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Characterization of Photosynthetic Events and Associated Changes in Various Clones of Tea (*Camellia sinensis* L.) under Low Temperature Conditions

L. A. Netto[1], K. M. Jayaram[2], P. Haridas[1], and Jos T. Puthur[3]*

[1]R&D Department, Tata Tea Ltd., Munnar, Kerala, India
[2]Department of Botany, Calicut University, Tenhipalam, Kozhikode, Kerala, India
[3]Postgraduate and Research Department of Botany, St. Thomas College, Pala, Arunapuram-686 574, Kottayam, Kerala, India

Low temperature is one of the major environmental factors determining the growth rate of *Camellia sinensis* (L.), and photosynthesis is one major metabolic process commonly used as a tool for identifying low temperature stress effects on plants. The Fv/Fm values at 6:30 a.m. (300-400 μmol $m^{-2} s^{-1}$) did not vary much between the clones of tea plants. Further, when the light incidence increased at mid noon hours (1700-1800 μmol $m^{-2} s^{-1}$), the Fv/Fm values recorded a decline, irrespective of the clones. Of the 10 clones of tea plants under investigation, 3 clones, namely CRA-6017, TTL-6 and SMP-1, recorded a sharp decrease in the Fv/Fm ratio by 2 p.m. The malondialdehyde (MDA) levels in all the clones increased from 9 a.m. (1500-1700 μmol $m^{-2} s^{-1}$) to 2 p.m. and from 4:30 p.m. (900-1100 μmol $m^{-2} s^{-1}$) onwards it started to decrease and reach the levels equivalent to 6:30 a.m. by 7 p.m. (5-10 μmol $m^{-2} s^{-1}$). But the clones of TTL-1, TTL-4 and UPASI-9 showed low temperature tolerance as appeared in chlorophyll a fluorescence response. They showed a higher percentage increase in MDA levels, as compared to TTL-6, CRA-6017 and SMP-1, which showed low temperature susceptibility. But the reduction in the level of MDA by 4:30 p.m. (recovery) was faster in the clones TTL-1, TTL-4 and UPASI-9, as compared to TTL-6, SM/OM/54 and SMP-1. The result indicates that in TTL-6, SMP-1 and SM/OM/54, the toxic oxygen species scavenging mechanisms may be less functional as compared to other clones. The percentage increase of proline and carotenoids was higher in clones TTL-1, TTL-4 and UPASI-9 as compared to TTL-6, CRA-6017 and SMP-1.

Keywords: Camellia sinensis, clones, low temperature, photosynthesis

Tea [*Camellia sinensis* (L.) O. Kuntze] is an important plantation crop of India that is grown in varied agro-climatic conditions. This particular plantation industry is facing two challenges at present. One is the high production cost that exceeds the present market rate of processed tea and the other is the high demand for quality tea in domestic and world markets. In order to meet these challenges, high-yielding clones with maximum quality potential has been sought that usually paralleled with the effort to select clones suite to the specific climatic regions (Joshy and Palani, 1998; Vyas et al., 1998).

Low temperature is one of the major environmental factors determining the growth rate of tea. It has been shown that temperatures below 13°C adversely affect the biosynthetic activities in tea plant (Barua, 1989; Hudson and Muraleedharan, 1996). Tea bushes are susceptible to winter desiccation and frost damage (Fuchinoue, 1985). Frost damage results in visual symptoms such as scorching of flush shoots and maintenance foliage in the light incidence as well as defoliation near the plucking table under moderate occurrence of frost, and die back of branches and splitting of bark if it is severe (Hudson and Muraleedharan, 1996).

Most of the high yielding tea clones are taken up widely for plantations in high altitudes, where considerable seasonal and diurnal lowering in temperature is noticed. In south India an area of 2816 ha is affected by low temperature and frost, which corresponds to 15.6% of the area under tea cultivation in the Nilgris and 16% in the high ranges (Hudson and Muraleedharan, 1996). Concerning these major problems, it would be appropriate to select clones of tea, which are better suited to the low temperature conditions.

Photosynthesis is affected greatly by any stress and it is commonly used as a tool for identifying low temperature stress effects on plants (Berry and Björkman, 1980; Larcher, 1994). This physiological process is

*Corresponding author; fax +91-482-221-6313
e-mail jtputhur@rediffmail.com

considered primarily for the rapid selection of plants most suitable for different climatic conditions. As photosynthesis and yield are closely related, photosynthetic performance of plant species is counted as a direct relation to yield of a particular clone (Zelitch, 1982). Although clonal variations in photosynthetic features and other associated physiological functions in relation to low temperature stress have been reported in other plant species (Janssen et al., 1995), such information is lacking for tea clones (Joshi and Palani, 1998). Therefore the present investigation was undertaken to determine the clonal variations in various physiological functions of tea with relation to low temperature.

MATERIALS AND METHODS

Ten different clones of tea, *C. sinensis*, were selected for the present study; TTL-1, TTL-2, TTL-4, TTL-5, TTL-6, SMP-1 and SM/OM/54 (released by Tata Tea Limited, Munnar, Kerala, India), UPASI-9 and CRA-6017 (released by United Planters' Association of South India – UPASI, Valparai, Tamil Nadu, India), and TRI-2025 (released by Tea Research Institute of SriLanka). All the plants were in their 3rd year from planting and were planted at a spacing of 4 × 2.5 ft along a single hedge at Tata tea experimental plots, Madupetty, Kerala, India. The study was carried out in the winter months (December to February) of 2001, 2002 and 2003 during which the temperatures varied between 3-12°C. During the winter months, maximum damage to tea plants was recorded for the past 20 consecutive years in Madupetty region. The observations related to plant response against low temperature were recorded at 6:30 a.m. (300-400 μmol $m^{-2} s^{-1}$, 3°C), 9 a.m. (1500-1700 μmol $m^{-2} s^{-1}$, 7°C), 11:30 a.m. (1700-1800 μmol $m^{-2} s^{-1}$, 12°C), 2 p.m. (1700-1800 μmol $m^{-2} s^{-1}$, 10°C), 4:30 p.m. (900-1100 μmol $m^{-2} s^{-1}$, 5°C) and 7 p.m. (5-10 μmol $m^{-2} s^{-1}$, 3°C). The values given in the brackets represent photosynthetically active radiation (PAR) and temperature corresponding to the hour of data recording, respectively. Observations were repeated for twelve different days in the months of December to February.

Proline content was estimated according to the procedure of Bates et al. (1973). Malondialdehyde (MDA) content was determined as described by Heath and Packer (1968). For estimating photosynthetic pigments, samples of fresh leaf tissue were extracted with 80% acetone, and estimation carried out according to Arnon (1949).

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

RESULTS AND DISCUSSION

In both forest and plantation crop nurseries, frost or low temperature tolerant varieties are assessed by various traditional methods including visual assessment of damage (Ritchie, 1991; Vyas et al., 1998). In the present study, damage caused by low temperature

Table 1. Fv/Fm measurements recorded in leaves of tea plants growing in low temperature conditions at various time intervals. The data is an average of recordings from three independent experiments each with three replicates (n=9). The data represent mean±SE.

Clone	6:30 a.m.	9:00 a.m.	11:30 a.m.	2:00 p.m.	4:30 p.m.	7:00 p.m.
TTL-1	0.784 ± 0.06	0.750 ± 0.05	0.651 ± 0.05	0.660 ± 0.05	0.738 ± 0.07	0.796 ± 0.07
TTL-2	0.786 ± 0.05	0.758 ± 0.06	0.613 ± 0.06	0.645 ± 0.05	0.723 ± 0.07	0.788 ± 0.05
TTL-4	0.787 ± 0.06	0.770 ± 0.06	0.644 ± 0.06	0.673 ± 0.06	0.739 ± 0.05	0.796 ± 0.05
TTL-5	0.783 ± 0.04	0.756 ± 0.07	0.649 ± 0.05	0.634 ± 0.06	0.712 ± 0.04	0.791 ± 0.06
TTL-6	0.770 ± 0.05	0.713 ± 0.07	0.539 ± 0.05	0.573 ± 0.06	0.701 ± 0.05	0.725 ± 0.04
U-9	0.775 ± 0.05	0.729 ± 0.05	0.612 ± 0.06	0.651 ± 0.05	0.723 ± 0.06	0.781 ± 0.06
CRA-6017	0.785 ± 0.06	0.714 ± 0.05	0.565 ± 0.05	0.556 ± 0.04	0.689 ± 0.06	0.749 ± 0.05
TRI-2025	0.787 ± 0.07	0.732 ± 0.04	0.608 ± 0.06	0.605 ± 0.04	0.699 ± 0.06	0.765 ± 0.05
SMP-1	0.780 ± 0.05	0.670 ± 0.05	0.481 ± 0.04	0.562 ± 0.05	0.651 ± 0.05	0.739 ± 0.07
SM/OM/54	0.778 ± 0.04	0.737 ± 0.06	0.584 ± 0.04	0.673 ± 0.06	0.735 ± 0.07	0.793 ± 0.07

and frost was assessed by physiological responses of plants as evaluated by monitoring various parameters like chlorophyll a fluorescence and extent of lipid peroxidation.

Measurement of chlorophyll a fluorescence is non-destructive, easy and fast and has been used as a reliable method for assessing frost and low temperature tolerance in plants (Mohammad et al., 1995). 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 photosystem (PS) II (Bolhar-Norden-kampf and Öquist, 1993; Schreiber and Bilger, 1993). The Fv/Fm values of the tea clones recorded on each day at 2.5 h interval, starting from 6:30 a.m. to 7 p.m., are shown in Table1.

Fv/Fm values at 6:30 a.m. did not vary much between the clones and was in the range of 0.770 to 0.800. With increase in light incidence at 11:30 a.m. and 2 p.m., the Fv/Fm values recorded a decline in all the clones. In 3 clones, namely CRA-6017, TTL-6 and SMP-1, a sharp decrease in the Fv/Fm ratio was recorded by 11:30 a.m. to 0.565, 0.539 and 0.481, respectively. The observation suggested that the functioning of the photosynthetic apparatus is severely affected by the cumulative effects of low temperature and high light. Readings at 4:30 p.m. and 7 p.m. showed that the changes in the values of Fv/Fm ratio developed a reversing trend and tendency to recover. By 7 p.m., in all the clones, the Fv/Fm values were almost restored to the initial values at 6:30 a.m.. It is a well-known phenomenon that capacity of plants to utilize the light absorbed declines significantly upon exposure to environmental stresses such as drought, salt and low temperature (He et al., 1995; Dubey, 1997; Giardi et al., 1997). This condition wherein the plants are unable to utilize the light absorbed results in the phenomenon called photoinhibition (Puthur, 2000).

Photoinhibition is caused largely due to the production of toxic oxygen species (Scandalios, 1993; Asada, 1994; Alam and Jacob, 2002). The increase in the generation of toxic oxygen species leads to a substantial inactivation/destruction of lipids, proteins and nucleic acids (Halliwell and Gutteridge, 1986; Scandalios, 1993). An important target for the action of toxic oxygen species is PS II (Powles, 1984; Krause, 1988) and as a result a significant decline in PS II activity was noted in a wide variety of plants exposed to photoinhibitory conditions (Constant et al, 1997; Keren et al, 1997). Photoinhibition is more prominent in the conditions of high light and low temperature (Björkman and Powles, 1984; Huner, et al., 1993; Wise, 1995).

The faster recovery in Fv/Fm values observed in clones such as TTL-1, TTL-4 and UPASI-9 suggests that there may be an effective damage negating operations, such as free radical scavenging mechanism, functioning within them and enabling them to reduce the damage caused by toxic oxygen species. Malondialdehyde (MDA) levels indicate the extent of lipid peroxidation caused by toxic oxygen species (Halliwell and Gutteridge, 1990; Alia et al., 1995; Saradhi et al., 1995; Puthur, 2000). To understand the level of

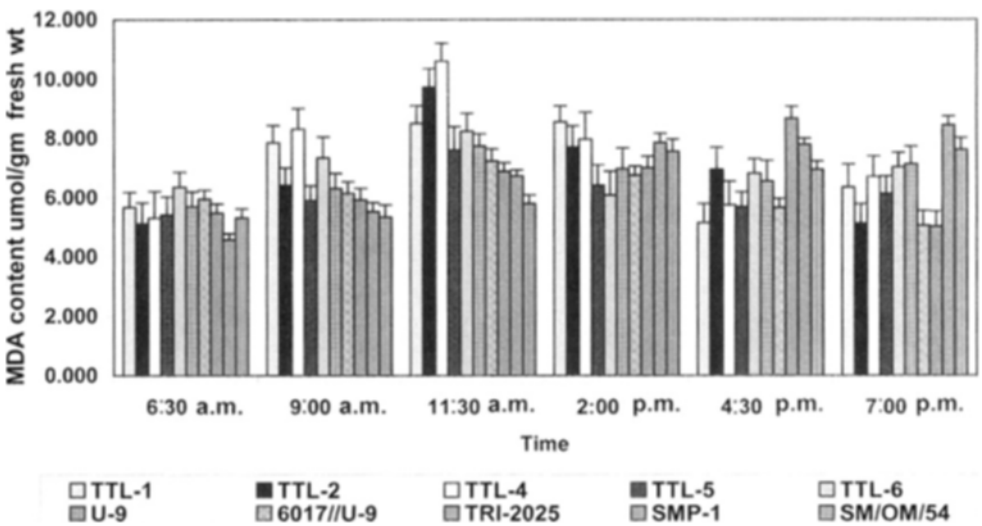

Figure 1. MDA content in leaves of tea plants growing in low temperature conditions. The vertical lines represent SE of the mean value of recordings from 3 independent experiments, each with a minimum of 3 replicates.

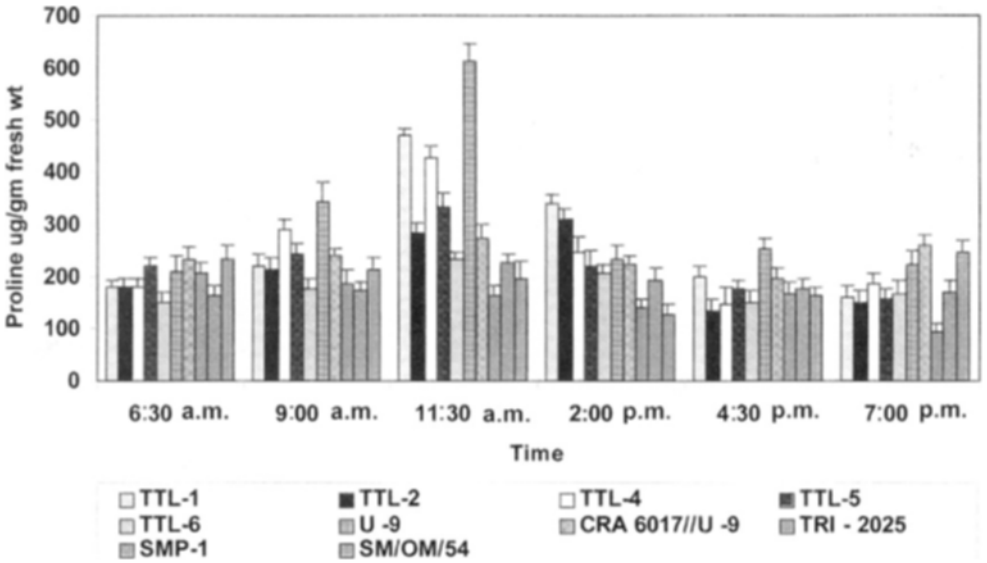

Figure 2. Proline content in leaves of tea plants growing in low temperature conditions. The vertical lines represent SE of the mean value of recordings from 3 independent experiments, each with a minimum of 3 replicates.

damage caused due to photoinhibition, the MDA content was measured at every 2.5 h interval from 6:30 a.m. to 7 p.m. The results showed a clear indication that MDA levels increased from 6:30 a.m. to 11:30 a.m. and then started to decrease and reached the levels equivalent to the level at 6:30 a.m. by 7 p.m. (Fig.1) in all the clones studied. But a surprising observation was made in the clones of TTL-1, TTL-4 and UPASI-9 that gave an indication of low temperature tolerance with regard to chlorophyll a fluorescence response. These clones showed a higher percentage increase in MDA levels (especially at 9 a.m., 11:30 a.m. and 2 p.m.), as compared to the clones TTL-6, CRA-6017 and SMP-1, which showed greater sensitivity to low temperature. But by 6 p.m. the levels of MDA were reduced in clones TTL-1, TTL-4 and UPASI-9, but in clones TTL-6, SM/OM/54 and SMP-1, the level of MDA remained almost the same as that of mid-noon hours when peak MDA levels were recorded. The results indicate that in TTL-6, SMP-1 and SM/OM/54, scavenging mechanisms against reactive oxygen species are less functional as compared to other clones.

Several scavenging mechanisms against toxic oxygen species are known to operate in plants. Proline (Saradhi et al., 1995) and carotenoid species (Siefermann-Hams,1987; Young et al.,1997) are known to play important roles in non enzymatic detoxification of toxic oxygen species. In our studies it was observed that proline is over-produced in clones TTL-1, TTL-4 and UPASI-9 (Fig. 2). Concomitant with a rise in the MDA levels in these three clones, an enormous increase in levels of proline and carotenoids was observed, especially in the hours of 9 a.m., 11:30 a.m. and 2 p.m. This over synthesized proline may be one of the means to take care of the excessive toxic oxygen species synthesized in those clones. Although the exact mechanism of scavenging by proline remains unelucidated (Puthur et al., 1996), it has been suggested that proline could be forming non-toxic hydroxyproline by reacting with $\cdot OH^1$ (Smirnoff and Cumbes, 1989; Matysik et al., 2002).

The increased levels of carotenoids recorded in clones TTL-1, TTL-4 and UPASI-9 (Fig. 3) could be an indication of yet another mechanism functional in these clones for nonenzymatic detoxification of toxic oxygen species. Carotenoids quench singlet oxygen and also avoid the generation of reactive oxygen species by absorbing excess excitation energy from chlorophyll by direct transfer (Arora et al., 2002). The concurrent increase and decrease of proline and carotenoids along with the MDA level shown in this study is a clear indication of their role in detoxifying reactive oxygen species. The plant system may be favouring for the overproduction of proline and carotenoids only when the situation warrants and the levels of these two entities decrease, along with a decrease in the level of reactive oxygen species.

Bisht et al. (1996) have reported that the clone UPASI-9 showed minimum photoinhibition in low

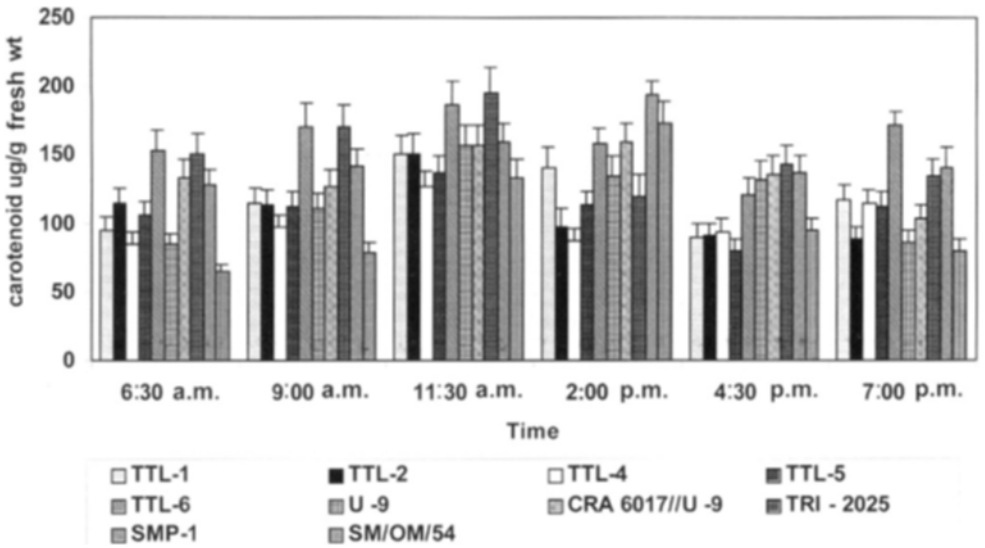

Figure 3. Carotenoid content in leaves of tea plants growing in low temperature conditions. The vertical lines represent SE of the mean value of recordings from 3 independent experiments, each with a minimum of 3 replicates.

temperature and frost conditions. Joshi and Palni (1996) have also confirmed the high thermo-tolerance of the clone UPASI-9 when compared to other clones in a study where measurement of photosynthetic rates at different temperatures was done. UPASI-9 is one of the parents of TTL-1 and it can be assumed that the trait of frost tolerance of UPASI-9 to pass on to the progeny.

The identification of tea clones such as TTL-1, TTL-4 and UPASI-9 as more low temperature and frost tolerant compared to others would be outmost importance for identifying clones suitable for a region frequented with low temperature conditions. Promotion of such low temperature and frost tolerant clones in such areas will ensure the production of estimated yield and quality of tea throughout the season.

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