Clonal variation of tea [*Camellia sinensis* (L.) O. Kuntze] in countering water deficiency

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Abstract Various clones of tea [*Camellia sinensis* (L.) O. Kuntze] such as TTL-1, TTL-2, TTL-4, TTL-5, TTL-6, UPASI-2 and UPASI-3 planted in the field were subjected to soil moisture stress conditions by withholding irrigation. A control set of the same clones were maintained by watering regularly. The soil water content of the irrigated and non irrigated plants was monitored through the soil moisture status. The extent of effect of drought on tea plants were monitored through various physiological parameters such as shoot weight, leaf water potential, chlorophyll and carotenoid content, chlorophyll fluorescence (Fv/Fm), net photosynthetic rate, transpiration rate, stomatal conductance and biochemical parameters such as extent of proline accumulation and free radical generation. These parameters were studied on the 30 d of non irrigation and on the 5 d during recovery from drought. The plants recovered when re-irrigated after 30 d of non-irrigation, which suggests that permanent wilting did not occur due to non-irrigation up to 30 d. On the 30 d of non-irrigation the clones TTL-1, TTL-6 and UPASI-2 showed lesser reduction of shoot weight, leaf water potential, chlorophyll fluorescence, photosynthetic rate, transpiration rate and stomatal conductance and increased proline and lesser lipid peroxidation as compared to the other clones. From these

results it can be concluded that the clones TTL-1, TTL-6 and UPASI-2 are comparatively more drought tolerant than the clones TTL-2, TTL-4, TTL-5 and UPASI-3.

Keywords *Camellia sinensis* · Drought · Photosynthesis · Water potential

Introduction

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; Marimuthu and Kumar 1998; Kigalu 2007). Generally, plants have an inbuilt mechanism to cope with stress, at the expense of photosynthates. If the moisture stress prolongs, defoliation and death of plants occurs (Marimuthu and Kumar 1998).

There are several reports that an increase in water stress may cause significant reduction in the photosynthetic rate, stomatal conductance and transpiration rate (Barbora 1994; Mengel and Kirkby 1996). Salisbury and Ross (1986) opined that the water potential within a leaf has a powerful effect on stomatal opening and closing, and with decrease in water potential (water stress increases) stomata close. The diversion of photosynthetic electrons to processes other than carbon reduction during abiotic stress will produce

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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.

Studies on moisture stress on different tea cultivars (Rajasekar et al. 1988; Singh and Handique 1993) 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. The results of this study further indicated that the clones susceptible to drought exhibited lower shoot water potential values than the tolerant ones. 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. The existence of correlation between free radical generation and proline accumulation during intensification of stress, provided a clue that proline accumulation is related to non-enzymatic detoxification of free radicals (Matysik et al. 2002).

Being a perennial crop the tea plant is capable of adapting to various types of stresses but the degree of tolerance varies with the cultivar (Chakraborty et al. 2000). Clonal differences of tea in responses to drought have been demonstrated by Carr (1977) and Othieno (1978) in Kenya, and by Nyirenda (1988) in Malawi, but these have not always been quantified in relation to the soil water deficit. According to Thomas et al. (2004) understanding physiological processes within the plant and their responses to environment are needed in view of exploiting the existing tea germplasm to evolve a drought tolerant planting material. Though drought is initially reversible, in its severest form it can lead to cell death and its ill effects can be minimized by the use of drought tolerant planting material (Jeyaramraja et al. 2003). Therefore the present study aims at exploring the possibility of evaluating various prominent clones of tea for their drought tolerance potential.

Materials and methods

The drought studies were carried out during the summer months of three consecutive years (2006–8). Two rows of 3 year old tea plants (20 each) of the clones TTL-1, TTL-2, TTL-4, TTL-5, TTL-6, UPASI-2 and UPASI-3 were selected for the study. One row was uniformly irrigated at 1 L per plant according to Verma and Palani (1997) using drip irrigation and the other row was non-irrigated starting from the month of March. The non irrigated plants were at

the upper part of the slopped terrain and irrigated plants at the lower part. Ample distance was maintained between the irrigated and non irrigated rows, so as to avoid the water sweeping into the non irrigated row. The soil water content of the irrigated and non irrigated plants was monitored through the soil moisture status.

The extent of effect of drought on tea plants were monitored through various physiological parameters such as shoot weight, leaf water potential, chlorophyll and carotenoid content, chlorophyll fluorescence (Fv/Fm), net photosynthetic rate, transpiration rate, stomatal conductance and biochemical parameters such as proline accumulation and free radical generation in the non-irrigated plants and the same was compared with irrigated plants. The observations were recorded on 0, 10, 20 and 30 d, as the drought was progressively increasing. The non-irrigated plants after 30 d were irrigated uniformly using drip irrigation and the same parameters were continued to be monitored on the 5 and 10 d after re-irrigation. The data is only shown for 30 d of non irrigation and 5 d after reirrigation

The soil moisture of the experimental plots was determined as per the method proposed by Rajasekar et al. (1988).

The shoots (10 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, Japan) and average values were recorded.

Leaf water potential (ψ_L) was measured using a dew point hygrometer (Model C-52 sample chamber connected to HR 33T microvoltmeter, Wescor Inc., Logan, USA) as described by Hajra (2002). ψ_L values were expressed as megapascals (MPa).

The extraction and estimation of total chlorophyll and carotenoids was done according to the method of Arnon (1949).

Chlorophyll fluorescence was monitored with a portable fluorescence induction monitor, FIM 1500 (Analytical Development Company Ltd. England). The photosynthetic plant parts were dark adapted for a duration of 30 min prior to fluorescence measurements. Original (Fo) and maximal (Fm) fluorescence yields were measured with weak modulated red light ($<0.5 \mu\text{mol m}^{-2}\text{s}^{-1}$) with a 0.8 pulse of saturating light ($>6.8 \mu\text{mol m}^{-2}\text{s}^{-1}\text{PAR}$).

Net photosynthetic rate (Pn) was measured by a closed system infrared gas analyser (portable photosynthesis system, CI-301 PS, CID, Inc., U.S.A.). The ambient CO₂ was 345 ± 15 ppm and all the measurements were taken under natural sunlight as described elsewhere (Dey et al. 1995). Dark green healthy mature leaves at the surface of the canopy and fully exposed to incident sunlight were used

for the observations. In the present study Pn was measured between 0900 and 1100 hours when the maximum values of Pn were observed in the diurnal study.

Proline estimation was carried out as per the method of Bates et al. (1973).

The malondialdehyde content was estimated according to the method of Heath and Packer (1968).

Results and discussion

The soil moisture decreased in the non-irrigated plots with increase in the period of drought, whereas in the irrigated plots the soil moisture level remained constant (Table 1). On the 10 and 20 d there was significant decrease in the soil moisture percentage in the non-irrigated plots and on the 30 d the soil moisture was 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, due to decline in the water availability. If the wilting is temporary the plants are able to recover when re-irrigated, whereas when the stage of permanent wilting has been reached wilting is irreversible and the plant dies (Mengel and Kirkby 1996). In the present study, when the clones under non-irrigation were irrigated, a speedy recovery occurred, indicating that the non-irrigation for 30 d may not have resulted in a state of permanent wilting.

Drought affecting the shoot weight and leaf water potential

The shoot weight of the non-irrigated plants was less than that of the same clones under irrigation. The maximum reduction in shoot weight in the non-irrigated plants of all the clones was observed on the 30 d without irrigation and the reduction was significant in all the clones studied ($p < 0.05$). The highest percentage of change in shoot weight on the 30 d without irrigation as compared to 0 d was observed in the clone UPASI-3 followed by TTL-2, whereas it was low in the clones TTL-1, TTL-6 and UPASI-2. The irrigated plants of all the clones maintained more or less same shoot weight throughout the study. When re-irrigation was done to the plants which were not irrigated for 30 d, there was an increase in the shoot weight of all the clones, which was significant and the highest increase was

recorded in the clone UPASI-2 on the 5 d compared to all the remaining clones (Fig. 1a).

A similar reduction in shoot weight due to water stress has been reported in various plant species subjected to drought stress (Mengel and Kirkby 1996; Thomas et al. 2004). According to Mengel and Kirkby (1996), when the water availability in the soil is poor and transpiration is high, a negative water balance occurs, resulting in loss of water by the plant, which is greater than its uptake. Further, they 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. Water stress also influences growth indirectly by influencing mineral uptake and allocation of photosynthates (Puthur 2000) which may result in reduced fresh weight. The comparatively higher shoot weight found in TTL-1, TTL-6 and UPASI-2 subjected to drought stress may be due to the capacity of these clones to maintain a better water status as compared to 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 5 d 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 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 near to total recovery within 5 d after re-irrigation indicating thereby that these clones were more elastic than the others studied.

In the non-irrigated plants the leaf water potential showed a reduction in all the clones on the 30 d of withholding irrigation and it was significant in TTL-2, TTL-4 and UPASI-3 (Fig. 1b) ($p < 0.01$). The leaf water potential on the 5 d of re-irrigating after 30 d of imposing water stress was more comparable to 0 d in the clones TTL-1, TTL-6 and UPASI-2, as compared to TTL-2, TTL-4, TTL-5 and UPASI-3. Wheat genotypes also showed such clonal variation in maintaining water potential, when grown under drought conditions (Sharma et al. 2003). They concluded that maintenance of higher internal water status is a character of the genotypes, which maintains high yields even when encountered with drought.

The leaf water potential in tea bushes is considered as an index for whole plant water status and maintenance of high

Table 1 Soil moisture percentage recorded in irrigated and non-irrigated plots. The values represent SE of the mean value of recordings from three different experiments, each with a minimum of three replicates

	Days without irrigation				Days after re-irrigation	
	0	10	20	30	5	10
Irrigated	22.7±1.8	22.8±2.1	22.4±1.9	22.9±1.8	22.7±1.9	22.7±2.1
Non-irrigated	22.7±2.2	18.30±1.8	11.5±1.0	8.01±0.7	22.90±1.9	23.01±2.1

Fig. 1 Effect of drought on shoot weight (a) and leaf water potential (b) in various clones of tea (*C. sinensis*) which was recorded on the 30 d without irrigation and on the 5 d during re-irrigation. The shoot weight and leaf water potential was measured in terms of g/fr. wt. and

MPa, respectively. The 0 d value was taken as 100%. The vertical lines represent SE of the mean value of recordings from three independent experiments, each with a minimum of three replicates (i.e. $n=9$)

leaf water potential is considered to be associated with dehydration tolerance mechanism and the ability to withstand drought in tea (Handique and Manivel 1986; Singh and Handique 1993) as well as in Kranti variety of rice (Kumar and Kujur 2003). The drought tolerant clones exhibited higher shoot water potential than the drought susceptible clones. Similar results were obtained in certain other clones of tea and according to these authors, the genotypes of tea susceptible to drought exhibited lower leaf water potential than the resistant ones.

The effect of drought on photosynthesis and allied features

A significant reduction in the total chlorophyll and carotenoid content was recorded in the non-irrigated plants, with the reduction increasing from the 0 d and reaching the maximum by the 30 d ($p<0.01$). In the clones TTL-2, TTL-4, TTL-5 and UPASI-3 as the moisture stress progressed from the 0 d to the 30 d, the chlorophyll a+b values lowered below 1,000 $\mu\text{g/g}$ fr. wt and less carotenoid content was observed in the same clones. Upon re-irrigating continuously for 5 d, the chlorophyll content started to build up from the lowered value and it significantly recovered in the clones TTL-1, TTL-6 and UPASI-2 as compared to the clones TTL-2, TTL-4, TTL-5 and UPASI-3. The increase in the values of carotenoid content from the 30 d of non-irrigation to the 5 d 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 (Table 2).

Shubhra et al. (2003) studied the effect of water stress on total chlorophyll content in cluster bean, and found that the total chlorophyll content of the leaf declined under water

stress. They opined that, 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 they opined that reduced ability to form proto-chlorophyll was considered to be primarily responsible for the inhibition of chlorophyll development under water stress. 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 due to the enhanced 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.

The Fv/Fm ratio of various clones of tea at the initial stage of the experiment did not show considerable variation between the clones. But the values decreased considerably at 30 d of non-irrigation as compared to that of the respective control plants. At 30 d of withholding irrigation the maximum reduction in Fv/Fm ratio was observed in TTL-2 and the minimum in TTL-1 (Fig. 2a). Jeyaramraja and coworkers (2003) reported that drought tolerant cultivars (ATK-1 and TRF-1) exhibited higher Fv/Fm ratio than UPASI-17 and they attributed low Fv/Fm ratio to the loss of primary photochemical efficiency of stressed leaves. In an earlier study carried out by Rajasekar et al. (1988), UPASI-3 was found to be susceptible and also reported that UPASI-2 was drought tolerant. But, in the present investigation, the clones TTL-1 and TTL-6 exhibited higher Fv/Fm values than UPASI-2 on the 30 d of non-irrigation. This

Table 2 Total chlorophyll and carotenoid (in parenthesis) content of tea (*C. sinensis*) leaves subjected to increasing drought stress of periods (0 and 30 d) and on 5 d of recovery from stress. The values represent SE of the mean value of recordings from three different experiments, each with a minimum of three replicates

	Total chlorophyll and carotenoid (in parenthesis) content ($\mu\text{g g}^{-1}$ fr. wt.)		
	Days without irrigation		Days after re-irrigation
	0	30	5
TTL-1 Irrigated	1,623 $\pm$ 97 (297 $\pm$ 18)	1,685 $\pm$ 101 (310 $\pm$ 19)	1,696 $\pm$ 93 (311 $\pm$ 22)
TTL-1 Non-irrigated	1,620 $\pm$ 186 (290 $\pm$ 17)	1,205 $\pm$ 121 (240 $\pm$ 22)	1,419 $\pm$ 85 (272 $\pm$ 22)
TTL-2 Irrigated	1,058 $\pm$ 69 (246 $\pm$ 15)	1,134 $\pm$ 51 (265 $\pm$ 16)	1,147 $\pm$ 57 (269 $\pm$ 22)
TTL-2 Non-irrigated	1,080 $\pm$ 92 (250 $\pm$ 15)	655 $\pm$ 92 (187 $\pm$ 17)	796 $\pm$ 72 (220 $\pm$ 19)
TTL-4 Irrigated	1,034 $\pm$ 93 (248 $\pm$ 15)	1,097 $\pm$ 99 (261 $\pm$ 20)	1,135 $\pm$ 102 (263 $\pm$ 18)
TTL-4 Non-irrigated	1,033 $\pm$ 98 (243 $\pm$ 15)	815 $\pm$ 73 (207 $\pm$ 20)	834 $\pm$ 101 (223 $\pm$ 16)
TTL-5 Irrigated	1,232 $\pm$ 105 (233 $\pm$ 14)	1,257 $\pm$ 94 (237 $\pm$ 23)	1,278 $\pm$ 89 (240 $\pm$ 17)
TTL-5 Non-irrigated	1,225 $\pm$ 123 (231 $\pm$ 16)	897 $\pm$ 63 (195 $\pm$ 19)	965 $\pm$ 116 (211 $\pm$ 20)
TTL-6 Irrigated	1,401 $\pm$ 126 (267 $\pm$ 16)	1,455 $\pm$ 131 (275 $\pm$ 21)	1,464 $\pm$ 102 (276 $\pm$ 19)
TTL-6 Non-irrigated	1,420 $\pm$ 121 (268 $\pm$ 20)	1,059 $\pm$ 90 (235 $\pm$ 18)	1,275 $\pm$ 102 (257 $\pm$ 18)
UPASI-2 Irrigated	1,346 $\pm$ 114 (299 $\pm$ 18)	1,379 $\pm$ 103 (303 $\pm$ 27)	1,379 $\pm$ 97 (304 $\pm$ 21)
UPASI-2 Non-irrigated	1,358 $\pm$ 122 (296 $\pm$ 18)	1,110 $\pm$ 105 (242 $\pm$ 18)	1,195 $\pm$ 84 (261 $\pm$ 13)
UPASI-3 Irrigated	1,131 $\pm$ 102 (257 $\pm$ 19)	1,174 $\pm$ 106 (261 $\pm$ 20)	1,190 $\pm$ 83 (266 $\pm$ 19)
UPASI-3 Non-irrigated	1,198 $\pm$ 102 (251 $\pm$ 15)	880 $\pm$ 114 (150 $\pm$ 16)	950 $\pm$ 124 (201 $\pm$ 15)

result indicates that the photochemistry associated with photosystem II in the clones TTL-1 and TTL-6 is more efficient than in UPASI-2.

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 and the photon yield of oxygen evolution under different environmental stresses (Bjorkman and Demmig 1987). They also reported that the Fv/Fm ratio of a normal functioning photosynthetic apparatus is 0.832 for a wide variety of plant species. 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. It is also well known that the ratio of Fv/Fm is a quantitative measure of photochemical efficiency (Jacob 1998) or optimal quantum yield of PS II (Bolhar-Nordenkamp and Oquist 1993). 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 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. 2b). 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, the photosynthetic rate was relatively less affected until the soil moisture reached 10.5 $\pm$ 1% range and it decreased progressively as the soil moisture reduced further. In the present study, on the 30 d of non-irrigation, the soil moisture was 8.01 $\pm$ 1% and there was drastic reduction in the photosynthetic rate in the clones TTL-2, TTL-4, TTL-5 and UPASI-3.

On the 30 d of non-irrigation a non-significant reduction of stomatal conductance was noticed in TTL-1, TTL-6 and UPASI-2, but it was significant in TTL-2, TTL-4, TTL-5 and UPASI-3 as compared to their control plants (Fig. 2d). A comparatively high stomatal conductance in TTL-1, TTL-6 and UPASI-2 may be due to the potential of these clones to maintain higher water potential so as to keep the stomata open, even during extreme water stress. According to Barbora (1994), the adverse effect of water stress on photosynthesis of tea was mainly due to impaired stomatal conductivity. Moreover on the 5 d of re-irrigation the clones TTL-1, TTL-6 and UPASI-2 showed the fastest recovery in the above parameters.

There are several reports that water stress induced reduction in the photosynthetic rate is accompanied by reduction in stomatal conductance and transpiration rate (Barbora 1994; Mengel and Kirkby 1996; Chen et al. 2010). 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 30 d as compared to the

Fig. 2 Effect of drought on Fv/Fm ratio (a), net photosynthetic rate (b), transpiration rate (c) and stomatal conductance (d) in leaves of various clones of tea (*C. sinensis*) which was recorded on the 30 d without irrigation and on the 5 d during re-irrigation. The Fv/Fm ratio, net photosynthetic rate, stomatal conductance and transpiration rate

was measured in terms of relative units, $\mu\text{mol m}^{-2}\text{sec}^{-1}$, $\text{mmol m}^{-2}\text{sec}^{-1}$, and $\text{mmol m}^{-2}\text{sec}^{-1}$, respectively. The 0 d value was taken as 100%. The vertical lines represent SE of the mean value of recordings from three independent experiments, each with a minimum of three replicates (i.e. $n=9$)

control plants (Fig. 2c) while in TTL-2, TTL-4, TTL-5 and UPASI-3 there was a significant reduction in the transpiration rate on the 30 d of non-irrigation as compared to the control plants. 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 (Puthur 2000).

Drought induced lipid peroxidation and the accompanied proline accumulation

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 10 d of non-irrigation itself (data not shown). After 30 d 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 as compared to TTL-2, TTL-4, TTL-5 and UPASI-3 (Fig. 3a). When irrigation was provided to the non-irrigated plants after the 30 d, the proline content decreased in all the clones and the reduction was significant. But in all the clones the final level of proline on the 5 d after re-irrigation was significantly higher than that recorded on the 0 d. A more rapid reduction in the level of proline was found in TTL-1, TTL-6 and UPASI-2 on the 5 d after re-irrigation as compared to the rest of the clones.

Fig. 3 Effect of drought on proline content (a) and MDA content (b) in leaves of various clones of tea (*C. sinensis*) which was recorded on the 30 d without irrigation and on the 5 d during re-irrigation. The proline content and MDA content was measured in terms of $\mu\text{g g}^{-1}\text{fr.}$

wt. and $\mu\text{mol g}^{-1}\text{fr. wt.}$, respectively. The 0 d value was taken as 100%. The vertical lines represent SE of the mean value of recordings from three independent experiments, each with a minimum of three replicates (i.e. $n=9$)

Proline is one of the organic molecules that accumulates in plants exposed to environmental stresses such as salt, drought, temperature etc. (Saraswathy et al. 1992; Saradhi et al. 1995; Puthur et al. 1996; Chakraborty et al. 2001; Matysik et al. 2002; Puthur and Rajan 2006; Szabados and Savouré 2010). Rajasekar et al. (1988) observed accumulation of higher levels of proline in the clones UPASI-2, UPASI-9 and UPASI-10 under prolonged drought stress, which was taken as indicative of their drought tolerant nature. 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 d 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 were responsible for its accumulation under stress condition. Proline is attributed with 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; Netto et al. 2005). Moreover, proline can act as a signaling molecule to modulate mitochondrial functions, influence cell proliferation or cell death and trigger specific gene expression, which can be essential for plant recovery from stress (Szabados and Savouré 2010). 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. Screening of ten green gram (*Vigna radiata*) genotypes for drought tolerance under

depleting soil moisture conditions (Naidu et al. 2001) reported highest proline accumulation in the drought tolerant genotypes K 851 and LGG 407. All these observations revealed that the accumulation of proline during stress is an indication of drought tolerance of a particular species.

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. 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. 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).

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 and is able to stabilize proteins, DNA and membranes, especially during stress conditions. Therefore, in the present investigation, efforts were also made to see whether there is any correlation between free radical generation and proline accumulation rate. The extent of free radical generation was monitored through the lipid peroxidation occurring in the tissues. Malondialdehyde (MDA) was measured considering it as a major cytotoxic product of lipid peroxidation and an indicator of free radical production (Saradhi et al. 1995). The level of MDA content

was found to increase significantly in the non-irrigated plants throughout the period of study and reached the maximum by the 30 d (Fig. 3b) ($p < 0.01$). The clones TTL-2, TTL-4, TTL-5 and UPASI-3 had higher MDA content on the 30 d of non-irrigation as compared to TTL-1, TTL-6 and UPASI-2. On the 5 d after re-irrigation, all the clones exhibited a significant decrease in the MDA content. The percentage decrease was higher in clones TTL-1, TTL-6 and UPASI-2, as compared to other clones.

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. According to Jia et al. (2003), under drought stress many metabolic processes can produce active oxygen species and that the hindrance to electron transport in mitochondria and chloroplast can result in the production 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.

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 (Puthur et al. 1996; Jacob 1998; Alscher et al. 2002; Gill and Tuteja 2010). 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 and this balance was achieved at a faster rate in TTL-1, TTL-6 and UPASI-2 than the other clones studied.

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.

Conclusion

Maintenance of high shoot weight, increased water potential, lesser reduction in total chlorophyll and carotenoid content, less reduction in Fv/Fm values, non significant reduction in stomatal conductance and transpiration rate, increased net photosynthetic rate, high levels of proline accumulation, lesser extent of lipid peroxidation in the clones TTL-1, TTL-6 and UPASI-2 as compared to other clones studied, under situations of less soil moisture content (8%) warrants the classification of these clones as drought tolerant types. Moreover re-irrigation after 30 d of stress

has resulted in a faster recovery in most of the parameters studied in these clones, which again acknowledges the drought tolerance potential of these three clones.

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