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Several progressions indicated by plant under heat stress

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Abstract
There are so many symptoms or indications exposed by plants to heat stress. High temperature stress indicators are signs of disturbance, either as visible, growth or morphological modification or invisible, physiological or biochemical changes that relate to repair and resistance mechanisms. Evaluating physiological and cellular parameters for assessing the severity of stress injury or level of heat stress tolerance is not always trivial. The current chapter reviews the methodology and principles of several major physiological and biochemical indicators commonly used to evaluate heat stress tolerance or injury: photosynthesis, water relations, cell membrane stability (CMS), lipid peroxidation, and stress-induced proteins. Multiple morphological, physiological and biochemical changes may occur simultaneously in response to heat stress, and sometimes are difficult to distinguish from one another. Each indicator is discussed independently in this chapter.

Keywords: Heat, photosynthesis, HSPs, membrane stability, stress

Introduction
Plants bare to environmental stress shows number of symptoms or indications. Stress indicators are signs of disturbance, visible as growth or morphological modification, physiological or biochemical changes. Plants experience high temperature in many different ways and adaptation or acclimation to high temperature occurs over different time scales and levels of plant organization. Exposure to high temperature can be chronic or long term, as experienced in hotter habitats, or it can be more acute, as a result of seasonal or daily temperature extremes. High temperatures alter lipid properties, causing membranes to become more fluid and thereby disrupting membrane processes. All proteins have an optimal temperature window for activity, so increased temperatures alter enzyme activity leading to imbalance in metabolic pathways, and eventually proteins denature. At high temperature membrane and protein damage leads to the production of active oxygen species (AOS). This may not be effectively controlled through antioxidants at high temperatures, resulting in heat-induced oxidative damage in addition to the direct effects of heating. At the physiological level, this damage translates into reduced efficiency of photosynthesis, impaired translocation of assimilates and loss of carbon gain. These factors in turn combine to cause altered phenology, reproductive failure and hasten senescence (Hall, 2001). Thus, it is to be expected that many different processes, involving many genes, are involved in plant responses to heat. Classic, response to acute heat stress is the production of heat shock proteins (HSPs), which function, at least in part, as molecular chaperones in cellular protein quality control (Boston *et al.,* 1996). HSPs, however, are only one component of the response to high temperatures, and current data indicate that there are multiple pathways that contribute to the ability of plants to tolerate heat. There has also been a recent explosion in information about the signalling molecules that may be involved in responses to heat, and these components begin to offer insight into events occurring in a plant as temperatures begin to rise. Altogether, genomics, genetic and transgenic experiments are helping to define the roles of individual and suites of genes in the response of plants to high temperatures. As the numerous interacting pathways become clearer it will be possible to understand the interactions of heat and other stresses and to consider the best strategies for improving plant heat tolerance.
Hence, in view of the above, the current reviews is to study several major physiological and biochemical indicators commonly used to evaluate hest stress tolerance or injury: photosynthesis, water relations, cell membrane stability (CMS), lipid peroxidation, and stress-induced proteins.

Multiple morphological, physiological and biochemical changes may occur simultaneously in response to the het stress, and sometimes are difficult to distinguish from one another. However, for an methodical discussion on some of indicator is presented in the review.

Photosynthesis

Photosynthesis is the fundamental process affecting plant growth, productivity, and its survival under heat stress. It is the reduction of CO_2 by plants that makes them autotrophic and virtually increases the complexity of life on earth. So, for the photosynthetic process under heat stress, we reassess the use of chlorophyll fluorescence and gas exchange rates as indicators of plant tolerance to high temperature. The basic principle behind fluorescence measurements relies on the fact that the light energy that is absorbed by chlorophyll molecules in photosynthetic organisms can be used in any one of three different methods of energy dissipation: it can be used to drive photosynthesis (photochemical quenching); excess energy can be dissipated as heat (non-photochemical quenching); or it can be re-emitted as light, chlorophyll fluorescence. Each quantum of light absorbed by a chlorophyll molecule raises an electron to an excited state. Upon de-excitation, a small proportion (1–4%) of the excitation energy is dissipated as red fluorescence. The use of fluorescence as an indicator arises from the fact that fluorescence emission is complementary to the other two dissipation mechanisms, i.e. photochemical quenching (photosynthesis) and the heat dissipation. Usually, fluorescence yield is highest when photochemistry and heat dissipation are lowest. The energy emitted from a leaf as fluorescence is lower than the light energy absorbed, and consequently, the fluorescence emission peak has a longer wavelength than that of the light absorbed. This phenomenon enables quantification of fluorescence yield by exposing a leaf to light. One of the most common parameters used for measuring chlorophyll fluorescence is the Fv/Fm ratio. When a leaf is kept in the dark all the residual energy is processed and the amount of fluorescence is small; this is considered the minimal fluorescence signal (Fo). After flashing the leaf with bright high-intensity light, the fluorescence signal will increase to a maximum (Fm), as it cannot photo chemically use all this energy. The difference between the maximum and minimum fluorescence is called the variable fluorescence (Fv). The ratio of Fv/Fm indicates the proportion of the maximum possible fluorescence that was used for photosynthesis, and is an estimate of the PSII maximum efficiency. This efficiency in healthy plants is usually about 80% or 0.8. Fv/Fm is considered to be inversely proportional to stress; therefore, values of Fv/Fm that are lower than ~0.8 are considered as injurious levels fluorescence methods have been used to evaluate heat stress effects on photosynthetic apparatus in many studies (Schreiber *et al.,* 1987) [63].

Fluorescence changes may reflect structural changes within the thylakoid membranes due to heat stress. Given that photosynthesis is considered to be the physiological process most sensitive to high-temperature damage and that PSII appears to be heat sensitive component of the photosynthetic apparatus, using chlorophyll fluorescence to measure thermotolerance of PSII in plants may provide early stress indication. Haldimann and Feller, 2004 [26] examined the impact of heat stress on the functioning of the photosynthetic apparatus in pea (*Pisum sativum* L.), and found chlorophyll fluorescence started to decrease when leaf temperature increased above 35 °C before the decline of net photosynthetic rate.

Gas exchange parameter

Gas exchange involves entry and loss of gases such as CO_2, H_2O, and O_2 in plant tissues. During photosynthetic gas exchange, CO_2 is taken up or consumed by leaves and O_2 is evolved or produced. During the respiratory gas exchange, O_2 is consumed whereas CO_2 is evolved. More CO_2 is consumed in photosynthesis simultaneously evolved respiratory processes, the net uptake of CO_2 is considered to be net photosynthesis, whereas, the total CO_2 assimilated in the chloroplasts is the gross amount of photosynthesis. Net photosynthetic rate is often used as a measure of plant activity. Otto Warburg Nobel Laureate pioneered measurements of photosynthetic rates using manometric techniques (Warburg, O., 1919) [77]. Photosynthesis is generally positively correlated to stress tolerance and, therefore, photosynthetic gas exchange parameters are widely used as stress indicators (Long and Woodward, 1988) [45].

A/Ci curves can be a useful tool for studying plant responses to temperature changes.

The CO_2 compensation point increases with increasing temperatures, mainly due to the increase in respiration. Extreme temperatures markedly stimulate the rate of photosynthesis and the Vmax is also usually stimulated (Sage and Reid, 1994) [56].

Many studies correlate net photosynthetic rate to stress tolerance. A study on the interactive effects of drought, heat, and elevated CO_2 on photosynthesis illustrated the importance of CO_2 gas exchange in stress tolerance (Hamerlynck, 2000) [29]. One-year-old creosote bush seedlings [*Larrea tridentata* (Sessé and Moc. ex DC.) Coville] were exposed to high temperatures (53°C) under three atmospheric CO_2 concentrations (360, 550, and 700 μ mol mol^{-1}) and two water regimes (well watered and drought subjected) (Hamerlynck, 2000) [29]. This study found that increasing CO_2 concentrations to 700 μ mol mol^{-1} improved net photosynthetic rate under heat stress.

Osmotic adjustment

In cellular dehydration results from various major environmental stresses, including high temperature, osmotic adjustment (OA) is a mechanism of acclimation, in which compatible solutes are actively accumulated within cells. This causes a reduction in osmotic potential, and leads to water movement into the cell or prevention of water efflux out of cells (Hare *et al.,* 1998, Ingram and Bartels, 1996, Bohnert and Jensen, 1996) [31, 36, 11]. In turn, plants maintain higher turgor potential and water retention. In plants that exhibit OA, loss in turgor can occur at lower water potentials than in nonosmotically adjusted leaf tissues (Zhang, 1999) [86]. It is important to note that this active accumulation of solutes triggered by decreases in cellular water content differs from accumulation of solutes simply due to loss of water and concentrating effects (Blum, 1988) [10]. Osmotically active solutes involved in OA include amino acids (e.g., proline), ammonium compounds (e.g., glycine betaine), sugars (e.g., fructans, sucrose), polyols (e.g., mannitol), inorganic ions (e.g., potassium), and organic acids (e.g., malate) (Zhang, 1999, Chaves *et al.,* 2003) [86, 15]. Determination of Osmotic adjustment several methods have been used. Babu *et al*., 1999 [7] conducted a comprehensive investigation on the comparison of different methods for evaluation of OA in rice

genotypes differing in capacity for OA. They found that absolute OA values and correlations of OA across genotypes varied among the different methods.

Membrane stability

A major impact of plant environmental stress is cellular membrane modification, which may result in impaired function or total dysfunction. The exact structural and functional modification caused by stress is not fully understood. However, the cellular membrane dysfunction due to stress is expressed as increased permeability and leakage of ions, which can be readily measured by the efflux of electrolytes. Hence, the estimation of membrane stability under stress by measuring cellular electrolyte leakage from affected plant tissues into an aqueous medium has been widely used as a screening tool for stress acclimation and tolerance. Stuart**,** N.W., 1939 recommended expressing electrolyte leakage as an index percentage of total electrolytes in the tested tissues. Based on Stuart's method, Flint *et al.*, 1966 developed an index of measuring electrolyte leakage for freezing injury. This method has since been used for evaluation of various stresses such as high temperature (Peck and Wallner, 1982, Shanahan *et al*., 1990) [47, 66], chilling injuries (King and Ludford, 1983, Yadava and Doud, 1978, Yelenosky, G., 1990) [40, 81, 82], dehydration (Premachandra *et al.,* 1989) [49], salinity (Sairam 2005) [58], and metal toxicity such as copper (Xiong and Wang, 2005) [80].

Electrolyte leakage

The procedure of electrolyte leakage analysis is quite simple. Fresh tissues (leaves or roots) are soaked in distilled, de-ionized water and solutes are allowed to efflux from the tissue into the solution until leakage ceases, at which time the electrical conductivity of the solution is measured (initial leakage, Ci). Following this measurement, samples are usually killed by autoclaving or rapid freezing in liquid nitrogen and are allowed time for complete leakage of solutes from the killed tissues to the solution. The conductivity of the solution is then measured and is considered as the maximum electrolyte leakage (Cm). Membrane stability or index of electrolyte leakage can be expressed as Ci/Cm × 100. This technique is simple; however, it has also been criticized (Whitlow, 1992) [78]. The two major problems with the method are that it does not measure membrane stability directly, and it does not take into account the effects of differences in anatomy between different species, which in turn can affect the conductance. However, the use of electrolyte leakage as an indicator for assessing the effect of environmental stresses on membranes remains a strong and an important tool, especially when comparing samples for the same species. Heat stress typically is characterized by direct damage to the integrity of cells, leading to various physiological changes (Larcher, 2003) [42]. Cell membranes, which are structurally made up of larger polyunsaturated fatty acids, are highly susceptible to high temperature and consequently changes in membrane fluidity, permeability, and cellular metabolic functions. Cell membranes become more fluid as temperatures rise, increasing the chance for membrane leakage (Behzadipour, 1998) [9].

Electrolyte leakage was used to measure ecotypic differences in heat tolerance of aspen (Populus tremuloides Michx.) leaves. Leaves were obtained from trees growing at different elevations from 1960 to 2454 m. Heat tolerance was greatest in trees growing in the lower sites and trees propagated from these sites that were grown in even lower elevations showed some increase in heat tolerance as measured by electrolyte leakage. Shanahan *et al.* (Shanahan, 1990) [66] found electrolyte leakage to be a useful screening procedure for selecting heat-tolerant genotypes of spring wheat (*Triticum aestivum*). Increase in electrolyte leakage was much more severe and occurred earlier for ryegrass than fescue subjected either to heat alone or combined with drought. Wallner *et al*., used electrolyte leakage to measure the effect of duration and level of imposed heat stress on turfgrass leaf segments. Electrolyte leakage revealed that differences between species in heat tolerance were most apparent when injury was monitored over time at 50°C. These studies suggest that electrolyte leakage could be used for selecting stress-tolerant grass species and cultivars. Quantitative differences in heat tolerance, evaluated using electrolyte leakage, were consistent with qualitative descriptions of drought resistance in some species (Wallner *et al.,* 1982) [47], but not in other plant species.

Lipid peroxidation

Oxidative injury in plants can be induced by many different biotic and abiotic stresses, including disease (Apostol *et al.,* 1989) [3], temperature extremes (Mishra and Singhal, 1992, Prasad, 1994, Schoner and Krause, 1990, Zhou and Zhao, 2004., Zhang, 1995) [85, 46, 48], drought (Zhang and Kirkham, 1996, Zhang and Kirkham, 1994, Smirnoff 1993, Sgherri and Navari-Izzo, 1995) [86, 84, 83, 67], salinity (Gossett *et al.,* 1994, Hernandez, 1993) [24, 33], nutrient deficiencies (Cakmak and Marschner, 1988) [14], high irradiance levels (Halliwell and Gutteridge, 1989) [27], and herbicides (Babbs *et al.,* 1989, Asada, 1999) [6]. These environmental factors are common in that they can all lead to formation of active oxygen and oxidative damage within cells. Excessive energy from reduced electron transport during stress can lead to an accumulation of excitation energy that can be dissipated by reducing molecular oxygen, whereby electrons leaked from chloroplasts and mitochondria interact with oxygen and generate active oxygen species (AOS) (Asada, 1999). AOS include singlet oxygen ($1O_2$), superoxide (O^{2-}), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH.). These species interact with cellular molecules and cause severe damage to lipids, nucleic acids, and proteins. Halliwell produced the first extensive review on active oxygen and its consequences, and several other overviews have subsequently been presented (Smirnoff 1993, Halliwell and Gutteridge, 1989, Asada and Takahashi, 1987, Cadenas, 1989, Foyer *et al*., 1994, Winston, 1990) [27, 67, 4, 13, 21, 79].

Antioxidant enzyme defence system

One assessment of the antioxidant system is through measurement of antioxidant enzymatic activities. Increases in enzyme activity may be induced by the presence of active oxygen species, thus providing indirect evidence for the extent of generation of active oxygen species or the greater requirement for this type of defense response (Smirnoff, 1993) [67]. Some common antioxidant enzymes include catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), glutathione reductase, and ascorbate peroxidase (APX). Tolerance to the aforementioned stresses is associated with maintenance or an increase in antioxidant enzyme activities (Bowler *et al.,* 1992, Gupta, 1993, Jagtap and Bhargava, 1995, Lascano, 2001, Price and Hendry, 1989, Sairam *et al.,* 2000) [59, 12, 25, 37, 43, 50]. However, not all

antioxidant enzymes change in their activities with the same pattern in response to stresses, or they may have differential effects on stress tolerance (Prasad, T.K. *et al.,* 1994, Sairam *et al.,* 2000, Anderson *et al.,* 1995, Saruyama and Tanida, 1995) [59, 48]. Sairam *et al.* indicated that there were differences in the increase in activities of various antioxidants among tolerant genotypes of wheat, such that one tolerant genotype had very high levels of ascorbic acid and APX, whereas another tolerant genotype exhibited higher SOD and CAT and intermediate ascorbic acid activities. They concluded that not all of the antioxidant enzymes may increase uniformly within tolerant selections, and that different antioxidant enzymes may be more significant for imparting tolerance in some genotypes compared to others. Queiroz *et al.* studied cold stress in *Coffea arabica* L. roots and found that APX and CAT did not change considerably, but POD increased to a greater extent under chilling stress compared to control plants. In some cases, antioxidant enzyme activity is not related to stress tolerance. Cavalcanti *et al.* concluded that SOD, POD, and CAT did not appear to contribute to survival under high salinity levels in salt-stressed cowpea leaves. Although antioxidant enzymes generally do play a role in the antioxidant capabilities of plants, there is obviously some variability in enzyme activities among species and genotypes. Therefore, this section focuses on lipid peroxidation, an indicator of stress injury that has a relatively consistent response among plant species.

Determination of lipid peroxidation

The extent of lipid peroxidation can be calculated by measurement of total thiobarbituric acid (TBA) reactive substances (TBARS) and expressed as equivalents of malondialdehyde (MDA) content, which is a secondary breakdown product of lipid peroxidation (Halliwell and Gutteridge, 1989) [27]. The more accurate estimation of lipid peroxidation is through direct quantification of the primary hydroperoxide products; however, this approach proves to be more difficult due to the labile nature of these primary products as well as a more involved protocol (Hodges, 1999) [34]. The TBARS assay has been utilized extensively in both plant and animal systems as a consistent means for estimation of lipid peroxidation. Many of the studies evaluating lipid peroxidation follow the original protocol of Heath and Packer, 1968. Briefly, leaf or root samples (typically 0.2–0.5 g fresh weight) are homogenized in a solution of approximately 0.1% trichloroacetic acid (TCA) at 4 °C, and then centrifuged at approximately 15,000 g for 10 minutes. For every 1 ml of supernatant, 4 ml of a thiobarbituric acid (TBA) solution (0.5% TBA in 20% TCA) is added for the reaction. Samples are incubated at 95°C for approximately 30 minutes, with the reaction then quickly terminated in an ice bath. The samples are centrifuged again at 10,000 g for 10 minutes. Finally, the specific absorbance of the product is recorded at 532 nm, with the value for nonspecific, background absorbance at 600 nm subtracted from 532 nm. MDA content is calculated using its extinction coefficient of 155 $mM^{-1}cm^{-1}$. Different modifications of this protocol exist, particularly in regard to tissue sample amounts and the reaction ratio of supernatant: TBA solution. For extraction of compounds in addition to MDA with limited plant tissue, different extraction methods are employed, such as those discussed by Ali *et al*. and Wang and Huang. These factors may be tested on an individual species basis to determine the most consistent levels of MDA. MDA can also be directly quantified using high-performance liquid chromatography or gas chromatography (Janero, 1990) [38]; however, the spectrophotometric method for testing the TBA–MDA complex may be less costly. Additional benefits for the TBARS assay include its convenient and straightforward protocol, reliability for estimation of small changes in MDA, and ease of processing large numbers of samples (Hodges, 1999) [34]. In some cases, however, there may be potential for interference of non-MDA substances in plant tissues resulting in an overestimation of MDA content and lipid peroxidation (Janero, 1990, Taulavuori 2001) [38, 72], such as in tissues containing high amounts of carbohydrates and pigments, or where additional compounds also contribute to absorbance at 532 nm (Hodges, 1999, Du and Bramlage, 1992, Stafford, 1994) [34]. Therefore, even though many current investigations utilize the original method, recent modifications in the Heath and Packer protocol have been made in order to improve the estimation of plant tissue lipid peroxidation. For example, Du and Bramlage (Du and Bramlage, 1992) adapted the basic TBARS measurement to subtract the sugar absorbance maximum at 440 nm from that at 532 nm in plant tissues high in sugar content. In order to correct for compounds that also absorb at 532 nm, Hodges *et al.* subtracted the absorbance of solutions extracted without TBA from the same plant extract reacted with TBA. The investigators found that TBA–MDA levels could be significantly over-estimated in plants such as red cabbage (*Brassica oleracea* L. var. capitata L.), eggplant (*Solanum melongena* L.), radish (*Raphanus sativus* L.), and highbush blueberry (*Vaccinium corymbosum* L.) by interfering compounds like anthocyanins if correction for these compounds were not made. Therefore, the modified protocol provided greater accuracy for quantification of MDA with little resulting loss in ease or rapidity of the original Heath and Packer procedure. Taulavuori *et al.* (Taulavuori, 2001) [72] tested the MDA correction method by Hodges *et al*. in bilberry (*V. myrtillus* L.) under field and growth chamber conditions and also found that the original, uncorrected method overestimated lipid peroxidation levels during cold acclimation.

Lipid peroxidation under high temperature

Tolerance to conditions resulting in oxidative stress has been associated with low levels of lipid peroxidation. Lipid peroxidation is caused by the oxidation of phospholipids and other unsaturated lipids when production of active oxygen species overwhelms the scavenging ability of the antioxidant defense system. Peroxidation leads to breakdown of these lipids and membrane function by causing loss of fluidity, lipid crosslinking, and inactivation of membrane enzymes. Lipid peroxidation is characterized by increasing production of MDA. Therefore, the level of lipid peroxidation is often expressed as MDA content, and is a commonly utilized measurement for assessing oxidative damage in both leaves and roots (Zhou, R. and Zhao, H., 2004, Cavalcanti, 2004) [87, 52], and its maintenance of low levels has been associated with increased stress tolerance in many species (Zhang and Kirkham, 1994, Sairam *et al.,* 2002) [84, 83]. Temperature-tolerant genotypes of wheat also had lower lipid peroxidation levels under high temperatures than did temperature-sensitive genotypes (Sairam *et al.,* 2000) [59].

Stress-induced proteins

To cope with environmental stress, plants activate a large set of genes leading to the accumulation of specific proteins,

which are generally considered stress induced proteins. Some stress-induced proteins, such as heat shock proteins (HSP) and late embryogenesis abundant proteins (LEA) are required for stress tolerance, and their accumulation has a role in protecting plant tissues from possible damages caused by environmental stresses (Sachs, 1996) [55]. The alteration of the protein level is a reflection of both transcriptional and translational regulation. The current section discusses the effect of environmental stress conditions on HSPs and LEAs, which function in protecting cells from stress injury and are the two major types of stress-inducible proteins that accumulate upon extreme temperature, water and salinity stress (Ingram and Bartels 1996, Wang *et al.,* 2003) [36, 80].

Heat shock proteins

Over two decades ago it was shown that when seedlings are shifted to temperatures 5°C or more above their optimal growth temperature, synthesis of most proteins and mRNAs is repressed, and the transcription and translation of a small set of proteins, which are said to be heat shock proteins (HSPs), are initiated by exposure to other environmental stress conditions, such as drought, cold, salinity, and hypoxia (oxygen deprivation). Consequently, HSPs are also referred to as stress proteins and their upregulation is sometimes described more generally as part of the stress response (Sabehat *et al.,* 1998) [54]. New HSP transcripts (mRNA) can be detected three to five minutes after heat stress (Sachs, 1996) [55]. Heat shock proteins also occur under nonstressful conditions, simply "monitoring" the cell's proteins. Some examples of their role as "monitors" are that they carry old proteins to the cell's "recycling bin" and they help newly synthesized proteins fold properly. These activities are part of a cell's own repair system, called the "cellular stress response." One of the important roles of HSPs involves stabilization of proteins in a particular state of folding. Through this mechanism, HSPs such as HSP90 and HSP70 facilatate a wide diversity of important processes including folding and transport of proteins across membranes and therefore these HSPs are also called "molecular chaperones" (Vierling, 1991, Ellis, 1987) [73]. The molecular masses of HSPs range from 15 to 104 kDA. Among five conserved families of HSPs—HSP100, HSP90, HSP70, HSP60, and small HSP (sHSP)—the small HSPs are found to be most prevalent in plants. sHSPs vary in size, with a molecular weight between 15 to 42 kDa (Sun, 2002) [71]. sHSPs are produced ubiquitously in prokaryotic and eukaryotic cells upon environmental stresses such as temperature, light, salinity, and drought (Wang *et al.*, 2003) [80]. Under unstressed growth conditions, most sHSPs cannot usually be detected in most plant tissues, however, upon stress there is an accumulation of sHSPs. The induction of sHSPs depends on the severity of the stress and its duration (Howarth, 1991) [35].

High levels of sHSPs, as a result of overproducing HSF's, increased the basal level of thermotolerance in Arabidopsis (Lee, 1995). The correlation between sHSP synthesis and stress response led to the hypothesis that sHSPs protect cells from detrimental effects of stress, however, the mechanisms in which they are involved in cell protection are still not fully understood (Sun, 2002) [71]. Hamilton and Heckathorn, 2001 suggested that sHSPs might act as antioxidants in protecting complex-I electron transport in the mitochondria during salinity stress. sHSPs as well as other HSPs are regarded as stress proteins with a potential to protect cells from stress damage. An increasing number of studies show the existence of cross tolerance in plants, where an exposure of tissue to moderate stress induces tolerance to other stresses. Although cross-tolerance has been demonstrated in several plant species, a common mechanism has not yet been found; however, HSPs have been demonstrated to play an important role in cross protection (Sabehat, 1998) [54].

Many publications have highlighted the importance of the molecular chaperone HSP90 complex in plant development and responsiveness to external stimuli. In particular, HSP90 is crucial for R-protein-mediated defense against pathogens. Other facets of the HSP90 function in plants include its involvement in phenotypic plasticity, developmental stability, and buffering of genetic variation (Sangster and 2005) [60]. Senthil-Kumar *et al*. 2003 [64], screened sunflower (Helianthus annuus) hybrid parents for high temperature tolerance. Seedlings of parental lines showed considerable genetic variability for thermotolerance. Thus, the existing variability formed the basis for identifying thermotolerant lines. The identified parental inbred lines were selected and established in the field and crossed to get F-1 hybrid seeds. The hybrid developed from selected variants of parental lines was compared with the original for thermotolerance. The selected hybrid was more tolerant compared with the original hybrid. The selected hybrid showed enhanced expression of HSP90 and HSP104 and also accumulated higher levels of the heat shock transcription factor. In another study, Srikanthbabu *et al.,* 2002 [68] exposed pea seedlings to a moderately high temperature prior to exposure to stressful high temperature. Plants that were acclimated to high temperature exhibited better growth compared to seedlings that were directly exposed to high temperature. The acclimated seedlings accumulated higher levels of hsp18.1 and hsp70 transcripts as well as HSP104 and HSP90 proteins.

Late embryogenesis abundant proteins

Late embryogenesis abundant (LEA) genes encode a diverse group of stress protection proteins during embryo maturation in all angiosperms (Bartels and Sunkar 2005) [36]. LEA proteins were first identified and characterized in cotton and represents one of the dominant proteins and mRNA species in mature embryos (Bartels and Sunkar 2005, Greenway and Galau, 1981) [36, 17]. Accumulation of LEA proteins correlates with increased levels of ABA and with acquisition of desiccation tolerance. (Galau, 1986) [22]. Similarly to HSPs, LEA proteins are usually not expressed in nonstressed tissues, but can be induced by osmotic stress or an exogenous application of ABA (Ingram and Bartels, 1996) [36]. Goyal *et al.* 2005 [23] proposed that LEA proteins might act as a novel form of molecular chaperone, a "molecular shield," to help prevent the formation of damaging protein aggregates during water stress. Figueras *et al.* (Figueras, 2004) [19] analyzed the effect of Rab17 expression, which is a LEA protein from maize, under a constitutive promoter in vegetative tissues of transgenic Arabidopsis plants. Gene products with a putative protective function such as LEA proteins have been identified; they were expressed at high levels in the cytoplasm or in chloroplasts upon dehydration and/or ABA treatment of vegetative tissue. Jayaprakash *et al.* 1998 reported the extent of genetic variability in the level of expression of LEA2 and LEA3 under stress in finger millet (*Eleusine coracana*) and rice seedlings. Tolerant genotypes exhibited higher expression of rab16A and M3 that code for LEA2 proteins than susceptible genotypes. A positive correlation was found between LEA2 and LEA3 protein levels and the growth of

seedlings during stress and recovery in both rice and finger millet, suggesting that the expression of LEA proteins could be a strong indication.

In nature, different plant species, populations and even individuals may employ different strategies to optimize growth and reproduction in the face of high temperature stress. Plants have evolved morphological, life history, physiological and cellular strategies not only to cope with temperature, but also to avoid high temperature damage. To date, the most successful strategies for enhancing agriculture in high temperature environments should be primarily involve sign of indicator and also avoidance mechanisms to heat tolerance.

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