Discussion

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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 (NO₃⁻) 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, CO₂ 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
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 Mg2+ 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 100th 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 14th 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 20th and 60th day there was significant decrease in the soil moisture % in the non-irrigated plots and on the 100th 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 100th 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 100th 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 25th 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 C3 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 100th 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 photoinhibition. 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
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 100th 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-1 and 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 C₃ 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 100th 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 \( \text{O}_2 \) 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 CO2, 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 CO2 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 14\textsuperscript{th} 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' 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 100th day of non-irrigation as compared to
The accumulation of proline in the former clones was much less compared to the latter. This supports the view of Saradhi el 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.