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Tea (*Camellia sinensis* (L.) O. Kuntze) clone with lower period of winter dormancy exhibits lesser cellular damage in response to low temperature

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Abstract

There is no literature available on the response of tea plant to low temperature. We studied the effect of low temperature on two clones of tea with contrasting periods of winter dormancy, a phenomenon in which the growth of apical shoots of tea is diminished during winter months. Clone 'Teenali 17/154' (TNL) showed shorter periods of winter dormancy than clone 'Kangra Jat' (KNJ). Low temperature (5 °C) resulted in increase of metabolic superoxide ($O_2^{\cdot-}$) content and cellular damage (as measured by tetrazolium chloride reduction test) in both the clones, however, the increase was lesser in the case of TNL compared to KNJ. Activities of superoxide dismutase (SOD; EC 1.15.1.1), ascorbate peroxidase (APX; EC 1.11.1.11) and glutathione reductase (GR; EC 1.6.4.2) increased in both the clones in response to low temperature however, GR activity exhibited significant differences ($P < 0.05$) between the two clones. Low temperature caused increase in the intensity of various isozymes of SOD, APX and GR. A new isozyme of SOD (Cu/Zn type) was induced in both the clones at low temperature. Significantly higher GR activity in both the clones suggested a role of this enzyme in imparting better protection to tea at low temperature. Also, clonal variation for GR isozyme was observed between the clones. Based on these results it appears that TNL, a clone with relatively lesser period of winter dormancy experiences lesser oxidative stress in response to low temperature compared to KNJ, a clone with relatively higher period of winter dormancy.

Keywords: *Camellia sinensis* (L.) O. Kuntze; Cellular damage; Oxidative stress; Superoxide anions; Winter dormancy

1. Introduction

Tea (*Camellia sinensis* (L.) O. Kuntze) is an important beverage crop of the world and is also known for its medicinal properties. It is a woody, perennial, evergreen plant grown in different agro-climatic zones across the world lying within 45°N to 34°S latitude [12]. Commercial production of tea depends upon the yield of apical buds and associated two leaves, commonly known as two and a bud (TAB), which is harvested at weekly/bi-weekly intervals depending upon the growth rate. TAB continues to grow throughout the year in areas near to equator. However, as the plants are grown beyond 16° north and south of equator, growth of the TAB is diminished during winters and plants are said to be dormant, a phenomenon known as winter dormancy [4]. Winter dormancy in tea affects yield, land use, infrastructure and labour.

Decline in temperature during periods of winter dormancy is one of the most obvious environmental factors experienced by tea plants. It is known that exposure of plants to abiotic factors including low temperature causes oxidative stress [6] in which increased production of reactive oxygen species (ROS) is evident. Low temperatures in light increase the levels of reactive oxygen species (ROS) in plants mainly because of chilling-induced photoinhibition [36], where molecular oxygen may act as an electron acceptor in place of $NADP^+$. ROS reacts with lipids, proteins and DNA, thus, causing immense cellular damage. Therefore, plants contain an array of enzymatic and non-enzymatic mechanisms to protect themselves from increased accumulation of ROS [10].

Abbreviations: APX, ascorbate peroxidase; GR, glutathione reductase; KNJ, Kangra Jat; LT, low temperature; NBT, nitroblue tetrazolium; $O_2^{\cdot-}$, superoxide; PAR, photosynthetic active radiations; ROS, reactive oxygen species; SOD, superoxide dismutase; TAB, two and a bud; TNL,Teenali 17/154; TTC, triphenyl tetrazolium chloride.

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Hypothesis by Allen [2] and transgenic studies by McKersie et al. [19] and Samis et al. [28] showed that overexpression of ROS scavenging enzymes had marked affect on yield enhancement. This suggests that the management of oxidative stress is key to plant performance under stress conditions. Oxidative stress mediated by low temperatures has been implicated in cellular and metabolic damage, thus, affecting growth and development of plants [8,24]. Therefore, it is pertinent to ask if tea also experiences cellular damage in response to low temperature and that if there is a correlation between the degree of damage and the tea clones exhibiting variable periods of winter dormancy. What happens to the accumulation of ROS in response to low temperature? How various enzymes involved with ROS scavenging respond to low temperature? Such questions have not been addressed in tea, which is an important crop of commerce throughout the world.

2. Results and Discussion

Tea is grown in different agro-climatic zones of the world and its yield depends upon the rate of growth of TAB. Regions lying between 16° north or south of equator show diminished growth of TAB during winter months, thus affecting commercial production of tea. Variability among clones is observed in duration of winter dormancy in tea. We estimated winter dormancy by monitoring the growth of apical bud [22] in the available clones growing in the experimental farm of the Institute. TNL showed 47 days of winter dormancy whereas KNJ experienced 77 days of winter dormancy (Fig. 1). Although, the growth of TAB never stopped completely, we have considered growth of less than 0.1 mm per 3 days as a condition of winter dormancy. Thus, apparently, clone TNL behaved in an efficient manner during winter periods than KNJ.

Environment during winter dormancy is characterized by the periods of low temperatures, which is known to affect physiological and metabolic dysfunctions in plants. It has been observed that leaves are more sensitive to low temperatures under irradiation than in darkness. Further, during low temperatures even the optimum light intensities become stressful [32]. Such a situation of low temperature is highly conducive for the imposition of oxidative stress [6]. Oxidative stress mediated by low temperatures has been implicated in cellular and metabolic damage, thus, affecting growth and development of plants [8,24].

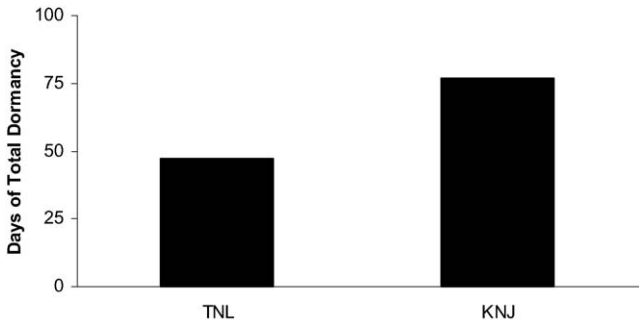

Fig. 1. Period of winter dormancy in clone 'TNL' and 'KNJ'. Growth rate of the apical buds were measured throughout the period of study. Dormant period for each clone was calculated as the total number of days when no appreciable growth (< 0.1 mm 3 days^{-1}) was observed for apical buds. For measuring rate of bud growth, twenty apical buds were tagged each from three separate plants of both the clones.

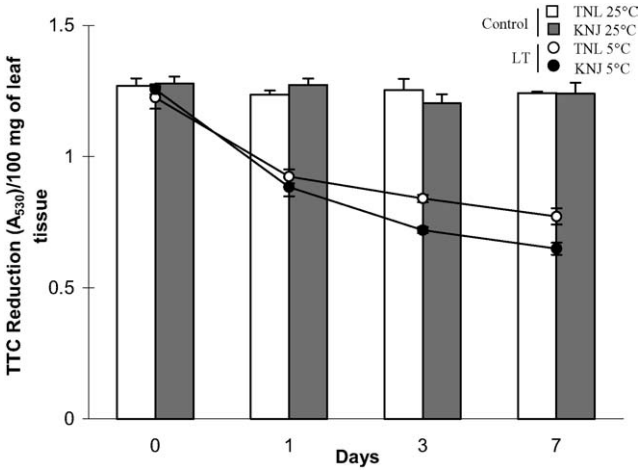

Fig. 2. Effect of low temperature on cellular damage in two different clones of tea. Cellular damage was assessed in terms of reduction of TTC by the leaf tissue. Reduced formazan was extracted from tissue and estimated by monitoring the absorbance at 530 nm. All the values are mean of four independent replicates ± SE.

Cellular damage caused by low temperature can be assessed by TTC reduction test [31]. When tea plants were exposed to low temperature, both the clones showed cellular damage within 1 day of exposure to 5 °C (Fig. 2). Although, both the clones showed damage in response to low temperature, TNL exhibited 12% lesser damage on day 7 of exposure to low temperature compared to KNJ, which was statistically significant ($P < 0.05$). It may be noted that the clone TNL, which exhibited lesser period of dormancy, also showed lesser damage in response to low temperature and the clone KNJ with longer winter dormancy suffered greater damage during low temperature.

Low temperature mediated cellular damage has largely been associated with the action of ROS [35]. Different methods have been used to estimate $O_2^{\cdot-}$, of which, tetrazolium based assay is preferred in spite of its limitations [13,33]. NBT test revealed that TNL had higher base levels of $O_2^{\cdot-}$ compared to KNJ (Fig. 3). On exposure to 5 °C, $O_2^{\cdot-}$ content increased in both the clones. However, in TNL the increase was 120% compared to control on day 7 whereas, in KNJ, $O_2^{\cdot-}$ increased by 153% compared to control during the same period. Although, significant differences ($P < 0.05$) were observed among clones and temperatures, their interactions did not show any statistically significant difference. Results suggested that low temperature mediated generation of ROS could be responsible for the observed cellular damage as exhibited in other plants as well [14,17,34].

Lesser cellular damage in TNL compared to KNJ suggested a relatively efficient anti-oxidative and cell components protecting system in TNL. In the present study, we