subjecting the seedlings to flashes of heat at 40 °C before exposing them to higher temperatures (45 °C) protects the seedlings (Lin et al., 1984).

Many types of Hsps have been identified in almost all organisms (Bharti and Nover, 2002). All Hsps are characterized by the presence of a carboxylic terminal called heat-shock domain (Helm et al., 1993). Heat-shock proteins having molecular weights ranging from 10 to 200 KD are characterized as chaperones where they participate in the induction of the signal during heat stress (Schöffl et al. 1999). Some researchers concluded that although there are some evidences for the genetic expression phenomenon in some specific cases, there are no final and conclusive evidence that this is what is happening in natural environment (Feder and Hofmann, 1999).

Heat-shock proteins of archaea have been classified on the basis of their approximate molecular weight into: (1) Heat-shock proteins 100 KD, i.e. Hsp100, (2) Hsp90, (3) Hsp70, (4) Hsp60, and small heat-shock proteins (sHsps) where the molecular weight ranges from 15 to 42 KD (Trent, 1996). These sHsps are usually a complex of small subunits where the molecular weight ranges from 200 to 800 KD (Kim et al., 1998).

In eukaryotic organisms, one of the reviews concluded that the principle heat-shock proteins of human beings do not differ from those of bacteria except for the presence of Hsp33 (Schlesinger, 1990). Later, the Hsps of human beings were grouped into five families (Kregel, 2002) as in Table 1.

In plants, general reviews (Schlesinger, 1990; Schöffl et al., 1998; Kotak et al., 2007) suggested five principal classes of Hsps characterized by their activities as molecular chaperones according to their approximate molecular weight: (1) Hsp100, (2) Hsp90, (3) Hsp70, (4) Hsp60, and (5) small heat-shock

Table 1 Families of Hsps in human beings, their site, and suggested functions (Kregel, 2002).

Hsp Families	Cellular location	Proposed functions
Hsp27 (sHsp)	Cytosol, nucleus	Microfilament stabilization, antiapoptotic
Hsp60	Mitochondria	Refolds proteins and prevent aggregation of denatured proteins, proapoptotic
Hsp70		Antiapoptotic
Hsp72(Hsp70)	Cytosol, nucleus	Protein folding, cytoprotection
Hsp73(Hsc70)	Cytosol, nucleus	Molecular chaperones
Hsp75(mHsp70)	Mitochondria	Molecular chaperones
Hsp78(GRP78)	Endoplasmic reticulum	Cytoprotection, molecular chaperones
Hsp90	Cytosol, endoplasmic reticulum, nucleus	Regulation of steroid hormone receptors, protein translocation
Hsp110/104	Cytosol	Protein folding

proteins (sHsps). Recently, another review (Gupta et al., 2010) put the heat-shock proteins into families according to their molecular weight, amino acid sequence homologies and functions: Hsp100 family, Hsp90 family, Hsp70 family, Hsp60 family, and the small Hsp family.

It appears that abbreviations of Hsps names of bacteria differ from those in eukaryotic cells as given below.

Escherichia coli	Eukaryotic cells
ClpB	Hsp100
HtpG	Hsp90
Dnak	Hsp70
GroEL	Hsp60

But for sHsps the nomenclature is the same (Kotak et al., 2007).

The roles played by heat-shock proteins in fungi have been reviewed lately (Panaretou and Zhai, 2008), with a general conclusion that they act as multi-component machines, playing roles in signaling and expansion of phenotypic plasticity, as well as their well-established function as molecular chaperones.

Plants vary greatly in the amount of expressed Hsps as well as their type (Hamilton et al., 1996). The most studied species of plants is *Arabidopsis thaliana* where the response to heat-shock treatment occurs through the participation of a number of different Hsps:

- 13 (Hsp20)
- 8 (Hsp70)
- 7 (Hsp90)
- 8 (Hsp100)
- 21 transcription factors (Hsfs) (Swindell et al., 2007), but in tomato there are at least 15 Hsfs (von Koskull-Döring et al., 2007).

Higher plants are characterized by the presence of at least 20 types of sHsps, but one species could contain 40 types of these sHsps (Vierling, 1991). sHsps, which are usually undetectable in plant cells under physiological conditions, are induced upon stress and plant tolerance to stress, including drought, salinity, oxidized species, and low temperatures (Löw et al., 2000; Hamilton and Heckathorn, 2001; Scharf et al., 2001; Zhang et al., 2008). It is believed that this diversification and abundance of the sHsps in a plant reflect an adaptation of the plant to heat stress (Waters et al., 1996). An example of this diversification of sHsps in plant species with their location is given in Table 2.

Furthermore, the sHsps of *A. thaliana* and *Lycopersicon esculentum* are divided into three subclasses (Scharf et al., 2001; Siddique et al., 2003). These included:

- Subclass CI represented by six proteins in *A. thaliana* and five proteins in *L. esculentum*
- Subclass CII represented by two genes in both plants
- Subclass CIII represented by one gene in both plants

A recent study (Siddique et al., 2008) reported the presence of other groups in the cytoplasm of *A. thaliana* cells and could be categorized into subclasses: CIV, CV, CVI, and CVII. Each subclass has its own distinct characteristics and role.

There are six groups of genes that encode for the sHsps. The grouping is based on the sequence similarity and the location of these proteins in the cell. There are two classes of proteins (Class I and Class II) in the cytoplasm encoded by two groups of genes. Other locations are chloroplasts, endoplasmic reticulum, mitochondria, and membranes (Vierling, 1991; Waters et al., 1996). The expression of genes for these sHsps is limited in the absence of environmental stress and occurs in some stages of growth and development of plants such as embryogenesis, germination, development of pollen grains, and fruit ripening (Sun et al., 2002).

The transcription of these genes is controlled by regulatory proteins called heat stress transcription factors (Hsfs) located in the cytoplasm in an inactive state. So these factors are considered as transcriptional activators for heat shock (Clos et al., 1990; Baniwal et al., 2004; Hu et al., 2009). Each factor has one carboxylic terminal (C-terminal) and three amino terminal (N-terminal) and has the amino acid leucine (Schuetz et al., 1991). Plants are characterized by a large number of transcriptional factors [at least 21 (Nover and Baniwal, 2006)]. These factors have been classified (Tripp et al., 2009) into three classes according to the structural differences in their aggregation in triples, i.e. oligomerization domains as follows:

- Plant HsfA such as HsfA1 and HsfA2 in *L. esculentum*
- Plant HsfB such as HsfB1 in *L. esculentum*
- Plant HsfC

Each factor has its role in the regulatory network in plants. However, all cooperate in regulating many functions and different stages of response to periodical heat stress (triggering, maintenance, and recovery). This role is represented in tomato system where HsfA1a is the master regulator that is responsible for the induced-stress gene expression including the synthesis of both HsfB1 and HsfA2 as these factors are found after

Table 2 Location of sHsps types in plant cell and the DNA accession number (after Waters et al., 1996).

Species	Protein	DNA accession number
Chloroplast-localized proteins		
Arabidopsis thaliana	HSP21	X54102
Glycine max	HSP22	X07188
Petunia hybrida	HSP21	X54103
Pisum sativium	HSP21	X07187
Triticum aestivum	HSP26A	X58280
Triticum aestivum	HSP26B	X67328
Zea mays	HSP26	L28712
Mitochondrial-localized protein		
Chenopodium rubrum	HSP23	X15333
Endoplasmic reticulum-localized proteins		
Arabidopsis thaliana	HSP22	U11501
Glycine max	HSP22	X63198
Pisum sativum	HSP22	M33898
Class I Cytocolically localized proteins		
Arabidopsis thaliana	HSP17.6	X16076
Arabidopsis thaliana	HSP17.4	X17293
Arabidopsis thaliana	HSP18.2	X17295
Chenopodium rubrum	HSP18.3	X53870
Daucus carota	HSP18.0	X53852
Daucus carota	HSP17.8	X53851
Glycine max	HSP17.5	M11318
Glycine max	HSP17.6	MI1317
Glycine max	HSP18.5	X07160
Helianthus annuus	HSP17.6	X59701
Lycopersicon esculentum	HSP17.8	X56138
Medicago sativa	HSP18.1	X58710
Medicago sativa	HSP18.2	X5871 1
Oryza sativa	HSP16.9	X60820
Oryza sativa	HSP17.4	D 12635
Pisum sativum	HSP18.1	M33899
Triticum aestivum	HSP16.9A	X13431
Triticum aestivum	HSP16.9B	X64618
Triticum aestivum	HSP16.9C	L 14444
Zea mays	HSP17.2	X65725
Class I1 Cytocolically localized proteins		
Arabidopsis thaliana	HSP17.6	X63443
Glycine max	HSP17.9	X07159
Ipomea nil (Pharbatis nil)	HSP18.8	M99430
Ipomea nil (Pharbatis nil)	HSP17.2	M99429
Lilium longiflorum	HSP17.6	D21816
Lilium longiflorum	HSP16.5	D21818
Pisum sativum	HSP17.7	M33901
Triticum aestivum	HSP17.3	X58279
Zea mays	HSP17.5	X54076
Zea mays	HSP17.8	X54075

the induction by heat treatment. These three factors are necessary for plant acquisition of heat tolerance (Baniwal et al., 2004). Mishra et al., 2002 have discussed the subject in detail in their paper. Hence, there is an Acquired Thermotolerance (AT) phenomenon which is supported by a study on *A. thaliana* that indicated the participation of HsfA2 (Charng et al., 2007). Furthermore, HsfA2 was finely regulated with Hsp17-CII during anther development of a heat-tolerant tomato genotype and was further induced under both short and prolonged heat stress conditions (Giorno et al., 2010). The study data suggested that HsfA2 may be directly involved in the activation of protection mechanisms in the tomato anther during heat stress and, thereby, may contribute to tomato fruit set under adverse temperatures.

In a study of molecular events, that is important to acquire thermotolerance in *A. thaliana,* the viability and transcription profiles were compared after three treatments. The first treatment was for the severe heat stress (45 °C) without acclimation; the second was a gradual increase from 22 °C to 45 °C over 6 h (one acclimation method); and the third treatment was 90 min at 38 °C plus 120 min at 22 °C before 45 °C, another acclimation method (Larkindale and Vierling, 2008). Results of this study indicated significant differences in the total spectrum of transcript changes in the two treatments (acclimation and without acclimation). There was also an increase in the transcript of specific genes involved in processes predicted to be required for thermotolerance (protection of proteins and translation and limiting oxidative stress). On the other hand, the study reported a decrease in transcripts (for programmed cell death, basic metabolism, and biotic stress responses). Finally the study reported the definition of eight genes involved in heat acclimation including cytosolic ascorbate peroxidase and the transcription factor HsfA7a and NF-X1.

Molecular pathway leading to the expression of genes to synthesize heat-shock proteins is composed of several mechanisms such as mechanism of sensing temperature that is connected to the mechanism of signal transfer to Hsfs where the activation of gene expression occurs by binding to the heat shock element (HSE) in DNA (Schöffl et al., 1998; Larkindale et al., 2005). HSE is a specific recognition sequence located in the region of gene activator in DNA. HSE was defined as alternating units of 5′-nGAAn-3′ and efficient binding requires at least three units (Morimoto 1998; Schöffl et al., 1998). In the absence of stressing factors, Hsfs are present in the cytoplasm as single and free as there is no binding activity with DNA, but when stress starts the factors aggregate in triplet and accumulate in the nucleus (Sorger and Nelson, 1989). The binding of Hsfs to DNA in tomato seedlings *Solanum lycopersicum* was promoted by salicylic acid (SA) that did not promote the transcription of *hsp70* mRNA or the expression of Hsfs such as *hsfA2* and *hsfB1*. This could indicate that SA has a role in modulating the Hsf for binding (Snyman and Cronje, 2008).

3. Role of heat-shock proteins

The function of any protein is determined by its formation and folding into three dimensional structure (Levitt et al., 1997). Formation of three dimensional structure requires 50% of principle amino acids sequence (Dobson et al., 1998). That is where the role of Hsps in the folding of other proteins is important. Morimoto and Santoro (1998) indicated that Hsps protect cells from injury and facilitate recovery and survival after a return to normal growth conditions. On the other hand, Timperio et al. (2008) specified that upon heat stress, the role of Hsp as molecular chaperones is without doubt, their function in non-thermal stress could be different: unfolding of proteins is not the main effect and protection from damage could occur in an alternative way apart from ensuring the maintenance of correct protein structure.

It has been suggested that Hsps general role is to act as molecular chaperones (Fig. 1) regulating the folding and accumulation of proteins as well as localization and degradation in all plants and animal species (Feder and Hofmann, 1999;

Schulze-Lefert, 2004; Panaretou and Zhai, 2008; Hu et al., 2009; Gupta et al., 2010). These proteins, as chaperones, prevent the irreversible aggregation of other proteins and participate in refolding proteins during heat stress conditions (Tripp et al., 2009). Each group of these Hsps has a unique mechanism and the role of each is briefed.

3.1. Class: sHsps

These proteins have a common alpha-crystallin domain containing 80–100 amino acid residues located in the C-terminal region (Seo et al., 2006). One of the characteristic functions of this class is the degradation of the proteins that have unsuitable folding. The representative protein is the sHsp ubiquitin (molecular weight is 8.5 KD) with its bounded enzymes (Ferguson et al., 1990). Another characteristic that distinguishes these sHsps from other chaperone classes is that their activity is independent from ATP (Miernyk, 1999). However, this paper gave more information about the structure, classification, and function of sHsps as well as the results of transcription of genes in *A. thaliana*. These results indicated the participation of other factors such as plant growth regulators and reactive oxygen species in plant heat tolerance.

The sHsps cannot refold non-native proteins, but they can bind to partially folded or denatured substrates proteins, preventing irreversible unfolding or wrong protein aggregation (Sun et al., 2002). Recent findings showed that the sHsp 18.1 isolated from *Pisum sativum*, as well as the sHsp 16.6 from *Synechocystis* sp. PCC6803 under in vitro conditions, binds to unfolded proteins and allows further refolding by Hsp70/Hsp100 complexes (Mogk et al., 2003).

It was noticed that there was a positive qualitative relation between the accumulation of sHsps in the plastids and thermotolerance of heat shock (from 28 to 40 °C) in six divergent Anthophyta species, including C_3, C_4, CAM, monocot, and dicot species. Similar results were obtained separately with four non Anthophyta species (Downs et al., 1998). Another study (Downs and Heckathorn, 1998) indicated that the mitochondrial sHsp protected NADDH: ubiquinone oxidoreductase (complex I) during heat stress in apple fruit of *Pyrus pumila* (P. Mill.) K. Koch var. McIntosh. This information might indicate some role of these proteins in adaptation of plants to heat stress. A recent review (Nakamoto and Vígh, 2007) concluded that there were some indications that small heat-shock proteins play an important role in membrane quality control and thereby potentially contribute to the maintenance of membrane integrity especially under stress conditions.

3.2. Class: Hsp60

This class Hsp60 is called in some of the literature as chaperonins and it is generally agreed that they are important in assisting plastid proteins such as Rubisco (Wang et al., 2004). Some studies pointed out that this class might participate in folding and aggregation of many proteins that were transported to organelles such as chloroplasts and mitochondria (Lubben et al., 1989). These Hsps60 bind different types of proteins after their transcription and before folding to prevent their aggregation (Parsell and Lindquist, 1993). Functionally, plant chaperonins are limited and the general idea is that stromal chaperones (Hsp70 and Hsp60) are involved in attaining functional conformation of newly imported proteins to the chloroplast (Jackson-Constan et al., 2001).

3.3. Class: Hsp70

In almost all organisms, the Hsp70 functions as chaperones for newly synthesized proteins to prevent their accumulations as aggregates and folds in a proper way during their transfer to their final location (Sung et al., 2001; Su and Li, 2008). Furthermore, Hsp70 and sHsps primarily act as molecular chaperone and play a crucial role in protecting plant cell from the detrimental effects of heat stress (Rouch et al., 2004) and Hsp70 and sHsp17.6 might play a crucial role in the development of cross-adaptation to temperature stress induced by heat acclimation (HA)- or cold acclimation (CA) pretreatment in grape plants (Zhang et al., 2008). Cooperation in the activities of this class (folding of proteins) and small heat-shock proteins such as sHsp18.1 (prevention of aggregation of proteins) was reported in a study of *P. sativum* (Lee and Vierling, 2000). Hsp70 participates, also, as a part of guidance complex import (translocon) that bound to protein precursor to be transferred through the membranes into the organelles such as chloroplast (Jackson-Constan et al., 2001; Soll, 2002).

There is some indication that Hsp70B found in the stroma of chloroplasts participate in photo protection and the repairing of photosystem II during and after the photoinhibition (Schroda et al., 1999). A more recent study on *A. thaliana* indicated the necessity of Hsp70 found in the stroma of chloroplast for the differentiation of germinating seeds and its tolerance of heat (Su and Li, 2008).

3.4. Class: Hsp90

The class Hsp90 shares with other classes, the role being molecular chaperones as Hsp90 can bind Hsp70 in many chaperone complexes and has important role in signaling protein function and trafficking (Pratt and Toft, 2003). This class, also, plays another important role as they regulate the cellular signals such as the regulation of glucocorticoid receptor (GR) activity (Pratt et al., 2004). Cytoplasmic Hsp90 is responsible for pathogen resistance by reacting with resistance protein (R) which is the signal receptor from the pathogen. The reaction between Hsp90 and resistance protein is very critical for the functioning of the latter as indicated from a study on *A. thaliana* and two species of tobacco namely *Nicotiana tabacum* and *Nicotiana benthamiana* (Hubert et al., 2003; Liu et al., 2004). This mechanism resembles the regulating mechanism of steroid receptor in animals (Schulze-Lefert, 2004). Thao et al. (2007) have reported that Hsp90 was an essential component of innate-immune response and pathogenic resistance in rice. In *A. thaliana*, there were some indications that Cytoplasmic Hsp90 negatively inhibited hsf in the absence of heat stress, but under heat stress this role is suspended temporarily, so that hsf is active (Yamada et al., 2007).

3.5. Class: Hsp100

One unique function of this class is the reactivation of aggregated proteins (Parsell and Lindquist, 1993) by resolubilization of non-functional protein aggregates and also helping to degrade irreversibly damaged polypeptides (Bösl et al., 2006; Kim et al., 2007). One cytoplasmic member of this class was

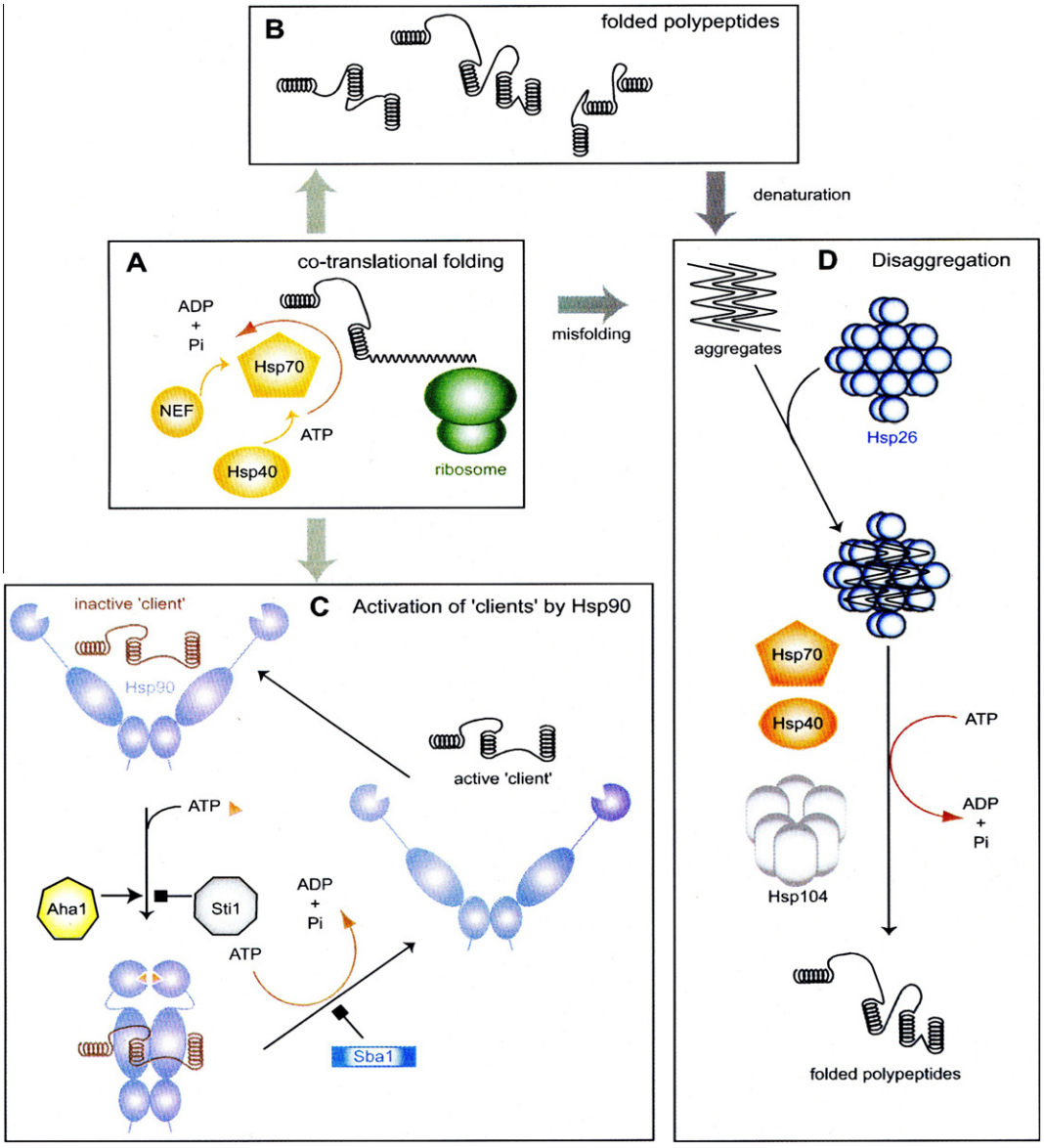

Figure 1 Simple illustration of part of the chaperone machines that operate in the cytosol: (A) Folding of proteins by Hsp70 is co-translational, nucleotide exchange factors (NEFs) and Hsp40 facilitate this process. (B) Once protein synthesis is complete. Homologues of Hsp70 promote folding in other cellular comparetments. (C) Certain proteins are presented in a largely folded though inactive state, to the Hsp90 chaperosome, the ATP-dependent action of which leads to activation of the substrate protein. Co-chaperones act as adaptors between Hsp70 and Hsp90, with specific co-chaperones acting as inhibitors (e.g. Sti1) or stimulators (e.g. Aha1) of the Hsp90 ATPase. (D) Misfolding and cellular stress lead to aberrant protein conformations, which can lead to aggregation. Hsp104 catalyses disaggregation, a process facilitated by Hsp's 70, 40 and 26. (From Panaretou and Zhai, 2008).

necessary for high heat tolerance by the plant, but not necessary for germination and growth in the absence of stress (Queitsch et al., 2000; Hong and Vierling, 2001). The function of this class is not restricted to acclimation to high temperatures, but a specific member of the family provides housekeeping functions that are essential to chloroplast development (Lee et al., 2006). It seems that this class participates also in facilitating the normal situation of the organism after severe stress (Gurley, 2000).

In general, principal Hsps that are expressed in large quantities during stress have related functions as they ameliorate the problems of unsuitable folding and aggregation (Queitsch et al., 2000).

There are large number of reviews about Hsps and their importance, and one extensive review (Feder and Hofmann, 1999) about physiological, ecological, and evolutionary aspects concluded that:

1. The expression of Hsps could occur in natural environment
2. The *hsp* genes are found in all species but they vary in patterns of expression
3. The expression of Hsps could be correlated with resistance to stress
4. The threshold of species for Hsps expression are correlated with the strength of stress prevailing in the environment

Previous conclusions about the roles of Hsps as molecular chaperone put them in three main roles: (1) induce (refold) denatured proteins, (2) participate in the finalization of the *de novo* synthesized proteins and (3) reduce the protein aggregation (Trent, 1996). Simply, the Hsps are known for their roles in the maturation of protein complexes and the degradation of damaged or misfolded peptides, and for regulating the activity of many signal transduction proteins (Pratt and Toft, 2003; Rutherford, 2003).

4. Phenomena of induction of Hsps in plants

Presence of Hsps in higher plants was discovered in tobacco and soybean using cell culture technique (Barnett et al., 1980). When soybean was subjected to 40 °C for four hours, ten new proteins were found, but disappeared after 3 h treatment at 28 °C (Key et al., 1981). Studying the gene expression of Hsp90 in rice plant (*Oryza sativa*) indicated that the heat-shock protein Hsp87 was present after 2 h of heat shock (from 28 to 45 °C), and its quantity was high and stable even after long heat stress (4 h) and the return to normal conditions (no stress). It was found, also, that Hsp90 (Hsp85 and Hsp87) could be induced by other kind of stress such as salinity, drought, and cold. This study reported the accumulation of different levels of these proteins in fifteen wild species of rice (Pareek et al., 1998).

In this context is what another study indicated about the importance of Hsp90 and the definition of the gene (*rHsp90*, GenBank Accession No. AB037681) that encodes them in rice plant, and the finding that they participate in plant tolerance of other abiotic stresses such as salinity (NaCl, $NaHCO_3$, and Na_2CO_3), desiccation (of polyethylene glycol, PEG), high pH (8.0 and 11.0), and high temperatures, viz 42 and 50 °C (Liu et al., 2006).

Several studies on other plants (Singla et al., 1997) indicated that Hsps synthesis qualitatively and quantitatively was dependent on cell/tissue type and/or the degree of differentiation and development. Earlier, the presence of a cytoplasmic class of proteins (Class 1) in seeds of wild and commercial legumes was reported (Hernandez and Vierling, 1993). This indicated the expression of this class under natural environment. A further field study of the expression of this class in leaves, flowers and developing seed pods in *Medicago sativa* was carried out. Results indicated the repeated formation of these proteins in flowers and buds, even in plants that did not have these proteins expressed in their leaves (Hernandez and Vierling, 1993). During storage of beech (*Fagus sylvatica* L.) seeds, a sHSP with molecular mass of approximately 22 kDa was identified (Kalemba and Pukacka, 2008).The largest content of this protein was observed in the oldest seeds, especially in embryonic axes.

A natural habitat near geysers in the National Yellowstone Park, Wyoming, has some plants (monocots and dicots) growing where the soil temperature is more than 40 °C. To evaluate the role of Hsps in the adaptation of these plants to such harsh environments, Hsps content of shoot and root systems of these plants were estimated. The presence of sHsp of Cytoplasmic Class 1 was reported, but they were not expressed in the shoot system. On the other hand, Hsp100 (Hsp101) was detected in both leaves and root system (Stout and Al-Niemi, 2002).

There were some studies about the presence of Hsps in plants subjected to two or more stress factors mimicking natural field conditions. In the field, heat stress was usually accompanied by one or more of stress factors such as drought, high irradiation, salinity, or others, but studies of this kind are scarce. One of these studies was performed on irrigated and non irrigated cotton plants (*Gossypium hirsutum* L.) where most growth parameters decreased (80% to 85%) in non irrigated plants (Burke et al., 1985). The study, also, indicated reduced photosynthesis (two folds) at midday compared to irrigated plants where the temperature under the canopy of irrigated plants reached 30 °C while that was 40 °C under the canopy of non irrigated plants. These differences between the two treatments were accompanied by differences in protein content too. Plant leaves of non irrigated accumulated a steady level of proteins that have molecular weight of 100, 94, 89, 75, 60, 58, 37, and 21 KD after several weeks, and these proteins were not detected in leaves of irrigated plants. Pursuing these results, leaves of cotton plant that was grown in growth chamber were incubated at 40 °C with the labeled amino acid [^{35}S]methionine and after three hours, the same proteins but labeled appeared. The final conclusion of this study was that cotton plants accumulated heat-shock proteins under natural conditions of drought stress and 40 °C temperature.

Day/night temperatures cycle affects plant growth and to test the effect of changing that from 20/30 °C to 40/50 °C in three desert succulent plants, *Agave deserti*, *Carnegiea gigantean*, and *Ferocactus acanthodes*, an experiment was performed (Kee and Nobel, 1986). It was reported that there was an increase in thermal tolerance (6–8 °C) after 10 days and all the three species accumulated protein with a molecular weight of 25–27 KD only at the cycle (40/50 °C), while other types of Hsps accumulated according to the species.

Photoinhibition is a limiting factor in photosynthesis and in natural environment the light intensity is very high, at least in some areas. Light induces the synthesis of the Hsps and they therefore might ameliorate the damage caused by high intensity of light. This possibility was investigated by comparing the content of Hsps of leaves exposed to direct sun light with shaded leaves of *Solidago altissima*, family Asteraceae in cold days and warm ones in the field (Barua and Heckathorn, 2006). The results indicated that the Hsps content in the leaves exposed to direct sun light and at natural heat stress was higher significantly. Both light and temperature significantly affected accumulation of Hsps in the laboratory.

Another field study on the desert legume *Retama raetam* and the interaction of stress factors in arid regions indicated the presence of daily periodism of photosynthesis (Merquiol et al., 2002). One period was between 07:00 and 10:00, while the second was between 15:00 and 17:00. Similar periodism was reported for another desert legume *Prosopis chilensis* growing in a desert of Chile (Ortiz and Cardemil, 2001). During the reduction in photosynthesis rate (from 11.00 to 15.00) there was an induction of transcripts of enzymes participating in defense (removal of reactive oxygen intermediates) and the synthesis of Hsps. The final conclusion was that *R. raetam* used a combination of avoidance and active defense mechanisms to withstand the stressful conditions that prevail within desert land.

Plant response to abiotic stress factors is controlled by complex net of genes. In the project of gene expression database of *A. thaliana* with the title of AtGenExpress, the effect of several abiotic stresses (heat, cold, drought, salinity, osmotic, UV-B, light, and wounding) was studied under similar conditions

on the seedlings of *A. thaliana* (Kilian et al., 2007), and the results were analyzed by the DNA Microarray Technology. This study provided the types of gene expression induced by abiotic stresses including models for the analysis of the information of gene expression in response to UV-B, drought, and cold stresses. Results indicated that the first reaction to stress on the level of transcription in this plant included a group of stress-response genes. These genes might have a crucial role in the response to different stresses as well as the main role of systemic signals generated by the tissue exposed to stress.

The interaction of different biotic and abiotic stresses with heat stress was studied and analyzed, and the information in respect of transcription of Hsps and Hsfs in the plant *A. thaliana* was deposited in the database AtGenExpress Consortium (Schmid et al., 2005). Results of the analysis indicated that all stresses interacted in the response pathways of heat-shock proteins and their factors, but the degree of interaction was different which suggested a cross-talk in the regulating net. Hu et al. (2009) examined a global expression profiling with heat stressed rice seedling, and then compared their own results with the previous rice data under cold, drought, and salt stresses. They concluded that Hsps and Hsfs might be important elements in cross-talk of different stress signal transduction networks.

In general, the expression of Hsps and its factors Hsfs was induced largely by heat, cold, salinity, and osmotic stresses. The response to other stress factors depended on protein class and tissue. For example, under all types of stresses, high expression response for class Hsp20 was recorded with high similarity of their information. Wounding the roots of the plant stimulated (after 12 h) the expression of several genes from the classes Hsp20, Hsp70, and Hsp100. High response of expression of the genes for Hsps and its factors Hsfs was observed under UV-B stress in aerial tissues (shoot), but in non aerial tissues (root system) there were no expression (Swindell et al., 2007).

There are some indications about the relationship between the response to heat stress and the response to oxidative stress as both stresses induce the pathways leading to the expression/accumulation of Hsps (Dat et al., 1998; Lee et al., 2000). On the other hand, heat stress induced the expression of antioxidant enzymes (Gong et al., 1998; Lee et al., 1999). One of these important enzymes is ascorbate peroxidase (APX) which uses ascorbate to nullify the toxicity of hydrogen peroxide (one of reactive oxygen species). Different isozymes of this enzyme were found in the cytoplasm of the plant cell and in some of its organelles (Panchuk et al., 2002). It was reported that there was an interdependence signaling for both stresses.

5. Heat stress tolerance and genetic engineering in plants

More than 52 plant species have been genetically modified. Some of these species are crop plants such as maize, soybean, cotton, and potato. They were modified for some desired traits such as increase in yield, resistance for some herbicides, resistance to insects, and change in sugars and starch. They were tested in the field (Dunwell, 2000). Other transgenic non-crop plants being resistant to stress were produced in the laboratory (Wang et al., 2003).

Now, different approaches are made to produce stress resistant plants to tackle the global warming (Report of the Working Group 2, 2007). The mechanism of molecular control of abiotic stress tolerance depends on activation and regulation of genes related to a particular stress. Abiotic stress tolerance in crop plants could practically be achieved by the combination of molecular techniques and traditional plant breeding in one program (Wang et al., 2003; Vinocur and Altman, 2005). Field evaluation of one transgenic plant *Agrostis* for salinity tolerance was reported by Dunwell, 2000. The plant contained betaine aldehyde dehydrogenase gene.

Temperature stress (high temperature) is considered as one of the major stresses on crop plants (Grover et al., 2000). The response of plants to heat shock resulted in changes in the level of enzymes, cellular membrane structure, photosynthesis activity, and protein metabolism (Singla et al., 1997). It has been reported that high temperature changed the properties of membranes of nucleus, endoplasmic reticulum, mitochondria, and chloroplasts of rice plant *O. sativa* (Pareek et al., 1998). Lipids in the thylakoid membranes of the chloroplast are very important to improve photosynthesis and hence stress tolerance. In 1992, scientists have modified plant cells with an increase in cold stress tolerance (Murata et al., 1992). This was achieved by increasing the gene expression of glycerol 3-phosphate acyltransferase from *Cucurbita maxima* and *A. thaliana* in tobacco plant cells, resulting in an increase in the degree of unsaturation of the lipids. Therefore, increasing the degree of unsaturation of fatty acids leads to an increase in cold tolerance. The opposite situation is that increasing the degree of saturation could lead to heat tolerance (Grover et al., 2000). In support of this hypothesis was what had been published about the possibility of using genetic engineering to have more tolerant plants to high temperatures by reducing the degree of saturation of fatty acids in membranes (Murakami et al.,2000) through silencing the enzyme φ3-fatty acid desaturase that involves in the synthesis of triple bonds in fatty acids. There was some indications that changing the levels of expressing Hsps by changing the transcription factor of *Arabidopsis* (AtHsf) led to an increase in heat tolerance (Lee et al., 1995). Consequently, the increase in the synthesis of osmolytes in the cell could participate in an increase in heat tolerance (Alia et al., 1998). Later, Sanmiya et al. (2004) reported that mitochondrial sHsp enhances thermotolerance in the transgenic plants of *N. tabacum* by the *MT-sHSP* gene from *L. esculentum*.

In an attempt to increase salinity tolerance of wheat plant, one report mentioned that transgenic plants were subjected to water stress, high salinity, and heat stresses under operating greenhouse conditions and in the field. Stress conditions were withholding watering the plant for two weeks (water stress), watering in the presence of 400 mM NaCl (salinity stress), and subjecting the plants to 46 °C for two hours followed by a 3-day period recovery at 28 °C (heat stress). Transgenic plants contained a gene (CtHSR1) from the yeast *Candida tropicalis*]. The results showed improvement of growth under both drought and heat stresses and lesser but still significant to salinity stress (Blumwald and Arif, 2007). Many attempts were made to have genetically engineered plants for stress tolerance especially crop plants. Most of these attempts were for one trait, while in natural conditions the prevailing conditions were more than one stress, hence the stress combination should be dealt with as a new state of abiotic stress as mentioned earlier (Mittler, 2006). However, some examples of these attempts are shown in Table 3.

Table 3 Transgenic attempts to enhance plant temperature stress tolerance.

Phenotypes	Function	Plant	Gene
HS tolerant; HS sensitive	TF	Tomato	HsfA1
HS tolerant	TF	*Arabidopsis*	Hsf3
HT sensitive	HSP	*Arabidopsis*	Hsp70
Abolished acquired thermotolerance	HSP	Maize	Hsp101
HT tolerant	HSP	Carrot	Hsp17.7
HT tolerant	Fatty acid desaturation	Tobacco	Fad7
HS tolerant	AOS metabolism	Barley	Hvapx1

Source: sample of a larger table of Sung et al. (2003).
Abbreviations: AOS, active oxygen species; HS, heat shock; Hsf, heat-shock factor; HSP, heat-shock protein; HT, high temperature; TF, transcription factor; APX, ascorbate peroxidase; fad7; fatty acid desaturation.

Preconditioning of the plants for acclimation of physiological processes under stress has been exploited. Vásquez-Robinet et al. (2010) investigated the behavior of heat-shock proteins during photosynthetic acclimation and different levels of water stress in loblolly pine seedlings. Their results suggested that a cycle of mild stress conditioned the trees to adapt to a more severe stress. Moreover, their results indicated specific patterns in needles in the expression of Hsp70, Hsp90, and sHSP genes.

In summary, the response to and survival of stress are complex phenomena in plants. However, there is substantial information about heat-shock proteins. Some literature describe their induction by different stresses, their arbitrary classification, and the function of various heat-shock proteins as chaperones. Some other literature deal with molecular biology and biochemistry that include cloning genes, determining the primary sequences of these proteins, and probing the regulatory factors affecting their induction.

The induction of transcription of these proteins is a common phenomenon in all living things. These proteins are grouped in plants into five classes according to their approximate molecular weight. It is believed that the diversification of these proteins reflects an adaptation to tolerate stress. Heat-shock proteins have some kind of related roles in regulating a range of effect or components, all of which contribute to survival under abiotic stress by solving the problem of misfolding and aggregation, as well as its role as chaperones.

Until now there are more than 52 plant species (including crop ones) that have been genetically engineered for different traits such as increased yield, herbicide, and insecticide resistance, and some metabolic changes. Finally, it is very important to study stress combinations to end up with tolerant plants.

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