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MOLECULAR ECOLOGY OF THE PLANT ADAPTATION TO HIGH TEMPERATURES

Bratislav STANKOVIĆ[1] & Ana GARIĆ-STANKOVIĆ[2]

[1]Department of Plant Biology, Ohio State University, Columbus, OH 43210, USA
[2]Department of Molecular Genetics, Ohio State University, Columbus, OH 43210, USA

ABSTRACT

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Plants respond to high temperature stress by activating a set of biochemical and physiological processes designed to hasten the thermo tolerance. The adaptation mechanisms include phenotypic, cellular and molecular modifications.The cellular responses involve alterations in amino acid metabolism, photosynthesis, carbohydrate metabolism, assimilation, and respiration Cells accumulate solutes having protective function on the plasma membrane. Biochemical changes in lipid composition lead to increased membrane stability. The translational machinery becomes redirected toward preferential synthesis of stress-induced proteins, with concomitant cessation of synthesis of the steady-state proteins.

With a few notable exceptions, most of the work related to molecular ecology of the heat stress response has concentrated on the rapid transcriptional activation of *hsp* genes and the ensuing production of heat shock proteins (HSPs) The high molecular weight HSPs are constitutively expressed at low levels. In contrast, the low molecular weights HSPs are produced *de novo* following stress. Advances have been accomplished in understanding the regulation of the *hsp* genes Correlation between the production of heat shock proteins and increased thermo tolerance has been demonstrated in a few cases. The purpose of this review is to present recent information concerning the cellular and molecular mechanisms underlying plant acclimation to high temperature, and to address some of the responses underlying thermo tolerance.

Key words; heat shock proteins, heat stress, high temperature

Abbreviations **(кратенки):** ABA - abscisic acid; HMW - high molecular weight; HSP - heat shock protein; LMW - low molecular weight; TKW - thermal kinetic window

ИЗВОД

Станковиќ, Б. и Гариќ-Станковиќ, А. (1997). Молекуларна екологија на адаптацијата на растенијата кон високи температури. Екол. Зашт. Живот Сред., Том 5, Бр. 1, Скопје.

Во услови на стрес предизвикан од високи температури на околината, растенијата реагираат со активирање на група биохемиски и физиолошки процеси чијашто цел е зголемување на топлотната отпорност. Адаптивните механизми вклучуваат промени на фенотипско, клеточно и молекуларно ниво. Клеточните промени опфаќаат промени во метаболизмот на аминокиселините, јаглените хидрати, нивото на фотосинтезата, асимилацијата и дишењето. Клетките акумулираат материи чијашто улога е заштита на плазмалемата. Биохемиските промени во составот на мастите ја стабилизираат

плазмалемата. Транслациониот механизам се фокусира кон синтеза на стрес протеини, додека во исто време синтезата на нормални протеини е прекината.

Со неколку исклучоци, најголем дел од истражувањата на молекуларната екологија на стресот предизвикан од високи температури се однесува на активирање на транскрипцијата на hsp (heat shock protein) гените, како и на последователното производство на HSP протеини. HSP протеините со голема молекуларна тежина се активирани конститутивно. За разлика од нив, HSP протеините со мала молекуларна тежина се синтетизираат само под услови на стрес. Во неколку испитувања прикажана е корелација помеѓу синтезата на HSP протеини и зголемената отпорност на растенијата кон топлотен стрес. Цел на овој труд е да ги соопшти поновите сознанија во однос на клеточните и молекуларните механизми коишто го детерминираат прилагодувањето на растенијата кон високи температури.

Клучни зборови: протеини на топлотниот шок, топлотен стрес, високи температури

INTRODUCTION

Higher plants have an optimum temperature for growth and development in a given habitat. The window of optimal temperatures varies during different stages of development, and plants have evolved adaptive mechanisms to reduce the impact of high solar radiation and the ensuing elevated temperatures in a dynamic environment. High temperature markedly reduces the growth and development of plants, and can have lethal consequences. In an agricultural context, it is often the largest limiting factor determining the agricultural yield. This fact has inspired workers to develop empirical methods for identification of the optimal temperatures for plant growth (Burke 1990).

The balance between absorbed and emitted solar and infrared radiation, heat conduction, heat convection, and transpiration, is the factor governing plant temperature (Nobel 1983). Lethal temperatures for vegetative tissues of flowering plant species typically range between 40°C and 60°C. Sub lethal leaf temperatures lead to metabolic and phenotypic changes which reflect acclimation mechanisms, designed to increase the thermo tolerance. The traits governing thermo toler-ance are species and strain-dependent, and the heat shock responses are a consequence of the activation of a complex set of ultrastractural, transcriptional, and translational adjustments and modifications.

Being sessile organisms, plants have to adapt their metabolism to changes in atmospheric temperature, minimizing the ensuing damage. High temperatures have multiple effects. They reduce nutrient ion uptake, enzyme activity, photosynthesis, and translocation of metabolites. They alter the rate of respiration leading to reduced growth and poor yield (Bjorkman et al. 1980). To survive supraoptimal temperatures of the habitat, plants have evolved adaptation and protection mechanisms. These consist of both short and long-term responses. Some short-term responses are probably involved in minimizing the impact of stress until it is alleviated. They are physiologically manifested as osmotic adjustments, directed toward increasing the intra-plant water flow. This is accomplished through reduction in the resistance to water flow and keeping the stomata open, the longer-term responses to hyperthermia most likely entail survival-related mechanisms. They are manifested through closure of stomata, reduction of the overall leaf area through abscission, and hastening or deferring reproduction. In an agricultural context, the benefit of various mechanisms of acclimation depends on the species and on the environment in which it is being produced.

The response to elevated high temperatures is complex. Heat stress is frequently accompanied with high light intensity stress. Both environmental factors increase the evaporative demand, indirectly contributing to water deficiency or salt stress. In terms of physiological and biochemical responses, a host of other a biotic agents can mimic the high temperature stress: water deprivation, anoxia, heavy metal ions, increased salt concentration, abscisic acid, auxin, ethylene, ethanol, uncouplers of oxidative phosphorylation, and metabolic inhibitors (Czarnecka et al. 1984; Vierling 1991; Van Breusegem et al. 1994), Thus the plant response to high temperature must be considered in view of the complex interaction and cross-talk of various a biotic stresses and their effects on the plant.

Recent advances in analytical chemistry and molecular biology have helped reveal re-

sponses and decipher parts of the mechanisms by which plants perceive and adapt to temperature changes (Fig. 1),. Segments of the signal transduction pathways leading to high temperature-induced changes in transcription and translation are now known, Certain biochemical and physiological roles of the genes and proteins involved have also been discovered, and they are outlined below

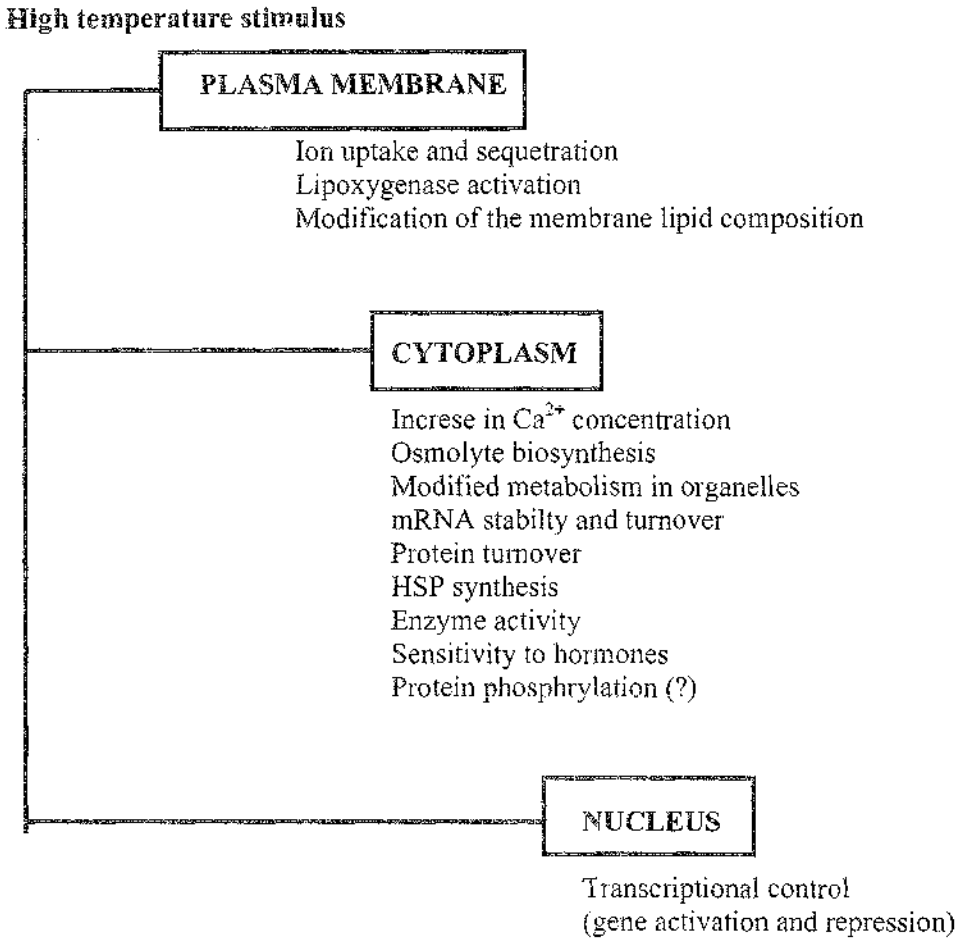

Fig. 1 Schematic pathways of the cellular and molecular acclimation to high temperature stress. Interaction and cross-talk of biochemical, functional and structural components from different cellular compartments is balanced to achieve thermo tolerance *in planta.*

Сл. 1 Шематски патишта на клеточната и молекуларната аклимација кон стрес од високи температури. Интеракцијата и "cross-talk" на биохемиските, функцио-налните и структурните компоненти од различни клеточни делови. е балансирана да се постигне толеранција кон топлина кај растенијата

MOLECULAR RESPONSES TO HIGH TEMPERATURE

Plant cells respond to elevated temperatures by dramatically altering both their transcriptional and translational patterns of gene expression, a common and well-documented molecular theme during the heat stress response is almost complete cessation of synthesis of the "normal", steady-state proteins. Instead, a limited number of specific genes, previously either silent, or active at very low levels, become upregulated. The newly-activated genes encode tissue-specific heat-shock proteins, which become so intensely synthesized and accumulated during heat stress, that eventually become major cellular constituents. All plant species examined, when shifted from ambient temperatures to temperatures five or more degrees higher, in a ma-

jority of the cell types produce HSPs. This response occurs in response both to abrupt and to gradual temperature changes, which are equivalent to those found under field conditions (Ho and Sacks 1989).

Initiated in *Drosophila,* studies over the past thirty years have revealed a large degree of HSPs evolutionary conservation among kingdoms, Both field and laboratory studies have demonstrated transient- and longer-term HSPs induction in plants following increase in temperature, and extensive reviews describing different facets of this phenomenon are available (Nagao et al, 1990; Vierling 1991; Hendrick and Hartl 1993; Parsell and Lindquist 1994), Similar to other organisms, the plant heat shock proteins have been categorized according to their approximate size, i.e. molecular weight, into five classes: high molecular weight (HMW) HSP110 (110 kDa); HSP90 (80-95 kDa); intermediate HSP70 (63-78); HSP60 (53-62 kDa); and the small, low molecular weight (LMW) (14-30 kDa). The far majority of plant heat shock proteins consist of HSP90, HSP70, and low molecular weight HSPs.

Regulation of the synthesis of high temperature-induced HSPs is performed at both transcriptional and translational level. The mPvNA for high molecular weight HSP is often constitutively expressed, albeit at low levels. However, even though the high molecular weight HSP transcripts are present in *Sorghum* embryos prior to treatment, they are not readily translated (Howarth 1990). In marked contrast, RNA transcripts for LMW cytoplasmic HSPs are absent from control tissues. Hence synthesis of low molecular weight HSPs requires transcription *de novo.* The LMW *hsp* genes become transcription ally activated within five minutes of exposure to high temperature (Nagao et al. 1990). Low molecular weight *hsp* transcript levels can increase as much as 200-fold compared to the unstipulated controls. The mRNA turnover time varies with the species and the severity of experienced stress, and the newly-synthesized proteins are present at significant levels up to several hours or days following stress.

Analyses of the promoter sequences have revealed bipartite promoter structure containing two consensuses 5'-upstream regulatory sequences. The heat shock promoter regulatory elements appear to have multiple copies (6-9) with overlapping elements (Schoffl et al. 1990). Such a structure enables differential levels of expression of various *hsp* gene family members following heat shock (Vierling 1991; Van Breusegem et al. 1994). Since the *hsp* genes are also activated following other stresses, it is plausible that the multitude of regulatory sequences is correlated to the number and/or types of environmental stresses the plant is experiencing Hence, the up regulation of *hsp* genes might be just a part of a general molecular mechanism protecting the plant cells from the potentially lethal effects of environmental stress.

An ascribed putative function of HSPs is their role in development of thermo tolerance through a conditioning treatment at lower, HSP-inducing temperatures. Indeed, through gradual exposure to higher temperature, plants can be conditioned and acclimated to withstand heat stress, and burgeoning evidence suggests correlation between the degree of HSP production and plant adaptation to high temperatures (Nagao et al. 1990; Lee et al. 1994; Parsell and Lindquist 1994). On the other hand, HSP production may not be essential for adaptation to heat stress. Some reports imply acquisition of thermo tolerance in the absence of newly-synthesized HSPs (Xiao and Mascarenhas 1985; Bonham-Smith et al. 1987). Such a discrepancy is not altogether surprising, since the exact functions of numerous cloned HSPs remain largely unknown. Only a few plant HSPs have been characterized in greater detail. One of them is the cytosolic 110-kDa ubiquitin, involved in ATP-dependent proteolysis. Ubiquity covalently attaches to proteins destined for degradation. Similarly, the mitochondria and chloroplast-localized GroEL (i.e. HSP60) is believed to function as a molecular chaperone. HSP60 is involved in assembly of macromolecular complexes in organelles. Other high molecular weight HSPs are also shown to function as molecular chaperones (Hendrick and Hartl 1993), they are probably involved in minimizing high-temperature stress damage through association with partially denatured proteins, preventing their further breakdown or nonspecific aggregation.

The majority of research related to heat shock-related transcriptional activation has concerned up regulation *of hsp* genes, However, heat stress has profound effects on other facets of the plant genome. Waters and Schaal (1996) showed that high temperature influenced the genomic components of *Brassica,* reducing the amount of nuclear ribosomal gene-copy number (rDNA). This effect was stable and even heritable, i.e. it was passed on to the F_1 generation.

Another whole family of heat stress-induced genes is the one comprising calmodulin-related touch *(TCH)* genes (Braam 1992). Several members of this gene family are unregulated following various a biotic stresses including heat. The significance, if any, of calmodulin-related enzymes in heat stress and in acquiring thermotolerance is unknown. High temperature causes release of calcium from intracellular stores. Activation of these genes might occur as a reaction to the heat stress-increased cytoplasmic Ca^{2+} levels, and it may or may not be causally related to the heat shock response.

CELLULAR RESPONSES TO HEAT STRESS

The effects of supraoptimal temperatures on the physiology and biochemistry of plant cells and tissues have been examined through studies both *in vitro* and *in vivo.* Suspension cultured cells of cowpea respond dramatically to elevated temperature. The ultra structural changes involve almost complete loss of polyribosome, rough endoplasmic reticulum, and dictyosomes. Dylewski et al. (1991) also observed nucleolar swelling and migration of intracellular waste material into the vacuole. When analyzed at amino acid level, heat shock differentially influenced the amino acid synthesis rate in cowpea cells. While proline, valine, leucine and isoleucine synthesis rates were increased several-fold in response to heat shock, phenylalanine synthesis rate dropped to 50% of the initial value (Mayer et al. 1990). Selective amino acid availability under temperatures stress will thus redirect numerous biochemical and metabolic pathways.

Dupuis and Dumas (1990) studied the effect of temperature stress on *in vitro* fertilization efficiency in maize. The fertilization rate was highly reduced when pollinated spike lets were exposed to temperatures above 36°C. Reportedly, the mature pollen, but not the female spike lets, was the tissue sensitive to heat stress.

Growing plant at high temperatures influences the intracellular levels of various plant hormones. Heat shock increases the content of abscisic acid (ABA) (Daie and Campbell 1981). ABA and cytokinins appear to have opposite effects and roles during high temperature stress. Whilst ABA levels rise during heat stress, cytokinin levels fall. This is not altogether surprising, since both hormonal changes favor stomata closure and reduce resistance to water flow.

High temperature stress inhibits the rate of carbohydrate synthesis, and plants adapt by adjusting the rates of photosynthesis and respiration (Pearcy 1977). The starch content in heat-stressed tomato leaves decreases, primarily due to increased starch hydrolysis and inhibition of its biosynthesis (Dinar et al. 1983). Similarly, starch accumulation in developing barley endosperm is decreased under' heat stress conditions. This reduction was attributed to the sharply decreased activity of the UDP sucrose synthase (MacLeod and Duffus 1988). The import of photosynthates is reduced under high temperatures. Thorne (1982) demonstrated that the phloem unloading in soybean is a temperature dependent process, which could be related to the heat-induced disruptions in membrane integrity.

Characterization of the intracellular localization of HSPs provides information concerning their putative functions, and can help clarify some of the observed variation in relation to thermo tolerance. The variations in the amount, array, and kinetics of HSP production can be a function of cell type, developmental stage, availability of nutrients (particularly nitrogen compounds), degree of thermal stress the plant is experiencing, and the habitat (Somers et al. 1991; Vierling 1991; Parsell and Lindquist 1994; Heckathorn et al 1996). Various plant heat shock proteins appear to be differentially localized in the nuclei, cytoplasm, chloroplasts, and mitochondria (Lin et al. 1984; Vierling 1991).

Leicht et al. (1986) suggested that there are at least three functionally distinct classes of HSPs: ones that enter the nucleus upon heat shock; others that are cytosolic (soluble HSPs); third that associate with the cytoskeleton. Indeed, certain *Drosophila* and mouse HSPs are cytoskeleton-binding proteins (Koyasu et al. 1986; Leicht et al. 1986). Due to the high degree of homology and evolutionary conservation, it would not be surprising if at least some plant HSPs turn out to be associated with the cytoskeleton. Obviously, an actin-HSP interaction in a nonmuscle actomyosin system has key biological signe ract with the outer chloroplast envelope membrane, enhancing formation of saturated lipids, in particular digalactosyl diacyl-

glycerols (Suss and Yordanov 1986). Chlorophyll fluorescence measurements indicate that heat-stressed chloroplasts undergo significant injury (Kraus et al. 1995). The known heat shock-induced chloroplast responses involve: (*i*) structural changes - disorientation of the lamellar systems, decreased synthesis of the chlorophyll *a/b* light harvesting complex, dissociation of the light-harvesting pigments and the photosystem reaction centers; *(ii)* functional modifications - decrease in the quantum yield of electron transport; *(iii)* alterations in photosynthetic electron transport; (i*v*) reduced carbon fixation through inhibition of ribulose-l,5,-bisphosphate carboxylase; (*v*) changes in carbohydrate metabolism (Burke 1990). A consequence of the above is a significantly decreased photosynthetic efficiency of plants subjected to elevated temperatures.

In the process of acclimation to heat stress, the lipids and proteins in the plasma membrane undergo structural changes designed to increase plasma membrane stability. These involve increased saturation content of membrane lipids, and have been observed both in the plasma membrane (Raison et al. 1980), and in the chloroplast outer envelope and thylakoid membranes (Suss and Yordanov 1986). Thermal injury induces expression of genes encoding for lipoxygenases LOX-1 and LOX-2 Lipoxygenases have multiple physiological roles in plant responses to stress such as pathogen attack, wounding, and anoxia. They are involved in membrane deterioration during aging, which led to the suggestion that they might be involved in thermo tolerance (Maccarone et al 1992). The effects of heat stress on endomembrane organization are poorly understood, and the reports on protein synthesis on the endoplasmic reticulum (ER) are sometimes conflicting. Using barley aleurone, Sticher et al. (1990) showed heat stress-induced arrest in synthesis of a-amylase, a protein translated in the ER. At the same time, the ER was not destroyed by heat-shock treatment. It is possible that the arrest in a-amylase synthesis occurs due to disassociation of a-amylase mRNA and ER-bound polysomes, and is ABA-mediated. Contrary to these observations, ER-mediated storage protein synthesis in soybean cotyledons was unaffected by heat stress (Hughes and Dunn 1990).

PHENOTYPIC ADAPTATION TO HIGH TEMPERATURE

Growth perturbation and inhibition occurs in plants experiencing high temperatures. The extent of decline in growth varies among species. Thin-barked woody species are susceptible to bark burn, which occurs due to overheating of the cambium. The particularly sensitive plant part is a narrow strip of bark at the soil level. In deciduous plants, the heat injury initially takes place in the larger' leaves that absorb a lot of irradiation. Hence some shrub species have developed the strategy of shedding the larger[1] leaves and producing smaller ones with the onset of the dry season (Newton and Goodin 1989).

Morphological acclimation to hyperthermal stress is performed through combined mechanisms involving both heat avoidance and tolerance. The physiological responses include decrease in respiration, increased cooling through transpiration, and decrease in absorption of irradiation. Avoidance is accomplished through phenotypic modifications designed to absorb less irradiation and decrease transpiration, while increasing the light reflectance. The adaptations involve enhancement in mesomorphic leaf structure, increased pubescence (hairiness), production of articular wax, shift toward vertical leaf orientation, decrease in leaf size, and increased salt content in the leaves (Nobel 1983, Burke 1990).

Thermoto lerance is the ability of plants to survive an otherwise lethal temperature by means of initial exposure to a hyper thermal shock at a nonlethal temperature. Studies conducted with soybean seedlings have led to conclusions that thermo tolerance develops in a time - and temperature - dependent manner, and is correlated to HSP synthesis (Lin et al. 1984; Key et al. 1985; Heckalhorn et al. 1996). A strong support for the role of HSPs in acquisition of thermo tolerance was recently demonstrated: a soybean 101-kDa 11SP was able to rescue a yeast thermosensithe mutant (Lee et al. 1994).

FUTURE PROSPECTS

To better understand the heat shock response, methods for determining the degree of stress the plant is experiencing have to be established. It is possible to determine the limits of high temperature stress in individual plant species through the use of a thermal kinetic window (TKW), which is based on the thermal dependence of the apparent Michaelis constant (Km) for a given enzyme (Burke 1990). Other physiological methods for assessment of experienced heat stress and heat tolerance involve measurement of electrolyte leakage (Binelli and Mascarenhas 1990), net $C0_2$ assimilation, and chlorophyll fluorescence (Kraus et al. 1995). Combined use of a few methods should provide accurate assessment of the degree of acquired thermo tolerance.

Although plant species vary in regard to their sensitivity and response to thermal stress, it is safe to assume that all plants have evolved capabilities for high temperature perception, signaling, and response, Improvement of thermoto lerance has large agricultural implications and has been a goal in crop breeding for lone time. The heat tolerance in plants is genetically determined. However, a larger number of genes is probably involved in acquisition of heat tolerance, which has rendered the selection schemes for plants exhibiting increased productivity under high temperatures largely unsuccessful (Marshall 1982).

In recent years, the studies of temperature stress tolerance have shifted from monitoring the physiological status of stressed plants compared with unstressed controls, to deducing biochemical and molecular mechanisms underlying sensitivity and tolerance. Understanding of the molecular ecology of the stress response is an initial step in manipulating and constructing transgenic plants with increased fitness in the stress environment. In relation to heat stress, the future research prospects are exciting. A lot of progress can be done on further characterization of the HSPs. The functions, subcellular localization, and modes of regulation for many of the HSPs have yet to be determined. To facilitate this, functional complementation and rescue of thermosensitive yeast and plant mutants provides a powerful yet elegant approach (Lee et al. 1994). Advantage can be taken of the heat sensitive genotype of *Arabidopsis* (Binelli and Mascarenhas 1990), and transgenic *Arabidopsis* plants can be used as an assay system for identification of genes related to thermotolerance. Identification of transcription factors interacting with the *hsp* regulatory elements will further our understanding of the plant stress transcriptional responses, both in relation to high temperature and in general. The nature of high temperature-induced genomic changes can be characterized in greater detail. Since plants do not sequester a germ line, heat stress-induced mutations are easily inherited, and being passed on to future generations, they could have significant evolutionary and agricultural implications.

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