LITERATURE REVIEW

2.1 Biochemical responses of plants to elevated temperature

Elevated temperatures are defined as the temperatures above the optimal growth temperature of plants and animals. The exposure of plants to elevated temperatures leads to heat stress that is often defined as the situation where temperatures are hot enough for sufficient time to cause irreversible damage to plant function or development. Different plant species and cultivars differ in their sensitivity to high temperatures and may be damaged to different extent by either high day or high night temperatures and by either high air or soil temperature. Plant responses to stressful environmental factors can be part of the mechanisms that permit the plant to withstand the stress. The response depends on the severity and duration of the stress, the developmental stage of the affected plant, the tissue type, and the interactions of multiple stresses. Progress in understanding plant responses to stress has been impressive. Many workers have worked on various crop plants to elucidate the biochemical and physiological responses of plants to elevated temperature stress. A brief review of literature in the line of investigation is presented below.

Blumenthal *et al.* (1995) designed experiments to identify wheat genotypes that might be tolerant to the effects of heat stress on grain quality and to further assess the molecular basis of these changes. Diverse set of 45 wheat genotypes was exposed to10h of 40°C on each of three consecutive days in a phytotron. Mean values of all the genotypes tested showed highly significant changes in 1000 kernel weight (-17%) and difference for heat resistance breakdown (17%). The general weakening of dough due to heat stress and decrease in protein content was accompanied by a decrease in glutenin and gliadin ratio and in the percentage of very large glutenin polymers. Bound lipid content increased, and there was a general reduction in the proportion of small starch granules. For all these attributes, reactions for individual genotype range from little change (tolerance to heat stress) to considerable change (susceptible to heat stress). They thus identified groups of genotypes that should be useful in breeding attempts to stabilize wheat against heat related variations in grain quality. Markers identified as potentially useful in

breeding for tolerance include the presence of Glu- Dld allele, and increase in glutenin to gliadin ratio and in the percentage of very large glutamine polymers.

Lafta and Lorenzen (1995) determined the role of sucrose-metabolizing enzymes in altered carbohydrate partitioning caused by heat stress. Potato (*Solanum tuberosum* L.) genotypes characterized as susceptible and tolerant to heat stress were grown at 19/17°C, and a subset was transferred to 31/29°C. Data were obtained for plant growth and photosynthesis. Enzyme activity was determined for sucrose-6-phosphate synthase (SPS) in mature leaves and for sucrose synthase, ADP-glucose pyrophosphorylase, and UDP-glucose pyrophosphorylase in developing tubers of plants. High temperatures reduced growth of tubers more than of shoots. Photosynthetic rates were unaffected or increased slightly at the higher temperature. Heat stress increased accumulation of foliar sucrose and decreased starch accumulation in mature leaves but did not affect glucose. SPS activity increased significantly in mature leaves of plants subjected to high temperature. Changes in SPS activity were probably not due to altered enzyme kinetics. The activity of sucrose synthase and ADP-glucose pyrophosphorylase was reduced in tubers, albeit less quickly than leaf SPS activity. There was no interaction of temperature and genotype with regard to the enzymes examined; therefore, observed differences do not account for differences between genotypes in heat susceptibility.

According to Stone and Nicolas (1995) short periods of very high temperature (>35°C) are common in many of the world's wheat growing areas and can be a significant factor in reducing yield and quality of wheat. A study was conducted by them to determine the stage at which the grain growth was most sensitive to a short period of high temperature and to examine whether varietal difference in heat tolerance were expresses in whole grain filling period. Two varieties of wheat differing in heat tolerance (cvv. Egret and Oxley) were exposed to a short (5 days) period of very high temperature (40°C max. for 6h each day) at 5 days interval throughout grain filling, starting from 15 days after anthesis (DAA) and concluding at 50 DAA. Response of grain dry matter accumulation and water content to high temperature was monitored throughout grain filling, and the result compared with the control maintained at 21/16°C day/night. Varietal difference was expressed throughout the grain filling period. Mature individual kernel mass was

most sensitive to heat stress applied early in grain filling and became progressively less sensitive throughout grain filling, for both varieties Reduction in mature kernel mass resulted primarily from reductions in duration rather than the rate of grain filling. To study the fractional protein accumulation in same experimental conditions, grain samples were taken through grain growth and analysed for protein content and composition (albumin/globulin, monomer, SDS soluble polymer and SDS insoluble polymer) using size exclusion high performance liquid chromatography (Stone and Nicholas, 1996). The timing of heat stress exerts a significant influence on the accumulation of total wheat protein and its fractions, and protein fractions differed in their responses to the timing of heat stress. Furthermore wheat genotypes influenced both the sensitivity of fractional protein accumulation to heat stress and the stage during grain filling at which maximum sensitivity to heat stress occurred.

Experiments were carried out with two wheat cultivars Marzak and Oum-rabia, which were subjected to three temperature regimes (20/15, 28/21and 36/29°C) beginning 10 days after anthesis to maturity. High temperature resulted in low values of seed yield and physical traits of seed quality. The effect of temperature on seed germination was not consistent between the two cultivars. High temperature during seed development and maturity had no effect on seed germination of Oum-rabia, whereas it decreased seed germination of Marzak. In contrast to seed germination, seed vigour was adversely affected by heat stress. This decline in seed germination vigor was reflected in reduced shoot and root dry weight, in increased shoot/root ratio, reduced root length, low root number per seedling, and high seed conductivity. Excised embryo culture showed marked differences in embryo growth potential. Although embryo from all treatments had germinated, a delay of 24-48 h was observed in the germination of embryos excised from seeds grown under high temperature conditions. Also their shoot and radical development over time lagged behind that of embryos isolated from seeds grown under cool temperature conditions. Exposing seeds to high temperature during development and maturity also resulted in low oxygen uptake. They also determined the effect of heat stress incase of nucleotide level and respiratory activity of mitochondria. Embryos from low temperature treatment showed rapid accumulation of ATP and higher levels and

rate of oxygen uptake then embryos from high temperature treatment. Embryos from medium temperature treatment exhibited intermediate values. Mitochondria from low temperature regimes were well developed with visible membranes and cristae; those from high temperature regimes were degenerating (Grass and Burris, 1995a & b).

High temperature tolerance of the pollen of P*etunia hybrida* L. and *Nicotiana sylvestis* L. was investigated by Rao *et al.* (1995) by treating dry pollen to temperature up to 75°C for 6-48 hrs and by studying their viability (by fluorochromatic reaction test), vigor and their ability to set fruits and seeds. In Petunia, temperature upto 60°C for 48 hrs did not affect pollen viability, vigour and their fruit and seed setting ability. A temperature of 75°C for 24 hrs reduced the pollen viability and vigour, but fruit and seed-setting ability existed. However, at 75°C exposure for 48 hrs proved lethal for *Petunia* pollen. In *Nicotiana*, pollen exposed to temperature of up to 75°C for 6-12 hrs were able to set seed. With a longer exposure the majority of pollen was FCR-positive, but they were unable to set seed. This result showed that pollen grains of *Petunia* and *Nicotiana* could withstand exposures of temperatures as high as 75°C and retain pollen function. This study also indicated that FCR test might not reflect true viability in pollen subjected to extreme stresses.

A system for the controlled expression of a foreign gene in the cultured tobacco cells (*Nicotiana tabacum*, BY2) by temperature shift was constructed by Yoshida *et al*, (1995). A 925 base pair DNA fragment containing the 5′ flanking region of a low-molecular mass heat shock protein gene (HSP 18.2) of *Arabidopsis thaliana* was inserted upstream of the β-glucuronidase reporter gene (GUS). The resulting HSP 18.2 GUS construct was introduced into BY2 cells by electroporation or *Agrobacterium* mediated transformation. Transient expression of HSP 18.2 promoter in protoplast was very low regardless of the heat shock. Although expression of the HSP 18.2 GUS chimeric gene in the stable transformants of BY2 was hardly detected in culture at 25°C, the expression increased rapidly on the transcriptional level when the incubation temperature was shifted to 35-37°C. After 2 hrs incubation at 37°C, GUS activity was about 1000 fold greater than that before

heat shock. The amount of GUS mRNA was maximum 2 hrs after heat shock, and then decreased gradually.

The responses of the photochemical apparatus of photosynthesis to low and high temperarures were compared by Verlag (1995) in leaves of the frost-sensitive *Solanum tuberosum* (cv. Haig) and of a frost-tolerant Andean potato, *Solanum* x *juzepczukii (*cv. Lucki). The main observations and conclusions of this study are that: (i) Photosystem II (PS II) is noticeably more heat-resistant in *S.* x *juzetczukii* than in *S. tuberosum*, indicating an enhanced generalized stress tolerance of the former genotype to extremes of temperature. (ii) T he higher thermostability of PS II in *S. x juzetczukii* leaves is not associated with any enhancement of the sensitivity of PS II photochemistry to chilling temperature. In both species, the chilling-induced inhibition of electron transport through PS II is closely correlated with the inhibition of the PSII-to-PSI electron flow, the rate of which is determined by the reoxidation of reduced plastoquinone. A slowdown of the latter reaction at low temperature can be attributed to the accumulation of protons in the thylakoid lumen associated with the inhibition of the Calvin cycle activity in chilled leaves, as suggested by the strong non-photochemical quenching of chlorophyll fluorescence. (iii) The photochemical activities of both species are similarly impaired by chilling treatments in the light, indicating that frost resistance does not preclude susceptibility to photoinhibition damage at temperature. (iv) A striking difference between *S. tuberosum* and *S. x juzeiczukii* is the high plasticity of the PS II thermotolerance in the latter species, with low (8°C) and high (35°C) temperature treatments respectively decreasing and increasing the heat-tolerance of PS II. These changes are not observed or are very limited in the Haig variety of S. tuberosum. (v) In contrast to the constitutive thermotolerance of PSII (measured in 23°C-grown plants), 35°C-induced thermotolerance has a dramatic effect on the photochemical activity at chilling temperature. When placed at 5°C, the intersystem electron flow of 35°C-treated leaves is dramatically inhibited as compared with non-treated leaves whereas triangle pH-related quenching of chlorophyll fluorescence is unchanged. These findings indicate independent control of non-acclimated heat-tolerance and thermally induced heat-tolerance of the photosynthetic membranes. Taken together, the presented data show that the photosynthetic apparatus of the cultivated Andean

hybrid, *S.* x *juzetczukii* though sensitive to chilling injury in the light, is adapted to the changing temperature conditions prevailing in the natural habitat of its wild progenitor where night frosts are associated with warm and sunny days.

Based on partial or complete sequence of 14 plant heat shock transcription factors from tomato, soybean, *Arabidopsis* and maize, Nover *et al.* (1996) proposed a general nomenclature with two basic classes, i.e. classes A and B containing two or more types of Hsfs (HsfA1and HsfA2). Despite some plants' specific peculiarities, essential functional domains and modules of these proteins are conserved among plants, yeast, *Drosophila* and vertebrates. Similar to the situation with the small heat shock proteins, the complexity of the hsf gene family in plants appears to be higher than in other eukaryotic organisms.

The expression of the heat shock protein (HSP) genes in the developing pollen and in the mature male gametophyte was surveyed by Mascarenhas and Crone (1996). In general, mature pollen lacks a normal heat shock response. In mature pollen of several species either no heat shock proteins are synthesized in response to heat stress, or if synthesized, only a subset are made and the response is weak both at transcriptional and translational level, compared with the response in the vegetative tissues. In developing pollen however, a subset of hsp is induced in response to heat stress. In addition, certain hsp genes or heat shock cognate genes are activated during normal pollen development in the absence of heat stress, indicating that these genes are likely to have important developmental functions.

Lipid composition of microsomes of heat stresses suspension culture was studied by Stryer *et al.* (1996). Heat stressed (30°C) cell suspension cultures of carrot attained a lower maximum cell density and showed browning earlier when compared with control cultures (22°C) over a 16 day growth period. Phospholipid class profile did not differ between cell grown at 30°C and 22°C. The fatty acid of phosphotidylcholine (PC) and phosphotidylethanolamine (PE) from microsomes of heat stressed cells were much less saturated than those of PC and PE from microsomes of control cells. In particular, there was a marked increase in the proportion of oleate [18:1 (9)] at the expense of linoleate [18:2 (9,12)] at the higher growth temperature. This difference could result from inhibition or loss of

microsomal lipid linked desaturase that inserts the double bond between carbon 12 and 13 of oleate anesterified to the glycerol moiety of PC and PE.

Heat tolerance in 23 tropical and one temperate fruit crop was evaluated by Yamada *et al.*(1996) by determining chlorophyll fluorescence [the ratio of the vriable fluorescence to the maximum fluorescence (F-v/ F-m), and the basal fluorescence (F-O)]. The ratio [R(v)] of F-v/F-m in leaves exposed to high temperature (45°C for 20 min.) to F-v/F-m to control temperature (25°C for 20 min.) was found to be highly and negatively correlated to the ratio [R(O)] of F-O exposed to the high temperature to F-O exposed to the control temperature. Leaves (3.5 months old) sampled in mid July were slightly but significantly more tolerant to heat than 2.5 months old leaves sampled in early to mid June. The ratio of the genetic variance to the total variance in the measurements was 0.90 for R(v), and 0.89 R (O). Pineapple, coconut palm and *Annona* species were heat tolerant, java apple rose apple, longan, and peach was sensitive.

De *et al.* (1996) showed the accumulation of proline in the seedlings as well as cultured cells of tomato as a consequence of short time heat shock (45°C for 4 and 8 hrs) and cold shock (4°C for 4 and 8 hrs) treatment. The involvement of calcium ion in the proline accumulation was demonstrated by using specific calcium chelator. EDTA and channel blockers. $CaCl_3$ and $CaCl_2$ pretreatment stimulated the accumulation of proline both in high and low temperature treated cultured cells and seedlings of tomato.

According to Bacci *et al.* (1996) in the leaves of herbaceous plants, sub-optimal temperatures influence the content and efficiency of the photosynthetic pigments and in more severe cases, alter mesophyll thickness. They examined the possibility of detecting the degree of alternation in sorghum leaf characteristics by indices of stress calculated from remotely sense data. Reflectants, colorimetric and ecophysiological measurements were performed on two cultivars of sorghum [*Sorghum bicolor* (L.) Moench.], grown at 15°C, 21°C and 32°C. Compared to plants growing at 21°C, the other two temperatures reduced the chlorophyll content and PS II efficiency in the leaves, which were less at 15°C than at 32°C.Slight differences, in these responses to temperature was also observed between the two

cultivars. Colorimetric coefficients detected a significant discoloration at 32°C and a marked reddening at 15°C. The indices calculated from the colorimetric data were able to to distinguish the differences between treatments, but they did not show a strict relationship with the trend of ecophysiological parameters.

The effect of heat stress on subcellular localization of Ca^{2+} in tomato fruits was studied by Garbaczewska *et al.*(1998). The tomato plants Robin cv., relatively tolerant to heat stress, were grown under uncontrolled greenhouse conditions to the stage of fruiting. The plants were placed for 20 hrs in two temperature regimes: 23°C (optimal temperature) and 40°C (heat stress) in darkness, under water vapour saturated atmosphere. Immediately after heat stress the fruits were harvested to estimate the water soluble and insoluble calcium contents and sub-cellular localization of Ca^{2+}. After heating, the concentration of calcium in tomato fruits increased about twice. In both temperature treatments the water-soluble fractions were lower than insoluble ones how ever, smaller differences between soluble and insoluble fractions were obtained after heat stress. The shapes and localization of Ca^{2+} detected with the use of potassium antimonate method showed that in fruits of control plants the precipitates were numerous, small and oval shape. They were dispersed in cytosol or adjoined to endoplasmic reticulum or to external membrane of chloroplast. In the fruits of heated plants the precipitates were irregular in shape, amorphous and singly dispersed in cytosol. They also observed some cytosolic changes in the structure of membranes and organelles of the plants of both experimental treatments. The heat induced increase of calcium content and changes in sub-cellular localization of Ca^{2+}under heat stress showed that calcium ions may be involved in avoiding heat injury.

Reproductive processes and pod yield in cowpea [(*Vigna unguiculata* (L.) Walp] an important crop grown in semi-arid sub-Saharan Africa were reported to be adversely affected by high temperature. Genotypic differences in heat tolerances have been identified under hot, long days, but it was not known if this tolerance was also exhibited in hot, short day environments typical of sub-Saharan Africa. The authors conducted the study to determine whether heat tolerance identified under hot, long days were expressed at the same stages of development under hot, short days, and whether responsiveness to temperature was additive and quantitative. A

heat tolerant (Prima), and heat susceptible (IT 84S-2246) cultivar of cowpea were grown in controlled environments under short days (12 h/day) initially at 30°C/24°C (Mod-T), where they remained for 0,10,20,30,40 days after emergence (DAE) to 36°C/27°C (High-T), where they remained for 5,10, or 20 days duration before returning to moderate temperature (Mod-T). Control plants were examined at Mod-T or High-T for 50 d when the first pods were mature and the experiments were terminated. There were significant effects of duration (D) and timing (T), and interactions between DxT, Tx genotypes (G) and DxTxG on pod weight plant (-1). Prima was significantly more tolerant to heat stress during flowering than IT 84S-2246 confirming that heat tolerance was expressed under hot, short days. The greater heat tolerance of Prima was associated with an ability to maintain peduncle and flower production at High-T and with greater podset. The sensitive period in IT 84S-2246 started at floral bud initiation (15-20 DAE), and effects of High-T thereafter were additive and quantitative (Craufurd *et al.*,1998).

Storozhenko *et al.* (1998) carried out experiments to better understand the role of ascorbate peroxidases in oxidative stress tolerance in which, the transcriptional regulation of the apx 1 gene from *Arabidopsis* was studied. The apx1 gene was expressed in all the tested organs of *Arabidopsis*; mRNA level were low in roots, leaves and stems and high in flowers. Steady state mRNA levels in leaves or cell suspensions increased after treatment with methyl viologen, etherphone, high temperature and illumination of etiolated seedlings. A putative heat shock element found in the apx1 promoter, was shown to be recognized by the tomato heat shock factor *in vitro* and to be responsible for the *in vivo* induction of the gene. The heat shock cis element also contributed partially to the induction of the gene by oxidative stress. By using *in vivo* dimethyl sulphate footprinting, they showed that protein interacted with the G/C rich element found in apx1 promoter.

Pareek *et al.*(1998) reported that while the rice 87 KDa protein was transiently synthesized within initial two hours of heat shock, high steady state level of the protein was retained even under prolonged high temperature stress condition or recovery following 4 hrs of heat shock. It was further shown that, fifteen different wild rice accumulated different levels of these proteins in response to heat shock treatment.

Presence of a high molecular weight protein in pea (*Pisum sativum* L.) seedlings was detected by means of Western Blotting by Chen and Su (1998).The protein consisted of an α (60.4 KDa) and a β (65.5 KDa) subunit. The protein had low ATPase activity. Its expression could be enhanced by 3 to 4 fold by under heat shock stress, but was not affected by exogenous application of ABA. The result of localization and 35 S-met labeling showed that it was a cytoplasmic protein and its synthesis was not inhibited by chloromphenicol.

Chen *et al.*(1998) also investigated the temperature, heat shock proteins and fertility changes in sorghum. They reported that sorghum sterile 3A line was induced to be fertile when it was heat shocked. By comparing mitochondria heat shock proteins of 3A line with 3B line they found that HSPs were encoded by nuclear DNA and were transported into mitochondria after being synthesized in the cytoplasm. When heat shocked for 2 hrs, 3A line produced 5 protein bands which weighed 70 KDa, 31 KDa, 24 KDa, 18 KDa and 16 KDa respectively whereasin 3B line, additional 94 KDa and 96 KDa bands appeared and the amount of HSP 70 was greater than in 3A line. When heat shock was given for 4 hrs, 94 KDa and 96 KDa HSPs in 3B line disappeared and 3B tended to be identical with 3A in HSPs.After heat shock treatment, the amount of mitochondrial total proteins increased greatly in both 3A and 3B. Then there was a sudden drop of HSPs. On the 8th hour 3B line had only four bands weighing 70 KDa, 32 KDa, 24 KDa and 16 KDa respectively and 70 KDa HSP was especially obvious while in 3A line, all HSPs disappeared. This indicates that HSPs are stable in 3B line but unstable in 3A line. Perhaps the difference is relevant to the stability of fertility of 3B line as well as infertility of 3A line.

Schraf *et al.* (1998) used Hsf knock out strains of yeast and transient reporter assays in tomato protoplast for functional analysis of HSF- coding cDNA clones and mutants derived from them. Hsf A2, which in tomato cell cultures was expressed after heat shock induction, tended to form large cytoplasmic aggregates together with other Hsps. In the transient expression assay its relatively low activator potential was evidently due to the inefficient nuclear import. However, the intermolecular shielding of the NLS could be released either by deletion of short

C-terminal fragment or by co expression with HsfA1, which form hetero-oligomers with HsfA2.

High temperature is a major determinant of wheat (*Triticum aestivum* L.) development and growth, decreasing yields by 3 to 5% per 1°C increase above 15°C in plants under controlled conditions. Even greater yield differences have been reported between favorable and unfavorable temperature conditions in the field. Gibson *et al.* (1999) studied the yield components of the hard red winter wheat cultivar Karl 92 that are affected by controlled high temperature during maturation of intact plants under simulated field populations. Day/night temperatures of 20/20, 25/20, 30/20, and 35/20°C were imposed from 10 and 15 d after anthesis until ripeness in two experiments, and temperatures of 25/20, 30/20, and 35/20°C were applied from 20 d after anthesis until ripeness in a third experiment. Grain yield was reduced by 78%, kernel number was reduced by 63%, and kernel weight was reduced by 29% at 35/20°C compared with 20/20°C from 10 d after anthesis until ripeness. The yield loss from high temperature applied during this period was much greater than for previous controlled-environment studies. Kernel numbers in treatments applied during early reproductive growth in our study were as sensitive to high temperature as wheat plants in previous field studies. High temperature applied 15 d after anthesis until ripening reduced grain yield 18%. Since kernel number was set by this time, the loss was exclusively due to decreased kernel weight. High temperature imposed from 20 d after anthesis decreased kernel weight by 18%.

The impact of simultaneous environmental stresses on plants and how they respond to combined stresses compared with single stresses is largely unclear. By using a transgene (*RD29A-LUC*) consisting of the firefly luciferase coding sequence (*LUC*) driven by the stress-responsive *RD29A* promoter, Xiong *et al.* (1999) investigated the interactive effects of temperature, osmotic stress, and the phytohormone abscisic acid (ABA) in the regulation of gene expression in *Arabidopsis* seedlings. Results indicated that both positive and negative interactions exist among the studied stress factors in regulating gene expression. At a normal growth temperature (22°C), osmotic stress and ABA act synergistically to induce the transgene expression. Low temperature inhibits the response to osmotic stress or to combined treatment of osmotic stress and ABA, whereas low temperature and ABA

treatments are additive in inducing transgene expression. Although high temperature alone does not activate the transgene, it significantly amplifies the effects of ABA and osmotic stress.

David and Steven (1999) carried out experiments by increasing the leaf temperature of intact cotton (*Gossypium hirsutum* L.) and wheat (*Triticum aestivum* L.) plants, which caused a progressive decline in the light, saturated CO_2 exchange rate (CER). CER was more sensitive to increased leaf temperature in wheat than in cotton and both species demonstrated photosynthetic acclimation when leaf temperature was increased gradually. Inhibition of CER was not a consequence of stomatal closure, as indicated by the positive correlation between leaf temperature and transpiration. The activation state of Ribulose bis-phosphate oxygenase/carboxylase, which is regulated by Rubisco activase, was closely correlated with temperature-induced changes in CER. Non-photochemical chlorophyll fluorescence quenching increased with leaf temperature in a manner consistent with inhibited CER and Rubisco activation. Both non-photochemical fluorescence quenching and Rubisco activation were more sensitive to heat stress than the maximum quantum yield of photochemistry of photosystem II. Heat stress led to decreased 3- phosphoglyceric acid content and increased Ribulose 1,5 bis-phosphate content which is indicative of inhibited metabolic flow through Rubisco. They concluded that heat stress inhibited CER primarily by decreasing the activation state of RUBISCO via inhibition of Rubisco activase. Although Rubisco activation was more closely correlated with CER than the maximum quantum yield of photochemistry of photosystem II, both processes could be acclimated to heat stress by gradually increasing the leaf temperature.

A band of heat shock proteins of 45 KD in leaf tissue of drought and heat tolerant maize line, ZPBL 1304 was reported by Ristic *et al.* (1999). This band has not been previously described in maize line and did not appear to be common in higher plants. It is not known how many polypeptides comprised this 45 KD band. For heat shock polypeptide study, plants were exposed to two environmental stress conditions, soil drying and high temperature (45°C) and high temperature (45°C) alone. Generally the pattern of heat shock polypeptide synthesis in both conditions was same. 2D electrophoresis revealed 3 heat shock polypeptides of 45 KD with

isoelectric points ranging from 5 to 5.5 and 2 heat shock polypeptides of 46 KD slightly above 5.5. Drought alone did not induce the synthesis of protein of 45 KD.

Two cDNAs, Ta HSP 23-5 and Ta HSP 23-6, encoding proteins with homology to mitochondrion localized (MT) small heat shock proteins (sHSPs) were isolated from heat shock cDNA library from *Triticum aestivum*. Ta HSP 23-5 specified a 214 amino acid protein and Ta HSP 23-6 specified a 216 amino acid protein. Amino acid sequence identity was only 45.7% between the two proteins. However, both proteins showed greater identity to MT sHSPs of other plant species than to any other sHSPs from wheat. Amino acid sequence alignments with other MT sHSPs identified the putative amino terminus of the mature proteins and consensus regions specific to this class of sHSPs. Transcripts of both genes were absent from control tissue, but strongly induced by heat stresses. Phylogenetic analysis indicated that these two wheat genes arose by duplication after the divergence of monocot and dicots (Basha *et al.*, 1999).

Heat shock protein 101 (HSP 101) cDNA and genomic clones were isolated by Nieto-Sotelo *et al.* (1999) from maize. The structure of maize HSP 101 revealed the presence of exons interrupted by 5 introns. Maize HSP 101 contained a predicted open reading frame that translated into a 912 amino acid sequence with a mass of 101 KD. Initiation of transcription was mapped 146 bases upstream of the AUG codon. Five HS element boxes were found. A protein sequence comparision showed that maize HSP 101 belonged to the heat shock 100 KD and caseno lytic protease B protein family that plays an important role in bacteria and yeast in survival to extremely high temperature and control of proteolysis. Accumulation of HSP 101 mRNA was strong under heat shock conditions, but was not detectable after cold or osmotic stress treatment or by application of ABA.

Lin *et al.* (1999) observed that 70 stress molecular chaperones are found in all the major sub-cellular compartments in plant cells and a multigene family encodes them. Twelve members of this family have been identified in spinach. The expression of the stress 70 molecular chaperones in response to heat shock is well known and it appears that low temperature exposure can also stimulate their expression. However, it was difficult to determine which members of the family are

specifically responsible to low temperature. This study concluded the levels of expression of the stress 70 family members and other selected chaperones in response to high and low temperature exposure. During heat shock of spinach, of the ten stress 70 family members that were examined all ten showed increased RNA levels after 1h, and all showed down regulation at longer duration of high temperature exposure.

Degradation of ribulose-1,5 bisphosphate carboxylase/oxygenase (Rubisco, EC 4.1.1.39) due to elevation in atmospheric temperature and extent of enzyme damage due to varietal differences were studied in two rice cultivars by Bose *et al.*(1999) using 35 methionine pulse- chase.The cultivars N-22 and IR-8 were certified by the authors as thermotolerant and thermosensitive respectively. Differential response was observed in both cultivars with the N-22 showing greater thermostability of Rubisco protein upto 45°C, while IR-8 was found to be thermolabile.Elevation of temperature to 50°C favoured degradation of proteins in both the cultivars. Protease activity as measured by Western Blot analysis using purified Rubisco revealed the thermosensitive nature of IR-8 and this could be correlated with the protein turn over by 35 S.methionine and Northern blot analysis. The results indicate that genetic differences exist in two cultivars and that heat tolerant cultivars have a protective mechanism against thermal degradation of Rubisco.

Timmusk *et al.* (1999) addressed changes in plant gene expression induced by inoculation with plant-growth-promoting rhizobacteria (PGPR). They established gnotobiotic system with *Arabidopsis thaliana* as model plant, and isolates of *Paenibacillus polymyxa* as PGPR. Subsequent challenge by either the pathogen *Erwinia carotovora* or induction of drought (abiotic stress) indicated that inoculated plants were more resistant than control plants. With RNA differential display on parallel RNA preparations from *P. polymyxa* treated or untreated plants changes in gene expression were investigated. From a small number of candidate sequences obtained by this approach, one mRNA segment showed a strong inoculation dependent increase in abundance. The corresponding gene was identified as *ERD15*, previously identified to be drought stress responsive. Quantification of mRNA levels of several stress-responsive genes indicated that *P. polymyxa* induced mild biotic

stress. This suggests that genes and/or gene classes associated with plant defenses against abiotic and biotic stress may be co-regulated.

Proline accumulation in response to drought and heat stress in cotton (*Gossypium hirsutum*) in six different cultivars was studied by De Ronde *et al.*(2000). They induced drought and a combination of drought and heat stress in three-week-old seedlings in the greenhouse. Results revealed that with decreasing water content there was a progressive increase in free proline in all six cultivars, as well as differences in the proline level between the different cultivars. Maximum accumulation of free proline in drought stressed cotton occurred at 11 days without water. The combination of heat and drought stress exhibited an increase in proline concentrations in five cultivars. Different proline profiles were observed for the different treatments and different mechanisms for heat and drought. Therefore, they proposed that proline may be used as an index for heat and drought tolerance.

Jiang and Huang (2000) conducted a study to determine physiological responses of Kentucky bluegrass (*Poa pratensis* L.) to drought and heat alone or together, and the effects of drought preconditioning on plant responses to subsequent heat stress. Kentucky bluegrass (cv. Mystic) was subjected to drought and/or heat stress (35°C/30°C, day/night) in growth chambers for 40 days. Canopy photosynthetic rate (P_n) and leaf photochemical efficiency (Fv/Fm) decreased under drought and heat stress. The decline in P_n was more severe under heat than under drought stress during the first 12 days of treatment. The reduction in Fv/Fm ratio was more severe under drought stress than under heat stress after 20 days of treatment. The combined heat and drought stresses (H+D) caused more dramatic reductions in P_n and Fv/Fm than either heat or drought alone, starting at 3 and 9 days after treatment, respectively.Drought or heat alone, or H+D, significantly reduced root dry weight. However, reduction was more severe under heat alone than under drought stress, particularly in the top 20 cm of soil. Drought preconditioning enhanced plant tolerance to subsequent heat stress but had no influence on plant tolerance to H+D. Drought-preconditioned plants maintained higher water status, stomatal conductance, and transpiration rate, and had significantly higher P_n and root dry weight than non-preconditioned plants during subsequent heat stress. No significant difference in Fv/Fm was observed between drought-preconditioned and non-preconditioned plants

under either heat alone or H+D. The results indicated that simultaneous drought and heat stresses were more detrimental than either stress alone. Drought preconditioning could improve Kentucky bluegrass tolerance to subsequent heat stress.

The effect of high temperature stress on wild and spring wheats has been studied by Waines (2000) in wild wheats that includes species in the genera *Aegilops* L. and *Triticum* L. Species exist in a polyploid series, diploid, tetraploid and hexaploid, based on the genome formula, n = x = 7 chromosomes. Commercial durum wheat is tetraploid with the genome formula BBAA, while bread wheat is hexaploid (BBAADD).Wheats grown at Riverside, California, from June to October exhibit heat stress at the vegetative and reproductive stages. Under high temperatures (28/15°C day/night) during the vegetative stage, many diploid species do not grow well. Wild diploid *T. urartu* (AA) and *T. monococcum* ssp. *boeoticum* (AA) exhibited more effects of heat stress than the goat grasses *A. speltoides* (SS = BB?) or *A. tauschii* (DD). Wild tetraploid *T. turgidum* L. ssp. *dicoccoides* Korn (BBAA) exhibited more vegetative-phase stress tolerance than the diploid wheats. Modern Mexican cultivars of durum and bread wheats showed good establishment under high field temperatures, but often tiller number was reduced, and the developmental stages were reduced in time. All the spring durum and bread wheats tested flowered and set seed. They produced anthers with fertile pollen, and they had reproductive heat tolerance. Many wild *Aegilops* and *Triticum* accessions did not boot for lack of vernalisation, or they showed reproductive heat stress. Ten wild accessions, including *A. speltoides*, *A. longissima* and *A. searsii*, showed normal vegetative and reproductive development and were considered heat tolerant. They came from the same geographic area in Palestine, which should be searched for landraces of wheats that show heat tolerance.

Changes in photosystem II (PSII) thermotolerance during drought and recovery were studied under controlled conditions in three Mediterranean cedar species (*Cedrus brevifolia* Henry, *C. libani* Loudon and *C. atlantica* Manetti) by Ladjal *et al.* (2001). The temperature at which the quantum yield of PSII photochemistry was reduced by 15% of its value at 25°C was 3 to 4°C higher in drought-treated plants than in well-watered plants. The drought-induced increase in PSII thermotolerance was already evident 8 days after water had been withheld from

the seedlings, when net CO_2 assimilation was still at 80% of its initial value, and was visible for up to 12 days after re-watering. When seedlings of the three species were exposed to temperatures above 45°C for 5 h, both maximal quantum yield of PSII photochemistry and net CO_2 assimilation rate were significantly reduced in unconditioned seedlings, whereas drought-preconditioned seedlings were almost unaffected by the heat treatment. Drought-preconditioned seedlings still exhibited a higher tolerance to heat stress than unconditioned seedlings 60 days after re-watering, although the transient, drought-induced osmotic adjustment had fully disappeared. Among species, *C. atlantica* was the most heat sensitive, whereas the heat treatment had no significant effect on the parameters measured in *C. brevifolia*.

In *Nicotiana attenuata*, systemic induction of heat-shock proteins (Hsps) was detected by Hamilton and Coleman (2001) in response to the treatment of single leaves by heat shock, mechanical damage, or exogenous application of methyl jasmonate (MJ). All treatments increased the abundance of members of the 70-kD Hsp (Hsp70) family and induced synthesis of one or more of the small Hsps (sHsp) (16–23 kDa) in both treated and untreated leaves. These results provide the first evidence that Hsps can be systemically induced in plants and suggest that systemic induction of Hsps may be important in pre-adapting leaves to stress.

Sharkova (2001) analyzed the effect of heat shock on the capacity of wheat plants to restore their photosynthetic electron transport after photoinhibition or repeated heating. The shoots of 16-day-old spring wheat plants (*Triticum aestivum* L., cv. Albidum 29) were subjected to heat shock (HS) at 40, 41, or 43°C for 10 min. The activity of the Hill reaction in chloroplasts isolated immediately after HS was 83, 61, and 30% of the initial value, respectively. The activity of the Hill reaction was also estimated after plant return to the initial growth conditions for one day. It was completely restored after heating at 40°C and achieved 82 and 30-33% of the initial level after heating at 41 and 43°C, respectively. Thereafter, the shoots were heated repeatedly at 42, 43, or 43.5°C for 10 min, and the activity of the Hill reaction was measured immediately or one day after this heating. Immediately after the second heating, the activity decreased again as compared to its value before heating. The percent of inhibition of the Hill reaction was similar in the control plants not subjected to preliminary HS and HS-treated plants independently of the temperature

used. However, after one-day growth under normal conditions, the activity of the Hill reaction was restored almost completely in HS-treated plants but not more than by 10% in the control plants. The conclusion is that different mechanisms underlie the development of the tolerance to HS and recovery. Some plants were tested for the effect of HS (40°C) on their tolerance to photoinhibition. The degree of the Hill reaction inhibition after plant exposure to the light of 65-75 klx for 3 hrs was essentially similar in detached leaves from the HS-treated and unheated plants and comprised about 40% of the activity before light stress. After the leaves were returned to the low-light conditions for 3 hrs, the Hill reaction was restored and attained about 75% of that before photoinhibition in both HS-treated and untreated plants. The lack of the HS-induced stimulation of the Hill reaction recovery after photoinhibition is evidently related to the fact that heating and excess light damage different sites of photosystem II, which implies the different pathways for the recovery of its functional activity.

Dalal and Khannna–Chopra (2001) investigated the activities of the antioxidant enzymes in the leaves of necrotic wheat hybrids, Kalyansona×C306 (K×C) and WL711×C306 (WL×C) and their parents at different developmental stages. The K×C hybrid exhibited more severe necrosis than WL×C. In K×C, superoxide dismutase (SOD) activity showed no increase over the parents, while WL×C showed an early increase, but it was possibly insufficient to scavenge increased superoxide. Activities of guaiacol peroxidase, ascorbate peroxidase and glutathione reductase were enhanced, while catalase exhibited a decrease in activity, with the appearance of visible necrosis in both the hybrids. The isozyme profile of the antioxidant enzymes was similar in the hybrids and their parents. One existing isoform of guaiacol peroxidase showed an early appearance in the hybrid and increased in intensity with the progression of necrosis. The results reveal a differential response of antioxidant enzymes in necrotic wheat hybrids as compared to their parents. The response differed in magnitude at developmental stages of the leaves, which might be related to the intensity of necrosis expressed by the hybrids.

The genetic control of heat tolerance through diallel analysis of selected wheat (*Triticum aestivum* L.) germplasm was determined by Ibrahim and Quick (2001). Heat-induced damage of plant membranes was assayed by the membrane thermal stability (MTS) assay, which measures electrolyte leakage from leaf tissue after exposure to high temperature. Six wheat genotypes ('TAM 107', 'TAM 108', 'Arlin',' Kauz', 'Glennson 82', and 'Siete Cerros') were hybridized in a complete diallel, and MTS was measured on 12 days old F_1 seedlings. The mean square for general combining ability (GCA) was four times that of specific combining ability (SCA), indicating the importance of additive gene effects in acquired thermal tolerance. Maternal effects accounted for 67% of reciprocal variation, suggesting that maternal seed-source effects may be important in hybrid seed. These results suggest that heat tolerance based on MTS can be improved using the existing genetic variability available within the germplasm.

Pressman *et al.* (2002) stated that continuous exposure of tomato 'Trust' to high temperatures (day/night temperatures of 32/26°C) markedly reduced the number of pollen grains per flower and decreased viability. The effect of heat stress on pollen viability was associated with alterations in carbohydrate metabolism in various parts of the anther during its development. Under control, favourable temperature conditions (28/22°C), starch accumulated in the pollen grains, where it reached a maximum value 3 days before anthesis; it then diminished towards anthesis. During anther development, the concentration of total soluble sugars gradually increased in the anther walls and in the pollen grains (but not in the locular fluid), reaching a maximum at anthesis. Continuous exposure of the plants to high temperatures (32/26°C) prevented the transient increase in starch concentration and led to decreases in the concentrations of soluble sugars in the anther walls and the pollen grains. In the locular fluid, however, a higher soluble sugar concentration was detected under the high-temperature regime throughout anther development. These results suggest that a major effect of heat stress on pollen development is a decrease in starch concentration 3 days before anthesis, which results in a decreased sugar concentration in the mature pollen grains. These events possibly contribute to the decreased pollen viability in tomato.

The molecular mechanisms by which plants acclimate to oxidative stress are poorly understood. To identify the processes involved in acclimation, Vranova *et al.*(2002) performed a comprehensive analysis of gene expression in *Nicotiana tabacum* leaves acclimated to oxidative stress. Combining mRNA differential display and cDNA array analysis, they estimated that at least 95 genes alter their expression in tobacco leaves acclimated to oxidative stress, of which 83% are induced and 17% repressed. Sequence analysis of 53 sequence tags revealed that, in addition to antioxidant genes, genes implicated in abiotic and biotic stress defenses, cellular protection and detoxification, energy and carbohydrate metabolism, *de novo* protein synthesis, and signal transduction showed altered expression. Expression of most of the genes was enhanced, except for genes associated with photosynthesis and light-regulated processes that were repressed. During acclimation, two distinct groups of coregulated genes ("early" and "late-response" gene regulons) were observed, indicating the presence of at least two different gene induction pathways. These two gene regulons also showed differential expression patterns on an oxidative stress challenge. Expression of "late-response" genes was augmented in the acclimated leaf tissues, whereas expression of "early-response" genes was not. Together, these data suggest that acclimation to oxidative stress is a highly complex process associated with broad gene expression adjustments. Moreover, the datas indicate that in addition to defense gene induction, sensitization of plants for potentiated gene expression might be an important factor in oxidative stress acclimation.

Plants respond to temperature stress by synthesizing a set of heat shock proteins (HSPs), which may be responsible for the acquisition of thermotolerance. The induction of small HSPs (sHSPs) in eight common bean varieties was evaluated by Simoes-Araujo *et al.* (2003) using Northern blot analysis and W HSP 16.9 cDNA as heterologous probe. Cowpea was used, as a positive control since this plant, as opposed to common bean, is known to grow well under high temperature regimes such as that found in the Brazilian semi-arid region. After the growth period, the plants were submitted to two h of heat shock at 40°C. All varieties tested were able to induce sHSP mRNAs that hybridized with W HSP 16.9 probe. However, significant kinetic differences were found when comparing different varieties. SHSP mRNA levels induced after heat shock in cowpea was higher than the levels

observed on the bean varieties displaying the highest expression of these proteins. Besides, the sHSP expression was also assessed at the protein accumulation level by Western-blot analysis for cowpea and both IPA 7 and Negro Argel varieties of bean plants. The revealed protein pattern confirmed that sHSPs are differentially expressed in distinct varieties of common bean according their heat stress tolerance.

Heat stress can detrimentally affect the reproductive capacity of many plants. The effect of a 7 or 14 days heat stress on flowering, seed set, pollen viability and germinability of flax (*Linum usistatissimum* L.) was assessed under growth chamber conditions by Cross *et al.* (2003). An incremental (2°C/h), cyclical (daytime high 40°C and night-time low 18°C) heat stress was applied 12 days after the initiation of flowering. Although flower formation in flax was not affected by heat stress, boll formation and seed set were reduced with onset of the heat stress. On removal of heat stress the stressed plants showed a compensatory response, flowering and producing bolls at a greater rate than the control plants. Heat stress significantly prolonged flowering by 17 days. Boll weight and seed weight were reduced with heat stress and the number of malformed, sterile seed increased three-fold after 14 days of heat stress. Pollen viability and appearance were negatively affected after 6 and 10 days of heat stress, respectively. Pollen germinability decreased by the sixth day of heat stress, with no pollen germinating by the tenth day. Effects of heat stress on pollen viability and germinability alone, which did not occur until after the sixth day of the stress, could not account for the decreased boll formation due to heat stress in flax. These observations suggest that a combined effect of heat stress on both pollen and ovules contributes to decreased boll formation and seed set in flax.

The accumulation of hydrogen peroxide (H_2O_2) in plants is typically associated with biotic or abiotic stresses. However, H_2O_2 is continuously produced in cells during normal metabolism. Yet, little is known about how H_2O_2 accumulation will affect plant metabolism in the absence of pathogens or abiotic stress. It has been reported that a deficiency in the H_2O_2-scavenging enzyme, cytosolic ascorbate peroxidase (APX1), results in the accumulation of H_2O_2 in *Arabidopsis* plants grown under optimal conditions. Knockout-Apx1 plants were characterized by suppressed growth and development, altered stomatal responses, and augmented induction of heat shock proteins during light stress. The inactivation of Apx1 resulted in the

induction of several transcripts encoding signal transduction proteins. These were not previously linked to H_2O_2 signaling during stress and may belong to a signal transduction pathway specifically involved in H_2O_2 sensing during normal metabolism. Surprisingly, the expression of transcripts encoding H_2O_2 scavenging enzymes, such as catalase or glutathione peroxidase, was not elevated in knockout-Apx1 plants. The expression of catalase, two typical plant peroxidases, and several different heat shock proteins was however elevated in knockout-Apx1 plants during light stress. The results demonstrate that in plants accumulation of H_2O_2 can suppress plant growth and development, interfere with different physiological processes, and enhance the response of plants to abiotic stress conditions. These findings also suggest that at least part of the induction of heat shock proteins during light stress in *Arabidopsis* is mediated by H_2O_2 that is scavenged by APX1 (Pnueli *et al.*, 2003).

Srivalli *et al.*(2003) have reported antioxidative defense system in an upland rice cultivar subjected to increasing intensity of water stress followed by recovery. They subjected rice (*Oryza sativa* L.) cv. Tulsi to three cycles of water stress of increasing stress intensity. Rewatering the plants for 48-hrs period terminated each stress cycle. The level of stress was measured by quantification of H_2O_2. The response of antioxidant metabolites such as ascorbate and glutathione, and enzymes such as superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), ascorbate peroxidase (APX, EC 1.11.1.11), glutathione reductase (GR, EC 1.6.4.2) and guaiacol peroxidase (POX, EC 1.11.1.7) was analysed in terms of activity and isozyme pattern for each cycle of stress and recovery. The differential response of the antioxidant enzymes with increasing stress intensity followed by recovery, highlight the different role of each in the drought acclimation process of upland rice. SOD and POX activity in stressed plants was higher than the controls in all the three cycles. The second level of stress saw an increase in all the enzymes with APX and GR showing its maximum activity and there was a better management of H_2O_2 levels. There was an induction of a new CAT isoform in stressed plants in the third cycle of water stress. The co-ordinated defense helped the plants to recover in terms of growth on rewatering after stress cycles.

Young *et al.* (2003) used table ADC-225-MK3 CO2 gas analyzer and PAM-2000 portable fluorescence measurement system to measure the net photosynthetic rate (Pn), initial fluorescence (Fo), maximal photochemical efficiency of PS II (Fv/Fm) and electron transport rate (ETR) of satsuma mandarin (*Citrus unshiu* Marc.) and navel orange (*C. sinensis* Osbeck) leaves. The results showed that exposure of citrus plants to high temperature (38-40° C) led to a lowering of Pn, Fv/Fm, and ETR, whereas Fo increased. After exposure to high temperature for 25 days, compared with normal temperature (25°C), the Pn of satsuma mandarin and navel orange leaves decreased by 55.6% and 39.8%, Fv/Fm decreased by 22.0% and 6.7% and ETR reduced by 55.0% and 41.5%, respectively. On the other hand, Fo increased 113.8% and 14.9%, respectively. With subsequent transfer to the 25°C culture room for 10 days, Pn,Fv/Fm, Fo and ETR recovered significantly. These results demonstrated that the reduction of Pn in *Citrus* was related to the inactivation of PS II reaction center.

Although the catalytic activity of Rubisco increases with temperature, the low affinity of the enzyme for CO_2 and its dual nature as an oxygenase limit the possible increase in net photosynthesis with temperature. For cotton, comparisons of measured rates of net photosynthesis with predicted rates that take into account limitations imposed by the kinetic properties of Rubisco indicate that direct inhibition of photosynthesis occurs at temperatures higher than about 30°C. Inhibition of photosynthesis by moderate heat stress (i.e.30–42°C) is generally attributed to reduced rates of RuBP regeneration caused by disruption of electron transport activity, and specifically inactivation of the oxygen evolving enzymes of photosystem II. However, measurements of chlorophyll fluorescence and metabolite levels at air-levels of CO_2 indicate that electron transport activity is not limiting at temperatures that inhibit CO_2 fixation. Instead, recent evidence shows that inhibition of net photosynthesis correlates with a decrease in the activation state of Rubisco in both C3 and C4 plants and that this decrease in the amount of active Rubisco can fully account for the temperature response of net photosynthesis. Biochemically, the decrease in Rubisco activation can be attributed to: (1) more rapid de-activation of Rubisco caused by a faster rate of dead-end product formation; and (2) slower re-activation of Rubisco by activase. The net result is that as temperature increases

activase becomes less effective in keeping Rubisco catalytically competent (Salvuccia *et al.*, 2004).

High temperature stress (HTS), during flowering decreases seed production in many plants. Young *et al.* (2004) determined the effect of a moderate HTS on flowering, fruit and seed set in *Brassica napus*. They exposed plants to a HTS (8/16 h dark/light, 18°C night, ramped at 2°C h^{-1}, over 6 hrs, to 35°C for 4 hrs, ramped at 2°C h^{-1} back to 23°C for 6 hrs) for 1 or 2 weeks after the initiation of flowering. Although flowering on the HTS-treated plants, during both the 1-week and 2-week HTS treatments, was equal to that of control-grown plants, fruit and seed development, as well as seed weight, were significantly reduced. Under HTS, flowers either developed into seedless, parthenocarpic fruit or aborted on the stem. At the cessation of the HTS, plants compensated for the lack of fruit and seed production by increasing the number of lateral inflorescences produced. During the HTS, pollen viability and germinability were slightly reduced. *In vitro* pollen tube growth at 35°C, from both control pollen and pollen developed under a HTS, appeared abnormal; however, *in vivo* tube growth to the micropyle appeared normal. Reciprocal pollination of HTS or control pistils with HTS or control pollen indicated that the combined effects of HTS on both micro-and megagametophytes were required to knock out fruit and seed development. Expression profiles for a subset of heat shock proteins (HSP101, HSP70, HSP17.6) showed that both micro- and megagametophytes were thermosensitive despite HTS-induced expression from these genes.

The effects of water deficit and high temperature on the production of α-amylase inhibitor 1 (α-AI-1) were studied in transgenic peas (*Pisum sativum* L.) that were developed by Majer *et al.* (2004) to control the seed-feeding pea weevil (*Bruchus pisorum* L., Coleoptera: Bruchidae). Transgenic and non-transgenic plants were subjected to water-deficit and high-temperature treatments under controlled conditions in the glasshouse and growth cabinet, beginning 1 week after the first pods were formed. In the water-deficit treatments, the peas were either adequately watered (control) or water was withheld after first pod formation. The high-temperature experiments were performed in two growth cabinets, one maintained at 27/22°C (control) and one at 32/27°C day/night temperatures, with the vapour

pressure deficit maintained at 1.3 kPa. The plants exposure to high temperatures and water deficit produced 27% and 79% fewer seeds, respectively, than the controls. In the transgenic peas the level of α-AI-1 as a percentage of total protein was not influenced by water stress, but was reduced on average by 36.3% (the range in two experiments was 11–50%) in the high-temperature treatment. Transgenic and non-transgenic pods of plants grown at 27/22°C and 32/27°C were inoculated with pea weevil eggs to evaluate whether the reduction in level of α-AI-1 in the transgenic pea seeds affected pea weevil development and survival. At the higher temperatures, 39% of adult pea weevil emerged, compared to 1.2% in the transgenic peas grown at the lower temperatures, indicating that high temperature reduced the protective capacity of the transgenic peas.

Ali *et al.* (2005) studied the effects of temperature on oxidative stress defense systems, lipid peroxidation and lipoxygenase activity in *Phalaenopsis*. The thermal dependencies of the activities of protective enzymes, photosynthetic efficiency (Fv/Fm), protein, non-protein thiol (NP-SH), cysteine content, lipoxygenase (LOX) activity (EC 1.13.11.12) and malondialdehyde (MDA) content at 25-40°C were determined for 4, 24 and 48 hrs in leaf and root segments of *Phalaenopsis*. Temperature-stress induced not only activities of active oxygen species (AOS) scavenging enzymes but also protein, NP-SH and cysteine content in both leaf and root segments at 30°C for 4 and 24 hrs (except for 48 h in some cases) compared to 25°C and greenhouse-grown leaf and root segments indicating that antioxidants enzymes played an important role in protecting plant from temperature-stress. However, activities of dehydroascorbate reductase (DHAR, EC 1.8.5.1), glutathione peroxidase (GPX, EC 1.11.1.9) and glutathione-*S*-transferase (GST, EC 2.5.1.18) in leaf and root, glutathione reductase (GR, EC 1.6.4.2) in leaf and guaiacol peroxidase (G-POD, 1.11.1.7) in root segments were induced significantly at 40°C compared to 25°C and greenhouse-grown plants suggesting that these enzymes play protective roles at high temperature. In contrast, activities of superoxide dismutase (SOD, EC 1.15.1.1) and monodehydroascorbate reductase (MDHAR, EC 1.6.5.4) in leaf and root, catalase (CAT, EC 1.11.1.6) in root, GR in root, and protein, cysteine, NP-SH content in both root and leaf and Fv/Fm ratio were diminished significantly at 40°C compared to 25°C and greenhouse-grown plants.

The close relation between activities of enzymes with their metabolites at 30°C than 40°C indicated that the antioxidants enzymes and metabolites both may play an important role in protecting cells against the temperature-stress.

Photosynthesis is particularly sensitive to heat stress and recent results provide important new insights into the mechanisms by which moderate heat stress reduces photosynthetic capacity. Perhaps most surprising is that there is little or no damage to photosystem II as a result of moderate heat stress even though moderate heat stress can reduce the photosynthetic rate to near zero. Moderate heat stress can stimulate dark reduction of plastoquinone and cyclic electron flow in the light. In addition, moderate heat stress may increase thylakoid leakiness. At the same time, rubisco deactivates at moderately high temperature. Relationships between effects of moderate heat on rubisco activation and thylakoid reactions are not yet clear. Reactive oxygen species such as H_2O_2 may also be important during moderate heat stress. Rubisco can make hydrogen peroxide as a result of oxygenase side reactions and H_2O_2 production by rubisco was recently shown to increase substantially with temperature. The ability to withstand moderately high temperature can be improved by altering thylakoid lipid composition or by supplying isoprene. Sharkey (2005) opined that this indicates that thylakoid reactions are important during moderate heat stress. The deactivation of rubisco at moderately high temperature could be a parallel deleterious effect or a regulatory response to limit damage to thylakoid reactions.

Net photosynthesis (Pn) is reversibly inhibited at moderately high temperature. To investigate this further, Kim and Portis (2005) examined the effects of heat stress on *Arabidopsis* plants in which Rubisco activase or thylakoid membrane fluidity had been modified. During heating leaves from 25 to 40°C at 250 ppm CO_2 and 1% O_2, the wild-type (WT), plants expressing the 43 kDa isoform only (rwt43), and plants accumulating activase 40% of WT (R100) exhibited similar inhibitions in the Pn and Rubisco activation state. Despite better membrane integrity than WT, plants having less polyunsaturation of thylakoid lipids (*fad7/8* double mutant) failed to maintain greater Pn than the WT. Plants expressing the 46 kDa isoform only (rwt46) exhibited the most inhibition, but plants expressing a 46 kDa isoform incapable of redox regulation (C411A) were similar to the WT. The null mutant (*rca*) exhibited a continuous decline in Pn. As measured by fluorescence,

electron transport activity decreased concomitantly with Pn but PSII was not damaged. Following a quick recovery to 25 from 40°C, whereas most lines recovered 90% Pn, the rwt46 and *rca* lines recovered only to 59 and <10%, respectively. As measured by NADP-malate dehydrogenase activation, after an initial increase at 30°C, stromal oxidation in the WT and rwt46 plants did not increase further as Pn decreased. These results provide additional insight into the role of Rubisco activation and activase in the reversible heat inhibition of Pn.

Gupta and Gupta (2005) studied the high temperature induced antioxidative defense mechanism in seedlings of contrasting wheat genotypes. Leaf discs of 15 d old seedling of wheat genotypes C-306(temperature tolerant) and HD 2329(widely adapted) were incubated at 25, 35 and 45°C to analyze the extent of membrane injury and antioxidative defense mechanisms. It is suggested that the tolerant genotype C-306 exhibited lower accumulation of MDA and H_2O_2 content owing to increased activities of superoxide dismutase, peroxidase and catalase under high temperature conditions. The higher water retention capacity and lower membrane injury in C-306 further helped in impairing high temperature tolerance. The HD 2329 was also able to resist high temperature stress to some extent via above adjustments.

Yang *et al.* (2005) genetically engineered tobacco (*Nicotiana tabacum*) with the ability to synthesis glycinebetaine by introducing the BADH gene for betaine aldehyde dehydrogenase from spinach (*Spinacia oleracea*). The genetic engineering enabled the plants to accumulate glycinebetaine mainly in chloroplasts and resulted in enhanced tolerance to high temperature stress during growth of young seedlings. Moreover, CO_2 assimilation of transgenic plants was significantly more tolerant to high temperatures than that of wild-type plants. The analyses of chlorophyll fluorescence and the activation of Rubisco indicated that the enhancement of photosynthesis to high temperatures was not related to the function of photosystem II but to the Rubisco activase-mediated activation of Rubisco. Western-blotting analyses showed that high temperature stress led to the association of Rubisco activase with the thylakoid membranes from the stroma fractions. However, such an association was much more pronounced in wild-type plants than in transgenic plants. The results in this study suggest that under high temperature stress, glycinebetaine maintains the activation of Rubisco by preventing the sequestration of Rubisco

activase to the thylakoid membranes from the soluble stroma fractions and thus enhances the tolerance of CO_2 assimilation to high temperature stress. The results seem to suggest that engineering of the biosynthesis of glycinebetaine by transformation with the BADH gene might be an effective method for enhancing high temperature tolerance of plants.

Seedlings of two tomato genotypes, *Lycopersicon esculentum* Mill.var.amalia and the wild thermotolerant type Nagcarlang, were grown under a photoperiod of 16 hrs light at 25°C and 8 hrs dark at 20°C. At the fourth true leaf stage, a group of plants were exposed to a heat-shock temperature of 45°C for 3 hrs, and measurements of chlorophyll fluorescence, gas-exchange characteristics, dark respiration and oxidative and antioxidative parameters were made after releasing the stress. The heat shock induced severe alterations in the photosynthesis of Amalia that seem to mitigate the damaging impact of high temperatures by lowering the leaf temperature and maintaining stomatal conductance and more efficient maintenance of antioxidant capacity, including ascorbate and glutathione levels. These effects were not evident in Nagcarlang. In Amalia plants, a larger increase in dark respiration also occurred in response to heat shock and the rates of the oxidative processes were higher than in Nagcarlang. This suggests that heat injury in Amalia may involve chlorophyll photooxidation mediated by activated oxygen species (AOS) and more severe alterations in the photosynthetic apparatus. All these changes could be related to the more dramatic effect of heat shock seen in Amalia than in Nagcarlang plants (Camejo *et al.*, 2006).

Hikosaka *et al.* (2006) reported that growth temperature alters temperature dependence of the photosynthetic rate (temperature acclimation). In many species, the optimal temperature that maximizes the photosynthetic rate increases with increasing growth temperature. Based on the biochemical model of photosynthesis, change in the photosynthesis–temperature curve was found to be attributable to four factors: intercellular CO_2 concentration, activation energy of the maximum rate of RuBP (ribulose-1,5-*bis*phosphate) carboxylation ($V_{c\ max}$), activation energy of the rate of RuBP regeneration (J_{max}), and the ratio of J_{max} to $V_{c\ max}$. In the survey, every species increased the activation energy of $V_{c\ max}$ with increasing growth temperature. Other factors changed with growth temperature, but their responses were different

among species. Among these factors, activation energy of $V_{c\ max}$ may be the most important for the shift of optimal temperature of photosynthesis at ambient CO_2 concentrations.

The impact of heat stress on the functioning of the photosynthetic apparatus in pea (*Pisum sativum* L.) plants grown at control (25°C; 25°C-plants) or moderately elevated temperature (35°C; 35°C-plants) was analyzed by Haldimann and Feller (2006). In both types of plants net photosynthesis (Pn) decreased with increasing leaf temperature (LT) and was more than 80% reduced at 45°C as compared to 25°C. In the 25°C-plants, LTs higher than 40°C could result in a complete suppression of Pn. Short-term acclimation to heat stress did not alter the temperature response of Pn. Chlorophyll a fluorescence measurements revealed that photosynthetic electron transport (PET) started to decrease when LT increased above 35°C and that growth at 35°C improved the thermal stability of the thylakoid membranes. In the 25°C-plants, but not in the 35°C-plants, the maximum quantum yield of the photosystem II primary photochemistry, as judged by measuring the Fv/Fm ratio, decreased significantly at LTs higher than 38°C. A post-illumination heat-induced reduction of the plastoquinone pool was observed in the 25°C-plants, but not in the 35°C-plants. Inhibition of Pn by heat stress correlated with a reduction of the activation state of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). Western-blot analysis of Rubisco activase showed that heat stress resulted in a redistribution of activase polypeptides from the soluble to the insoluble fraction of extracts. Heat-dependent inhibition of Pn and PET could be reduced by increasing the intercellular CO_2 concentration, but much more effectively so in the 35°C-plants than in the 25°C-plants. The 35°C-plants recovered more efficiently from heat-dependent inhibition of Pn than the 25°C-plants. The results show that growth at moderately high temperature hardly diminished inhibition of Pn by heat stress that originated from a reversible heat-dependent reduction of the Rubisco activation state. However, by improving the thermal stability of the thylakoid membranes it allowed the photosynthetic apparatus to preserve its functional potential at high LTs, thus minimizing the after-effects of heat stress.

Borjigidai *et al.* (2006) studied the effect of elevated CO_2 on temperature dependence of photosynthetic rates in rice (*Oryza sativa*) grown in a paddy field, in

relation to seasons in two years. Photosynthetic rates were determined monthly for rice grown under free-air CO_2 enrichment (FACE) compared to the normal atmosphere (570 vs 370 μmol mol^{-1}). Temperature dependence of the maximum rate of RuBP (ribulose-1,5-bisphosphate) carboxylation (V_{cmax}) and the maximum rate of electron transport (J_{max}) were analysed with the Arrhenius equation. The photosynthesis–temperature response was reconstructed to determine the optimal temperature (T_{opt}) that maximizes the photosynthetic rate. There was both an increase in the absolute value of the light-saturated photosynthetic rate at growth CO_2 (P_{growth}) and an increase in T_{opt} for P_{growth} caused by elevated CO_2 in FACE conditions. Seasonal decrease in P_{growth} was associated with a decrease in nitrogen content per unit leaf area (N_{area}) and thus in the maximum rate of electron transport (J_{max}) and the maximum rate of RuBP carboxylation (V_{cmax}). At ambient CO_2, T_{opt} increased with increasing growth temperature due mainly to increasing activation energy of V_{cmax}. At elevated CO_2, T_{opt} did not show a clear seasonal trend. Temperature dependence of photosynthesis was changed by seasonal climate and plant nitrogen status, which differed between ambient and elevated CO_2.

Heat-shock proteins (HSPs) protect cells from abiotic stresses. However, most work on HSPs in plants has been carried out in laboratory-grown crop or model species. Few studies have examined field expression of HSPs or HSP expression in response to multiple stresses that often occur simultaneously in nature. Heat stress in nature is frequently accompanied by high light, and photoinhibition is a major limitation for photosynthesis. Barua and Heckathorn (2006) reported that light induction of HSPs may help ameliorate damage from excess light. They analyzed whether accumulation of representative HSPs differed in naturally occurring *Solidago altissima* (goldenrod) in contrasting light microclimates (open sun vs. shade) and on cool vs. warm days. Their results show that HSP content in field-grown plants, undergoing natural temperature stress, was greater in open sun than shaded environments. Supporting these results, both light and temperature significantly affected accumulation of HSPs in the laboratory. This was the first study to show that the interaction of light microclimate and temperature can significantly influence HSP accumulation in field-grown plants.

Porch (2006) screened 14 genotypes of common bean for heat tolerance in both the greenhouse and field in Puerto Rico using previously developed stress indices. A total of three sets of paired trials were conducted in the field and in the greenhouse under high temperature (stress) and lower temperature (low-stress) conditions. The geometric mean (GM), stress tolerance index (STI) and stress susceptibility index (SSI) were used to evaluate the genotypic performance under stress and low-stress conditions. The results indicate that it was possible to identify superior genotypes for heat tolerance based on their stress indices. Porch (2006) also suggested that in this evaluation of heat tolerance indices, STI and GM, although correlated, were found to be effective stress indices for the selection of genotypes with good yield potential under stress and low-stress conditions.

Oxidative damage resulting from temperature extremes was studied by Mahan and Mauget. (2006) in cotton (*Gossypium hirsutum* L.) cultivar Fibermax 958. Cultivars were planted at Lubbock, TX, in 2003 and 2004 to investigate the effect of low and high temperatures on oxidative stress and antioxidant metabolism in seedlings exposed to normal thermal variation. Early and late plantings in 2003 provided seedlings of different ages for comparisons. Malondialdehyde was slightly increased in response to low temperatures indicating some oxidative damage in the seedlings. The activities of ascorbate peroxidase and glutathione reductase were not altered in response to low or high temperatures. The glutathione pool was predominately reduced in all plantings in both years indicating sufficient reduced glutathione. It was therefore concluded that antioxidant metabolism in the seedlings was sufficient to mitigate oxidative damage with only minor alterations.

Wang *et al.* (2006) determined the effect of high temperature stress during reproductive development on pod fertility, seed set, and seed yield of chickpea (*Cicer arietinum* L). They grew 'Myles' desi and 'Xena' kabuli chickpea in a controlled environment under 20/16°C day/night air temperatures (control). High (35/16°C) and moderate (28/16°C) temperature stresses were imposed for 10 days during early flowering and pod development. Compared to the control, the early flower high temperature stress decreased ($P < 0.01$) pod production by 34% for Myles and 22% for Xena, whereas high temperature stress during pod development decreased ($P < 0.05$) seeds per plant by 33% for Myles and 39% for Xena.

Consequently, the high temperature stress during pod development decreased ($P < 0.01$) seed yield by 59% for Myles and 53% for Xena. Yield reduction was greater due to the stress during pod development compared to the stress during early flowering. Plants recovered to a greater degree from the early flower stress compared to the pod development stress. The Myles desi produced 40 seeds per plant and the Xena kabuli produced 15 seeds per plant, whereas the Myles had smaller individual seed size than the Xena. Consequently, the Myles desi produced 26% greater seed yield than the Xena kabuli under the same conditions. Minimizing the exposure of chickpea to high temperature stress during pod development will increase pod fertility, seed set, and seed yield of the crop.

2.2 Metabolic changes associated with induction of thermotolerance

Heat stress responses are widely conserved among different organisms. Thermotolerance can be developed as plants acclimate to a non lethal high temperature. During heat stress in plants, as in other organisms, gene expression patterns, including transcription and translation, are altered to promote the accumulation of HSPs. The induced expression of HSPs is correlated with development of a thermotolerant state. Thermotolerance represents a property of all living cells and refers to the capacity of cells to survive or recover from normally lethal exposures to abrupt, severe heat shock, if, before the lethal stress, the cells are exposed to milder or short period of heat stress condition. Previous studies have shown that plants acquire thermotolerance under conditions that induce the accumulation of HSPs. Timed temperature treatment which induced a thermotolerant state also induced the expression of HSPs. In a study with *Arabidopsis thaliana* plants containing antisense DNA sequence that reduces HSP 70 synthesis Lee and Schoeffl (1996) showed that the high temperature extreme at which the plants could survive was reduced by 2°C compared with controls, although the mutant plants grew normally at optimum temperature. Presumably failure to synthesize the entire range of HSPs that are usually induced in plants would lead to a much more dramatic loss of thermotolerance. Other studies with both *Arabidopsis* mutants and transgenic plants demonstrate that at least HSP 101 is a critical component of both induced and constitutive thermotolerance in plants.

Rao *et al.* (1997) investigated how salicylic acid (SA) enhances H_2O_2 and the relative significance of SA enhanced H_2O_2 in *Arabidopsis thaliana*. SA treatments enhanced H_2O_2 production, lipid peroxidation, and oxidative damage to proteins, and resulted in the formation of chlorophyll and carotene isomers. SA-enhanced H_2O_2 levels were related to increased activities of Cu, Zn-superoxide dismutase and were independent of changes in catalase and ascorbate peroxidase activities. Prolonging SA treatments inactivated catalase and ascorbate peroxidase and resulted in phytotoxic symptoms, suggesting that inactivation of H_2O_2 degrading enzymes serves as an indicator of hypersensitive cell death. Treatment of leaves with H_2O_2 alone failed to invoke SA-mediated events. Although leaves treated with H_2O_2 accumulated *in vivo* H_2O_2 by 2 fold compared with leaves treated with SA, the damage to membranes and proteins was significantly less, indicating that SA can cause greater damage than H_2O_2. However, pretreatment of leaves with dimethylthiourea, a trap for H_2O_2, reduced SA-induced lipid peroxidation, indicating that SA requires H_2O_2 to initiate oxidative damage.

Hormones govern all aspects of plant metabolism. When plants are subjected to heat stress during vegetative growth stage, among other things, it alters hormone homeostasis, including hormone stability, content, biosynthesis and compartmentalization. Abscisic acid (ABA) is implicated in induction of HSPs, plant osmotic stress response and mediates one of the intracellular dehydration signaling pathways. In the field, where heat and drought stresses frequently occur simultaneously, ABA induction can be an important component of thermotolerance. Indeed, in maize (*Zea mays*), exogenously applied ABA has been reported by Gong *et al.* (1998a) to mimic water stress in increasing thermotolerance of photosystem II; additional application of calcium in maize also acted synergistically with ABA (Gong *et al.*, 1998a).

Exposure of plants to elevated temperatures results in a complex set of changes in gene expression that induce thermotolerance and improve cellular survival to subsequent stress. Gong *et al.* (1998b) reported that pretreatment of young tobacco (*Nicotiana plumbaginifolia*) seedlings with Ca^{2+} or ethylene glycol-bis (aminoethylether)-N,N,N,N-tetraacetic acid enhanced or diminished subsequent thermotolerance, respectively, compared with untreated seedlings, suggesting a

possible involvement of cytosolic $^{Ca2+}$ in heat-shock (HS) signal transduction. Using tobacco seedlings transformed with the Ca^{2+}-sensitive, luminescent protein aequorin, they observed that HS temperatures induced prolonged but transient increases in cytoplasmic but not chloroplastic Ca^{2+}. A single HS initiated a refractory period in which additional HS signals failed to increase cytosolic Ca^{2+}. However, throughout this refractory period, seedlings responded to mechanical stimulation or cold shock with cytosolic Ca^{2+} increases similar to untreated controls. These observations suggest that there may be specific pools of cytosolic Ca^{2+} mobilized by heat treatments or that the refractory period results from a temporary block in HS perception or transduction. Use of inhibitors suggests that HS mobilizes cytosolic Ca^{2+} from both intracellular and extracellular sources.

Lopez-Delgado *et al.* (1998) reported the induction of thermotolerance in potato microplants by acetylsalicylic acid and H_2O_2. They subjected potato microplants propagated as nodal explants to heat treatments *in vitro* similar to those employed in the thermotherapy step of virus eradication procedures.Low concentrations (10^{-6}-10^{-5} M) of acetyl salicylic acid (ASA) in the culture medium improved (by 3-7 fold) tolerance of a 5 week high temperature (35°C) treatment. Furthermore, tissues subcultured on ASA-free medium following several weeks of growth on ASA were more thermotolerant (by 3-8 folds) to a 7 week 35°C treatment, and (by 3-8 folds) to 15h 42°C heat-shock. Stems of microplants grown on ASA contained significantly less catalase activity and higher levels of H_2O_2 than controls. Explanting and heat treatment, however, reduced catalase activity to similar levels in ASA-treated and control microplant tissues. To confirm the role of H_2O_2 in induction of thermotolerance, nodal explants were incubated for 1hr in H_2O_2 (0.1-50 mM), and then cultured under standard conditions. The microplants that grew from H_2O_2-treated explants showed concentration dependent decreases in stem height, but were significantly more thermotolerant than control, more than 1 month after the H_2O_2 treatment further confirming the direct roles of ASA and H_2O_2 in thermotolerance.

Heat acclimation and salicylic acid treatment were earlier shown to induce thermotolerance in mustard (*Sinapsis alba* L.) by Dat *et al.*(1998a). In seedlings subjected to 1 hour of heat acclimation treatment glucosylated SA increased 5.5 fold

and then declined during the next 6 hrs. Increases in SA were smaller (2 fold) but significant. Changes in antioxidants revealed that the reduced to oxidized ascorbate ratio was 5 fold lower than in controls after 1 hr of treatment. Glutathione reductase (GR) activity in treated samples was found to be more than 50% higher during the first 2 hrs of treatment. Activities of dehydroascorbate reductase decreased by at least 25% during the first 2 hrs but were 20% to 60% higher in treated samples than in control leaves after 3-6 hrs. Ascorbate peroxidase (APOX) activity was found to be 30% higher after one hour of heat treatment. Young leaves appeared to be better protected by antioxidant enzymes following heat acclimation than the cotyledons or stem. Changes in endogenous SA and antioxidants may be involved in heat acclimation.

Dat *et al.* (1998b) also reported that spraying mustard (*Sinapis alba* L.) seedlings with salicylic acid (SA) solutions between 10 and 500 µm significantly improved their tolerance to a subsequent heat shock at 55°C for 1.5 hr. The effects of SA were concentration dependent, with higher concentrations failing to induce thermotolerance. The time course of thermotolerance induced by 100 µm SA was similar to that obtained with seedlings acclimated at 45°C for 1 hr. Heat shock at 55°C caused a significant increase in endogenous H_2O_2 and reduced catalase activity. A peak in H_2O_2 content was observed within 5 min of either SA treatment or transfer to the 45°C acclimation temperature. Between 2 and 3 hrs after SA treatment or heat acclimation, both H_2O_2 and catalase activity significantly decreased below control levels. The lowered H_2O_2 content and catalase activity occurred in the period of maximum thermoprotection. It is suggested that thermoprotection obtained either by spraying SA or by heat acclimation may be achieved by a common signal transduction pathway involving an early increase in H_2O_2.

Trofimova *et al.* (1999) studied the effect of Ca^{2+} on heat shock induced changes in the cell protein synthesis incorporation of 35 S methionine into protein in cultured sugarbeet (*Beta vulgaris* L.) cells incubated in the media containing different Ca^{2+} concentrations. Heat shock inhibited the synthesis of non-heat shock proteins and promoted the synthesis of set of HSPs, typical of plants. The synthesis of non-HSPs was found to be greatly inhibited by external Ca^{2+} removal by treatment of the cells with ethylene glycol-bis (beta aminoethylether)–N, N, N', N' tetra acetic

acid. In contrast, extracellular Ca^{2+} appeared to be not strictly required for the de novo synthesis of HSPs. Cell injury increased if the cells were exposed simultaneously to high temperature and Ca^{2+} deficent medium. Recovery of HSP synthesis and reduced cell injury were observed after addition of exogenous Ca^{2+} to Ca^{2+} depleted cells. These findings were consistent with the findings of the other workers who also mentioned the requirement of Ca^{2+} for the survival of cells under heat shock and the involvement of Ca^{2+} ions in the development of thermotolerance.

It has been demonstrated that treatment with 24-epibrassinolide, a brassinosteroid increases the basic thermotolerance of *Brassica napus* and tomato seedlings. Recent studies have shown that brassinosteroids are essential for proper plant development. Several lines of evidences suggest that in addition to their role in plant development brassinosteroids exert anti stress effects on plants. However the mechanisms by which they modulate plants' stress responses are not known. *Brassica napus* and tomato seedlings grown in presence of 24-epibrassinolide (EBR) were found to be more tolerant to lethal temperature treatment than control plants grown in absence of EBR. Since a pre- conditioning treatment with this compound was not required to observe the effect, it was concluded that EBR treatment incrases the basic thermotolerance of seedlings. Analysis of HSPs in seedlings by Western Blot analysis indicated that HSPs did not accumulate preferentially in EBR treated seedlings at control temperature. However, after heat stress, HSP accumulation was higher in EBR treated seedlings than in untreated seedlings. The results of present study provide the first direct evidence for EBR induced accumulation of HSPs. The higher accumulation of HSPs in EBR treated seedlings raises the possibility that HSPs contribute at least in part to thermotolerance in EBR treated seedlings. A search for factors other than HSps, which may directly or indirectly contribute to brassinosteroid mediated increase in thermotolerance is underway (Dhaubadel *et al.*,1999).

Hardin *et al.*(1999) demonstrated root membrane thermostability in *Cornus florida* L. using flowering dogwood seeds from different heat zones. The unsuberised, current season, fine root tissues were subjected to temperature treatment ranging from 20°C to 60°C for 30 minutes and analyzed for cellular electrolyte leakage. Electrolyte leakage from root tissue exhibited a sigmoidal

response to temperatures for trees from each location. Critical mid point temperature (T_m) was found to be greater for seedlings native to USDA hardiness zone 6b (AHS heat zone 7, 52.4 ± 0.6°C) than T_m for seedlings originating from USDA zone 7a (AHS heat zone 51.2 ± 0.5°C). However seedlings from USDA zone 8a (AHS 8 zone) at 51.5 ± 0.4°C were smaller to those collected in USDA zones 6b (AHS zone 7). The results of this study found little genetic variability across this part of the native range of flowering dogwood regarding root thermotolerance.

Queitsch *et al.* (2000) reported that transgenic *Arabidopsis* plants expressing less than usual amounts of HSP101, a result of either antisense inhibition or cosuppression, grew at normal rates but had a severely diminished capacity to acquire heat tolerance after mild conditioning pretreatments. The naturally high tolerance of germinating seeds, which express HSP101 as a result of developmental regulation, was also profoundly decreased. Conversely, plants constitutively expressing HSP101 tolerated sudden shifts to extreme temperatures better than did vector controls. Therefore, it was concluded that HSP101 plays a pivotal role in heat tolerance in *Arabidopsis*.

A screening method, based on hypocotyl elongation, for mutants of *Arabidopsis thaliana* that were unable to acquire thermotolerance to high-temperature stress was developed by Hong *et al.* (2000). They have defined four separate genetic loci, *hot1-4*, required for this process *hot1* was found to have a mutation in the heat shock protein 101 (Hsp101) gene, converting a conserved Glu residue in the second ATP-binding domain to a Lys residue, a mutation that is predicted to compromise Hsp101 ATPase activity. In addition to exhibiting a thermotolerance defect as assayed by hypocotyl elongation, 10-day-old *hot1* seedlings were also unable to acquire thermotolerance, and *hot1* seeds had greatly reduced basal thermotolerance. Complementation of *hot1* plants by transformation with wild-type Hsp101 genomic DNA restored *hot1* plants to the wild-type phenotype. The *hot* mutants are the first mutants defective in thermotolerance that have been isolated in a higher eukaryote, and *hot1* represents the first mutation in an Hsp in any higher plant. The phenotype of *hot1* also provides direct evidence that Hsp101, which is required for thermotolerance in bacteria and yeast, is also essential for thermotolerance in a complex eukaryote.

Jiang and Huang (2001) suggested that calcium (Ca^{2+}) may be involved in plant tolerance to heat stress by regulating antioxidant metabolism or/and water relations. They examined whether external Ca^{2+} treatment would improve heat tolerance in two C(3), cool-season grass species, tall fescue (*Festuca arundinacea* L.) and Kentucky bluegrass *(Poa pratensis* L.), and determined the physiological mechanisms of Ca^{2+} effects on grass tolerance to heat stress. Grasses were treated with $CaCl_2$ (10 mM) or H_2O by foliar application and then exposed to heat stress (35/30°C) in growth chambers. Some of the Ca^{2+} untreated plants were maintained at 20/15°C as the temperature control. Heat stress reduced grass quality, relative water content (RWC), and chlorophyll (Chl) content of leaves in both species, but Ca^{2+} treatment increased all three factors under heat stress. The Ca^{2+} concentration in cell saps increased with heat stress and with external Ca^{2+} treatment in both species. Osmotic potential increased with heat stress, but external Ca^{2+} treatment had no effect. Osmotic adjustment increased during short-term heat stress, but then decreased with a prolonged period of stress; it was not influenced by Ca^{2+} treatment. The activity of superoxide dismutase (SOD) in both species increased transiently at 12 days of heat stress and then remained at a level similar to that of the control. External Ca^{2+} treatment had no effect on SOD activity. The activities of catalase (CAT), ascorbate peroxidase (AP), and glutathione reductase (GR) of both species decreased during heat stress. Plants treated with Ca^{2+} under heat stress had higher CAT, GR and AP activities than untreated plants. Lesser amounts of malondialdehyde (MDA) accumulated in Ca^{2+} treated plants than in untreated plants during extended periods of heat stress. The results suggested that exogenous Ca_2, treatment enhanced heat tolerance in both tall fescue and Kentucky bluegrass. This enhancement was related to the maintenance of antioxidant activities and a decrease in membrane lipid peroxidation, but not to the regulation of osmotic potential and osmotic adjustment.

Effect of abscisic acid on active oxygen species, antioxidative defence system and oxidative damage were studied in leaves of maize (*Zea mays* L.) seedlings by Jiang and Zhang (2001). Seedlings were supplied with different concentrations of abscisic acid (ABA) and its effects on the levels of superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and the content of catalytic Fe, the activities

of several antioxidative enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR), the contents of several non-enzymatic antioxidants such as ascorbate (ASC), reduced glutathione (GSH), alpha-tocopherol (α-TOC) and carotenoid (CAR), and the degrees of the oxidative damage to the membrane lipids and proteins were examined. Treatment with 10 and 100 μM ABA significantly increased the levels of O^{2-} and H_2O_2 followed by an increase in activities of SOD, CAT, APX and GR, and the contents of ASC, GSH, α-TOC and CAR in a dose- and time-dependent pattern in leaves of maize seedlings. An oxidative damage expressed as lipid peroxidation, protein oxidation, and plasma membrane leakage did not occur except for a slight increase with 100μM ABA treatment for 24 hrs. Treatment with 1,000μM ABA led to a more abundant generation of O^{2-} and H_2O_2 and a significant increase in the content of catalytic Fe, which is critical for H_2O_2 dependent hydroxyl radical production. The activities of these antioxidative enzymes and the contents of α-TOC and CAR were still maintained at a higher level, but no longer further enhanced when compared with the treatment of 100 μ M ABA. The contents of ASC and GSH had no changes in leaves treated with 1,000 μM ABA. These results indicate that treatment with low concentrations of ABA (10 to 100 μM) induced an antioxidative defence response against oxidative damage, but a high concentration of ABA (1,000 μM) induced an excessive generation of AOS and led to an oxidative damage in plant cells.

Roles of abscissic acid (ABA) in water stress-induced oxidative stress were further investigated by Jiang and Zhang (2002) in leaves of maize (*Zea mays* L.) seedlings exposed to water stress induced by polyethylene glycol (PEG 6000). Treatment with PEG at -0.7 MPa for 12 and 24 hrs led to a reduction in leaf relative water content (RWC) by 7.8 and 14.1%, respectively. The mild water stress caused significant increases in the generation of superoxide radical (O2-) and hydrogen peroxide (H_2O_2), the activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) and the contents of ascorbate (ASC), reduced glutathione (GSH). The moderate water stress failed to further enhance the capacity of antioxidant defense systems, as compared to the mild water stress. The contents of catalytic Fe, which is critical for H_2O_2-dependent hydroxyl radical (OH) production, and the oxidized forms of ascorbate and

glutathione pools, dehydroascorbate (DHA) and oxidized glutathione (GSSG), markedly increased a significant oxidative damage to lipids and proteins took place under the moderate water stress. Pretreatment with ABA caused an obvious reduction in the content of catalytic Fe and significant increases in the activities of antioxidant enzymes and the contents of non-enzymatic antioxidants, and then significantly reduced the contents of DHA and GSSG and the degrees of oxidative damage in leaves exposed to the moderate water stress. Pretreatment with an ABA biosynthesis inhibitor, tungstate, significantly suppressed the accumulation of ABA induced by water stress, reduced the enhancement in the capacity of antioxidant defense systems, and resulted in an increase in catalytic Fe, DHA and GSSG, and oxidative damage in the water-stressed leaves. These effects were completely prevented by addition of ABA, which raised the internal ABA content. These data indicate that ABA plays an important role in water stress-induced antioxidant defense against oxidative stress.

Protection against heat stress-induced oxidative damage in *Arabidopsis* involving calcium, abscisic acid, ethylene and salicylic acid were reported by Larkindale and Knight (2002). Heat caused increased thiobarbituric acid reactive substance levels and reduced survival. Both effects required light and were reduced in plants that had acquired thermotolerance through a mild heat pre-treatment. Calcium channel blockers and calmodulin inhibitors increased the effects of heating and added calcium reversed them, implying that protection against heat-induced oxidative damage in *Arabidopsis* requires calcium and calmodulin. Similar to calcium, SA, 1- aminocyclopropane-1-carboxylic acid and ABA added to plants protected them from heat-induced oxidative damage. The ethylene-insensitive mutant etr-1, the ABA-insensitive mutant abi, and a transgenic line expressing nahG (consequently inhibited SA production) showed increased susceptibility to heat. These data suggest that protection against heat-induced oxidative damage also involves ethylene, ABA and SA. Measurement of cytosolic calcium level during heating revealed that calcium peaks were greater in thermotolerant plants, implying that these calcium signals might play a role in mediating the effects of acquired thermotolerance.

Results of growth responses to cytokinin indicate that heat stress injury in creeping bentgrass (*Agrostis palustris* L.) can be alleviated to some extent by injection of cytokinin to the root zone (Liu and Huang,,2002). Creeping bentgrass was exposed to three air/soil temperature regimes for 56 days in growth chambers: (i) low air and soil temperature control (20/20°C); (ii) high soil temperature (20/35°C); and (iii) high air/soil temperatures (35/35°C). Four different concentrations (0.01, 0.1, 1, and 10 μmol) of zeatin riboside (ZR) or water (control) were injected into the root zone (0 to 5 cm depth) of plants on the day before heat stress was imposed (0 day) and 14 days after. Leaf electrolyte leakage (EL) and the content of a lipid peroxidation product, malondialdehyde (MDA), increased, whereas leaf chlorophyll content and activities of superoxide dismutase (SOD) and catalase (CAT) decreased at 20/35°C or 35/35°C for ZR-untreated plants. Exogenous ZR significantly suppressed these responses under both high temperature regimes. Application of 10 μmol ZR was most effective in slowing leaf senescence and alleviating heat-induced lipid peroxidation of cell membranes, followed by 1 μmol at 35/35°C. Applying 0.1 and 0.01 μmol ZR had no effects on creeping bentgrass responses to 20/35 or 35/35°C. The results therefore, suggested the alleviating effects of ZR at 1and 10 μmol in heat injury to creeping bentgrass was related to the inhibition of lipid peroxidation and slowing leaf senescence.

Mitogen-activated protein kinases (MAPKs) appear to be ubiquitously involved in signal transduction during eukaryotic responses to extracellular stimuli. In plants, no heat shock-activated MAPK has so far been reported. Also, whereas cold activates specific plant MAPKs such as alfalfa SAMK, mechanisms of such activation are unknown. Sangwan *et al.* (2002) reported the presence of heat shock-activated MAPK (HAMK) immunologically related to ERK (Extracellular signal-Regulated Kinase) superfamily of protein kinases. Molecular mechanisms of heat-activation of HAMK and cold-activation of SAMK were investigated. It was found that cold-activation of SAMK requires membrane rigidification, whereas heat-activation of HAMK occurs through membrane fluidization. The temperature stress and membrane structure-dependent activation of both SAMK and HAMK is mimicked at 25°C by destabilizers of microfilaments and microtubules, latrunculin B and oryzalin, respectively; but is blocked by jasplakinolide, a stabilizer of actin

microfilaments. Activation of SAMK or HAMK by temperature, chemically modulated membrane fluidity, or by cytoskeleton destabilizers is inhibited by blocking the influx of extracellular calcium. Activation of SAMKor HAMK is also prevented by an antagonist of calcium-dependent protein kinases (CDPKs). In summary, the datas indicate that cold and heat are sensed by structural changes in the plasma membrane that translates the signal via cytoskeleton, Ca^{2+} fluxes and CDPKs into the activation of distinct MAPK cascades.

Bowen *et al.* (2002) demonstrated that the heat shock response is also involved in thermotolerance in suspension- cultures cells of apple fruit. When cultured apple cells (*Malus domestica*) were heat-treated at temperatures from 24 to 42°C , an increase in expression of heat shock protein mRNA transcripts was detected within 5°C of the culture growth temperature. An increase in the expression of HSP transcripts was also associated with a 1hr 38°C heat pre-treatment that made the apple cells tolerant to a subsequent 1h 42°C lethal heat treatment.

Panchuk *et al.* (2002) studied the effects of elevated growth temperatures and heat stress on the activity and expression of ascorbate peroxidase (APX). They compared wild-type Arabidopsis with transgenic plants over expressing heat shock transcription factor 3 (HSF3), which synthesize heat shock proteins and are improved in basal thermotolerance. Following heat stress, APX activity was positively affected in transgenic plants and correlated with a new thermostable isoform, APX^S. This enzyme was present in addition to thermolabile cytosolic APX1, the prevalent isoform in unstressed cells. In HSP3-transgenic plants, APX^S activity was detectable at normal temperature and persisted after severe heat stress at 44°C. In nontransgenic plants, APX^S was undetectable at normal temperature, but could be induced by moderate heat stress. The mRNA expression profiles of known and three new Apx genes were determined using real-time PCR. Apx1 and Apx2 genes encoding cytosolic APX were heat stress and HSF dependently expressed but only the representations of Apx2 mRNA met the criteria that suggest identity between APX^S and APX2: not expressed at normal temperature in wild type, strong induction by heat stress, and HSF3-dependent expression in transgenic plants. Their data suggest that Apx2 is a novel heat shock gene involved in thermoprotection and

that the enzymatic activity of APX2/APX^{s} is required to compensate heat stress dependent decline of APX1 activity in the cytosol.

Hu and Kloepper (2003) tested the hypothesis that some Plant growth-promoting rhizobacteria (PGPR) strains can activate heat stress tolerance. Two-week old tomato plants with or without PGPR treatment were subjected to heat stress with or without heat pre-treatment, which can activate thermotolerance. Some PGPR strains increased the tomato seedling survival rate and enhanced the shoot weight under heat stress conditions. These results suggest that the response of PGPR-treated plants subjected to heat stress mimicked the classic heat- shock response.

Salicylic acid (SA) is reported to protect plants from heat shock (HS), but insufficient is known about its role in thermotolerance or how this relates to SA signaling in pathogen resistance. Clarke *et al.* (2004) tested thermotolerance and expression of pathogenesis-related (PR) and HS proteins (HSPs) in *Arabidopsis thaliana* genotypes with modified SA signaling: plants with the SA hydroxylase NahG transgene, the non expresser of PR proteins (npr1) mutant, and the constitutive expressers of PR proteins (cpr1 and cpr5) mutants. At all growth stages from seeds to 3-week-old plants, role of SA-dependent signaling in basal thermotolerance (i.e. tolerance of HS without prior heat acclimation) was evident. Endogenous SA correlated with basal thermotolerance, with the SA-deficient NahG and SA-accumulating cpr5 genotypes having lowest and highest thermotolerance, respectively. SA promoted thermotolerance during the HS itself and subsequent recovery. Recovery from HS apparently involved an NPR1-dependent pathway but thermotolerance during HS did not. SA reduced electrolyte leakage, indicating that it induced membrane thermoprotection. PR-1 and Hsp17.6 were induced by SA or HS, indicating common factors in pathogen and HS responses. SA-induced Hsp17.6 expression had a different dose–response to PR-1 expression. HS-induced Hsp17.6 protein appeared more slowly in NahG. However, SA only partially induced HSPs. Hsp17.6 induction by HS was more substantial than by SA, and we found no SA effect on Hsp101 expression. All genotypes, including NahG and npr1, were capable of expression of HSPs and acquisition of HS tolerance by prior heat acclimation. Although SA promotes basal thermotolerance, it is not essential for acquired thermotolerance. Salicylic acid

(SA) is reported to protect plants from heat shock (HS), but insufficient is known about its role in thermotolerance or how this relates to SA signaling in pathogen resistance. Clarke *et al.* (2004) tested thermotolerance and expression of pathogenesis-related (PR) and HS proteins (HSPs) in *Arabidopsis thaliana* genotypes with modified SA signaling: plants with the SA hydroxylase *NahG* transgene, the nonexpresser of PR proteins (*npr1*) mutant, and the constitutive expressers of PR proteins (*cpr1* and *cpr5*) mutants. At all growth stages from seeds to 3-week-old plants, for SA-dependent signaling in basal thermotolerance (i.e. tolerance of HS without prior heat acclimation) was evident. Endogenous SA correlated with basal thermotolerance, with the SA-deficient *NahG* and SA-accumulating *cpr5* genotypes having lowest and highest thermotolerance, respectively. SA promoted thermotolerance during the HS itself and subsequent recovery. Recovery from HS apparently involved an NPR1-dependent pathway but thermotolerance during HS did not. SA reduced electrolyte leakage, indicating that it induced membrane thermoprotection. PR-1 and Hsp17.6 were induced by SA or HS, indicating common factors in pathogen and HS responses. SA-induced Hsp17.6 expression had a different dose–response to PR-1 expression. HS-induced Hsp17.6 protein appeared more slowly in *NahG*. However, SA only partially induced HSPs. Hsp17.6 induction by HS was more substantial than by SA, and we found no SA effect on Hsp101 expression. All genotypes, including *NahG* and *npr1*, were capable of expression of HSPs and acquisition of HS tolerance by prior heat acclimation. Although SA promotes basal thermotolerance, it is not essential for acquired thermotolerance.

Larkindale and Huang (2004) investigated whether pre-treating plants with specific putative signaling components and heat acclimation would induce tolerance of a cool-season grass, creeping bentgrass (*Agrostis stolonifera* var. palustris), to subsequent heat stress and whether thermotolerance induction of those pretreatments was associated with the regulation of antioxidant regenerating enzymes. The treatments included foliar application of salicylic acid (SA), abscisic acid (ABA), calcium chloride ($CaCl_2$), hydrogen peroxide (H_2O_2), 1-aminocyclopropane-1-carboxylic acid (ACC, a precursor of ethylene prior to the exposure of plants to heat stress (35°C) in a growth chamber. Physiological

measurements including turf quality, leaf photosynthetic rate, and levels of oxidative damage demonstrated that all treatments increased heat tolerance. The better heat tolerance for pre-treated plants as compared to controls was related to the protection of oxidative damage under heat stress. APX activity increased over the first 2 days and 5 days of heating for ACC and $CaCl_2$ respectively, but for only 12 hrs for H_2O_2. SA and ABA pre-treatments had no effects on APX activity earlier, but maintained APX activity at a significantly higher level than in controls after 24 hrs of heating. SA and ABA pre-treatments had no effects on POX activity. ACC treatment significantly increased POX activity. Pre-treatment with $CaCl_2$, H_2O_2, and HA reduced POX activity, particularly during the later phase of heating. Plants treated with SA, $CaCl_2$, H_2O_2 and HA had lower CAT activity than their control plants prior to heating and within 48 hrs of heat stress. ABA and ACC pre-treatments maintained higher CAT activity than the controls after 48 hrs of heating. ACC, $CaCl_2$, or HA pre-treatments increased SOD activity only before 5 days of heat stress. SA and ABA pre-treatments had less effect on APX activity earlier under heat stress. These results suggest that specific groups of potential signaling molecules may induce tolerance of creeping bentgrass to heat stress by reducing oxidative damage.

Larkindale and Huang (2005) suggested that signaling molecules like abscisic acid (ABA), salicylic acid (SA), ethylene, and hydrogen peroxide (H_2O_2) are involved in the regulation of plant responses to heat stress in creeping bentgrass (*Agrostis stolonifera*). The effects of treatment with ABA, SA, H_2O_2, and ACC (an ethylene precursor) on physiological damage occurring in creeping bentgrass during heat stress (35°C for 1 month) and the effects of chemical application and the induction of thermotolerance using moderate heat stress (30°C for 24 hrs) were compared by the authors. All of the pre-treatments (heat or chemical) resulted in increased tolerance to prolonged heat stress (1 month) compared to control plants. All treated samples showed more green leaves, decreased membrane leakage and reduced oxidative damage compared to control plants. An oxidative burst was detected 5 min after the initiation of heat treatment, with the increase in H_2O_2 being detected primarily in the apoplast of the cells in both leaf and root tissues. Free SA was detected only an hour after the initiation of heat stress, and concentration

remained low subsequently. Neither ABA nor ethylene concentrations rose during heat stress, but the concentration of both increased during subsequent cooling. These results suggest that the signaling components of interest are involved in thermotolerance in creeping bentgrass, but that the different chemicals are likely to be involved in separate signaling pathways. An oxidative burst and SA may be bona fide heat stress signals, but ABA and ethylene appear to be involved in signaling pathways in response to recovery from heat stress in this species.

Larkindale *et al.* (2005) also further investigated the importance of different processes to heat stress tolerance, in 45 *Arabidopsis thaliana* mutants and one transgenic line for basal and acquired thermotolerance at different stages of growth. Plants tested were defective in signaling pathways (abscisic acid, salicylic acid, ethylene, and oxidative burst signaling) and in reactive oxygen metabolism (ascorbic acid or glutathione production, catalase) or had previously been found to have temperature-related phenotypes (e.g. fatty acid desaturase mutants, uvh6). Mutants were assessed for thermotolerance defects in seed germination, hypocotyl elongation, root growth, and seedling survival. Fifteen mutants showed significant phenotypes. Abscisic acid (ABA) signaling mutants (abi1 and abi2) and the UV-sensitive mutant, uvh6, showed the strongest defects in acquired thermotolerance of root growth and seedling survival. Mutations in nicotinamide adenine dinucleotide phosphate oxidase homolog genes (atrbohB and D), ABA biosynthesis mutants (aba1, aba2, and aba3), and NahG transgenic lines (salicylic acid deficient) showed weaker defects. Ethylene signaling mutants (ein2 and etr1) and reactive oxygen metabolism mutants (vtc1, vtc2, npq1, and cad2) were more defective in basal than acquired thermotolerance, especially under high light. All mutants accumulated wild-type levels of heat shock protein 101 and small heat shock proteins. These data indicate that, separate from heat shock protein induction, ABA, active oxygen species, and salicylic acid pathways are involved in acquired thermotolerance and that UVH6 plays a significant role in temperature responses in addition to its role in UV stress.

He *et al.* (2005) reported that application of salicylic acid (SA) to the shoots and soil could improve heat tolerance of Kentucky bluegrass (*Poa pratensis* L.). Effects of SA at different concentrations (0, 0.1, 0.25, 0.5, 1, and 1.5 mmol) on heat

tolerance were examined in Kentucky bluegrass exposed to 46°C for 72 hrs in a growth chamber. Influences of SA on the production of active oxygen species (AOS), superoxide anion ($O_2^{\cdot-}$), and hydrogen peroxide (H_2O_2), and activities of antioxidant enzymes, superoxide dismutase (SOD), and catalase (CAT), were also examined. Among SA concentrations, 0.25 mmol was found to be most effective in enhancing heat tolerance in Kentucky bluegrass, which was manifested by improved regrowth potential following heat stress of 72 hrs and maintenance of leaf water content at 77% during the 12 hrs stress period similar to that under normal temperature conditions. The $O_2^{\cdot-}$ generating rate increased significantly at 6 hrs of heat stress, and SOD activity increased significantly at 2 h but decreased to the control level at 6 hrs of heat stress in SA-untreated plants. The SA application suppressed the increase of $O_2^{\cdot-}$ generating rate and enhanced SOD activity significantly at 2 and 6 hrs of heat stress, respectively. The SA application decreased H_2O_2 level significantly at 2 and 12 hrs of heat stress, and increased CAT activity significantly within 12 hrs of heat stress. The results suggest that SA application enhanced heat tolerance in Kentucky bluegrass and SA could be involved in the scavenging of AOS by increasing SOD and CAT activities under heat stress. The effect of abscisic acid (ABA) on *Stylosanthes guianensis* (Aublet) Sw. and its relation to antioxidant systems under chilling stress was studied by Zhou *et al.* (2005). *Stylosanthes guianensis* seedlings were sprayed with 10 mg L^{-1} ABA or water. One day later, the plants were transferred to a 10°C growth chamber and grown for 7 days with a 12 hrs photoperiod at 160 μmol m^{-2} s^{-1} photosynthetic photon flux density. The chilling treated plants were then rewarmed to 28°C for 2 days. During the 9-days treatment, a series of enzyme activities, relative water content (RWC), and electrolyte leakage were measured on sampled leaflets. The results showed that chilling increased electrolyte leakage of both water- and ABA-treated plants, while RWC decreased under chilling conditions. ABA-treated plants had lower electrolyte leakage and higher RWC than those of water-treated plants. Activities of ascorbate peroxidase (APX) and catalase (CAT) and contents of reduced glutathione (GSH) and ascorbic acid (AsA) were transiently enhanced by ABA treatment before the plants were subject to chilling. ABA-treated *S. guianensis* retained higher levels of superoxide dismutase (SOD), APX, GSH, and AsA than

water-treated ones under chilling conditions. The results suggested that ABA-increased chilling resistance in *S. guianensis* is partially associated with enhanced scavenging systems.

Thermotolerance induced by isoprene has been assessed by Velikova and Loreto (2005), during heat bursts but there is little information on the ability of endogenous isoprene to confer thermotolerance under naturally elevated temperature, on the interaction between isoprene-induced thermotolerance and light stress, and on the persistence of this protection in leaves recovering at lower temperatures. Moderately high temperature treatment (38°C for 1.5 hrs) reduced photosynthesis, stomatal conductance, and photochemical efficiency of photosystem II in isoprene-emitting, but to a significantly lower extent than in isoprene-inhibited *Phragmites australis* leaves. Isoprene inhibition and high temperature independently, as well as together, induced lipid peroxidation, increased level of H_2O_2, and increased catalase and peroxidase activities. However, leaves in which isoprene emission was previously inhibited developed stronger oxidative stress under high temperature with respect to isoprene-emitting leaves. The heaviest photosynthetic stress was observed in isoprene-inhibited leaves exposed to the brightest illumination (1500 μmol m^2 s^{-1}) and, in general, there was also a clear additive effect of light excess on the formation of reactive oxygen species, antioxidant enzymes, and membrane damage. The increased thermotolerance capability of isoprene-emitting leaves may be due to isoprene ability to stabilize membranes or to scavenge reactive oxygen species. Irrespective of the mechanism by which isoprene reduces thermal stress, isoprene-emitting leaves are able to quickly recover after the stress. This may be an important feature for plants coping with frequent and transient temperature changes in nature.

Methionine biosynthesis has taken different evolutionary pathways in bacteria, fungi and plants. To gain insight into these differences and to search for new ways of manipulating methionine biosynthesis in plants, the yeast (*Saccharomyces cerevisiae*) Met2 gene and the bacteria (*Leptospira meyeri)* MetX gene, both encoding homoserine O-acetyltransferase, were expressed in tobacco plants by Gamrasni *et al.* (2005). They found protein aggregates in extracts of these transgenic plants, whose levels were much higher in plants grown at 35°C than at 25°C. It appears that the yeast and the

bacterial proteins are heat labile and tend to change their intracellular conformation. These conformational changes of the transgenic proteins were more prominent at high temperature and most probably triggered aggregation of the yeast and the bacterial proteins. Moreover, plants expressing the yeast gene that grew at 35°C over-accumulated stress-associated metabolites, such as phenolic compounds, including tannins, as well as the amino acid arginine. In addition, the transgenic plants expressing high levels of the foreign genes showed growth retardation, which further suggests that, these plants suffer from internal stress. The changes in protein conformation and the consequent triggering of stress response may account for the ability of these transgenic plants to tolerate more extreme heat stress (60°C) than the wild-type plants.

A primary economic concern of sod producers is loss of sod quality during the transportation and storage phases of a sale. Previous research and field experience indicate that soil and plant respiration rates, and thus the rate of pallet heating, may be reduced by harvesting in the morning, lowering mowing heights and removing clippings, and minimizing tissue nitrogen and soil moisture before harvest. However, even when proper cultural guidelines are followed, excessive sod heating and tissue damage often occurs. Various pre-and post-harvest chemical treatments aimed at protecting leaf tissue integrity during and after supraoptimal heating have shown promise for increasing transplant success. One of these compounds is the natural plant growth regulator salicylic acid (SA). This study was conducted by Ervin *et al.* (2005) to investigate the influence of pre-harvest foliar application of SA on transplant injury and root strength of tall fescue (TF; *Festuca arundinacea* Schreb.) and Kentucky bluegrass (KBG; *Poa pratensis* L.) sod following supraoptimal heating. Salicylic acid was applied at 0.5 kg ha^{-1} to the turfgrass 10 days before harvest and canopy photochemical efficiency was measured 1 day before harvest. Harvested and rolled sod was subjected to high temperature stress (38–40°C for 72 or 96 hrs), transplanted into the field, and injury and root strength were determined. Application of SA enhanced the pre-harvest canopy photochemical efficiency of KBG and TF sod in both years. Averaged over years and heat duration, SA increased canopy photochemical efficiency by 12% for KBG and 14% for TF. Salicylic acid reduced visual injury and enhanced post-harvest root strength in both

years. Averaged over years and heat duration, SA increased transplant root strength by 26% for KBG and 9% for TF. These data suggest that pre-harvest foliar SA application may improve shelf life and transplant success of supraoptimally heated cool-season sod.

Lakindale *et al.*,2005 investigated the importance of different processes to heat stress tolerance in 45 *Arabidopsis* (*Arabidopsis thaliana*) mutants and one transgenic line. Plants tested were defective in signaling pathways (abscisic acid, salicylic acid, ethylene, and oxidative burst signaling) and in reactive oxygen metabolism (ascorbic acid or glutathione production, catalase) or had previously been found to have temperature-related phenotypes (e.g. fatty acid desaturase mutants, *uvh*6). Mutants were assessed for thermotolerance defects in seed germination, hypocotyls elongation, root growth, and seedling survival. Fifteen mutants showed significant phenotypes. Abscisic acid (ABA) signaling mutants (*abi 1 and abi 2*) and the UV-sensitive mutant, *uvh6*, showed the strongest defects in acquired thermotolerance of root growth and seedling survival. Ethylene signaling mutants (*ein2 and etr1*) and reactive oxygen metabolism mutants (*vtc1, vtc2, npq1, and cad2*) were more defective in basal than acquired thermotolerance, especially under high light. All mutants accumulated wild-type levels of heat shock protein 101 and small heat shock proteins. Their data therefore, indicate that, separate from heat shock protein induction, ABA, active oxygen species, and salicylic acid pathways are involved in acquired thermotolerance and that UVH6 plays a significant role in temperature responses in addition to its role in UV stress.

The relationship between the accumulation in endogenous free salicylic acid (SA) induced by heat acclimation (37°C) and the activity of PIP2-phospholipase C (PIP2-PLC; EC 3.1.4.3) in the plasma membrane fraction was investigated in pea (*Pisum sativum* L.) leaves by Liu *et al.* (2006). Heat acclimation induced an abrupt elevation of free SA preceding the activation of PLC toward PIP2. Immunoblotting indicated a molecular mass with 66.5 kDa PLC plays key role in the development of thermotolerance in pea leaves. In addition, some characterizations of PLC toward PIP2 isolated from pea leaves with two-phase purification containing calcium concentration, pH and a protein concentration were also studied. Neomycin sulfate, a well-known PIP2-PLC inhibitor, was employed to access the involvement of PIP2-

PLC in the acquisition of heat acclimation induced-thermotolerance. They were able to identify a PIP2-PLC, which was similar to a conventional PIP2-PLC in higher plants was identified from pea leaves suggesting that PIP2-PLC was involved in the signal pathway that leads to the acquisition of heat acclimation induced-thermotolerance. On the basis of these results, it was concluded that the free SA may function as the upstream event in the stimulation of PIP2-PLC in response to heat acclimation treatment.

Plants and animals share similar mechanisms in the heat shock (HS) response, such as synthesis of the conserved HS proteins (Hsps). However, because plants are confined to a growing environment, in general they require unique features to cope with heat stress. Charng *et al.* (2006) analyzed the function of a novel Hsp, heat-stress-associated 32-kD protein (Hsa32), which is highly conserved in land plants but absent in most other organisms. The gene responds to HS at the transcriptional level in moss (*Physcomitrella patens*), *Arabidopsis* (*Arabidopsis thaliana*), and rice (*Oryza sativa*). Like other Hsps, Hsa32 protein accumulates greatly in *Arabidopsis* seedlings after HS treatment. Disruption of Hsa32 by T-DNA insertion does not affect growth and development under normal conditions. However, the acquired thermotolerance in the knockout line was compromised following a long recovery period (>24 hrs) after acclimation HS treatment, when a severe HS challenge killed the mutant but not the wild-type plants, but no significant difference was observed if they were challenged within a short recovery period. Quantitative hypocotyl elongation assay also revealed that thermotolerance decayed faster in the absence of Hsa32 after a long recovery. Similar results were obtained in *Arabidopsis* transgenic plants with Hsa32 expression suppressed by RNA interference. Microarray analysis of the knockout mutant indicates that only the expression of Hsa32 was significantly altered in HS response. Taken together, the results suggest that Hsa32 is required not for induction but rather maintenance of acquired thermotolerance, a feature that could be important to plants.

The heat shock protein ClpB is a member of the Clp family and functions as molecular chaperones. ClpB is related to the acquired thermotolerance in organisms. A cDNA of 3144 bp was screened out of a tomato cDNA library. The polypeptide deduced from the longest ORF contains 980 amino acid residues, and was classified

into HSP100/ClpB family based on the result of molecular phylogenesis analysis. Thus it was named as LeHSP110/ClpB according to its calculated molecular weight. LeHSP110/ClpB was characteristic of heat-inducibility but no constitutive expression, and was demonstrated to locate in chloroplastic stroma. An antisense cDNA fragment of LeHsp110/ClpB under the control of CaMV 35S promoter was introduced into tomato by *Agrobacterium tumefactions*-mediated method. At high temperature, the mRNA levels of LeHsp110/ClpB in antisense transgenic plants were lower than those in control plants. The PS II of transgenic plants is more sensitive to high temperature than that of control plants according to data of Fv/Fm. These results of Gong and Bao (2006) clearly showed that HSP110/ClpB plays an important role in thermotolerance of high plants.

Chen *et al.* (2006) revealed an important role of galactolipids in thermotolerance To identify the various mechanisms that plants have evolved to cope with high temperature stress, they isolated a series of *Arabidopsis* mutants that are defective in the acquisition of thermotolerance after an exposure to 38°C, a treatment that induces acquired thermotolerance in wild-type plants. One of these mutants, *atts 02*, was not only defective in acquiring thermotolerance after the treatment, but also displayed a reduced level of basal thermotolerance in a 30°C growth assay. The affected gene in *atts 02* was identified by positional cloning and encodes digalactosyldiacylglycerol synthase 1 (DGD1) (the *atts02* mutant was, at that point, renamed *dgd1-2*). An additional dgd1 allele, *dgd1-3*, was identified in two other mutant lines displaying altered acquired thermotolerance, *atts100* and *atts104*. Expression patterns of several heat shock proteins (HSPs) in heat-treated *dgd1-2* homozygous plants were similar to those from identically treated wild-type plants, suggesting that the thermosensitivity in the *dgd1-2* mutant was not caused by a defect in HSP induction. Lipid analysis of wild type and mutant plants indicated a close correlation between the ability to acquire thermotolerance and the increases in digalactosyldiacylglycerol (DGDG) level and in the ratio of DGDG to monogalactosyldiacylglycerol (MGDG). These results suggest that the DGDG level and/or the ratio of DGDG to MGDG may play an important role in basal as well as acquired thermotolerance in *Arabidopsis*.