Review of Literature
2.1 Nutrients

Watson and Wettasinghe (1982) compared three types of N fertilizers, sulphate of ammonia, urea and calcium ammonium nitrate for the content of N, P, K, Ca, Mg and Mn in leaves of different clones of tea. According to the authors, nitrogenous fertilizers form the major component of fertilizers applied to tea in both quantity and cost. Sulphate of ammonia produced higher yields compared to urea and calcium ammonium nitrate and it also resulted in higher leaf N, P, Mn and lower leaf Mg. It was observed that N, P, Mg and Mn contents in the leaf were affected by the N fertilizer used while the Ca content was independent of the type of fertilizer used. The plants, which received sulphate of ammonia, had the highest amount of N, P and Mn and the lowest amount of Mg in the leaf. The higher N content in plants, which received sulphate of ammonia, suggests a greater efficiency of this fertilizer in N uptake when compared to Urea and calcium ammonium nitrate.

Young tea plants were pruned, with and without foliage, to study the uptake and utilization of N (Krishnapillai, 1983). The author found that there was a drop in N uptake immediately after pruning and this drop corresponded to the leaf area removed from the plants. In cases where foliage was retained, N uptake continued although reduced and increased as new leaves were
formed during recovery. The N uptake stopped when there were no leaves in
the bush and the plants failed to recover.

Studies were made to find out the relationship between yield and leaf
nutrient content in the mature leaves of various tea clones (Wanyoko and
Njuguna, 1983). The nutrients analysed in the mature leaves were N, P, K,
Ca, Mg and Mn. The authors found that among the nutrients studied only K
had a significant direct relationship with yield. Though N is the most
important nutrient with regard to the yield of the tea bush, as a substantial
amount of this nutrient is removed during plucking, it was found that there
was no relationship between N and yield. One reason for this could be that
the amount of N (200 kg N/ha/year) applied was sufficient for the tea bush
and as N is very mobile in the soil, its absorption by the root is easy. It was
noted by the authors that the second important nutrient removed during
plucking is K and this nutrient has a limiting effect on yield only during
stress. According to them, application of K by foliar method especially prior
to drought or any other stress would be useful.

Wickremasinghe et al. (1984) compared the efficiency of utilization of
urea and ammonium sulphate fertilizer in mature tea plants using $^{15}$N labelled
urea and ammonium sulphate fertilizer. The authors found that the percentage
of N derived from fertilizer in the flush and third leaf were directly related to
the availability of fertilizer N in the soil, while the mature leaf continued to
steadily draw and store fertilizer N and served as a sink in both urea and
ammonium sulphate treatments. It was also observed that the effect of urea
and ammonium sulphate fertilizers on crop growth and new shoot production
was very similar and evident after about seven weeks from fertilizer
application. The study revealed that both urea and ammonium sulphate were
equally efficient sources of fertilizer N for mature tea.
According to Rahman (1988) for efficient and adequate absorption of nutrients certain conditions were to be fulfilled in tea and they were: i) nutrients must be available in adequate quantity and in a balanced proportion, ii) conditions for absorption must be favourable and iii) roots must be active. Since absorption is through the roots they must be well developed and conditions for absorption, mainly aeration and moisture should be favourable. The author also stated that N is mostly applied as urea or sulphate of ammonia.

Hettiarachchi et al. (1997) studied the leaf nutrient concentrations in tea leaves in particular and soil nutrient status for the interpretation of crop performances in relation to clonal teas grown under different climatic conditions in Sri Lanka. They observed that when the N, P and K concentrations in the leaves decreased, the Ca, Mn, Fe and Al concentrations increased with leaf maturity and the highest concentration of N, P and K was found in the flush (bud, first and second leaves).

Studies were carried out by Gogoi et al. (1994) to determine the N uptake and the activities of nitrate and nitrite reductase enzymes in the shoots and feeder roots of tea cultivars at different doses of P. The results indicated that while P upto 50 kg ha\(^{-1}\) enhanced N uptake, translocation and activities of nitrate reductase and nitrite reductase with concomitant increase in yield of tea, when P was applied at 75 and 100 kg ha\(^{-1}\) it depressed the activities of these enzymes and adversely affected the N metabolism of the tea plant.

The uptake, distribution and redistribution of \(^{15}\)N in young tea plants during the first flush season were studied (Okano et al., 1994). The authors observed that the rate of N uptake was at a relatively lower level before bud break and it increased to 1.5 fold after bud break. Most of the N absorbed before bud break was distributed to the stem and roots while after bud break they were retranslocated to the sprouting shoots. It was also seen that more
than 75% of N absorbed after bud break was partitioned to the sprouting shoots. Further, they found that approximately 30% of N, which accumulated in sprouting shoots, was newly absorbed and the remaining 70% was retranslocated from the vegetative parts, which was expected to play an important role in the growth of sprouting shoots in both quantity and quality.

To find out the seasonal changes in N uptake of tea plants, Okano and Matsuo (1996) applied $^{15}$N on young hydroponically grown plants, every month throughout the year. It was observed that the N uptake was active in April and May and then declined during the summer season. The uptake was vigorous in October and November and decreased again during the winter season. The authors found that a large amount of N was partitioned to the leaves, especially to the sprouting shoots during the active growing period of April to September. During the dormant period from November to February most of the absorbed N stayed in the roots and after bud break it was translocated to the first flush shoots.

The K content in different plant parts (shoots, mature leaf, branchlets, twigs, thick wood and root) of tea and in the made tea was estimated (Sud, 1996), and it was found that the shoots contained high concentration of K (2.51-2.71%), the amount of K assimilated in the production of 1000 kg of commercial made tea was 26-27 kg and the quantity of K present in tea liquor was 48-53 mg/cup. It was also reported by the author that 45% of the exchangeable K was depleted at the end of the cropping season and potassium deficiency led to defoliation and death of the tea bush.

Studies were carried out to determine responses of N-S on the yield and nutrient uptake in four year old rejuvenated tea plants (Sharma et al., 2002). The authors found a significant positive correlation between N uptake and yield of first season and uptake of N, K and Ca of the first season with the yield of the second season. They also noted that the K uptake of the first
season correlated with the yield of the third season and N and K uptake during the second season correlated with the yield of the same season. Further it was noted that the yield of tea in the third season was correlated with the N, P and S uptake of that season.

The major nutrient content of leaves, photosynthetic CO₂ exchange rate, stomatal conductance, transpiration rate and biomass production in *Hevea brasiliensis* after application of equal doses of N, P and K to all the plants were investigated (Sobhana *et al.*, 1996). The authors noticed considerable variation in the leaf N, P and K content, which indicate that there is genetic variation in the absorption of mineral nutrients. According to them nutrient deficiencies suppress photosynthetic rate and there is a positive correlation between leaf N content and photosynthetic rate. When symptoms of elemental deficiency or toxicity are evident, structure of the chloroplasts is usually altered which affects photosynthesis. These authors also reported that the leaf N, P and K per unit area exhibited significant positive correlations with CO₂ exchange rate, stomatal conductance and transpiration rate while the leaf nutrient content showed positive relationship with the water use efficiency of leaves.

Physiological response of pot-grown tea plants to various levels of N fertilizer was studied to establish the suitable level of N for tea (Okano *et al.*, 1997). The study was carried out by using an arbitrary unit of N application as 1 N plot (200 mg N pot⁻¹ year⁻¹ which corresponds to 10 kg N 10 a⁻¹ year⁻¹) and experimental plots from 0 to 27 N were created using ammonium sulphate. The authors observed that absorption of N was increased with increase in concentration of N applied, but the capacity of uptake gradually became saturated. The optimum concentration for maximum growth and yield was found to be 6N.
2.2 Leaf water potential

It was pointed out by Acevedo *et al.* (1971) that with adequate water, the elongation of young maize leaves was constant; it slowed down when the water potential of the soil dropped from −0.1 to −0.2 bar and stopped when it dropped to −2.5 bars. It was observed that when the water potential of the root medium suddenly decreased below 0 bar, growth stopped initially and resumed at a lower rate; when the water potential was suddenly increased back to 0 bar, growth accelerated to a high rate before slowing to a steady state rate. The results indicated increased cell extensibility during water stress. The leaves stressed for one or more days, after rewatering, attained almost the length of the control leaves. The authors then concluded that the direct role of water in growth was proved by the sensitivity and rapidity of response to changes in water status and the uptake of water provided the physical force for cell enlargement.

The effect of drought on the fixation and translocation of labelled C in potato (*Solanum tuberosum*) was studied (Munns and Pearson, 1974). The authors noticed that irrespective of whether tubers were present or absent low leaf water potentials resulted in a decrease in translocation, which was proportional to the decline in net photosynthesis. They observed preferential supply of photosynthates to the lower parts of the tuberous plants during drought, which indicated the strength of the tuber as a 'sink'. One of the most striking results of drought on the plants bearing tubers was the very rapid cessation of leaf expansion. It was concluded by the authors that drought-induced changes in the distribution of photosynthates in potato was due to direct effects on photosynthesis and not due to either vein loading or movement of photosynthates within the conducting vessels.

Sung and Krieg (1979) studied the effect of water stress intensity and stage of plant development on photosynthesis and translocation rate changes
in cotton and sorghum plants. At midday as leaf water potentials declined in both species, the photosynthetic rates were reduced and this indicated that photosynthesis is more sensitive to moisture stress. The authors also found that severe water stress corresponding to leaf water potentials of \(-27\) bars, did not completely inhibit either photosynthesis or translocation.

The photosynthesis in *Coffea arabica* plants under conditions of water stress was studied by Kumar and Tieszen (1980). It was observed that at fixed temperature (25°C) low leaf water potential reduced photosynthesis through its influence on stomata. But under field conditions low leaf water potential and an associated rise in temperature could lower the rate of photosynthesis by lowering both mesophyll and stomatal conductances.

The genotypic differences in leaf osmotic potential among grain sorghum (*Sorghum bicolor*) cultivars were investigated by Shackel *et al.* (1982). The authors observed that seasonal changes in osmotic and total water potential indicated progressively increasing levels of predawn turgor potential for both frequently irrigated and water limited treatments but progressively decreasing levels of mid-afternoon turgor potential. The maintenance of turgor under water-limited compared to frequently irrigated conditions was exhibited at predawn but not at midday. The authors came to a conclusion that the differences in leaf osmotic potential may be used to select sorghum genotypes that exhibit contrasting water relations, and the selection might be more effective under frequently irrigated conditions and during grain filling than under water-limited environments or during early stages of growth.

Screening of different clones of tea for drought tolerance under field stress conditions were done by Handique and Manivel (1986) and they found that the drought tolerant clones exhibited higher and drought susceptible ones lower shoot water potential values. These authors also stated that the shoot
water potential ($\Psi$ shoot) in mature tea bushes under moisture stress was an index of the plant water status and hence their ability to withstand drought. According to them, based on the existence of a strong correlation between shoot water potential and drought tolerance, shoot water potential studies may be used as a criterion for screening drought tolerant cultivars in tea. Therefore it is clear that better plant water status under conditions of moisture stress will contribute to drought tolerance in tea.

Uprety and Sirohi (1986) studied water potential, osmotic potential and relative water content in flag leaves of wheat plant under field conditions at high nitrogen (250 kg N per ha) and low nitrogen (110 kg N per ha), while phosphorus and potassium were given as basal dose and nitrogen was applied in split doses. The authors found that under high nitrogen, water potential of leaf significantly increased and the osmotic potential decreased but the relative water content remained unaffected by nitrogen application.

Flower et al. (1990) studied the influence of osmotic adjustment on growth, stomatal conductance and light interception of various sorghum cultivars in extreme environmental conditions. The resistant lines adjusted at a faster rate and had a higher potential for adjustment than the susceptible lines. It was found that most of the osmotic adjustment occurred within three weeks after withholding water and at high predawn leaf water potentials. As a result the resistant lines were able to maintain a positive turgor to lower leaf water potentials than susceptible lines. Throughout the drought period the leaf area of all the water stressed plants was similar when expressed relative to the control regardless of the level of osmotic adjustment. On the basis of these turgor-related processes, it was concluded that there would be little advantage in selecting plants with a higher capacity for osmotic adjustment in the semi-arid tropics of India where the study was carried out.
In order to study the effect of stress, leaf water potential, canopy temperature and transpiration rate were measured in irrigated and non-irrigated wheat (Kumar and Tripathi, 1991). The authors noticed that the non-irrigated plants had consistently higher canopy temperature, lower leaf water potential and transpiration during the day. In non-irrigated wheat the leaf water potential declined at a faster rate until the peak stress period (14.00 hours) approached and recovered slowly in the late afternoon as compared to irrigated wheat. The canopy temperature of non-irrigated plants increased earlier during forenoon and remained higher later in the afternoon than that of irrigated plants. At the same transpiration rate, lower values of leaf water potential and higher values of canopy temperature were seen in the afternoon than in the forenoon due to which the phenomenon of hysteresis was exhibited. It was also observed that the degree of hysteresis increased with increasing plant water deficit and crop age. Finally the authors came to a conclusion that hysteresis is the combined effect of water deficit and seasonal changes in plant behaviour and may be used to assess crop water status.

According to Premachandra et al. (1992), lower osmotic potential helped sorghum (Sorghum bicolor) plants to maintain turgor and decreased the sensitivity of turgor-dependent processes. It was seen that sugar and K were the major solutes contributing to osmotic potential in sorghum and their concentration increased by 37.4% and 27% respectively under water deficit conditions. In the non-irrigated plants stomatal conductance and cuticular conductance were found to be lower. In the leaves of water deficient plants the epicuticular wax load increased and it was found to be positively correlated with cuticular conductance and cell membrane stability.

2.3 Photosynthetic pigments under nutrient and stress conditions

Sharma and Tripathi (1994) carried out experiments on barley at deficient, optimum and excess levels of Fe. It was observed that Fe
deficiency increased water potential and transpiration and decreased water saturation deficit and diffusive resistance. The Fe deficient plants had lower concentration of sugars and starch in the leaves and exhibited a marked decrease in the net photosynthesis. The reduction in photosynthesis was primarily due to decrease in leaf chlorophyll concentration. While Fe deficiency caused significant decrease in water use efficiency, excess supply led to decreased plant water status, transpiration and photosynthetic activity.

Iron deficiency in banana, guava, stargoose berry, chilly, crossandra and bougainvillea were studied (Balakrishnan et al., 2000). The authors found that the Fe deficient plants had reduced chlorophyll a, b, a+b and carotenoid content. The Fe deficiency also affected the Fv/Fm ratios also indicating an involvement of Fe in chlorophyll biosynthesis as well as in components of photophosphorylation. The authors concluded that there was possibility of using chlorophyll fluorescence as a diagnostic tool to identify Fe deficiency in crop plants.

The effect of P on growth, chlorophyll and proline in clusterbean under conditions of moisture stress was studied (Shubhra et al., 2003). The experiment was carried out with three levels of P 15, 30 and 60 mg kg⁻¹ soil supplied in two split doses at a week’s interval in the form of KH₂PO₄ one week after sowing. The results of their study revealed that while moisture deficit caused reduction in the dry weight of plant parts and chlorophyll content of the leaf, there was accumulation of large amount of proline in the leaf. The P application was effective in improving the chlorophyll content and dry weight of plant parts and the proline content also decreased by P treatment.

The effect of different sources and doses of K on biochemical parameters like chlorophyll, carotenoid, polyphenol, catechin, amino acid and nutrient content such as P, Mg, Zn, K and N in three leaves with a bud of tea
were studied (Venkatesan et al., 2005). It was found that leaf N had a positive and significant correlation coefficient with amino acids. Though a linear increase in the biochemical parameters was seen against rate of K application, in the case of sulphate of potash a comparatively small quantity was sufficient to improve the same parameter. Irrespective of their dosage over control both the sources of K, muriate of potash and sulphate of potash, did not influence the chlorophyll content of the shoot. The authors concluded that the ideal N:K ratios would be 1:0.83 or 1:0.62 if the source of K is muriate of potash and it would be 1:0.21 or 1:0.42 for sulphate of potash for achieving the maximum productivity and biochemical parameters.

The effect of water stress on the content and organization of chlorophyll in mesophyll and bundle sheath chloroplasts of maize (Zea mays) was investigated (Alberte et al., 1977). It was observed that the majority of chlorophyll lost due to water stress occurred in the mesophyll cells and lesser amount was lost from the bundle sheath cells. The reasons for this preferential loss could be due to the fact that the mesophyll cells are farther removed from the vascular supply of water than the bundle sheath cells, and hence develop greater cellular water deficits, which led to greater loss of chlorophyll.

Kapur (1999) studied the impact of light intensity on Andrographis paniculata and found that increasing light intensity reduced the chlorophyll content on unit leaf area, land area as well as per gram dry weight basis. The chlorophyll content was significantly affected both by light as well as age of plants. A significant negative correlation was observed between light and chlorophyll a/b ratio, with age the ratio declined up to 212 days and then increased steadily.

Nine different groundnut genotypes were subjected to study for their relationship of mineral ash and total chlorophyll content of leaves with
transpiration efficiency under three moisture regimes (Reddy et al., 2000). It was found that there was a strong positive relationship between mineral ash content and chlorophyll content with transpiration efficiency. According to the authors, under conditions of limited water, the mineral ash content and chlorophyll content of leaves could be used for selection and breeding programmes for higher water use efficiency.

Estimation of leaf chlorophyll content in different tea clones by using Minolta chlorophyll meter and chlorophyll quantification by the conventional method was compared (Kumar et al., 2002). They found a linear relationship between the values obtained by the chlorophyll meter and total chlorophyll content. There was a wide variation in the chlorophyll content between the clones and varieties studied. The authors found a positive correlation between the values of the chlorophyll meter and the rate of photosynthetic carbon assimilation and therefore the photosynthetic rate could be predicted using the chlorophyll meter values.

Pandey (2002) studied the possibility of using chemicals other than plant growth regulators to increase the content of photosynthetic pigments in pruned tea. The study proved that ‘Jibika’ (mixture of GA3, GA4 and GA7) increased total chlorophyll, chlorophyll a, chlorophyll b and chlorophyll a/b ratio in the leaves during mid-May. During mid-July methanol treated bushes produced maximum total chlorophyll and chlorophyll a, while succinic acid and thiourea recorded the highest chlorophyll b and chlorophyll a/b ratio respectively. Another observation was that the amount of total chlorophyll and its fractions were found to be higher in mid-July than in mid-May and in both these periods the carotenoid content was maximum after ‘Jibika’ treatment. The leaf area was significantly higher after methanol treatment during mid-July and mid-May as compared to other treatments. The author concluded there was a possibility of enhancing chlorophyll biosynthesis in tea
by application of ‘Jibika’, methanol and succinic acid while ‘Jibika’ and sucrose application could improve carotenoid biosynthesis.

Nair et al. (2004) studied chlorophyll distribution in leaves of drought tolerant and susceptible clones of *Hevea brasiliensis*. The studies showed that during drought conditions the drought susceptible clone exhibited a relatively higher degree of chlorophyll degradation than the drought tolerant clones. They also reported that the chlorophyll content of *Hevea brasiliensis* decreased as the level of tissue moisture deficit increased.

The effect of severe drought on leaf photosynthesis of *Hevea brasiliensis* showed inhibition of leaf photosynthetic rate during drought (Jacob et al., 1999). There was diversion of more photosynthetic electrons away from carbon to oxygen reduction, leading to oxidative damage of photosystem II and loss of total soluble proteins as well as proteins associated with photosystem II. The authors also stated that the drought induced oxidative stress led to senescence as indicated by loss of chlorophyll.

Kumar et al. (2000) examined the effect of photon flux densities on the photosynthetic behaviour of genotypes of rice, ragi, barnyard millet and soybean in relation to the photosynthetic pigments. In all the genotypes of the different crops, an increase in photosynthetic rate was recorded with increase in photon flux densities. The soybean genotypes possessed high photosynthetic rate under normal light and maintained relatively higher photosynthesis under low light conditions. The genotypes of rice, ragi and barnyard millet, which showed higher photosynthesis rate under low light, could not maintain the higher photosynthesis under normal light conditions. The photosynthetic rate recorded at low photon flux densities (400 μmolm⁻²s⁻¹) had significant positive correlation with chlorophyll b, total carotenoids and significant negative association with chlorophyll a/b and chlorophyll a/total carotenoids ratio, while photosynthesis recorded at normal light (1500
μmolm⁻²s⁻¹) showed significant positive correlation with chlorophyll a/total carotenoids ratio. The authors suggested that for selection of rice, ragi and barnyard genotypes suitable for low light/cloudy conditions, high contents of chlorophyll b, total carotenoids and high photosynthetic efficiency under low light conditions might comprise important criteria instead of high photosynthetic efficiency under normal light conditions for hills.

2.4 Chlorophyll fluorescence under nutrient and stress conditions

Maxwell and Johnson (2000) have detailed the uses of chlorophyll fluorescence as a practical guide to measure the photosynthetic performance of plants. According to them, light energy absorbed by chlorophyll molecules in a leaf can undergo one of three fates: it can be used to drive photosynthesis, excess energy can be dissipated as heat or it can be re-emitted as light which is chlorophyll fluorescence. As these three processes occur in competition, any increase in the efficiency of one will result in a decrease in the yield of the other two. The fluorescence can give information about the ability of a plant to tolerate environmental stresses and the extent to which those stresses have damaged the photosynthetic apparatus. The decrease in dark adapted Fv/Fm and increase in F0 indicate photoinhibitory damage in response to high temperature, low temperature, excess photon flux density (PFD) and water stress.

The effects of extreme phosphate deficiency during growth on the contents of adenylates and pyridine nucleotides and the in vivo photochemical activity of photosystem II (PS II) were determined in the leaves of Helianthus annuus and Zea mays grown under controlled environmental conditions (Jacob and Lawlor, 1993). The authors observed that phosphate deficiency decreased the amounts of ATP and ADP per unit leaf area and the amounts of oxidized pyridine nucleotides per unit leaf area, but not those of pyridine nucleotides. There was a slight increase in the initial fluorescence (F0) and a
decrease in maximum fluorescence (Fm) in phosphate deficient leaves as compared to the control. It was also noted that deficiency of inorganic phosphate leads to increased photoinhibition of PS II.

Experiments were carried out in sunflower (*Helianthus annuus*) and maize (*Zea mays*) plants grown in controlled environment chambers with either adequate supply or no external supply of inorganic phosphate (Jacob, 1995). Chlorophyll fluorescence from photosystem II (PS II) was measured using a modulated fluorescence measuring system at various photon flux densities at room temperature.

De Costa et al. (2000) studied the effect of three different levels of N (0, 52.5 and 105 ppm) on the photosynthesis in clonal tea and found that light saturated photosynthetic rate and photochemical efficiency increased significantly with increasing nitrogen supply at low irradiance. The authors observed that the photosynthetic rate was positive above a threshold of 2% leaf N content and reached a plateau at around 2.9% of leaf N. When the leaf N increased above 2.8% the photochemical efficiency increased rapidly and reached a plateau at around 3.2% of leaf N. After application of N the response of photosynthesis was immediate, but the photochemical efficiency responded only after one month.

Plants of *Phaseolus vulgaris* were subjected to increasing water deficit and combination of water deficit and high temperature (Yordanov et al., 1997). The photosynthetic gas exchange and chlorophyll fluorescence induction kinetics excited by low and saturating photon flux densities at 25 and 45°C were studied. While water deficit caused significant decrease in the rates of CO₂ uptake and O₂ evolution, the combination of water deficit with high temperature led to inhibition of both CO₂ uptake and O₂ evolution. According to the authors, the combination of water stress and high temperature induces considerable functional and structural changes in the
photosynthetic apparatus, the limit of which depends on its genetically determined capacity as well as on its physiological state. They also reported that, due to drought the oxidizing site of PS II was injured and the activities of ribulose-1, 5-biphosphate carboxylase/oxygenase (Rubisco) and other photosynthetic enzymes as well as protein content decreased.

The loss of photosynthetic capacity of leaves was one of the early symptoms of environmental stress and the primary effects of environmental stress was inhibition of photosynthesis at high light intensities (photoinhibition) (Jacob, 1998). Disturbances in the normal photosynthesis due to environmental perturbations increased the production of superoxide radicals, which was harmful to plants. According to the author, from simultaneous measurements of photosynthesis and chlorophyll fluorescence from the same leaf, partitioning of photosynthetic electrons between carbon reduction and other processes could be estimated. Pulse amplitude modulated chlorophyll fluorescence signals could be used to calculate the coefficients of useful photochemical quenching and wasteful nonphotochemical quenching of excitation energy present in the chlorophyll. The author further stated that Fv/Fm ratio (0.832±0.004) is an important parameter of the physiological state of the leaves and that severe environmental stresses decrease this ratio.

Jacob and Karaba (2000) made simultaneous measurements of chlorophyll fluorescence and gas exchange in leaves of *Hevea* experiencing drought stress. When leaves were excised, the rate of photosynthetic carbon assimilation and the rate of *in vivo* electron transport across photosystem II decreased while rate of photosynthetic electron diversion away from carbon reduction presumably for oxygen reduction leading to the production of reactive species of oxygen and free radicals, increased. In the case of plants with injured roots, a similar observation was made by them leading to an inhibition of the quantum yield of photosystem II activity and was related to
ageing as indicated by loss of chlorophyll content of leaf. From the studies they concluded that green leaves experiencing stress increased diversion of electrons away from carbon to oxygen and under conditions of high light intensity hastens leaf senescence possibly through production of reactive species of oxygen and free radicals.

Experimental work on three nursery grown tea cultivars using chlorophyll fluorescence showed that when the soil moisture deficit increases, the variable to maximal fluorescence ratio (Fv/Fm) decreases, indicating a loss in primary photochemical efficiency of the stressed leaves of tea (Jeyaramraja et al., 2003). These authors reported that chlorophyll fluorescence can be used as a non-invasive probe of photochemical events taking place in intact leaves and Fv/Fm ratio has been suggested as a quantitative measure of photochemical efficiency of the photosystem II (PS II) complex. They further stated that though initially reversible, drought in its severest form can lead to cell death and the ill effects of drought can be minimized by the use of drought tolerant planting material.

Discussing about the photoinhibition of photosynthesis Powles (1984) stated that the exposure of many plants to temperatures in the chilling range (0-20°C) or to freezing temperatures had adversely affected plant functions. The author noticed that photosynthesis was one of the first processes affected and the symptoms of low temperature damage to the photosynthetic apparatus were especially pronounced or only occur when substantial light accompanied the low temperature exposure. The capacity of plants to utilize the light energy absorbed by them declined significantly on their exposure to environmental stresses. Long-term exposure of plants or photosynthetic organelles to strong light can result in photodestruction of photosynthetic pigments caused by oxygen and light dependent bleaching which is defined as photooxidation. Photooxidation of pigments occurred only after a certain
degree of photoinhibition had occurred and therefore photoinhibition of photosynthesis was not a consequence of pigment destruction. Photooxidation causes oxygen-dependent bleaching of carotenoid and then chlorophyll pigments. The author also mentioned that one of the important targets for the action of the toxic oxygen species was the photosystem II and photoinhibition led to inhibition of photosynthesis due to changes at the reaction centers of photosystem II and photosystem I.

Baker (1991) investigated the effect of environmental perturbations on photosynthesis and found that the photosystem II played an important role in the response of photosynthesis in higher plants to environmental stresses. The identification of PS II as the primary site of photoinhibition in thylakoids led to considerable attention being focused upon PS II with respect to environmental stress effects on photosynthesis. The author also reported that the photochemical activity of the PS II population in leaf will be determined by both the ability of the PS II antennae to capture light energy and the efficiency with which captured excitation energy is utilised for photochemistry. As a result the changes in the antenna size and quantum efficiency of photosystem II may have implications on CO₂ assimilation.

The chlorophyll fluorescence technique was used for assessing the cold temperature tolerance of Picea plants (Adams and Perkins, 1993). According to Mohammad et al. (1995), Vyas et al. (1998), JongUn et al. (2000) and Alam et al. (2004) it is a non-destructive, easy, fast and more reliable method for assessing frost or low temperature stress tolerance. For assessing cold temperature tolerance using chlorophyll fluorescence of leaves of Picea plants collected from the field, these were exposed to controlled freezing temperatures (Adams and Perkins, 1993). No decrease in fluorescence at progressively lower temperatures, relative to the unfrozen control foliage was noticed until a critical temperature was reached, whereupon rapid, irreversible
decreases in fluorescence occurred. The authors found that chlorophyll fluorescence was an indicator of many plant stresses and can be used to quantify various stresses in plants including chilling/freezing injury. The authors ultimately came to a conclusion that rapid reductions in fluorescence reflect actual tissue injury caused to leaf tissues exposed to cold temperatures.

Winter depression of photosynthesis in tea leaves was studied by Okano and Matsuo (1994) studied and they observed that in the cold resistant variety, depression of photosynthesis in winter was relatively less and obtained favorable recovery. In the cold sensitive cultivar winter depression of photosynthesis was severe and showed only little recovery and the less cold sensitive cultivar showed intermediate response to low temperature.

According to Vyas et al. (1998) tea bushes are prone to winter dessication and frost damage. Chlorophyll a fluorescence technique was adopted for assessing cold stress tolerance in six different clones of tea obtained from different agro-climatic regions of India maintained in the open and under shade. The authors observed that the plants kept under shade maintained a uniform $F_v/F_m$ ratio of around 0.800, indicating that frost rather than low temperature was the major cause of damage. The results showed that under open condition the value of $F_v/F_m$ dropped below 0.650 in all the clones except in the clone UPASI-9 which showed minimum photoinhibition during winter and appeared to be most tolerant to frost.

Chlorophyll fluorescence technique was used to determine the chilling tolerance in 64 cultivars of strawberry flowers exposed to low temperature (Khanizadeh et al., 1999). The results obtained were compared with results obtained by traditional visual method (% of visual damage to the flower). A positive correlation was obtained between the chlorophyll fluorescence of the susceptible flowers and the degree of frost damage and these authors came to
a conclusion that chlorophyll fluorescence may be used as a tool to select frost hardy cultivars.

Alam and Jacob (2002) studied the effect of frost on *Hevea brasiliensis* plants and compared those with a parallel set of plants kept under frost free conditions. The experiment was repeated in certain other species of plants also grown at low and high temperatures. The photosynthetic rate, stomatal conductance and chlorophyll fluorescence in terms of Fv/Fm ratio were measured under both low and high photosynthetic photon flux densities (PPFD) and chlorophyll and malondialdehyde (MDA) content in the leaves were also estimated. According to the authors, low temperature stress inhibited photosynthetic rate more in *H. brasiliensis* than in other species naturally acclimated to the cool conditions and the low temperature induced inhibition was further aggravated at high PPFD and all the native species found in Madupetty, except Napier grass, had high photosynthetic rates in spite of low temperatures. Leaf net photosynthetic rate (Pn) is dependent on ambient temperature as Calvin cycle enzymes are temperature sensitive.

Chlorophyll a fluorescence technique was used for assessing cold stress tolerance in various clones of *Hevea brasiliensis* wherein two clones were grown under low temperature for critical assessment of metabolic responses in comparison with the control plants of the same clone grown in warm climate (Alam *et al.*, 2004). Though the activities of the photosystem II were reduced at low temperature, there was excess flow of photosynthetic electrons across photosystem II and it was the fate of these electrons that would decisively influence the photochemical activities right from photosystem II to leaf biochemistry and depending upon the intrinsic capacity of each clone to handle these electrons at low temperature, oxidative damage might occur. Out of the two clones studied (RRIM 600 and RRII 105) by the above authors, in the clone RRII 105 excess photosynthetic electrons-induced
photo oxidative damage was relatively more as reflected in less superoxide dismutase (SOD) activity and higher MDA/Chlorophyll ratio. The authors concluded that the effective photosystem II quantum yield could be a reliable tool for assessing cold stress tolerance and it can also be measured in a reasonably shorter time.

2.5 Photosynthesis

Aslam et al. (1977) studied the effect of age of plant and leaf on CO₂ exchange rates and transpiration rates in 15 genotypes of cassava (Manihot esculenta). The authors found that while the plant age had no effect on leaf CO₂ exchange rates, the transpiration rates in 14-week-old plants were significantly greater than those in seven-week-old plants. The CO₂ exchange rates and transpiration rates decreased with leaf age. The stomatal and residual resistances to the diffusion of CO₂ were found increased with leaf age in all the genotypes. It was also noted that the chlorophyll content decreased and specific leaf weight increased with leaf age.

Twelve clones of tea were subjected to a study of their rate of photosynthesis, assimilate partitioning and water holding capacity and it showed that there existed significant differences in net photosynthesis between the clones (Kumar et al., 1993). The Assam variety had higher rates of net photosynthesis. The authors found that the China variety of tea bushes exhibited significantly higher water holding capacity than the Cambod variety and the least was in the Assam variety. While studying the proline accumulation, the clone UPASI-3 had highest proline content as compared to other Assam variety plants and the China variety accumulated the least amount of proline compared to the Assam and the Cambod varieties. According to them, an assessment of the physiological responses of the tea plant is of utmost importance in order to identify traits, which could form a
basis for selection and plant improvement programmes and they identified the clone UPASI-3 as being susceptible to drought.

Palanisamy (1996) studied the effect of diurnal variations in net photosynthetic rate, transpiration rate, stomatal conductance and nitrate reductase activity in relation to environmental factors in field grown Pongamia pinnata trees. It was seen that the net photosynthetic rate and stomatal conductance were maximum at 8.00 am hours while photosynthetically active radiation (PAR), temperatures of leaf and air and transpiration rate were higher at 12 noon. The water use efficiency was maximum and minimum at 7.00 am and 12 noon respectively. The net photosynthetic rate was found to be much lower in shade leaves than in the sunlit ones. The author finally came to a conclusion that in Pongamia transpiration rate was correlated with photosynthetically active radiation (PAR) and air temperature, while the net photosynthetic rate was correlated with stomatal conductance.

According to Bai and Kelly (1999), there were significant differences in the net photosynthetic rate among eight genotypes (Asparagus officinalis) and the values ranged from 15.67 to 27.79 μmolm⁻²s⁻¹. The variability in the photosynthetic capacity was positively correlated with the long-term economic yields of these. The genotypes having high photosynthetic rates were found to have high specific leaf mass and therefore selecting them for high specific leaf mass could be adopted as a method for preliminary selection of genotypes with high photosynthetic rates. It was also seen that the daily photosynthetic rate patterns appeared to be related to the daily changes of stomatal conductance.

Rate of photosynthesis on a tea clone T78 during different seasons was studied (Hajra and Kumar, 1999) and it was demonstrated that the maximum value of photosynthetic rate was in the month of October when humidity was
very high, temperature, sunshine hours and soil moisture were moderate and photosynthetic photon flux density was highest. According to the authors, the low temperature along with the low soil moisture reduced photosynthetic rate during winter and an important limiting factor for photosynthesis was found to be moisture stress.

The bract photosynthetic and respiratory activity was compared with that of leaf in sunflower by Laxman and Srivastava (2000). The bract net photosynthetic rate during ontogeny was found to be negative in the initial stage and gradually increased in the later stages. The lower photosynthetic rate of bracts relative to leaves was due to lower stomatal conductance, low chlorophyll concentration and N content. They also found that stomatal CO₂ concentration in bracts was 20 to 27 % higher compared to leaf, which indicated lower mesophyll efficiency of the bracts in comparison to leaves.

Subrahmanyam and Dutta (2000) studied the relationship between leaf photosynthetic characteristics and yield in ten buckwheat (Fagopyrum esculentum and F. tatricum) cultivars. The photosynthetic rate was positively and significantly associated with stomatal conductance, transpiration rate and total biomass. The specific leaf mass exhibited a strong positive association with rate of photosynthesis and total biomass and, according to the authors, the specific leaf mass could be used in breeding programmes to select higher photosynthetic rate and higher biomass.

The photosynthetic rate and transpiration rate of twelve tree species were measured from June to November and it was noted that in most of the tree species the photosynthetic rate was significantly higher from July to November and the transpiration rate declined after August (Thakur and Kaur, 2001). A decline in transpiration with the ageing of the leaf was because leaf age is very important in regulating transpirational losses. The water use efficiency was low in all the twelve species, maximum value was in Albizia
and minimum in Morus. The authors found that the canopy of the tree species intercepted a significant amount of photosynthetically active radiation and allowed only 7-22% transmission of photosynthetically active radiation beneath the canopy. They concluded that the tree species, which had good growth up to ten years, had higher photosynthetic rate, light interception and water use efficiency over the other tree species.

According to Hajra and Kumar (2002), the highest value of photosynthesis in the tea clone T78 was between 10.00 am and 12 noon irrespective of the season. The midday decrease in photosynthetic rate was found to be due to a decline in stomatal conductance, which appeared to be a reflection of stomatal closure rather than photoinhibition. The authors found that the chlorophyll content was highest during autumn followed by rainy and winter seasons. While a positive correlation was found between chlorophyll a and chlorophyll b content, the correlation between photosynthesis and chlorophyll content was negative.

Studies were carried out by Burman et al. (2003) on the effect of kinetin on growth, dry matter production, seed yield, net photosynthetic rate, total chlorophyll and nitrate reductase activity in clusterbean (Cyamopsis tetragonoloba) under moisture deficit condition. It was observed that in kinetin treated plants, the net photosynthetic rate and nitrate reductase activity increased significantly. There was also more partitioning of photosynthates towards seeds, which resulted in higher harvest index in kinetin treated plants. The beneficial effects of kinetin were due to higher content of different leaf metabolites (starch, soluble protein etc.), which prolonged the active growth phase.

Field experiments were carried out in 12 cowpea genotypes belonging to different growth habit (determinate and indeterminate) under rainfed conditions by Kalpana et al. (2003). It was observed that the determinate
genotypes had higher values of photosynthetic rate, transpiration rate, and stomatal conductance in comparison to indeterminate genotypes. The photosynthetic rate, transpiration rate, and stomatal conductance were maximum at flowering stage and declined at pod development stage in all the genotypes and there existed a wide variation among the genotypes. The decline in photosynthetic rate at pod development stage could be due to the mobilization of leaf N for the development of protein rich seeds. The authors finally noted that the genotypes with higher photosynthetic rate and stomatal conductance, were high yielders.

Karuppaiah et al. (2003) studied the effect of different antitranspirants on growth, photosynthesis and yield characters in brinjal. All the antitranspirants affected the photosynthetic characters such as net photosynthetic rate, stomatal conductance, intercellular CO₂ concentration, transpiration and relative water content. The different antitranspirants produced different photosynthetic responses. The maximum net photosynthetic rate, relative water content and minimum transpiration were seen with kaolin (7.5%), which was at par with salicyclic acid (1000 ppm) and liquid paraffin (1.5%). According to the authors, the increased net photosynthetic rate in kaolin and salicyclic acid treatments might be due to the reduction in transpiration rate, stomatal conductance and intercellular CO₂ concentration. The fruit yield was found to be significantly increased with kaolin (7.5%) and salicyclic acid (1000 ppm).

The photosynthetic rates of seven cultivars of *Nicotiana tabacum* were studied by Kumar (1982). There was strong positive correlation between photosynthetic rate and nitrate reductase activity and leaf N content. It was seen that the mean leaf area of the fifth leaf and the time of emergence of the leaf were similar in all the cultivars, but all the cultivars showed significant differences in photosynthetic rate. According to the author there was no
significant correlation between photosynthetic rate and stomatal resistance or chlorophyll content.

The effect of phosphate deficiency on the composition and photosynthetic CO₂ assimilation rates of fully expanded leaves of sunflower, maize and wheat plants was studied by Jacob and Lawlor (1991). The rate of photosynthesis in leaves and stomatal conductance were lower in plants grown with inadequate phosphate when measured under any given light intensity or CO₂ partial pressure. It was observed that the mesophyll capacity for photosynthesis was greatly limited by phosphate deficiency and the leaves deficient in phosphate had large number of small size cells per unit leaf area than leaves with adequate phosphate. The leaf chlorophyll content decreased in sunflower and maize but not in wheat. According to the authors, phosphate is a constituent of many structural and functional components in the cell and its involvement in energy metabolism of cells as ATP, particularly in photosynthesis and respiration ensures a critical role in all plant functions.

Studies were carried out to determine the effect of irrigation and fertilizer in the tea clone 6/8 (Smith et al., 1993) and they found that irrigation and fertilizer application increased the photosynthetic rate by increasing both the photosynthetic rate per unit leaf area in the healthy leaves and the proportion of sunlight intercepted by photosynthetically efficient leaves. The authors also observed that when fertilizer was applied at 225 kg N ha⁻¹ per annum, it caused an increase in the photosynthetic rate with increase in stomatal conductance, but a further increase to 375 kg N ha⁻¹ per annum decreased photosynthesis in spite of increasing the stomatal conductance.

The effect of P deficiency in mulberry plants was investigated and decreased leaf area and chlorophyll content were observed (Sharma, 1995). It was noted that in P deficient plants there was significant decrease in leaf water potential, stomatal conductance, transpiration and degree of succulence and increase in water saturation deficit, which resulted in water deficit. The P
deficiency caused increased CO$_2$ compensation point, decreased net photosynthesis, saturation concentration of intercellular CO$_2$ and stomatal limitation, indicating that the effect of P deficiency on photosynthesis was non-stomatal.

The individual and combined effect of P and K on the morphophysiological traits and yield of chickpea (*Cicer arietinum*) cv. Pusa 417 was studied (Samiullah and Khan, 2003). It was found that a combination of 40 kg P$_2$O$_5$ and 20 kg K$_2$O per hectare produced the maximum yield. The authors also observed that at lower P levels (0 or 20 kg P$_2$O$_5$ per hectare), the requirement of K was high (40 kg K$_2$O per hectare) and at higher levels of P (40-60 kg P$_2$O$_5$ per hectare), the application of only 20 kg K$_2$O per hectare proved to be sufficient. The maximum net assimilation and crop growth rate were recorded during the 60-90$^{th}$ day interval. Though the crop attained the maximum leaf area index at 120$^{th}$ day, the optimum leaf area for most efficient utilization of photosynthetically active radiation was during 60-90$^{th}$ day interval. Thereafter shading of lower leaves started resulting in decrease in net assimilation and crop growth rate values at later growth intervals. The highest value of net assimilation rate was recorded during the 60-90$^{th}$ day interval at 20 kg K$_2$O per hectare and 40 kg P$_2$O$_5$ per hectare.

Field experiments were carried out to find the effect of date of sowing and levels of N, P and K on sunflower (Mandal *et al.*, 2003). Results of the experiment showed that sowing sunflower in the 1$^{st}$ week of March was beneficial. Application of 80 kg N, 17.5 kg P and 33.3 kg K ha$^{-1}$ resulted in higher dry matter production, leaf area index, number of seeds head$^{-1}$, 1000 seed weight and seed yield as compared to the other treatment combinations. This was due to the beneficial role played by NPK in increasing plant height and leaf area index due to better photosynthetic activity.
The effect of plant density and N on the physiological variations in French bean (*Phaseolus vulgaris*) was studied (Dhanjal et al., 2003). The leaf area index and crop growth rate were higher at higher plant density, whereas dry weight per plant, net assimilation rate and relative growth rate in general were higher at lower plant density. It was also observed that increasing levels of N fertilizer up to 120 kg ha$^{-1}$ increased dry weight, leaf area index, crop growth rate and relative growth rate but the net assimilation rate increased up to 60 kg N ha$^{-1}$ only.

Vyas et al. (2001) studied the effect of different levels of potassium (25, 50, 100 and 200 ppm) on the water relations, CO$_2$ assimilation, enzyme activities and plant performance under soil moisture deficits in clusterbean during vegetative, flowering and pod development stages. They observed that the reduction in the leaf relative water content and plant water potential due to water stress was less in plants grown at 200 ppm K, while water stress drastically reduced the rate of net photosynthesis at all growth stages and at all K levels. The photosynthetic rates of all concentrations of K treated plants were relatively higher both in control as well as water stressed plants at 200 ppm K level. Therefore it was seen that K application helped clusterbean plants in maintaining internal water and metabolic activities under water stress and also produced increased chlorophyll content in control and water stressed plants.

Jones (1973) studied the effect of water stress on various photosynthetic parameters of cotton plants. It was observed that the stressed plants had lower rates of potential photosynthesis and actual photosynthesis. The stomatal and intercellular resistances and the corresponding photosynthetic limitations were greater in stressed plants. Most of the photosynthetic parameters showed complete recovery 24 hours after
Rewatering and the author opined that the major cause of reduced photosynthesis was due to stomatal closure.

Studies on leaves of wheat plants for the rate of net photosynthesis at various temperatures at fixed O₂, CO₂ and photosynthetically active radiation were carried out (Keys et al., 1977). When the O₂ concentration was decreased from 21 to 2%, the rate of photosynthesis increased by 32% at the lowest temperature and by 54% at the highest temperature. From the results the authors concluded that photorespiration was relatively greater at higher temperatures.

The effect of humidity on photosynthesis, transpiration and water use efficiency under conditions of adequate soil moisture was studied in several plant species (Rawson et al., 1977). The photosynthesis, stomatal and internal diffusion resistances of whole, attached and single leaves were not found to be affected by changes in humidity. The transpiration rate increased linearly with increasing vapour pressure deficit. It was also reported by the authors that the water use efficiency was highest in the C₄ xerophytes and lowest in the C₃ mesophytes.

The irrigated and non-irrigated corn plants (Zea mays) fed with ¹⁴C were subjected to study the translocation of ¹⁴C to different plant parts (Brevedan and Hodges, 1973). It was found that the leaves of stressed plants retained more radioactive carbon in both the fed portion and non-fed portion of the leaf, than in nonstressed plants. The stressed plants as well as nonstressed plants continued to translocate photosynthetically assimilated ¹⁴C for 90 minutes, but between 90 and 120 minutes after labeling, there was a major reduction in the amount translocated in stressed plants as compared to the non-stressed plants.
An attempt was made to evaluate the plant measurements, stomatal conductance, leaf water potential and leaf area as indicators of water stress in soybean (*Glycine max*) (Sivakumar and Shaw, 1978). It was observed that the stomatal conductance and leaf water potential measured several times during the growing season were closely related to changes in soil water potential. The relative growth rate of soybeans showed negative correlation with stomatal conductance, leaf water potential and rate of leaf area expansion. The authors noted decreased leaf water potential with decreased soil water potential. The authors stated that decreased stomatal conductance led to a decrease in the photosynthetic activity, which resulted in a reduced rate of dry matter production. They finally concluded that plant measurements in addition to soil water measurements were useful to indicate and quantify water stress effects under field conditions.

Louwerse (1980) studied the effect of CO$_2$ concentration and irradiance on the stomatal behavior of maize, barley and sunflower plants. It was found that in maize the net photosynthetic rate was linearly related to the irradiance and independent of the ambient CO$_2$ concentration. In sunflower and barley the net photosynthetic rate and transpiration rate decreased with increasing ambient CO$_2$ concentration. In all the three species of plants studied, the internal CO$_2$ concentration was independent of the irradiance. In maize the internal CO$_2$ was independent of ambient CO$_2$, but in sunflower and barley the internal CO$_2$ concentration was proportional to the ambient CO$_2$ concentration with a ratio of 0.6.

Singh et al. (1982) studied the effect of radiation, temperature and humidity on the photosynthesis, transpiration and water use efficiency of chickpea. The peak rates of photosynthesis were recorded at 1800 µE m$^{-2}$ s$^{-1}$ photosynthetic active radiation (PAR), 22°C air temperature and 14 mb vapour pressure deficit (VPD) of air in the glass house. The rate of
transpiration increased due to increase in photosynthetic active radiation, air temperature and vapour pressure deficit of air. It was observed that the water use efficiency remained higher and almost static from 600 to 1800 \( \mu \text{E m}^{-2} \text{s}^{-1} \) photosynthetic active radiation, but declined at lower and upper end of radiation intensity curve. It could also be seen that the water use efficiency declined progressively as the air temperature and vapour pressure deficit of air in the chamber was elevated.

According to Jacob (1988) a variety of abiotic stress exists in the agroclimatic zones of the world which include drought stress, low or high light stress, nutrient stress and salinity stress out of which drought stress is the most important one. The author also stated that plant growth and productivity were very strongly correlated with moisture availability whereby drought is known to be the most important single variable, which reduced productivity in many parts of the world.

The influence of temperature on dry matter accumulation of maize (Zea mays) canopies were studied (Tollenaar, 1989). The maize plants were grown in controlled environment at photosynthetic photon flux density (PPFD) of 650 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) under five constant day/night temperature regimes (15, 19, 23, 27, 31°C) and five differential day/night temperature regimes (15/3, 19/7, 23/11, 27/15, 31/19°C). The leaf photosynthetic rates were measured at the 12-leaf stage at photosynthetic photon flux densities ranging from zero to 2500 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) and growth analysis was performed from planting to the 12 leaf stage. The leaf photosynthetic rate increased linearly from 15 to 27°C and the magnitude of the response was larger at high PPFD than at low PPFD. The \( Q_{10} \) for specific growth rate in the range 11 to 31°C was 1.93 from the fourth to the 12\(^{th} \) leaf stage. The leaf photosynthetic rate at 650 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) was found to be highly correlated with the specific growth rate across the temperature regimes studied. Finally the author opined that the
specific growth rate and net assimilation rate during the early phases of maize development were highly correlated with the leaf photosynthetic rates across the temperature regimes studied.

Prasad and Srivastava (1992) studied the effect of moisture stress on photosynthesis during the ontogeny of sunflower (*Helianthus annuus*). It was observed that the stomatal conductance and net photosynthetic rate declined sharply with increasing levels of moisture stress in the pre-flowering stage. The vegetative stage was found to be more susceptible to moisture stress with regard to stomatal conductance and net C exchange rate. From the results it was concluded that the reduction in the rate of electron transport through PS II and PS I was comparatively more in the flowering stage than at other developmental stages, at all levels of moisture stress.

Barbora (1994) studied six tea clones under different soil moisture regimes. The author found that moisture stress reduced rates of photosynthesis and respiration due to impaired stomatal conductivity. The transpiration rate was also reduced due to water stress and the transpiration response varied in different clones of tea.

The water relations, gas exchange, chlorophyll fluorescence and leaf abscisic acid content in apple trees during water stress and recovery period were studied (Fernandez *et al.*, 1997). The studies showed a reduction in CO₂ assimilation, transpiration and leaf conductance during stress while the variable and maximal chlorophyll fluorescence was not so sensitive to stress. The authors found that leaf water potential was consistently lower during drought stress and returned to control values upon irrigation. They also stated that chlorophyll fluorescence measurements can be used to know how electron transport through the photosystems is affected during water stress.
Jinke (1998) studied the effect of moisture stress on the photosynthesis of tea which showed that mild stress reduced net photosynthetic rate and transpiration rate due to reduced stomatal conductance. But, according to the author, the principal cause of reduction in net photosynthetic rate under severe water stress was attributed to the decline of photosynthetic capacity of mesophyll cells.

Rice genotypes kept under high water stress by submergence exhibited reduction in photosynthetic rate, stomatal conductance, intercellular CO₂ concentration, hill activity, chlorophyll fluorescence and chlorophyll and carotenoid content. The osmotic potential of fully submerged leaves above a critical point, led to lower stomatal conductance and intercellular CO₂ concentration, which became sub optimal for carboxylation in mesophyll cells (Adak and Gupta, 2000).

Sasaki et al. (2000) studied the effects of light during low temperature treatment and water stress on freezing tolerance and sugar contents in cabbage seedlings. The authors noticed that when cabbage seedlings were exposed to non-freezing low temperature (5°C) under a 12-hour photoperiod, they acquired freezing tolerance, while the plants exposed to non-freezing low temperature in the dark did not acquire this. The plants, which were subjected to water stress by withholding water, displayed a higher degree of freezing tolerance and increased sugar contents than the watered plants. It was concluded that cabbage seedlings required light to acquire freezing tolerance after exposure to low temperature, de-acclimation was induced without light and water stress increased the freezing tolerance to a certain extent.

The effect of salt and water stress on fruit quality, physiological responses, macro and micro element contents in the leaves of Satsuma mandarin (Citrus unshiu) grafted on trifoliate orange (Poncirus trifoliata) under greenhouse conditions were investigated (Morinaga and Sykes, 2001).
Though there was no significant effect on tree growth, salt and water stress treatments resulted in a reduction of photosynthetic rates. Water stress decreased leaf water potential and increased stomatal resistance compared to the control and salt stressed trees while the concentrations of Na and Cl of the leaves were not affected by salt or water stress treatments. But the K concentration of the leaves was reduced significantly by 10 mM NaCl and water stress. Water stress advanced fruit maturity though the fruit size and fresh weight were reduced. The internal fruit quality was improved for the trees irrigated with 10 mM NaCl solution or subjected to water stress.

The role of different secondary traits in improving the flowering stage drought tolerance in six varieties of rice was investigated (Kumar and Kujur, 2003). It was observed that the tolerant varieties had lower leaf temperature than air temperature under drought, which was related to higher transpiration rates with the help of higher root to shoot ratio. Under drought high leaf water potential was considered to be responsible for photosynthetic stability. In drying soil, the stomatal conductance also controls the photosynthetic rate and the transpiration rate was significantly reduced due to drought. The authors finally came to a conclusion that less delay in flowering, high leaf water status, high root to shoot ratio, low leaf temperature and high membrane stability under drought contributed significantly to flowering stage drought tolerance in rice.

Sharma et al. (2003) studied the effect of moisture stress on wheat genotypes grown under irrigated, mild stress and severe stress conditions. The authors observed that canopy temperature depression, relative water content and water potential decreased significantly with increase in moisture stress. The maximum relative water content was found in the clones which exhibited higher water potential. Though yield and its attributes for all the genotypes decreased significantly in the stressed environments, the yield
reduction over control was 23.8% and 57.6% in mild and severely stressed conditions respectively.

2.6 Proline and MDA content during drought and frost stress

Hanson et al. (1977) evaluated the accumulation of proline in two cultivars of barley (*Hordeum vulgare*) Proctor and Excelsior. The leaf water potential in the mid blade zone always fell more rapidly in Proctor than in Excelsior and consequently reached the critical −30 to −40 bar value earlier. In both the varieties as the leaf water potential fell, free proline accumulated in the leaf tissue and reached the highest concentration as leaf kill became severe; at this stage much of the free proline was localized in the non-viable leaf zone. The leaves of the variety Proctor always accumulated free proline more rapidly than Excelsior leaves. It was also seen that on relief of water stress, the free proline levels declined in viable leaf tissue within three days and approached control values in four to six days, but remained very high in the drought-killed desiccated leaf zone. The authors finally came to a conclusion that the positive correlation between proline accumulating potential and drought resistance in barley might be in error.

The changes caused by drought in the activities of superoxide dismutase (SOD) and catalase, level of lipid peroxidation and membrane permeability were studied in two mosses, the drought tolerant *Tortula ruralis* and the drought sensitive *Cratoneuron filicinum* (Dhindsa and Matowe, 1981). It was found that in *T. ruralis* the activities of SOD and catalase increased during slow drying and the level of lipid peroxidation consequently declined. On subsequent rehydration the enzyme activities declined and the level of lipid peroxidation gradually rose to normal levels. In the drought sensitive moss, *C. filicinum* the activities of SOD and catalase declined during drying as well as during subsequent rehydration. There was also rapid increase in lipid peroxidation during rehydration. It was concluded that a
capacity to limit membrane damage to a reparable level by controlling lipid peroxidation may be an important factor for drought tolerance in plants.

Kandpal et al. (1981) studied the effect of water stress on proline accumulation in Ragi and showed that free proline content increased (6 to 85 fold) in the leaves as the degree of water stress created by polyethylene glycol treatment, was prolonged. According to these authors, water stress stimulated the activities of ornithine aminotransferase and pyrroline-5-carboxylate reductase, the enzymes of proline biosynthesis and markedly inhibited the enzymes involved in proline degradation viz., proline oxidase and pyrroline-5-carboxylate dehydrogenase. The authors further suggested that the increase in free proline content of Ragi leaves could be due to enhanced activities of enzymes synthesizing proline and more importantly due to severe inhibition of the enzymes degrading proline.

Pot culture experiments were conducted by Patil et al. (1984) on five maize genotypes to study the effect of drought stress on free proline content and relative water content (RWC) in the root, leaf sheath and leaf blade. During the study the authors observed that with the advance of stress, relative water content decreased while free proline content increased.

The effect of water stress on leaf water content, stomatal conductance and proline accumulation in the leaves of ten potato (Solanum tuberosum) genotypes was studied (Bansal and Nagarajan, 1986). It was found that under stress conditions, water saturation deficit in leaves was significantly correlated with tuber weight and tuber number while in non-stressed plants it was correlated with tuber weight but not with tuber number. Stomatal conductance was negatively correlated with tuber weight and tuber number under stress conditions. In the stressed plants proline accumulation in leaves showed a significant negative correlation with tuber weight and tuber number. In the non-stressed plants proline accumulation in the leaves was negatively
correlated with tuber number. The authors indicated that stomatal conductance was a better parameter as an index for drought resistance than water saturation deficit and proline content in leaves of potato.

Kuo et al. (1986) examined the proline contents of anthers, pollen, pistils and leaves of several tomato (Lycopersicon esculentum) cultivars under different temperature conditions. High temperatures reduced the proline content in the anther regardless of the stage of development and in the pistils of later floral bud stage. Though the proline content in the leaves was lower than that of anthers or pistils, high temperature increased proline level in the leaves. The authors concluded that the low proline accumulation in the anthers and pollen at high temperature might be due to the high accumulation in the leaves.

Proline and dry matter accumulation in different varieties of sugarcane under moisture stress was studied (Singh and Singh, 1986). The results showed that certain varieties had higher accumulation of proline than the others especially during moisture stress and these varieties also produced more dry matter than the ones which had lesser proline. The authors also stated that high proline and its further accumulation under stress was known to act as storage compound for carbon and nitrogen.

Studies on moisture stress on different tea cultivars (Rajasekar et al., 1988) have shown that proline content increased in all the clones and the drought tolerant clones had significantly higher amounts of proline and relative water content than the susceptible clones under prolonged stress. They also found that the rate of moisture loss under stress was lower in the tolerant clones and that the stress resulted in a decline in the total chlorophyll content of all the clones. The authors have reported that proline accumulation could be a defence mechanism to overcome moisture stress and have also mentioned that it serves as a compatible solute to maintain the osmotic
balance between cytoplasm and vacuole. They found that the clones UPASI-2, UPASI-9 and UPASI-10 accumulated higher levels of proline under prolonged water deficit, which is indicative of their drought tolerance, and the clones UPASI-3, UPASI-8 and UPASI-17 were found to be drought susceptible in comparison to them. Their study also revealed that water stress led to a decline in total chlorophyll content in all the clones.

Narayan and Misra (1989) investigated the varietal differences in free proline accumulation under water stress in bread wheat and durum wheat. All the varieties accumulated free proline under unirrigated condition. The results indicated that the varieties accumulating higher free proline under unirrigated condition also yielded higher under stress. The varieties having different degree of drought resistance differed in their capacity to accumulate free proline and the resistant varieties accumulated higher levels of free proline than susceptible ones under stress. The authors finally came to a conclusion that proline works as a source of energy, carbon and nitrogen and also protects several enzymes against the inactivating effects of heat during water stress and therefore proline accumulation under water stress helps to resist drought.

Coconut genotypes under moisture stress simulated by an osmoticum of 30% polyethylene glycol and air desiccation, for a period of twenty four hours had different effects on the varieties studied (Voleti et al., 1990). The relative water content of leaves was reduced more due to air desiccation than the osmotic stress. Between the two types of stress created, the accumulation of proline did not differ much except in the dwarf varieties which exhibited higher proline content in the polyethylene glycol treated leaves than in the air desiccated ones. These authors found an inverse relationship between relative water content and proline accumulation and the data of this study revealed that proline content was not associated with drought tolerance in coconut.
The relationship between the osmoregulation capability of various pea cultivars (*Pisum sativum* L.) grown in climatic chambers (subjected to one dehydration cycle) and their yields in the field under drought conditions were investigated (Rodriguez-Maribona *et al*., 1992). It was noted by the authors that there was a linear relationship between yield and osmotic adjustment; the genotypes which showed the best osmoregulation capability also had the best yield under stress. The authors opined that a genotype which showed the highest yield had a high capacity for osmoregulation and high water use efficiency and these are two characteristics associated with drought resistance.

In order to carry out evaluation for drought tolerance in coffee, eight robusta accessions, one local robusta cultivar and an improved series of diallelic crosses of robusta were studied for their pattern of solute accumulation (Saraswathy *et al*., 1992). Before inducing stress, at wilting and after alleviation of moisture stress, solutes such as free proline, N, P, K, Ca and total carbohydrates were estimated. The authors found that the accessions 1932 and 1979 had accumulation of free proline, N, P, K and Ca during stress and decrease of the contents after alleviation of stress as compared to the other accessions. This indicated that these accessions were capable of making osmotic adjustments during stress, which were observed in drought tolerant coffee cultivars. Therefore it was concluded that among the exotic robusta accessions 1932 and 1979 were more drought tolerant than the local cultivar.

Singh and Handique (1993) found that higher quantity of proline was found in the leaves of drought tolerant cultivars than in the susceptible ones while studying different clones of tea for their drought tolerance under conditions of soil moisture stress. The results of this study indicated that the clones susceptible to drought exhibited lower shoot water potential values than the tolerant ones. The authors found that the value of stomatal diffusion resistance-transpiration rate could be used as a screening parameter for
identifying drought tolerant and susceptible genotypes. They stated that the stomatal diffusion resistance values differ significantly between clones and are higher in drought tolerant cultivars than in susceptible ones, and transpiration rate values were lower in drought tolerant cultivars than in susceptible ones. The conclusion of this particular study was that soil moisture stress increases stomatal diffusion resistance and decreases transpiration rate, and these two interrelated physiological processes help in building up of plant water status. Another observation of this study was that increased stomatal diffusion resistance exhibited by drought tolerant cultivars shows that the stomata responded quickly to moisture stress by reducing the transpiration rate and thereby helped the plant in building up of plant water status. As such enhanced plant water status could be reflected through higher water potential, leaf water content, relative turgidity and lower water saturation deficit, it was suggested by them that these parameters could be used as selection criteria for drought tolerance in selection and breeding in tea.

In light grown cotyledons of radish (*Raphanus sativus* cv National) Hervieu *et al.* (1994) observed that the concentration of proline and the level of ornithine aminotransferase activity increased with decrease of relative water content. The treatment with Gabaculine, which is an irreversible inhibitor of ornithine aminotransferase, reduced the water stress induced proline accumulation considerably. It was concluded that, in addition to the glutamate pathway, the ornithine aminotransferase pathway contributed through an increase of ornithine aminotransferase activity to proline synthesis in water stressed cotyledons.

Saradhi *et al.* (1995) studied the effect of UV radiation on proline accumulation in the seedlings of Rice, Mustard and Mung Bean. The authors found that the level of proline increased with increase in exposure time of UV
radiation in the shoots of seedlings of Rice, Mustard and Mung Bean. The malondialdehyde (an indice of lipid peroxidation) was also higher in the shoots of seedlings exposed to UV radiation as compared to control seedlings, indicating that UV radiations increased lipid peroxidation. From the results the authors concluded that UV radiation induced proline accumulation protects plants against the UV radiation caused peroxidative processes.

Two cultivars of soybean (Glycine max) after germination under different concentrations of NaCl salinity were observed for their protein, amino acid, proline and protease activity (Durgaprasad et al., 1996). It was found that in both the varieties the amino acid and proline content increased with increasing salinity while the protein content decreased in both control and treated plants. The protease activity increased gradually upto 96 hours of germination in the control and treatment of both varieties. It was noted that the accumulation of amino acids and proline might serve as an osmoticum to protect cell organelles and enzymes under saline condition.

Puthur et al. (1996) reported that proline accumulation was one of the strategies plants have evolved to tackle environmental stress. According to the authors, proline acts as a reservoir of C and N, protects proteins/enzymes, scavenges free radicals, regulates cytosolic pH and NAD (P)^+ /NAD (P) H ratio. Generally with increase in the intensity of stress, the plant cells show an increase in the extent of free radical generation as well as proline accumulation. The existence of correlation between free radical generation and proline accumulation provided a clue that proline accumulation is related to non-enzymatic detoxification of free radicals.

Studies were carried out in Triticum durum seedlings to demonstrate the effect of water or saline stress on proline content (Mattioni et al., 1997) and, according to the authors, proline increased more rapidly than other amino acids with the induction of either water or salt stress. Finally the authors
came to a conclusion that drought and salinity are the most important environmental factors that cause osmotic stress and a reduction in plant growth and crop productivity.

During drought season elevated temperatures are a usual feature. Chakraborty et al. (2000) studied different clones of tea at temperatures ranging from 40 to 60° C, which showed that the proline content increased significantly in all the varieties in response to temperature stress while the protein content decreased. They have further reported that accumulation of proline in tea leaves at elevated temperature may be due to the slow utilization of proline for protein synthesis and stimulation of glutamate conversion to proline.

Tripathi et al. (2000) studied the effect of cold stress on two varieties of Betel (Desavari and Bangla) by monitoring the lipid peroxidation (MDA), proline content, relative water content etc. According to them, cold stress in Betel is known to cause cessation of growth, tip drying, loss of chlorophyll, drying of leaf margins and sometimes the whole vine with the extent of damage ranging from 15% to total crop failure. The authors also found that in Betel the level of proline was more in the cold damaged leaves as compared to leaves without any injury. A consequence of cold stress was the build up of peroxide levels in the system, which is known to be toxic. While the peroxidases played an important role in the detoxification of peroxides in the system, its activity was found to be higher in Desavari than in Bangla variety. They came to a conclusion that the variety Desavari was relatively better adapted to cold stress than Bangla, which may be due to certain structural and biochemical features.

Chakraborty et al. (2001) studied the effect of moisture stress on proline content of six varieties of one-and-a-half year old tea plants and found that the free proline content increased after 7 and 14 days of moisture stress.
created by withholding irrigation. According to these authors, slow utilization of proline for protein synthesis and stimulation of glutamate conversion to proline during stress are considered to be responsible for its accumulation under stress. They also found that as water stress increases from mild to moderate, cell biochemical processes are increasingly affected.

Screening of ten greengram (*Vigna radiata*) genotypes for drought tolerance under depleting soil moisture conditions was done by Naidu *et al.* (2001). The relative water content of leaves and leaf area plant\(^{-1}\) decreased in all the genotypes under stress, while the proline content in the leaf increased with water stress. Among the genotypes K 851 and LGG 407 accumulated more proline and possibly this proline contributed towards osmotic adjustment, which plays a major role in maintaining turgor over fluctuating soil water potentials. The seed yield also decreased drastically in all the genotypes as the plants were subjected to progressive drought stress under receding soil moisture situation. From the results it was concluded that the genotypes K 851, Pusa 9072 and LGG 407 did well under drought stress by maintaining leaf area, leaf relative water content and high proline which resulted in less reduction in yield.

The leaf water status and proline content of coconut seedlings were determined during non-stress, stress and recovery periods (Bai and Rajagopal, 2004). Though the seedling combinations did not show significant differences in the leaf water potential during the non-stress and stress periods, proline concentrations exhibited significant differences between the seedling combinations and the treatments. There was no correlation between the leaf water potential and proline content and the recovery from stress indicated that some seedlings were more tolerant to water stress than others. The proline accumulation capacity did not show any relationship with the recovery potential of the seedlings. According to the authors, the proline concentration was not a good indicator of leaf water status in coconut.