Literature Review

Normal growth and development of plants is greatly dependent on the capacity to overcome environmental stresses. Environmental stress conditions like high salinity, drought, high incident light and low or high temperature cause major crop losses worldwide. A common denominator in all these adverse conditions is the production of reactive oxygen species (ROS) within different cellular compartments of the plant cell. Since plants are immobile and remain rooted to the soil, they have developed robust mechanisms including enzymatic or non-enzymatic scavenging pathways to counter the deleterious effects of ROS production. Temperature stress has turned out to be the most important abiotic stress to which a plant is exposed. Temperature stresses experienced by plants can be classified into three types: those occurring at (*a*) temperatures below freezing, (*b*) low temperatures above freezing, and (*c*) high temperatures (Iba, 2002). Because of the overall impact of abiotic stresses in general and temperature stress in particular, on plant metabolism, it is an area where current research is focussed. Hence there are a number of general reviews on oxidative stress in plants and the response of plants in general to temperature stress (Yoshida ,1999; Apel and Hirt, 2004; Jithesh *et al.*;2006; Ahmad *et al.* 2008; Badea and Basu, 2009). The review of literature presented below gives an insight into work done in the line of the investigation.

Low temperature stress

Several reports are available on the response of different varieties of maize to chilling. Marc *et. al.* (1995) examined the response of antioxidants to acclimation and chilling in various tissues of dark-grown maize *(Zea mays)* seedlings in relation to chilling tolerance and protection from chilling induced oxidative stress. They reported that chilling caused an accumulation of H_2O_2 in both the coleoptile + leaf and the mesocotyl (but not roots), and acclimation prevented this accumulation. None of the antioxidant enzymes were significantly affected by acclimation or chilling in the coleoptile + leaf or root. However, elevated levels of glutathione in acclimated seedlings may contribute to an enhanced ability to scavenge H_2O_2 in the coleoptile + leaf. In the mesocotyl (visibly most susceptible to chilling), catalase3 was elevated in acclimated seedlings and may represent the first line of defence from mitochondria-generated H_2O_2. Nine of the most prominent peroxidase isozymes were induced by acclimation, two of which were located in the cell wall, suggesting a role in lignifications. Lignin content was elevated in mesocotyls of acclimated seedlings,

probably improving the mechanical strength of the mesocotyl. One cytosolic glutathione reductase isozyme was greatly decreased in acclimated seedlings, whereas two others were elevated, possibly resulting in improved effectiveness of the enzyme at low temperature. When taken together, these responses to acclimation illustrate the potential ways in which chilling tolerance may be improved in pre-emergent maize seedlings. In another study, antioxidant enzyme activities were determined at the first, third and fifth leaf stages of four inbred lines of maize *(Zea mays* L.) exhibiting differential sensitivity to chilling. Plants were exposed to a photoperiod of 16:8 L: D for one of three treatments: (a) control (25 °C), (b) control treatment plus an exposure to a short-term chilling shock (11 °C 1 d prior to harvesting), and (c) long-term (11 C constant) chilling exposure. Catalase (CAT; EC 1.11.1.6), ascorbate peroxidase (ASPX; EC 1.11.1.11), superoxide dismutase (SOD; EC 1.15.1.1), glutathione reductase (GR; EC 1.6.4.2), and monodehydroascorbate reductase (MDHAR; EC 1.6.5.4) activities were assessed. Reducing and non-reducing sugars and starch concentrations were determined general metabolic indicators of stress. Reduced activities of CAT, ASPX, and MDHAR may contribute to limiting chilling tolerance at the early stages of development in maize. Changes in levels of sugar and starch indicated a more rapid disruption of carbohydrate utilization in comparison to photosynthetic rates in the chilling-sensitive line under short-term chilling shocks and suggested a greater degree of acclimation in the tolerant lines over longer periods of chilling (Hodges,1997). The potential role of antioxidant enzymes in protecting maize (*Zea mays* L.) seedlings from chilling injury was also examined by analyzing enzyme activities and isozymes profiles of chilling-susceptible (CO 316) and chilling-tolerant (CO 328) inbreds(Pinhero *et.al. 1997*).. Leaf superoxide dismutase (SOD) activity in CO 316 was nearly one-half that of CO 328, in which the high activity was maintained during the chilling and post chilling periods. Activity of glutathione reductase (GR) was much higher in roots than in leaves. CO 328 also possessed a new GR isozymes that was absent in roots of CO 316. Ascorbate peroxidase (APX) activity was considerably lower in leaves of CO 328 than in CO 316, and nearly similar in roots. Paclobutrazol treatment of CO 316 induced several changes in the antioxidant enzyme profiles and enhanced their activities, especially those of SOD and APX, along with the induction of chilling tolerance. These results suggest that increased activities of SOD in leaves and GR in roots of CO 328, as well as SOD and

APX in leaves and roots of paclobutrazol-treated CO 316, contribute to their enhanced chilling tolerance

In a further study, the mechanisms of chilling acclimation and the role of antioxidant enzymes, catalase in particular, in inducing chilling tolerance in pre-emergent maize (*Zea mays* L.) seedlings have been investigated by Prasad (1997). Seedlings were acclimated to chilling stress in two different ways. Three-day-old seedlings did not survive 7 d of 4°C stress unless acclimated by exposure to either 14°C for 1 d or 4°C for 1 d followed by recovery at 27°C for 1 d. Although no changes in superoxide dismutase and ascorbate peroxidase activities were observed, both kinds of acclimated seedlings had higher catalase (CAT), glutathione reductase, and guaiacol peroxidase activities compared with nonacclimated seedlings during low-temperature stress and recovery conditions. To study the role of CAT in chilling tolerance, aminotriazole (AT) was used as a tool to artificially inhibit CAT activity and to initiate oxidative stress in the seedlings. Treatment of acclimating seedlings with 3 mM AT for 18 h abolished the acclimation phenomenon. AT treatment was found to be specific to CAT inhibition, because the total activities or isozymes profiles of the other investigated antioxidant enzymes were not altered in AT-treated seedlings. Protein carbonyl content, an indication of oxidative damage, was increased 2-fold in nonacclimated and AT-treated acclimated seedlings. These results collectively indicate that acclimation to prolonged chilling stress can be achieved by briefly pre-exposing the seedlings to 4°C chilling stress and that acclimation-induced (oxidative stress-induced) CAT seems to play a major role, probably along with other antioxidant enzymes, in inducing chilling tolerance in pre-emergent maize seedlings. In another study,tolerance to low temperature and parquet-mediated oxidative stress was investigated by Lannelli et al. (1999) in two *Zea mays* genotypes, VA 36 and A619, grown at 25/22 °Cand 16/14 °Cfor 50 d after germination. VA36, the tolerant genotype, showed an enhanced resistance to parquet as compared to A619, the sensitive genotype, when grown at low temperature. In VA36, superoxide dismutase and ascorbate peroxidase activities increased during growth at both 25/22 °Cor 16/14 °C. In A619, superoxide dismutase activity was similar in plants grown at both 16/14 °C or 25/22 °C. Ascorbate peroxidase activity was always significantly lower in plants grown at low temperature than in plants grown at 25/22 °C. The total ascorbate peroxidase activity was correlated with the cytosolic ascorbate peroxidise protein

content in all but A 619 plants grown at low temperature for 25 d. The isozymes pattern of SOD showed a higher abundance of Mn SOD in VA36 than in A619 and of Fe SOD in A619 compared to VA 36. Growth at low temperature enhanced resistance to parquet infiltration more in VA 36 than in A619. SOD and APX activities were generally higher and more stable with the increase of parquet concentration in VA 36 than in A619. Damage indicated by Fv/Fm and ion leakage after parquet infiltration were generally higher in plants grown at 25/22 'C than at 16/14 C and higher in plants grown at 25/22 'C than at 16/14 C and higher in A 619 than in VA36. However, no causual link was proved between the extent of damage and the increase of SOD and APX activities alone. It was suggested that tolerance to oxidative stress requires an interacted enhancement of the antioxidant system. The distribution of antioxidants between bundle sheath and mesophyll cells of maize leaves was analysed by Pastori (2000) in plants grown at 20 °C, 18 °C and 15 °C. The purity of the isolated bundle sheath and mesophyll fractions was determined using compartment-specific marker enzymes. In plants grown at 15 °C, ascorbate peroxidase, CuZn-superoxide dismutase (CuZn-SOD) and monodehydroascorbate reductase activities were increased in the bundle sheath cells, and glutathione reductase, dehydroascorbate reductase and monodehydroascorbate reductase activities were enhanced in the mesophyll cells. SOD was absent from the mesophyll of plants grown at 20 °C but an Fe-SOD activity was found in the mesophyll of plants grown at 15 °C. Foliar Mn-SOD activities were decreased at 15 °C compared to 20 °C. Catalase was undetectable in the mesophyll extracts of plants grown at 15 °C. Ascorbate and glutathione contents were considerably higher in the mesophyll than the bundle sheath fractions of plants grown at 20 °C. The ratios of reduced to oxidized forms of these antioxidants were significantly decreased in the bundle sheath, but increased in the mesophyll of leaves grown at 15 °C. Foliar H_2O_2 accumulated at 15 °C compared to 20 °C. Most of the foliar H_2O_2 was localized in the mesophyll tissues at all growth temperatures. The differential distribution of antioxidants between leaf bundle sheath and mesophyll tissues, observed at 20 °C, was even more pronounced when plants are grown at 15 °C and according to the authors, these may contribute to the extreme sensitivity of maize to low temperatures.

Chilling shoot cultures from *Oryza sativa* L. cv. Taipei 309, to 4°C was reported to lead to conditions of oxidative stress. Tissue H_2O_2 was observed to increase more than fourfold by 8 d of chilling, and levels of reduced glutathione, which normally rise in growing shoot cultures at 25°C, were considerably repressed in chilled cultures. Whilst the activity of ascorbate peroxidase in chilled shoots remained similar to the activities in control cultures at 25°C, the most notable effects of chilling to 4°C were the very significant loss of catalase and glutathione reductase activity. Although prior exposure of shoot cultures to abscisic acid (ABA) at 25°C increased levels of catalase activity, such increased levels were not sustained when the pre-treated cultures were placed at 4°C. More over such pre-treatment with ABA did not increase the subsequent ability of shoot cultures to grow at 4°C (Fadzillah *et.al.* 1996).

French bean (*Phaseolus vulgaris* L. cv. Contender) plants at five developmental stages(4,8,16and 20 d after sowing) were exposed to one of three treatments:1-25 °C(control), 2- expose to chilling at 10 °C only for 2d prior to sampling , and 3- long term exposure to chilling at 10 °C. Short and long-term chilling decreased plant growth . Higher concentrations of ascorbate and glutathione were found in the chilling –treated plants throughout the different period of growth in comparison with those in the control plants. The activities of superoxide dismutase, ascorbate peroxidase, and glutathione reductase increased in the chilling-treated seedlings while activities of catalase and peroxidase and of β-carotene content decreased in young chilling –treated plants and slightly increased in older ones (Saht, 1998)

Exposure of coffee to low temperatures caused growth inhibition, changes in metabolic rates, and membrane alterations (Queiroz *et al.*, 1998).Root tissue exposed to 10°C evolved significantly lower rates of metabolic heat compared with controls grown at 25°C, and the values were closely associated with the observed root growth inhibition. Electron paramegnetic resonance spectra of intact tissues showed that the spin probe 5-deoxylstearic acid was capable to intercalate within the cellular membrane lipids. Indeed , at the depth of the 5^{th} carbon atomes of the alkyl chains, the nitroxide readical detected more rigid membranes in seedlings exposed to 10°C compared with 25°C treated samples. Ascorbate peroxidase and catalase activities did not show appreciable changes under chilling conditions, while guaiacol peroxidase activity increased 55% compared to the control. On the other hand, glutathione reductase activity decreased, in parallel to a significant decline in the capacity to

reduce tri phenyl-tetrazolium. Results showed a marked correlation between lipid peroxidation and root tissue damage, which seemed to be associated with increased membrane rigidity.

The effect of heat-shock (42°C or chilling –shock(5 °C) on growth and some relevant metabolic changes of broad bean (*Vicia faba* L.)were studied. Both heat and chilling stress induced a reduction in growth rate, membrane stability and content of photosynthetic pigments(chlorophyll a, b and carotenoids).K^+ efflux and UV absorbance increased at increasing or decreasing temperature. Considerable variations in the content of cellwall components (pectin, cellulose, hemicelluloses and lignin), cellwall associated proteins ,soluble sugars, starch , total lipids, glycolipids, phospholipids and sterols were induced by extreme temperature (Hamada, 2001).

Aerial parts of the chilling-sensitive young sal seedlings showed over production of reactive oxygen species (ROS) and thiobarbituric acid reactive substances (TBARS) in response to constant chilling exposure during November to March (9-14.1°C) in field conditions. Almost 4-6 fold increase in ROS was observed in aerial parts of chilling exposed seedlings than the control seedlings (maintained in greenhouse). Increased formation of ROS was found to be closely associated with the rise in TBARS in leaf (5.8 fold) and shoot (4.8 fold) tissues. On the contrary the leaf and shoot of control seedling and root of both control and chilling exposed seedlings exhibited relatively very low levels of superoxide and TBARS. The chilling exposed seedlings also showed striking weakening in the free radical processing enzymes systems. The low temperatures during November to March resulted in reduced activities of superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (POX) and ascorbate peroxidase (APX) almost by 49,26,7 and 78% in leaves and 65,46,9 and 85% in shoots respectively compared to leaves and shoots of control seedlings. Their results indicated that , substantially higher rates of liberation of superoxide and TBARS along with drastic failure of antioxidant enzyme system in chilling sensitive sal seedlings leads to oxidative bursts terminating into irreversible injury in leaves and shoots of these seedlings. (Keshavkant and Naithani, 2001)

Wheat (*Triticum aestivum* L., cv. Beloslava) seeds were imbibed for 24 h in water solutions containing 0, 25 and 50 mg. of paclobutrazol by Berova *et al.*(2002). The seedlings were grown as a substrate culture under controlled climatic conditions. Seven-day-old plants were exposed to low temperature stress by placing them in a

cold room at a temperature of 2±1°C for 10 days – the first phase of hardening, -4±1°C for 3 days – the second phase of hardening and freezing, -10±1°C for 1 day – the third phase of hardening and freezing. After exposure to stress, the seedlings were returned to a climatic chamber with controlled climatic conditions. Under stress conditions the growth rates of the PBZ-treated seedlings measured by height, fresh and dry weights were greater than the control. Low temperature stress (LTS) induced lipid peroxidation and increased peroxidase activity. It was also found that LTS decreased the chlorophyll and carotenoid levels. A decrease in fluorescence ratio (Fv/Fm) indicated lower photosynthetic efficiency. These deteriorative symptoms in the control seedlings were ameliorated by the PBZ treatment. Based on the results of triazole studies, authors presumed that the stress protection caused by PBZ probably contributes to some extent to the enhanced activity of the free-radical scavenging systems.

Chilling whole rice seedlings at 5°C significantly increased the time needed to recover linear growth and reduced the subsequent linear rate of radical growth. Subjecting non chilled seedlings to a 45°C heat shock for up to 20 min did not alter subsequent growth, where as a 3 min heat shock was optimal in reducing growth inhibition caused by 2 days of chilling. The activity of five antioxidant enzymes (superoxide dismutase (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 (GPX; EC 1.11.1.7), and DPPH(1,1- diphenyl -2-picrylhydrazyl)- radical scavenging activity were measured in heat –shocked and / or chilled radicles. Heat shock slightly increased the activity of CAT, APX, and GR and suppressed the increase of GR and GPX activity during recovery for, chilling. Increased CAT , APX, GR and DPPH – radical scavenging activity and protection of CAT activity during chilling appear to be correlated with heat shock-induced chilling tolerance (Kang and Saltveit, 2002).

The changes in antioxidant enzymes and polyamines were investigated in the leaves of watermelon plants in response to a short exposure to chilling temperatures by Kwon *et al.* (2002). Chilling temperatures not only reduced biomass but also caused an overall increase in antioxidant enzyme activities and polyamines in the leaves of watermelon. The antioxidant enzyme activities after chilling treatment were higher than those plants grown at 30 °C. The catalase (CAT) and peroxidase (POD)

activities in leaves were significantly increased, reaching a maximum at 2 days after chilling treatment, while they decreased slightly after 3 days. The means of antioxidant enzyme activities were higher in the leaves than in shoot apexes. In the native-gel assay of antioxidant enzymes, the low temperature treatment resulted in quantitative changes in CAT and superoxide dismutase (SOD) isozymes profiles, but they did not find any qualitative changes in the isozymes which were induced by chilling. In contrast, low temperatures induced the synthesis of 4 new POD band isozymes in watermelon leaves. Similarly increased polyamine contents of watermelon leaves were found to be associated with antioxidant enzyme activities under the chilling conditions. Exposure to low temperatures caused an increase in spermidine (SPD) and spermine (SPM), but not in putrescine (PUT) levels. One of the possible mechanisms of chilling resistance was an observed increase in polyamines with the marked increases in antioxidant enzyme activities. The results also indicate that SPD and SPM levels in watermelon leaves could have a protective role against chilling-induced active oxygen species.

The antioxidant effects, the levels of total phenol and the total phenol contents of volatile oils and plant extracts were determined in eight various Rosemary (*Rosmarinus officinalis* L.) clones by Bányai *et al.*(2003). Antioxidant activities and the total phenol contents were measured by spectophotometric method as well as the volatile oil content of the fresh plants with gas chromatograph. Their preliminary results clearly indicate that the antioxidant capacity of volatile oils and plant extracts closely related to the total phenol contents. Reason of the observed differences should be revealed by the determination of the quantity and quality of the individual volatile oil components.

The effect of elevated light treatment (25 °C, PPFD 360 μmol m^{-2} sec^{-1}) or chilling temperatures combined with elevated light (5 °C, PPFD 360 μmol m^{-2} sec^{-1}) on the activity of six antioxidant enzymes, guaiacol peroxidases, and glutathione peroxidase (GPx, EC 1.11.1.9) protein accumulation were studied in tobacco *Nicotiana tabacum* cv. Petit Havana SR1. Both treatments caused no photo oxidative damage, but chilling caused a transient wilting. The light treatment increased the activities of ascorbate peroxidase (APx, EC 1.11.1.11) and guaiacol peroxidases while catalase (EC 1.11.1.6), superoxide dismutase (SOD, EC 1.15.1.1), monodehydroascorbate reductase (MDHAR, EC 1.6.5.4), dehydroascorbate reductase

(DHAR, EC 1.8.5.1), and glutathione reductase (EC 1.6.4.2) were unchanged. In contrast, chilling treatment did not increase any of the antioxidant enzyme activities, but decreased catalase and to a lesser extent DHAR activities. Glutathione peroxidase protein levels increased sporadically under light treatment and constantly under chilling. Both chilling and light stress caused induction of glutathione synthesis and accumulation of oxidised glutathione, although the predominant part of the glutathione pool remained in the reduced form. Antioxidant enzymes from the chilling treated plants were measured at both 25 °C and 5 °C. Measurements at 5 °C revealed a 3-fold reduction in catalase activity, compared with that measured at 25 °C, indicating that the overall reduction in catalase after four days of chilling was approximately 10-fold. The overall reduction in activity for the other antioxidant enzymes after four days of chilling was 2-fold for GR and APx, 1.5-fold for MDHAR, 3.5-fold for DHAR. The activity of SOD was the same at 25 and 5 °C. These results indicated that catalase and DHAR were most strongly affected by the chilling treatment and may be the rate-limiting factor of the antioxidant system at low temperatures (Gechev *et al.*2003)

Funatsuki *et al.* (2003) investigated the isozyme profiles of antioxidant enzymes in soybean cultivars and lines with different seed productivity in cool climate conditions as a step towards understanding the physiological and genetical mechanisms underlying chilling tolerance in soybean. While no difference in superoxide dismutase, or catalase isozyme profiles was observed among the cultivars and lines tested, authors found polymorphism in the ascorbate peroxidase isozyme profile; there were two types, with or without a cytosolic isoform (APX1). The cultivars and lines lacking APX1 proved more tolerant to chilling temperatures, as evaluated by yielding ability. The genotype- dependent deficiency of APX1 was consistent in plants and tissues under various oxidative stress conditions including the exposure to low-temperatures. In addition, the genetic analysis of progeny derived from crossing between cultivars differing in the isozymes profile indicated that the APX1 deficiency is controlled by a single recessive gene (*apx1*), and is inherited independently of the genes that have previously been identified for their association with chilling tolerance. Molecular and linkage analyses suggested that the variant gene of the APX1-absent genotype coding for a cytosolic APX, which contained a single nucleotide substitution and a single nucleotide deletion in the coding region, is responsible for the genotype-dependent deficiency of APX1.

Rice (*Oryza sativa* L.) is a tropical crop, but is also grown in temperate regions in late spring to summer. Cold temperature damage is a common problem for early-planted rice in temperate countries. Physiological responses to chilling, including antioxidative enzyme activity, were investigated in rice to identify mechanisms of chilling tolerance. Plants were exposed to 15°C (cold-acclimated) or 25°C (nonacclimated) for 3 d, under 250 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR). All plants were then exposed to chilling temperature at 5°C for 3 d and allowed to recover at 25°C for 5 d. Leaf fresh weight, relative water content, lipid peroxidation, chlorophyll a fluorescence, and quantum yield showed that cold-acclimated leaves were less affected by chilling compared to nonacclimated leaves. Cold-acclimated leaves also recovered faster from chilling injury than nonacclimated leaves. Kuk *et al.*(2003) analyzed the isozymes profile and activity of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) in rice varieties. Significant induction of expression and activity of antioxidative enzymes CAT and APX in leaves and SOD, CAT, APX, and GR in roots were observed. They deduced that CAT and APX are most important for cold acclimation and chilling tolerance. Increased activity of antioxidants in roots was suggested to be more important for cold tolerance than increased activity in shoots. Chilling-sensitive rice plants can be made tolerant by cold acclimation.

Using Scots pine (*Pinus sylvestris* L.) as a model plant the environmental signals inducing frost hardening and dehardening, respectively were investigated. Over 2 years the changes in frost resistance of Scots pine needles were recorded together with the annual courses of day-length and ambient temperature. Both act as environmental signals for frost hardening and dehardening. Climate chamber experiments showed that short day-length as a signal triggering frost hardening could be replaced by irradiation with far red light, while red light inhibited hardening. The involvement of phytochrome as a signal receptor could be corroborated by respective night-break experiments. More rapid frost hardening than by short day or far red treatment was achieved by applying a short period (6 h) of mild frost which did not exceed the plant's cold resistance. Both types of signals were independently effective but the rates of frost hardening were not additive. The maximal rate of hardening was – 0×93°C per day and frost tolerance of < – 72°C was achieved. For dehardening, temperature was an even more effective signal than day-length. (Beck *et al.* 2004)

Lee *et al.* (2004) studied changes in biochemical and physiological status, level of oxidative damage, and antioxidant enzyme activities in detached leaves of cucumber plants *(Cucumis sativus* L. cv. Pyunggangnaebyungsamchuk) that were exposed to a low temperature of 4^0C. Chlorophyll fluorescence (Fv/Fm) declined during the chilling treatment, but was slowly restored after the tissues were returned to 25^0C. Likewise, the fluorescence quenching coefficient and relative water content decreased during the stress period, but then increased during recovery. In contrast, they detected a significant rise in protein and hydrogen peroxide contents in the chilled leaves, as well as higher activities for superoxide dismutase, ascorbate peroxidase, peroxidase, and glutathione reductase. However, the level of catalase decreased not only during chilling but also after 24 h of recovery. These results indicate that exposure to low temperatures acts as an oxidative stress. Moreover, they proposed that a regulating mechanism exists in the detached cucumber leaves and contains an antioxidant defense system that induces active oxygen species, thereby alleviating the effects of chilling stress within 12 h. In the course of the year perennial plants of the temperate climate zones undergo frost hardening in autumn and dehardening in spring.

According to Scott *et al.*(2004), the growth of *Arabidopsis* plants in chilling conditions could be related to their levels of salicylic acid (SA). Plants with the SA hydroxylase *NahG* transgene grew at similar rates to Col-0 wild types at 23°C, and growth of both genotypes was slowed by transfer to 5°C. However, at 5°C, *NahG* plants displayed relative growth rates about one-third greater than Col-0, so that by 2 months *NahG* plants were typically 2.7-fold larger. This resulted primarily from greater cell expansion in *NahG* rosette leaves. Specific leaf areas and leaf area ratios remained similar in both genotypes. Net assimilation rates were similar in both genotypes at 23°C, but higher in *NahG* at 5°C. Chlorophyll fluorescence measurements revealed no PSII photodamage in chilled leaves of either genotype. Col-0 shoots at 5°C accumulated SA, particularly in glucosylated form. SA in *NahG* shoots showed similar tendencies at 5°C, but at greatly depleted levels. Catechol was not detected as a metabolite of the *NahG* transgene product. Scott et al.(2004) also examined growth and SA levels in SA signalling and metabolism mutants at 5°C. The partially SA-insensitive *npr1* mutant displayed growth intermediate between *NahG* and Col-0, while the SA-deficient *eds5* mutant behaved like *NahG*. In contrast, the *cpr1* mutant at 5°C accumulated very high levels of SA and its growth was much

more inhibited than wild type. At both temperatures, *cpr1* was the only SA-responsive genotype in which oxidative damage (measured as thiobarbituric acid-reactive substances) was significantly different from wild type.

Mung bean (*Phaseolus radiatus* Linn.) and garden pea (*Pisum satium* Linn.), which were stressed 4 days under a low temperature of 10^0C, were used as materials by Chen *et al.* (2005) to study the cold tolerance of plant with different resistance. On the 2nd and 3rd day under 10^0C stress, both the malondialdehyde (MDA) content and the superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) activities increased significantly in hypocotylar cells of mung bean, so did SOD activity in garden pea, but other physiological indexes in garden pea were not different from the non-treatment groups . In hypocotylar cells of mung bean, SOD activity was always maintained at the highest level in a period of time, as also POD activity. Ultra structural results after stress indicated as follows: (1) Plastids in hypocotylar cells of mung bean accumulated much starch, whereas , the form of plastids in hypocotylar cells of garden pea changed markedly to become dumb-bell-shaped, round or irregular, with the last one being the most common form ; (2) In both mung bean and garden pea, central vacuole was divided into small vacuoles, and the number of mitochondria increased and became aggregated. Judging from the activities of protective enzymes and ultra structures, 10 ^{0}C low temperature caused non-lethal, temporary injuries to hypocotyls ultra structures in mung bean, but no visible injury at all, and even improved its cold tolerance to a certain degree in garden pea.

The responses of antioxidative system of rice to chilling were investigated in a tolerant cultivar, Xiangnuo-1, and a susceptible cultivar, IR-50. The electrolyte leakage and malondialdehyde content of Xiangnuo-1 were little affected by chilling treatment but those of IR-50 increased. Activities of suoperoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase, and ascorbic acid content of Xiangnuo-1 were remained high, while those of IR-50 decreased under chilling. The results indicated that higher activities of defense enzymes and higher content of antioxidant under stress were associated with tolerance to chilling.(Huang and Guo, 2005).

According to Mahan and Mauget (2005), early season temperature stress adversely affects the growth and development of cotton (*Gossypium hirsutum* L.) seedlings.

Oxidative damage resulting from temperature extremes was thought to be a cause of diminished seedling performance. Cotton (cv Fibermax 958) was 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. Authors concluded that the indicators of antioxidant metabolism varied in the seedlings but not in response to temperature variation. They proposed that antioxidant metabolism in the seedlings was sufficient to mitigate oxidative damage with only minor alterations.

Changes of activity antioxidant enzymes and of levels of isoflavonoids were studied in the roots and hypocotyls of the etiolated soybean (*Glycine max* (L.) Merr. var. Essor) seedlings, submitted to cold. Prolonged exposure to 1 °C inhibited hypocotyls and root elongation and limited their growth after seedlings were transferred to 25 °C. Roots were more sensitive to chilling than hypocotyls. At 1 °C a gradual increase in MDA concentration in roots but not in hypocotyls was observed. An increase in catalase (CAT, EC 1.11.1.6) and superoxide dismutase (SOD, EC 1.15.1.1) activity in hypocotyls was observed both at 1 °C and after transfer of plants to 25 °C. In roots, CAT activity increased after 4 days of chilling, while SOD activity only after rewarming. l-Phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) activity decreased in roots of chilled seedlings, but did not change in hypocotyls until activity increased after transfer to 25 °C. The content of genistein and daidzein increased after 24 h of treatment by low temperature and then decreased with prolonged chilling in hypocotyls and remained high in roots (Posmyk *et al.*2005).

Under circumstances where electron transport is restricted, low temperature condition oxidative stress may occur even at optimal or low-light intensities. Short term-effects of light intensities(20 or 100 μmol $m^{-2}sec^{-1}$), on the levels of 6 enzymatic, two non-enzymatic antioxidants, chla, chl b, total carotenoid and β-carotene , on the antioxidant response of *Dunaliella salina* under cold temperature (13°C)were quantified after 24h stress treatments. The activity of superoxide dismutase (SOD)

increased, under 13 °C/100 μmol $m^{-2}sec^{-1}$, whereas ascorbate peroxidase(APX), dehydroascorbate reductase(DHAR), superoxidase dismutase and pyrogallol peroxidase activities were induced under 13 °C/20 μmol $m^{-2}sec^{-1}$. The cells exhibited an increase in reduced ascorbate and reduced glutathione (GSH) coincident with a marked increase in oxidized glutathione (GSSG), at 13 °C/20 μmol $m^{-2}sec^{-1}$. There were no marked changes in ascorbate or glutathione pools at 13°C/20 μmol $m^{-2}sec^{-1}$, which are similar to those at 28 °C/100 μmol $m^{-2}sec^{-1}$ .Chlorophyll and carotenoids reduction were also observed under chilling treatements, which were more reduced by the higher light intensity (13 °C/100μmol $m^{-2}sec^{-1}$). The results of present study indicated various antioxidants responds to different combinations of chilling and low light intensities in *D. salina*. These responses are very sensitive to small increase in the light intensity (Haghjou *et al.* 2006)

Einset *et al.*(2007) identified genes up regulated by glycinebetaine that are involved in reactive oxygen species (ROS) metabolism and membrane trafficking processes. Direct evidence was provided for a role for a membrane trafficking protein (RabA4c) in GB's effect on ROS accumulation during chilling. Chilling elevates ROS levels and results in inhibited root growth upon transfer of plants back to normal growing conditions. During the 2–4 day recovery period, ROS levels decline in root tips and in leaves. If ROS accumulation in response to chilling is blocked by pre-treatment with GB, optimal root growth begins as soon as plants are transferred back to normal growing conditions without a recovery period, suggesting that chilling stress involves a ROS signalling pathway.

Jain *et al.* (2007) conducted a laboratory experiment to study the effect of low temperature stress on stubble bud sprouting and associated biochemical changes in sugarcane (*Saccharum* spp. Hybrid). At 25°C stubble bud sprouting was about 80%, whereas at 15 and 6 °C , it was 56% and 23% , respectively. In stubble buds the levels of reducing sugars and acid invertase were low, while IAA, total phenol and proline contents were high at low temperatures, as compared to normal temperature (25 °C). Similarly, the specific activities of antioxidant enzymes, viz., catalase and peroxidase in stable buds were higher at low temperature than at normal temperature. The results indicate that poor sprouting of stubble buds at low temperatures appears to be due to a reduced availability of reducing sugars concomitant with a lower activity of acid invertase. An increased level of IAA together with toxicity build –up in situ due to an

accumulation of total phenols may be responsible for the maintenance of dormancy in stubble buds at low temperatures. On the other hand, higher activities of catalase and peroxidase enzymes may protect stubble buds from an oxidative damage, while proline accumulation to act as an osmoprotectant under low temperature stress.

The effect of low temperatures on polyamines, jasmonates, abscisic acid (ABA), and antioxidant activities was investigated by Yoshikawa *et al.* (2007) in apple fruit lets. Although endogenous ABA concentrations were not significantly different between untreated control fruit kept at −2°C and those kept at 20°C, endogenous jasmonic acid (JA), putrescine, and spermidin concentrations at −2°C were generally higher than those at 20°C. Endogenous ABA concentrations increased in *n*-propyl dihydrojasmonate (PDJ)—or spermine-treated fruit in comparison to the untreated control at 20 and −2°C. The applications of PDJ or spermine decreased low-temperature injuries such as splitting and spotting in fruit. Although the IC_{50} of 1,1-diphenil-2-pycrylhydrazyl (DPPH)-radical scavenging activities was not significantly different among the treatments, the IC_{50} of O_2^--scavenging activities in PDJ-treated or Spm-treated fruit at 5 days after the low-temperature treatment was lower than in the untreated control at 20 and −2°C. The expression of *MdCHS* increased in Spm-treated fruit. The concentrations of ascorbic acid, catechin, chlorogenic acid, epi-catechin, and phloridzin in Spm-treated fruit were higher than in the untreated control at −2 or 20°C. These facts suggest that ABA, jasmonates and polyamines may be associated with low-temperature stress tolerance in apple fruitlets.

It was observed by Dutta *et al.* (2008) that temperature had a profound effect on chloroplast biogenesis and associated greening processes. Therefore, the import efficiency of *in vitro* translated precursor of nuclear coded small subunit of ribulose 1,5 bisphosphate carboxylase/ oxygenase (pRSS) into chloroplasts isolated from pea plants exposed to chill-stress (7°C), and heatstress (40°C) for 24–48 h was studied. The binding of precursor proteins to the envelope membranes was not affected in chill-stressed plants. The protein import into chloroplasts in chill-stressed plants was reduced. In heat-stress, binding of pRSS was impaired most likely due to reduced presence of the receptor. When isolated intact chloroplasts were given 10 min of heat stress at 35°C their protein import efficiency was severely inhibited implying that protein import apparatus in pea has a low thermal stability. Down-regulation of

plastid development in temperature stress could be partly attributed to reduced protein import into chloroplast.

The activity of peroxidase (PRX) isozyme, lipid peroxidation (Malondialdehyde, MDA content) and cell membrane injury were studied during low temperature treatment for different periods in strawberry (*Fragaria x ananassa* cv. Camarosa) leaf tissues. Seedlings were grown for six weeks (plants had 4-5 leaves) in a greenhouse then the plants were transferred to a climate chamber with constant 5oC, 60% relative humidity, 14/10 h (light/dark) photoperiod regime and 4 LS light intensity for 1, 4, 7 or 10 days to impose a low temperature stress. In general, low temperature application during 10 days caused a linear increase in MDA content. Native polyacrylamide gel electrophoresis (PAGE) of both acidic and basic peroxidase (PRX) isozymes yielded a single sharp protein band with Rf=0.23 and Rf=0.17, respectively. In addition data indicated a strong relationship between band intensities and the duration of the low temperature treatment. However, the considerable increase of PRX activities could not stop the deleterious effects of low temperature, but reduced severity of stress, thus showing a reduction in the percentage of injury on the 7th day which is correlated with cold-acclimation of strawberry leaf tissues under low temperature (Gulen *et al.*2008).

Soybean (*Glycine max*) is a tropical crop , but is also grown in temperate regions in middle spring to late summer (Yadeghari *et al.*2008).Cold temperature damage is a common problem for this plant in temperate regions. Physiological responses to chilling, including antioxidative enzyme activity, relative water content (RWC) and soluble sugar contents were investigated in soybean to identify mechanisms of chilling tolerance. Plants were exposed to 15°C (cold-acclimated) or 25 °C (nonacclimated) for 24h, under 250 µmol m-2s-1 Photosynthetically Active Radiations(PAR). Then all plants were exposed to 4 °C (chilling temperature) for 24h and allowed to recover at 25 °C for 24h. They analyzed the activity of Ascorbate Peroxidase(APX) , catalase(CAT) and Guaiacol Peroxidase(GPX) and soluble sugar content and RWC in both shoots and roots of soybean seedlings . It was revealed that the activity of APX and CAT and GPX were induced in leaves and roots. Increased activity in roots is important for cold tolerance as compared to shoots. The amount of RWC decreased in both roots and shoots, but soluble sugar content increased,

especially in shoots as compared to control plants. Chilling sensitive soybean plants can be made tolerant to cold by cold acclimation.

Dose-dependent effects of selenium on growth and physiological trait of wheat seedlings (*Triticum aestivum* L. cv Han NO.7086) exposed to cold stress were reported Chu *et al.* (2009). Responses of seedlings were different depending on the Se concentration. The treatments with 0.5 and 1.0 mg Se kg^{-1} significantly increased biomass and chlorophyll content of seedlings. However, the treatments at 2.0 and 3.0 mg Se kg^{-1} only induced an evident increase in chlorophyll content and did not promote biomass accumulation of seedlings. Antioxidant compounds content (anthocyanins, flavonoids, and phenolic compounds) and antioxidant enzymes' activities (peroxidase and catalase) increased by different Se treatments, while only the treatment with 1.0 mg Se kg^{-1} induced a significant reduce in malondialdehyde content and the rate of superoxide radical production of wheat seedlings. The results of this study demonstrated that Se supply could increase antioxidant capacity of seedlings, and optimal Se supply reduced production of free radicals, membrane lipid peroxidation, and promoted biomass accumulation.

The influence of proline and betaine exposure on antioxidant and methylglyoxal (MG) detoxification system during cold stress in *Camellia sinesnis* (L.) O. Kuntze was investigated by Kumar and Yadav (2009). Cold stress enhanced MG and lipid peroxidation levels in tea bud (youngest topmost leaf). This increase was resisted upon the exposure of tea bud to proline and betaine. Exposure of tea bud with proline and betaine also help in maintaining thiol/ disulfide ration during cold stress. Proline exposure enhanced glutathione-S- transferase and glutathione reductase (GR) activity, while betaine exposure increased only GR activity during cold stress. Furthermore, effect of proline / betaine was studied on glyoxalase pathway enzymes that are involved in MG detoxification and comprise and betaine showed protective effect on glyoxalase I and activationg effect on glyoxalase II during cold stress in tea bud. This investigation , therefore suggest that proline and betaine might provide protection to cold stress in tea by regulating MG and lipid peroxidation formation as well as by activating or protection some of antioxidant and glyoxalase pathway enzymes.

Radyuk *et al.* (2009) studied the effect of low about – zero temperature (2°C) on the content of low-molecular antioxidants (ascorbic acid, glutathione and

carotenoids) and also activities of antioxidant enzymes (ascorbate peroxidase, APO; catalase , CAT ; glutathione reductase, GR; and superoxide dismutase, SOD) in green barley (*Hordeum vulgare* L.) seedlings. Under stress conditions, the content of low molecular antioxidants, especially that of reduced ascorbate form, increased. Low-temperature stress activated APO, CAT, GR and SOD. First enzymes responding to the action of stress factor were APO and CAT. i.e., enzymes neutralizing hydrogen peroxide in plant cells, which indicated H_2O_2 active generation at low temperature. Cytoplasmic SOD was more active than its chloroplast isoforms. This indicated that oxidative process initiation under low-temperature stress occurred more actively in the cytosol. After termination of stress- factor action, the content of total ascorbate, glutathione, and carotenoids reduced rapidly to the level close to the initial one. During post-stress period, the amount of reduced ascorbate declined as well; however, it remained at the level higher than the initial one . Activities of APO and CAT dropped sharply; activities of GR and SOD reduced gradually. Thus, reduced ascorbate, APO and CAT play an important role in plant cell defense against above zero temperatures close to zero; reduced ascorbate, GR, and SOD are especially important during post-stress period.

To understand the adaptability of alfalfa (*Medicago sativa* L.) to chilling stress, Wang *et. al.*,(2009) analyzed the antioxidative mechanism during seed germination. The germination rates of six alfalfa cultivars were studied comparatively at 10^0C. Xinmu No. 1 and Northstar were selected as chilling stress-tolerant and stress-sensitive cultivars for further characterization. After chilling treatment, Xinmu No. 1 showed higher seedling growth than Northstar. Xinmu No. 1 exhibited low levels of hydrogen peroxide and lipid peroxidation compared with Northstar. In addition, shoots in Xinmu No. 1 treated with chilling showed higher activities of the superoxide dismutase,ascorbate peroxidase (APX), and catalase than those of Northstar, whereas Xinmu No. 1 showed higher APX activity in roots that Northstar. These results indicated that high antioxidation activity in Xinmu No. 1 under chilling stress is well associated with tolerance to chilling condition during germination.

Popov *et al.* (2010) studied low temperature adaptation of cold sensitive tobacco plants in relation to peroxidation of lipids (POL) in their leaves and roots. Experiments were performed with tobacco plants (*Nicotiana tabacum* L., cv. Samsun). Cold hardening (6 days at 8°C) exerted principally different action on

tobacco leaves and roots. In the leaves, the contents of dienoic conjugates and MDA was reduced, and tissue cold tolerance, even to below zero temperatures, was improved. In contrast, in the roots, POL was activated and root cold tolerance decreased. It is suggested that an incapability of the tobacco root system to adapt to low temperature was a limiting factor determining the low potential of this and other cold sensitive plants to hypothermia.

Elevated Temperature Induced Stress

According to Tewari and Tripathy (1998), chlorophyll (Chl) biosynthesis in chill (7°C)- and heat (42°C)-stressed cucumber (*Cucumis sativus* L. cv poinsette) seedlings was affected by 90 and 60%, respectively. Inhibition of Chl biosynthesis was partly due to impairment of 5-aminolevulinic acid biosynthesis both in chill- (78%) and heat-stress (70%) conditions. Protochlorophyllide (Pchlide) synthesis in chill- and heat-stressed seedlings was inhibited by 90 and 70%, respectively. Severe inhibition of Pchlide biosynthesis in chill-stressed seedlings was caused by inactivations of all of the enzymes involved in protoporphyrin IX (Proto IX) synthesis, Mg-chelatase, and Mg-protoporphyrin IX monoester cyclase. In heat-stressed seedlings, although 5-aminolevulinic acid dehydratase and porphobilinogen deaminase were partially inhibited, one of the porphyrinogen-oxidizing enzymes, uroporphyrinogen decarboxylase, was stimulated and coproporphyrinogen oxidase and protoporphyrinogen oxidase were not substantially affected, which demonstrated that protoporphyrin IX synthesis was relatively more resistant to heat stress. Pchlide oxidoreductase, which is responsible for phototransformation of Pchlide to chlorophyllide, increased in heat-stress conditions by 46% over that of the control seedlings, whereas it was not affected in chill-stressed seedlings. In wheat (*Triticum aestivum* L. cv HD2329) seedlings porphobilinogen deaminase, Pchlide synthesis, and Pchlide oxidoreductase were affected in a manner similar to that of cucumber, suggesting that temperature stress has a broadly similar effect on Chl biosynthetic enzymes in both cucumber and wheat.

Molecular chaperones, including the heat-shock proteins (Hsps), are a ubiquitous feature of cells in which these proteins cope with stress-induced denaturation of other proteins. Hsps have received the most attention in model organisms undergoing experimental stress in the laboratory, and the function of Hsps at the molecular and cellular level is becoming well understood in this context. A

complementary focus is now emerging on the Hsps of both model and nonmodel organisms undergoing stress in nature, on the roles of Hsps in the stress physiology of whole multicellular eukaryotes and the tissues and organs they comprise, and on the ecological and evolutionary correlates of variation in Hsps and the genes that encode them. This focus discloses that (*a*) expression of Hsps can occur in nature, (*b*) all species have *hsp* genes but they vary in the patterns of their expression, (*c*) Hsp expression can be correlated with resistance to stress, and (*d*) species' thresholds for Hsp expression are correlated with levels of stress that they naturally undergo. These conclusions are now well established and may require little additional confirmation; many significant questions remain unanswered concerning both the mechanisms of Hsp-mediated stress tolerance at the organism level and the evolutionary mechanisms that have diversified the *hsp* genes(Feder and Hofmann,1999).

Understanding physiological and biochemical factors involved in heat-stress injury would help improve heat tolerance of cool-season grasses. The objective of a study by Liu and Huang (2000) was to investigate lipid peroxidation of cell membranes in relation to heat-stress tolerance in creeping bentgrass (*Agrostis palustris* Huds.) . Two creeping bent grass cultivars differing in heat tolerance, L-93 (heat tolerant) and Penn cross (heat sensitive) were grown under two temperature regimes: 22/16°C (day/night) and 35/25°C for 56 d in growth chambers. Photochemical efficiency (Fv/Fm) and chlorophyll content of leaves; and electrolyte leakage (EL); content of the lipid peroxidation product, malondialdehyde (MDA); and activities of antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) in leaves and roots were determined biweekly during heat stress. Leaf Fv/Fm ratio and chlorophyll content decreased, whereas EL and MDA contents of both leaves and roots increased under heat stress in both cultivars, but to a greater extent in Penncross. The activities of SOD and CAT decreased, whereas POD activity increased in both leaves and roots, which occurred to a greater extent for Penncross. The increases in MDA content and POD activity under heat stress were greater for leaves than for roots in both cultivars. These results suggest that decreased activities of antioxidant enzymes could result in an increased level of lipid peroxidation. Thus, decreased activities of antioxidant enzymes could contribute to damage of cell membranes and to leaf senescence as demonstrated by increased EL and reduced Fv/Fm, and by decreased chlorophyll content during heat stress. Cultivar

variations in antioxidant enzyme activities were associated with their differences in heat tolerance as evidenced by Fv/Fm ratio, chlorophyll content, and EL.

High temperature is a major factor limiting growth of cool-season grasses during summer months. A study was conducted to determine whether oxidative stress is involved in leaf injury induced by high soil temperatures in two creeping bentgrass (*Agrostis palustris* Huds.) cultivars, heat-tolerant L-93 and heat-sensitive Penncross. Shoots and roots were exposed to four differential temperature regimes in growth chambers and water baths: (i) 20/20°C (control); (ii) 20/35°C (high soil temperature); (iii) 35/20°C (high air temperature); and (iv) 35/35°C (high shoot/soil temperatures). Turf quality, leaf photochemical efficiency (Fv/Fm), electrolyte leakage (EL), content of a lipid peroxidation product (malondialdehyde, MDA), and activities of the antioxidants superoxide dismutase (SOD) and catalase (CAT) were determined. Turf quality and leaf Fv/Fm ratio decreased, whereas EL and MDA contents increased under high soil temperature alone or in combination with high air temperature regimes in both cultivars, but to a greater extent in Penncross than in L-93. Decreases in turf quality and Fv/Fm ratio and increases in EL and MDA were more pronounced at 20/35°C than at 35/20°C. The activities of SOD and CAT decreased with prolonged periods of high temperatures and to a greater extent for Penncross than for L-93. The reductions in SOD and CAT activities were more severe at 20/35 than at 35/20°C. These results suggest that high soil temperature caused more severe oxidative damage to leaves than high air temperature by limiting antioxidant activities and inducing lipid peroxidation. This oxidative stress was associated with accelerated leaf senescence under high temperature conditions. Maintenance of antioxidant activities and low levels of lipid peroxidation was related to the better tolerance of creeping bentgrass to high soil temperature stress imposed on roots or high air temperature on shoots. (Huang *et al.* 2001)

The effects of high temperature on antioxidant enzymes were investigated in three mulberry (*Morus alba* L.) cultivars (cv. K-2, MR-2 and BC2-59). High temperature was imposed by maintaining the plants at 40°C for 120, 240 and 360 min in an environmental plant growth chamber. The activities of superoxide disumutase (SOD), catalase (CAT), guaiacol peroxidase (POD), ascorbate peroxidase (APX) and glutathione reductase (GR) were assayed in the leaf extracts of control and high

temperature-treated plants. Antioxidant enzyme activities were high in all the mulberry cultivars in response to high temperature treatment. However, cv. BC2-59 showed significantly higher activities of all the five antioxidant enzymes in response to high temperature compared to those from the leaves of K-2, and MR-2 mulberry cultivars. The present study suggested that the cv. BC2-59 has an efficient antioxidant system among the three cultivars, which could prevent the oxidative damage in the leaves caused by high temperature stress.(Chaitanya *et al.2002*)

Panchuk *et al.* (2002) studied the effects of elevated growth temperatures and heat stress on the activity and expression of ascorbate peroxidase (APX). To find evidence for a connection between heat stress response, oxidative stress, and common stress tolerance, 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 thermo stable isoform, APXS. This enzyme was present in addition to thermo labile cytosolic APX1, the prevalent isoform in unstressed cells. In HSF3-transgenic plants, APXS activity was detectable at normal temperature and persisted after severe heat stress at 44°C. In nontransgenic plants, APXS 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 APXS 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 and that the enzymatic activity of APX2/APXS is required to compensate heat stress-dependent decline of APX1 activity in the cytosol.

Effects of high temperature on the activity of peroxidase (PRX) isozyme and leaf proteins were studied in strawberry (*Fragaria x ananassa* cv. Camarosa). Seedlings were grown using perlite for 3 weeks at 25/10 °C day/night temperature, and watered daily by 1/3 strength modified Hoagland nutrient solution. Half of the plants were transferred to a growth chamber with a constant 25 °C for a week to

acclimate the plants. Temperature was increased stepwise (5 °C/48 h) to 30, 35, 40 °C and finally to 45 °C. In addition to acclimated plants, new plants were transferred from outside to the growth chamber, at each temperature step to impose a heat shock. In general, effects of gradual heat stress (GHS) and shock heat stress (SHS) on the variables studied were significant. PRX activities were high in all the samples in response to high temperature treatment. Conversely, total protein content was decreased by heat stress. GHS plants showed significantly higher activities of PRX enzyme in response to high temperature compared to those from the leaves of SHS plants. One basic PRX band (rf=0.22) was detected in all the samples with different intensity in polyacrylamide gel electrophoresis (PAGE). In addition, plants exposed to GHS leaked less electrolytes from the leaves compared with the plants exposed to SHS (Gulen and Eris, 2004)

Metabolic profiling analyses were performed to determine metabolite temporal dynamics associated with the induction of acquired thermotolerance in response to heat shock and acquired freezing tolerance in response to cold shock. Low-M_r polar metabolite analyses were performed using gas chromatography-mass spectrometry. Eighty-one identified metabolites and 416 unidentified mass spectral tags, characterized by retention time indices and specific mass fragments, were monitored. Cold shock influenced metabolism far more profoundly than heat shock. The steady-state pool sizes of 143 and 311 metabolites or mass spectral tags were altered in response to heat and cold shock, respectively. Comparison of heat- and cold-shock response patterns revealed that the majority of heat-shock responses were shared with cold-shock responses, a previously unknown relationship. Coordinate increases in the pool sizes of amino acids derived from pyruvate and oxaloacetate, polyamine precursors, and compatible solutes were observed during both heat and cold shock. In addition, many of the metabolites that showed increases in response to both heat and cold shock in this study were previously unlinked with temperature stress. This investigation provides new insight into the mechanisms of plant adaptation to thermal stress at the metabolite level, reveals relationships between heat- and cold-shock responses, and highlights the roles of known signalling molecules and protectants(Kaplan *et.al.* 2004).

Within their natural habitat, plants are subjected to a combination of abiotic conditions that include stresses such as drought and heat. Drought and heat stress have

been extensively studied; however, little is known about how their combination impacts plants. The response of *Arabidopsis* plants to a combination of drought and heat stress was found to be distinct from that of plants subjected to drought or heat stress (Rizhsky, *et al.* 2004). Transcriptome analysis of *Arabidopsis* plants subjected to a combination of drought and heat stress revealed a new pattern of defense response in plants that includes a partial combination of two multigene defense pathways (i.e. drought and heat stress), as well as 454 transcripts that are specifically expressed in plants during a combination of drought and heat stress. Metabolic profiling of plants subjected to drought, heat stress, or a combination of drought and heat stress revealed that plants subject to a combination of drought and heat stress accumulated sucrose and other sugars such as maltose and glucose. In contrast, Pro that accumulated in plants subjected to drought did not accumulate in plants during a combination of drought and heat stress. Heat stress was found to ameliorate the toxicity of Pro to cells, suggesting that during a combination of drought and heat stress sucrose replaces Pro in plants as the major osmoprotectant. Their results highlight the plasticity of the plant genome and demonstrate its ability to respond to complex environmental conditions that occur in the field.

Drought and heat are two major factors limiting growth of cool season grasses. Rapid recovery from the combination of those stresses is important for the persistence of perennial turf grasses. A study was designed to examine physiological factors associated with the persistence and recovery of Kentucky bluegrass (*Poa pratensis* L.) exposed to combined drought and heat stress following rewatering and/or temperature drop. Two cultivars differing in drought and heat tolerance, 'Midnight' (tolerant) and 'Brilliant' (sensitive), were exposed to drought and heat stress (35°C) simultaneously in a growth chamber until most plants became brown and completely desiccated (14 d). Plants were then subjected to three recovery treatments: (i) rewatered but exposed to heat stress (rewatering); (ii) returned to optimum temperature (20°C) but unwatered (cooling), and (iii) rewatering and cooling. Leaf photochemical efficiency (Fv/Fm), chlorophyll content, and activities of superoxide dismutase (SOD) and catalase (CAT) declined, while electrolyte leakage (EL) and lipid peroxidation increased rapidly during the combined stress. The adverse impact of the combined stress was more severe for Brilliant than for Midnight. Following rewatering or in combination with cooling, all parameters except chlorophyll content fully recovered for Midnight. However, for Brilliant, most of the parameters did not recover completely; Fv/Fm

recovered partially. There was no recovery for any parameters of either cultivar when plants were returned to the optimum temperature but still unwatered. The results suggested that simultaneous drought and heat stress could cause permanent physiological damage for Kentucky bluegrass, particularly for the stress-sensitive cultivar. Rewatering was essential for physiological recovery from the combined stress, regardless of temperature conditions. Rapid resumption of Fv/Fm, cell membrane stability, and antioxidant activities were important factors contributing to the recovery of Kentucky bluegrass. (Wang and Huang ,2004)

Almeselmani *et al.* (2006) conducted an experiment to study the effect of high temperature stress on the antioxidant enzyme activity in five wheat genotypes viz., PBW 343, PBW 175, HDR-77, HD 2815 and HD 2865. There was significant increase in the activity of superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) in the late and very late planting and at all stages of plant growth, i.e., vegetative, anthesis and 15 days after anthesis (DAA), however glutathione reductase (GR) and peroxidase (POX) activity decreased under late and very late plantings compared to normal planting. In general HD 2815, HDR-77 showed relatively higher SOD, APX, GR, CAT and POX activity in the late plantings compared to PBW 343, PBW 175 and HD 2865. Significant reduction in chlorophyll content and increase in membrane injury index were observed in all genotypes with age, and also under late and very late sowings at all the stages of plant growth. However HD 2815 and HDR-77, which showed highest activity of various antioxidant enzymes under late and very late sowing also showed minimum reduction in chlorophyll content and lower membrane injury index, indicating the amelioration of high temperature stress induced oxidative stress by antioxidant enzymes. Various antioxidant enzymes showed positive correlation (r) with chlorophyll content and negative with membrane injury index at most of the stages in the five wheat genotypes.

Minimizing the exposure of an annual crop to abiotic stresses may increase seed yield. A study was conducted to determine the effect of high temperature stress during reproductive development on pod fertility, seed set, and seed yield of chickpea (*Cicer arietinum* L). 'Myles' desi and 'Xena' kabuli chickpea were grown 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 d 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 (Wang *et al.* 2006).

Xu and Zhou(2006) determined the photosynthetic gas exchange, chlorophyll fluorescence, nitrogen level, and lipid peroxidation of the leaves of a perennial grass (*Leymus chinensis* (Trin.) Tzvel.) Subjected to three constant temperatures (23, 29 and 32°C), and five soil-moisture levels (75–80%, 60– 65%, 50–55%, 35–40% and 25–30% of Weld capacity, respectively). High temperature significantly decreased plant biomass, leaf green area, leaf water potential, photosynthetic rate (*A*), maximal efficiency of PSII photochemistry (*Fv*/*Fm*), actual PSII efficiency (_PSII), the activities of nitrate reductase (NR; EC 1.6.6.1) and glutamine synthetase (GS; EC 6.3.1.2), but markedly increased the ratio of leaf area to leaf weight (SLA), endopeptidase (EP; EC 3.4.24.11) activity, and malondialdehyde (MDA) content, especially under severe water stress conditions. The *A* and *Fv*/*Fm* were significantly and positively correlated with leaf-soluble protein content, and the activities of NR and GS. However, both photosynthesis parameters were significantly and negatively correlated with EP activity and MDA content ($P < 0.05$). It is suggested that high temperature, combined with severe soil drought, might reduce the function of PSII, weaken nitrogen anabolism, strengthen protein catabolism, and provoke lipid peroxidation. The results also indicate that severe water stress might exacerbate the adverse effects of high temperature, and their combination might reduce the plant productivity and distribution range of *L. chinensis* in the future.

Plant and animals share similar mechanisms in the heat shock (HS) responses, 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 32KD protein (HSa32) , which is highly conserved inland plants but absent in most other organisms. The gene responds to HS at the transcriptional level in moss (*Physcomitrella patens*), Arabidopsis (*A. thaliana*), and rice (*Oriza sativa*) . Like other HSPs, Has 32 protein accumulates greatly in *Arabidopsis* seedlings after HS treatment. Disruption of Has 32 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 (>24hrs) after acclimation HS treatment , when a server 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 hypocotyls elongation assay also revealed that thermotolerance decayed faster in the absence of Has 32 after a long revovery. Similar results were obtained in transgenic plants with Has expression suppressed by RNA interference. Micro array analysis of the knockout mutant indicates that only the expression of Has 32 was significantly altered in HS responses. Taken together the result suggests that Has 32 is required not for induction but rather maintenance of acquired thermotolerance, a feature that could be important to plants.

The response of understory species to elevated temperatures is not well understood but is important because these plants are highly sensitive to their growth conditions. Three-year-old plants of *Panax quinquefolius*, an understory herb endemic to the eastern deciduous forests of North America, were grown in a greenhouse at 25/208C (day/night) or 30/258C for one growing season and analyzed each month. Plants grown at high temperatures had an early onset of leaf senescence and therefore accumulated less carbon. From May to July, P. *quinquefolius* grown at high temperatures had decreased photosynthesis (52%), stomata conductance (60%), and root and total biomass (33% and 28%, respectively) compared to plants grown at low temperatures. As *P. quinquefolius* prepared to overwinter, plants grown at high temperatures had less root biomass (53%) than plants in low temperatures. The amount of storage-root ginsenosides was unaffected by temperature, and differences in storage root size may explain why plants grown at high temperatures had greater concentrations of storage root ginsenosides (49%) than plants grown at low

temperatures. *Panax quinquefolius* is clearly sensitive to a 58^0 C increase in temperature, and therefore other understory species may be negatively impacted by future increases in global temperature. (Jochum *et al.*2007)

The heat stress – induced dehydrin proteins (DHNs) expression and relationship with the water relations of sugarcane (*Saccharum officcinarum* L.) leaves were studied. Sugarcane seedlings were subjected to heat stress (day/ night temperature of 40/ 35°C) under relative humidity 60/65% to avoid aerial desiccation and determinations made at 4, 12, 24, 36, 48 ,60 and 72 h. The leaves showed a sharp decline in the water and osmotic potentials, and relative water content during first 12h of heat stress but a regain in their values in 24h. The pressure potential ($¥_p$) decreased initially but increased later and approached control leaves. The increase in $¥_p$ was tightly correlated t the accumulation of free proline, Glycinebetaine and soluble sugars, indicating their possible involvement in the osmotic adjustment under heat stress. Immunological detection revealed the expression of three DHNs with an apparent molecular mass of 21,23 and 27 kDa under heat stress(48to 72h) and their expression was independent of the changes in the water relations of leaves. (Wahid and Close, 2007).

Adebooye *et al*(.2008) investigated the influence of root zone temperature (RZT) and the aerial application of paraquat on stress defence mechanisms of *Trichosanthes cucumerina* L. To achieve this objective, *T. cucumerina* cv Green was grown with roots at 25 and 30°C root zone temperature and maintained at 20 ± 1°C air temperature in a growth chamber. These RZT and air temperature had earlier been shown to favor growth and fruit production in T. cucumerina. Plants at each RZT were subjected to paraquat treatment (+P) and without paraquat treatment (-P). Paraquat (0.2 mmol/L) was applied as aerial spray. Results showed that the individual main effects of RZT and parquet treatments significantly affected he chlorophyll fluorescence and gas exchange parameters, while the interaction of both reatments had no significant effect. Results showed that the total phenolics and ascorbic acid contents of T. cucumerina at 30°C were significantly higher than at 25°C. The *T. cucumerina* plants in +P treatment recorded significantly lower maximum photochemical efficiency (Fv/Fm), net photosynthesis (A), transpiration rate (E), intercellular CO2 oncentration (Ci) and stomatal conductance (gl) compared to untreated plants. Also, plants raised at 30°C recorded significantly higher Fv/Fm, A,

E, Ci and gl compared to plants raised at 25°C. Plants that were sampled at 48 h after paraquat treatment recorded a higher degree of oxidative damage compared to those sampled at 24 h after treatment. They showed that the degree of damage suffered by *T. cucumerina*, when treated with paraquat either at 25 °C or RZT was similar at 48 h after treatment. Either at 25 or 30°C, exposure of *T. cucumerina* to paraquat would impose the same degree of oxidative damage.

Abiotic stresses, such as high temperature, and salt stress are major factors which reduce crop productivity. Effects of high temperature (46-48° C) and salt stress (0.4 M) on French bean (*Phaseolus vulgaris*), a major vegetable crop, were evaluated in terms of antioxidants and antioxidant enzymes in S-9 cultivar. Both stresses caused similar responses in the plant. Oxidative stress indicators such as H_2O_2, TBARS, glutathione, ascorbic acid, and proline were significantly elevated. Similarly, antioxidant enzyme, guaiacol-specific peroxidase (POX) was significantly elevated. Other enzymes, β-amylase and acid phosphatase (AP) activities were marginally enhanced. However, stresses had contrasting effects on glutathione reductase (GR) and catalase (CAT), which were drastically reduced in temperature stress, and elevated in salt stress. No variations were observed in AP, POX, and CAT isozymes. Patterns of GR and β-amylase isozymes differed between temperature and salt stress. SDS-PAGE indicated entirely different sets of proteins in temperature and salt stressed seedlings. Growth rate and fresh mass were affected to same extent, relative to their respective controls. DNA damage was more pronounced under temperature stress than under salt stress. Response mechanism of French bean appears to involve some players which are common to both the stresses, and few specific to individual stress (Nagesh and Devaraj, 2008).

Wheat crop was exposed to continual heat stress throughout the crop growth period or terminal heat stress, i.e. during grain growth period. Characterization of genotypes in both continual and terminal heat stress environments is necessary to identify the sources of heat tolerance for these environments. Hence in this study, 20 genotypes of *T. aestivum* (hexaploid, BBAADD genome) and 16 genotypes of *T. durum* (teraploid, BBAA genome) were evaluated for terminal and continual heat stress tolerance at Delhi and Madhya Pradesh (MP), India, respectively. Normal and late sowing were done at both the locations to assess the genotypes under normal and heat stress environments, respectively. The late sown crop of Delhi experienced

higher temperatures during grain development, while in MP environments, the crop experienced moderately higher temperatures during normal sowing and extremely higher temperatures during late sown conditions throughout the crop growth period. Wide variation for continual or terminal heat tolerance for yield and yield components was found between *T. aestivum* and *T. durum* genotypes. Heat tolerance of genotypes varied in continual or terminal heat stress environment. *T. aestivum* genotypes such as LOK1, HUW 234, Raj 3777, C306, NI5439, NP846 and Kalyansona and *T. durum* genotype DHT15 showed heat tolerance in both terminal and continual heat stress environments. Under both heat stress environments, high biomass production and grains m^{-2}apppear to be the two important traits for achieving heat tolerance in yield. The reduction in grain number at continual heat stress environments was due to persistently higher temperature during pre-heading and post – heading period, while in terminal heat stress environment the reduction in grain number was due to sudden increase in temperature during grain growth period. Photothemal quotient correlated positively and significantly with both grain yield and grains per unit area under both continual and terminal heat stress environments. Reduction in grain number under continual but moderate heat stress environment acts as a compensation mechanism to maintain grain weight in wheat genotypes. *T. aestivum* genotypes Kindan and Lok1 and *T. durum* genotypes III8498 and DHT15 maintained grain weight of >45g 1000 $grains^{-1}$ under heat stress environments. This study shows that wheat cultivars differ in their heat tolerance depending upon the nature of heat stress, i.e. continual of terminal heat stress. (Patil *et al.* 2008)

An experiment was conducted with three wheat genotypes differing in their sensitivity to moisture and/or temperature stress to study the relationship of the chloroplast antioxidant system to stress tolerance. Both moisture stress and temperature stress increased glutathione reductase and peroxidase and decreased membrane stability, chlorophyll content and chlorophyll stability index in all genotypes. Under moisture stress. DL 153–2 showed the highest membrane stability index, chlorophyll content, chlorophyll stability index, glutathione reductase activity and peroxidase activity. However, under elevated temperature conditions, HD 2285, and to a lesser extent DL 153–2, showed higher membrane stability, chlorophyll content and chlorophyll stability index and activities of glutathione reductase and peroxidase. Raj 3077, which is sensitive to both drought and temperature stress, showed the lowest membrane stability, chlorophyll content and chlorophyll stability

index and glutathione reductase and peroxidase activity under elevated temperature as well as drought conditions. Thus, authors concluded that tolerance of the genotype to moisture and/or temperature stress is closely associated with its antioxidant enzyme system. (Sairam ,2008).

Two wheat genotypes, C 306 (tolerant) and PBW 343 (susceptible to temperature stress) were grown in growth chambers in the phytotron facility of IARI, New Delhi. The plants were maintained at 18/23°C (control) and 25/35°C (temperature stress) night/day temperatures after maximum tillering. Water potential was significantly reduced at anthesis, and at 7 and 15 days after anthesis in both genotypes in the heat stress treatment, and a greater reduction was recorded in PBW 343. The membrane stability index was also lower in the heat stress treatment in both genotypes at the vegetative stage, at anthesis andat 15 days after anthesis, and a greater reduction was observed in PBW 343 than in C 306. The hydrogen peroxide content increased as the plants advanced in age, and higher hydrogen peroxide content was recorded in PBW 343 than in C 306 at different stages of growth in the heat stress treatment. The superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR) and peroxidase (POX) activities increased significantly at all stages of growth in C 306 in response to heat stress treatment, while PBW 343 showed a significant reduction in catalase, glutathione reductase and peroxidase activities in the high temperature treatment. Northern blot showed a significant increase in the *APX*-mRNA level under heat stress at the vegetative and anthesis stages, and the expression was greater in C 306. From the results it is apparent that the antioxidant defence mechanism plays an important role in the heat stress tolerance of wheat genotypes. (Almeselmani *et al.* ,2009)

Even though high temperatures significantly reduced both vegetative growth and yield in cotton, very little is known about the effects of heat stress on cotton antioxidant system. Thus, the effects of gradual heat stress on cotton growth in controlled conditions were investigated in the present study. At squaring stage, cotton plants were subjected to two different temperatures, 38 and 45 °C t determine the influence of heat stress on the plants. The results of the present study showed that heat stress did not significantly altered the levels of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) in the leaves , whereas there was a remarkable decline in proline quantity of the leaves of plants subjected to 45°C heat stress. AS for the

amount of total chlorophyll content, a slight increase at plants treated with 38°C temperature was observed. Furthermore, the activities of some enzymes such as superoxide dismutase (SOD) , which were associated with heat stress response in other plants was also investigated. For example , there was decline in the activity of SOD in the plants exposed to high temperatures . On the contrary, catalase (CAT) activity increased at 45°C ; peroxidase (POX) activity increased at 38°C and ascorbate peroxidase (APX) activity increased at 38°C and 45°C. The results from this study suggest a potential role for CAT, POX and APX in the reduction of elevated levels of H_2O_2 in cotton plants grown under heat stress condition. To sum up, it could be concluded that, diurnal gradual heat stress caused a low oxidative injury in cotton.(Gur *et al.* 2010)

Chemical Pre-treatments for Stress Amelioration

Dat *et al.* (1998) investigated changes in endogenous SA and antioxidants in relation to induced thermotolerance in *Sinapis alba* L. Thirty minutes into a 1-h heat-acclimation treatment glucosylated SA had increased 5.5-fold and then declined during the next 6 h. Increases in free SA were smaller (2-fold) but significant. Changes in antioxidants showed the following similarities after either heat-acclimation or SA treatment. The reduced-to-oxidized ascorbate ratio was 5-fold lower than the controls 1 h after treatment but recovered by 2 h. The glutathione pool became slightly more oxidized from 2 h after treatment. Glutathione reductase activity was more than 50% higher during the first 2 h. Activities of dehydroascorbate reductase and monodehydro ascorbate reductase decreased by at least 25% during the first 2 h but was 20% to 60% higher than the control levels after 3 to 6 h. One hour after heat acclimation ascorbate peroxidase activity was increased by 30%. 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.

The addition of 0.5 mM salicylic acid (SA) to the hydroponic growth solution of young maize (*Zea mays* L.) plants under normal growth conditions provided protection against subsequent low-temperature stress. This observation was confirmed by chlorophyll fluorescence parameters and electrolyte leakage measurements. In addition, 1 d of 0.5 mM SA pre-treatment decreased net photosynthesis, stomatal conductivity and transpiration at the growth temperature (22/20 °C). Since there was

only a slight decrease in the ratio of variable to maximal fluorescence (Fv/Fm) the decrease in photosynthetic activity is not due to a depression in photosystem II. The analysis of antioxidant enzymes showed that whereas SA treatment did not cause any change in ascorbate peroxidase (EC 1.11.1.11) and superoxide dismutase (EC 1.15.1.1) activities, there was a decrease in catalase (EC 1.11.1.6) activity, and an increase in guaiacol peroxidase (EC 1.11.1.7) and glutathione reductase (EC 1.6.4.2) activities after the 1- d SA treatment at 22/20 °C. In native polyacrylamide gels there was, among the peroxidase isoenzymes, a band which could be seen only in SA-treated plants. It is suggested that the pre-treatment of maize plants with SA at normal growth temperature may induce antioxidant enzymes which lead to increased chilling tolerance.(Janda *et al.* 1999)

A study was designed to examine whether external Ca^{2+} treatment would improve heat tolerance in two C3, cool-season grass species- tall fescue (*Festuca arundinacea* L.) and Kentucky bluegrass (*Poa pratensis* L.) and to determine the physiology 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 increase 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 12d 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), ascrobate peroxide (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 malonialdehyde (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 Kentuncky 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 (Jiang and Huang *al* 2000).

The hypothesis that physiologically active concentrations of salicylic acid (SA) and its derivatives can confer stress tolerance in plants was evaluated using bean (*Phaseolus vulgaris* L.) and tomato (*Lycopersicon esculentum* L.). Plants grown from seeds imbibed in aqueous solutions (0.1-0.5 mM) of salicylic acid or acetyl salicylic acid (ASA) displayed enhanced tolerance to heat, chilling and drought stresses. Seedlings acquired similar stress tolerance when SA or ASA treatments were applied as soil drenches. The fact that seed imbibition with SA or ASA confers stress tolerance in plants is more consistent with a signaling role of these molecules, leading to the expression of tolerance rather than a direct effect. Induction of multiple stress tolerance in plants by exogenous application of SA and its derivatives may have a significant practical application in agriculture, horticulture and forestry (Seneratna *et al.* 2000)

Borsani *et al.* (2001) studied the responses of wild-type *Arabidopsis* and an SA-deficient transgenic line expressing a salicylate hydroxylase (*NahG*) gene to different abiotic stress conditions. Wild-type plants germinated under moderate light conditions in media supplemented with 100 mM NaCl or 270 mM mannitol but showed extensive necrosis in the shoot. In contrast, *NahG* plants germinated under the same conditions remained green and developed true leaves. The lack of necrosis observed in *NahG* seedlings under the same conditions suggested that SA potentiates the generation of reactive oxygen species in photosynthetic tissues during salt and osmotic stresses. This hypothesis is supported by the following observations. First, the herbicide methyl viologen, a generator of superoxide radical during photosynthesis, produced a necrotic phenotype only in wild-type plants. Second, the presence of reactive oxygen-scavenging compounds in the germination media reversed the wild-type necrotic phenotype seen under salt and osmotic stress. Third, a greater increase in the oxidized state of the glutathione pool under NaCl stress was observed in wild-type seedlings compared with *NahG* seedlings. Fourth, greater oxidative damage occurred in wild-type seedlings compared with *NahG* seedlings under NaCl stress as measured by lipid peroxidation. Their data support a model for SA potentiating the stress response of the germinating *Arabidopsis* seedling.

Roles of abscisic acid (ABA) in water stress-induced oxidative stress was investigated in leaves of maize (*Zea mays* L.) seedlings exposed to water stress induced by polyethylene glycol (PEG 6000) by Jiang and Zhang (2002). Treatment with PEG at -0.7 MPa for 12 and 24 h led to a reduction in leaf relative water content (RWC) by 7.8 and 14.1%, respectively. Duration of the osmotic treatments is considered as mild and moderate water stress. The mild water stress caused significant increases in the generation of superoxide radical (O 2 -) and hydrogen peroxide (H 2 O 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 2 O 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. Their data indicate that ABA plays an important role in water stress-induced antioxidant defense against oxidative stress.

Plants, in common with all organisms, have evolved mechanisms to cope with the problems caused by high temperatures. Larkindale and Knight (2002) examined specifically the involvement of calcium, abscisic acid (ABA), ethylene, and salicylic acid (SA) in the protection against heat-induced oxidative damage in *Arabidopsis*. Heat caused increased thiobarbituric acid reactive substance levels (an indicator of oxidative damage to membranes) 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 these 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 (a precursor to ethylene), and ABA added to plants protected them from heat-induced oxidative damage. In addition, the ethylene-insensitive mutant *etr-1*, the ABA-insensitive mutant *abi-1*, and a transgenic line expressing *nahG* (consequently inhibited in SA production) showed increased susceptibility to heat. These data suggest that protection against heat-induced oxidative damage in *Arabidopsis* also involves ethylene, ABA, and SA. Real time measurements of cytosolic calcium levels during heating in Arabidopsis detected no increases in response to heat per se, but showed transient elevations in response to recovery from heating. The magnitude of these calcium peaks was greater in thermotolerant plants, implying that these calcium signals might play a role in mediating the effects of acquired thermotolerance. Calcium channel blockers and calmodulin inhibitors added solely during the recovery phase suggest that this role for calcium is in protecting against oxidative damage specifically during/after recovery.

Sakhabutdinova *et al.* (2003) investigated the effect of salicylic acid (SA) on plant resistance to environmental stress factors. Treatment of wheat plants with 0.05mM SA increased the level of cell division within the apical meristem of seedling roots which caused an increase in plant growth. Phytohormones are known to play a key role in plant growth regulation. It was found that the SA treatment caused accumulation of both ABA and IAA in wheat seedlings. However, the SA treatment did not influence cytokinin content. According to the authors, the protective and growth promoting effects of SA are due to the phenomenon described above. The SA treatment reduced the damaging action of salinity and water deficit on seedling growth and accelerated a restoration of growth processes. Treatment with SA essentially diminished the alteration of phytohormones levels in wheat seedlings under salinity and water deficit. The SA treatment prevented the decrease in IAA and cytokinin content completely which reduced stress-induced inhibition of plant growth. Also, high ABA levels were maintained in SA treated wheat seedlings which provided the development of antistress reactions, for example, maintenance of proline accumulation. Thus protective SA action includes the development of antistress programs and acceleration of normalization of growth processes after removal stress factors.

Efficacy of heat acclimation and salicylic acid (SA) treatment in induction of thermotolerance was tested in six different genotypes of *Cicer arietinum* L. Remarkable reduction in relative injury of membrane was observed in plants treated with SA in comparison to heat - acclimatized and untreated control seedlings subjected to lethal temperature treatment .Both treatment resulted in increased in protein and proline content over control seedlings, which was more significant in SA pre- treatments, with the maximum increase being recorded in ICC 4918 and 1852. Both treatments led to the induction of peroxidase (POX), ascorbate peroxidase (APOX) and catalase (CAT) activities. Activities of POX and APOX increased remarkably , while CAT showed a reduction in activity.(Chakraborty and Tongden, 2005)

A 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 .SA was applied at 0.5Kg ha^{-1} to the turfgrass 10days before harvest and canopy photochemical efficiency was measured 1day before harvest. Harvested and rolled sod was subjected to high temperature stress(38-40°C for 72-96hrs), 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 .SA 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.

Effects of Ca^{2+} ions on the intensity of lipid peroxidation, activities of guaiacol peroxidase, superoxide dismutase (SOD), and catalase, as well as on heat resistance of winter wheat (*Triticum aestivium* L.) coleoptiles were examined. A preliminary incubation of coleoptiles segments in a 5 mM $CaCl_2$ solution was shown to improve their survival rates after an injuring heat treatment (43.5°C). The effect of Ca2+ was suppressed by the inhibitor of Ca2+channels (1 mM $LaCl_3$). An incubation of coleoptiles in the presence of 5 mM CaCl2prior to the stress treatment elevated the content of lipid peroxidation product, malondialdehyde (MDA) and

stimulated the activities of guaiacol peroxidase, SOD, and catalase. After the heat exposure of untreated and Ca^{2+} -treated seedlings, differential changes in MDA content and in activities of guaiacol peroxidase, SOD, and catalase were observed. It is concluded that a short-term oxidative stress arising in Ca2+ -enriched plant tissues after the heat treatment is unrelated to their irreversible damage (Kolupaev *et al.*2005)

He *et al.* (2005) reported that application of salicylic acid (SA) to the shoots and soil could improve heat tolerance of Kentucky bluegrass, and investigated whether SA-induced heat tolerance is related to changes in antioxidant activities. 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 h in a growth chamber. Influences of SA on the production of active oxygen species (AOS), superoxide anion (O_2^-), and hydrogen peroxide (H_2O_2), and activities of antioxidant enzymes, superoxide dismutase (SOD), and catalase (CAT), were examined. Among SA concentrations, 0.25 mmol was most effective in enhancing heat tolerance in Kentucky bluegrass, which was manifested by improved re- growth potential following heat stress of 72 h and maintenance of leaf water content at 77% during the 12-h stress period similar to that under normal temperature conditions. The O_2^-, generating rate increased significantly at 6 h of heat stress, and SOD activity increased significantly at 2 h but decreased to the control level at 6 h of heat stress in SA-untreated plants. The SA application suppressed the increase of O_2 generating rate and enhanced SOD activity significantly at 2 and 6 h of heat stress, respectively. The SA application decreased H2O2 level significantly at 2 and 12 h of heat stress, and increased CAT activity significantly within 12 h 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 ABA on *Stylosanthes guianensis* (Aublet) Sw. and its relation to antioxidant systems under chilling stress was studied by Zhou *et al.* (2005). *Stylosanthes guianesis* seedlings were sprayed with 10mg 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 12h photoperiod at 160μmol $m^{-1}S^{-1}$ photosynthetic photon flux density. The chilling treated plants were then re-warmed to 28°C for 2days. During the 9 days treatment, a series of enzyme activities, related water content (RWC) and electrolytic leakage were

measured on samples 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. 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 subjected 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 suggest that ABA increased chilling resistance in *S guianensis* is partially associated with enhanced scavenging systems.

It is now widely accepted that salicylic acid (SA) signaling is mediated by reactive oxygen species (ROS) production. Faravardeh and Rabbani (2006), studied the effect of SA on peroxidase activity and superoxide an ion production in potato leaf cell suspension. The results showed that potato cells are in sensitive to low concentrations of exogenous SA (< 1 mM) and the effect is observed at 1-5 mM SA. The cells exposed to SA exhibit higher peroxidase activity and show different peroxidase pattern when analyzed on native gels compared to the control. Superoxide an ion production was enhanced after two hours oftreatment and 2.5 mM SA gives the highest value. The results suggest peroxidase-mediated detoxification of ROS elicited by SA.

Thermotolerance and related antioxidant enzyme activities induced by both heat acclimation and exogenous salicylic acid (SA) application were studied in grapevine (Vitis vinifera L. cv. Jingxiu) by Wang and Li(2006) . Heat acclimation and exogenous SA application induced comparable changes in thermotolerance, ascorbic acid (AsA), glutathione (GSH), and hydrogen peroxide (H2O2) concentrations, and in activities of the antioxidant enzymes superoxide dismutase (SOD), peroxidase (POD), glutathione reductase (GR), ascorbic peroxidase (APX) and catalase (CAT) in grape leaves. Within 1 h at 38°C, free SA concentration in leaves rose from 3.1 μg g^{-1} FW to 19.1 μg g^{-1} FW, then sharply declined. SA application and heat acclimation induced thermotolerance were related to changes of antioxidant enzyme activities and antioxidant concentration, indicating a role for endogenous SA in heat acclimation in grape leaves.

Exogenous salicylic acid has been shown to confer tolerance against biotic and abiotic stresses. In the present work the ability of its analogue, 4-hydroxybenzoic acid

to increase abiotic stress tolerance was demonstrated: it improved the drought tolerance of the winter wheat (*Triticum aestivum* L.) cv. Cheyenne and the freezing tolerance of the spring wheat cv. Chinese Spring. Salicylic acid, however, reduced the freezing tolerance of Cheyenne and the drought tolerance of Chinese Spring, in spite of an increase in the guaiacol peroxidase and ascorbate peroxidase activity. The induction of cross tolerance between drought and freezing stress was observed: drought acclimation increased the freezing tolerance of Cheyenne plants and cold acclimation enhanced the drought tolerance. The induction of drought tolerance in Cheyenne was correlated with an increase in catalase activity (Horváth *et al.*,2007).

Chilling tolerance of salicylic acid (SA) in banana seedlings (*Musa acuminata* cv., Williams 8818) was investigated by changes in ultra structure in this study. Pretreatment with 0.5 mmol/L SA under normal growth conditions (30/22 °C) by foliar spray and root irrigation resulted in many changes in ultrastructure of banana cells, such as cells separation from palisade parenchymas, the appearance of crevices in cell walls, the swelling of grana and stromal thylakoids, and a reduction in the number of starch granules. These results implied that SA treatment at 30/22 °C could be a type of stress. During 3 d of exposure to 7 °C chilling stress under low light, however, cell ultrastructure of SA-pretreated banana seedlings showed less deterioration than those of control seedlings (distilled water-pre-treated). From the above experiment Kang *et al.(2007)* concluded that the SA could provide some protection for cell structure of chilling-stressed banana seedling.

Salicylic acid pre-treatment also improved the acclimation of tomato to high salinity observed by Szepesi *et al.* (2008). The aim of their study was to investigate the effect of salicylic acid (SA) pre-treatment on the salt stress acclimation of tomato plants (*Lycopersicon esculentum* Mill. L. cv. Rio Fuego). The antioxidant defence and detoxifying capacity of the tissues were analysed by measuring the accumulation of soluble, non-enzymatic antioxidants (anthocyanins) and the activities of glutathione S-transferases (GSTs) at low (10^{-7} M) and high (10^{-4} M) SA concentrations in plants exposed to 100 mM NaCl. GSTs are a diverse group of enzymes that catalyse the detoxification of xenobiotics and other toxic organic compounds, and anthocyanins are among the few endogenous substrates that bind to GSTs and are sequestered to the vacuole. It was found that 10^{-4} M SA pre-treatment improved the acclimation of tomato to high salinity. SA pre-treatments increased the accumulation of anthocyanins

both in the presence and absence of 100 mM NaCl. The extractable GST activity of tissues increased under salt stress in young leaves and roots of the control and in plants pre-treated with 10^{-4} M SA, while the extractable GST activity in these organs was reduced by 10^{-7} M SA. It is suggested that elevated GST activity is a prerequisite for successful acclimation to high salinity in tomato plants pre-treated with A, but it may also be a symptom of tissue senescence.

In plants, salicylic acid (SA) is a signalling molecule regulating disease resistance responses such as systemic acquired resistance (SAR) and the hypersensitive response (HR), and has been implicated in both basal and acquired thermotolerance. It has been shown that SA enhances heat-induced Hsp/Hsc70 accumulation in plants. To investigate the mechanism of how SA influences the heat shock response (HSR) in plants, tomato seedlings were treated with SA alone, heat shock, or a combination of both before analyses of *hsp70* mRNA, heat shock factor (Hsf)–DNA binding, and gene expression of *hsp70, hsfA1, hsfA2*, and *hsfB1*. SA alone led to activation of Hsf–DNA binding, but not induction or transcription of *hsp70* mRNA. SA had no significant effect on *hsfA2* and *hsfB1* gene expression, but potentiated the basal levels of *hsfA1*. In heat-shocked plants, Hsf–DNA binding was established, and increased *hsfA1*, *hsfA2*, and *hsfB1* expression was followed by accumulation of Hsp70. SA plus heat shock showed enhanced Hsf–DNA binding, enhanced induction of *hsp70* mRNA transcription, and gene expression of *hsfA1, hsfA2,* and *hsfB1*, resulting in potentiated levels of Hsp/Hsc70. Since increased *hsp70* and *hsf* gene expression coincide with increased levels of Hsp70 accumulation, it is concluded that SA-mediated potentiation of Hsp70 is due to modulation of these Hsfs by SA. In our efforts to understand the role of Hsp70 in heat-related disease susceptibility, the degree of the complexity of the cross-talk between the pathways in which SA is involved, *inter alia*, the plant defence response, the HSR and thermotolerance, was further underscored.(Snyman and Cronje, 2008)

Using the technique of *in vivo* incubation of the grape berry (*Vitis vinifera* L. cv. Cabernet Sauvignon) tissue in the SA-contained medium, the effects of exogenous SA on the gene expression of PAL and the accumulation of polyphenols during high temperature stress were investigated by Wen *et al.(*2008). The results showed that SA could induce the accumulation of PAL mRNA and the synthesis of new PAL protein, and increase the activity under high temperature stress. A significant accumulation of

phenolics was also observed in the SA-treated berries. But, the activation of PAL by SA could be blocked by the pretreatments of berry tissues with the protein synthesis inhibitor cycloheximide, and mRNA transcription inhibitor, actinomycin D, respectively. It is thus speculated that SA may induce the activation of PAL and the accumulation of phenolics leading to the development of thermotolerance.

Two rice (*Oryza sativa* L.) cultivars differing in chilling sensitivity, Changbaijiu (chilling-tolerant) and Zhongjian (chilling-sensitive) were pre-treated with 0.5, 1.0 and 2.0 mM salicylic acid (SA) for 24 h before chilling at 5 °C for 1 d. Chilling induced SA accumulation, particularly conjugated SA in both leaves and roots of the two rice cultivars. After SA administration, SA accumulated in the roots of both cultivars at a concentration-dependent manner, whereas only a slight increase was observed in their leaves. Conjugated SA accounted for most of the increase. The beneficial effect of SA treatment on protecting rice seedlings from chilling injury was not observed at any concentration in either cultivar. Pre-treatment with SA even decreased their chilling tolerance confirmed by increased electrolyte leakage and lipid peroxidation. Further, most of the activities of antioxidant enzymes decreased or remained unchanged in leaves and roots of SA pre-treated seedlings after chilling. These results implied that down-regulation of antioxidant defence might be involved in the reduction of chilling tolerance in SA-pre-treated plants (Wang *et al.* 2009)

Zhou and Guo (2009) tested whether Ca^{2+}, a second messenger in stress response, is involved in ABA-induced antioxidant enzyme activities in *Stylosanthes guianensis*. Plants were sprayed with abscisic acid (ABA), calcium channel blocker, LaCl3, calcium chelator, ethylene glycol-bis (β-amino ethyl ether)-*N,N,N',N'*-tetraacetid acid (EGTA), and ABA in combination with LaCl3 or EGTA. Their effects on superoxide dismutase (SOD) and ascorbate peroxidase (APX) activities and chilling resistance were compared. The results showed that ABA decreased electrolyte leakage and lipid peroxidation but increased maximum photochemical efficiency measured as variable to maximum fluorescence ratio (Fv/Fm) under chilling stress. Treatment with $LaCl_3$ or EGTA alone and in combination with ABA increased electrolyte leakage and lipid peroxidation, decreased Fv/Fm, suggesting that the block in Ca^{2+} signalling decreased chilling resistance of *S. guianensis* and the ABA-enhanced chilling resistance. ABA-induced SOD and APX activities were suppressed by $LaCl_3$ or EGTA. The results

suggested that Ca^{2+} is involved in the ABA-enhanced chilling resistance and the ABA-induced SOD and APX activities in *S. guianensis*.

The alternative pathway is a cyanide-resistant and non-phosphorylatory electron transport pathway in mitochondria of higher plants. Alternative oxidase (AOX) is the terminal oxidase of this pathway. Lei *et al.* (2010) investigated the effect of exogenous salicylic acid (SA) on alternative pathway in cucumber (*Cucumis sativus* L.) seedlings under low temperature stress. Results showed that during the process of low temperature stress, the alternative pathway capacity was enhanced as AOX expression increased in SA pre-treated seedlings. Compared with seedlings without SA pre-treatment, slower decrease of relative water content and lower levels of electrolyte leakage, H2O2 and malonyldialdehyde content were detected in SA pre-treated seedlings. These results indicated that SA could alleviate the injury caused by low temperature on cucumber seedlings. Since the special protective functions of alternative pathway and AOX in plants, we suggested that the alternative pathway was related to SA-mediated plant resistance to environmental stresses such as low temperature.