LITERATURE REVIEW

The role of environment in regulating a plant's metabolism is undisputed. Plants respond to different environmental variations by adjusting their metabolic processes to suit the particular conditions. Among the most commonly encountered stresses in nature, and, consequently, most widely studied, are the temperature, drought, as well as attacks by pathogens. A number of previous workers have carried out researches to elucidate the biochemical responses of plants to these important stresses. A brief review of literature, relating to the biochemical responses of plants to elevated temperature, drought and fungal infections have been presented in the following pages.

2.1. Biochemical responses of plants to elevated temperature

A rapid increase in the incubation temperature of whole leaves of the arctic plant *Saxifraga cernua*, grown at 10^0 C or 20^0C, resulted in a noncoordinate expression of 21 novel and (or) enhanced heat shock polypeptides. Unlike other organisms, the expression of most of the normal polypeptides was not reduced. Incorporation of radioactivity during hypothermic shifts was similar in both groups of plants at all temperatures investigated, with peak incorporation recorded at 33^0C. The temperature at which the expression of the heat shock polypeptide was at a maximum level was also 33^0C. Protein synthesis was reduced after a heat shock at 35^0C and completely inhibited at 38^0C. These data of Mason-Apps *et al.*, (1990) suggested that the pre-shock growth temperature had no effect on the temperature of either maximum expression of the heat shock polypeptides or the level of protein synthesis during the heat-shock. Recovery at 10^0C from a 30^0C heat shock was completed within 7-9 h. Extended time periods (up to 24 h) at the shock temperature of 33^0C demonstrated that the heat shock response in this arctic plant was multiphasic, consisting of an early group and five sets of late heat-shock polypeptides.

Heat shock protein synthesis and accumulation in diploid wheat was studied by Vierling *et. al.*, (1990). The objective of their study was to determine if genotype differences exist in the synthesis and the accumulation of HSPs in diploid wheat *Triticum monococcum.* Plants of three*T. monococcum* accessions were heat shocked at 37^0C in a controlled condition for one hour and HSPs ware separated by 2D gel

electrophoresis. Qualitative and quantitative diversity of HSPs synthesis in *T.monococcum* was noticed. Optical density of 24 HSPs ranging from 0.33 to 44.64 kD on silver stained gels were identified. The authors concluded that the identification of genetic variability in heat shock protein synthesis within diploid wheat might provide a useful tool for genetic and physiological studies of the role of heat shock proteins in higher plants.

Halle *et al.*(1990), carried out experiments with the leaf segments of *Brassica napus*, which were exposed to 22, 35, 38 or 40^{0}C for upto 4 h. Analysis of radiolabeled proteins by two dimensional sodium dodecyl sulfate – polyacryalmide gel electrophoresis and fluorography revealed two major groups of heat shock proteins (HSPs). One group comprised HSPs 70, 76 and 87, with (pIs) isoelectric points ranging from 5, 7, to 6.1, where as the second group had molecular masses ranging from 23 to 26 kDas and pIs from 5.6 to 6.9. Immunoblot analysis, using antibodies directed against the large (RLSU) and small (RSSU) subunits of RUBISCO showed that increasing temperatures from 35 to 38^{0}C or 40^{0}C for the duration of thermal stress (i.e., from 1 to 5 h), did not affect levels of the RSSU (15kDa), whereas the levels of the RSLU (52kDa) fell sharply. Nevertheless, the activity of RUBISCO was not adversely affected at 37^{0}C for periods of upto 5 h. Northern blot analysis revealed that the increase observed in HSP70 synthesis during heat shock may be transcriptionally regulated, but the decrease in the RLSU was not accompanied by a corresponding reduction in levels of its mRNA.

Barley heat shock protein have been cloned and characterized by Kruse *et al.*, (1993) by hybrid release translation and sequenced. Clones coding for proteins of 17, 18, 30, 32 and 70 Kda have been obtained. Out of these the 30 and 32 kDa proteins have been characterised as precursors to plastidic proteins of 26 kDa by post translational transport and by cDNA sequencing. The coding regions of these two transcribed genes are highly homologous. Accumulation of the plastid HSP and of HSP 70 as well as their corresponding mRNA has been studied in 2-6 days old seedlings and in the 7-day-old leaf. The mRNA for all investigated proteins were only found after a heat shock; the mRNA level increased towards the tip of the leaf and with development. Furthermore, under the conditions used the mRNAs for all investigated heat shock proteins accumulated in parallel. Unexpectedly both proteins,

HSP 70 and HSP 26, were found by Western blotting in the 2-day-old control plants in the absence of any inducing heat shock. At later stage of development and in the leaf gradient only immunoreactivity with HSP 70 was observed. In contrast to the levels of their mRNAs the highest levels of HSP 30-26 and 70 have been observed in the basal segments indicating that translational control plays a role during HSP expression. Under severe heat shock a protein of 30kDa was induced whose identity was not known but which reacted with the antibody to HSP 30-26 and might represent the accumulating processors of the plastidic proteins.

Blumenthal *et al.*, (1995) designed experiments to identify wheat genotypes that might be tolerant to the effects of heat stress on grain quality and to further assess the molecular basis of these changes. A diverse set of 45 wheat genotypes were exposed to 10 hours of 40^0C on each of three consecutive days in a phytotron. Mean values of all the genotypes showed highly significant changes in 1,000 kernel weight (-17% difference for heat stress minus control), protein content (17% increase), dough mixing time in a 2g Mixograph and resistance breakdown (17%). The general weakening of dough due to heat was accompanied by a decrease in glutenin to gliadin ratio and in the percentage of very large glutenin polymers. Bound lipid content increased, and there was a general reduction in the proportion of small starch granules. For all these attributes, reactions for individual genotype ranged from little change (tolerance to heat stress) to considerable change (susceptible to heat stress). They thus identified groups of genotypes that should be useful in breeding attempts to stabilize wheats against heat related variations in grain quality. Markers identified as potentially useful in breeding for tolerance include the presence of the Glu-Dld allele, and increase in glutenin- to- gliadin ratio and in the percentage of very large glutenin polymers.

According to Stone and Nicolas (1995) short periods of very high temperature (>35^0C) are common in many of the world's wheat growing areas and can be a significant factor in reducing yield and quality of wheat. A study was conducted by them to determine the stage at which the grain growth was most sensitive to a short period of high temperature and to examine whether varietal difference in heat tolerance were expressed in whole grain filling period. Two varieties in wheat differing in heat tolerance (cvv. Egret and Oxley) were exposed to a short (5 days)

period of very high temperature (40^{0}C max. for 6 hours each day) at 5 day interval throughout grain filling, starting from 15 days after anthesis (DAA) and concluding at 50 DAA. Response of grain dry matter accumulation and water content to high temperature were monitored throughout grain filling, and the result compared with the control maintained at 21/16 ^{0}C day/night. Varietal difference in heat tolerance was expressed throughout the grain filling period. Mature individual kernal mass was most sensitive to heat stress applied early in grain filling and became progressively less sensitive throughout grainfilling, for both varieties. Reduction in mature kernel mass resulted primarily from reductions in duration rather than the rate of grain filling. To study the fractional protein accumulation in same experimental conditions, grain samples were taken through grain growth and analysed for protein content and composition (albumin/globulin, monomer, SDS soluble polymer and SDS insoluble polymer) using size exclusion high performance liquid chromatography (Stone and Nocholas, 1996). The timing of heat stress exert a significant influence on the accumulation of total wheat protein and its fractions, and protein fractions differed in their responses to the timing of heat stress. Furthermore wheat genotype influenced both the sensitivity of fractional protein accumulation to heat stress and the stage during grain filling at which maximum sensitivity to heat stress occurred.

Gagliardi *et al* (1995) analysed the expression of heat shock protein 70 (HSP70) and heat shock factor (HSF) gene during maize pollen development. They showed that in the absence of heat stress, HSP70 genes were highly expressed in late-bicellular pollen as compared to the other stages. HSP70 transcript were significantly accumulated in response to heat stress at the late microspore stage but to a much lower extent than in vegetative tissues. The latest stage of pollen development, i.e. mid-tricellular and mature pollen, did not exhibit heat induced accumulation of HSP70 transcripts. Therefore, they analysed the expression of hsf genes throughout pollen development. They demonstrated that at least three hsf genes were expressed in maize and that transcripts to one hsf gene, whose expression was independent of temperature in somatic as well as in microgametophytic tissues, were present at similar levels throughout pollen development. In addition, they showed that, the expression of two other hsf genes is heat inducible in maize vegetative tissues and is not significantly increased after heat shock in any stage of pollen development. This result indicated

that the loss of hsf gene expression at late stage of pollen development was not due to a modification of hsf gene expression at the mRNA level and that hsf gene expression was differently regulated in vegetative and microgametophytic tissue.

Grass and Burris (1995a) carried out experiments with two wheat cultivars Marzak and Oum-rabia, which were subjected to three temperature regimes (20/15, 28/21,36/29 degrees C) beginning 10 d after anthesis to maturity. High temperature resulted in low values of seed yield and physical traits of seed quality. The effect of temperature on seed germination was not consistent among the two cultivars. High temperature during seed development and maturity had no effect on seed germination of Oum-rabia, whereas it decreased seed germination of Marzak. In contrast to seed germination, seed vigor was adversely affected by heat stress. This decline in seed vigor was reflected in reduced shoot and root dry weight, in increased shoot/root ratio, reduced root length, low root number per seedling, and high seed conductivity. Excised embryo culture showed marked differences in embryo growth potential. Although embryo from all treatments had germinated, a delay of 24-48 h was observed in the germination of embryos excised from seeds grown under high temperature conditions. Also their shoot and radical development over time lagged behind that of embryos isolated from seed grown under cool temperature conditions. Exposing seeds to high temperature during development and maturity also resulted in low oxygen uptake. They (Grass and Burris, 1995b) also determined the effect of heat stress in case of nucleotide level and respiratory activity of mitochondria. Embryos from low temperature treatment showed rapid accumulation of ATP and higher energy levels and rates of oxygen uptake than embryos from high temperature treatment. Embryos from medium temperature treatment exhibited intermediate values. Mitochondria from low temperature regimes were well developed with visible membranes and cristae; those from the high temperature regimes were degenerating.

High temperature tolerance of the pollen of *Petunia lybrida* L. and *Nicotiana sylvestris* L. was investigated by Rao *et al.*,(1995) by treating dry pollen to temperature of up to 75^{0}C for 6-48 h and by studying their viability (by flurochromatic reaction test), vigor, and their ability to set fruits and seeds. In *Petunia*, temperatures of upto 60 ^{0}C for 48 h did not affect pollen viability, vigour and their fruit and seed setting ability. A temperature of 75^{0}C for 24 h reduced the pollen viability and vigour,

but fruit and seed-setting ability existed. However, at 75^{0}C exposure for 48 h proved lethal for *Petunia* pollen. In *Nicotiana*, pollen exposed to temperature of up to 75^{0}C for 6-12 h were able to set seed. With a longer exposure the majority of pollen were FCR –positive, but they were unable to set seed. This result showed that pollen grains of *Petunia* and *Nicotiana* could withstand exposures of temperatures of as high as 75^{0}C and retain pollen function. This study also indicated that FCR test may not reflect true viability in pollen subjected to extreme stresses.

Using antibody raised against two-sunflower small heat shock proteins, Alamillo *et al* (1995) detected immunologically related proteins in unstressed vegetative tissues from the resurrection plant *Craterostigma plantagineum.* In whole plants, further accumulation of these polypeptides was induced by heat- shock or water stress. In desiccation intolerant *Craterostigma* callus tissue, they failed to detect small heat shock protein related polypeptides, but their expression, and the concurrent acquisition of desiccation tolerance was induced by exogenous ABA treatment. In unstressed plants, the cross-reacting polypeptides were abundant in the roots and lower part of the shoots, where they showed homogeneous tissue distributions. This constitutive expression was novel for vegetative tissues of higher plants, and resembled the expressions of small heat shock proteins in desiccation-tolerant zygotic embryos and germinating seeds.

A system for the controlled expression of a foreign gene in the cultured tobacco cells (*Nicotiana tabacum*, BY2) by temperature shift was constructed by Yoshida *et al*, (1995). A 925 base pair DNA fragment containing the 5' flanking region of a low-molecular-mass heat shock protein gene (HSP18.2) of *Arabidiopsis thaliana* was inserted upstream of the beta-glucoronidase reporter gene (GUS). The resulting HSP18.2 GUS construct was introduced into BY2 cells by electroporation or *Agrobacterium* mediated transformation. Transient expression of HSP18.2 promoter in protoplast was very low regardless of the heat shock. Although expression of the HSP 18.2 .GUS chimeric gene in the stable transformants of BY2 was hardly detected in culture at 25^0 C, the expression increased rapidly on the transcriptional level when the incubation temperature was shifted to 35-37^0 C. After a 2 hour incubation at 37^{0}C, GUS activity was about 1000 fold greater than that before heat shock. The amount of GUS mRNA was maximum 2 hour after heat shock, and then decreased gradually.

Lipid composition of microsomes of heat stressed suspension culture was studied by Styer *et al.* (1996). Heat stressed (30^0 C) cell suspension cultures of carrot attained a lower maximum cell density and showed browning earlier when compared with control cultures (22^0 C) over a 16 day growth period. Phospholipid class profile did not differ between cell grown at 30^0C and 22^0C. The fatty acid of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) form microsomes of heat stressed cells were less saturated than those of PC and PE from microsomes of control cells. In particular, there was a marked increase in the proportion of oleate [18:1(9)] at the expense of linoleate [18:2 (9,12)] at the higher growth temperature. This difference could result from inhibition or loss of the microsomal lipid linked desaturase which inserts the double bond between carbon 12 and 13 of oleate esterified to the glycerol moiety of PC and PE.

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

Based on partial or complete sequence of 14 plant heat shock transcription factors from tomato, soybean, *Arabidopsis* and maize, Nover *et al.*, (1996) proposed a general nomenclature with two basic classes, i.e. classes A and B containing two or more types of Hsfs (HsfA1,HsfA2). Despite some plant's specific peculiarities, essential functional domains and modules of these proteins are conserved among plants, yeast, *Drosophila* and vertebrates. Similar to the situation with the small heat shock proteins, the complexity of the hsf gene family in plants appears to be higher than in other eukaryotic organisms.

A flash-induced trans-thylakoid electric field was measured by Havaux *et al.*, (1996) at 515nm as an electrochromic absorbance shift in intact potato leaves using a

double flash defferential spectrophotometter. The decay rate of the electrochromic shift in dark adopted samples was used to examine the conductance to ions of thylakoid membranes. Heat stress (39.5^{0}C for 15 min.) was found to accelarate drastically the electric field decay, with the half decay time falling from more than 200ms to less than 45ms. Heat induced accelaration of the electric field breakdown was insensitive to the PSII electron donor hydroxylamine and to the ATPase inhibitor.

De *et al.* (1996) showed the accumulation of proline in the seedlings as well as cultured cells of tomato as a consequence of short time heat shock (45^{0}C for 4 and 8 hours) and cold shock (4^{0}C for 4 and 8 hour) treatment. The involvement of calcium ion in the proline accumulation was demonstrated by using specific calcium chelator, EGTA and channel blockers. $CaCl_3$ and $CaCl_2$ pretreatment stimulated the accumulation of proline both in high and low temperature treated cultured cells and seedlings of tomato.

Heat tolerance in 23 tropical and one temperate fruit crop was evaluated by Yamada *et al.*, (1996) by determining chlorophyll fluorescence [the ratio of the variable fluorescence to the maximum fluorescence (F-v/F-m), and the basal fluorescence (F-O)]. The ratio [R(v)] of F-v / F-m in leaves exposed to high temperature (45^{0}C for 20 min.) to F-v /F-m to control temperature (25^{0}C for 20 min.) was found to be highly and negatively correlated to the ratio [R(O)] of F-O exposed to the high temperature to F-O exposed to the controled temperature. Leaves (3.5 months old) sampled in mid July were slightly but significantly more tolerant to heat than 2.5 months old leaves sampled in early to mid June. The ratio of the genetic variance to the total variance in the measurements was 0.90 for R(v),and 0.89 R(O). Pineapple, coconut palm, and *Annona* species were heat tolerant, java apple ,rose apple, longan, and peach was sensitive.

According to Bacci *et al* (1996) in the leaves of herbaceous plants, sub optimal temperatures influence the content and efficiency of the photosynthetic pigments and, in more severe cases, alter mesophyll thickness. They examined the possibility of detecting the degree of alteration in sorghum leaf characteristics by indices of stress calculated from remotely sense data. Reflectants, colorimetric and ecophysiological measurements were performed, on two cultivars of sorghum [*Sorghum bicolor* (L.) Moench.], grown at 15^{0}C, 21^{0}C and 32^{0}C. Compared to plants growing at 21^{0}C, the

other two temperatures reduced the chlorophyll content and PSII efficiency in the leaves, which were less at 15^{0}C than at 32^{0}C. Slight differences, in these responses to temperature was also observed between the two cultivars. Colorimetric coefficients detected a significant discoloration at 32^{0}C and a marked reddening at 15^{0}C. The indices calculated from the colorometric data were able to distinguish the differences between treatments, but they did not show a strict relationship with the trend of ecophysiological parameters.

Longitudinal halves of freshly harvested avocado fruit (*Persea americana* Mill, 'Hass') were pretreated at 38^{0}C for 1 hour in a water bath, while the other halves remained at 20^{0}C in air. Then the entire fruit was either treated from 1-10 min. at 50^{0}C. or held at 20^{0}C (as control). Fruit quality (daily evaluation of browning and internal quality when ripe), and pulse amplitude modulated (PAM) fluorescence measurements, were made on the skin of each fruit half 1 hour after hot water treatment (HWT), 3 hours later, and each subsequent day after ripening. The pretreated half of the fruit showed almost no development of external browning during the ripening period, while the nonpretreated halves were severely damaged by HWTs. External browning increased with longer HWT duration. Heat damage was also evident as hardening of the skin when fruit ripened, and such damage was reduced by heat treatment and increased with longer HWT duration. HWT had a rapid and marked effect on chlorophyll fluorescence (F-V /F-M ratio) of avocado skin. Whereas fluorescence of the control fruit remained constant over the first 5 days, in both pretreated and nonpretreated fruit, within 1 hour of HWT, the F-V /F-M ratio had dropped to the minimal levels, with little further change. The value of F-V /F-M ratio 3 to 6 hours after the HWT was directly related to the duration of the HWT. Although pretreatment almost eliminated the browning, little effect of pretreatment could be detected in the F-V /F-M ratio. There was a strong negative correlation between external browning and F-V/ F-M ratio, for nonpretreatted fruit, but this correlation was not significant for pretreated fruuit. Woolf and Laing (1996) concluded after this study that, chlorophyll fluorescence clearly reflects the effects of heat on the photosynthetic systems in avocado fruit, but dose not detect the alleviation of heat damage by heat pretreatments.

According to Owuor and Obanda (1996) high leaf temperature during the withering process of black tea manufacture decreases the theaflavins, brightness, flavor index, and sensory evaluation scores of black tea. Black tea manufactured with withering temperature 30^0C have high thearubigins and total color levels but lack briskness. Their results suggested a need to control the withering temperatures to below 30^0C.

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

The effect of heat stress on subcellular localization of Ca^{2+} in tomato fruits was studied by Garbaczewska *et al.*, (1998). The tomato plants Robin cv., relatively tolerant to heat stress, were grown under uncontrolled greenhouse conditions to the stage of fruiting. The plants were placed for 20 hours in two temperature regimes: 23^0C (optimal temperature) and 40^0C (heat stress) in darkness, under water vapor saturated atmosphere. Immediately after heat stress the fruits were harvested to estimate the water soluble and insoluble calcium contents and subcellular localization of Ca^{2+}. After heating, the concentration of calcium in tomato fruits increased about twice. In both temperature treatments the water-soluble fractions were lower than insoluble ones however, smaller differences between insoluble and soluble fractions were obtained after heat stress. The shapes and localization of ca^{2+} detected with the use of potassium antimonate method showed that in fruits of control plants the precipitates were numerous, small and oval shape. They were dispersed in cytosol or adjoined to endoplasmic reticulum or to external membrane of chloroplast. In the fruit

of heated plants the precipitates were irregular in shape, amorphous and singly dispersed in cytosol. They also observed some cytosolic changes in the structure of membranes and organelles of the plants of both experimental treatments. The heat induced increase of calcium content and changes in subcellular localization of ca^{2+} under heat stress showed that calcium ions may be involved in avoiding heat injury.

According to Craufurd *et al* (1998) reproductive processes and pod yield in cow pea (*Vigna unguiculata* (L.) Walp), an important crop grown in semi – arid sub-Saharan Africa, are adversely affected by high temperature. Genotypic differences in heat tolerances have been identified under hot, long days, but it was not known if this tolerance was also exhibited in hot, short day environments typical of sub-Saharan Africa. The authors conducted the study to determine whether heat tolerance identified under hot, long days was expressed at the same stages of development under hot, short days, and whether responsiveness to temperature was additive and quantitative. A heat tolerant (Prima), and heat susceptible (IT84S-2246) cultivar of cowpea, were grown in controlled environments under short days (12 h day (-1), initially at 30^0C / 24^0C (Mod-T), and then transferred at 0, 10, 20, 30, 40 days after emergence (DAE) to 36^0C /27^0C (High –T), where they remained for 5, 10 or 20 days duration before returning to Mod-T.Control plants remained at Mod-T or High-T for 50 days, when the first pods were mature and the experiments were terminated. There was significant effects of duration (D) and timing (T), and interactions between D x T, T x genotype (G) and D x T x G on pod weight plant (-1). Prima was significantly more tolerant to high temperature during flowering than IT84S-2246 confirming that heat tolerance was expressed under hot, short days. The greater heat tolerance of Prima was associate d with an ability to maintain peduncle and flower production at High-T, and with greater podset. The sensitive period in IT84S-2246 started at floral bud initiation (15-20 DAE), and effects of High-T thereafter were additive and quantitative.

A cDNA library from a mixed population of female and male gametophyte of *Griffithsia japonica* Okamura was constructed, and a cDNA clone, designated as GJFP-1 (G. *japonica* female predominant -!), was isolated by differential screening of the cDNA library. The transcript corresponding to GJFP-1 was abundant in female gametophytes, but only basal levels of the transcript were detected in male and

tetrasporangial thalli. Determination of the nucleotide sequence of GJFP –1 identified a putative open reading frame encoding 313 amino acids and a 3' untranslated region of 116 nucleotides. The deduced amino acid sequence of GJFP-1 for the putative open reading frame shared high homology with the previously reported amino acid sequences of heat shock protein 90. RNA blot hybridization analysis for the transcript of the heat shocked G.*japonica*. *In situ* hybridization for the GJFP-1 transcripts again showed a differential specificity of the transcripts in female gametophytes. The data reported here suggest a possibility of hsp 90 function linked to the development of the female gametophyte in G. *japonicum* (Lee *et al.*,1998).

Pareek *et al.*, (1998) reported that while the rice 87 kDa protein was transiently synthesized within initial 2 hours of heat shock, high steady state level of this protein were retained even under prolonged high temperature stress condition or recovery following 4 hour heat shock. It was further shown that, fifteen different wild rice accumulated differential levels of these proteins in response to heat shock treatment.

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

Schraf *et al.*, (1998) used Hsf knock out strains of yeast and transient reporter assays in tobacco protoplast for functional analysis of HSF – coding cDNA clones and mutants derived from them. Hsf A2, which in tomato cell cultures was expressed after heat shock induction, trended to form large cytoplasmic aggregates together with other Hsps. In the transient expression assay its relatively low activator potential was evidently due to the inefficient nuclear import. However, the intermolecular shielding of the NLS could be released either by deletion of short C-terminal fragment or by coexpression with HsfA1, which form hetero- oligomers with HsfA2.

Sorghum sterile 3A line was induced to be fertile when it was heat shocked. By comparing mitochondria heat shock proteins of 3A line with 3B line, it was found that

hsps were encoded by nuclear DNA and were transported into mitochondria after synthesized in the cytoplasm. When heat shocked for 2 hours, 3A line produced 5 protein bands which weighed 70, 31, 24, 18 and 16 Kda respectively, whereas in 3B line, additional 96 Kda and 94 Kda bands appeared, and the amount of Hsp 70 was greater than that in 3A line. When heat shock went on for 4 hours, 96 Kd and 94 Kd Hsps in 3B line disappeared and 3B tended to be identical with 3A on Hsps. After heat shock treatment, the amount of mitochondria total protein increased greatly in both 3A and 3B. Then, there was a sudden drop of Hsps. On the 8th hour, 3B line had only 4 bands, which weighed 70, 31, 24, and 16 Kda respectively and 70 Kda was especially obvious, while in 3A line, all Hsps disappeared. This indicates that Hsps are stable in 3B line but deficient or unstable in 3A line. Chen *et al* (1998) concluded that the difference could be relevant to the stability of fertility of 3B line as well as the infertility of 3A line.

Edreva *et al.* (1998), analyzed non acclimate bean plants in which heat shock induced oxidative damage (increase of free radical concentration and drop of bound thiols, indication of aggregation of proteins) which was regulated by the enhanced activity of peroxidase and superoxide dismutase, as well as by the accumulation of polyphenols and especially of polyamines. In the plants acclimated to high temperature no oxidative damage occurred following heat shock.

The involvement of kinases in heat stress signaling in tomato cells was studied by gel kinase assay using myelin basic proteins as substrate, and by *in vitro* phosphorylation assays in Mono O fractions of tomato cell lysates (Heider *et al.* 1998). A kinase with an apparent molecular mass of approximately 509 kDa was rapidly deactivated upon heat stress as judged from in gel kinase assays. Cycloheximide treatment increased kinase activity, but concomitant heat treatment abolished cycloheximide-induced activation.

In order to test the effect of Ca^{2+} on heat shock induced changes in cell protein synthesis, the incorporation of (35-S) methonine into protein was studied in cultured sugar beet (*Beta vulgaris* L.) cells incubated in media containing different calcium concentrations. Heat shock inhibited the synthesis of non-heat shock proteins and promoted the synthesis of HSPS, typical of plants. The synthesis of non-heat shock proteins was greatly inhibited by external Ca^{2+} removal by the treatment of the cells

with ethylene glycol–bis (β–aminoehtylether)-N, N, N', N'- tetraacetic acid. In contrast, extracellular ca^{+} appeared not to be strictly required for the *de novo* production of Hsps, but their cation exerted different effects on the synthesis of individual Hsps. Cell injury increased if ht cells were exposed simultaneously to high temperature and Ca^{2+} deficient medium. Recovery of Hsps synthesis and reduced cell injury were observed after addition of exogenous calcium to Ca^{2+} depleted cells. These findings are consistent with a Ca^{2+} requirement for the survival of the cells under heat shock and likely for the development of the cell thermotolarance (Trofimova *et al*, 1999).

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

Ristic *et al* (1999) showed a band of heat shock proteins of 45 KD in a leaf tissue of drought and heat tolerant maize line, ZPBL 1304. This band has not been previously described in maize line and did not appear to be common in higher plants.

It was not known how many polypeptides comprised this 45 KD band. For heat shock polypeptide study, plants were exposed to two environmental stress conditions, soil drying and high temperature (45^0C) and high temperature (45^0C) alone. Generally the pattern of heat shock polypeptide synthesis in both conditions was same. 2D electrophoresis revealed 3 heat shock polypeptides of 45 KD with isoelectric points ranging from 5 to 5.5 and 2-heat shock polypeptides of 46 KD slightly above 5.5. Drought alone did not induce the synthesis of protein of 45 KD.

Two cDNAs, TaHSP23-5 and TaHSP 23-6, encoding proteins with homology to mitochondrion localized (MT) small heat shock proteins (sHSPs) were isolated from a heat shock cDNA library from *Triticum aestivum.* Ta HSP 23-5 specified a 214 amino acid protein and Ta HSP 23-6 specified a 216 amino acid protein. Amino acid sequence identity was only 45.7% between the two proteins. However, both proteins showed greater identity to MT sHSPs of other plant speices than to any other sHSPs from wheat. Amino acid sequence alignments with other MT sHSPs identified the putative amino terminus of the mature proteins and consensus regions specific to this class of sHSPs. Transcripts of both genes were absent from control tissue, but strongly induced by heat stresses. Phylogenetic analysis indicated that these two wheat genes arose by duplication after the divergence of monocots and dicots (Basha *et al,* 1999).

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

Lin *et al* (1999) observed that 70 stress molecular chaperones are found in all the major subcellular compartments in plant cells and a multigene family encodes them. Twelve members of this family have been identified in spinach. The expression

of the stress 70 molecular chaperone in response to heat shock is well known and it appears that low temperature exposure can also stimulate their expression. However, it was difficult to determine which members of the family are specifically responsible to low temperature. This study was concluded to determine the levels of expression of the stress 70 family members and other selected chaperones in response to high and low temperature exposure. During heat shock of spinach, of the ten stress 70 family members that were examined, all 10 showed increased RNA levels after 1 hour, and all showed down regulation at longer duration of high temperature exposure.

2.2. Biochemical response of plants to water stress

Mesophyll and bundle sheath cells of maize leaves (*Zea mays* L.) both contain the enzymes ascorbate peroxidase (AP) and glutathione reductase (GR) which are involved in hydrogen peroxide detoxification. Since bundle sheath cells of maize are deficient in photosystem II and high CO_2 levels, oxidative stress may be less severe in these cells than in mesophyll cells. Brown *et al.* (1995) conducted a study to determine if AP and GR activity levels preferentially increase in mesophyll cells relative to bundle sheath cells when plants are subjected to moderate drought. Although drought inhibited the growth of greenhouse grown plants, it did not affect the levels of proteins, chlorophyll, AP. GR was unaffected by drought in whole leaf tissue and mesophyll cells, but did increase slightly in bundle sheath cells. This slight increase is of questionable biological importance. AP and GR activity levels were similar in mesophyll cells, bundle sheaths cells and whole leaf tissue. This data suggest that moderate drought has little effect on the enzymes of the hydrogen peroxide scavenging system and that mesophyll and bundle sheath cells may be exposed to similar levels of hydrogen peroxide.

Wheat genotypes DS-4, Chakwal –86, (better performer) Pavon, DS-17 (poor performer) were raised under 0, and –0.6 MPa (PEG –6000) for 14 days and harvested by Ashraf *et al.* (1995) to study growth parameter; activities of selected enzymes and nucleic acid metabolism. It was observed that root and shoot lengths, root shoot dry weights and activities of enzymes, except that of peroxidase decreased in all the genotypes subjected to water stress. However, reduction in activities of enzymes was comparatively less in better performing genotypes.

The relationship between the changes in putrescine (Put) and polyamine (PA) contents and proline accumulation induced by osmotic stress were investigated by Aziz and Larher (1995) using rape leaf discs (RLD) as an experimental model. The aliphatic PAs, Put, Spermidine(Spd) and Spermine (Spm), were detected in fresh cut RLD and the most abundant among them was Spd. In addition, the HPLC profile of the benzoylated PAs presented an unknown peak X23 that eluted after that of Spm and did not correspond to any commercially available PAs. X23 occurred also under conjugated forms which were not destroyed in acidic conditions. The study of the time courses of the changes in the levels of free PAs, X23, and proline demonstrated that in response to a moderate osmotic shock (-1.5 MPa) enhancement of PAs were detected after 2 h lag phase, while the onset of proline accumulation started 2 h later. When RLD were submitted to wilting or to increased osmotic stress, using a range of osmotica, they exhibited osmotic adjustment which can be associated with an increase in Put and Spd contents followed by a moderate rise of Spm. Both free and conjugated PA levels changed.

The effect of phosphorus (P) and soil water availability (W) on the growth and development of wheat plants (*Triticum aestivum* L.cv. Minaret) was studied by Rodriguez and Goudriaan (1995) in a pot experiment. Four levels of P supply (0, 15, 30, and 100 mu gP /g soil) were applied before sowing. Thirty-four days after sowing (DAS), the pots were kept near 100% of field capacity (FC). From 34 DAS until one week before anthesis (67DAS), half of the pots were maintained between 60-70% FC. Control pots were kept at 85-95% FC by weighing and watering the pots every two to three days. Shoots were harvested four times before anthesis and twice after. At each harvest, dry matter and P accumulation was measured in leaves, stems and ears. In this study, thermal time until anthesis was inversely related to the level of P application. Phosphorus additioning affected the allocation of biomass and P in aerial plant organs. Plants growing only with soil P showed a delay in the allocation of dry matter and P into leaves and stem with respect to plants fertilized with 100mu g P/ g of soil. In this study, the authors showed that the final composition of the grain was dependent on re-mobilization from other plant organs.

Changes in amino acid composition of alfalfa (*Medicago sativa* L.) phloem sap were studied in response to a water deficit. Sap was collected by stylectomy. As

the leaf water potential (Psi) decreased from –0.4 to –2.0 MPa, there was a significant increase in the total amino acid concentration, due to that of some amino acids: proline, valine, isoleucine, leucine, glutamic acid, aspartic acid, and threonine. Asparagine concentration, which is the main amino acid assayed in the phloem sap of alfalfa, did not vary with the plant water status. The other amino acid concentration remained stable as Psi varied; in particular, gamma amino butyric acid concentration remained unchanged, whereas it varied in response to wounding. The more striking change in the sieve tube was the accumulation of proline, which was observed below a Psi threshold value of about –0.9 to -2.0 MPa (concentration x 60 for a decrease of Psi from –0.9 to –2.0 MPa). The role of such ranges in phloem sap amino acid concentration in osmotic adjustment of growing tissue was discussed by Girousse *et al.*(1996).

Seedling survival of drought during the first days following germination can be one of the most critical factors in successful establishment of the spices. Seventy–two hour old seedlings of *Cerastium fontanum* Bamug. and *Lotus corniculatus* L., were exposed to severe desiccation for 36 h and the recovery of the whole plants was monitored over 17 days by Olsson *et al.* (1996). The analysis exposed the very different responds to water stress in the two species. The effect of water stress on the less drought tolerant *L. corniculatus* within the first 5-10 days after drought treatment included a two fold larger loss in dry weight than in *C. fontanum* and a two fold rise in lipid peroxidation, in triacyl glycerols and in free fatty acids. The ratio of monogalactosyl diacylglycerols/ digalactosyl diacylglycerols (MGDG/ DGDG) declined 3 fold, while the proportion of MGDG was some twelve fold lower. In contrast, in the relatively drought tolerant seedlings of *C. fontanum* no change of this order were recorded in the days immediately following rehydration.

The effect of water stress on growth of *Triticum durum* L., was investigated by Kameli and Losel (1996) in relation to sugar accumulation and water status of wheat plants before, during and after a period of water stress. The slight decrease in water potential in the first few days after withholding water had no detectable effect on growth. Inhibition of growth was only apparent when the water content started decline. Dry weight continued to increase during water stress, even under severe water stress (after day 27) which was associated with a sharp rise in sugar content

accounting for 20% of the grain in dry matter between days 27 and 31. The increase in leaf length and leaf area of stressed plants following re-watering, from day 37, was owing to the leaves regaining turgidity after wilting. Growth inhibition coincided with a considerable increase in sugar content. Photosynthesis rather than reserve starch might be the major source of sugar accumulated under water stress in durum wheat.

Potatoes grown for processing in irrigated regions of the Pacific Northwest sometimes develop undesirably high concentration of reducing sugars in tuber stem ends due to hot weather and water stress during tuber development. Such tubers usually produce French fries with dark stem ends or sugar ends. In order to better quantify the relationship between water stress and stem end sugar levels for Russet burbank, single episodes of transitory water stress were established by Eldredge *et al.* (1996), by delaying irrigation until soil water potential ranging from –32 to –107 kPa were reached during early tuber bulking. To determine when the increase in reducing sugar occurred, tubers were sampled before transitory stress, at maximum stress, after stress were relieved with sprinkler irrigation, and post harvest. Reducing sugar concentration did not increase in tuber stem end until two weeks or longer after the plant water stress was relieved. Increased reducing sugar concentrations were positively associated with decreased soil crater potential (drier soil). Tubers were sliced and fried at harvest and six weeks post harvest. Decreasing soil water potential (drier soil) was associated with progressively darker fry colors at harvest and post harvest. The effect of imposed water stress on tuber stem end reducing sugar concentrations was most pronounced in post harvest.

Muller *et al.*, (1996) carried out an experiment, to examine how the pool of non-structural carbohydrates in soybean root nodules are affected under water stress conditions depending on the nature of symbiont strains with particular emphasis on the plant borne carbohydrates sucrose and pinitol, and on tetrahalose, a compatible solute synthesized by the bacteroids. Soybean plants were inoculated with the nitrogen fixing strains *Bradyrhizobium japonicum* 61-A-101 or USDA 110 spc4 and cultivated axenically under conditions in which nodules formed in an upper soil compartment while roots for water supply grew into a compartment filled with nutrient solution. When the nodules were well established (1 month post inoculation), 10% w/v PEG 6000 was added to the nutrient solution. This led to a slowly progressing, moderate

water stress, as determined by measuring the decrease of transpiration, and to a decrease in nitrogen fixation. The pool sizes of the major non-structural carbohydrates changed during progression of water stress. Sucrose, the major soluble carbohydrate in nodules of unstressed plants (2 and 4%, respectively of nodule dry weight depending on symbiont strain), strongly increased in nodules of stressed plants, reaching nearly 10% of dry weight. The activities of two major sucrose consuming enzymes, sucrose synthase and alkaline invertase, decreased markedly in nodules of stressed plants. Starch decreased only transiently upon water stress. Pinitol increased more than 4 times, reaching about 1% of nodule dry weight during the stress. Tetrahalose, the major soluble carbohydrate synthesized by the bacteroids, increased in nodules colonized by USDA 110 spc4 from about 0.2 to 0.8% of nodule dry weight, while in nodules colonized by 61-A-101 it amounted to more than 1.5% of dry weight of both with and without stress.

The sensitivity of seed composition to drought was compared by Bouchereau *et al.* (1996) among three spring glass- house grown rapeseed genotypes by applying water shortage treatments at various stages of development. All traits under study associated with the biochemical composition of the seed were drastically modified in plants subjected to drought during flowering. Water shortage during a restricted period in the early stage of vegetative growth was also important for seed quality. Despite limited fluctuations in total lipid content, changes in fatty acid compositions were found, especially in the erucic acid metabolic pathway (i.e. oleic, gadoleic and erucic acid). A slight increase in seed protein concentration was observed after early vegetative and flowering drought treatments. The total sugar contents of seeds were not significantly affected by water deprivation. Significant effects of drought stress, depending on its timing, were observed in the accumulation of secondary metabolites (i.e. phenolics and glucosinaolates) which are of major importance for rapeseed meal quality.

According to Champolivier and Merrien (1996) yield and yield components were mainly affected by water shortage occurring from flowering to the end of seed set in oil seed rape. The greatest reduction (48%) was observed when only 37% of full water requirement were supplied to the plant during this stage. The number of seeds per plant was the main yield component affected. Some compensation occurred when

the water supply was restored. The 1000- seed weight was only affected by a water stress from the stage when the pods were swollen until the seeds colored stage. The results demonstrated a marked reduction in oil concentration when water deficit occurred from anthesis to maturity. There was an inverse relationship between oil and protein concentration. The most marked effect observed in this experiment, was on the glucosinolate concentration where increase of up to 60% was observed.

Long term drought stress on photosystem II (PSII) was studied by Giardi *et al.* (1996) in pea seedlings. Drought stress (reduction of water content by 35-80%) led to a considerable depletion of the PSII core, and the remaining PSII complex appeared to be functional and reorganized, with a unit size (LHCP/PSII core) two fold greater than that of well irrigated plants. By immunoblotting analysis of the PSII proteins from grana and stroma lamellae, the enhanced degradation of CP43 and D1proteins was observed in water stressed plants. Also water stress caused increased phosphorylation of the PSII core and increased D1 protein synthesis. Water stress mediated increase in D1 protein synthesis did not occur when plants were exposed to photoinhibitory light. The depletion of the PSII core was essentially reversed when water stressesd plants grown at low visible irradiance were watered. They suggested that the syndrome caused by the effect of long term water stress on photosynthesis is a combination of at least two events: a reduction in the number of active PSII core and a PSII reorganization with enhanced D1 turn over to counteract the core depletion.

According to Epron (1997), the rate of net CO_2 assimilation (A) in cedar needles declined during drought progression, while the quantum yield of electron transport measured under moderate light and high CO_2 (Delta F/F – m) remained almost constant. A slight but significant decrease in Delta F/F – m were observed in severely droughted plants. This suggested that the decline in A during drought progression was mostly due to stomatal closure, and that the photosynthetic apparatus of cedar was only impaired by severe water deficits. A Turkish provenance of *C. libani* displayed a significantly higher thermotolerance than a French provenance of *C.atlantica* at whatever levels of drought. Nevertheless, there was a strong interaction between drought and heat stress in both cases. The temperature at which F-v/F-m was lowered to 15% of its original value was increased by more than 3 to 4 degrees in

drought plants. This drought induced shift in the plants PSII thermotolerance was still evident when heat treatments were done under high light rather than in darkness.

In an extensive breeding program based on biotechnological approaches several alfalfa regenerants were selected by Djilianov *et al.*, (1997) *in vitro* for PEG tolerance. A system in which severe osmotic shock is applied to detached leaves was developed and some physiological traits were followed. No significant differences in relative water content (RWC) decrease, proline accumulation and ion leakage between the tested genotypes were found with 40% PEG stress. When the samples were allowed to recover on osmotic free buffer clear distinction could be made. The osmotic –tolerant genotype were able to restore their RWC after 24 h on PEG, while the sensitive plants (the explant source genotype) lost this capability. The well-defined tendency to restore relatively low values of proline content and ion leakage during recovery could be related to osmotic tolerance. Electrophoretic mobility appears to be sensitive test for assessment of osmotically induced changes of the cell surface. The surface charge density of the leaf thylakoids in two of their regenerants was not greatly affected by the osmotic stress and they were able to restore their initial values

The effect of rapidly vs., gradually induced soil water depletion in leaf gas exchange parameters was investigated by Socias *et al,* (1997) in subterranean clover leaves in three different environments: growth room, outside late autumn (lower irradiance and evaporative demand) and late spring (higher irradiance and evaporative demand). The stomatal closure is the early response to soil water depletion and it showed a common pattern for both water stress methods in all three contrasting environments. This early response (around 40% reduction in response to irrigated plants) was developed before any substantial change in leaf water relations was achieved. During all of the experimental periods in the three contrasting environments, stomatal conductance was shown to be highly dependent on soil water availability (R (2) 0.94, 0.55 and 0.74). The photosynthesis rate reductions were mainly explained by stomatal closure variations (R (2) of 0.97- 0.99). In the three contrasting environments, the slowly induced drought plants showed higher leaf water content and water potential for similar soil water availability. This was reflected in higher stomatal conductance and photosynthesis.

Wheat plants grown on vermiculite and treated with nutrient solutions either NO_3^- or NH_4^+ form of nitrogen, were subjected to slow developing drought. During the drought, leaf water potential, chlorophyll content and chlorophyllase activity were monitored by Mihailovic *et al.* (1997). The results indicated that with the decreasing water potential there occur changes both in chlorophyll content and chorophyllase activity. These changes are dependent on the type of nitrogen ion applied. Comparing chlorophyll content changes with cholorophyllase activity changes in both N treatments they concluded that chlorophyllase may not have only hydrolytic function under drought conditions.

Rodrigues *et al.*, (1997) evaluated the effects of water deficits on some parameters associated with yield, and defined critical deficiency periods and their intensities in relation to wheat yield. The experiment was set up in a greenhouse in pots, under 18^0C and 65% humidity. Water deficiency levels of –1.0, -2.0 and –3.0 MPa were applied when plants reached the phonological stages of the fourth leaf flag leaf, anthesis and milking stage. The plants were hydrated as soon as the stress levels were reached. Flag leaf stage was most sensitive to water deficiency, resulting in the greatest yield reduction, followed by anthesis and fourth leaf stage. A water deficit of –2.0 MPa, applied at anthesis, reduced leaf area without affecting the grain yield. The water deficit imposed at flag leaf stage was associated with reduction in spike, as well as with late tillering. Grain yield reduction in wheat occurred only when the water deficiency level exceeded –2.0 MPa, affecting mainly the number of seeds per spike.

Baruah *et al.*, (1998) conducted Green house experiments with upland rice varieties Rasi, Annanda, Govind, Fapori ahu, Padumani ahu and Rangadaria to test their moisture stress tolerance at seedling stage by withholding water supply. High yielding variety maintained high leaf water potential, relative water content, nitrate reductase activity, leaf chlorophyll and proline accumulation during stress. Although there was significant difference in N, P and K contents in the shoots due to stress in different varieties, Govind had higher tissue K even under moisture stress conditions. According to the author, these physiological and metabolic components of cultivar Govind indicate its suitability for growing under water stress condition.

The changes in endogenous proline levels of *Raphanus sativus* L. seedlings were monitored by Khanna and Rai (1998) in presence of exogenous amino acids in

normal and osmotically stressed seedlings. In unstressed seedlings, proline uptake was detected only at higher (1mM) concentration of applied L-proline; however, proline uptake was promoted at all (1 μM to 100μM) concentrations of applied proline under osmotic stress conditions. Amongst other exogenous amino acids, L-leucine, L-glutamic acid, L-alanine, and L-histidine enhanced endogenous levels of proline, while exogenous hydroxyproline and gamma amino butyric acid reduced it.

The response of 49 pea cultivars with different drought tolerance was studied by Sanchez *et al.* (1998). The tolerance to stress was determined according to the grain yield or the harvest index in rainfed fanning. In this conditions variability among the genotypes in turgor maintenance, measured as the slope of the turgor potential (psi (p)) function against water potential (psi (w)), was observed. The cultivars, which best maintain turgor, were those which were more drought tolerant. Turgor maintenance was significantly related to osmotic adjustment (OA). However OA could not explain all the variability observed in d psi (p) /d psi (w). Therefore, the authors suggested that, other factor, such as tissue elasticity may also be influential. Soluble carbohydrate concentration increased (from 1.5 to 7 times) when the studied cultivars were subjected to water stress. The lines with a conventional leaf type, showed a greater sugar content than semi leafless line when watered as well as subjected to desiccation. The stimulation of sugar levels induced by drought was proportional to OA. During stress, the average soluble sugar content of all cultivars would be equivalent to 17.3% or 8.6% of psi (s100), if all carbohydrates were present in the tissue as monosaccharides or disaccharides respectively. These suggested that sugars play an important role in OA in peas. The free proline levels also increased (from 4 to 40 times) in response to water stress. However, the contribution of this amino acid to psi (s100) was small (approximately 1%) and no significant relationship were observed between proline content and OA. The cultivars, which accumulate more proline, had lower water contents upon turgor loss. This seems to indicate that proline may play a role in minimizing the damage caused by dehydration.

The cDNA clone ERD5 (early responsive to dehydration), isolated from 1 h dehydrated *Arabidoipsis*, encodes precursor of proline dehydrogenase (ProDH), which is a mitochondrial enzyme involved in the first step in the conversion of proline to glutamic acid. The transcript of the erd5 (ProDH) genes were undetectable when the

plants were dehydrated, but large amount of transcript accumulated when plants were subsequently rehydrated. Accumulation of the transcript was also observed in plants that had been incubated under hypoosmotic conditions in media that contained L or D proline. The authors (Jose and Puuigdomenech, 1998) isolated 1.4 Kb DNA fragment of the putative promoter region of the ProDH gene. The beta glucuronidase (GUS) reporter gene driven by the 1.4 kb ProDH promoter was induced not only by rehydration but also by hypoosmolarity and L-and D-proline at significant levels in transgenic *Arabidopsis* plants. The promoter of ProDH gene directs strong GUS activity in reproductive organs such as pollen and pistils and in the seeds of the transgenic plants. GUS activity was detected in vegetative tissue such as in veins of leaves and root tips when the transgenic plants were exposed to hypoosmolarity and proline solutions. GUS activity increased during germination of the transgenic plant under hypoosmolarity.

Two experiments were conducted by Huang and Fry (1998) to investigate genotypic variations in morphological, anatomical, and physiological reponses of roots to drought stress in tall fescue (*Festuca arundinacea* Schreb.). Tall fescue cultivars 'Kentucky-31' (forage type), 'Mustang' (turf type), and 'MIC18' (dwarf, turf type) were examined under well watered or drought stressed conditions in a greenhouse. Root systems of MIC18 were much slower and smaller than those of Kentucky-31 and Mustang under well-watered condition. After 7 days of soil drying, root dry weight was significantly lower than that of well-watered plants for both MIC18 and 'Kentucky-31'. The reduction in root dry weight, relative to control plants, were greater for MIC18 than'Kentucky-31'. Root water potential and turgor pressure were reduced, with soil drying, leading to cortical cell shrinkage. Kentucky-31 root suffered less turgor loss than those of MIC18 under drying condition. Specific root length (SRL) and root/ shoot ration increased with soil drying. Greater increase in SRL occurred for Kentucky-31 than for MIC18. More extensive root hairs developed in plants of Kentucky-31 and MIC18 stressed for 14d after 28 day of drying. After 14 d than in well watered plants. Root hairs became lee extensive after 28 d of drying, Kentucky-31 roots had significantly lower electrolyte leakage than those of MIC18.

Plants of *Argyranthemum coronopifolium* were submitted to water stress (preconditioned by watering for 3 days, two dry-wet cycles were imposed) and salt

stress (15 day of exposure to 140mm of NaCl followed by a recovery period of 11 days), independently. Effect of water and salt stresses on gas exchange, water relations, and growth parameters were investigated by Deherralde *et al.* (1998) to know the resistance of *A. coronopifolium* to these kinds of stress. Water and salt stress promoted reduction in leaf biomass due to both senescence and death of leaves, what has been considered to an avoidance mechanism that allows minimizing water loss. The degree of osmotic adjustments reached by the plants was very similar in both cases studies however, the maintenance of turgor did not maintain growth. Probably, the osmotic adjustment reached via salinity treatment induced some damage to the photosynthetic apparatus that was not induced by the water stress. In this sense, reduction in the photosynthetic rate and chlorophyll content were observed even when the salts were removed. It suggested that there was a toxic effect of salt concentration that could also explain the greater effect on the growth of the salinized plants.

Water-soluble carbohydrates contributing to genotypic differences to consecutive drought and salinity stress in wheat seedlings were investigated by Kerepesi *et al.* (1998). Total water soluble carbohydrates, glucose, fructose, sucrose, and fructan contents and the distribution of degree of polymerization of fructans were measured in wheat seedlings exposed to 18% PEG induced drought stress followed by an equiosmolar salinity. Tolerant genotypes accumulated higher soluble carbohydrate levels than the sensitive ones. Both ionic and nonionic stresses increased the percentage distribution of reducing sugars. The concentration of principal component of soluble carbohydrate content increased in response to drought stress and conversely, decreased due to salt stress. PEG- induced fructan accumulation was highest in leaves and showed a positive correlation with the drought tolerance of the varieties. Fructan contents in stems increased in tolerant genotypes but decreased in sensitive ones under NaCl treatment. Increment of polyfructan percentage distribution was greater in tolerant varieties than in sensitive ones.

Lu and Neumann (1998) investigated the possible occurrence of species diversity in mechanisms underlying leaf –growth inhibition by water stress, in related cereal plants. Water stress was generated by additions of PEG-6000 to the root medium. Effects of external water potentials ranging from 0 to –0.6 Mpa, on early growth parameters of emerging leaves were measured under controlled environmental

conditions, using pairs of maize, barley or rice genotypes with differing resistance to water stress under field conditions. Water potentials of –0.4MPa for 24 h similarly reduced leaf growth, comparative production rates of leaf epidermal cells and cell size in all genotypes. These reductions did not appear to be caused by reduction in the osmotic potential gradients between the expanding leaf cells and their external water source. However, growth inhibition in maize and barley, was accompanied by significant reductions in comparative leaf and cell wall extensibility. Moreover, regression plots revealed good linear correlation's ($r = 0.83^{**}$ for maize and $r = 0.77^{**}$ for barley) between the reductions in leaf growth induced by a series of extensibility. In contrast, the reduction in growth of rice leaves, was not accompanied by any significant changes in leaf or cell wall extensibility. Similarly, regression plot revealed poor correlation between leaf growth and leaf extensibility in both paddy and upland rice ($r = 0.17$ and $r = 0.07$, respectively). Thus they concluded that despite numerous inter-spices similarities, biophysical changes associated with stress induced leaf growth inhibition in maize and barley, differed from those in rice.

The effect of water stress on flag leaf senescence and protease activity during grain development was examined by Srivalli *et al.* (1998) in a wheat variety. Total chlorophyll content and soluble protein content were used as marker for monocarpic senescence. Endopeptidase activity and exopeptidase activities were assayed using RuBisCo as a physiological substrate at acidic (pH 4.8), neutral and alkaline (pH 8.5) values at three stages during grain filling period. Water stress enhanced the rate of monocarpic senescence and concomitantly increased the endopeptidase and exopeptidase activities at all pH tested in the leaves. The above observation showed that different proteolytic enzymes might come into play under water stress, which are independent of the reproductive sink effect.

According to Joshee *et al.*, (1998) one of the water stress specific cDNA clones of rice wsi18 gene can be induced by water stress conditions such as manitol, NaCl, and dryness, but not by ABA, cold or heat. A genomic clone for wsi18, pwsi18, contained about 1.7kbp of the 5' upstream sequence, two introns, and the full coding sequence. The 5' upstream sequence of pwsi18 contained putative cis-acting elements, namely an ABA-responsive element (ABRE), three G-boxes, three E boxes and MEF-2 sequence, for direct and two inverted repeats, and four sequence similar to DRE,

which ids involved in the dehydration process of Arabidiopsis genes. The gusA reporter gene under the control of the pwsi18 promoter showed transient expression in response to water stress. Deletion of the down-stream DRE like sequence between the distal G-boxes –2 and 3 resulted in rather low GUS expression.

Three week old plants of two unrelated lines of maize and their hybrid were submitted to progressive water tress for 10 d. Changes induced in leaf proteins were studied by 2D electrophoresis and quantitatively analysed by image analysis by Riccardi *et al.* (1998). Seventy-eight proteins out of a total of 413 showed significant quantitative variations, with 38 of them exhibiting a different expression in the two genotypes. Eleven proteins that increased by a factor of 1.3 to 5 in stressed plants and 8 proteins detected only in stressed plants were selected for internal amino acid microsequencing, and by similarity search 16 were found to be closely related to the previously reported proteins. In addition to proteins already known to be involved to response to water stress (e.g. Rab17), several enzymes involved in basic metabolic cellular pathways such as glycolysis and the Krebs cycle (e.g. enolase, triose phosphate isomerse) were identified, as well as several others including caffeate O-methyl transferase, the induction of which could be related to lignification.

Observation on the remobilization of proline and other nitrogen compounds from senescing leaves of maize under water stress were made by Carcellor *et al.*(1999). The effects of water stress around anthesis on proline accumulation and translocation from leaves of two maize cultivars (DA 4F-37 and DA XL 636) were studied. Water stress increased leaf proline content only in DA 4F 37, while proline in leaf exudates was detected only in DA XL 636 water stressed plants. Proline translocation was not associated with increased nitrogen remobilization from leaves. The accumulation proline cultivar DA 4F 37 showed a higher osmotic adjustment capacity than DA XL 636. Leaf proline in water stress DA 4F 37 plants varied with daytime. High proline concentrated during the morning was found in leaves with high relative water content. This evidence would support the hypothesis that proline is involved in osmotic adjustment.

Studies were conducted by Lazcano and Lovatt (1999), to observe the relationship between relative water content, nitrogen pools, and growth of *Phaseolus vulgaris* L. and *P.acutifolius* A.Gray during water deficit. *P.acutifolius* A.Gray is a

potential source of stress tolerant traits for *Phaseolus vulgaris* L. through interspecific hybrids. Replicate pots, each containing three 5 d old plants in 18.9 g of soil with 4L of available water, were subjected to water deficit by withholding water (terminal drought) or were maintained under well watered (control) conditions. Compared with controls, stressed plants of both species accumulated approximately 55% less shoot dry matter. Root dry matter accumulation was inhibited to a greater degree in P.acutifolius (approx. eq. 70% for two genotypes) than in *P.vulgaris* (14 and 27% for two genotypes). *P. acutifolius* maintained greater shoot RWC than *P.vulgaris*. In droughted plants *P.acutifolius*, leaf arginine and proline concentrations did not change, total polyamine concentration decreased, and ammonia increased compared with controls. In *P.vulgaris*, water deficit increased concentration of arginine and proline, while total polyamine and ammonia concentrations did not change as compared with the control. In all four varieties, proline concentration was inversely proportional with RWC. The authors concluded that leaf proline concentration could be an indicator of plant water status in *Phaseolus* but not of tolerance or sensitivity of vegetative growth to water deficit.

Studies on the water deficit on growth, yield and eco-physiological responses of four tomato cultivars were conducted by Lutfor *et al.* (1999). The response of water stress on growth, yield and various morphological characters were studied in two drought tolerant cultivars TM 0126 (TM) and VF-134-12 (VF) and two drought sensitive ones Kyokko (KK) and Ratan (RT). Water stress decreased yield, flower number, fruit set percentage and dry matter production in all cultivars, but the reduction was greater in drought sensitive cultivars than the tolerant ones. Photosynthetic rate (Pr), transpiration rate (Tr), leaf water potential (LWP), water use efficiency (WUF) were reduced, and leaf temperature (LT) and stomatal resistance (ST) were increased by water stress in all cultivars. The reduction of Pr, Tr, LWP and WUF, were less prominent in tolerant cultivar than the resistant one, whereas the increase of Tr and Sr was more conspicuous in tolerant cultivars than in sensitive ones. The better performance of tolerant cultivars under water stress and recovery after re-watering are attributed to the ability of plants to maintain a better water status and minimize the reduction of photosynthesis.

2.3. Biochemical response of plants to biotic stress

Stem injection of tobacco cultivar Ky14 with *Peronospora tabacina* or leaf inoculation with tabacco mosaic virus induced systemic resistance to both pathogens. The treatment also elicited a systemic increase in preoxidase activity, which was positively correlated with induced resistance. Increase was evident in cytosol, intercellular fluid, and cell wall fractions. Upon challenge with *P.tabacina*, peroxidase activity further increased in the induced plants and remained higher after challenge compared to the control plant. The isozyme patterns of peroxidase on isoelectric focussing gels showed an increase of two anionic peroxidases. Both peroxidsaes were positively correlated with induced resistance (Ye *et al.*1990). They further reported that inoculation of three leaves of tobacco cv. Ky 14 with TMV and incubation at 23^0C for 3-12 days caused localized necrosis on the incubated leaves whereas plants held at 28^0C after inoculaiotn developed systemic mosaic symptoms. Pathogenesis related (PR) proteins and activities of peroxidase, β-1, 3 glucanase and chitinase systemically increased in the inoculated plants at 23^0C. When TMV inoculated leaves were removed 12 days after inoculation at 23^0C and plants were challenged with TMV or *P.tabacina* and held at 23^0C, induced resistance to P.tabacina and TMV was apparent. However, resistance to blue mold but not to TMV was apparent when the procedure was repeated, except that plants were transferred to 28^0C one day after challenge with TMV or *P.tabacina.* PR proteins and activities of peroxidase, β 1,3 glucanase and chitinase were further increased after challenge with *P.tabacina* or TMV in induced plants at both temperatures. Tn 86, a culitvar systemically susceptible to TMV, was protected against blue mould but not against TMV by stem inoculation with *P.tabacina.* PR proteins were induced and the activities of the enzymes were gradually increased in the induced plants.

Nemestothy and Guest (1990) reported that no.2326, a cultivar of tobacco resistant to race O of the blank shank pathogen *Phytopthora nicotianae* var. *nicotianae* responded to stem inoculation by rapidly accumulating sesquiterpenoid phytoalexins and activating phenyl alanine ammonia lyase activity in the infection front. In cv. Hicks, a near isogenic susceptible cultivar, both responses were slower. Pretreatment of leaf disc with propylene oxide, which killed the cells, mevenollin, a specific

inhibitor of sesquiterpenoid biosynthesis, or non-specific amino transferase inhibitor, aminoxyacetic acid (AOA), inhibited post infection phytoalexin accumulation in both cultivars, and induced susceptibility in cv. NC 2326. Amino hydrazinophenyl propionic acid (AHPP), a specific inhibitor of phenylalanine ammonia lyase enzyme and aminoethoxyvinylglycine (AVG), an inhibitor of ethylene biosynthesis, did not affect the susceptibility of either culitvar.

Kumar *et al.*, (1990) studied the biochemical changes in pearl millet shoot infected with downy mildew pathogen *Sclerospora graminicola* (Sacc). Decrease in total phenol content, total chlorophyll and total free amino acid was reported whereas increase of the specific activity of the enzyme nitrate reductase was reported in both the diseased shoots and roots of pearl millet (*Pennisetum americanum* L. leck).

Alfalfa (*Medicago sativa* L.) cell suspension cultures accumulated high concentration of the pterocarpon phytoalexin medicarpin, reaching a maximum within 24 hours after exposure to an elicitor prepared from cell walls of the phytopathogenic fungus *Colletotrichum lindemuthianum* (Dalhim *et al.*, 1990). This was preceeded by increase in the extractable activities of the isoflavonoid biosysnthesis enzymes, L-phenylalanine ammonia lyases, cinnamic acid 4-hydroxylase, 4-coumarate CoA-ligase, chalcone synthease, chalcone isomearse and isoflavone O-methyl tranferase. Pectic polysaccharides are weak elicitors of PAL activity but did not induce medicarpin accumulation, whereas reduced glutathione was totally inactive as an elicitor in this system. The fungal cell wall extract was a weak elicitor of the lignin biosynthetic enzymes caffeic acid O-methyl tranferase and coniferyl alcohol dehydrogenase, but did not induce appreciable increase in the activity of the hydrolytic enzymes chitinase and β1,3 glucanase. Accumulation of polyphenoloxiadse, peroxidase and PAL, from cucmber (*Cucumis sativus* L.) leaves, were studied during the period of 22 days after inoculation with cucumber powdery mildew. Results indicated that early rapid increase of polyphenol oxiadse, peroxiadse and PAL activities was of great significance in the disease resistance.

Estabrook *et al.*, (1991) have used conserved and non-conserved regions of cDNA clones for PAL and chalcone synthase (CHS) isolated from a soybean nodule cDNA library to monitor the expression of the members of two gene families during the early stages of the soybean *Bradyrhizoboum japonicum* symbiosis. Their results

demonstrated that subsets of PAL and CHS gene families are specially induced in soybean roots after infection with *B. japonicum*. Furthermore, by analyzing a super nodulating mutant line from soybean that differs from the wild type parent in the number of successful infections, the induction of PAL and CHS was shown to be related to post infection events. Nodulated root formed by a Nod^+ Fix^+ strain of *B.japonicum*, resembling a pathogenic association, displayed induction of another distinct set of PAL and CHS genes. The author suggested that symbiosis specific PAL and CHS gene family in soybean, are not induced by stress or pathogen interaction. PAL in activating factor (IF) prepared from chloroplast isolated from the sunflower leaves was utilized to study its inactivating effect of PAL from *Rhodotorula glutinis, in vitro*. The effect of inactivation by inactivating factor were compared with those caused by chemicals such as sodium borohydride and nitromethane. The sunflower inactivator acted as an enzyme and enzymatic inactivation caused irreversible loss of PAL catalytic activity accompanied by the shortening of the enzyme molecule. However, the capacity of the IF inactivated PAL to bind to L-phenyl alanine, the enzyme substrate was maintained. These effect of inactivating factor from sunflower leaves were quite different from those inhibitor isolated from different sources by another marker (Gupta and Creasy, 1991).

Healthy pea plants were found to contain a substance, tentatively called "endogenous suppressor", which specifically suppressed the accumulation of pisatin in pea plants that was induced by treatment with $CuCl_2$ or an elicitor from *Mycosphaerella pinodes*. This suppressor elicited the accumulation of phytoalexins in other legumes, such as kidney bean, soybean and cowpea. The endogenous suppressor functioned to delay the accumulation of pisatin, the activation of phenylalanine ammonia lyase (PAL) and the accumulation of mRNAs of PAL and chalchone synthase induced by the elicitor from *M.pinodes*. The substance specifically induced the susceptibility to nonpathogenic, such as *Mycosphaerella ligulicola, Mycosphaerella melonis*. in pea out of four species of legume tested, but the effect was not cultivar specific. Thus the endogenous suppressor, in healthy pea plants suppressed a series of self-defense reactions and induced susceptibility in a species-specific manner, being similar to the exogenous fungal suppressor from the pea pathogen, *Mycosphaerella pinodes* (Nasu *et al.*, 1992).

Corcuera (1993) discussed possible resistance factors of this plant, such as morphological defenses and natural chemicals that have been shown, or suggested to be involved in protection of barley against aphids. The available evidence for the role-played by waxes gramine, aconitic acid, phenolics and amino acids presented. Environmental stress also affects plant–aphid interactions because the chemical composition of the plant changes. Water stress increases susceptibility, and NaCl and temperature increase resistance to aphids. The compatible solute glycine betaine, which accumulates under several types of stress, increases reproduction of aphids. Temperature and availability of nitrates increase gramine content of the leaves and, therefore, resistance to the aphids.

Chaudhuri and Nair (1991) reported that changes in proteins and polyphenols were observed in plants and calli when they are infected by rhizobia. Protein content increased with age and was greater in infected than in the uninfected plants/ callus. Infestation of Chinia and Malbhog varieties of banana by six dominant fungi viz. *Botryodiplodia theobromae, Fusarium oxysorium, Helminthosporium spiciferrum, Curvularia lunata, Aspergillus flavus* and *Trichothecium roseum* exhibited a gradual fall in all the forms of proteins under pathogenesis. The maximum reduction of proteins was observed due to B.theobromae at the end of the incubation period in both the varieties of bananas. Healthy Chinia and Malbhog varieties of banana showed an initial increase upto 2nd day which subsequently decreased with the increase in the incubation period.

Peroxidase and phenylalanine ammonia lyase (PAL) activity was determined in leaves of healthy and inoculated *Brassica napus* cultivars, showing differential disease reaction towards a virulent and a weakly virulent strain of *Leptosphaeria maculans*, the black leg pathogen by Chakraborty *et al.* (1993). Both enzymes show increased activity as a result of inoculation; PAL activity increasing as early as 12 hours after inoculation. The most significant increase in PAL and peroxiadse was observed when the moderately resistant cultivar, cresor, was challenged with the weakly virulent strain. Highest activity of these two enzymes was detected 2 day after inoculation. Very low peroxidase activity was detected in both strains of L.*maculans*, while no PAL activity was detectable in either of the strain. Cytochemical test

revealed peroxidase activity following inoculation mainly in the epidermal and guard cell.

Subcellular localization of the pathogenesis-related PR-1 proteins of unknown function was studied in roots of resistant *Nicotiana tabacum* cv.xanthi nc uninfected or infected *in vitro* by the black root rot fungus *Chalara elegans*. Using polyclonal or monoclonal antibody raised against PR-1a (or b), protein (Tahire-Alaoui *et al.*, 1993). In healthy tobacco roots, the PR-1 proteins were found to be present in lower amounts in intercellular space material, over cell walls and secondary thickenings of xylem vessels. All these cell compartments were significantly enriched in the PR-1 proteins in infected tobacco root tissues. Cell wall outgrowths, typically induced in hypodermal cells by *Chalara elegans* infection and wall appositions formed in cortical parenchyma cells in response to infection, were also the sites of PR-1 protein accumulation. In contrast, very little occurred in electron translucent intercellular spaces. Pr-1 proteins were also detected in the wall of both inter and intracellular fungal hyphae invading root tissues, but not in axenically cultured *Chalara elegans* or hyphae developing outside roots.

Carver *et al.*, (1994) reported that the seedling leaves of oat cvs Maldwyn and Selma have no known major resistant genes to powdery mildew caused by *Erysiphe graminis* f.sp.*avenae*, but their susceptibility to infection is quantitative. Thus only a portion of fungal germlings successfully overcame cell defenses to penetrate host epidermis to form haustoria OH-PAS([[(2-hydroxyphenyl) amino] sulphinyl]acetic acid,1,1-dimethyl ethyl ester) a potent, specific suicide inhibitor of CAD (cinnamyl alcohol dehydrogenase), an enzyme specifically involved with synthesis of lignin precussors, was shown to inhibit CAD from oat *in vitro*. For *in vivo* assays of effects on epidermal cell defenses, the cut ends of excised seedling leaves were immersed in OH-PAS solution for 24 hours to allow uptake before inoculation with *E.graminis* conidia. Inoculated leaves were allowed OH-PAS uptake during a further 36 hours incubation period. Initial experiments established that OH-PAS at 10^{-3} M decreased the frequency and intensity of localized auto fluorescent host epidermal cell responses associated with primary germ tubes (PGTs) and appressoria. Concurrently, OH-PAS treatment doubled the proportion of appressoria forming haustoria; i.e. it increased quantitative susceptibility by suppressing host cell defenses. Similar results were

obtained with 10^{-3}M AOPP (L-aminoxy B-phenyl propionic acid), a competitive inhibitor of PAL. Both inhibitors doubled the proportion of appressoria penetrating epidermal cells and forming haustoria. Both inhibitors reduced the frequency and intensity of localized auto fluorescence epidermal host cell responses to PGTS and appressoria, although the effect of AOPP was somewhat greater than that of OH-PAS. Neither OH-PAS nor AOPP had any deleterious effects on fungal development. Results support the idea that host autoflurogens accumulating at sites of fungal germ tube contact with epidermal cells are phenolic compounds. In addition the study provided experimental evidence pointing to involvement of product synthesized as part of the lignin biosynthetic pathway in oat epidermal cell defense against attempted penetration by appressoria of *E.graminis* f.sp.*avenae.*

Seedling leaves of two pairs of near isogenic barley lines were inoculated with the conidia of powdery mildew fungus, *Erysiphe graminis* D.C.f.sp.*hordei* Marchal, race 3. by Clark *et al.*, (1994). One set of isolines (RISQ 5678 R and RISQ 5678 S) differed at the ML-O locus, where the recessive allele (ml-o) confers a high degree of race non-specific, penetration based and papilla associated resistance to *E.graminis,* while the dominant allele (MI-O) allows a proportion of attacking fungal germlings to succeed in infection. The second isoline set (Algerian R and Algerian 5) differed at the Ml-a locus where the dominant allele confers race-specific, epidermal cell death resistance visible only by light microscopy. The recessive allele (Ml-a) allows a proportion of attacking fungal germlings to succeed in infection. Leaf samples were taken at 0, 2, 6, 8, 10, 12, 15, 18, 21 and 24 hours after inoculation , to examine the timings of host epidermal cell cytoplasmic aggregate responses (visible by light microscopy) relative to PAL mRNA transcript accumulation (determined by quantitative northern blots), and PAL enzyme activity (using radiolablled phenylalanine). *E.graminis* produced primary germ tubes (PGTs) within two-hours and appressorial germ tubes within 6 to 10 h of inoculation. In all isolines, host epidermal cell cytoplasmic aggregates formed and subsequently dispersed beneath PGTs, between 2 and 10 h and beneath appresoria between 6 and 15h concurrently biphasic patterns of PAL transcript accumulation, typed by peaks at 4 and 12 h occurred in all isolines. Temporal patterns of PAL enzyme activity were roughly similar to those of PAL transcript accumulation. Fungal germ tube contact initiated

host epidermal cell cytological responses common to all isolines, induced PAL transcript accumulation, and increased PAL activity regardless of the Mendelian inheritance of "major gene resistant factors" in the barley isoline sets. Thus there was PAL induction associated with a general defense to infection, but no unusually strong correlation between PAL induction and major gene resistance was found.

Three antifungal proteins have been identified by Kumari *et al.*, (1994) in sorghum endosperm acting on the grain mold pathogen *Fusarium moniliforme* Sheldon causing inhibition of growth. Both morphological and biochemical changes in fungal hyphae were observed on treatment with antifungal proteins. The 18kDa antifungal protein caused sloughing of cell wall polysaccharides without much release of cytoplasmic materials as seen by a slight increase in absorbance at 280 and 265 nm, and by microscopic examination. The decrease in pH or electrical potential of the medium as the fungi respired was not altered by the presence of 18 kDa antifungal protein in the incubation mixture. The 26 and 30 kDa-protein fraction on the other hand caused leakage of cytoplasmic contents as observed microscopically without releasing polysaccharides from the cell walls. Addition of 26 and 30 kDa protein in the incubation mixture reduced the rate of the rise in pH or fall in the electrical potential of the medium. Immunoflorescent microscopy indicated that the 18 and 26 kDa proteins bound to discrete areas of the fungal hyphal walls whereas, the binding of the 30 kDa antifungal proteins was not specific.

Sharma and Kaul (1995) reported that the nine apple cultivars were analyzed for the phenolics and related enzymatic activities in healthy and scab inoculated leaves throughout the pathogenesis. There was no significant difference in the total phenol content in the younger healthy leaves of test cultivars. However an upsurge in total and O-phenols was noticed after inoculation; the increase was however more conspicuous in the resistant combinations. Higher enzymatic activity of β glucosidase, polyphenol oxidase, and peroxidase was associated with the resistant cultivars, which increased in the leaves when challenged by scab pathogen, *Venturia inaequalis.*

Various phenols were examined in root exudates from 10 day old seedlings of chickpea varieties grown in sand culture and in root, stem and leaf tissue of different stage grown in wilt sickplot. Effect of these phenols on spore germination and mycelial growth of fungus was also examined. Hydroquinone and Chlorogenic acid

were higher in root exudates. Salicylic acid was higher in leaf tissues of resistant varieties at preinfection stage. Also at disease initiation stage, chlorogenic acid and p-Coumaric acid were higher in root and leaf tissue and hydroquinone and umbelliferone were higher in stem tissue of resistant variety. Most of the phenols inhibited spore germination and mycelial growth of the fungus under *in vitro* condition, but salicylic acid was most effective (Mandavia *et al* 1995).

Chowdhury (1995) studied the biochemical changes associated with the induction of resistance to rust infection in ground nut caused by *Puccinia arachidis* speg. Results revealed that all the chemicals provided significant protection. Best result was achieved with IAA. When infected plants were analyzed for biochemical changes the treated plants always recorded higher phenolics, proteins and oxidase activity as compared to untreated plants.

Gupta *et al.*, (1995) reported changes in the level of total phenol and specific activity of peroxidase, polyphenol oxidase and catalase in healthy and *Alternaria* leaf blight susceptible leaves of *Brassica* species. *Brassica* sp which are tolerant *(B.carinata, B.napus)* and susceptible (*B.juncea, B.campestris*) to *Alternaria brassicae* and *Alternaria brassicola* were used in the study. Healthy and infected leaves were collected at 20 days intervals after 40 days of sowing for various biochemical analyses. Result indicated an initial increase in the level of total phenol followed by continuous decrease with the ages of plants in all *Brassica* spices. Tolerant spices of *Brassica* registered considerable higher amount of total phenol compared to susceptible ones at all stages of plant development.

Wheat kernels that were lightly or moderately infected by *Fusarium graminearum* were analyzed by Boyacioglu *et al.*, *(*1995) in terms of their carbohydrate, lipid and protein contents to determine any compositional changes. The significant composition changes in lightly infected wheat were increases in reducing sugars (24%), non-starch lipids (5%), and decreases in cellulose (17%) and hemicellulose (20%) components. In moderately infected wheat, the increase in protein (6%), total sugars (26%), reducing sugars (14%), non-starch (20%) and starch lipids (8%), and decreases in apparent and total amylose (11-20%), cellulose (43%), and hemicellulose (37%) components were also significant. Furthermore, infection with *F.graminearum* decreased the proportion of water extractable protein (albumin)

and storage protein (glutenin) by 33 and 80% respectively, in moderately infected wheat.

The changes in biochemical contents of vitamin C, total carotenoids, chlorophyll, protein and amino acid profile in CMV infected leaves/ seeds of *Amaranthus* and *Chenopodium* species were studied by Prakash *et al.*, (1995). The amount of protein was higher (9-20%), while vitamin C (37-79%), carotenoids (19-52%) and chlorophyll (5-45%) were lower in all the tested species. Amino acid composition of infected leaves/ seeds also showed some significant changes compared with healthy specimen.

Rate of lipid peroxidation (malonaldehyde formation), levels of ascorbic acid and nonprotein thiols, and activities of ascorbate peroxidase (AP), dehydroascorbate reductase (DHAR), glutathione reductase (GR), glutathione S-transferase (GST), and quinone reductase (QR) were determined in the leaves of three barley cultivars inoculated with a Hungarian isolate of *Erysiphe graminis* f. sp. *hordei.* Markedly increased malonaldehyde levels (enhanced lipid peroxidation) were observed in the leaves of resistant cultivar Amsel after infection but not in two susceptible clutivars. In the diseased susceptible cultivars Emir and GK-Omega, however, the ascorbic acid levels substantially decreased. A substantial increase in AP and decline of DHAR activities also were observed in the mildewed susceptible plants. A dramatic induction of NADPH-consuming activity was found in the inoculated leaves of highly susceptible culitvar Emir concomitantly with decreasing 1-electron QR activity. Less pronounced changes in the parameters were found in the resistant cultivar Amsel. Thiol level increased moderately in the culitvar Amsel and in susceptible culitvarGK-Omega. No significant change in activity of GR was found in either cultivar. GST activity was induced in each inoculated cultivar, most significantly in highly susceptible Emir (up to about 360% of the control). Several antioxidative processes seemed to be activated in compatible host –parasite relationship, which may diminish the damaging effect of oxidative stress. This supposition was confirmed by infecting one cultivar (Amsel) with compatible and incompatible mildew races. These antioxidative processes led to an early necrotization in the resistant host (El-Zahaby *et al.*, 1995).

Changes in phenolic compounds, carbohydrates and mineral elements in resistant (M-147, G-201) and susceptible (NC-13, Gk-19) cultivars of groundnuts were studied. Total phenols and O-phenols were high, whereas reducing and nonreducing sugar was low in resistant cultivars in comparison to susceptible ones. Amount of phenolic compounds increased whereas carbohydrates decreased after infection in all the cultivars. Ten amino acids were detected in resistant cultivars in comparison to none in susceptible cultivars. N, Mn, and Fe contents were low whereas P, K, Cu, Zn were higher in resistant as compared to susceptible cultivars. N, P, Cu and Mn decreased and K, Zn, Fe increased in all the cultivars after infection (Sindhan and Parashar, 1996).

In a histochemical study comparing seedlings of races C and D of *Orobanche cumana* Wallr. (syn. O. cernua Loefl.0) attacking sunflower (*Hellianthus annuhtus* L.) in southern Russia, Antonova and Terborg (1996) distinguished three groups of *O.cumana* seedlings according to the peroxidase content of the cells in the radicles: 1) those with neither extracellular nor intarcellular peroxiadse and whose radicals have a smooth apex (these were classified as non-infective. 2) those with a peroxiadse content of the nuclei and the cytoplasm layer adjacent to the cell wall, as well as excretion of the peroxidase from the apex of the radicles; 3) those with a similarly high activity of peroxiadse in the parasite cells, but without extracellular excretion. The extracellular peroxidase in *O.cumana* race C reacts with phenolic compounds, which are lignin precursors of the host, resulting in host resistance due to the formation of the lignin layers in sunflower possessing the OR3 gene for resistance. The absence of extracellular peroxidase in *O.cumana* race D prevents lignin formation and enables the parasite to attach to host vascular system.

Healthy plant of chickpea genotypes ICC 1096, resistant to *Botrytis cinerea* had less amount of total soluble sugar and free amino acid but higher amount of total phenol than in the susceptible genotype BGM 408 irrespective of plant parts tested. The amount of sulfur containing amino acid, methionine and cystine was almost double in the resistant genotype compared to susceptible ones. Shoot tips had higher amount of sugar and free amino acid but lower phenol content compared to middle and lower leaves irrespective of genotypes. Sugar and phenol decreased after inoculation by *B. cinerea.* The decrease in phenol content was more in susceptible

genotypes. Free pool of amino acid increased by 280% in BGM 408 after inoculation as compared to only 22% in ICC 1069. All the amino acids except asaprtic acid decreased after inoculation (Mitter *et al.*, 1997)

Genes encoding phenylalnine ammonia lyase (PAL) from a small multigene family with atleast three members in pea. Tissue specific expression of the promoter of a member of PAL gene family (PSPAL1) was investigated in the transgenic tobacco transformants carrying the different modes of chimeric fusion between the PSPAL1 promoter and a bacterial beta- glucuroniadse (GUS) gene. In stems, strict correlation was found between steady state levels of GUS mRNA and enzyme activity. Significantly high level of GUS activity was observed in roots, particularly in meristematic tissues, and the pigmented region of the petal of trangenic tobacco carrying the translational fusion type B (-1,394 to +140 of PSPAL1 connected to GUS), followed by moderately high level of GUS activity carrying the translational fusion type A (-1, 394 to _ 117). GUS expressions of tissues of mature leaves, however, were very low in these constructs. Extremely low GUS activity was observed in the transformants of transcriptional fusion type (-1, 394 to +5), whilst no activity was detected carrying non-transcriptional fusion type (-1, 394 to –27). Furthermore, the pattern of the PSPAL1 expression was characterized in response to pathogen in wounding in transgenic tobacco carrying the translational fusion type B. Woundings itself triggered the marked expression of PSPAL1- driven GUS expression at the wounded sites. Inoculation of non-pathogens, *Phytophthora capsici, P. boehmeriae* and, *Erysiphe gramins f. sp.hordei*, caused rapid and very clear GUS expression zone along with the development of hypersensitive cell death area where callose was accumulated; however, the inoculation of a pathogen, *P.nicotiana* caused slow and hazy GUS expression zone along with the lesion development. These results of Kawamata *et al.*, (1997) suggest that the expression of pea PSPAL1 promoter be regulated in a similar fashion, at least in a part, in pea and trasngenic tobacco, under the plant development and various environmental cues.

The influence of the gall aphid *Adelges abietis* on the metabolism of phenolic compounds were studied by Kraus and Spiteller (1997) comparing extracts of spruce shoots and galls of the same tree during one vegetation period. Phenolic and phenolic glucosides, 47 in total, were identified, and quantified as their trimethylsilylated

derivatives by CC-mass spectroscopy. Shoots contained two to ten times more free phenolics than galls. Their amount increased over the vegetation period. The main compounds, besides cinnamic acids, were acetophenone derivatives in shoots and 4-hydroxyphenylpropionic acid in galls. The amount of phenolic glucosides in shoots exceeded that in galls by a factor of 60 to100. Shoots contain preferentially acetophenone glucosides. Derivatives of cinnamic alcohol glucosides prevailed in galls.

In higher plants, sugars are required not only to sustain heterotrophic growth but also to regulate the expression of a variety of genes. Environmental stress such as pathogen infection and wounding, activate a cascade of defense responses and may also affect carbohydrate metabolism. In a study, the relationship between sugar and stress activated signal transduction pathways and the underlying regulatory mechanisms were analyzed by Ehness *et al.*, (1997). Photoautotrophically growing suspension culture cells of *Chenopodium rubrum* were used as a model system to study the effects of metabolic regulator D-glucose and of different stress related stimuli on photosynthesis, sink metabolism, and defense response by analyzing the regulations of mRNAs for representative enzymes of these pathways. Glucose as well as the fungal elicitor chitosan, the phosphatase inhibitor endothall, and benzoic acid were shown to result in a coordinated regulatory mechanism. The mRNAs for phenylalnine ammonia lyase, a key enzyme for defense response, and for the skin-specific extracellular invertase were induced. In contrast, mRNA for the calvin cycle enzyme ribulose bisphosphate carboxylase was repressed. This inverse regulatory pattern was also observed in experiments with wounded leaves of *C. rubrum* plants. The differential effect of the protein kinase inhibitor staurosporine on mRNA regulation demonstrated that the carbohydrate signals and the stress-stimuli independently activated different intracellular signal pathways that ultimately are integrated to coordinately regulate source and sink metabolism and activate defense responses. The various stimuli triggered the transient and rapid activation of protein kinases that phosphorylate the myelin basic protein. The involvement of phosphorylation in signal transduction was further supported by the effect of the protein kinase inhibitor staurosporine at the mRNA level.

In sunflower leaves higher amount of chlorophyll, amino acid and O-dihydroxy phenol was observed in cultivars tolerant to *Puccinia calcitrapae* var. *centaureae* whereas in susceptible cultivars total soluble and reducing sugar were more. Total chorophyll, a and b, total amino acid and soluble sugar declined while the total O-phenol content increased after rust infection (Singh *et al.*, 1998b). In further study Singh *et al.* (1998a) reported that the studies on the effect of the CMV on chlorophyll content and mineral elements were carried out in two resistant and two susceptible chili varieties. Resistant genotype in general recorded higher content of cholorophyll, phosphorous, and magnesium and lower of zinc and manganese than susceptible genotypes. The level of Mg was found to be relatively higher in resistant varieties but it showed very little increase after infection.

Induction of peroxidase has been correlated with the resistant interactions between rice and *Xanthomonas oryzae* pv. *oryzae*. To assist in analysis of role of rice peroxidases on plant defense against the bacterial pathogen, three peroxiadse genes, POX22.3,POX8.1 and POX 5.1, were identified from a rice cDNA library that was constructed from the leaves of plants undergoing a resistant reaction. These genes were highly similar in nucleic acid and amino acid sequences and belonged to a gene family. The three genes showed differential expression in infiltrated rice leaves during pathogen interaction and mechanical stress. Only two peroxidase genes, POX8.1 and POX22.3, were predominately expressed during resistant interactions. These two genes were also expressed in susceptible interactions, but induction was delayed compared with resistant interactions. POXgX9, a fourth peroxiadse gene that was isolated from a genomic library, was adjacent to POX22.3 in the rice genome and had greater than 90% similarity in nucleotide and amino acid sequence identity to POX22.3. Interestingly, POXgX9 was expressed only in the root of rice plants. While POX22.3 was expressed both in roots ands leaves, POX8.1 and POX5.1 were not detected in roots but were induced in leaves by mechanical wounding different times after treatment. POX22.3, POX8.1 and POX5.1 were estimated to be present in single copy in rice haploid genome. These results of Chittoor *et al.* (1997) indicated that different members of rice peroxidase gene family are distinctly regulated in response to various environmental cues.

An experiment was conducted by Yubedee (1998) to find out the possible relationship between phenolic contents and peroxidase activities of different *Dioscorea* spp to *Fusarium moniliforme* Sheldon. *Dioscorea cayenensis* Lam and *D. dumetorum* (Knuth) Pax had very high content of polyphenol oxidase, peroxidase and total phenol and was resistant to *F. moniliforme, D. rotundata pooir, D.alata* L. *and D. esculenta* (Lour) Burk had low contents of polyphenol oxidase, peroxidase and total phenol contents and were susceptible to *F. moniliforme*. Phenolic contents and peroxidase activities decreased with increase in the age of yam tubers and with the increase in the rate of infection. There was a sharp rise in the amount of phenolic compounds and peroxiadse activities at the start of infection. They later dropped as the infection progressed.

Leaves of two barley (*Hordeum vulgare* L.) isolines, Alg-R, which has the dominant Mlal allele conferring hypersensitive race-specific resistance to avirulent races of *Blumeria graminis*, and Alg-S, which has the recessive mlal allele for susceptibility to attack, were inoculated with *B.graminis* f. sp. *hordei.* Total leaf and apoplastic antioxidant was measured 24 hour after inoculation when maximum members of attacked cells showed hypersensitive death in Alg-R. Cytoplasm contamination of the apoplastic extracts, judged by the marker enzyme glucose 6 phosphate dehydrogenase, was very low (less than 2%) even in inoculated plants. Dehydroascorbate, glutathione, superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase, monodehydroascorbate reductase and dehydroascorbate reductase were present in the appoplast. Inoculation had no effect on the total foliar ascorbate pool size or the redox state. The glutathion content of Alg-S leaves and apoplast decreased, whereas that of Alg-R leaves and apoplast increased after pathogen attack, but the redox state was unchanged in both cases. Large increase in foliar catalase activities was observed. Vanacker *et al.* (1998) with this study concluded that sustained oxidation did not occur and that differential strategies of antioxidant response in Alg-S and Alg-R may contribute to pathogen sensitivity.

A protein associated with citrus blight (CB), a disease of unknown cause, was partially characterized by Ceccardi *et al.* (1998). The 12kDa protein, designated as p12, was diagnostic of CB and was present in leaves xylem fluid from roots and stems of CB affected trees. The protein and upto six other CB specific proteins were readily

detected by SDS-PAGE of xylem fluid from CB affected trees. The partial N-terminal amino acid sequence of p12 was found to be unique based on databased searches. A cDNA library from CB affected root cambium was screened with a 60bp fragment, obtained by PCR amplification of cDNA with degenerate primers deigned using the amino acid sequence of p12, and two clones were selected. These cones were sequenced revealing 674-nucleotide cDNA with 393 nt ORF that included sequence predicted by the N-termial amino acid sequence of p12. The amino acid sequence based on the p12 BRF was found to be up to 49% similar and 31% identical to expansins. Bacterial expression of the cloned ORE, which encodes an 11.8Kda protein plus an N-terminal hydrophobic signal peptide, produced an immunoreactive protein of the expected size. By northern blot analysis, it was determined that p12 transcripts are present in root and stem cambium, but not in leaves of CB affected trees, suggesting transport of the protein to leaves. Southern hybridization analysis of citrus genomic DNA indicated that p12 is a citrus encoded protein.

Cell suspension culture of parseley (*Petroselium crispum*) has previously been used as a suitable system for studies of the nonhost resistance response to *Phytopthora sojae*. In this study, Gus-Mayer *et al.* (1998) replaced the penetrating fungus by local mechanical stimulation by using a needle of same diameter as a fungal hypha, by local application of a structurally defined fungus derived elicitor, or by a combination of the two stimuli. Similar to the fungal infection hyphae, the local mechanical stimulus alone induced the translocation of cytoplasm and nucleus to the site of stimulation, the generation of intracellular reactive oxygen intermediates (ROI), and the expression of the some, but not all, elicitor responsive genes. When the elicitor was applied locally to the cell surface without mechanical stimulation, intracellular ROI also accumulated rapidly, but morphological changes were not detected. A combination of the mechanical stimulus with simultaneous application of low doses of elicitor closely stimulated early reactions to fungal infection, including cytoplasmic aggregation, nuclear migration, and ROI accumulation. By contrast, cytoplasmic rearrangements were impaired at high elicitor concentrations. Neither papilla formation nor hypersensitive cell death occurred under the conditions tested. These results suggested that mechanical stimulation by the invading fungus is responsible for the observed intracellular rearrangements and may trigger some of the previously demonstrated

changes in the activity of elicitor responsive genes, whereas chemical stimulation is required for additional biochemical processes.

Leaf phenolic composition in three *Salix myrsinifolia* Salisb. clones (V8, V45 & V43) inoculated with *Melamspora* rust, was analysed by Hakulinen *et al.*, (1999) to detect local rust induced alterations during different stages of infection (2, 7 & 21 days after inculation). Phenolic levels and percentage of uredial area varied significantly between clones, the level of some phenolic compounds were lower in rust infected plants than in control plants at the initial stage of rust infection suggesting a rapid response of phenolic metabolism to rust attack. Moreover the clone V8 contained the highest constitutive (+)catechin level. In clone V45, rust infection caused the most pronounced increase in th elevels of individual phenolics at 7 DAI. This increase may have been effective in ragarding the subsequent spead and development of rust. In the most suceptible clone V43, rust induced phenolic responses were less pronounced and delayed. The result suggested that in specific yellow rust interactions, constitutive levels of phenolics,as well as, induced phenolic responses, may contribute to the expression of rust resistance.