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Heat Shock Proteins and Heat Shock Response in Plants

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ABSTRACT

Prokaryotic and eukaryotic cells respond potentially harmful stimulations like heat stress by inducing synthesis of stress proteins so called heat shock proteins (Hsps) besides other metabolites. Heat stress response is a reaction when tissues and cells of an organism were exposed to sudden heat stress and is characterized by temporary expression of Hsps. Primary protein structures of Hsps and heat shock response are highly conserved in every organism which has been sought. Therefore it has been considered that Hsps might be closely involved in protection of organisms against heat stress and keeping homeostasis. Most of Hsps are known as molecular chaperons whose biological role is to maintain and shield the unfolded state of newly synthesized proteins thus preventing them from misfolding or aggregating. Here it was summarized the significance of Hsps and heat shock response in plants.

Key Words: Heat stress, Heat shock proteins, Plants, Thermal tolerance.

1. INTRODUCTION

Stress is generally defined as all effects that have negative impact on plants [46]. Active plant growth in mesophile organisms is in between 10-40°C. Any temperature which is under and below that range creates heat stress on metabolic activities of plants [48].

Plant growth is a function of biochemical reactions and those reactions are controlled by enzymes. Most of the chemical reactions increase as two fold by every 10°C increase in between 20-30°C. Temperatures above that range, reaction speed decreases since enzymes step by step denatured or inactivated. In addition, reactions catalyzed by enzymes depend on completeness of tertiary structure of enzymes. Increasing molecular collisions as temperature increase, harms tertiary structures and reduce enzyme activities and reaction rates. Faster increase in temperature brings faster denaturation. When an enzyme is inactivated, growth processes related to that enzyme is depressed [48]. Photosynthesis is an important reaction affected by heat. Photosynthesis is performed in between 10-30°C range in temperate climate plants. Above 10°C photosynthesis increases as temperature increases, it decreases above 30°C [48]. Respiration generally adapt a normal course at 5-25°C, it is accelerated up to 30-35°C and decelerated above 35°C [23]. It was reported that heat stress disrupts water, ion and organic solute movement across plant membranes [21], reduces chemical reactions, gas solubility, mineral absorption and water take up [48], impairs photosynthetic electron transport system, and increases oxidative degeneration of membrane lipids [10]. Toxic material formation has been detected as a result of high temperature. It was suggested that heat injury is due to the toxic effect of NH_3 produced at high temperatures and that this effect is counteracted by the respiratory production of organic acids. This may help to explain the heat tolerance of succulents, since they posses a highly acid metabolism. On the other hand, their acidity is at a maximum when danger of heat is minimal (at night) and at a minimum when the danger is maximal (in the afternoon) [28]. Heat stress is a major factor limiting the productivity and adaptation of crops, especially when temperature extremes coincide with critical stages of plant development. The rate of temperature change and the duration and degree of high temperatures all contribute to the intensity of heat stress. Where heat stress occurs, it is important that plants possess a certain degree of heat tolerance to survive the stress period. In addition, plant response to heat stress depends on the thermal adaptation, the duration of the exposure and the stage of growth of the exposed tissue [6].

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2. HEAT SHOCK PROTEINS IN PLANTS

Prokaryotic and eukaryotic organisms respond potentially harmful stimulations such as high and low temperature, radiation (UV), heavy metals, pesticide, salinity, bacterial and viral infections, parasitism, drought, oxidative stress, senescence, harmful mutations and reduction of cellular energy by inducing the synthesis of protein family called Stress Proteins. Heat shock proteins (Hsps) and other stress proteins have been known to protect cells against deleterious effects of stress [4, 13, 20, 30, 44, 56]. Hsp homologs were found in every species that have been studied [13]. Hsps are also expressed in some cells either constitutively or under cell cycle or developmental control [51, 54]. Hsps and their cognates are found in every organism at ordinary growth temperature and play an important role in cellular functions related with growth [30, 54]. The major stress proteins occur at low to moderate levels in cells that have not been stressed but accumulate to very high levels in stressed cells [56].

The major Hsps are found in different molecular weights and synthesized by eukaryotes belong to six structurally distinct classes: Hsp100, Hsp90, Hsp70, Hsp60 (or chaperonins), ~ 17-to 30 kDa small Hsps (sHsps) and ubiquitin (8,5 kDA) [51, 54]. Hsps are characterized as structurally unstable proteins. They serve important physiological functions. These functions of Hsps are closely related to resistance to heat and the other stresses [20, 42]. In all organisms, the induction of Hsps is remarkably rapid and intense. Concentrations of some proteins might reach 1% of total leaf and root cellular proteins. Temperatures above 10-15°C of optimal growth temperature induct Hsp response [30, 31, 45].

Heat shock response is an evolutionary conserved reaction that cells stop cellular activities and normal protein synthesis in order to temporarily reprogram Hsp synthesis [56]. There is a striking relationship between temperature inducing Hsp synthesis and organisms. While in the fruit fly *Drosophila melanogaster*, induction occurs between 33-37°C in termophilic bacteria growing at 50°C, the proteins are induced when temperatures are raised to 60°C, in arctic fishes growing at 0°C, they are induced at 5-10°C. [30]. In general, threshold temperature for Hsp induction is correlated with the typical temperatures at which species live. For example, termophilic species have higher threshold than other species. These results suggest that translation itself may have an upper thermal maximum that varies among species adapted to different temperature environments [13]. Stress sensitivity of plants varies during different stages of life cycle [52]. Most of the species show different and characteristic Hsp expression during different stages of their growth [13]. It was observed that cell groups or organisms die when temperature increase suddenly above optimum growth temperature of organisms studied up to date. However, Hsp synthesis is induced and cells die slowly when mild degree high temperature was introduced beforehand [30].

Distribution of Hsps differs among tissue, organ and genetic variability of plant [40]. It was reported that sHsps were differentially expressed in distinct varieties of common bean according their heat tolerance [3]. Mariamma et al. [34] reported that the antibodies against pea Hsp18.1 cross reacted with rice Hsp19 and 19.5 while antibodies against the carboxyl end of pea Hsp21 cross reacted with rice Hsp27. Ledesma et al. [26] studied proteins of leaves and flowers of strawberry plants (*Fragaria* x *ananassa* Duch.) exposed to 20, 33 and 42°C for 4 hours. In leaves and flowers of both cultivars, the content of most proteins decreased, but a few new proteins appeared in response to heat stress. These Hsps were detected in the range of 19-29 kDa in leaves and 16-26 kDa in flowers. The intensity of a 43 kDa protein spot increased in flower of one cultivar but, not in the other cultivar. These results show that the effects of heat stress on Hsp synthesis in strawberry plants differ between plant organs and plant cultivar. Temperature of 30-40°C combined with salt stress was introduced on lentil seeds during germination. While expression of sHsps declines at 30 and 40°C, this declining was higher in 40°C than 30°C [11].

It was reported that there is genetic variability in the synthesis of Hsps in wheat, barley, maize and potato [1, 8, 9, 25, 36, 49, 55, 58]. Heat responsive proteins of total endosperm composed mainly of prolamins were characterized in wheat. Of the 43 heat-changed proteins, 24 were found to be up-regulated whereas 19 spot proteins were down-regulated. 42 spots out of 43 were identified. Some enzymes of them were found to be involved in different metabolic pathways [32]. It was shown that there are quantitative and qualitative differences in especially sHsp synthesis [50] after heat stress in diploid [49] and hexaploid [25]. Protein synthesis was investigated in durum and bread wheat exposed to different temperatures during ripening. The result showed that wheat plants respond to thermal stress by triggering the typical mechanisms of the heat shock response including activation of the heat shock genes [47]. Embryos of *T. aestivum* L. simultaneously heat shocked following several different periods of prior imbibition up to 12 hours synthesized many groups of Hsps typical of other plant and animal systems [18]. Quantative and qualitative Hsp variations were observed in three spring wheat (*T.aestivum* L.) [58] and in two winter wheat (*T. aestivum* L.) and three genetically different three lines of *T. monococcum* [37]. Specific Hsps were identified for each line.

Temperature which maximum Hsps were synthesized changes according to species and the optimum temperature that species grow in plants. Plants synthesize Hsps proportionally with severity of heat until maximum level. Hsp synthesis is completely induced for surviving with maximum activation of other protection mechanisms at near deadly temperatures. However plants probably synthesize middle level Hsps at mild heat stress conditions at first, but if heat stress continues they synthesize more Hsps [1]. Seasonal variation in heat shock proteins Hsp70 and Hsp90

expression in an exposed and shaded habitat of *Iris pumila* has been detected. The concentrations of Hsp70 and Hsp90 were found significantly greater at the open area than shaded under storey, reaching their maximum in the summer, especially in plants experiencing full sunlight [33].

If proteins which are found in optimal growth temperature, also are found in heat stress conditions; these proteins are found in two forms: either their synthesis resumes again after shifting control temperature [39]. However, Hsp synthesis decreases in some organisms. In addition, sometimes both Hsp and normal protein synthesis are halted. Stability of relationship between Hsp transcription factor and heat shock elements can explain the transient nature of heat shock response [57]. Synthesis of some proteins insignificantly changes in heat stress comparing to control temperature. However, it was declared that protein synthesis is significantly inhibited in dicotyledonous plants comparing to control [8]. For example it was reported that when growing temperatures of seedlings of soybean were shifted from 28°C control temperature to 40°C, Hsp synthesis is significantly increased but normal protein synthesis is decreased [29]. Necchi et al. [36] reported that 5 different crop species (*T. aestivum, T. durum* Desf, *Hordeum vulgare* L., *Secale cereale* L. ve *Triticale*) had 13 strong protein bands, in addition to proteins found in control temperature.

Hsp synthesis appear naturally when plants are exposed to heat shock Chen et al. [7] reported that when pea plant was exposed to heat shock gradually up to 40°C, number of sHsps were recorded. It was observed that when these plants were returned to control temperature, these proteins were disappeared in 50h half life. Since half life of these proteins is long, they could have an important role during recovery. Synthesis of sHsps in heat stress is a characteristic of heat shock response in plants [1].

Studies have shown that also age impacts on the expression of inducible Hsps. Mostly, a decrease of Hsp expression level after induction is observed with age. The main reason seems to be a lower capacity to up-regulate expression at an older age. However at senescence, a low level of inducible Hsp70 is continuously expressed in *Drosophila*. It was suggested that the low Hsp induction was caused by a more pronounced need for chaperones in order to maintain optimal cell function and homeostasis [44].

3. FAMILIES OF HEAT SHOCK PROTEINS

Major Hsps synthesized by eukaryotes belong to six structurally conserved distinct classes: Hsp100, Hsp90, Hsp70, Hsp60 (Chaperonins), approximately 17-30 kDa molecular weight small Hsps (sHsps) and ubiquitin (8,5 kDa) [51, 54]. High molecular weight Hsps are characterized by high sequence homology in plants. But even closely related Hsp gene families show difference in terms of their specific function. In addition Hsp homologs which belong to same family also function in different cell departments. sHsps show great difference in plants among eukaryotes [51].

Hsp100 family: Members of this family are constitutively expressed, but they are also up regulated by environmental stresses. Proteins of this family generally function to protect protein denaturation and/or aggregation of proteins [53]. It was shown that Hsp104 in *Arabidopsis* and Hsp104 in yeast play an important role in heat stress tolerance [5, 31]. It was stated that over expression of Hsp101 in *Arabidopsis* has positive effect on growth during recovery period [52]. It was recently found that Hsp101 homologue in *Arabidopsis* is involved in plastid differentiation mediating internal thylakoid membrane formation and conferring thermotolerance to chloroplasts during heat stress [27, 35].

Hsp90 family: This family consists of proteins which have highly conserved amino acid sequence [30]. Proteins of this family especially are rich in cytoplasmic phosphoproteins [40]. The major role of Hsp90 is to manage protein folding. But it also plays a key role in signal transduction networks, cell cycle control, protein degradation and protein trafficking. In addition, it might also play a role in morphological evolution and stress adaptation in *Drosphila* and *Arabidopsis*. ATP is required its functions. It is among the most abundant proteins in cells: 1-2% of total cellular protein. Although Hsp90 chaperones are constitutively expressed in most organisms, their expressions increase in response to stress in both prokaryotes and eukaryotes. Decreasing the level of functional Hsp90 in *Drosophila* by genetic mutation or by treatment with an Hsp90 inhibitor causes developmental abnormalities and morphological changes [53].

Hsp70 family: Members of the Hsp70 family have been highly conserved through evolution. In addition to Hsp70s strongly inducible by heat shock and other forms of cellular stress, constitutively expressed Hsps70s have essential functions under no stressful conditions. These proteins include the *E.coli* dnaK, the yeast cytosolic proteins Ssa1p and Ssa2p and the Hsc70 of mammalian cells, the so called clathrin uncoating ATPase [16]. Homologs of these proteins are found within mitochondria, cytoplasma, nucleus, ER and chloroplasts in eukaryotic cells. Hsp70s of Mammalian and *Drosophila* cells are major translation products of heat shocked cells. Hsp70 accumulates in nucleus after heat shock exposure [19]. Organellar Hsps have specific amino terminal target sequences. They provide Hsp70 to translocate appropriate membrane sites. It has been shown that all Hsps have structural and functional properties following below:

1. They bind to ATP and highly conserved amino terminal ATP binding (~450 amino acid) and following more variable carboxyl terminal substrate binding domain. This is most likely responsible for peptide binding.
2. They bind to unfolded or partially denatured polypeptides.

3. They use the energy coming from ATP hydrolysis to release the substrates [16].

Members of Hsp70 family are at least 50% homolog at amino acid sequence level. ATPase domain is placed in amino terminal region. Some members of Hsp70 family are phosphorylated and/or methylated in vertebrates, yeast, bacteria and plants [40]. *Arabidopsis* genome has at least 18 genes coding proteins of Hsp70 family. There has been at least 12 Hsp70 in spinach genome Hsps70 members are also expressed in environmental stresses like heat, cold, chemicals. Over expression of Hsp70 genes induces thermal tolerance and increase resistance to environmental stresses [53].

***Hsp60 family*:** This family of highly conserved proteins of approximately 60 kDa is chaperones helping protein folding and subunit assembly. Their prokaryotic and eukaryotic members are dimeric and have phosphorylated isoforms. Members of this family are 14-subunit oligomers. They are found in bacteria cytoplasm. While members of their stress-inducible and non-inducible members found in bacteria cytosol and in the inner space of mitochondria and chloroplast, they have not been detected in ER and cytoplasm [19]. The Hsp60s functionally cooperate with a smaller co-chaperonin protein of 10 kDa subunit. Their eukaryotic homologs constitute majority of total cellular proteins [2]. They are developmentally controlled and found in mitochondria and chloroplast [40]. 61 and 60 kDa of Rubisco subunit binding proteins, which are nuclear encoded, synthesized on cytoplasmic ribosomes and imported into chloroplasts and bind to large subunit encoded in chloroplast of Rubisco [16, 40]. Chloroplast chaperonin is required for the assembly of Rubisco, hence the name rubisco binding protein [19]. This assembly is not covalent and requires ATP hydrolysis [16].

***Small Heat Shock Proteins (sHsps)*:** In somatic tissues of plants sHsps are the most abundant group of Hsps and 17-30 kDa are unique to higher plants. The abundance and heterogeneity of sHsps suggest that they may have unique physiological functions [50]. Mammalian and yeast cells have only one sHsp located in cytosole. However, plants usually produce more than 20 Hsps and they are often the most abundant and stress responsive group of Hsps in plants [17]. Heterogeneity of sHsps is unique to plants and plants are only eukaryotes in which organelle localized sHsp have been described. sHsps show very low sequence similarity. This applies not only to comparisons of sHsps between divergent species, but also to comparisons between different classes of plant sHsps [54]. In addition sHsps from different organisms are related with each other and even their sequence structure show difference; their structural properties are conserved [30]. Plant sHsps are all encoded by nuclear and are divided into 6 classes: 3 classes of (classes CI, CII and CIII) of sHsps are localized in the cytosole or in the nucleus and the other three in the plastids, the endoplasmic reticulum and the mitochondria (CIV, CV and CVI). The organellar forms of sHsps appear to be unique to plants with the exception of the mitochondrial Hsp22 in *Drosophila melanogaster*. Classes CI and CII of cytosolic/nuclear sHsps are generally encoded by multigene families within the same class, different members of sHsps share high sequence homology at the amino acid level. sHsps of the same class are also homologous among different plant species; however sHsps from different classes share low sequence similarities [45]. All Hsps share a conserved 90 amino acid carboxyl-terminal domain called the α-crystalline domain or heat shock domain. This domain distinguishes sHsps from other small proteins induced by heat and might have an important role in chaperon activity. This domain can be subdivided into consensus I and consensus II domains separated by a hydrophilic domain of variable length. It was determined that consensus I and II have similar hydrophobicity and secondary structure [54]. Amino-terminal domains of the plant sHsps are quite divergent between classes. For the chloroplast, endoplasmic reticulum and mitochondria-localized proteins, amino-terminal sequences typical of organelle targeting peptides are present. Within the amino-terminal regions of the mature proteins there are consensus domains that are unique to each class of sHsps. There is methionine rich 28 amino acid long consensus III domain in sHsps targeted to chloroplast and represents the most highly conserved domain in sHsp. The presence of these highly conserved domains unique to different classes of sHsps suggests that they serve important roles in the function of these proteins [54, 59]. It has been shown that sHsps located in mitochondria and chloroplasts protect respiratory electron transport in mitochondria and PSII electron transport in chloroplast. A chloroplastic 22 kDa Hsp from *Chenopodium album*, which is localized in thylakoid lumen, interacts specifically with the thermo labile oxygen evolving complex of PSII. Therefore protecting it from heat stress damage but fails to reactivate the heat denatured PSII [17, 45]. Some sHsps have been demonstrated to act as molecular chaperone *in vitro* and *in vivo*. 18.1 kDa sHsp has been shown to prevent protein aggregation and maintain them refolding by other chaperons. There is no evidence that sHsps are required for normal cellular functions. They are typically found in heat stressed plants [45]. sHsps are also produced in other environmental stresses and some developmental stages (embryogenesis, germination, pollen growth and fruit maturation). Inducing sHsp gene expression and accumulation of sHsps in environmental stresses indicate important roles of these proteins in stress [11, 15, 26, 45]. The extent of sHsp accumulation depends on temperature and the duration of the stress period [45]. sHsps accumulate significantly in couple of hours. They show a heterogenic structure according to their molecular weight, isoelectric point and stainability [22]. After the heat stress has been released, the sHsps are quite stable with half-lives of 30-50 h. suggesting that sHsps may be important for recovery as well. sHsp genes are expressed under control of the heat shock transcription factor (HSF). It has been believed that sHsps play role in translational control on heat stress [45]. It is also unique for plant sHsps to form heat shock granules

(HSGs), which are approximately 40 nm in diameter, 200-400 kDa molecular weights during long term heat stresses. Ongoing heat stress induces accumulation of unfolded proteins bound to sHsp oligomers in the cytoplasm. The complexes of denatured proteins-sHsp oligomers can be stored transiently in HSGs that disintegrate during the recovery period. HSGs contain mainly cytosolic sHsps, Hsp70 and heat stress transcription factor HSFA 2 are also present in HSG complexes. Oligomers formed by sHsps go under structural modifications and change their surface hydrophobicity; thus increasing affinity of substrate binding [45].

***Ubiquitin (Hsp8.5 group)*:** Ubiquitin is a highly conserved, low molecular weight protein with 75-76 amino acid residues and found in every eukaryotic cell. It is either found as free or bound to various proteins by their terminal Glycin amino acid [30]. Heat stress induced synthesis of ubiquitin may have a vital function for stress tolerance and recovery [40]. Plants use a repertoire of methods to control the level and activity of their constituent proteins. One method is selective protein breakdown by the ubiquitin/26S proteasome pathway. Remarkably, recent analyses of the near-complete *Arabidopsis thaliana* genome identified >1300 genes, or ,5% of the proteome, involved in the ubiquitin/26S proteasome pathway, making it one of the most elaborate regulatory mechanisms in plants [61].

4. MOLECULAR REGULATION OF HEAT SHOCK RESPONSE

Threshold temperature for inducing Hsp synthesis is generally related to temperature at which each species ordinarily grows [13]. Major prokaryotic Hsps are constitutively expressed by special genes at all temperatures. After switching temperature or treatment with any agent harming to proteins, expression of these genes speeds up and reaches the level that is characteristic for that species in a few minutes. Heat shock response is controlled by α^{32} polypeptide (rpoH gene product) at transcriptional level. There is an analog situation in eukaryotes. Expression of Hsp genes are primarily regulated at transcriptional level [20, 55]. Heat shock response is controlled by heat shock transcription factors (HSF) binding to specific DNA sites (HS elements) at transcriptional level [2]. HSF specifically binds to heat shock elements (HSEs) in promoters and activates the transcription of HS genes [20, 60]. HSFs, once plant has sensed an increase in temperature, go from a monomeric state in the cytoplasm to a trimeric state in the cell nucleus where they can bind the HSEs. HSF binding recruits other transcription components, resulting in gene expression within minutes. Unlike animals and yeasts, which may have 4 or fewer HSFs, plants have been shown to have multiple copies of these genes: tomato has at least 17 and *Arabidopsis* has 21 different HSF genes [24]. HSFA2 of *Arabidopsis* has been shown as a key regulator in response to several types of environmental stress [38].

An important component of thermotolerance is heat tolerance of gene expression. Heat stress is known to swiftly alter the pattern of gene expression, inducing the Hsp complement and inhibiting expression of many genes expressed under temperature. The mRNAs encoding non-heat stress-induced proteins are destabilized during heat stress. Heat stress inhibits splicing and it was hypothesized that Hsp encoding mRNAs can be processed properly due to the absence of introns in the corresponding genes [31]. The heat shock response is connected with profound changes of the intracellular protein distribution in general. This is observed in "microscale" e.g. the migration of RNAPII from control genes to heat shock-activated genes or binding of the activated cytoplasmic heat shock transcription factor to nuclear heat shock promotor sites, but also in macroscale. Examples are the reorganization of the cytoskeleton, the massive accumulation of nonhistone proteins in the chromatin or the formation of HSG. Restoration of the normal protein pattern of protein distribution is an essential part of the recovery period. It is improved in thermo tolerant cells and precedes the recovery of normal cellular functions [41].

Plant sHsps are controlled at transcriptional level in heat stress. sHsps might protect and keep stable untranslated normal cellular mRNAs during heat stress [45, 54]. Describing some isoforms of sHsps indicates those proteins are coded by different post-translational modifications [43].

The high selectivity of Hsp synthesis depends on the massive synthesis of new mRNAs encoded by the Hsp genes and the number of alterations of the translation apparatus leading to an efficient discrimination of non-Hsp mRNAs. The selectivity of Hsp synthesis in heat shocked cells does not correspond to the composition of the mRNA fraction. Most of the "control" mRNAs, though not translated, are maintained intact and can be reactivated in the recovery period. Heat shock mRNAs rapidly accumulate during heat shock and preferentially occupy the polysomal apparatus [40].

A hypothetic multistep model of essential translation events during heat shock can be put forward:

In 0-5 min, an immediate initiation deficiency and the collapse of the cytoskeleton lead to the destruction of polysomes, ***15 min. after stress,*** formation of Hsgs (nontranslated control mRNAs are protected from degradation by increasing deposition of Hsps); ***in late phase of the (heat shock) hs or in the recovery period***, stored mRNAs are reactivated [40].

Slow adaptation to heat or pre-stimulation increase translation at strong heat shock situations. Many modifications of translation process were described in microorganisms, plants and animals. Storing of transiently untranslated mRNAs is needed to efficiently recover synthesis of control proteins [40].

5. THERMAL TOLERANCE AND MOLECULAR CHAPERONES

Biologically function of proteins is critically dependent on the formation/dissolution of weak chemical bonds. This requirement irrevocably synthesizes organisms to environmental factors that affect weak bonds. If these factors are present in excess or in insufficient quantity, the result is departure from the native structure of proteins, with ultimately devastating consequences for protein functions. Nonnative proteins expose regions that are satisfied in the native structure; these exposed unsatisfied regions can bind other such regions in other proteins and so lead to aggregations of proteins that at worst are cytotoxic and at best reduce the pool of functional protein in the cell. This problem is ancient and widespread as life itself- and so is one major biological solution: molecular chaperones. Molecular chaperons are a class of proteins that function to minimize the problems that arise when other proteins are in nonnative conformations. Molecular chaperons can recognize and bind to nonnative and release them in highly related fashion, allowing the bound proteins to attain/reattain their native conformation and/or be targeted for degradation and removal from the cell [14]. Chaperons are responsible for protein folding, assembly, translocation and degradation in many cellular processes. They stabilize proteins and membranes and can assist in protein refolding under stress conditions. Hsps function as molecular chaperones; i.e. they interact with other proteins and, in so doing, minimize the probability that these other proteins will interact inappropriately with one another. Hsps recognize and bind to other proteins when these other proteins are in nonnative conformations, whether due to protein denaturing stress or because the peptides they comprise have not yet been fully synthesized, folded, assembled or localized to an appropriate cellular compartment (Figure 1). Typically Hsps function as oligomers. They are responsible for maintaining "Hsps" partner proteins in a folding competent, folded or unfolded state; organellar localization, import, and/or export; minimizing the aggregation of non-native proteins; and targeting non-native or aggregated proteins for degradation and removal from the cell. Chaperones generally should recognize structural elements exposed by non-native proteins [13, 19, 54].

Five major families of Hsps are known as chaperones: the Hsp70 (DnaK), the chaperonins (GroEL and Hsp60), the Hsp90, the Hsp100 (Clp) and the smallHsp (sHsp) family. Aside from these major families, there are other proteins with chaperone functions, such as protein disulfide isomerase and calnexin/calreticulin, which assist in protein folding in the endoplasmic reticulum [53].

Evident differences of the thermotolerant states observed:

a) After a short and mild heat shock

b) After a moderate to severe heat shock applied to few hours

c) After a long term adaptation to growth or at least survival at elevated temperatures.

Experimental heat shock research mostly concentrates on conditions a and b. If both are compared two different states of thermotolerance can be defined. Long term heat shock (c) has two important effects: destruction of the chloroplast structure connected with bleaching observed in the virus infections from growing meristems of many cultural plants [40].

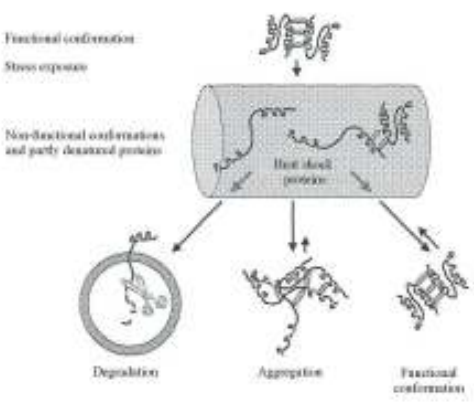

Figure 1. Cellular function of heat shock proteins. The fate of proteins with non-functional conformations after stress exposure may be either to re-obtain the functional conformation, form aggregations with other misfolded proteins or become degraded. Hsps play a helper role in shifting the equilibrium in the direction of more functional proteins or degradation of damaged proteins [44].

For thermotolerance induction in plants, tissues or cell cultures are either subjected to a continuous, moderate heat shock, to a preinduction procedure or they are slowly adapted to increasingly severe heat shock [40]. Preinduction heat shock procedures also induce tolerance to other stresses and other types of stresses induce tolerance to heat stress [30].

Two tightly related aspects have to be considered:

1. the protection of cellular fine structure during the stress period

2. the effective repair of eventual damages with restoration of the normal metabolic and gene expression activities mainly in the recovery period [40].

Similar to animal systems, the 3 phases of heat shock induction (few minutes), expression (1-3 hrs) and decay (12-48 hrs) of Hsps and thermotolerance are very similar also in plants. Generally it is not justified to conclude from analyses of growth of survival curves that Hsps are required or not required for

thermotolerance. For example, growing pollen tubes of *Tradescantia paludosa*, though unable to Hsp synthesis, exhibit certain aspects of induced thermotolerance simply demonstrate that other protective agents contribute as well [40].

Variation in Hsp production among closely related species from thermally contrasting habitats has been observed even when these species are grown and heat stressed under identical conditions. This variation in Hsp production is often correlated with organismal thermotolerance [12]. There are many studies showing quantitative variations for their tolerance degrees among genotypes. The acquisition of thermotolerance appears to depend on not only upon the synthesis of Hsps but also on their selective cellular localization. Lin et al. [29]. reported that in the soybean seedlings, several Hsps become selectively localized in or associated with nuclei, mitochondria and ribosomes at 40°C, they were absent in other parts. The selective localization of Hsps is temperature dependent. Genotypic variations have been observed for thermosensitivity in studies up to date. Heat shock sensitive genotypes synthesized Hsps earlier than tolerant genotypes [25]. Especially very early detected (in a few minutes in heat shock) specific Hsp transcripts might have effect on order of Hsp gene expression and cause different tolerance levels among species [55]. Tolerance to heat shock can not be controlled by only one gene. The heat shock tolerance is determined by different gene sets in different stages of life cycle and in different tissues. Hsp70 was shown to be causally involved in the capacity to acquire thermotolerance in *Arabidopsis*. Hsp27 in another thermosensitive *Arabidopsis* mutant. Hsp17 was identified as a factor of acquired thermo tolerance in the study of transgenic cells [31].

6. CONCLUSIONS

Abiotic stresses especially temperature, salinity and drought, are the primary causes of plant loss worldwide. Plants can experience wide fluctuations of temperature on a daily or seasonal basis and they are constantly exposed to changing environments and genetic threats. The Hsps buffer this environmental variation and are therefore important factors for the maintenance of homeostasis across environmental regimes. Heat shock proteins are important in relation to stress resistance and adaptation to the environment. Understanding the role of Hsps in relation to stress resistance in a more applied perspective as a potential indicator of stress is important. New technological developments make it possible to investigate the role of genes coding for Hsps in greater detail. A combination of genomics (e.g. quantitative trait loci, microarray and quantitative PCR studies) and proteonomics (e.g. 2-D gel electrophoresis, X-ray crystallography and nuclear magnetic resonance studies) will further elucidate the effects of stress on expression patterns at the DNA, RNA and protein level. In combination with more traditional methods of protein expression analysis, much more detailed understanding of Hsp regulation and expression will be obtained. Thereby, the role of Hsps in relation to heat and other environmental stresses will be better understood.

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