EFFECT OF PLANT NUTRIENTS AND VARIOUS STRESSES ON SOME PHYSIOLOGICAL ASPECTS OF TEA, *CAMELLIA SINENSIS* (L.) O. KUNTZE

Thesis submitted to the University of Calicut in partial fulfilment of the requirements for the Degree of
DOCTOR OF PHILOSOPHY

LEONARD ALLEN NETTO

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Department of Botany

UNIVERSITY OF CALICUT

Dr. K.M. JAYARAM, Ph.D.
Lecturer

Division of Plant Physiology
and Biochemistry
Department of Botany
University of Calicut
Kerala, India – 673 635
Phone: 0494-2401144 * 406, 407
Fax : 0494 – 2400269
Grams: UNICAL
e-mail: jayaramkm@yahoo.co.in

17- 09 - 2007

CERTIFICATE

This is to certify that the thesis entitled "**Effect of plant nutrients and various stresses on some physiological aspects of tea, *Camellia sinensis* (L.) O. Kuntze** " submitted by **Mr. Leonard Allen Netto** in partial fulfilment of the requirements for the degree of **Doctor of Philosophy** in Botany, University of Calicut, is a bonafide record of research work undertaken by him in this Department under my supervision during the period 2000-2007 and that no part thereof has been presented before for any other degree or diploma.

DR. K.M. JAYARAM

'GAEA', P.O. KANHANGAD SOUTH, KASARAGOD DT, KERALA – 671 315 Phone: 04672 200439, 9447691751

DECLARATION

I hereby declare that the thesis entitled "**Effect of plant nutrients and various stresses on some physiological aspects of tea, *Camellia sinensis* (L.) O. Kuntze** " submitted by me in partial fulfilment of the requirements for the degree of **Doctor of Philosophy** in Botany, University of Calicut, has not been submitted before for any other degree or diploma.

Calicut University Campus
17-9-2007

LEONARD ALLEN NETTO

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Contents

Introduction

Tea [*Camellia sinensis* (L.) O. Kuntze], belonging to the family Theaceae, is grown under a wide range of soil and climatic conditions. The commercial cultivation of tea extends from 44° N to 34°S latitudes and it is grown at altitudes varying from about 700 metres to 2400 metres above sea level. Tea prefers an acidic soil with a pH around 5.0 being ideal (Hudson *et al.,* 2002). In South India, tea is grown on the hill slopes of the Western Ghats having latosols (Ranganathan and Natesan, 1985). Ambient temperatures above 30° C and below 13° C are not favourable for the growth of the tea bush. The relative humidity should be around 80% most of the time and should never be less than 40% for luxuriant growth of tea (Hudson *et al.,* 2002). The recommended limit of soil temperature is about 20° and the upper limit is about 29° C. Tea is mainly cultivated as a rain-fed crop which grows well in areas where annual rainfall varies from 1150 to 6000 mm and the distribution of rainfall over the year is as vital as the total annual rainfall (Hajra, 2001).

Tea is a unique crop and, incidentally, a very interesting and attractive one. Its cultivation and harvesting do not fit into any typical cropping pattern and its processing as well as marketing are very specific. To meet the

increasing demand for tea in the national and international market, production has to be augmented by planting improved clones, by increasing the acreage under tea and also by enhancing the yield of existing and future plantations. The non-availability of land with suitable climatic conditions has imposed restrictions on the horizontal growth of the tea industry and therefore increase of production per unit land appears to be a viable solution (Vyas *et al.,* 1998). This can be achieved by studying the physiological competence of different clones towards various stresses.

Being perennial species, plantation crops are exposed to periodic cycles of various environmental stresses during their economic life span. Cultivated and harvested throughout the year, tea is perturbed throughout its life cycle by biotic or abiotic stress leading to a certain decline in production, which depends on the individual potentiality of the clone towards that stress. The ideal situation would be to have clones which are versatile to adverse climatic conditions, thereby making them suitable for planting in places which are either suitable or unsuitable. The most important, effective and economical means of improving productivity is by planting the best suitable cultivars. The tea plant is capable of adapting itself to various types of stresses but the degree of tolerance varies with the cultivar (Chakraborty *et al.,* 2000).

A number of clones of tea have been released in recent years. Most of the clones released are based on their yield and quality attributes. Though there are several tea clones, their individual capabilities in relation to stress tolerance towards various stresses, such as drought and frost, and responses towards nutrients have not been properly elucidated.

Normally tea is mainly grown as a long-term monoculture and fertilization is an important part of its normal intensive production (Bonheure and Willson, 1992). Without the application of fertilizer, the supply of

nutrients available in the soil will become exhausted, leading to mineral deficiencies in the plants, severe reduction in yield and ultimately to the death of plants and a degraded plantation. Fertilizer application can also correct deficiencies of minerals arising from an inadequate supply in the soil. In favourable circumstances a yield response commences three to four weeks after application of fertilizer. Fertilizers are the costliest input in the tea industry.

Rainfall has an influence on the nutrient absorption of a plant. If rainfall occurs within 24 hours of fertilizer application, there is considerable loss of nitrogen and potassium fertilizers by run off and leaching. The light intensity and length of day increase the fertilizer requirement of a crop and the higher the light intensity the better will be the fertilizer utilization (Verma, 1993). Nutrients are taken up by the plant primarily from the soil solution and direct absorption from soil-solid phase by contact exchange and by root interception occurs only to a limited extent. Plants absorb nutrients in the form of ions, which are transported to the roots in soil by mass flow and diffusion, which is regulated by water. It is known that under comparable conditions the nitrogen and organic matter increase, as the effective moisture becomes greater. Therefore maintenance of optimum soil moisture regime, either from rainfall or irrigation, becomes essential for efficient utilization of fertilizers (Verma, 1993).

Tea is grown in acidic soil with low available nitrogen and the leafy tissues of the crop have a high content of nitrogen and this makes nitrogen one of the important fertilizers for tea (Hajra, 2001). According to Hajra (2001) with very high level of nitrogen, the response of plants may become reduced and uneconomical. It is also found that the response at any level is dependent on soil and climatic factors and also on the adequate availability of other nutrients. Continuous plucking of tea leaves causes regular removal of

nutrients, mainly nitrogen, from the soil, so the soil becomes depleted of this nutrient and should be replenished.

Drought, a complex phenomenon in nature, is caused by the combination of soil moisture status, high ambient temperature and dry wind. It is a recurring phenomenon in South India and is one of the major constraints for tea cultivation (Satyanarayana and Cox, 1994; Jeyaramraja *et al.,* 2003; Thomas *et al.,* 2004). When moisture stress sets in, the available water in the soil is rapidly depleted through evapotranspirational losses mediated by extreme environmental conditions. A number of reports have been published on yield reduction in tea experiencing drought (Handique and Manivel, 1986; Satyanarayana and Cox, 1994; Varadan, 1996; Marimuthu and Kumar, 1998). Generally, plants have an inbuilt mechanism to cope with stress to an extent at the expense of photosynthates, but under severe drought, carbohydrates stored in the roots are utilized by the plants while defoliation and death occur under prolonged moisture stress (Marimuthu and Kumar, 1998).

Most of the tea plantations in the world are located in high altitudes and plants growing there are facing low temperature stress or frost, which is one of the most important climatic factors limiting crop productivity (Sharma *et al.,* 2000; Hudson and Muraleedharan, 1996). Frost may damage the photosynthetic mechanism, thus resulting in a reduced yield.

The objectives of the present study are to elucidate the response of various clones of tea to the lower and higher concentrations of fertilizers recommended by UPASI. The study also concentrated on the effect of various stresses like drought and frost on various clones of tea. The cost of fertilizers is increasing year after year and recurrence of drought and frost are the major constraints experienced by the tea industry. A number of clones are now available and the response of these clones to the different concentrations

of fertilizers, drought and frost conditions has not been elucidated so far. So the present study aims to unveil the response of various clones to different concentrations of fertilizers and abiotic stresses like drought and frost. To determine the effect of these factors on different clones of tea, various physiological and biochemical parameters were studied.

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 CO_2 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 CO_2 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 (Ψ 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 KH_2PO_4 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 $\mu molm^{-2}s^{-1}$) 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

$\mu molm^{-2}s^{-1}$) 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 CO_2 uptake and O_2 evolution, the combination of water deficit with high temperature led to inhibition of both CO_2 uptake and O_2 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 CO_2 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 Fv/Fm 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 Fv/Fm 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 CO_2 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 CO_2 exchange rates, the transpiration rates in 14-week-old plants were significantly greater than those in seven-week-old plants. The CO_2 exchange rates and transpiration rates decreased with leaf age. The stomatal and residual resistances to the diffusion of CO_2 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 $\mu molm^{-2}s^{-1}$. 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 CO_2 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. tatatricum*) 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 upto 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 CO_2 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 CO_2 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 CO_2 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 CO_2 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_2O_5 and 20 kg K_2O per hectare produced the maximum yield. The authors also observed that at lower P levels (0 or 20 kg P_2O_5 per hectare), the requirement of K was high (40 kg K_2O per hectare) and at higher levels of P (40-60 kg P_2O_5 per hectare), the application of only 20 kg K_2O per hectare proved to be sufficient. The maximum net assimilation and crop growth rate were recorded during the 60-90th day interval. Though the crop attained the maximum leaf area index at 120th day, the optimum leaf area for most efficient utilization of photosynthetically active radiation was during 60-90th 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 60-90th day interval at 20 kg K_2O per hectare and 40 kg P_2O_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 1st 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 O_2, CO_2 and photosynthetically active radiation were carried out (Keys *et al.*, 1977). When the O_2 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 C_4 xerophytes and lowest in the C_3 mesophytes.

The irrigated and non-irrigated corn plants (*Zea mays*) fed with ^{14}C were subjected to study the translocation of ^{14}C 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 ^{14}C 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 $\mu E\ m^{-2}\ s^{-1}$ 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 μE m^{-2} 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 μmol $m^{-2}s^{-1}$ 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 μmol $m^{-2}s^{-1}$ 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 μmol $m^{-2}s^{-1}$ 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 CO_2 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 CO_2 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 CO_2 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.

Materials and Methods

3.1 Plant material

The different clones of tea (*Camellia sinensis*) selected for various experiments in the study were TTL-1, TTL-2, TTL-4, TTL-5, TTL-6, SMP-1, SM-OM-54, (developed by Tata Tea Limited, Munnar, Kerala) UPASI-2, UPASI-3, UPASI-9 (developed by United Planters' Association of Southern India-UPASI, Valparai, Tamil Nadu), TRI-2025 (developed by Tea Research Institute of Sri Lanka) and CR-6017. The clones TTL-1, TTL-2, TTL-4, TTL-5 and TTL-6 are newly released and their capabilities/ adaptability with regard to their stress tolerance potential and nutrient requirements have not been studied. All the plants were of three years age and were planted at a spacing of 1.2 metres x 0.75 metre along a single hedge with a plant population of 10760 plants per hectare.

3.2 Nutrient studies

The clones selected for the nutrient studies were TTL-1, TTL-2, TTL-5 and TTL-6. The fertilizers were applied in five split doses in a tea field with the above mentioned clones in their third year from planting during the months of April, May, August, September and November. For providing Nitrogen and Potassium to plants, Ammonium Sulphate and Muriate of

Potash mixture was applied during April and Urea and Muriate of Potash mixture was applied in May, August, September and November. Phosphorus was applied in the form of Rock Phosphate and was only a one-time application (Verma and Palani, 1997).

3.2.1 Preparation of different concentrations of nutrients

The three different concentrations of fertilizers applied to various clones of tea (*Camellia sinensis*) were 50%, 100%, and 150%, in which 100% is the UPASI recommended levels of fertilizer for tea plantations.

3.2.1.1 Preparation of N and K mixture

(1) For preparing 50% of the actual concentration (considered as half dose of the UPASI recommended concentration) - 150 kg of N and 225 kg of K_2O per hectare in the form of Ammonium Sulphate and Muriate of Potash in April and Urea and Muriate of Potash in May, August, September and November respectively was applied. This is equivalent to 13.94 g of Ammonium Sulphate and 6.94 g of Muriate of Potash mixture per bush in April and 6.13 g of Urea and 6.94 g of Muriate of Potash mixture per bush for the months of May, August, September and November.

(2) For preparing 100% - 300 kg of N and 450 kg of K_2O per hectare in the form of Ammonium sulphate and Muriate of potash in April and Urea and Muriate of Potash in May, August, September and November respectively was applied (Verma and Palani, 1997). This is equivalent to 27.88 g of Ammonium sulphate and 13.88 g of Muriate of Potash mixture per bush in April and 12.26 g of Urea and 13.88 g of Muriate of Potash mixture per bush for the months of May, August, September and November.

(3) For preparing 150% of the actual concentration (considered as 50% more than the UPASI recommended concentration) - 450 kg of N and 675 kg

of K_2O per hectare in the form of Ammonium Sulphate and Muriate of Potash in April and Urea and Muriate of Potash in May, August, September and November respectively was applied. This is equivalent to 41.82 g of Ammonium Sulphate and 20.82 g of Muriate of Potash mixture per bush in April and 18.40 g of Urea and 20.82 g of MOP mixture per bush for the months of May, August, September and November.

3.2.1.2 Preparation of P fertilizer

One application of P at 50% (considered as half dose of the UPASI recommended concentration) - 40 kg of P_2O_5 per hectare in the form of Rock Phosphate, which is equivalent to 12.26 g of Rock Phosphate per bush was applied.

One application of P at 100% (this represents the UPASI recommended level of P) - 80 kg P_2O_5 per hectare in the form of Rock Phosphate (Verma and Palani, 1997) which is equivalent to 24.53 g of Rock Phosphate per bush was applied.

One application of P at 150% (considered as 50% more than the UPASI recommended concentration) - 120 kg P_2O_5 per hectare in the form of Rock Phosphate, which is equivalent to 36.80 g of Rock Phosphate per bush was applied.

3.2.2 Application of nutrients

For the application of fertilizers (N, K and P) to tea bushes the method proposed by Verma and Palani (1997) was followed. The fertilizer containing N and K was applied by broadcasting Ammonium Sulphate and Muriate of Potash mixture in April and as Urea and Muriate of Potash mixture for the remaining four applications namely as May, August, September and November. The P application was done once a year by placement method at

15-25 cm depth in the month of November. The P application was done separately and not along with the N and K application.

The experiment was laid out in randomized block design with seven replications. Each plot consisted of 20 bushes established at 1.2 metres x 0.75 metre. The data presented in later chapters are an average of the five different NK applications and one P application done during the years 2002, 2003 and 2004.

The various parameters monitored at 5-day intervals for thirty days after each fertilizer application were NPK content in leaves, single shoot weight, chlorophyll and carotenoid content, chlorophyll fluorescence and net photosynthetic rate.

3.2.3 Analysis of N, P and K

The shoots consisting of three leaves and a bud were plucked from different clones of tea such as TTL-1, TTL-2, TTL-5 and TTL-6 treated with 50, 100 and 150% NPK fertilizer. The shoots collected from ten different bushes of a clone of a treatment were pooled together and brought to the laboratory for further studies.

The shoots were first washed in tap water and then in distilled water. The samples were then kept in a hot air oven for drying. The temperature of the oven was adjusted to 65° C and the samples were kept in the oven for 48 hours till constant weight was obtained. Dry grinding of the sample shoot was done using a sample mill as suggested by Tandon (1993). After grinding, the powder was put in a clean dry bottle and placed in a hot air oven at 65^0 C for 24 hours for additional drying to remove moisture accumulated during grinding. The N, P and K content were analysed according to Tandon (1993).

3.2.3.1 Analysis of N content

Following the method of Tandon (1993), the nitrogen content was estimated using 2200 Kjeltec Auto Distillation Unit, manufactured by Foss Tecator, Sweden. Five hundred milligram of the dried sample was taken in a dry digestion tube and 20 ml of concentrated sulphuric acid was added to it. The same was digested at 400^0 C to obtain a clear solution in a fume chamber. The clear solution obtained was placed in the 2200 Kjeltec Auto Distillation Unit and 150 ml of 40% sodium hydroxide was added to the solution. The ammonia evolved was collected in a conical flask containing 20 ml of 0.1 N sulphuric acid and a few drops of Methyl Red indicator. To determine the excess unreacted acid remaining in the conical flask, the collected samples were subjected to titration against 0.1N sodium hydroxide. The colour change from pink to golden yellow was taken as the end point. A blank was prepared by mixing 0.1 N sulphuric acid and a few drops of Methyl Red indicator and the mixture was titrated against 0.1N sodium hydroxide. The end point was noted and the difference in end points of the blank and the sample was used for the calculation of percentage of N in the samples, adopting the following formula:

$$\text{Percentage of N} = \frac{\text{Titre value of (blank- sample) x Normality of NaOH x 0.014x100}}{\text{Weight of sample}}$$

Where 0.014 is the milli equivalent of nitrogen.

3.2.3.2 Analysis of P content

The phosphorus content was estimated by using vanado-molybdo phosphoric yellow colour method (Tandon, 1993). Five hundred milligram of the dried leaf sample was digested with 20 ml of diacid mixture (Nitric acid

(HNO_3): Perchloric acid ($HClO_4$) – mixed in 9:4 ratio) at a temperature of 400^0C till a clear solution was obtained. The digested sample was quantitatively transferred to a 100 ml volumetric flask using distilled water and made up to the volume. From this solution, 5 ml was pipetted out and transferred to a 25 ml volumetric flask and 5 ml of ammonium metavanadate was added. The volume was made upto 25 ml with distilled water and the absorbance of the solution was measured at 430 nm after keeping for 30 minutes (for the colour to stabilise) using U-2000 (Hitachi) Spectrophotometer. The standard solution was prepared by dissolving 4.4 g of potassium dihydrogen phosphate (KH_2PO_4) in 1 litre of distilled water, which is equivalent to 1000 ppm solution. From this 1000 ppm solution 5 ml was pipetted out and made upto 100 ml with distilled water to get 50 ppm solution. The concentration of the sample was obtained from a standard graph, which was developed by feeding different solutions of known P concentration such as 0, 0.5, 1.0, 2.5, 5, 7.5 and 10 ml of 50 ppm solution of phosphate standard, each of them containing 5 ml of ammonium metavanadate, and measured at 430 nm after keeping for 30 minutes (for the colour to stabilise). The percentage of P content was calculated using the following formula:

$$\text{Percentage of P} = \frac{\text{O.D. at 430 nm x volume made up x volume made up x 100}}{\text{Weight of sample taken x Aliquot taken x } 10^6}$$

3.2.3.3 Analysis of K content

Five hundred mg of the dried leaf sample was digested with 20 ml of diacid mixture (Nitric acid (HNO_3): Perchloric acid ($HClO_4$) – mixed in 9:4 ratio) at a temperature of 400^0C till a clear solution was obtained. The same was quantitatively transferred to a volumetric flask and made up to 100 ml using distilled water. From this five ml of the sample solution was taken and

made up to 25 ml. The standard solution was prepared by dissolving 0.191 g of KCl in 1 litre of distilled water from which 0, 1, 2, 3, 4 and 5 ppm solutions were prepared and fed into the flame photometer (Model CL 22D, Elico Limited, Hyderabad, India). After adjusting the needle of the flame photometer to zero by feeding blank, the needle was adjusted to 100 by feeding the highest concentration of K solution. Then the other standards were fed to obtain the flame photometer readings. The flame photometer readings were plotted against the concentration of the standards to obtain the standard curve. Then the sample solution was fed into the flame photometer and the reading was recorded. The K content in the sample was calculated using the following formula:

$$\text{Percentage of K} = \frac{\text{K concentration from graph x volume made up x 100}}{\text{Weight of sample taken x Aliquot taken x } 10^6}$$

3.2.4 Weight of shoots

The shoots consisting of three leaves and a bud from ten different bushes of a clone were collected. Immediately after collection the samples were brought to the laboratory. Fresh weight of individual shoots were determined separately by using an electronic balance (Shimadzu) and average values were recorded.

3.2.5 Estimation of photosynthetic pigments

The photosynthetic pigments like chlorophyll and carotenoids were estimated according to the method of Arnon (1949).

3.2.5.1 Extraction

For estimating total chlorophyll and carotenoid pigments, young tea leaf samples (shoots consisting of three leaves and a bud) were collected from ten different bushes of a clone subjected to a treatment selected randomly.

The leaves were cut into small pieces and thoroughly pooled together. One gram material was weighed and homogenized in 10 ml of 80% chilled acetone using a clean mortar and pestle. The homogenate was transferred to centrifuge tubes and centrifuged at 12000 xg for 10 minutes at 4^0C. The supernatant was collected and the sediments were re-extracted with 80% chilled acetone until a clear supernatant was obtained. The supernatant collected after re-extraction was mixed together and used for estimation.

3.2.5.2 Estimation

From the combined supernatant 1 ml was pipetted out and added to 9 ml of 80% chilled acetone, which was used for quantification of the pigments. Absorbance of the resultant supernatant was measured against 80% acetone as blank at 645, 646, 663 and 750 nm using a U-2000 (Hitachi) Spectrophotometer. The amount of chlorophyll a, chlorophyll b and total chlorophyll were calculated using the following formula (Arnon, 1949):

$$\text{Chl a } (\mu g / g \text{ tissue}) = \frac{12.69\,(A_{663}-A_{750}) - 2.96\,(A_{645}-A_{750})}{W} \times V$$

$$\text{Chl b } (\mu g / g \text{ tissue}) = \frac{22.9\,(A_{645}-A_{750}) - 4.68\,(A_{663}-A_{750})}{W} \times V$$

$$\text{Chl a+b } (\mu g / g \text{ tissue}) = \frac{20.12\,(A_{646}-A_{750}) + 8.02\,(A_{663}-A_{750})}{W} \times V$$

For estimation of carotenoids the absorbance of the supernatant was measured against 80% acetone as blank at 750, 663, 646 and 470 nm using a U-2000 (Hitachi) Spectrophotometer. The carotenoid content was calculated by using the following formula (Arnon, 1949):

$$\text{Carotenoids } (\mu g / g \text{ tissue}) = \frac{(1000 x A_{470}) - (3.27 x \text{Chl a}) - (104 x \text{Chl b})}{229 \times W} \times V$$

Where A represents absorbance of the pigments at suffixed wavelength.

Chl a = 12.69 $(A_{663}-A_{750})$ – 2.96 $(A_{646}-A_{750})$

Chl b = 22.9 $(A_{646}-A_{750})$ – 4.68 $(A_{663}-A_{750})$

W = weight of the extracted material

V= Volume of the extraction medium

3.2.6 Measurement of chlorophyll fluorescence

Chlorophyll fluorescence of leaves of various tea clones treated with different concentrations of fertilizers (50, 100 and 150%) was monitored with a portable Fluorescence induction monitor, FIM 1500 (Analytical Development Company Ltd., England) equipped with data transfer software (TAGA 1.02 & DATS 1.02 Versions). The light level used was 100% LED at 650 nm. To measure the fluorescence, the third leaf from the young bud of a shoot in the centre of the tea bush was dark adapted for a duration of 30 minutes prior to the measurement of Fv/Fm with a small, light weight leaf clip. The clip has a small shutter, which was closed over the leaf, when the clip was attached, so that light is excluded and dark-adaptation takes place. The portion of the dark adapted leaf was illuminated after 30 minutes with a flash of saturated light, the initial fluorescence (F0) and maximum fluorescence (Fm) were measured. The variable fluorescence yield (Fv) is the difference between Fm-F0. The PS II activity was measured in terms of the Fv/Fm ratio. The photochemical yield of PS II is equal to the ratio between Fv and Fm (Fv/Fm). The readings were repeated on ten different leaves in different bushes, which were randomly selected for each treatment.

3.2.7 Measurement of CO_2 exchange

Net photosynthetic rate of different clones of tea subjected to various concentrations of fertilizer application, such as 50, 100 and 150% was measured by a closed system infrared gas analyser (portable photosynthesis system, Cl-301 PS, CID, Inc., U.S.A.). All the measurements were taken under natural sunlight. The third leaf from the bud fully exposed to incident sunlight was used for taking measurements and the readings of photosynthetic rate were measured between 9.00 am and 11.00 am. The instrument had internal programmes to calculate the rate of net photosynthesis from the measurements taken. The net photosynthetic rate was expressed in $\mu mol^{-1}m^{-2}second^{-1}$. The readings were repeated on ten different leaves in different bushes, which were randomly selected for each treatment.

3.3 Drought studies

3.3.1 Method of creation of drought

The drought studies were carried out during the summer months in the years 2002, 2003 and 2004. Two rows of tea plants (20 each), which were three years old, of the clones TTL-1, TTL-2, TTL-4, TTL-5, TTL-6, UPASI-2 and UPASI-3 were maintained for the study. One row was uniformly irrigated at one litre per plant according to Verma and Palani (1997) using drip irrigation and the other row was maintained as non-irrigated starting from the month of December to March.

Various parameters such as weight of shoots, soil moisture status, leaf water potential, relative water content, chlorophyll and carotenoid content, chlorophyll fluorescence, net photosynthetic rate, proline and malondialdehyde (MDA) content of plants grown under both irrigated and non-irrigated conditions were monitored on the 0, 20^{th}, 60^{th} and 100^{th} day from the beginning of the study. The non-irrigated plants after 100 days

were irrigated uniformly using drip irrigation and the same parameters in non-irrigated condition were monitored on the 7^{th} and 14^{th} day after re-irrigation.

3.3.2 Weight of shoots

The shoots (ten each of a clone selected randomly) consisting of three leaves and a bud were collected from the clones subjected to irrigated and non-irrigated conditions. The collected shoot samples were immediately brought to the laboratory and fresh weight of each shoot was determined separately by using an electronic balance (Shimadzu) and average values were recorded.

3.3.3 Soil moisture status

The soil moisture of the experimental plot was determined as per the method proposed by Rajasekar *et al.* (1998). The soil samples were collected at a depth of zero to nine inches and nine to eighteen inches, using a crowbar, from ten different locations in the irrigated and non-irrigated plots on the 0, 20^{th}, 60^{th}, 100^{th} day and on the 7^{th} and 14^{th} day after re-irrigation. The samples collected during each interval from the irrigated and non-irrigated plots were pooled and taken immediately to the laboratory. From this 100 gram was weighed and kept in a hot air oven and the temperature was maintained at 100° C. The dry weight was determined until constant weight was obtained. The soil moisture content was determined as follows:

Soil moisture content= Fresh weight – Dry weight

3.3.4 Measurement of leaf water potential

The leaf water potential is a measure of the water content inside the leaf and was determined by using HR 33T Dew Point Microvoltmeter supplied by Wescor Inc., USA. A small circular portion of green leaf

sample (leaf disc) taken from the third leaf from the young bud in the centre of the bush was enclosed in the leaf chamber and leaf water potential was monitored and these were expressed in megapascals (MPa). The readings were repeated on ten different leaves in different bushes, which were randomly selected for each treatment.

3.3.5 Measurement of relative water content of leaves

To determine the relative water content of leaves of plants grown under irrigated and non-irrigated conditions, fresh weight of third leaf from bud was collected and determined. After determining the fresh weight the samples were kept in a hot air oven, the temperature of which was maintained at 80° C for 48 hours. The dry weight of the samples was found out until constant weight was obtained. All the weights were taken using an electronic balance. By using this data, the relative water content of leaves was calculated as per the formula suggested by Johnson *et al.* (1997) and the values were expressed in percentage.

$$\text{Relative Water Content} = \frac{\text{Fresh weight-Dry weight}}{\text{Fresh weight}}$$

3.3.6 Estimation of photosynthetic pigments

For estimation of photosynthetic pigments, shoots consisting of three leaves and a bud of each clone under the non-irrigated and irrigated conditions were collected from randomly selected ten plants of each clone. The leaves were cut into small pieces and thoroughly pooled together and one gram was weighed out from the pooled mixture. The extraction and estimation of chlorophyll a, chlorophyll b, chlorophyll a+b and carotenoids was done according to the method of Arnon (1949) as described earlier.

3.3.7 Measurement of chlorophyll fluorescence

For determination of chlorophyll fluorescence, the clones of tea (*Camellia sinensis*) such as TTL-1, TTL-2, TTL-4, TTL-5, TTL-6, UPASI-2 and UPASI-3 maintained in irrigated and non-irrigated conditions were used. The measurement of chlorophyll fluorescence was done at a regular interval on the 0, 20^{th}, 60^{th} and 100^{th} day of irrigation and non-irrigation. After 100 days of non-irrigation the plants were irrigated as mentioned earlier and the chlorophyll fluorescence of leaves of these re-irrigated plants along with the irrigated plants were monitored on the 7^{th} and 14^{th} day. The chlorophyll fluorescence was measured as per the method described in nutrient studies.

3.3.8 Measurement of CO_2 exchange

Net photosynthetic rate, stomatal conductance and transpiration of leaves of tea clones selected for the study were measured on the 0, 20^{th}, 60^{th} and 100^{th} day of irrigation and non-irrigation and thereafter on the 7^{th} and 14^{th} day of irrigation of non-irrigated plants by a closed system infrared gas analyser (portable photosynthesis system, Cl-301 PS, CID, Inc., U.S.A.). All the measurements were taken under natural sunlight. The third leaf from the bud fully exposed to incident sunlight was used for taking measurements. In the present study photosynthetic rate was measured between 9.00 am and 11.00 am. The instrument had internal programmes to calculate the rate of net photosynthesis, transpiration and stomatal conductance from the measurements taken. The net photosynthetic rate was expressed in $\mu mol^{-1}m^{-2}second^{-1}$, the transpiration rate and stomatal conductance were expressed in $millimol^{-1}m^{-2}second^{-1}$. The readings were repeated on ten different leaves in different bushes, which were randomly selected for each treatment.

3.3.9 Estimation of proline

Proline was estimated in different clones of tea growing under irrigated and non-irrigated conditions during the intervals mentioned above. Leaf samples (consisting of three leaves and a bud) were collected from ten randomly selected plants of a clone under each treatment. The samples were pooled together, brought to the laboratory and cut into small pieces. Proline estimation was done based on the method of Bates *et al.* (1973).

3.3.9.1 Extraction

One gram of leaf material was weighed and homogenized in 10 ml of 3% aqueous sulphosalicylic acid (w/v) using a clean mortar and pestle. The homogenate was transferred to centrifuge tubes, centrifuged at 15000 xg for 10 minutes at room temperature and the supernatant was collected.

3.3.9.2 Estimation

Two ml of the supernatant was pipetted out to a clean test tube and reacted with 2 ml of acid ninhydrin (prepared by warming 1.25 g ninhydrin salt in 30 ml glacial acetic acid and 20 ml of 6 M orthophosphoric acid with agitation until dissolved) and 2 ml of glacial acetic acid. The resultant mixture was incubated in a boiling water bath maintained at 100° C for 1 hour and the reaction was terminated by placing the test tubes in an ice bath. Subsequently 4 ml of toluene was added to each reaction mixture. The solution was then mixed vigorously, using a cyclomixer for 15-20 seconds to facilitate quick diffusion/movement of chromophore from aqueous phase to non- aqueous phase. The toluene layer was separated from the aqueous layer using a separating funnel and the absorbance was measured at 520 nm using a U-2000 (Hitachi) Spectrophotometer. All the above mentioned materials except the plant material was used as the blank. Concentration of proline in

the sample was computed from a standard curve of L-proline and expressed in μg g^{-1} fresh weight (Bates *et al.*, 1973).

3.3.10 Estimation of malondialdehyde (MDA)

As described above, the leaf samples were collected, cut into small pieces and pooled together. The malondialdehyde content was estimated according to the method of Heath and Packer (1968).

3.3.10.1 Extraction

One gram of leaf material was taken and homogenized in 5 ml of 5% trichloroacetic acid (TCA) using a clean mortar and pestle. The homogenate was transferred to centrifuge tubes and centrifuged at 12000 xg for 15 minutes at 25^0C and the supernatant was collected.

3.3.10.2 Estimation

Malondialdehyde content was estimated according to the method of Heath and Packer (1968). From the supernatant two ml was pipetted out and mixed with an equal volume of 0.5% thiobarbituric acid (TBA) in 20% trichloroacetic acid (TCA). The mixture was heated at 95^0 C for 25 minutes in a water bath, cooled to the level of the room temperature and then centrifuged for 2 minutes to obtain a clear solution. The supernatant was collected and the absorbance was measured at 532 nm using a U-2000 (Hitachi) Spectrophotometer. The absorbance value at 532 nm was corrected for nonspecific turbidity by subtracting absorbance value at 600 nm. The amount of MDA was calculated by using an extinction coefficient of 155 mM^{-1} cm^{-1} and expressed in μmol g^{-1} fresh weight.

3.4 Frost studies

The frost studies were carried out during the winter months of 2002, 2003 and 2004. Ten different clones selected for the low temperature stress

studies were -TTL-1, TTL-2, TTL-4, TTL-5, TTL-6, SMP-1, SM-OM-54, UPASI-9, CR-6017 and TRI-2025. The observations relating to plant response against low temperature like chlorophyll and carotenoid content, chlorophyll fluorescence, proline and malondialdehyde (MDA) content of leaves were recorded at two-and-a-half-hour intervals starting from 6.30 am to 7 pm. The observations were repeated on different days in the months of December to February when low temperatures/frost occurred.

3.4.1 Estimation of photosynthetic pigments

Three leaves and a bud were collected from randomly selected ten plants of a clone at the intervals mentioned above. The leaves were brought to the laboratory, cut into small pieces and pooled together. These leaf samples were used for the estimation of chlorophyll a+b and carotenoid contents according to the method of Arnon (1949) as described earlier.

3.4.2 Measurement of chlorophyll fluorescence

The above mentioned clones were selected and the chlorophyll fluorescence was measured at a regular interval of 2.5 hours from 6.30 am to 7 pm. The chlorophyll fluorescence was measured as described in the nutrient studies.

3.4.3 Estimation of proline

The above mentioned clones were subjected to the estimation of proline content at the same intervals specified. The extraction and estimation was done as per the method of Bates *et al.* (1973) which was described in drought studies.

3.4.4 Estimation of malondialdehyde (MDA) content

The malondialdehyde in the above mentioned clones during the same intervals was estimated according to the method proposed by Heath and Packer (1968) which was described in detail in the drought studies.

3.5 Statistical analysis

For all physiological studies ten samples were collected and mean values were recorded. The biochemical analysis was carried out in duplicate and repeated thrice and the mean values were recorded. The standard deviation and standard error were calculated and data was analysed using 't' test of significance.

Table 3.1: Weather data for the period of study

Month & Year	Mean Min. Temp. (^{0}C) 8 a.m.	Mean Max. Temp. (^{0}C) 8 a.m.	Mean RH (%) 2 p.m.	Mean Wind Run (km/day)	Mean Sunshine (hours/day)	Total Evaporation loss (mm/month)	Total Rainfall (mm/month)
Apr-01	13	23	71	42	4.8	11	298.45
May-01	13	24	73	61	5.7	31	116.33
Jun-01	15	21	84	110	1.2	0	363.47
Jul-01	14	21	79	99	2.7	20	478.03
Aug-01	14	20	84	86	2.9	17	182.12
Sep-01	13	22	79	56	4.3	18	183.13
Oct-01	14	21	84	56	3.2	26	214.38
Nov-01	14	21	78	84	4.8	30	128.02
Dec-01	9	21	72	125	6.0	56	0.00
Jan-02	7	22	58	77	6.8	67	0.00
Feb-02	8	22	56	113	7.0	70	0.00
Mar-02	9	24	47	78	7.6	73	68.33
Apr-02	12	24	66	47	6.5	34	139.70
May-02	14	23	79	71	4.6	27	196.85
Jun-02	15	21	86	96	2.4	9	229.36
Jul-02	13	21	79	73	3.9	1	119.89
Aug-02	14	20	87	98	1.9	12	384.05
Sep-02	11	22	74	63	5.4	41	88.90
Oct-02	14	21	81	57	2.7	5	274.83
Nov-02	12	22	74	77	4.4	45	51.56
Dec-02	9	27	62	106	5.3	68	21.84
Jan-03	8	22	56	101	7.1	75	0.00
Feb-03	9	23	58	69	6.6	50	0.00
Mar-03	9	24	52	70	7.3	70	97.03
Apr-03	12	24	70	45	5.5	47	86.87
May-03	14	24	75	57	5.2	32	154.18
Jun-03	15	22	80	86	3.8	32	252.48
Jul-03	15	20	87	91	1.9	14	241.05
Aug-03	14	21	80	89	4.3	29	161.54
Sep-03	11	23	85	85	5.9	42	61.98
Oct-03	14	21	85	54	3.4	16	456.44
Nov-03	13	21	82	109	4	34	92.46
Dec-03	8	21	66	88	6.6	62	9.91
Jan-04	7	22	55	73	7	69	0.00
Feb-04	6	23	45	84	7.8	74	0.00
Mar-04	8	25	46	82	7.9	86	0.00
Apr-04	12	24	73	53	5.4	46	107.70
May-04	14	21	84	90	3.9	21	407.16

Results

4.1 Effect of different plant nutrient concentrations on physiological changes in tea clones

4.1.1 NPK content of the leaf

In the present study the leaf N and P content in all the clones showed a significant increase up to the 25th day and declined thereafter (Fig. 4.1 and 4.2) ($p<0.05$). The leaf K content showed a significant increase on the 10th day and subsequently declined (Fig. 4.3) ($p<0.05$). In general the N and P content in the three different concentrations declined from the 25th day onwards while the K content declined after the 10th day itself.

The highest increase in N levels on the 25th day after fertilizer application was in the clone TTL-6 and the increase was significant also (Fig. 4.1). In the clones TTL-1, TTL-2 and TTL-5, the highest increase of leaf N occurred in the 50% fertilizer concentration applied plants after 25 days. Whereas in TTL-6 the highest leaf N was recorded in the 150% fertilizer concentration applied plants. In the clone TTL-6, 50% fertilizer concentration recorded the lowest values, the 100% fertilizer applied plants had higher values than the 50% fertilizer applied plants and the highest values of leaf N were in the 150% fertilizer applied plants. The percentage increase

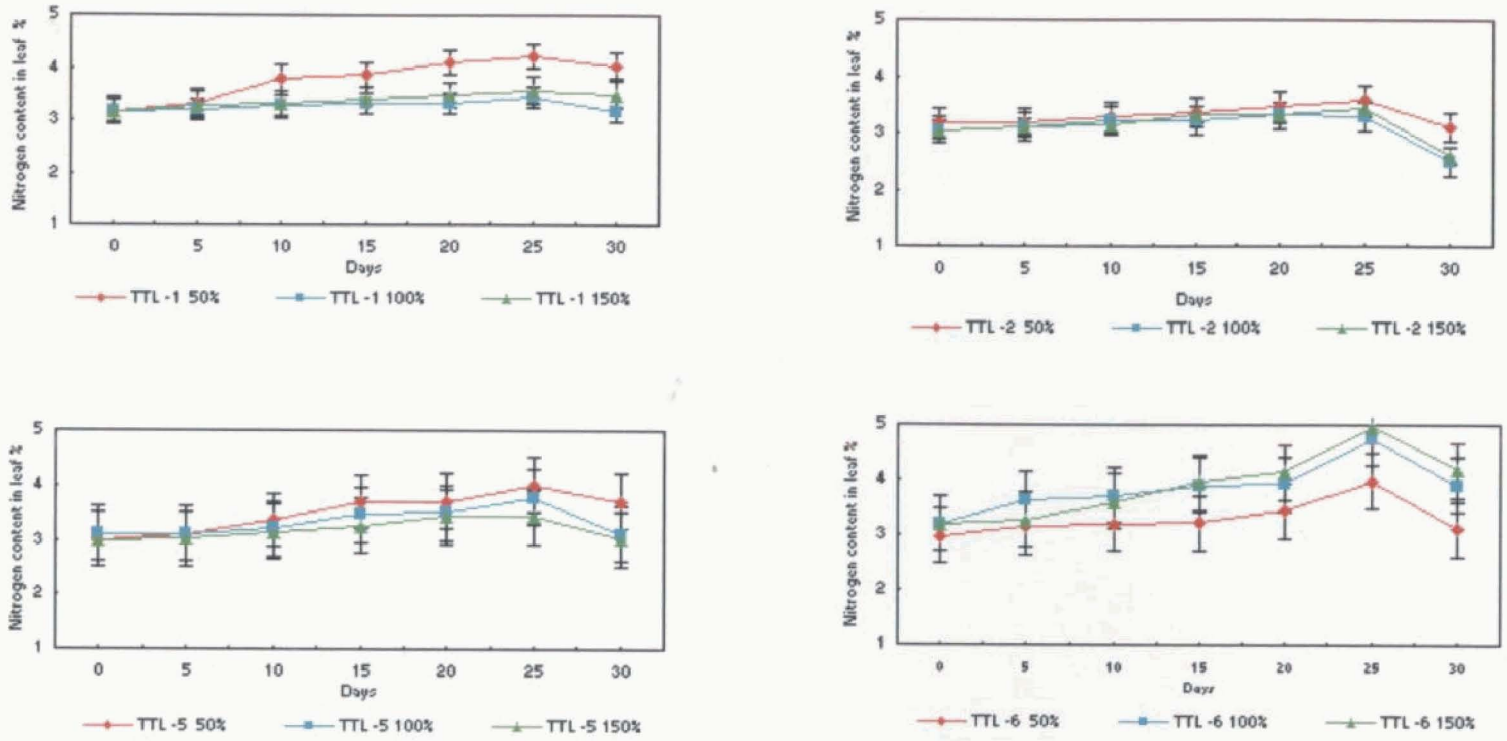

Fig. 4.1: Effect of various concentrations of fertilizers on N content in leaf in different clones of tea (*C. sinensis*).

of leaf N on the 25th day as compared to the 0 day was 34, 49 and 56% in the 50, 100 and 150% fertilizer applied plants respectively in the clone TTL-6.

In the case of P absorption there was an increase upto the 25th day and a reduction was observed after the 25th day (Fig. 4.2). The lowest leaf P values were recorded in the clone TTL-5 applied with 150% fertilizer concentration and the highest P values were recorded in the clone TTL-1 applied with 150% fertilizer concentration. In TTL-1, TTL-2 and TTL-5, the highest leaf P values were found in the 50% fertilizer applied plants. In TTL-1 and TTL-2 though the highest leaf P content was recorded in the 50% fertilizer applied plants, the next higher level of leaf P was observed in the 150% fertilizer applied plants. But in TTL-5, though the highest leaf P content was recorded in the 50% fertilizer applied plants, the next lower level of leaf P was observed in the 100% fertilizer applied plants and the 150% fertilizer applied plants recorded the lowest leaf P content.

In the clone TTL-6 the lowest leaf P content was found in the 50% fertilizer applied plants and the next higher level was in the 100% fertilizer applied plants and the highest values of leaf P was in the 150% fertilizer applied plants.

Unlike N and P absorption, the K levels were found to be maximum on the 10th day after fertilizer application and the increase was significant (Fig. 4.3). On the 10th day after application of fertilizer, the highest values of leaf K was in the clone TTL-2 with 150% fertilizer concentration and the lowest value was in the clone TTL-5 with 100% fertilizer concentration. In TTL-5 the highest level of leaf K was found in the 50% fertilizer applied plants whereas in the clone TTL-1 the highest level of leaf K was noted in the 100% fertilizer applied plants on the 10th day after fertilizer application. As observed in the case of leaf N and P, the leaf K also showed an increasing trend in the clone TTL-6 with increase in the fertilizer concentration.

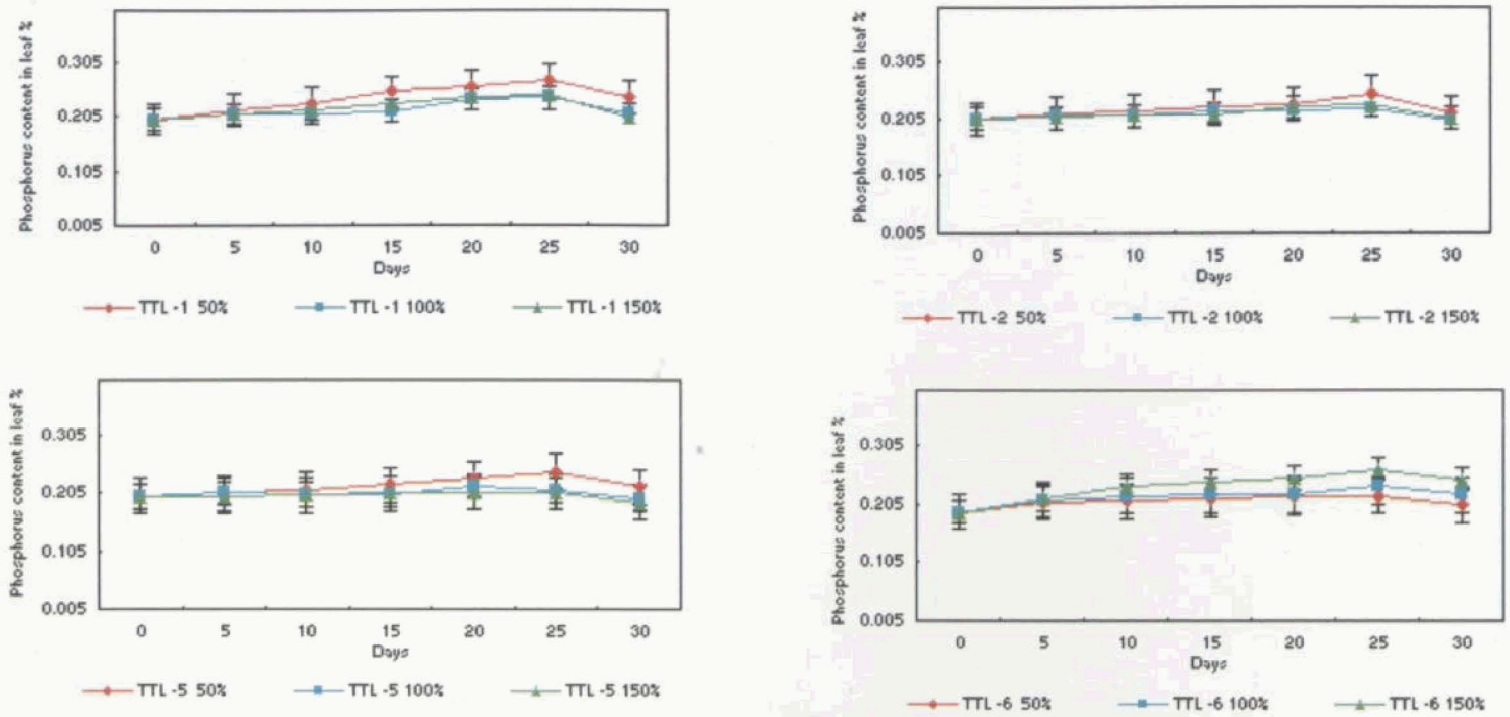

Fig. 4.2: Effect of various concentrations of fertilizers on P content in leaf in different clones of tea (*C. sinensis*).

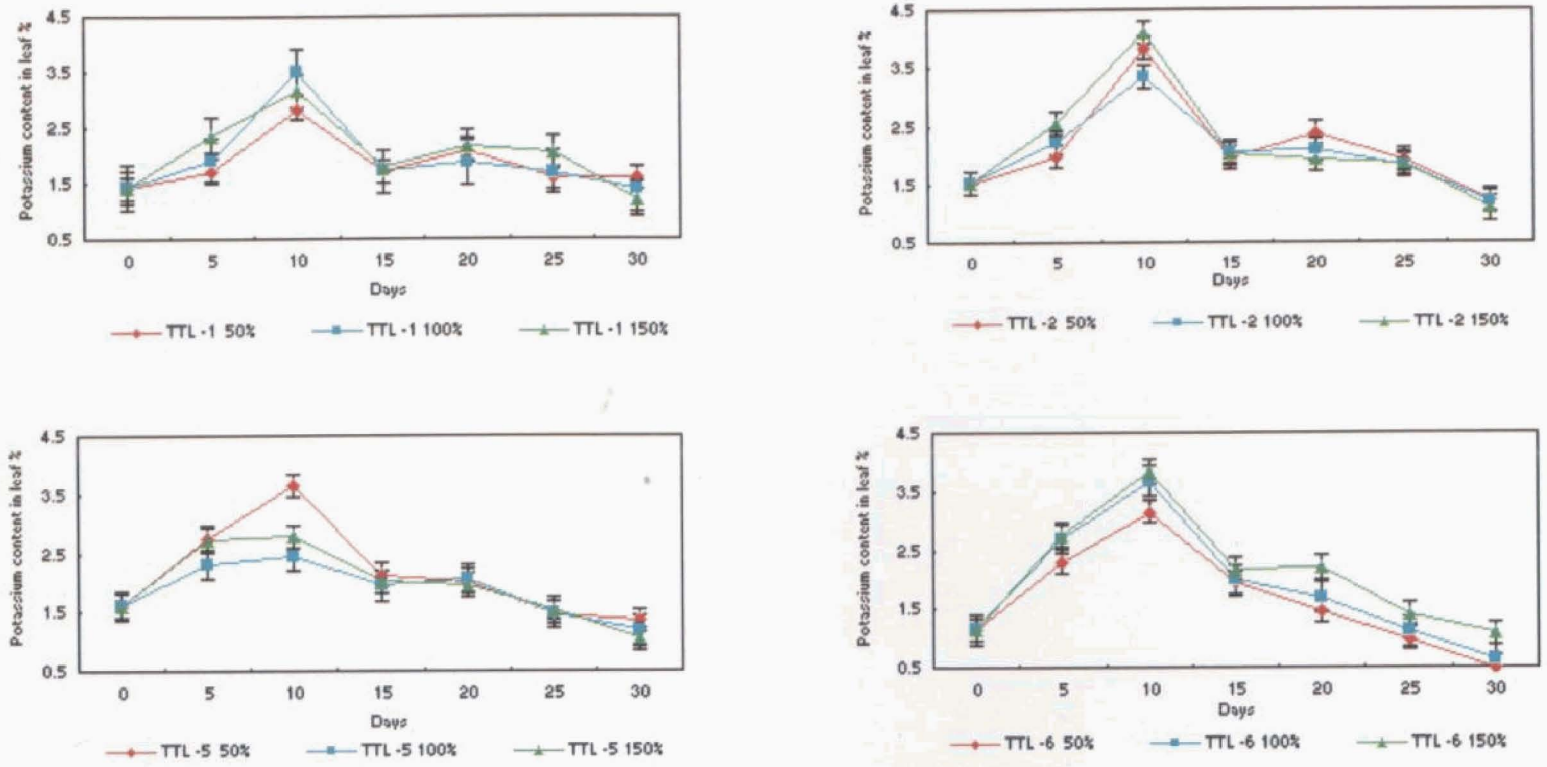

Fig. 4.3: Effect of various concentrations of fertilizers on K content in leaf in different clones of tea (*C. sinensis*).

As the maximum values of the nutrients N and P were found on the 25th day after fertilizer application, all the data given below on the effect of nutrients are presented based on the 0 day and the 25th day after fertilizer application.

4.1.2 Weight of shoot

In the present study, growth was measured in terms of weight of individual shoots. The weight of individual shoots showed a significant increase on the 25th day over the weight recorded on the 0 day (Fig. 4.4) ($p<0.01$). The increase seen in all the clones on the 25th day over the 0 day was significant. In the clones TTL-1, TTL-2 and TTL-5, the highest increase in the shoot weight was observed in the 50% fertilizer applied plants while in the clone TTL-6 the highest value was obtained in the 150% fertilizer applied plants.

In the case of the clone TTL-6 the lowest shoot weight was in the 50% fertilizer applied plants, the 100% fertilizer applied plants showed an increase over the 50% fertilizer applied plants and the 150% fertilizer applied plants showed an increase over the 100% fertilizer applied plants. The increase from 50 to 100% and from 100 to 150% was significant. The maximum value in the case of TTL-1, TTL-2 and TTL-5 was in the 50% fertilizer applied plants and the increase over the 100 and 150% fertilizer applied plants was significant in these clones.

4.1.3 Chlorophyll and carotenoid content

During the course of the present study, in all the clones the chlorophyll a+b and carotenoid content exhibited a significant increase on the 25th day when compared to the 0 day (Fig. 4.5 and 4.6) ($p<0.05$). The total chlorophyll content as well as the carotenoid content increased with application of NPK, irrespective of the concentration provided. In TTL-1,

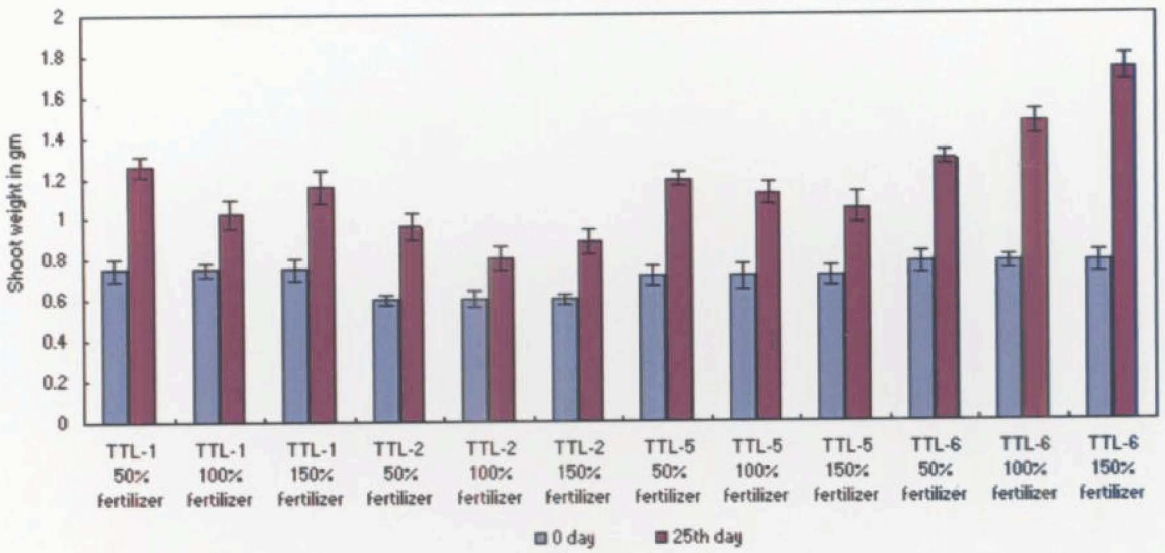

Fig. 4.4: Effect of various concentrations of fertilizers on shoot weight in different clones of tea (*C. sinensis*).

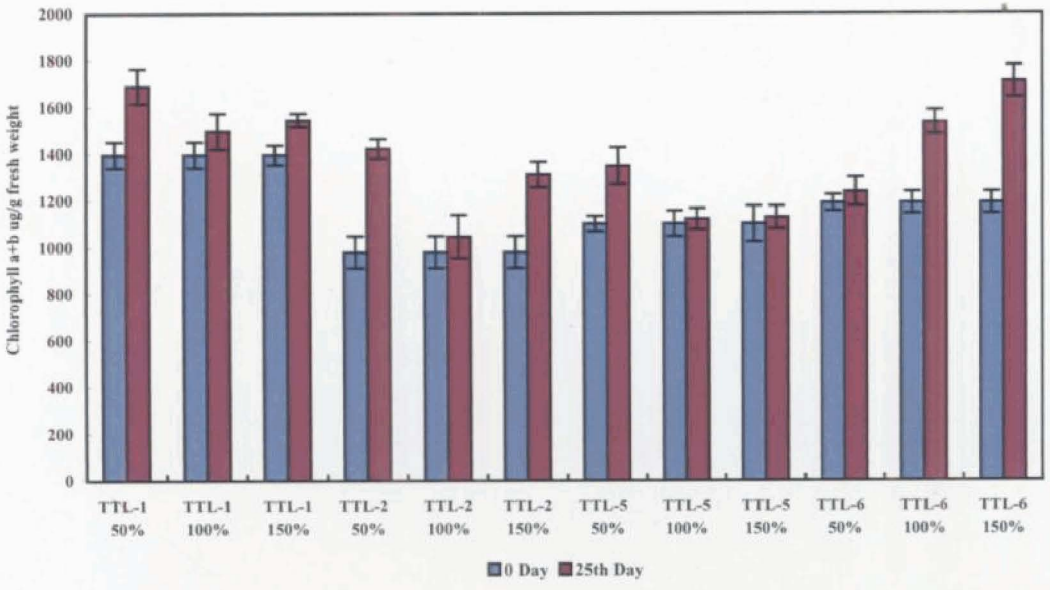

Fig. 4.5: Effect of various concentrations of fertilizers on chlorophyll a+b content in different clones of tea (*C. sinensis*).

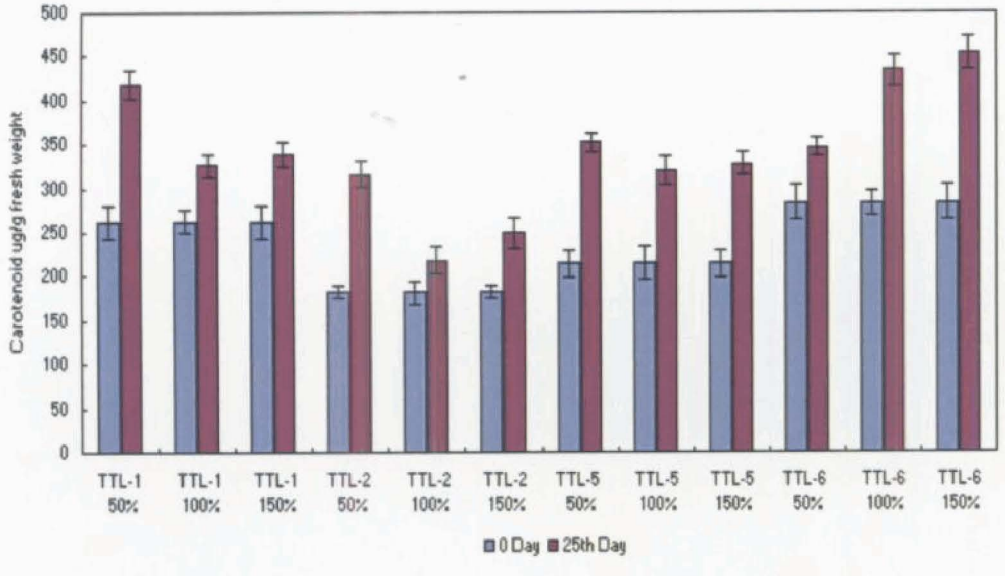

Fig. 4.6: Effect of various concentrations of fertilizers on carotenoid content in different clones of tea (*C. sinensis*).

TTL-2 and TTL-5, the highest values were observed in the 50% fertilizer applied plants as compared to the 100 and 150% fertilizer applied plants. In contrast to this observation, in TTL-6 as the fertilizer concentration was increased from 50 to 100 and 150%, there was a significant increase in the total chlorophyll and carotenoid content with the increasing concentration of NPK. In the clone TTL-6 the 100% fertilizer applied plants had higher values than the 50% fertilizer applied plants and the 150% fertilizer applied plants had higher values than the 100% fertilizer applied plants.

Therefore the highest values of total chlorophyll and carotenoid content in the clone TTL-6 were observed in the 150% fertilizer applied plants while in TTL-1, TTL-2 and TTL-5 the highest values were obtained in the 50% fertilizer applied plants.

4.1.4 Chlorophyll fluorescence

In the present study, in all the clones there was a significant increase in the Fv/Fm values on the 25th day compared to the 0 (day of fertilizer application) (Fig. 4.7) ($p<0.05$). In the case of the clone TTL-1 the highest increase was noticed in the 50% fertilizer applied plants and similarly in TTL-2 and TTL-5 also the highest increase was observed in the 50% fertilizer applied plants. In the clone TTL-1 the least increase in Fv/Fm ratio was noticed in 100% fertilizer applied plants and the second highest Fv/Fm ratio was in the 150% fertilizer applied plants. In the clone TTL-2 also the same trend was observed. In the clone TTL-5 as the fertilizer concentration increased, the Fv/Fm ratio decreased and the lowest value of Fv/Fm ratio was observed in the 150% fertilizer applied plants.

In contrast to this observation, in the clone TTL-6 there was an increasing trend with increasing fertilizer concentration, the 50% fertilizer applied plants had the least increase while the 100% fertilizer applied plants

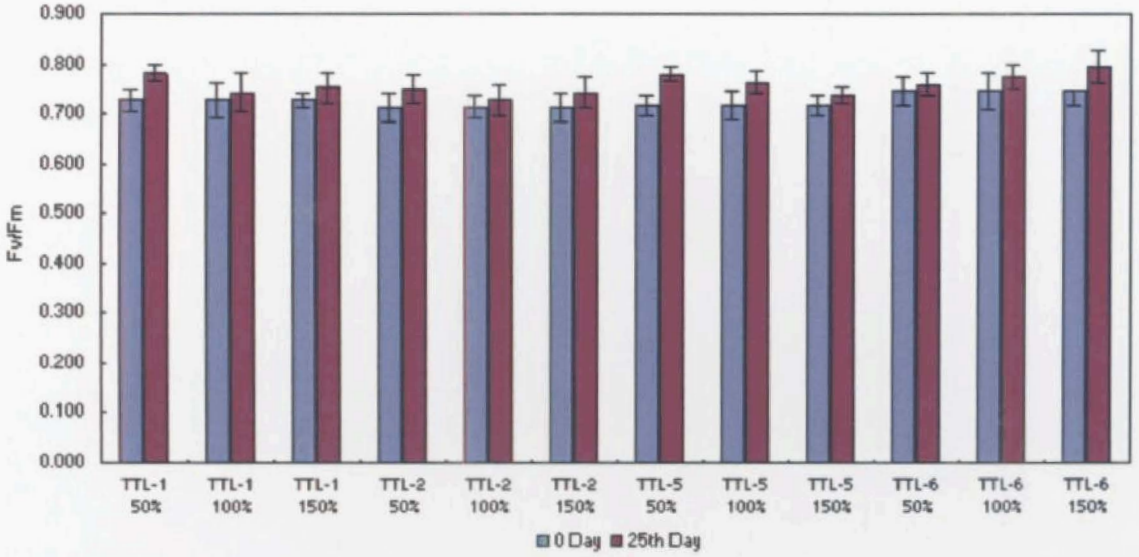

Fig. 4.7: Effect of various concentrations of fertilizers on Fv/Fm ratio in different clones of tea (*C. sinensis*).

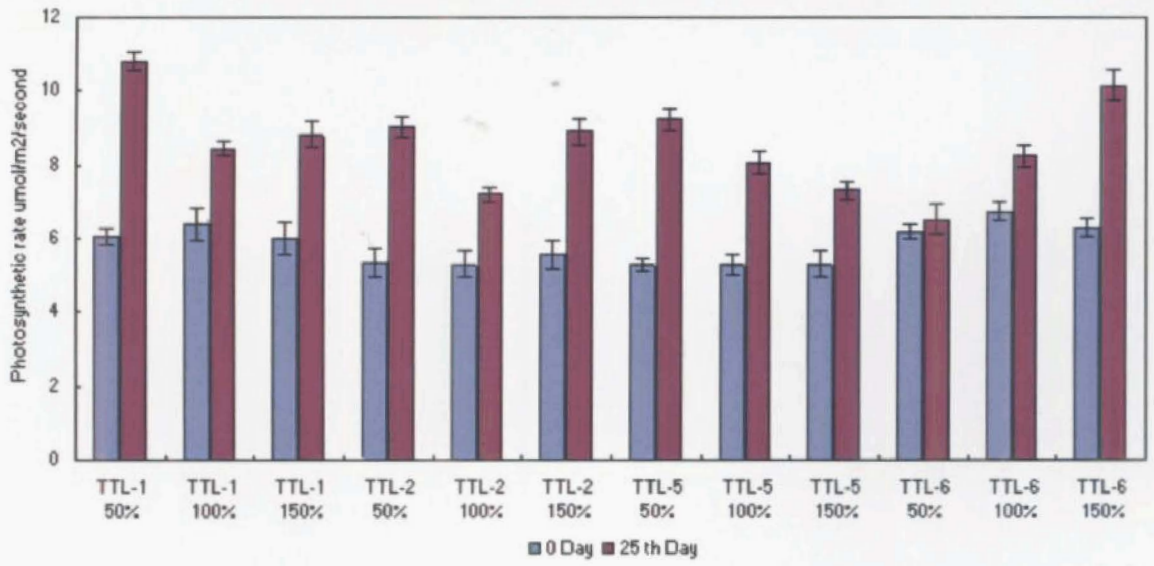

Fig. 4.8: Effect of various concentrations of fertilizers on net photosynthetic rate in different clones of tea (*C. sinensis*).

exhibited more increase than the 50% fertilizer applied plants and 150% fertilizer applied plants exhibited the highest increase on the 25th day. On comparing all the four clones and the three different fertilizer concentrations applied, it was found that the highest Fv/Fm ratio was observed in the clone TTL-6 which received 150% fertilizer.

4.1.5 Photosynthetic rate

Similar to the fluorescence values, during the present study the photosynthetic rate also exhibited a significant increase on the 25th day compared to the 0 day (Fig. 4.8) ($p<0.01$). Among the clones studied TTL-1, TTL-2 and TTL-5 exhibited the highest increase in the 50% fertilizer applied plants. When treated with 100% and 150% fertilizer concentrations, these clones had lower photosynthetic rate than the 50% fertilizer concentration applied plants. The highest photosynthetic rate on the 25th day was recorded in the clone TTL-1 applied with 50% fertilizer. The lowest photosynthetic rate in the clones TTL-1 and TTL-2 were in the100% fertilizer applied plants and the second highest photosynthetic rate was in the 150% fertilizer applied plants. The increase in photosynthetic rate from the 100% to the 150% fertilizer applied plants in TTL-1 and TTL-2 was significant.

In the clone TTL-5, as the fertilizer concentration was increased there was a decrease in the photosynthetic rate. The highest photosynthetic rate in this clone was in the 50% fertilizer applied plants, the next highest was in the 100% and the lowest rate was in the 150% fertilizer applied plants. The decrease in photosynthetic rate between the fertilizer concentrations in the clone TTL-5 was a significant one.

In the clone TTL-6 there was an increase in the photosynthetic rate with increase in the fertilizer concentration. The lowest values were noticed in the 50% fertilizer applied plants, the 100% fertilizer applied plants

exhibited higher values than the 50% fertilizer applied plants and the highest values in this clone were in the 150% fertilizer applied plants.

4.2 Effect of drought on physiological changes in tea clones

4.2.1 Weight of shoot

In the present study the plucked shoots of irrigated and non-irrigated plants of various clones of tea were weighed separately and the average weight of a single shoot was determined. The shoot weight of the non-irrigated plants was less than that of the same clones when irrigation was provided and equalled or was comparable only during the recovery period i.e. after providing irrigation to the non-irrigated plants (Fig. 4.9). The maximum reduction in shoot weight in the non-irrigated plants of all the clones was observed on the 100^{th} day without irrigation and the reduction was significant in all the clones studied ($p<0.05$). The irrigated plants of all the clones maintained more or less same shoot weight throughout the study.

The individual shoot weight of all the non-irrigated clones showed a decrease, which was significant throughout the period of study, as compared to their irrigated control. When the non-irrigated plants were irrigated after 100 days, an increase in the individual shoot weight was noticed on the 7^{th} and 14^{th} day of irrigation. When re-irrigation was done to the plants which were not irrigated for 100 days, there was an increase in the shoot weight which was significant and the highest value was recorded in the clone UPASI-2 on the 7^{th} day compared to all the remaining clones.

The lowest single shoot weight on the 100^{th} day without irrigation was noticed in the clone UPASI-3 (0.63 g) followed by TTL-2 (0.67 g). The highest values in the same period were in the clone UPASI-2 (0.95 g) followed by TTL-1 (0.94 g) and TTL-6 (0.93 g) respectively.

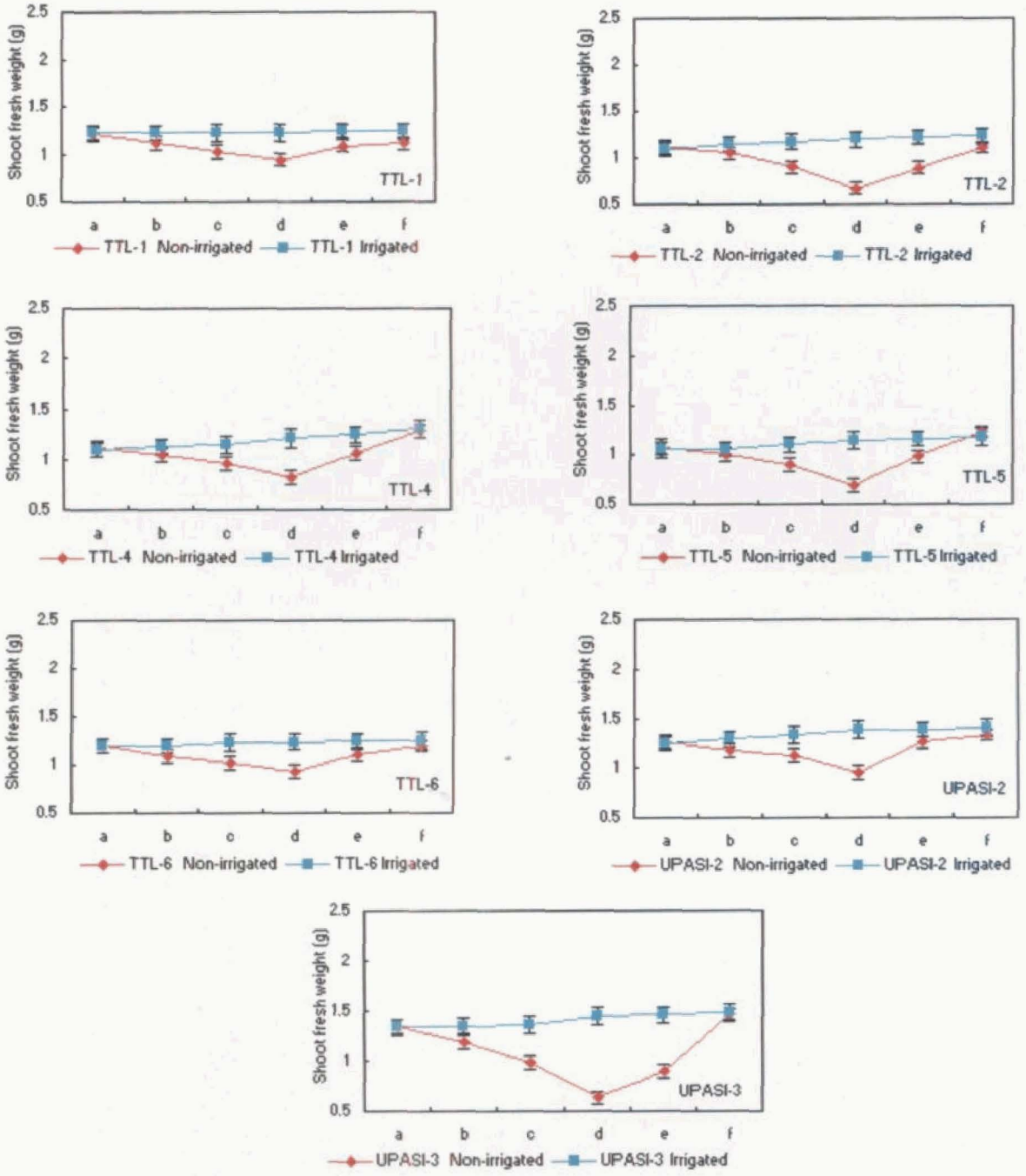

Fig. 4.9: Effect of drought on shoot weight in various clones of tea (*C. sinensis*) which was recorded on the 0, 20th, 60th, 100th day without irrigation and on the 7th and 14th day during re-irrigation. The 0, 20th, 60th, 100th day without irrigation and the 7th and 14th day during re-irrigation are represented by a, b, c, d, e and f respectively.

4.2.2 Soil Moisture Status

Under the regime of regular irrigation just prior to the start of water stress, the soil moisture status in the field remained nearly close to the value of 22.7% (Fig. 4.10). Under water stress there was a decline in soil moisture status to 18.3% by the 20^{th} day and further to 11.5% by the 60^{th} day. The decrease in soil moisture % was significant throughout the study. It was on the 100^{th} day after withholding irrigation that the soil moisture dropped below the wilting coefficient of about 8%. When recovery was attempted by watering the soil, moisture status returned back to 23%, approximately equivalent to the soil moisture on the day when the stress was initiated. The soil moisture % in the irrigated plots remained more or less constant.

4.2.3 Leaf water potential

In the irrigated plants of all the clones the leaf water potential was more or less the same. In the non-irrigated plants the leaf water potential showed a significant reduction in all the clones on the 100^{th} day of withholding irrigation (Fig. 4.11) ($p<0.01$). The highest value on the 100^{th} day of moisture stress was observed in the clones TTL-1, TTL-6 and UPASI-2 in the non-irrigated plants. The lower values were in the clones TTL-2, TTL-4, TTL-5 and UPASI-3. The lowest value in the non-irrigated plants was seen in the clones TTL-2 and UPASI-3.

After providing irrigation to the non-irrigated plants, on the 14^{th} day the leaf water potential values were found to increase and a significant increase was noticed in all the clones studied.

4.2.4 Relative water content

In the present study all the irrigated clones exhibited a slight increase in the relative water content from the beginning to the end of the experiment,

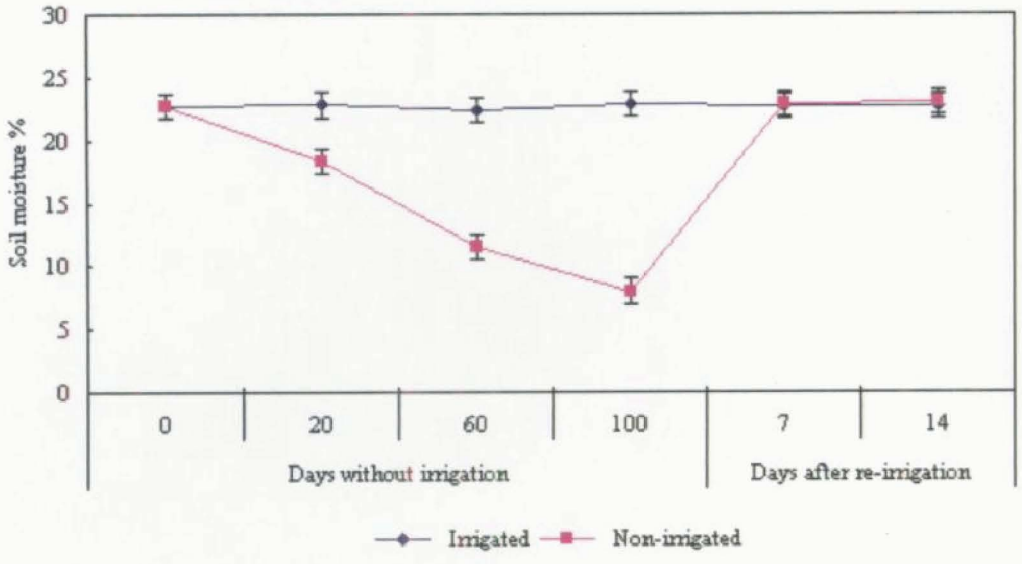

Fig. 4.10: Mean soil moisture % recorded in irrigated and non-irrigated plots.

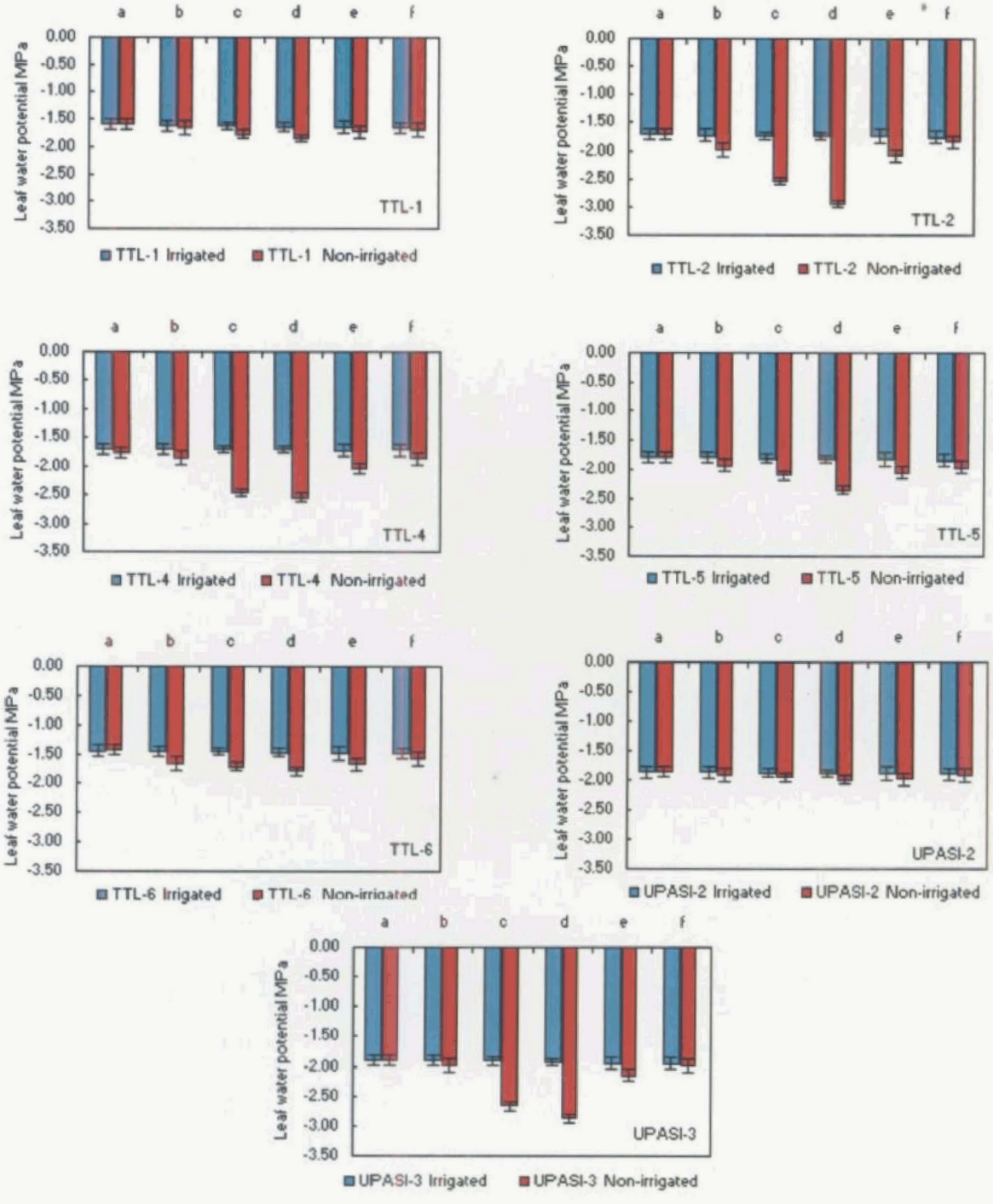

Fig. 4.11: Effect of drought on leaf water potential in various clones of tea (*C. sinensis*) which was recorded on the 0, 20th, 60th, 100th day without irrigation and on the 7th and 14th day during re-irrigation. The 0, 20th, 60th, 100th day without irrigation and the 7th and 14th day during re-irrigation are represented by a, b, c, d, e and f respectively.

which was not significant. In the case of the non-irrigated clones there was a significant reduction in the relative water content by the 100th day of non-irrigation (Table 4.1). Though all the clones showed a reduction in the relative water content by the 100th day, the maximum reduction was observed in UPASI-3 (63%) followed by the clones TTL-2, TTL-4 and TTL-5. The least reduction in relative water content on the 100th day of non-irrigation was in TTL-1 (76%), TTL-6 (77%) and UPASI-2 (78%).

The non-irrigated plants after 100 days were subjected to irrigation and a rapid increase in relative water content was observed on the 14th day after irrigation as compared to the 100th day of non-irrigation. The non-irrigated plants showed a tendency to recover and reached almost the values seen at the beginning of the experiment. On the 14th day of re-irrigation of the non-irrigated plants, the clones which achieved relative water content values nearest to that on the 0 day of the experiment were TTL-1, TTL-6 and UPASI-2.

4.2.5 Chlorophyll and carotenoid content

In the present study the pigment composition was found to be more in the leaves of all the clones in the irrigated state than in the non-irrigated state (Fig. 4.12). A significant reduction in the chlorophyll a+b content was seen in the non-irrigated plants, with the reduction increasing from the 0 day and reaching the maximum by the 100th day ($p<0.01$) (Fig. 4.12). In the clones TTL-2, TTL-4, TTL-5 and UPASI-3 as the moisture stress progressed from the 0 day to the 100th day, the chlorophyll a+b values reached less than 1000 µg/g fresh weight on the 100th day.

In the irrigated condition the clones TTL-1, TTL-2, TTL-4, TTL-5, TTL-6, UPASI-2 and UPASI-3 exhibited an increase in chlorophyll a+b content throughout the period of study, which was not significant. In the

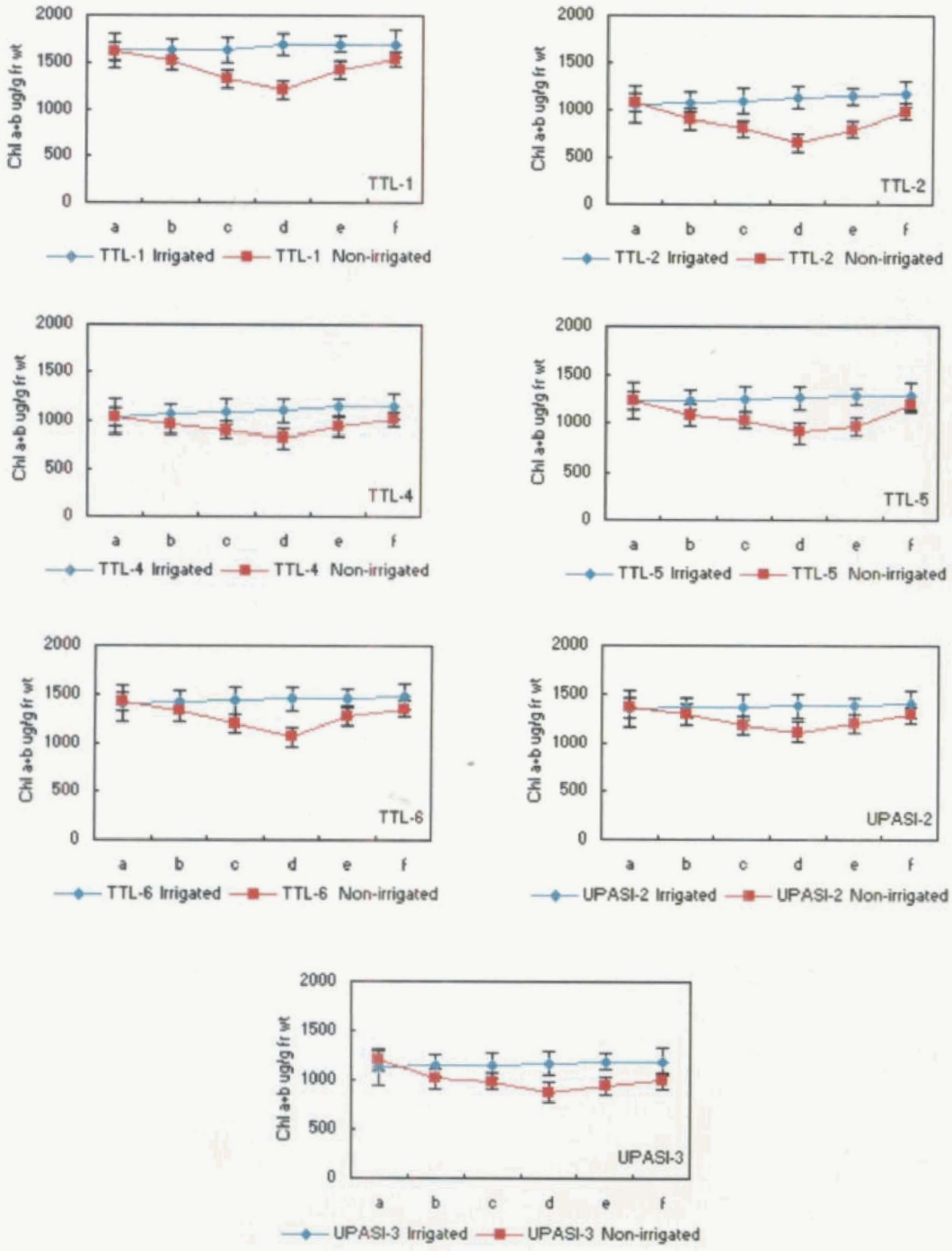

Fig. 4.12: Effect of drought on chlorophyll a+b content in various clones of tea (*C. sinensis*) which was recorded on the 0, 20th, 60th, 100th day without irrigation and on the 7th and 14th day during re-irrigation. The 0, 20th, 60th, 100th day without irrigation and the 7th and 14th day during re-irrigation are represented by a, b, c, d, e and f respectively.

Table 4.1: Relative water content in various clones of tea (*C. sinensis*) with and without irrigation on the 0, 20th, 60th and 100th day and during re-irrigation on the 7th and 14th day.

Clone with treatment	Days without irrigation				Days after re-irrigation	
	0	20	60	100	7	14
TTL-1 Irrigated	86% ± 2.8	86% ± 3.5	87% ± 3.5	87% ± 3.2	88% ± 3.4	89% ± 3.5
TTL-2 Irrigated	87% ± 3.7	87% ± 3.4	88% ± 3.5	89% ± 3.4	90% ± 2.9	90% ± 3.4
TTL-4 Irrigated	86% ± 3.8	86% ± 3.7	87% ± 3.2	88% ± 3.1	89% ± 3.3	89% ± 3.1
TTL-5 Irrigated	86% ± 3.1	87% ± 3.8	89% ± 3.4	89% ± 3.1	90% ± 3.5	90% ± 3.1
TTL-6 Irrigated	88% ± 3.0	88% ± 3.7	89% ± 3.2	90% ± 3.4	90% ± 3.5	91% ± 3.3
UPASI-2 Irrigated	88% ± 3.5	89% ± 3.7	90% ± 4.1	91% ± 3.3	94% ± 3.2	94% ± 2.9
UPASI-3 Irrigated	89% ± 3.4	89% ± 3.1	90% ± 3.3	91% ± 3.2	92% ± 3.4	92% ± 3.7
TTL-1 Non-irrigated	86% ± 2.5	85% ± 3.1	81% ± 3.3	76% ± 3.4	78% ± 3.0	85% ± 3.3
TTL-2 Non-irrigated	88% ± 3.3	85% ± 3.1	75% ± 3.2	66% ± 3.7	71% ± 3.3	84% ± 3.1
TTL-4 Non-irrigated	86% ± 2.9	82% ± 2.7	70% ± 3.0	69% ± 3.2	72% ± 3.0	83% ± 3.3
TTL-5 Non-irrigated	85% ± 3.1	83% ± 2.9	75% ± 3.4	70% ± 3.1	79% ± 3.0	83% ± 3.2
TTL-6 Non-irrigated	88% ± 2.9	84% ± 2.7	79% ± 3.2	77% ± 3.3	80% ± 3.5	87% ± 3.1
UPASI-2 Non-irrigated	88% ± 3.2	85% ± 3.3	80% ± 3.5	78% ± 3.0	82% ± 3.1	87% ± 3.6
UPASI-3 Non-irrigated	89% ± 2.7	84% ± 2.9	72% ± 3.3	63% ± 3.5	70% ± 3.1	85% ± 3.7

irrigated condition the chlorophyll a+b content was maximum in leaves of the clone TTL-1 and was minimum in TTL-2 and TTL-4 throughout the period of study.

In all the clones, in the non-irrigated state a significant reduction in chlorophyll a+b content was noticed by the 60th day itself. The maximum reduction on the 100th day without irrigation was observed in the clones TTL-2, TTL-4 and UPASI-3. A significant reduction in chlorophyll a+b content was noticed in the clone UPASI-3 throughout the period of study.

The leaf chlorophyll a+b content of all non-irrigated plants was found to have increased by the 7th day after re-irrigation but was not significant in all the clones. The increase was significant by the 14th day after re-irrigation. The highest values of chlorophyll a+b on the 14th day after re-irrigation were observed in the clone TTL-1, TTL-6 and UPASI-2 and the lowest values were in the clones TTL-2, TTL-4, TTL-5 and UPASI-3.

All the clones under irrigation showed an increase in carotenoid content throughout the period of study, which was not significant (Fig. 4.13). The highest values of carotenoid content were observed in the clone TTL-1, as noticed in the case of chlorophyll a+b content.

The carotenoid content in the leaves of the clones under non-irrigated conditions exhibited a reduction throughout the period of study and was lowest on the 100th day without irrigation. Among the clones studied, the lowest value of carotenoid content was observed in the clone UPASI-3 followed by TTL-2, TTL-5 and TTL-4 on the 100th day after withholding irrigation. The reduction in the level of carotenoid content in the non-irrigated plants from the 0 day to the 100th day in all the clones was a significant one. Also, the reduction in the carotenoid content in the non-irrigated plants on the 60th day itself was significant.

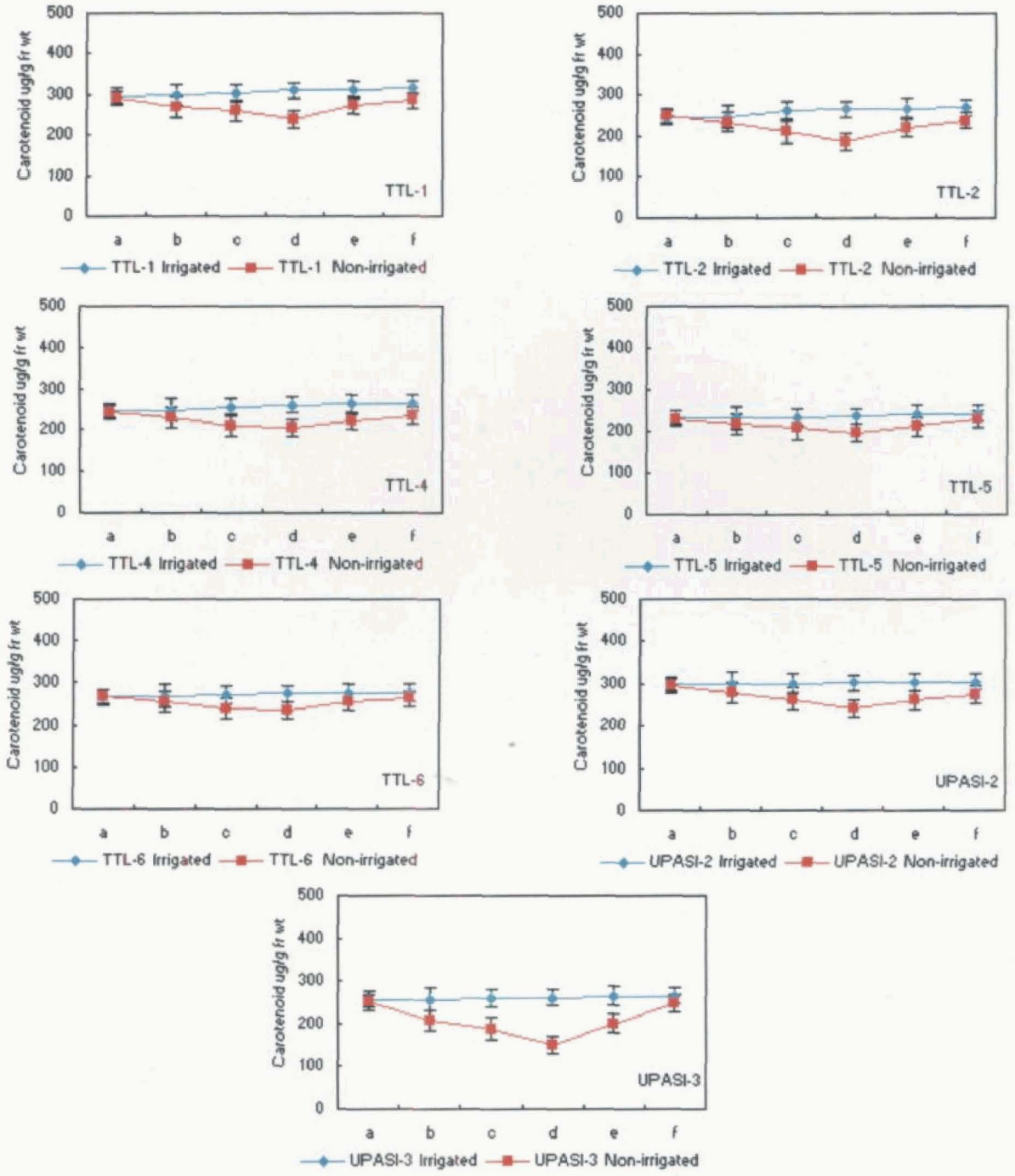

Fig. 4.13: Effect of drought on carotenoid content in various clones of tea (*C. sinensis*) which was recorded on the 0, 20th, 60th, 100th day without irrigation and on the 7th and 14th day during re-irrigation. The 0, 20th, 60th, 100th day without irrigation and the 7th and 14th day during re-irrigation are represented by a, b, c, d, e and f respectively.

The increase in the values of carotenoid content from the 100th day of non-irrigation to the 7th day of re-irrigation was significant in the case of TTL-1, TTL-2, TTL-6, UPASI-2 and UPASI-3, while it was non-significant in TTL-4 and TTL-5. The increase in carotenoid content on the 14th day of re-irrigation was significant in all the clones and was highest in the clone TTL-1 (285 µg/g fresh weight), followed by UPASI-2 (275 µg/g fresh weight) and TTL-6 (263 µg/g fresh weight).

4.2.6 Chlorophyll fluorescence

In the present study, withholding irrigation resulted in a significant reduction in the Fv/Fm values throughout the period of non-irrigation, which was lowest by the 100th day ($p<0.05$). A similar pattern of reduction was not observed in the irrigated clones. However, a slight increase in Fv/Fm values of the irrigated clones was observed which was not significant (Fig. 4.14). The reduction of Fv/Fm was maximum in the clones UPASI-3 (0.497) and TTL-2 (0.510) and minimum in the clone TTL-1 (0.643) on the 100th day without irrigation.

When subjected to irrigation, the non-irrigated clones exhibited a rapid and significant increase in their Fv/Fm values. The increase in Fv/Fm values of the various clones on the 14th day after re-irrigation was significant when compared to that on the 100th day without irrigation. The highest values on the 14th day after irrigation were observed in the clones TTL-1 (0.736), UPASI-2 (0.731) and TTL-6 (0.721).

4.2.7 Net photosynthetic rate

In the present study the net photosynthetic rate of various clones of tea were measured and recorded under irrigated and non-irrigated conditions on the 100th day without irrigation and on the 14th day after providing irrigation (Fig. 4.15). It was found that there was a significant reduction in the

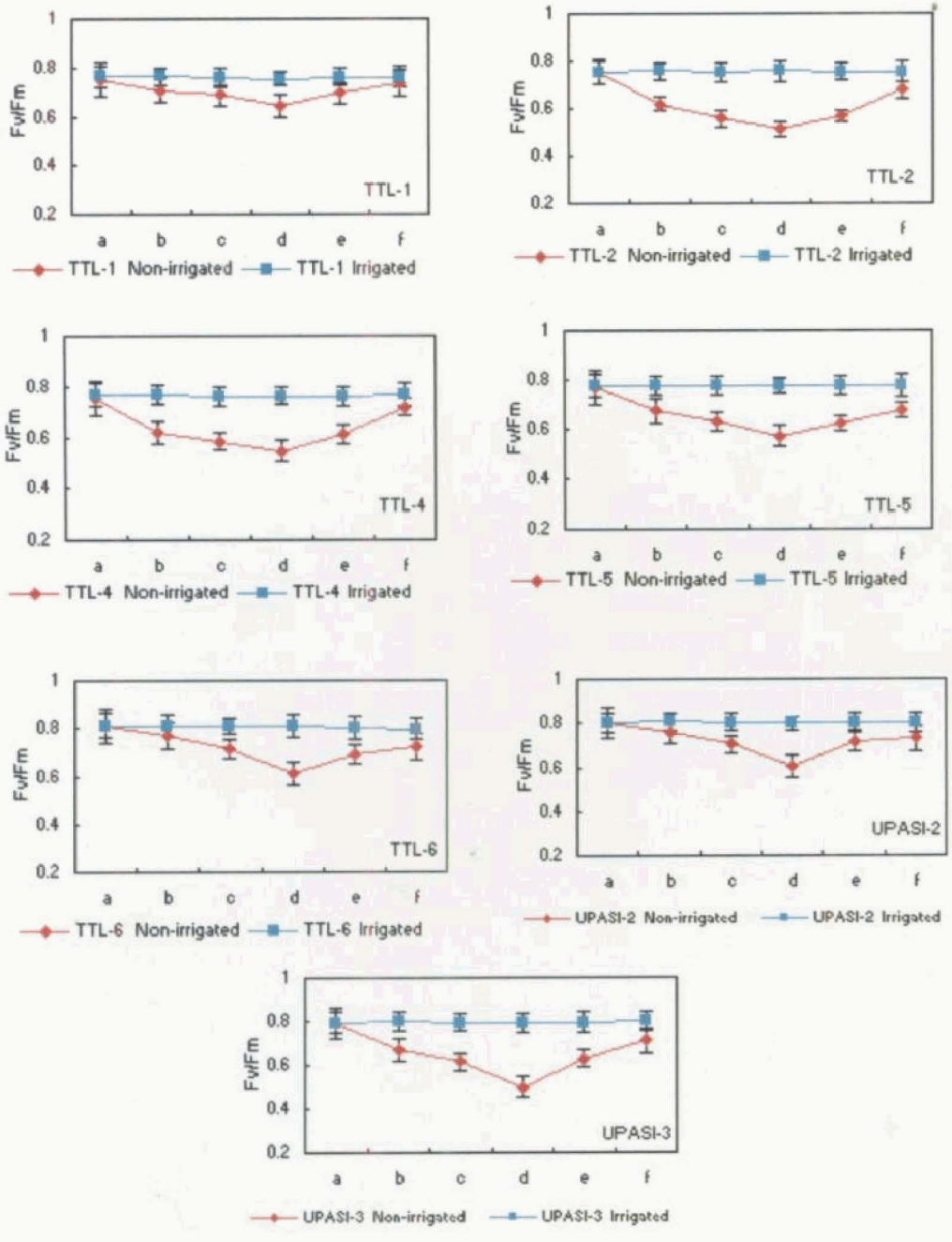

Fig. 4.14: Effect of drought on Fv/Fm ratio in various clones of tea (*C. sinensis*) which was recorded on the 0, 20th, 60th, 100th day without irrigation and on the 7th and 14th day during re-irrigation. The 0, 20th, 60th, 100th day without irrigation and the 7th and 14th day during re-irrigation are represented by a, b, c, d, e and f respectively.

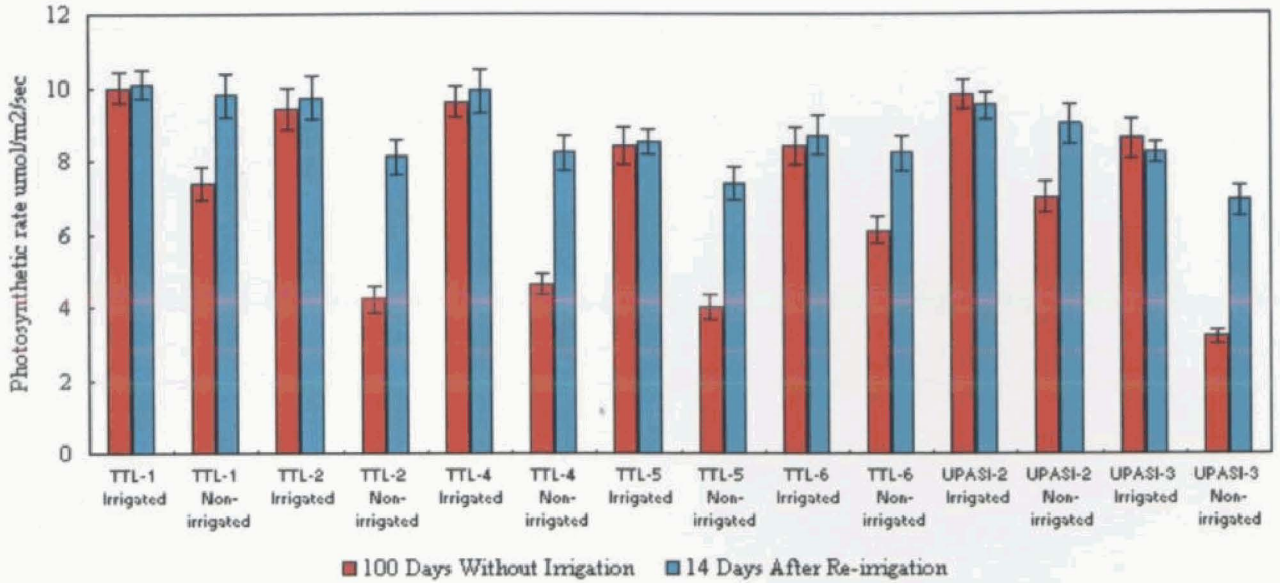

Fig. 4.15: Effect of drought on net photosynthetic rate in various clones of tea (*C. sinensis*). The data was recorded on the 100th day without irrigation and on the 14th day during re-irrigation.

photosynthetic rate of all the clones under non-irrigated conditions on the 100th day. The highest values of photosynthesis in the non-irrigated condition were observed in the clone TTL-1 and the lowest in the clone UPASI-3. Under irrigation also the clone TTL-1 exhibited the highest value of photosynthesis. Among the clones studied, the minimum reduction in photosynthesis was in the clones TTL-1, TTL-6 and UPASI-2 and the maximum reduction was observed in the clones TTL-2, TTL-4, TTL-5 and UPASI-3.

After 14 days of irrigation a rapid increase in the rate of photosynthesis was exhibited by non-irrigated plants as compared to the values in non-irrigated condition on the 100th day. The clone TTL-2 and UPASI-3 recorded a significant increase in the photosynthetic rate on the 14th day after re-irrigation as against the 100th day without irrigation. The difference in the values between the irrigated and non-irrigated condition on the 14th day was minimum in the clones TTL-1, TTL-6 and UPASI-2. On the 14th day of re-irrigation the highest value of photosynthesis was noted in the clone TTL-1.

In the present study the transpiration rates in the clones TTL-2, TTL-4, TTL-5 and UPASI-3 exhibited a significant reduction on the 100th day of withholding irrigation when compared to the same clones in the irrigated condition (Fig. 4.16). The clones TTL-1, TTL-6 and UPASI-2 had only a non-significant reduction in the transpiration rates at the same time as compared to its irrigated condition. In the irrigated condition on the 100th day the highest transpiration rate was achieved in the clone TTL-6.

On the 14th day after providing irrigation to the non-irrigated plants, the transpiration rates in the clones TTL-2, TTL-4, TTL-5 and UPASI-3 did not reach the levels of transpiration rate of the same clones in the irrigated condition. The clones TTL-1, TTL-6 and UPASI-2 in the non-irrigated

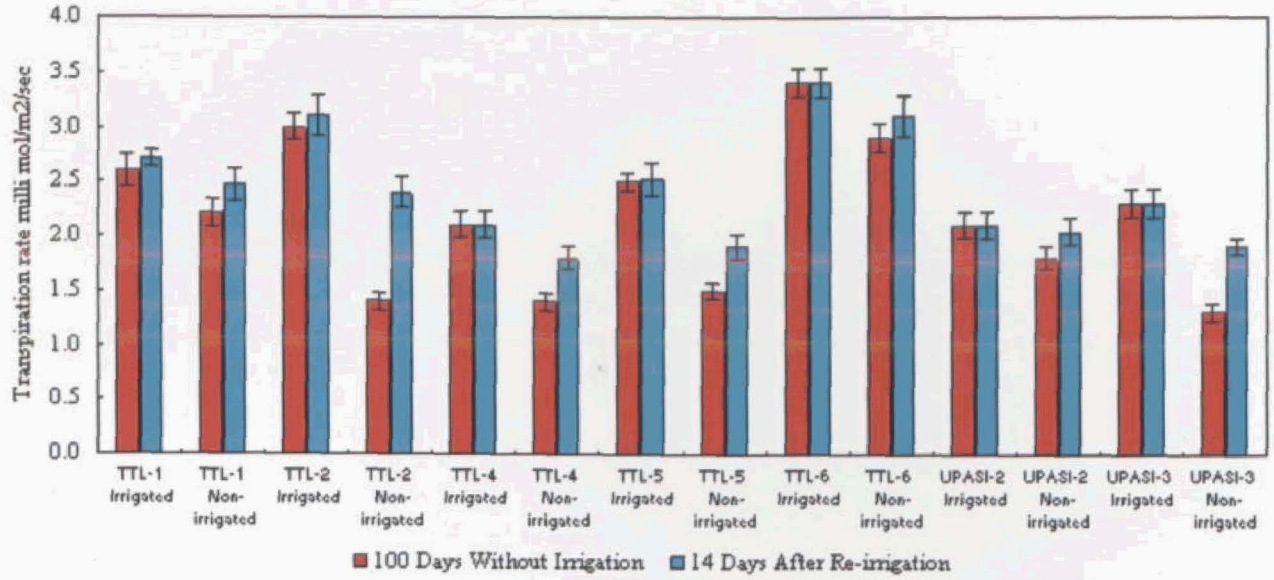

Fig. 4.16: Effect of drought on transpiration rate in various clones of tea (*C. sinensis*). The data was recorded on the 100th day without irrigation and on the 14th day during re-irrigation.

condition did not show any significant difference with the irrigated condition of the same clones on the 14th day.

In the present study, the stomatal conductance of the leaves of non-irrigated clones of tea showed a significant decrease in TTL-2, TTL-4, TTL-5 and UPASI-3 compared to the irrigated condition (Fig. 4.17). Among these clones the highest reduction was observed in the clone TTL-5. Though the clones TTL-1, TTL-6 and UPASI-2 in the non-irrigated condition exhibited a reduction when compared to the irrigated plants, the reduction was non-significant in these clones. The minimum reduction among these clones was in TTL-6 and the maximum reduction was in TTL-1.

On the 14th day after providing irrigation to the non-irrigated plants, the stomatal conductance in the clones TTL-1, TTL-6 and UPASI-2 reached the level of its irrigated condition and the difference was non-significant. Though the clones TTL-2, TTL-4, TTL-5 and UPASI-3 showed an increase on the 14th day of re-irrigation over that recorded on the 100th day without irrigation the values did not reach the levels of their continuously irrigated counterparts.

4.2.8 Proline Content

In the present study the proline content of leaves of the irrigated and non-irrigated plants was determined (Fig. 4.18). At the initial stage of the experiment, on the 0 day the highest proline content was in the clone TTL-4 and the lowest in the TTL-5. Thereafter, as the days of moisture stress progressed, the level of proline also increased in all the clones. The increase in proline content in the non-irrigated clones was significant on the 20th day of non-irrigation itself.

A significant increase in the proline content was found in non-irrigated plants throughout the period of investigation and was highest on the 100th day

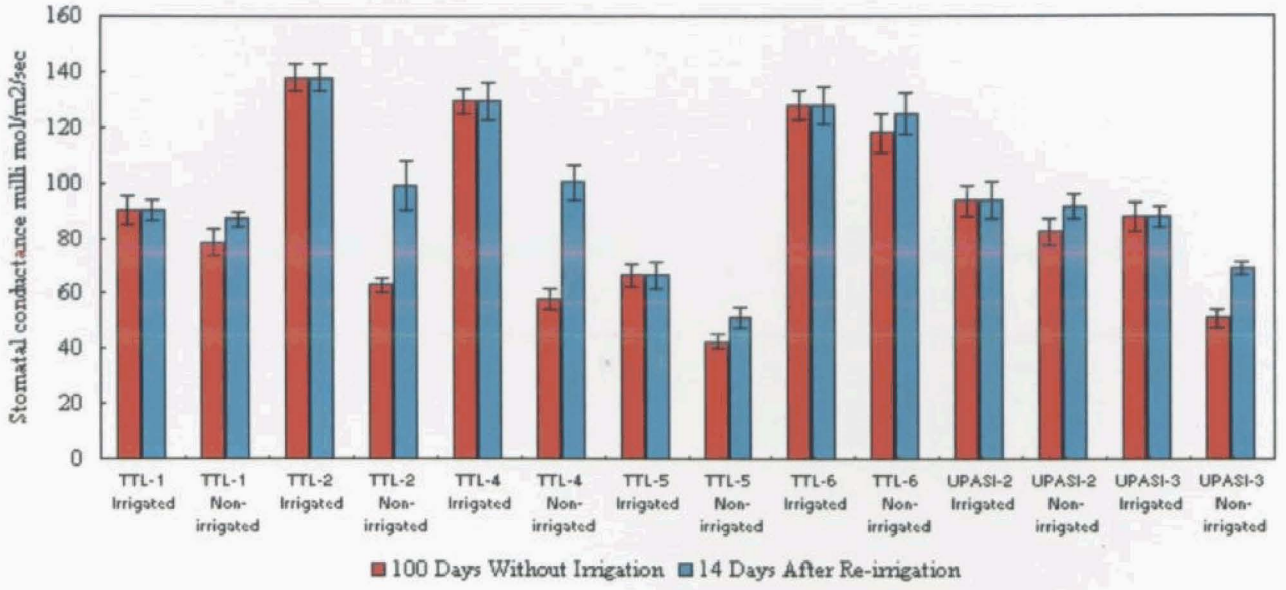

Fig. 4.17: Effect of drought on stomatal conductance in various clones of tea (*C. sinensis*). The data was recorded on the 100th day without irrigation and on the 14th day during re-irrigation.

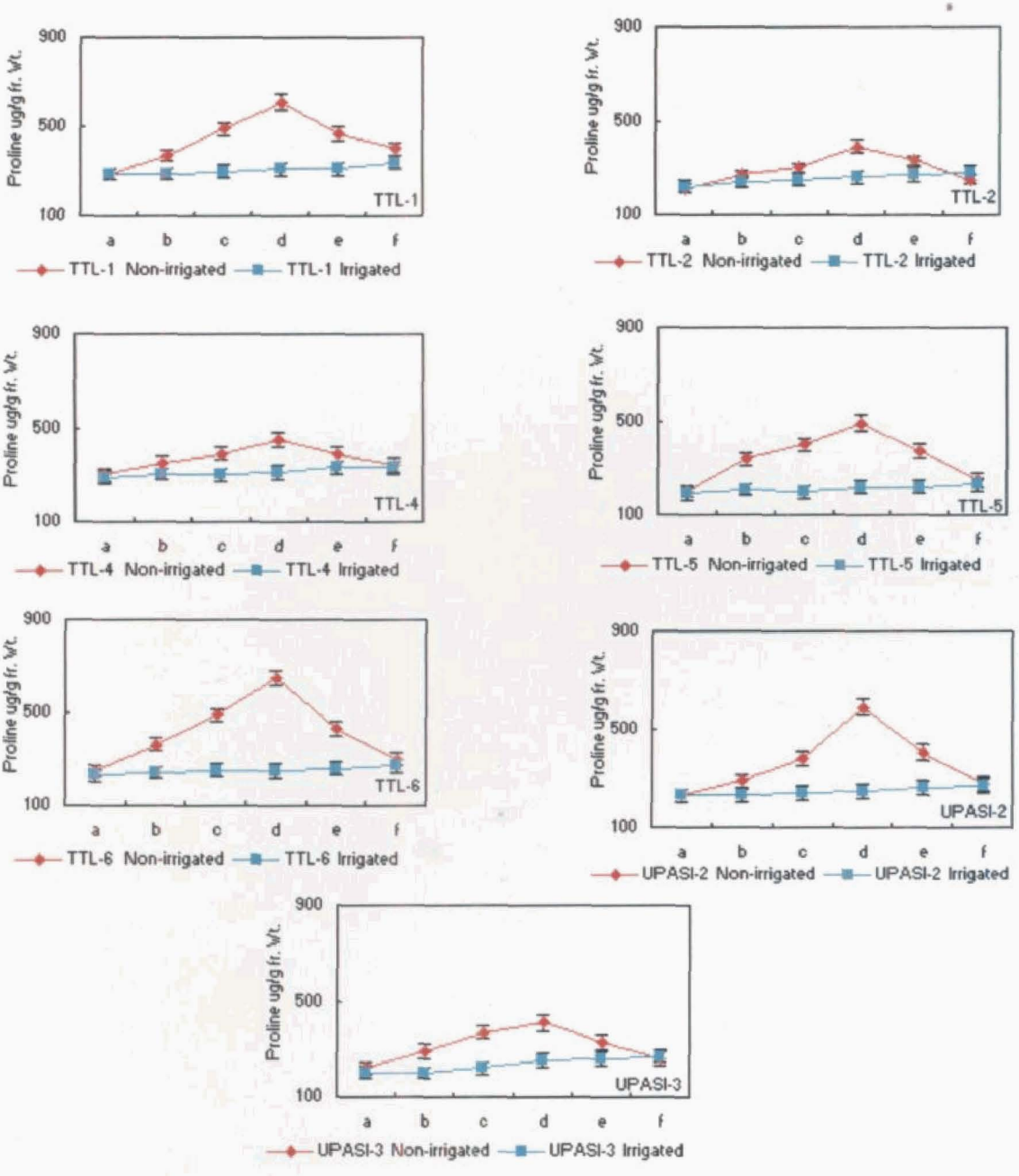

Fig. 4.18: Effect of drought on proline content in various clones of tea (*C. sinensis*) which was recorded on the 0, 20th, 60th, 100th day without irrigation and on the 7th and 14th day during re-irrigation. The 0, 20th, 60th, 100th day without irrigation and the 7th and 14th day during re-irrigation are represented by a, b, c, d, e and f respectively.

of non-irrigation ($p<0.01$). After 100 days of non-irrigation a high level of proline content was found to accumulate in all the clones and it was highest in TTL-1, TTL-6 and UPASI-2 and lowest in TTL-2, TTL-4, TTL-5 and UPASI-3.

When irrigation was provided to the non-irrigated plants after the 100th day, the proline content immediately decreased in all the clones and the reduction was significant. But in all the clones the final level of proline on the 14th day after re-irrigation was significantly higher than that recorded on the 0 day. A more rapid reduction in the level of proline was found in TTL-1, TTL-6 and UPASI-2 on the 14th day after re-irrigation as compared to the rest of the clones.

4.2.9 MDA Content

In the present study all the clones in the irrigated state maintained a steady level of MDA content. The level of MDA content was found to significantly increase in the non-irrigated plants throughout the period of study and reached the maximum by the 100th day (Fig. 4.19) ($p<0.01$). Even by the 20th day of non-irrigation the MDA content exhibited a significant increase in all the clones. The clones TTL-2, TTL-4, TTL-5 and UPASI-3 had higher MDA content on the 100th day of non-irrigation as compared to TTL-1, TTL-6 and UPASI-2. The highest value of MDA in the non-irrigated plants on the 100th day of withholding irrigation was recorded in TTL-2 (10.39 µmol/g fresh weight) and in UPASI-3 (9.73 µmol/g fresh weight) and the lowest values were in TTL- 6 (7.23 µmol/g fresh weight), TTL-1 (7.61 µmol/g fresh weight) and UPASI-2 (7.82 µmol/g fresh weight).

On the 14th day after re-irrigation, all the clones exhibited a significant decrease in the MDA content. Except in the case of the clone TTL-1 and

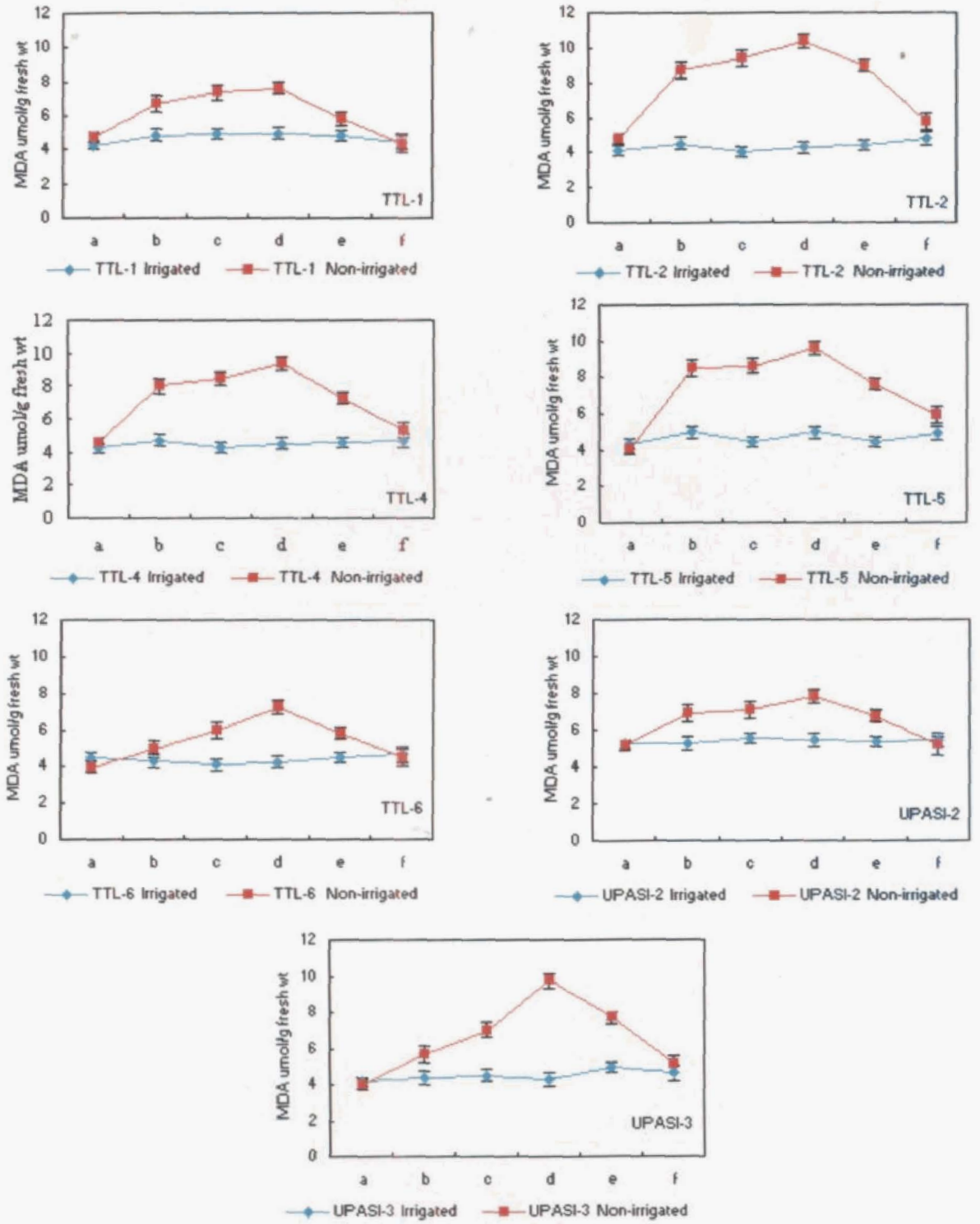

Fig. 4.19: Effect of drought on MDA content in various clones of tea (*C. sinensis*) which was recorded on the 0, 20th, 60th, 100th day without irrigation and on the 7th and 14th day during re-irrigation. The 0, 20th, 60th, 100th day without irrigation and the 7th and 14th day during re-irrigation are represented by a, b, c, d, e and f respectively.

UPASI-2, all the other clones had higher MDA values than that recorded on the 0 day of non-irrigation.

4.2.10 MDA Chlorophyll ratio

In the present study, it was noticed that on the 100th day of non-irrigation the irrigated plants of all the clones maintained more or less similar values (Fig. 4.20). Among the non-irrigated plants, the clones TTL-1, TTL-6 and UPASI-2 exhibited the lowest MDA chlorophyll ratio. The increase in the non-irrigated plants of all the clones over the irrigated plants was significant. On the 100th day of non-irrigation the highest values of MDA chlorophyll ratio were observed in the clones TTL-2, TTL-4, TTL-5 and UPASI-3.

The difference in MDA chlorophyll ratio between the irrigated and the non-irrigated plants was minimum in the clones TTL-1, TTL-6 and UPASI-2 on the 14th day of recovery from drought and was highest in the clone TTL-2 (Fig. 4.21). The clones UPASI-3, TTL-4 and TTL-5 exhibited slight difference between the irrigated and non-irrigated plants on the 14th day of recovery from drought.

4.2.11 Proline MDA content

On the 100th day of non-irrigation, the highest values of proline were found in the clones TTL-1, TTL-6 and UPASI-2 (Fig.4.22). It could be observed that in the clones TTL-1, TTL-6 and UPASI-2 there was a proportionate increase in the proline content with increase in the MDA which was not found in the rest of the clones. The highest MDA content was seen in the clone TTL-2 and the lowest in TTL-6.

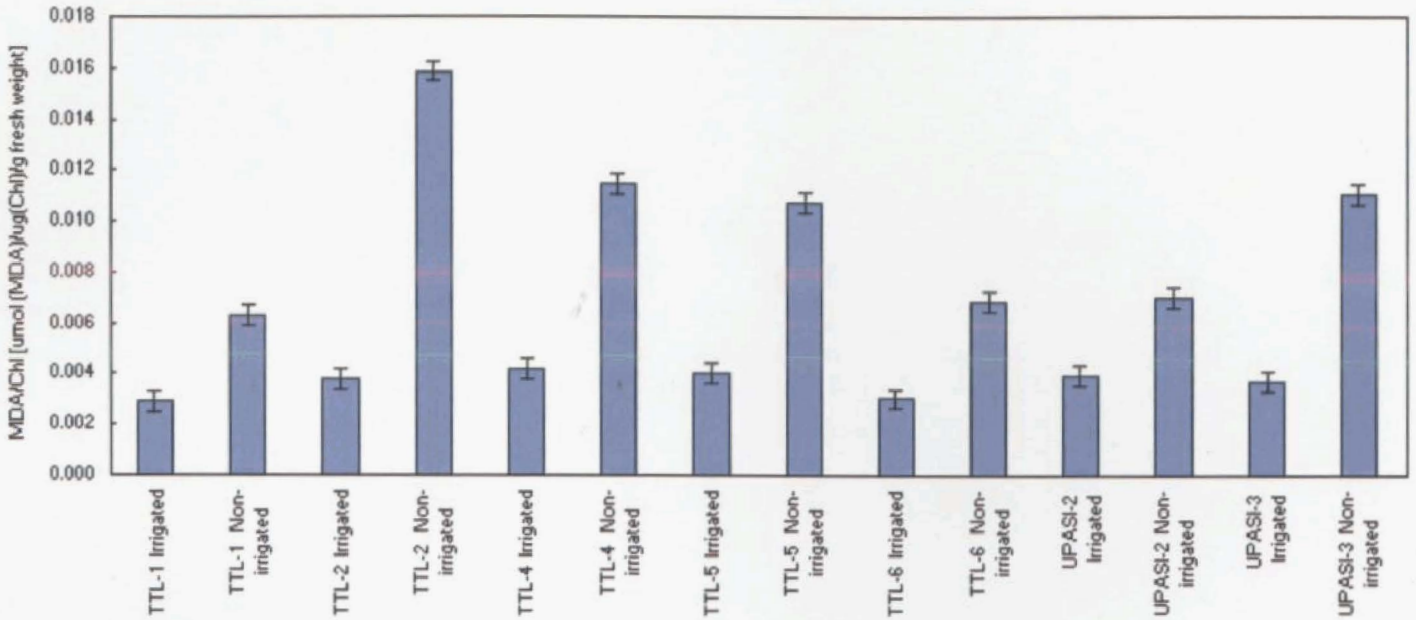

Fig. 4.20: Effect of drought on MDA chlorophyll ratio in various clones of tea (*C. sinensis*) which was recorded on the 100th day without irrigation.

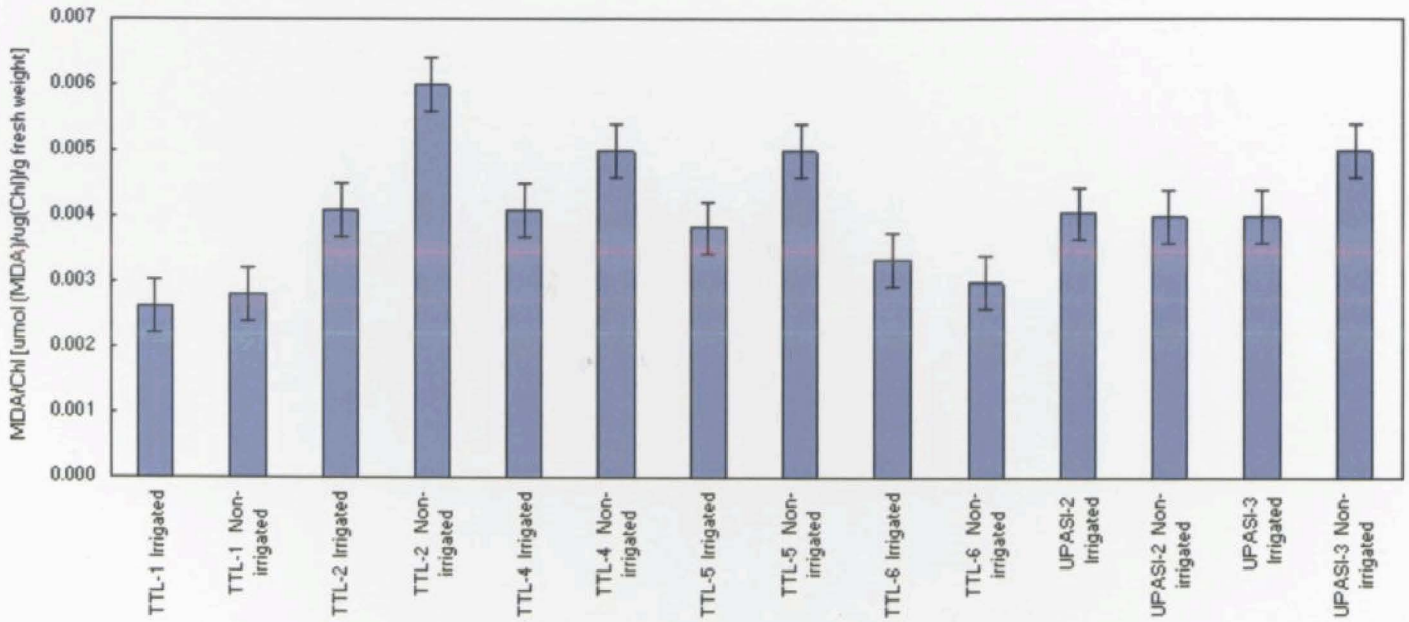

Fig. 4.21: Effect of re-irrigation on MDA chlorophyll ratio in drought affected clones of tea (*C. sinensis*) which was recorded on the 14th day during re-irrigation.

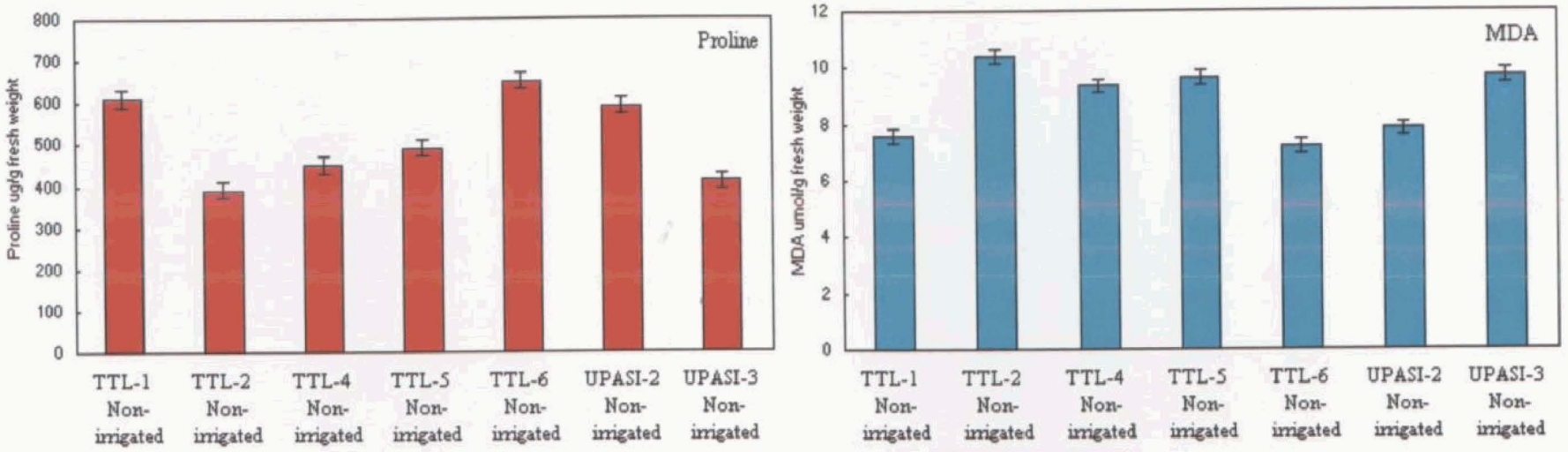

Fig. 4.22: Effect of drought on proline and MDA content in various clones of tea (*C. sinensis*) which was recorded on the 100th day without irrigation.

4.3 Effect of frost on physiological changes in tea clones

4.3.1 Carotenoid content

At the initial stage of the experiment (at 6.30 am) the carotenoid content was less and was found to increase significantly in the subsequent stage (at 11.30 am) (Fig. 4.23) ($p<0.05$). The highest values of carotenoid content at 11.30 am were observed in the clones TTL-1 (191 µg/g fresh weight), UPASI-9 (186 µg/g fresh weight) and TTL-4 (176 µg/g fresh weight) and the lowest values at the same time were in TTL-5 (117 µg/g fresh weight) and SM-OM-54 (133 µg/g fresh weight).

It was noted at 4.30 pm that the values recorded by the clones TTL-1, TTL-2, TTL-4, TTL-5, TTL-6 and UPASI-9 were less than those recorded at 11.30 am. At the final stage of the experiment the clones TTL-1, TTL-4, TTL-5, TTL-6, SMP-1 and SM-OM-54 exhibited an increase in the carotenoid content over the values recorded at 6.30 am. But the values recorded by TTL-1, TTL-2, TTL-4, UPASI-9, CR-6017 and SM-OM-54 at 7 pm were significantly less than those recorded at 11.30 am.

4.3.2 Chlorophyll fluorescence

At the initial stage of the experiment (i.e. at 6.30 am) the Fv/Fm values between the clones did not show much variation (Fig. 4.24). The lowest value of Fv/Fm was recorded in the clone TTL-6, and the highest in TTL-4.

Further, with increasing light intensity, the Fv/Fm values were found to decline significantly until 11.30 am irrespective of the clones ($p<0.01$). Of the 10 clones studied, 3 clones CR-6017, TTL-6 and SMP-1 exhibited a sharper decrease than the other clones in the Fv/Fm ratio by 11.30 am and the maximum reduction was shown by SMP-1 (0.481). Then all the clones showed an increase in the Fv/Fm value upto 2 pm except for the clones CR-

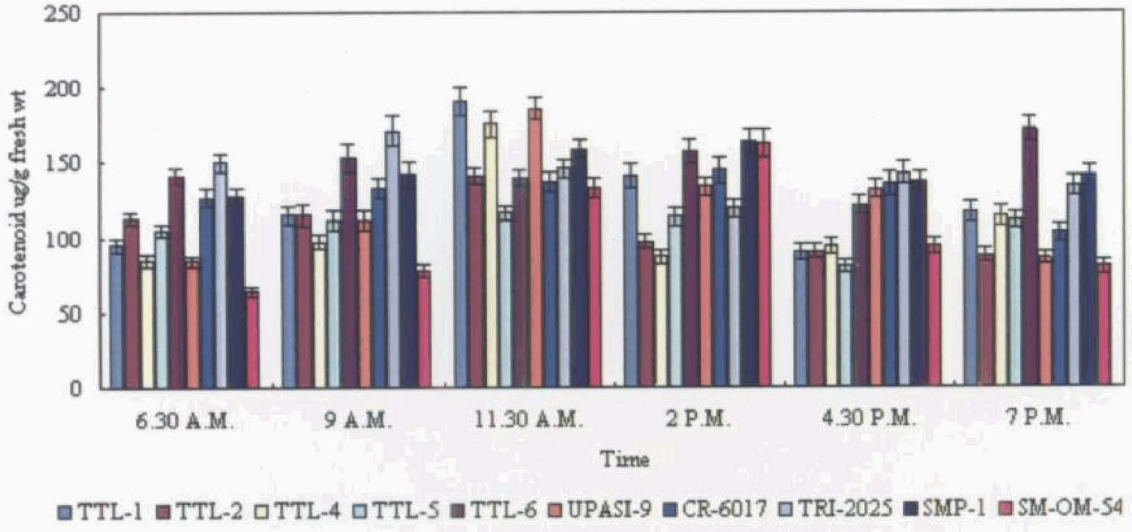

Fig. 4.23: Effect of frost on carotenoid content in various clones of tea (*C. sinensis*).

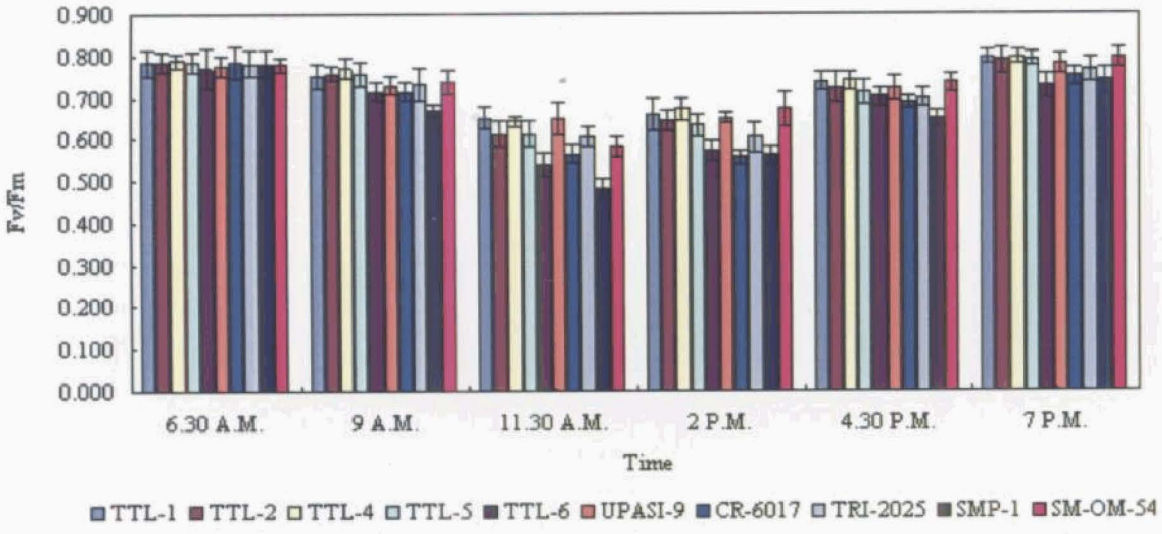

Fig. 4.24: Effect of frost on Fv/Fm ratio in various clones of tea (*C. sinensis*).

6017 and TRI-2025. The highest values at 11.30 am were recorded in the clones TTL-1 (0.651), UPASI-9 (0.649) and TTL-4 (0.644).

After 2 pm i.e. at 4.30 pm and 7 pm, the Fv/Fm values increased significantly in all the clones. At the last stage i.e. at 7 pm the Fv/Fm values obtained were higher than the initial values recorded at 6.30 am except in the clones TTL-6, CR-6017, TRI-2025 and SMP-1. These clones exhibited a reduction in the values as compared to those obtained at the initial stage.

4.3.3 Proline content

Proline content in the leaves of all the clones studied, when measured at 2.5 hour intervals from 6.30 am to 7 pm, indicated an increase at the first two stages with a maximum at 11.30 am except in the clone SM-OM-54 (Fig. 4.25). The highest proline content was recorded in the clones TTL-1 (470 µg/g fresh weight), TTL-4 (426 µg/g fresh weight) and UPASI-9 (612 µg/g fresh weight) at 11.30 am and the increase was significant ($p<0.01$).

After 2.5 hours i.e. at 2 pm a significant reduction in the proline content was noticed in all the clones, which was drastic in UPASI-9. But the clone TTL-2 exhibited a further increase during this period and only thereafter a decrease was observed.

At the end of the experiment the clones TTL-1, TTL-2, TTL-5 and SM-OM-54 exhibited lesser amount of proline content than that observed at the initial stage, but it was found increased in the other clones. The reduction in proline content was maximum in SM-OM-54 and minimum in TTL-1. The clone SM-OM-54 exhibited a rapid and significant reduction in proline content in all the subsequent stages upto 2 pm, then showed a rapid increase and finally it was found to decrease at 7 pm.

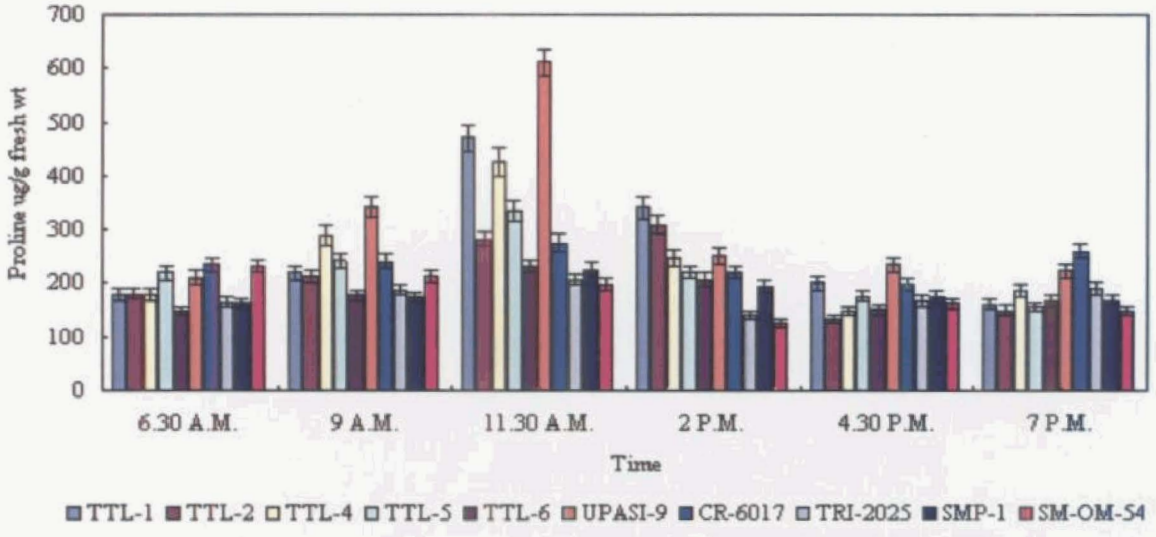

Fig. 4.25: Effect of frost on proline content in various clones of tea (*C. sinensis*).

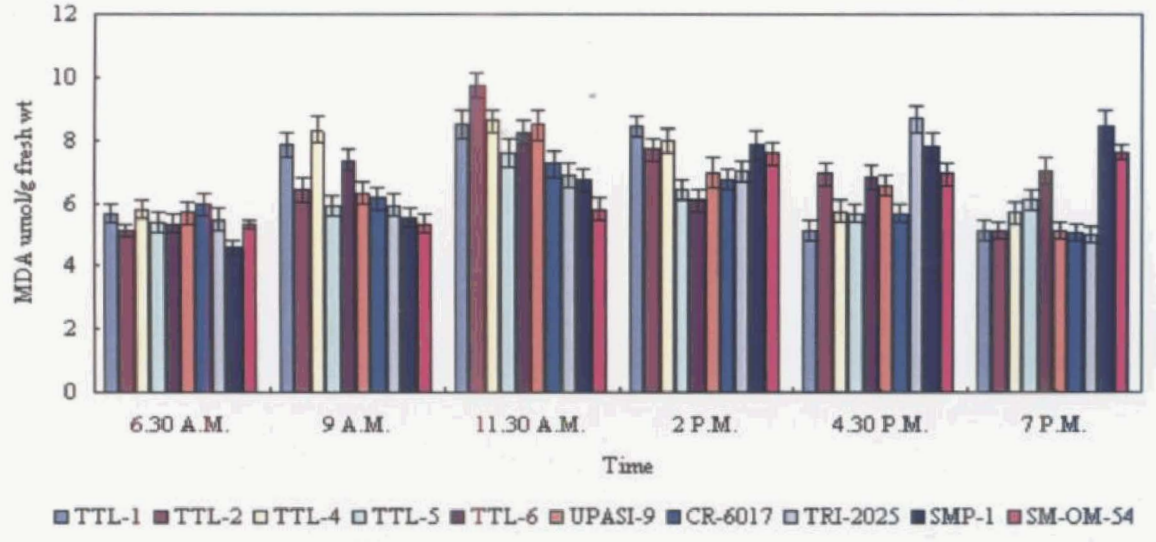

Fig. 4.26: Effect of frost on MDA content in various clones of tea (*C. sinensis*).

4.3.4 MDA content

In all the clones studied, a significant increase in the MDA content was observed from 6.30 am to 11.30 am (Fig. 4.26) ($p<0.01$). At the initial stage of the experiment all the clones possessed more or less the same MDA levels. The increase between 6.30 am and 11.30 am was maximum in TTL-2 and this clone possessed the highest amount of MDA at 11.30 am. The clones TTL-1, TTL-2, TTL-4, TTL-6 and UPASI-9 had high MDA content at 11.30 am compared to the remaining clones.

After 11.30 am, i.e. at 2 pm the clones TRI-2025, SMP-1 and SM-OM-54 showed a further increase but the other clones exhibited a rapid and significant decrease. The reduction in the MDA content continued in the next stage also. But the clones TTL-6 and TRI-2025 exhibited a significant increase during this period.

A further reduction in the MDA content was observed in the last stage of the experiment in the clones TTL-1, TTL-4, UPASI-9, CR-6017 and TRI-2025 and the other clones exhibited an increase. During the period of the experiment the highest MDA content was observed at 11.30 am in most of the clones except in TRI-2025, SMP-1 and SM-OM-54. The MDA content in the clone TRI-2025 continued to increase upto 4.30 pm and then showed a drastic reduction. In the clones SMP-1 and SM-OM-54 the maximum MDA content was observed at 2 pm and 7 pm.

At the initial stage of the experiment, the highest MDA content was observed in the clone CR-6017 and the lowest in SMP-1. At the end of the experiment all the clones (except TTL-1, TTL-4, UPASI-9, CR-6017 and TRI-2025) exhibited an increase in the MDA content over that of the initial stage; the maximum increase was obtained in SMP-1 and the minimum in TTL-2.

4.3.5 MDA Chlorophyll ratio

The lowest MDA chlorophyll ratio was recorded in the clones TTL-1, TTL-4 and UPASI-9 at 11.30 am while all the remaining clones exhibited significantly higher values as compared to these clones (Fig. 4.27). The lowest value was recorded in the clones TTL-1 and UPASI-9 and the highest in TTL-2.

4.3.6 Proline MDA content

In the present study the clones TTL-1, TTL-4 and UPASI-9 exhibited significantly high values of proline at 11.30 am compared to the remaining clones (Fig. 4.28). The highest value was recorded in the clone UPASI-9 and the lowest in SM-OM-54. In the case of MDA content, the highest value was obtained in the clone TTL-2 and the lowest in SM-OM-54 at 11.30 am.

From the results it could be observed that in the clones TTL-1, TTL-4 and UPASI-9 there was a proportionate increase in the proline content with increase in the MDA (Fig. 4.28).

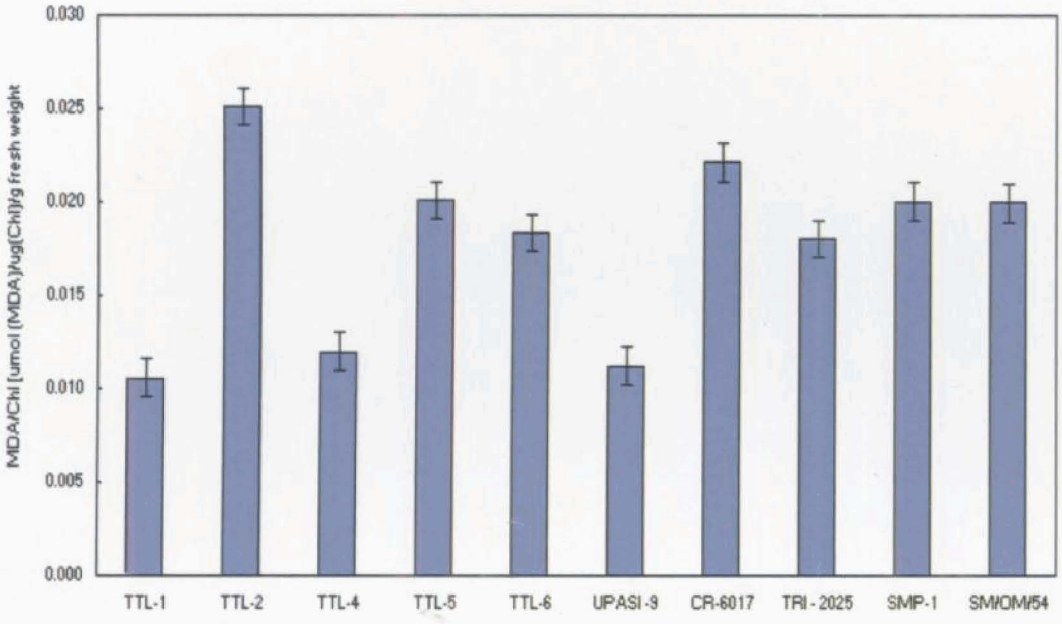

Fig. 4.27: Effect of frost on MDA chlorophyll ratio in various clones of tea (*C. sinensis*) recorded at 11.30 am.

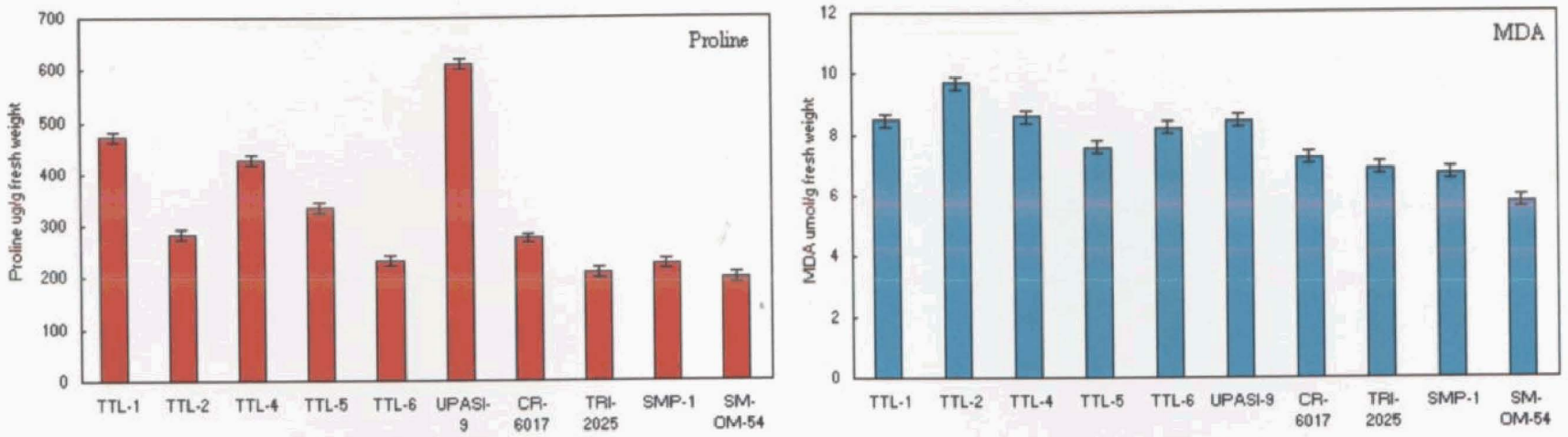

Fig. 4.28: Effect of frost on Proline and MDA content in various clones of tea (*C. sinensis*) recorded at 11.30 am of the day.

Discussion

In the present study it was observed that the N and P levels in the leaves of all the clones investigated increased gradually and attained maximum values on 25th day after application of fertilizers (Fig. 4.1 and 4.2), while the maximum level of K content was found on the 10th day and decreased thereafter (Fig. 4.3). Of the various clones studied the highest N content was obtained in the 50% fertilizer applied plants of TTL-1, TTL-2 and TTL-5. But in TTL-6 the leaf N content was found to increase with increase in concentration of fertilizer applied. Though nitrate and ammonia form of N can be taken up and metabolized by plants, nitrate is often the preferred source for crop growth, but it also depends on plant species and environmental factors (Noggle and Fritz, 1979; Salisbury and Ross, 1986; Mengel and Kirkby, 1996). Therefore the difference in the uptake of N in the different clones of tea may be due to the preference to nitrate or ammonia form of N. Further Mengel and Kirkby (1996) also reported that the nitrate content of the soil solution is of major importance in plant N nutrition. In field experiments Bartholomew (1971) found that uptake of ^{15}N fertilizer by maize was closely related to the total amount of rainfall during the experimental period.

According to Nair *et al.* (1993) the nitrate uptake is temperature sensitive and the sensitivity varies with the species. In barley plants the uptake is more at low temperature whereas in *Pennisetum* and corn it is more at high temperature. These authors also suggested that nitrate uptake is affected by plant age, root mass and genotype. The rate of uptake of nitrate (NO_3^-) is generally very high as plants require large amounts of N. Nitrate is the major source of N available to plants and before it can be metabolized it must be reduced to nitrite (Mengel and Kirkby, 1996). According to these authors response to N depends on soil conditions, the particular crop species and the plant nutrient supply in general. They further added that the response of plants to N also depends on how well the crop is supplied with other nutrients.

The clones TTL-1, TTL-2 and TTL-5 exhibited the maximum values of leaf P in the 50% fertilizer applied plants. In TTL-6, leaf P content increased with increase in concentration of fertilizer. The difference in the P uptake between the clones may be due to differences between the plant species as reported by Mengel and Kirkby (1996). The authors opined that the capability of P uptake differs between plant species and may even differ between cultivars of the same species. According to these authors, placement of P fertilizer ensures that a high concentration of fertilizer comes in contact with a more limited volume and the phosphate concentration of the soil solution is thus higher in the placed zone. Further these authors suggested that as the mobility of phosphate in the soil profile is comparatively low, the absorption of P depends much on the root growth and the root morphology of the crop. As plant roots push their way through the soil they come in contact with the phosphate of the soil solution. If the roots have a high demand for P, phosphate is absorbed by the roots at a high rate and the soil solution in the direct root vicinity is depleted of phosphate. The difference in

accumulation of P in TTL-6 and TTL-1, TTL-2 and TTL-5 may also be due to the difference in root growth and root morphology of these clones.

Barber and Thomas (1972) reported that the capability of plants to take up P is genetically controlled. Therefore the clone TTL-6 may be capable of absorbing more P from the soil with increase in P concentration, while TTL-1, TTL-2 and TTL-5 need only a minimum quantity (50%) to attain maximum absorption levels, which might be regulated by the genetic nature of these clones. In addition to the phosphate concentration of the soil solution, the phosphate buffer power of the soil plays a crucial role in determining the rate of P supply to crops (Nair and Mengel, 1984). According to these authors, the optimum soil solution phosphate concentrations differ for individual crops, cropping systems and particular cultivation sites.

Plants are capable of absorbing phosphate from solutions of very low phosphate concentrations (Loneragan and Asher, 1967). According to Mengel and Kirkby (1996), the uptake of phosphate is an active process and it is taken up by plant cells against a very steep concentration gradient. The authors further stated that the ATPase activity is expected to have an impact on phosphate uptake, which is in accordance with the close relationship observed between root respiration and phosphate uptake and relates to the provision of respiratory ATP to the ATPase. According to Noggle and Fritz (1979), phosphate is assimilated in plant cells mostly by incorporation into ATP. These authors stated that some of the phosphate absorbed by the roots of plants is taken upward in the transpiration stream to the leaves and therefore it can be expected that phosphate is assimilated in leaves as well as in roots.

In the case of leaf K content, TTL-6 maintained the same trend as N and P accumulation, i.e. there was an increase in leaf K content with

increase in concentration of fertilizer. This may be due to the particular nature of the rooting system in TTL-6 as proposed by Mengel and Kirkby (1996). According to the authors, when legumes and grasses were growing together, K uptake was considerably higher in grasses and low level K conditions can lead to the disappearance of legumes. These authors opined that K retention in the cell depends mainly on the negative potential of the cell and if the negative potential is lowered, the retention capacity for K is also decreased. They further stated that the rapid uptake of K^+ is because of the relatively high permeability of plant membranes to K^+, which may be due to the ionophores located in the membrane, which enable facilitated diffusion. Therefore, based on the above factors, it can be concluded that the clone TTL-6 would have taken up more K and retained or accumulated it in the leaf with increase in the concentration of fertilizer.

There are reports that K is of utmost importance for the maintenance of water status of the plants. Lauchli and Pfluger (1978) reported that the uptake of water in cells and tissues is frequently the consequence of active K^+ uptake. The turgor of young leaf cells of *Phaseolus vulgaris* was dependent on the K^+ content (Mengel and Arneke, 1982). In the low K^+ treatment the turgor was significantly lower than that of high K^+ treatment and under this condition growth rate, cell size and water content of the tissue were reduced. Supporting this view in the present study the shoot weight of TTL-6 increased with concentration of K applied to the plants.

Mengel and Kirkby (1996) reported that the response to K depends to a considerable extent on the level of N nutrition. Similarly, in TTL-6 leaf K content increased with increase in N content. Maximum accumulation of K and N in leaves of plants of TTL-5 applied with 50%

fertilizer also showed that the accumulation of both K and N are interdependent. This indicates that TTL-5 requires only 50% fertilizer to accumulate maximum K and N. This may be due to an active K^+ uptake process as proposed by Cheeseman and Hanson (1979). According to these authors, an active uptake process is supposed to play a role at low K concentrations in corn plants.

The relationship between leaf nutrient content and leaf photosynthetic capacity has been reported by many workers (Barker, 1979; Sobhana *et al.*, 1996). The occurrence of increased accumulation of N, P and K in leaves of clone TTL-6 with increase in concentration of fertilizer applied along with increased photosynthesis is in agreement with the results obtained by DeJong and Doyle (1985). These authors suggested that in peach leaves the leaf N content is highly correlated with CO_2 assimilation rate. De Costa *et al.* (2000) opined that the rate and efficiency of photosynthesis of tea are highly dependent on leaf N. In the present study also the highest N content was noticed in TTL-6 and in this clone there was an increase in the rate of photosynthesis with increase in fertilizer concentration.

In TTL-5 the highest photosynthetic rate was observed in the 50% fertilizer level, followed by the 100% and the lowest in the 150% fertilizer concentration. This showed that the photosynthetic rate is directly proportional to the level of P in the leaf and according to Sivak and Walker (1986) the P level is well documented to promote photosynthesis. Similarly, the clone TTL-5 when treated with 50% fertilizer exhibited high photosynthetic rate but it was less in the 150% fertilizer applied plants. In the clone TTL-6 a reverse of this i.e. an increase in photosynthetic rate with an increase in concentration of fertilizer was observed.

In the clone TTL-6 applied with various concentrations of fertilizers, highest K content was noticed in 150% fertilizer applied plants. But the clone TTL-5 applied with the same concentration of fertilizer showed lower K content in the leaves. Samarappuli (1992) suggested that K sufficient rubber plants close stomata and reduce transpiration more rapidly than K deficient plants. It could be assumed that in TTL-6 there was no deficiency of K, which led to an efficient functioning of the stomata and thereby improved photosynthesis.

On the 25th day after fertilizer application, shoot weight was found to be maximum in all the clones of tea studied (Fig. 4.4). Samiullah and Khan (2003) reported that application of nutrients to chickpea plants enhanced the branch number and leaf area index through its role in increasing cell size. According to the authors, the increased leaf area index resulted in accumulation of photosynthates and enhanced shoot dry matter accumulation. The clones TTL-1, TTL-2 and TTL-5 exhibited maximum shoot weight in 50% fertilizer applied plants. This result showed a correlation with that of leaf N and P contents in these clones. Sivasankar *et al.* (1993) opined that increased N supply increased the leaf area and photosynthetic productivity of a crop canopy, which ultimately led to dry matter production. According to these authors, development of adequate leaf area index is essential for a crop canopy with regard to light interception, utilization, CO_2 fixation and dry matter production. The authors also stated that inadequate N reduced plant growth by restricting leaf area and dry matter production as reported by many authors (Singh and Anderson, 1973; Edwards and Barber, 1976; Dutta and Sharma, 2000; Mandal *et al.*, 2003). Therefore, the increased shoot weight found in the 50% fertilizer applied plants of TTL-1, TTL-2 and TTL-5 may be due to the increased N and high photosynthate accumulation.

In the clone TTL-6 there was an increase in shoot weight with increase in the concentration of NPK fertilizer and increase in the leaf NPK content

(Fig. 4.4). Sivasankar *et al.* (1993) suggested that one of the key roles of N in producing high crop yields is through the establishment of fully grown crop canopy. The authors also suggested that N fertilization led to an increased leaf size and area in crops like maize, sorghum, rice and wheat. Further in support of this, it has been stated by Mengel and Kirkby (1996) that the level of N nutrition required for optimum growth during the vegetative period must also be balanced by the presence of other plant nutrients in adequate amounts. The synthesis of organic N compounds depends on a number of inorganic ions including Mg^{2+} for the formation of chlorophyll and phosphate for the synthesis of nucleic acids. According to these authors, the uptake of nitrate and especially its assimilation into protein are considerably influenced by plant K status. Potassium is important for growth and elongation probably in its function as an osmoticum and may react synergistically with indole acetic acid. Therefore the high shoot weight found in the clone TTL-6 was probably not only due to the increased N content but also due to the increased P and K content found in this clone.

In the present study, the increase of single shoot weight in all the fertilizer applied clones may be due to the increased accumulation of N, P or K at various levels and this may have enhanced the turgidity of the young leaf tissues and ultimately leads to increased shoot weight of the tea clones studied. According to Mengel and Arneke (1982), the turgidity of young leaf cells of *Phaseolus vulgaris* was regulated by the K^+ ion in the soil as well as in the leaves.

All the clones of tea plants subjected to moisture stress conditions exhibited the most reduction in shoot weight on the 100^{th} day of non-irrigation (Fig. 4.9). The highest shoot weight among the non-irrigated plants was observed in TTL-1, TTL-6 and UPASI-2 as compared to the remaining clones. Similar pattern of reduction in growth due to water

stress has been reported in various plant species (Hsiao, 1973; Salisbury and Ross, 1986; Mengel and Kirkby, 1996; Thomas *et al.*, 2004). Salisbury and Ross (1986) opined that plant growth is primarily caused by an uptake of water, water pressure drives growth by forcing the cell wall and membranes to expand. The rate of water movement into a cell is governed by the water potential gradient and the permeability of the membrane to water. According to Mengel and Kirkby (1996), when the water availability in the soil was poor and transpiration was high, a negative water balance resulted in which case the loss of water by the plant is greater than its uptake. If the water loss was excessive, water stress inhibited growth. These authors suggested that during water stress the turgor pressure in the plant cell falls and cell expansion is decreased. Thus there is a close correlation between decrease in cell size and the degree of water stress in plant tissues.

According to Kalpana *et al.* (2003), the plant productivity to a large extent is determined by the efficiency of photosynthesis under a given environmental condition and available resources. The comparatively higher shoot weight found in TTL-1, TTL-6 and UPASI-2 subjected to drought stress may be due to the better photosynthetic rates in these clones as compared to TTL-2, TTL-4, TTL-5 and UPASI-3.

Water stress influences growth by affecting cell elongation directly and indirectly by influencing mineral uptake, allocation and photosynthesis (Hsiao, 1973) which may result in reduced fresh weight. In the present investigation a decrease in shoot weight occurred in all the clones throughout the period of non-irrigation. Among the clones, TTL-1, TTL-6 and UPASI-2 showed a comparatively higher shoot weight than that of the other clones. This indicates a tendency of these clones to withstand drought conditions better than TTL-2, TTL-4, TTL-5 and

UPASI-3. In the irrigated plants of all the clones there was no significant reduction in the shoot weight throughout the period of study. The reason for this may be because there was no moisture stress and thus the cell elongation was not adversely affected as reported by Hsiao (1973).

The drought stress may affect water uptake, cell expansion and photosynthesis, which may ultimately result in reduced shoot weight. However, the clones TTL-1, TTL-6 and UPASI-2 exhibited high shoot weight which indicates that these clones were less affected by drought, and comparatively higher water uptake, cell expansion and photosynthesis occurred in them than in TTL-2, TTL-4, TTL-5 and UPASI-3.

The non-irrigated plants of the tea clones after re-irrigation showed a speedy recovery by the 14^{th} day of re-irrigation. Salisbury and Ross (1986) opined that plants under stress usually recover if irrigated when stresses are –1.0 to –2.0 MPa, which indicates that the biological strain was elastic or somewhat elastic. The re-irrigation may lift the strain and thus try to function normally. Of the various clones, TTL-1, TTL-6 and UPASI-2 showed signs of recovery within seven days after re-irrigation and this indicates that the strain caused by drought is somewhat more elastic in these clones than in the other clones. So these clones may have quick recovery potential and can be considered as drought-tolerant.

The soil moisture status studies showed a decrease in the soil moisture % in the non-irrigated plots with increase in the period of drought (Fig. 4.10). Whereas in the irrigated plots the soil moisture level remained constant as there was no shortage of water. On the 20^{th} and 60^{th} day there was significant decrease in the soil moisture %. in the non-irrigated plots and on the 100^{th} day the value dropped below 8% which was considered as the wilting coefficient as suggested by Rajasekar *et al.* (1988). At this stage the clones UPASI-3 and TTL-2 had shown the signs

of wilting. This may be because when soil started to dry out during non-irrigated condition, the water availability declines finally reaching a point at which the water is so strongly held by adsorption that plant roots are not able to utilize it and the plants growing in the soil begin to wilt (Mengel and Kirkby, 1996). According to these authors, if the wilting is temporary the plants are able to recover when water is supplied to the soil, whereas when the stage of permanent wilting has been reached wilting is irreversible and the plant dies. In the present study also when the clones under non-irrigation were irrigated a speedy recovery occurred. This indicates that the non-irrigation for 100 days may not have seriously affected the soil water status and thus permanent wilting did not occur.

The leaf water potential of all the non-irrigated clones of tea (*C. sinensis*) was found to decrease from the zero day to the 100^{th} day (Fig. 4.11). Identical results were obtained in different wheat genotypes grown under drought conditions (Sharma *et al.*, 2003). They concluded that the high yielding genotypes should maintain cooler canopy, higher internal water status and yield attributing characters under the depleting soil moisture condition. Sivakumar and Shaw (1978) reported that the decreased soil water potential also decreased the leaf water potential of soybeans (*Glycine max*). In the present study the irrigated plants exhibited not much variation in leaf water potential and they maintained more or less constant values throughout the experimental period.

According to Manivel and Handique (1983) water potential is directly proportional to the turgidity of a cell and hence is connected with the water deficit of a plant. Farquhar and Sharkey (1982) reported that the changes in stomatal conductance cause changes in leaf water potential by changing the transpiration rate. When the water availability in the soil is poor and transpiration is high, a negative water balance results i.e. the loss of water by

the plant is greater than its uptake. If the loss becomes excessive, the plants at first wilt and water stress inhibits growth (Mengel and Kirkby, 1996).

The leaf water potential in tea bushes (Handique and Manivel, 1986) and Kranti variety of rice (Kumar and Kujur, 2003) is considered as an index for whole plant water status and maintenance of high leaf water potential is considered to be associated with dehydration mechanism and the ability to withstand drought. According to those authors, maintenance of relative turgor is essential for all growth processes, which is affected by moisture stress. These authors stated that water potential is a measure of the plant water status that imparts relative turgor. The drought tolerant clones exhibited higher shoot water potential than the drought susceptible clones. Similar results were obtained in some other clones of tea (Singh and Handique, 1993) and according to these authors, the genotypes of tea susceptible to drought exhibited lower leaf water potential than the resistant ones. In agreement with these views, the clones TTL-1, TTL-6 and UPASI-2 exhibited higher and TTL-2, TTL-4, TTL-5 and UPASI-3 lower leaf water potential values (Fig. 4.11), and can be considered as drought tolerant and susceptible clones respectively.

As the photosynthetic rate is very much dependent on the internal water status of the plant, it is also affected by the reduced leaf water potential. Low leaf water potential reduced photosynthesis through its influence on stomata. Under field conditions of low shoot water potential and rise in temperature, it could lower the rate of photosynthesis by lowering both mesophyll and stomatal conductances (Kumar and Tieszen, 1980). In the present investigation the clones with comparatively high leaf water potential, i.e., TTL-1, TTL-6 and UPASI-2 exhibited higher photosynthetic rate than the remaining clones. This indicates that these clones are more resistant to drought than the other clones.

In the non-irrigated plants of all the clones there was a significant reduction in the relative water content on the 100th day of water stress compared to the control plants (Table 4.1). The clones TTL-1, TTL-6 and UPASI-2 exhibited least reduction in the relative water content as compared to the clones TTL-2, TTL-4, TTL-5 and UPASI-3. In studies carried out in coffee cultivars under soil moisture stress conditions, Saraswathi *et al.* (1996) noticed a significant decrease in relative water content in the susceptible cultivar (S.274), while such a kind of decrease did not occur in the tolerant cultivar (Sln. 7.3). Similarly, higher relative water content in drought tolerant cultivars of tea has been reported by Chakraborty *et al.* (2002). These studies revealed that the reduction of relative water content in drought tolerant plants was comparatively lesser than that of the drought susceptible ones. In support of these views, in the present study also a meagre reduction of relative water content was noticed in tea clones such as TTL-1, TTL-6 and UPASI-2 and hence these clones may be considered to be drought tolerant as compared to TTL-2, TTL-4, TTL-5 and UPASI-3.

On providing irrigation to the plants, which were not irrigated, the relative water content showed a significant increase on the 14th day. The clones TTL-1, TTL-6 and UPASI-2 showed the highest relative water content on the 14th day after re-irrigation and the values were nearest to that of the initial day. Similar results were obtained by Patil *et al.* (1984) in maize genotypes and they found that though the plants wilted under moisture stress, after re-irrigation the plants could recover, which was evident from the relative water content of the plants. According to these authors, the recovery was possible because of the leaf sheath succulence as revealed by the higher relative water content in it.

According to Salisbury and Ross (1986), cellular growth is most sensitive to water stress; decreasing the external water potential by only –0.1 MPa or less results in a decrease in cellular growth. Therefore the response of cellular growth to water stress appears to reduce the shoot and root growth. According to these authors, this is usually followed closely by a reduction in cell wall synthesis. As the clones TTL-1, TTL-6 and UPASI-2 exhibited the least reduction in relative water content and shoot weight on the 100^{th} day of withholding irrigation, it could be assumed that in these clones the cellular growth was not as adversely affected as in the clones TTL-2, TTL-4, TTL-5 and UPASI-3.

The shoot weight of tea clones showed a close relationship with the relative water content. When the relative water content decreased, the shoot weight also decreased considerably and vice versa. The clones TTL-1, TTL-6 and UPASI-2 showed comparatively higher relative water content and shoot weight values and therefore these clones can be considered as more drought tolerant than the rest.

The chlorophyll a+b and carotenoid content of all the clones treated with various concentrations of fertilizers showed an increase on the 25^{th} day after fertilizer application as compared to zero day (Fig. 4.5 and 4.6). But in the clone TTL-6 these pigments increased with increase in concentration of fertilizer applied and the N content in it was also high. Mader and Volfova (1984) reported that the level of N in leaf of barley affected the size and morphology of chloroplasts. Therefore the increase in the pigments of TTL-6 with increased N application might be due to the increased leaf N content. Sivasankar *et al.* (1993) reported that approximately 75% of the N in a plant leaf with C_3 photosynthesis is invested in chloroplasts and most of it is used in photosynthesis. The leaf N plays a key role in determining a crop's photosynthetic capacity and the major effect of N application on crop

photosynthesis is through increased light interception. The authors mentioned that N being an essential component of proteins, it can also have a direct effect on the rate of photosynthesis per unit leaf area.

A similar trend as seen in the clone TTL-6 was not noticed in TTL-1, TTL-2 and TTL-5 (Fig. 4.5 and 4.6). In these clones though the fertilizer concentration was increased from 50 to 150%, no corresponding increase in the chlorophyll and carotenoid pigment values was observed. This may be due to the lower absorptive potential of N by these clones at higher fertilizer concentrations. Lavon *et al.* (1999) reported that N is a major constituent of numerous chloroplast components and a large amount of reduced N is stored in the RuBPcase protein and in the light-harvesting chlorophyll protein complexes. The RuBPcase which forms nearly 50% of the soluble protein of the leaf may account for one third to one half of the total N in that organ. According to these authors, N is also a constituent of the chlorophyll molecule and the balance between RuBPcase and chlorophyll may be important for maintaining efficient photosynthesis. In the present study high chlorophyll and carotenoid content was noticed in the 50% N fertilizer applied plants of the clones TTL-1, TTL-2 and TTL-5 with a concomitant increase in leaf N content. As already discussed, the inability of these clones when treated with 100 and 150% fertilizers to absorb and accumulate N, may be responsible for the low levels of leaf chlorophyll and carotenoid content.

Tea clones with high P accumulation showed increased chlorophyll and carotenoid contents (Fig. 4.5 and 4.6). Shubhra *et al.* (2003) observed that P improved the chlorophyll content in control as well as water stressed plants of clusterbean (*Cyamopsis tetragonoloba*). In support of this view, higher chlorophyll a+b and carotenoid pigments were noticed at higher leaf P concentrations and was maximum in TTL-6, which may be due to the increased accumulation of P in them.

When symptoms of elemental deficiency or toxicity are evident, structure of the chloroplasts is usually altered, which affects photosynthesis (Sobhana *et al.*, 1996). This proves the relationship between elemental concentration and the chloroplasts. The maximum chlorophyll a+b and carotenoid values on the 25th day after application of NPK fertilizers (Fig. 4.5 and 4.6) in tea clones subjected to the study are in agreement with the views of Sobhana *et al.* (1996). According to Pandey (2002), the chlorophyll and carotenoids are structural and functional components of the chloroplasts.

As a result of the increased chlorophyll content there were higher Fv/Fm values in the clones (Fig. 4.7). It has been reported by Pandey (2002) that an increase in the chlorophyll content of the leaves in tea enhances the photosynthetic rate, which in turn increases the biomass production. Similar results were obtained in *Hordeum vulgare* (Sharma and Tripathi, 1994) and in banana, guava, stargoose berry and chillies (Balakrishnan *et al.*, 2000). In the present study also a high photosynthetic rate was observed in TTL-6 in which high leaf chlorophyll content was recorded.

In all the non-irrigated clones of tea (*C. sinensis*) the chlorophyll a+b and carotenoid content was found reduced with increase in the period of drought (Fig. 4.12 and 4.13). The irrigated plants of the same clones maintained more or less same values throughout the period of the study. The maximum reduction of total chlorophyll and carotenoid content was seen on the 100th day of moisture stress, and from the 0 day to the 100th day the decline was progressive. Shubhra *et al.* (2003) studied the effect of water stress on total chlorophyll content in clusterbean, and found that the total chlorophyll content of the leaf declined under water stress. According to the authors, it may be due to decreased synthesis and

increased degradation of chlorophyll in leaves under water stress. Similar results were obtained in tea leaves by Rajasekar *et al.* (1988) and the authors opined that reduced ability to form proto-chlorophyll was considered to be responsible for the inhibition of development of chlorophyll under water stress.

The clones TTL-1, TTL-6 and UPASI-2 had the maximum values of chlorophyll a+b and carotenoid content on 100^{th} day of non-irrigation while the remaining clones showed lesser values and the lowest value was recorded in the clones TTL-2 and UPASI-3 (Fig. 4.12 and 4.13). It has been reported by Nair *et al.* (2004) in *Hevea brasiliensis* that as the level of tissue moisture deficit increased, the chlorophyll content decreased. According to the authors, the decrease in chlorophyll content may be due to degradation of chlorophyll, which is more prominent in drought susceptible clones than the drought tolerant clones. In the present investigation, TTL-1, TTL-6 and UPASI-2 exhibited comparatively higher values of chlorophyll pigments than the remaining clones and this could be due to the non/or less degradation of chlorophyll pigments during drought, which can be considered as drought tolerant nature of these clones compared to the other clones.

More or less identical results were obtained in maize (Alberte *et al.*, 1977) and Swiss chard (Poljakoff-Mayber, 1981). According to Poljakoff-Mayber (1981) the reduced Hill reaction during water stress was restored on rehydration almost to the level of continuously turgid leaves. Further the author noticed that 24 hours after re-watering the chloroplast structure returned to normal.

The clones TTL-1, TTL-6 and UPASI-2 exhibited a rise in their chlorophyll a+b and carotenoid content compared to the other clones immediately after re-irrigation of plants grown under drought (Fig. 4.12 and 4.13). In the present study the faster recovery of the clones TTL-1, TTL-6

and UPASI-2 may also be due to normalisation of chloroplast structure on re-irrigation, which was exhibited by increased chlorophyll content. So the ability for less degradation of chlorophyll and immediate recovery on re-watering showed the drought tolerant nature of these clones as compared to the rest.

Under low temperature conditions an increase in carotenoid content was observed in the clones TTL-1, TTL-4 and UPASI-9 at 11.30 am as compared to the remaining clones (Fig. 4.23). Similar results were obtained by Siefermann-Hams (1987), Young *et al.* (1997) and Puthur (2000) and according to them this may be an indication of a nonenzymatic detoxification mechanism of toxic oxygen species. According to Knox and Dodge (1985) and Arora *et al.* (2002), the carotenoids, which are essential components of thylakoid membranes, quench singlet oxygen and also protect by absorbing excess excitation energy from chlorophyll by direct transfer. Functioning of chloroplasts is considered to be first affected when plants are exposed to stress conditions (Puthur *et al.*, 1996). These authors suggested that chloroplast is the major site for generation of toxic oxygen species and synthesis of proline under stress. They also opined that the increased level of proline protects the function of chloroplast under stress. In the present study the proline content increased when the tea clones were exposed to low temperature conditions (Fig. 4.25). A more pronounced increase was seen in TTL-1, TTL-4 and UPASI-9 as compared to the remaining clones, and the increased level of proline may protect the function of chloroplasts under low temperature stress.

Puthur (2000) reported that in *Sesbania sesban* plant embryos exposed to light of high photon flux density, there was significant increase in the production of MDA. In general MDA is widely considered to be an index of lipid peroxidation resulting due to excessive generation

of toxic oxygen species. In support of this view, in the present study an increased MDA content and a proportionate increase in proline content also occurred at 11.30 am in the clones TTL-1, TTL-4 and UPASI-9 (Fig. 4.26 and Fig. 4.25). But in the remaining clones, though there was an increase in MDA content at 11.30 am, the carotenoid and proline content was comparatively low and thus failed to protect chloroplasts from toxic oxygen species; hence the increase of MDA was not proportionate to that of proline and carotenoid.

From the results it is evident that the plant system may be favouring the over production of proline and carotenoids only when the situation warrants and the level of proline and carotenoids decrease along with a decrease in the level of reactive oxygen species (Fig. 4.25 and 4.23). This was seen only in the case of TTL-1, TTL-4 and UPASI-9 and therefore it could be concluded that they are more low temperature or frost tolerant as against the other clones. The frost tolerant nature of the clone UPASI-9 was reported by various authors (Bisht *et al.*, 1996; Vyas *et al.*, 1998). In the present study the frost tolerant nature of UPASI-9 was once again confirmed and it was noticed that the same level of tolerance was shown by the clones TTL-1 and TTL-6 compared to the remaining clones subjected to the study.

The chlorophyll fluorescence (Fv/Fm) studies showed that the values increased from the 0 day to the 25th day after application of various concentrations of NPK fertilizers to different clones of tea (Fig. 4.7). The increase was significant and maximum in 50% fertilizer applied plants of TTL-1, TTL-2 and TTL-5 but was not significant in higher concentrations (i.e. 100 and 150%). But the clone TTL-6 showed an increased rate of fluorescence with increase in the concentration of fertilizer application. Selvendran and Selvendran (1973) as well as the present study found that the

application of N fertilizer increased the accumulation of N content in tea leaves.

De Costa *et al.* (2000) opined that N accumulation in tea leaves is closely associated with all stages of photosynthetic process. Further, the authors noted an increased photochemical efficiency due to the accumulation of N. It is a well established fact that Fv/Fm is a quantitative measure of the photochemical efficiency (Kitajiima and Butler, 1975). In the present study a correlation was obtained between the quantity of N accumulation and the Fv/Fm ratio. The clones TTL-1, TTL-2 and TTL-5 accumulated more N in 50% fertilizer applied plants which also showed high Fv/Fm values. This showed that these clones need only 50% of UPASI recommended concentration of fertilizer to acquire the saturated condition and hence high Fv/Fm ratio.

In the clones TTL-1, TTL-2 and TTL-5 the Fv/Fm values were less in 100 and 150% NPK applied plants compared to 50%. It is evident from the results of N and P accumulation that these clones exhibited minimum level of leaf N and P in 100 and 150% fertilizer applied plants. Jacob and Lawlor (1993) and Jacob (1995) suggested that inorganic P deficiency causes photoinhibition of PS II; and the lesser Fv/Fm values may be due to photoinhibiton. According to Jacob (1995), the efficiency of excitation energy captured by open PS II reaction centers and quantum yield of PS II photochemistry were decreased by phosphate deficiency. It was also found that phosphate deficiency decreased the rate of PS II photochemistry as well as the probability of excitation energy transfer from PS II antenna to PS II reaction center.

Fluorescence is a measure of the activity of PS II. Phosphorus deficiency causes photoinhibition of PS II, which ultimately resulted in an impaired Fv/Fm ratio (Jacob and Lawlor, 1993). But in the present study, the

application of various concentrations of fertilizers increased the Fv/Fm ratio of tea clones. The Fv/Fm value of the clones was found to increase with increase in accumulation of P.

Drought is one of the major constraints for tea cultivation and though initially reversible, in its severest form it can lead to cell death (Jeyaramraja *et al.*, 2003) and according to Jacob (1998) the ratio of Fv to Fm is an important parameter of the physiological state of the leaves and severe environmental stresses decrease this ratio. In the present investigation, the Fv/Fm ratio of various clones of tea (*C. sinensis*) at the initial stage of the experiment did not show considerable variation between the clones (Fig. 4.14). But the values decreased considerably after 100 days of non-irrigation as compared to that of the respective control plants. After 100 days of withholding irrigation the maximum reduction in Fv/Fm ratio was observed in UPASI-3 and the minimum in TTL-1. Similar results were obtained in certain other tea clones such as ATK-1, TRF-1 and UPASI-17, subjected to different periods of water stress (Jeyaramraja *et al.*, 2003) and revealed that the reduction in the Fv/Fm ratio with increased moisture stress might be due to the loss of primary photochemical efficiency of stressed leaves. The authors also noticed a higher Fv/Fm ratio in drought tolerant cultivars (ATK-1 and TRF-1) and lower Fv/Fm ratio in the drought susceptible clone (UPASI-17). According to these authors, the reduction of Fv/Fm ratio became evident after 7 days of drought and it may be due to drought induced decrease in variable fluorescence resulting from reduced maximal fluorescence.

Studies on some physiological changes in various tea (*C. sinensis*) clones under water stress were carried out by Rajasekar *et al.* (1988). From the results, they came to a conclusion that the clone UPASI-2 was drought tolerant, while the clone UPASI-3 was drought susceptible. But, in the

present investigation, the fluorescence studies showed that the clones TTL-1 and TTL-6 exhibited higher Fv/Fm values (i.e., 0.643 and 0.614 respectively) than that of UPASI-2 (0.604) on the 100th day of non-irrigation. This result indicates that the two clones TTL-1 and TTL-6 may have more drought tolerance potential than UPASI-2 and can be considered as drought tolerant along with UPASI-2. This is in agreement with the views of Jeyaramraja *et al.* (2003) and according to them the Fv/Fm values are higher in the drought tolerant tea clones and lower in the susceptible clones. The authors further reported that the fluorescence ratio came down to 0.590 in the susceptible clone UPASI-17. In the present study also, the Fv/Fm values were very low in the clones TTL-2, TTL-4, TTL-5 and UPASI-3, which was identical to UPASI-17 as reported by Jeyaramraja *et al.* (2003). So the clones TTL-2, TTL-4, TTL-5 and UPASI-3, in the present study, can be considered as drought susceptible.

The inhibitory effect of low temperature stress on leaf photosynthesis is well documented (Powles, 1984; Huner *et al.*, 1993; Alam and Jacob, 2002). Vyas *et al.* (1998) noticed that with the onset of frost during extreme winters, the Fv/Fm values decreased for tea clones kept in open conditions. Similar observations were made by Adams and Perkins (1993) in tree species of *Picea* with the onset of winter. It is well known that the ratio of Fv/Fm is a quantitative measure of photochemical efficiency (Kitajiima and Butler, 1975) or optimal quantum yield of PS II (Schreiber and Bilger, 1993; Bolhar-Nordenkampf and Oquist, 1993). The studies carried out by Alam and Jacob (2002) revealed that the maximum potential yield of PS II in the dark adapted state and effective quantum yield at a given photosynthetic photon flux density (PPFD) were markedly decreased in low temperature sensitive species when they were exposed to low temperature stress.

In the present study during low temperature conditions it was observed that the Fv/Fm values at 6.30 am, did not show much variation between the clones and the values were in the range of 0.770 to 0.787 (Fig. 4.24). With increase in light intensity at 11.30 am and 2 pm, all the clones exhibited a decline in the Fv/Fm values. According to Fryer *et al.* (1998), strong irradiance inhibits photosynthesis in green leaves experiencing an abiotic stress such as chilling. Of the 10 clones investigated in the present study, the clones TTL-1, TTL-4 and UPASI-9 showed lesser reduction in the Fv/Fm ratio (0.651, 0.644 and 0.649 respectively) at 11.30 am compared to the remaining clones. All the other clones exhibited a considerable reduction in the Fv/Fm ratio which was around 0.600 and below at 11.30 am, which indicates that these clones can be considered as frost susceptible and the rest as tolerant ones as discussed earlier in accordance with the views of Jeyaramraja *et al.* (2003).

Observations at 4.30 pm and 7.00 pm showed that the changes in the values of Fv/Fm ratio developed a reversing trend and tendency to recover from low temperature and high light intensity stress condition (Fig. 4.24). At 7.00 pm the Fv/Fm values of all the clones were almost identical to that of the initial values recorded at 6.30 a.m. It is a well-established fact that photoinhibition is reversible in most cases and the photosynthetic rates are restored when the plant is exposed to less intense irradiance. The ultra structural studies carried out in other plant species also revealed that no permanent damage occurred to the thylakoid membranes during photoinhibition, except where prolonged stress occurs (Hall and Rao, 1999).

According to Vyas *et al.* (1998), the freezing temperature adversely affected the photosynthetic apparatus in tea plants and resulted in a reduction

in the Fv/Fm ratio. Mohammad *et al.* (1995) and Fernandez *et al.* (1997) reported that chlorophyll fluorescence is a reflection of PS II activity and any reduction in chlorophyll fluorescence indicates the reduction of photochemical activity of PS II. The reduction of chlorophyll fluorescence in frost affected tea plants (Fig. 4.24) in the present study may also be due to the reduced photochemical efficiency of PS II. So, the observations in the present investigation suggested that at the initial stage of the experiment the photosynthetic mechanism in all the clones was not adversely affected, but was affected severely at the later stages of the experiment by the cumulative effects of low temperature and high light intensity.

The Fv/Fm values of the clones TTL-1, TTL-4 and UPASI-9 at 11.30 am showed that these clones were not affected to the same extent as the other clones (Fig. 4.24). This indicates that the photochemical efficiency of leaves of these clones was not affected considerably when subjected to frost. So these clones can be considered as low temperature tolerant. A similar observation was made by Alam and Jacob (2002) in *Hevea* and Napier grass. These authors suggested that the maximum potential quantum yield of PS II (dark adapted Fv/Fm) showed a decrease in response to low temperature stress in *Hevea* and Napier grass, which were susceptible to low temperature stress.

Bjorkman and Demmig (1987) reported that the Fv/Fm ratio of a normal functioning photosynthetic apparatus is 0.832 for a wide variety of plant species. In the various clones of tea studied the Fv/Fm ratio of the control plants ranged from 0.752 to 0.813, which remained more or less unchanged throughout the period of study. But the plants subjected to drought stress exhibited a decline in Fv/Fm ratios to the range of 0.497 to 0.643 on the 100^{th} day and for low temperature stress affected plants the range was 0.481 to 0.651 at 11.30 am. This is in agreement with the views of

various authors (Bjorkman and Demmig 1987; Jacob 1998; Jeyaramraja *et al.*, 2003). According to Jeyaramraja *et al.* (2003), any unusual change in the overall bioenergetic status of the plant (including changes in the photosynthetic apparatus, stomatal opening etc.) can be detected by a change in the chlorophyll fluorescence.

Baker (1991) opined that PS II plays an especially important role in the response of photosynthesis in higher plants to environmental perturbations and stresses. The fluorescence emission by chlorophyll molecules changes depending upon the degree to which the photochemistry is affected during stress (Jacob, 1998). Physiologically the decrease in Fv/Fm ratio indicates a reduction in the photochemical efficiency of PS II complex which could be due to inefficient energy transfer from the light harvesting Chlorophyll a/b complex to the reaction center (Briantais *et al.*, 1986). The reduction of Fv/Fm ratio in different tea clones may be due to decrease of photochemical efficiency of PS II complex because of the inefficient energy transfer from the light harvesting complex to the reaction centre.

It is a well-known phenomenon that the capacity of plants to utilize the light absorbed declined significantly upon exposure to environmental stresses such as drought, salt and low temperature (He *et al.*, 1995; Dubey, 1997; Giardi *et al.*, 1997). When the absorbed light energy is greater than that utilized for photochemistry, a phenomenon called photoinhibition is found to occur (Puthur, 2000) and is caused largely due to the production of toxic oxygen species. In the present study also increased MDA content occurred in various frost affected tea clones (Fig. 4.26), which may have led to photoinhibition and would have resulted in a decline in Fv/Fm ratio. It was established that one of the important targets for the action of toxic oxygen species is PS II (Powles, 1984; Krause, 1988; Constant *et al.*, 1997; Keren *et al.*, 1997) and in the

present study also the decrease in Fv/Fm values of plants under stress conditions may be due to the over production of toxic oxygen species which make the PS II less functional.

From the drought and frost studies it is evident that the Fv/Fm values of the severely affected plants were comparatively lower than that of the other clones. This may be due to the reduced photochemical efficiency of PS II as discussed earlier. So in the present study, low Fv/Fm values were obtained in TTL-2, TTL-4, TTL-5 and UPASI-3 under drought and TTL-2, TTL-5, TTL-6, CR-6017, TRI-2025, SMP-1and SM-OM-54 under frost which indicates that the photochemical efficiency of PS II of these clones was reduced considerably and they can be considered susceptible to drought and frost respectively. The clones TTL-1, TTL-6 and UPASI-2 under drought and TTL-1, TTL-4 and UPASI-9 under frost exhibited comparatively high Fv/Fm values and this may be due to higher photochemical efficiency of PS II than in the other clones and these can be considered as drought and frost tolerant clones respectively. While removing the stress condition these clones exhibited a rapid increase in Fv/Fm values and hence showed quick recovery.

Irrespective of the concentration of fertilizer applied, an increase in the photosynthetic rate was observed in all the clones on the 25th day after applying fertilizer (Fig. 4.8). Identical results were obtained in peach (DeJong and Doyle, 1985) and in rubber (Sobhana *et al.*, 1996). According to these authors, the mineral N either directly or indirectly played an important role in changing the photosynthetic efficiency. In support of this view, the clones TTL-1, TTL-2, TTL-5 and TTL-6 also produced an increased content of N in the leaves after 25 days of fertilizer application (Fig. 4.1).

The clones TTL-1, TTL-2 and TTL-5 recorded maximum photosynthetic rate in 50% fertilizer applied plants as compared to the

remaining two concentrations. A decreasing trend in photosynthesis occurred in TTL-5 with increase in concentration of fertilizer. This may be due to low rate of absorption of N in higher concentrations and hence this clone can be considered as highly sensitive to high concentrations of N fertilizers and even the normal concentrations recommended by UPASI. This indicates that with 50% N fertilizers itself the leaves accumulate sufficient quantity of N required for the plant and it may ultimately result in increased photosynthetic rate.

DeJong and Doyle (1985) observed a significant positive correlation between leaf N content and the photosynthetic rate. According to Sivasankar *et al.* (1993), approximately 75% of the N in a plant leaf with C_3 photosynthesis is invested in chloroplasts and most of it is used in photosynthesis. The leaf N plays a key role in determining a crop's photosynthetic capacity. The major effect of N application on crop photosynthesis is through increased light interception. The authors have mentioned that N being an essential component of proteins, it can also have a direct effect on the rate of photosynthesis per unit leaf area. So, it can be assumed that the increased leaf N content might have played a significant role in the increased photosynthetic rate of these clones.

The clone TTL-6 showed an increased photosynthetic rate with a concomitant increase of leaf N and P content (Fig. 4.1 and 4.2). According to Sobhana *et al.* (1996), P may have direct effect on photosynthesis by modifying the energy metabolism. Moreover, in *Hevea brasiliensis* these authors noticed a positive correlation of leaf P per unit area with stomatal conductance. The authors have also mentioned that N being a constituent of proteins, it could have many general effects on photosynthesis through its effects on protein synthesis.

Humble and Raschke (1971) reported that K^+ was found accumulated in the guard cells of open stomata, whereas in closed stomata no K^+

accumulation was seen. As the fertilizer application enhanced the accumulation of minerals in the plant parts particularly K, this helps in the regulation of stomatal opening and thus leads to increased uptake of photosynthetic C which may ultimately produce high rate of photosynthetic assimilates. It is already reported that photosynthesis is largely dependent on stomatal regulation (Hsiao, 1973). In the present study also increased accumulation of K and increase in photosynthetic rate was seen in tea plants applied with fertilizer, which may also be due to increased regulation of stomatal opening.

The leaf photosynthesis in *Camellia sinensis* (Barbora, 1994), *Hevea brasiliensis* (Jacob *et al.*, 1999) and *Coffea arabica* (Kumar and Tieszen, 1980) was found affected when grown under soil moisture deficit conditions. In accordance with these observations, in the present study also a decrease in the net photosynthetic rate occurred in the non-irrigated plants of all the clones of tea as compared to the irrigated control plants (Fig. 4.15). According to Barbora (1994), there were clonal variations in the photosynthetic rate of six clones of tea in response to soil moisture and it was also observed that the photosynthetic rate declined with decreased soil moisture. Further, it was reported that the photosynthetic rate was relatively less affected until the soil moisture reached 10.5±1% range and it decreased progressively as the soil moisture reduced further. In the present study on the 100^{th} day of non-irrigation the soil moisture was 8.01±1% and there was drastic reduction in the photosynthetic rate in the clones TTL-2, TTL-4, TTL-5 and UPASI-3.

The studies carried out by Jacob (1998) showed that a significant proportion of photosynthetic electrons generated by PS II are used for various processes other than photosynthetic C reduction, which include photorespiration, nitrate assimilation etc. Photorespiration and nitrate

assimilation help to drain photosynthetic energy which is in surplus of C reduction and thus prevent photoinhibition of PS II. However, when the leaves are experiencing extreme abiotic stress and the C assimilation capacity has decreased substantially, the rates of photorespiration and nitrate assimilation may not be large enough to take care of the excess electrons. This leads to increased diversion of excited electrons to processes such as Mehler reaction where molecular O_2 will act as the terminal electron acceptor of the photosynthetic electron transport chain instead of NADP. Thus, diversion of photosynthetic electrons to processes other than C reduction during abiotic stress will produce large amounts of free radicals in the leaf especially in the presence of high light intensity. According to Sengupta *et al.* (1993), the ability of a leaf to scavenge these free radicals determines its photosynthetic response to abiotic stress.

As already discussed, the photosynthetic efficiency of tea plants grown under drought conditions is regulated by the production of very damaging free radicals during abiotic stress. This revealed that during stress condition C reduction cycle becomes inefficient due to non-availability of sufficient electrons. After 100 days of non-irrigation there was not much reduction in the photosynthetic rate in the clones TTL-1, TTL-6 and UPASI-2 as compared to TTL-2, TTL-4, TTL-5 and UPASI-3. The lower photosynthetic rates in TTL-2, TTL-4, TTL-5 and UPASI-3 may be due to the excess diversion of excited electrons to sites other than C reduction, but the occurrence of comparatively lesser diversion of electrons may have resulted in the higher photosynthetic rate in TTL-1, TTL-6 and UPASI-2.

There are several reports that an increase in water stress may cause significant reduction in the photosynthetic rate, stomatal conductance and

transpiration rate (Santarius, 1967; Salisbury and Ross, 1986; Barbora, 1994; Mengel and Kirkby, 1996). In the present study the non-irrigated plants of TTL-1, TTL-6 and UPASI-2 exhibited a non-significant reduction of transpiration rate on the 100th day as compared to the control plants (Fig. 4.16) while in TTL-2, TTL-4, TTL-5 and UPASI-3 there was a significant reduction in the transpiration rate on the 100th day of non-irrigation as compared to the control plants. It is well established that water stress inhibits stomatal opening and photosynthesis. Mild water stress, however, appears to have little effect on stomatal closure (Hsiao, 1973; Mengel and Kirkby, 1996). But when the water stress is more severe, there is a reduction in the uptake of CO_2, as a result of stomatal closure; moreover photophosphorylation and photolysis are also impaired (Santarius, 1967). Similarly, Brevedan and Hodges (1973) observed that maize plants grown under water stress resulting in a water potential of –1.7 to –2.2 MPa showed an inhibited CO_2 assimilation rate due to early stomatal closure. In the present study the earlier closure of stomata may have helped to reduce the heavy loss of water through transpiration in clones TTL-2, TTL-4, TTL-5 and UPASI-3 as compared to TTL-1, TTL-6 and UPASI-2 when the plants were facing a drought situation.

The stomatal conductance also showed a decrease in the tea plants grown under conditions of non-irrigation (Fig. 4.17). On the 100th day of non-irrigation a non-significant reduction of stomatal conductance was noticed in TTL-1, TTL-6 and UPASI-2, which was significant in TTL-2, TTL-4, TTL-5 and UPASI-3 as compared to their control plants. As mentioned earlier TTL-1, TTL-6 and UPASI-2 had higher relative water content on the 100th day of non-irrigation as compared to the rest of the clones (Table 4.1). A comparatively high stomatal conductance in TTL-1, TTL-6 and UPASI-2 may be due to the high relative water content in the leaves of these clones. In support of this view, Hsiao (1973) reported that photosynthesis is largely dependent on stomatal regulation. Similarly

Salisbury and Ross (1986) opined that the water potential within the leaf has a powerful effect on stomatal opening and closing, as water potential decreases (water stress increases) the stomates close. Of the various clones studied, TTL-1, TTL-6 and UPASI-2 exhibited high relative water content, transpiration and stomatal conductance which indicates the more tolerant nature of these clones as compared to the others.

According to Rajasekar *et al.* (1988) UPASI-2 is a drought tolerant clone. The clones TTL-1 and TTL-6, in common with UPASI-2, had a relatively higher rate of photosynthesis and therefore these two clones can also be considered drought tolerant. Similarly, UPASI-3 is considered to be a drought susceptible clone (Rajasekar *et al.*, 1988) and the reduced rate of photosynthesis in TTL-2, TTL-4, TTL-5 and UPASI-3 in the present study confirmed their comparatively drought susceptible nature.

According to Barbora (1994), the adverse effect of water stress on photosynthesis of tea was mainly due to impaired stomatal conductivity, resulting from the decreased hydration of the protoplasm. Similarly in the present study the stomatal conductance reduced significantly in clones such as TTL-2, TTL-4, TTL-5 and UPASI-3 as compared to that of the other clones, which may be due to the adverse effect of water stress on photosynthesis. It was earlier mentioned that the clones with less photosynthetic rate under drought conditions may be drought susceptible and others may be drought tolerant. The rate of stomatal conductance once again ascertained the susceptible and tolerant nature of different clones of tea subjected to the study. Transpiration rates of the clones under drought conditions are also more or less comparable to that of photosynthesis and stomatal conductance. These results are in agreement with the views of Salisbury and Ross (1986) and Barbora (1994). So it can be concluded that the clones TTL-1, TTL-6 and UPASI-2 are comparatively more drought tolerant than the remaining

clones. Moreover on the 14th day of re-irrigation the clones TTL-1, TTL-6 and UPASI-2 showed the fastest recovery in all the above parameters, which once again re-confirms the comparatively more drought tolerant nature of these clones.

The proline content in leaves of all the clones of tea (*C. sinensis*) was less, immediately before the commencement of moisture stress (Fig. 4.18). But, when the days of moisture stress prolonged, the level of proline accumulation in all the clones was found to increase. Proline is one of the organic molecules that are found to accumulate in plants exposed to environmental stresses such as salt, drought, temperature etc., (Saraswathy *et al.*, 1992; Saradhi *et al.,* 1995; Puthur *et al.*, 1996; Puthur, 2000; Chakraborty *et al.*, 2001; Matysik *et al.*, 2002; Puthur and Rajan, 2006). Rajasekar *et al.* (1988) evaluated various tea clones such as UPASI-2, UPASI-3, UPASI-8, UPASI-9, UPASI-10 and UPASI-17 for drought tolerance and found significant accumulation of proline in all the clones and came to a conclusion that, proline accumulation is a defensive mechanism to overcome the conditions of water stress. Similarly, Chakraborty *et al.* (2001) studied the drought induced biochemical changes in young tea leaves and observed a rapid increase in the accumulation of proline content in all the varieties investigated after 7 days of drought over their respective control plants. According to these authors, the slow utilization of proline for protein synthesis and stimulation of glutamate conversion to proline during stress are responsible for its accumulation under stress condition. Durgaprasad *et al.* (1996) noticed an increased proline content with a concomitant decrease of protein content in stress-affected plants.

Aspinall and Paleg (1981) opined that proline is considered to be involved in adaptation mechanisms of plants subjected to various stresses. It may have multiple functions; osmotic adjustment, maintenance of protein

stability and as storage of N and C to overcome the unfavourable conditions resulting from stress (Andrade *et al.*, 1995). Puthur *et al.* (1996) felt that whatever be the actual reason behind proline accumulation, the plants exposed to stress seem to be very well benefited by it.

In the present study the clones TTL-1, TTL-6 and UPASI-2 exhibited high proline accumulation on 100th day of water stress (Fig. 4.18). Rajasekar *et al.* (1988) estimated the proline content in various tea clones under prolonged water stress and noticed accumulation of higher levels of proline in the clones UPASI-2, UPASI-9 and UPASI-10 which is indicative of their drought tolerant nature. Similar pattern of proline accumulation occurred in certain other clones of tea, (Singh and Handique, 1993) and some accessions of robusta coffee (Saraswathy *et al.*, 1992), which were also drought tolerant. According to Blum and Ebercon (1976), plants with high proline accumulation during moisture stress are known to be drought tolerant. Similarly, Narayan and Misra (1989) reported that certain wheat varieties, which had different degrees of drought resistance, differed in their capacity to accumulate proline and the resistant varieties accumulated higher levels of proline than susceptible ones under water stress. All these observations revealed that the accumulation of proline during stress is an indication of drought tolerance of a particular species. In agreement with these views, in the present investigation also higher proline accumulation was noticed in TTL-1, TTL-6 and UPASI-2 than the other clones of tea studied and they could be considered as drought tolerant.

Saraswathy *et al.* (1992) noticed a decrease in the proline content in the tolerant robusta coffee accessions after relief from the stress while there was no decrease in the other accessions. A similar result was obtained in the present study also during the days after re-irrigation of the non-irrigated tea plants (Fig. 4.18). The non-irrigated tea plants after re-irrigation exhibited a

decrease in the accumulation of proline content in all the clones. But the decrease was rapid in TTL-1, TTL-6 and UPASI-2 and gradual in the other clones. Andrade *et al.* (1995) reported that the proline accumulated in plants performs multiple functions such as osmotic adjustment, maintenance of protein stability and storage of N and C to overcome the unfavourable conditions resulting from stress.

From the results it is clear that accumulation of proline during non-irrigated conditions in various tea clones can be reversed by re-irrigation. At the peak stage of non-irrigation the high proline content as compared to that of its irrigated control takes care of the survival of the plants under the stressed condition. When the stress condition was removed, the level of proline content declined to that of the control plants which may be due to the oxidation of proline to glutamate as suggested by Blum and Ebercon (1976). As the clones TTL-1, TTL-6 and UPASI-2 exhibited high accumulation of proline during non-irrigated condition, these clones can be considered to be comparatively more drought tolerant than the rest.

Tea (*C. sinensis*) clones grown under low temperature conditions showed very low proline content at the beginning of the experiment, but increased thereafter and attained maximum values at 11.30 am (Fig. 4.25). Similar results were obtained in cold damaged betel leaves (Tripathi *et al.*, 2000) and the results showed that the proline content in cold damaged betel leaves was more than that of the leaves without frost injury. According to Powles (1984), photosynthesis is one of the first processes affected and the symptoms of low temperature damage to the photosynthetic apparatus occur only when substantial light accompanies the low temperature exposure. This could be the reason for the occurrence of low proline content noticed at the initial stages in all the tea

clones as the intensity of light was low at these intervals and when the intensity of light was increased a corresponding increase in proline accumulation was noticed at 11.30 am.

Similarly, Puthur and Rajan (2006) studied the effect of various light intensities on the accumulation of proline in *Vanilla planifolia.* According to them, the proline content in the leaves was found to increase with increase in light intensity. In a variety of plants the concentration of proline increases under stress upto 100 times to that of the normal level, which makes upto 80% of the total amino acid pool (Matysik *et al.*, 2002). Therefore proline produced in more quantities during conditions of stress takes care of the stressful situation created either biotically or abiotically.

Of the ten clones of tea subjected to the study, the clones TTL-1, TTL-4 and UPASI-9 exhibited highest proline content at 11.30 am compared to the rest of the clones (Fig. 4.25). According to various authors, the accumulation of proline during stress is considered to be an adaptive mechanism to overcome the stressful condition and the plants, which accumulate high quantity of proline, are considered to be stress tolerant plants (Blum and Ebercon, 1976; Rajasekar *et al.*, 1988; Saraswathy *et al.*, 1992). Similarly, in the present study the higher accumulation of proline under low temperature conditions in TTL-1, TTL-4 and UPASI-9 may also be due to the low temperature tolerant nature of these clones.

Though the remaining clones exhibited an increase in the proline content at 11.30 a.m. it was lesser than that observed in TTL-1, TTL-4 and UPASI-9. At the subsequent intervals the proline level decreased considerably in these clones and by 7.00 pm the values became identical to that obtained at the initial stage of the experiment. The high

concentration of proline content in TTL-1, TTL-4 and UPASI-9 might be to protect the plants against damage caused by reactive oxygen species. Matysik *et al.* (2002) have reported that in addition to its role as an osmolyte and a reservoir of C and N, proline has been shown to protect plants against free radical induced damage. These authors also opined that proline is capable of forming charge transfer complex and can quench singlet oxygen effectively. According to these authors, due to its action as singlet oxygen quencher and scavenger of $OH^{\cdot}$ radicals, proline is able to stabilize proteins, DNA and membranes, especially during stress conditions.

The plants, which are exposed to various environmental stresses, show a marked decline in the mitochondrial electron transport activity (Puthur *et al.*, 1996). According to Alia and Saradhi (1993), the suppression in the mitochondrial electron transport is the prime cause behind proline accumulation. The excessive synthesis of proline during various environmental stresses may be an appropriate adaptive mechanism evolved by the plants to reduce excess NADH resulting due to inhibition of mitochondrial electron transport activity (Alia and Saradhi, 1993).

All the above views showed that proline is capable of protecting plants grown under various stresses in many ways. So in the present study the high production of proline in the clones TTL-1, TTL-6 and UPASI-2 under moisture stress and TTL-1, TTL-4 and UPASI-9 under low temperature conditions protect the plants from abiotic stress related damage and hence these clones can be considered to be comparatively more drought and frost tolerant than the remaining clones investigated.

Scandalios (1993) reported that factors like UV light, other forms of radiation, herbicides, pathogens, certain injuries, hyperoxia, ozone,

temperature fluctuations and various other stresses are known to induce free radical formation in most aerobic organisms. Malondialdehyde (MDA) is a major cytotoxic product of lipid peroxidation and acts as an indicator of free radical production (Saradhi *et al.*, 1995).

A progressive increase in the MDA content was observed in all the non-irrigated clones of tea from the zero day to the 100th day (Fig. 4.19). Similar results were obtained by various authors (Scandalios, 1993; Saradhi *et al.*, 1995; Jia *et al.*, 2003). According to Jia *et al.* (2003), under drought stress many metabolic processes can produce active oxygen species and the authors have suggested that the electron transport in mitochondria and chloroplast is the main source of active oxygen species in plants. In support of this view, Puthur *et al.* (1996) observed high MDA content in the chloroplasts of leaves exposed to high light intensities.

Of the various clones studied, maximum value of MDA was obtained in TTL-2, TTL-4, TTL-5 and UPASI-3 and minimum in TTL-1, TTL-6 and UPASI-2 (Fig. 4.19). After re-irrigation the MDA values decreased in all the clones, but the clones TTL-1, TTL-6 and UPASI-2 exhibited a considerable reduction of MDA content on 7th day of re-irrigation as compared to the other clones. Puthur *et al.* (1996), Jacob (1998) and Alscher *et al.* (2002) opined that free radicals are generated due to the unusual distribution of electrons from electron transport chains of chloroplast and mitochondria to molecular oxygen and during unstressed condition the formation and removal of free radicals are in balance. The decreased MDA content during re-irrigation of non-irrigated tea plants indicates that the formation and removal of free radicals may be in balance. So in the present study, the balance was achieved at a faster rate in TTL-1, TTL-6 and UPASI-2 which showed

that these clones can recover more speedily on re-irrigation than TTL-2, TTL-4, TTL-5 and UPASI-3.

During low temperature conditions in the present study the MDA content was found to increase and reached maximum values at 11.30 am and 2 pm in all the clones (Fig. 4.26). The increased MDA content at 11.30 am and 2 pm could be due to the occurrence of high irradiance at those intervals as stated by Alam and Jacob (2002). According to these authors, strong irradiance aggravates the effect of low temperature stress by diverting more electrons for active oxygen species production and that results in oxidative stress.

The clones subjected to low temperature conditions exhibited an increased MDA content at 11.30 am which was high in TTL-1, TTL-2, TTL-4, TTL-6 and UPASI-9 as compared to other clones (Fig. 4.26), but at 7.00 pm, with the exception of TTL-6, the MDA content was reduced to the level at 6.30 am. Similar results were obtained in rice plants (Saradhi *et al.*, 1995) and *Vanilla planifolia* (Puthur and Rajan, 2006) under stress and showed that though high MDA content was produced, it was regulated by the high proline content and other free radical detoxifying mechanisms produced during stress. According to these authors, proline has an important role in protecting plants against free radical damage. In support of this view, in the present study high proline accumulation was noticed in TTL-1, TTL-4 and UPASI-9 at 11.30 am (Fig. 4.25). The high proline content found in TTL-1, TTL-4 and UPASI-9 during low temperature stress may have a protective role against free radical damage. Even though TTL-2 and TTL-6 produced a high MDA content at 11.30 am, the proline content was comparatively less and the clones may have experienced free radical damage during low temperature stress. Similarly all the other clones also produced low proline but considerably high MDA content.

Jacob (1998) opined that the carbon assimilation capacity of leaves under abiotic stress decreased considerably, and the rates of photorespiration and nitrate assimilation mechanism may not be large enough to take care of the excess electrons. This resulted in an increased diversion of excited electrons to other electron accepting sites on the electron transport chain, where molecular oxygen might act as the terminal electron acceptor of the photosynthetic electron acceptor chain instead of NADP. This results in production of super oxide, which is an active oxygen species that can lead to production of very damaging other radicals, if the oxygen scavenging mechanisms are not effective. Thus, diversion of photosynthetic electrons to process other than carbon reduction during abiotic stress will produce large amount of free radicals in the leaf, particularly in the presence of high intensity of light. In the present investigation also diversion of photosynthetic electrons to sites other than carbon reduction may have taken place in the leaves of plants under low temperature stress and resulted in an increased production of MDA.

As proline accumulation was found to be the highest in the clones TTL-1, TTL-6 and UPASI-2 (Fig. 4.18), it could be assumed that the high levels of proline in these clones might play a significant role in detoxifying the reactive oxygen species. It has been suggested by Matysik *et al.* (2002) that proline accumulates in high amounts in several plants under stress and this protects plants against damage by reactive oxygen species. Besides, the antioxidant defence system of the plant comprises a variety of antioxidant molecules and enzymes (Arora *et al.*, 2002). According to Saradhi *et al.* (1995), proline might have the capacity to scavenge and / or to reduce the production of free radicals.

The MDA content was high in the clones such as TTL-2, TTL-4, TTL-5 and UPASI-3 on the 100^{th} day of non-irrigation as compared to

TTL-1, TTL-6 and UPASI-2 (Fig. 4.19). The accumulation of proline in the former clones was much less compared to the latter. This supports the view of Saradhi *et al.* (1995) that proline might have the capacity to reduce the production of free radicals. Besides, Arora *et al.* (2002) opined that any mechanism, which reduces the oxidative stress, might play an important role in drought tolerance. So the clones TTL-1, TTL-6 and UPASI-2 which accumulated higher proline with lower malondialdehyde may be considered as drought tolerant and the others as drought susceptible. Similarly the fast recovery of these clones from drought stress, after re-irrigation once again confirmed the drought tolerant nature of these clones.

However, in frost affected tea plants higher MDA content was found in TTL-1, TTL-2, TTL-4, TTL-6 and UPASI-9 but the concomitant increase of high proline content in TTL-1, TTL-4 and UPASI-9 might have played a crucial role in regulating the damaging activity of free radicals. The low level of proline accumulation and high MDA content in TTL-2 and TTL-6 may have affected the clones adversely because of the lack of protective mechanism provided by proline and thus these clones can be considered as frost susceptible along with the other clones. Even though TTL-1, TTL-4 and UPASI-9 had enhanced levels of free radicals during stress, a correspondingly high concentration of proline accumulation extended a protective role and hence these clones can be considered as frost tolerant as suggested by Scandalios (1990).

As free radicals can affect the chlorophyll molecules (Puthur *et al.*, 1996), the MDA chlorophyll ratio was determined to evaluate the stress tolerance of the clones and it showed a very interesting trend. Of the various clones subjected to drought studies, the clones TTL-1, TTL-6 and UPASI-2 exhibited a low MDA chlorophyll ratio (Fig. 4.20 and 4.21) as

compared to TTL-2, TTL-4, TTL-5 and UPASI-3. This is in agreement with the views of Annamalainathan *et al.* (2006). According to these authors, the MDA chlorophyll ratio in rubber plants is a typical reflection of the degree of susceptibility to drought. The authors compared the MDA chlorophyll ratio of a drought tolerant clone with that of a drought susceptible clone. The results showed that the drought affected plants of RRII-105, a drought susceptible clone, exhibited highest MDA chlorophyll ratio while RRIM-600, a tolerant clone, recorded the lowest ratio. Thus it once again confirmed the drought tolerant nature of the clones TTL-1, TTL-6 and UPASI-2 due to low MDA chlorophyll ratio compared to TTL-2, TTL-4, TTL-5 and UPASI-3 in which the MDA chlorophyll ratio was high.

Under frost conditions it was noticed that the clones TTL-1, TTL-4 and UPASI-9 exhibited low MDA chlorophyll ratio compared to the remaining clones (Fig. 4.27). Similar results were obtained in two clones of rubber (RRIM-600 and RRII-105) under low temperature stress conditions (Alam *et al.*, 2004). The results showed that the clone RRIM-600 had lesser MDA chlorophyll ratio as compared to RRII-105. The authors noted that the clone RRIM-600 performed better than RRII-105 in the cold prone areas, and thus proved the cold temperature tolerant nature of the clone RRIM-600. Alam and Jacob (2002) reported that the MDA chlorophyll ratio is an index of oxidative damage caused by impaired photosynthetic photochemistry. In low temperature tolerance studies done by the authors, high MDA chlorophyll ratio was recorded in the two low temperature susceptible species, *Hevea* and Napier grass, which indicated that there was enhanced active oxygen species production in them.

Therefore in the present study also, as the clones TTL-1, TTL-4 and UPASI-9 exhibited high proline content and low values of MDA chlorophyll

ratio, the protective mechanism provided by proline may have resulted in the reduction of the level of free radicals in these clones. Therefore these clones could be considered as more low temperature tolerant than the other clones studied.

Summary and Conclusions

Various clones of tea (*Camellia sinensis*) were subjected to different nutrient levels and environmental stresses, such as drought and frost and were used for determination of different physiological changes. All the experiments were carried out in 2002 and repeated in 2003 and 2004.

The clones selected for the nutrient studies were TTL-1, TTL-2, TTL-5 and TTL-6. The fertilizers were applied in five split doses during the months of April (Ammonium Sulphate and Muriate of Potash (MOP) mixture), May, August, September and November (Urea and MOP mixture). Three different concentrations of fertilizers applied to the tea clones were 50, 100 and 150%, in which 100% is the UPASI recommended level of fertilizer for tea plantations. One application of P at 50, 100 and 150% during each year was also done proportionately. The Ammonium Sulphate and MOP mixture and Urea and MOP mixture were applied by broadcasting method and P application by placement method at 15-25 cm depth of soil in the month of November. The P application was done separately and was not along with the N and K application.

Following standard procedures, NPK content of leaf, weight of shoots, chlorophyll and carotenoid content, chlorophyll fluorescence (Fv/Fm) and photosynthetic rate were determined at a regular interval of five days for 30 days.

The drought studies were carried out during the summer months of the period of study. The clones TTL-1, TTL-2, TTL-4, TTL-5, TTL-6, UPASI-2 and UPASI-3 were selected for the study and the plants were maintained in two rows. Of the two rows, one row was uniformly irrigated using drip irrigation and the other row was left unirrigated starting from the month of December to March. The weight of shoots, soil moisture status, leaf water potential, relative water content, chlorophyll and carotenoid content, Fv/Fm, photosynthetic rate, stomatal conductance, transpiration rate, proline and malondialdehyde (MDA) content were monitored in both irrigated and non-irrigated plants on the 0, 20^{th}, 60^{th} and 100^{th} day from the beginning of drought. After 100 days, the non-irrigated plants were irrigated and the same parameters were studied on the 7^{th} and 14^{th} day after re-irrigation.

The frost studies were carried out during the winter months in the same years. Ten different clones selected for the low temperature stress studies were TTL-1, TTL-2, TTL-4, TTL-5, TTL-6, UPASI-9, SMP-1, SM-OM-54, CR-6017 and TRI-2025. The observations, relating to plant response against low temperature, like carotenoid content, Fv/Fm, proline and MDA content of leaves were recorded at a regular interval of 2½ hours from 6.30 am to 7.00 pm. The observations were repeated on different days in the months of December to February when low temperatures/frost occurred.

All the plants selected for the experiments were in their third year from the date of planting and were planted at a spacing of 1.2 metres x 0.75 metre along a single hedge with a plant population of 10760 plants per hectare.

The following observations and conclusions were made from the present investigation:

A correlation between shoot weight and leaf N, P and K content was established in the clone TTL-6 but in TTL-1, TTL-2 and TTL-5 the shoot weight was found correlated with N and P only.

High shoot weight during moisture stress in the clones TTL-1, TTL-6 and UPASI-2 indicates that these clones were less affected by drought. The reason for this could be that comparatively higher water uptake, cell expansion and photosynthesis occurred in them than in TTL-2, TTL-4, TTL-5 and UPASI-3.

The soil moisture studies revealed that by the 100^{th} day of non-irrigation the soil moisture percentage dropped below 8% which is considered as the wilting coefficient. When the plants were re-irrigated after 100 days of non-irrigation, they recovered which suggests that permanent wilting did not occur.

Higher leaf water potential was established in the clones TTL-1, TTL-6 and UPASI-2 than in TTL-2, TTL-4, TTL-5 and UPASI-3 during drought stress. This indicates the ability of the former clones to withstand drought better than the latter. Moreover the genotypes tolerant to drought exhibited higher and those susceptible to drought lower leaf water potential values.

TTL-1, TTL-6 and UPASI-2 showed higher relative water content than the rest of the clones which is indicative of their drought tolerant nature.

A correlation between leaf N, P content and chlorophyll a+b and carotenoid content was established in all the clones investigated.

Comparatively increased synthesis and decreased degradation of chlorophyll a+b and carotenoid was established in the clones TTL-1, TTL-6 and UPASI-2 under drought and thus these clones can be considered as drought tolerant than the rest which are drought susceptible.

In the low temperature studies, the clones TTL-1, TTL-4 and UPASI-9 showed an increase in their carotenoid content at 11.30 am compared to the rest. This may be an indication of their non enzymatic detoxification mechanism of toxic oxygen species and therefore their frost tolerant nature.

The clones TTL-1, TTL-2 and TTL-5 exhibited higher Fv/Fm values when applied with 50% fertilizer concentration as against TTL-6 which showed an increase in the Fv/Fm values with increase in concentration of fertilizer. This is in tune with the N and P accumulation in these clones.

During moisture stress, though there was reduction, the clones TTL-1, TTL-6 and UPASI-2 exhibited comparatively higher Fv/Fm values than the remaining clones. The reduction in Fv/Fm ratio with increased moisture stress might be due to the loss of primary photochemical efficiency of stressed leaves. The higher Fv/Fm ratio in TTL-1, TTL-6 and UPASI-2 suggests their drought tolerant nature and lower values found in TTL-2, TTL-4, TTL-5 and UPASI-3 suggests their drought susceptible nature.

Among the clones studied under frost conditions, TTL-1, TTL-4 and UPASI-9 exhibited minimum reduction in their Fv/Fm values as compared to the others. This indicates that the primary photochemical efficiency of leaves of these clones was not affected considerably when subjected to low temperature stress. Therefore these clones are considered to be comparatively more low temperature tolerant than the rest.

The clones TTL-1, TTL-2 and TTL-5 showed an increased photosynthetic rate in 50% fertilizer concentration and in TTL-6 the photosynthetic rate increased with fertilizer concentration. This is probably due to the increased N and P accumulation found in the leaves.

During moisture stress the clones TTL-1, TTL-6 and UPASI-2 exhibited only a slight reduction in the photosynthetic rate, transpiration rate

and stomatal conductance in comparison to TTL-2, TTL-4, TTL-5 and UPASI-3. This indicates the lesser diversion of electrons to sites other than C reduction and non-closure of stomata in TTL-1, TTL-6 and UPASI-2. Thus the clones TTL-1, TTL-6 and UPASI-2 can be considered to be more drought tolerant than the rest.

In drought studies, the clones TTL-1, TTL-6 and UPASI-2 had the minimum MDA and highest proline content. So it is assumed that the high levels of proline in these clones might play a significant role in detoxifying the reactive oxygen species and thus they can be considered to be drought tolerant.

During low temperature studies, TTL-1, TTL-2 TTL-4, TTL-6 and UPASI-9 exhibited higher MDA content. But in the clones TTL-1, TTL-4 and UPASI-9 a concomitant increase in the proline content was observed which might have played a crucial role in regulating the damaging activity of free radicals. Therefore the clones TTL-1, TTL-4 and UPASI-9 can be considered to be frost tolerant.

In the moisture stress studies the clones TTL-1, TTL-6 and UPASI-2 exhibited low MDA chlorophyll ratio while in the low temperature stress studies the clones TTL-1, TTL-4 and UPASI-9 showed low MDA chlorophyll ratio. The clones with low MDA chlorophyll ratio are considered to be more stress tolerant. Therefore the clones TTL-1, TTL-6 and UPASI-2 can be considered as drought tolerant and the clones TTL-1, TTL-4 and UPASI-9 as frost tolerant. From the studies it is clear that the clone TTL-1 is tolerant to both drought and frost.

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