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Photoassimilatory and photorespiratory behaviour of certain drought tolerant and susceptible tea clones

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Abstract

Net photosynthetic rate (P_N) in the mother leaves was higher in the drought tolerant (DT) clones of tea (*Camellia sinensis*) while liberation of the fixed ^{14}C in light from the mother leaves was higher in the drought susceptible (DS) clones. The DT clones translocated more photosynthates to the crop shoots (three leaves and a bud) from the mother leaf than the DS clones. Concentrations of RuBP carboxylase (RuBPC) or oxygenase (RuBPO) had no relationship with the drought tolerant nature of tea clones but their ratio correlated with the same. DT tea clones had higher catalase activity that could scavenge the hydrogen peroxide formed in the photorespiratory pathway and thereby reduced photorespiration rate (P_R). The ratio of RuBPC/RuBPO had a positive correlation with P_N and catalase activity. Negative correlation between RuBPC/RuBPO and P_R and between catalase activity and RuBPO activity was established.

Additional key words: *Camellia sinensis*; catalase; net photosynthetic rate; photorespiration; RuBPC; RuBPO; translocation.

Introduction

Characterisation of tea clones with respect to quality and productivity is an important criterion in order to develop markers for plant breeding and improvement programmes. The primary anabolic and catabolic processes that determine productivity are photosynthesis and photorespiration. Inhibition of net photosynthetic rate (P_N) by O_2 in C_3 plants is caused by the O_2-dependent formation of CO_2 during photorespiration (Zelitch 1982). The photorespiratory processes are initiated by ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) (Ogren 1984) and are regulated by the O_2/CO_2 ratio and temperature. Increasing the O_2 concentration increases the RuBPO activity as well as that of glycollate oxidase, a flavoprotein that oxidizes glycollate to glyoxylate with the production of hydrogen peroxide (Zelitch and Ochoa 1953). Glycollate oxidase is located in leaf peroxisomes (Tolbert 1980). The non-enzymatic peroxidation of keto acids in the photorespiratory pathway by hydrogen peroxide rapidly decarboxylates substrates such as glyoxylate to CO_2 and formate and converts hydroxypyruvate to CO_2 and glycollate (Zelitch 1990a).

Although leaf peroxisomes contain large amounts of catalase (Tolbert 1980), the enzyme may be inefficient in breaking down low concentrations of hydrogen peroxide because of its kinetic characteristics (Halliwell 1984). Hence plants with enhanced catalase activity might evolve less photorespiratory CO_2 because the peroxidative decarboxylation of ketoacids is slowed. Such plants may, therefore, show increased P_N.

As there is no literature available on a perennial crop such as tea regarding the relationship of catalase with P_N and P_R, we undertook this experiment to determine the role of this enzyme. We studied also P_N and P_R in mother leaves and translocation of photosynthates to the crop shoots from the mother leaves in established drought-tolerant (DT) and -susceptible (DS) tea clones to characterise them physiologically. To characterise them biochemically, we measured the activities of RuBPCO and catalase. The relationship between RuBPCO and catalase and the relationship of these enzymes with P_N and P_R was also determined.

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Abbreviations: DS – drought susceptible; DT – drought tolerant; P_G – gross photosynthetic rate; P_N – net photosynthetic rate; P_R – photorespiration; RuBPC – ribulose-1,5-bisphosphate carboxylase; RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBPO – ribulose-1,5-bisphosphate oxygenase.

Materials and methods

Tea clones (*Camellia sinensis*) were planted during the year 1969 in the Experimental Farm of UPASI Tea Research Foundation, located in the Anamallais of Western Ghats in southern India (10° 30'N, 77°0'E, altitude 1 050 m a.s.l.). Some of these clones are drought tolerant (UPASI-1, UPASI-2, and UPASI-9) and some drought susceptible (UPASI-3, UPASI-8, and UPASI-17). This is based on the assessment of their survival and field performance during drought periods over several years in different tea growing areas of south India (Satyanarayana *et al*. 1992) and on morphological and physiological factors influenced by drought (Rajasekar *et al*. 1988). All the experiments were conducted during the dry season (January-March) of south India.

Physiological studies: Radiotracer (^{14}C) method of Hale and Weaver (1962) was used for analysing CO_2 assimilation in the mother leaves and translocation of photosynthates from the mother leaves to crop shoots. Photorespiratory loss was estimated following the method of Barbora and Barua (1988). In brief, a mother leaf subtending a growing axillary shoot (three leaves and a bud) was exposed to 25 mm^3 of labelled sodium carbonate (procured from the Board of Radiation and Isotope Technology, Government of India) in a closed transparent plastic bag on a bright sunny day under given conditions. Release of labelled carbon from the sodium carbonate was augmented by lactic acid followed by HCl. The photoassimilation of ^{14}C by the mother leaf was allowed for one hour. Then a plastic bag containing 0.1 M KOH solution replaced the plastic bag containing labelled sodium carbonate, and the set up was left over for another one hour. Then the KOH solution was collected for quantifying the ^{14}C released during photorespiration. The mother leaves and the subtending crop shoots were harvested on the next day and powdered. Radioactivity counting was carried out using a liquid scintillation counter (*Wallac Oy*, *LKB Rackbeta*, model *1214*, Turku, Finland). The ^{14}C activity recorded in the mother foliage and harvested shoot provided the data on P_N and translocation, respectively, while the counting noted in the KOH solution gave P_R. P_N and P_R were added to get P_G.

Biochemical studies: Young shoots comprising three leaves and a bud were harvested immediately before the analysis of enzymes. Activity of RuBPCO (E.C.4.1.1.39) was determined by measuring the incorporation of $^{14}CO_2$ into acid-insoluble material according to Gimenez *et al.* (1992) with certain modifications. Leaves were ground in a pre-chilled mortar and pestle with 20 mM Tris-HCl (pH 8.0). Aliquots of the extract were assayed at 25 °C in a stirred oxygen electrode chamber (*Orion Research*, model *290A*, Beverly, USA). Activation of enzymes in the crude extract was allowed to proceed for three minutes at 25 °C and the reaction was started by addition of RuBP substrate. $^{14}CO_2$ incorporation continued for 30 s before the addition of formic acid. The assay mixture was dried overnight at 80 °C and ^{14}C activity determined using the scintillation counter. Protein content in the aliquot was quantified following the standard method of Lowry *et al.* (1951).

Catalase (E.C. 1.11.1.6) was assayed following the method of Zelitch (1990b). Leaf tissue was homogenised in a pre-chilled mortar and pestle with 7.5 cm^3 of ice-cold 0.05 M K-phosphate buffer (pH 7.4) and 11.3 mg dithiothreitol. The suspension was centrifuged at 16 000×*g* for 3 min and the clear supernatant was assayed for catalase activity by measuring the rate of decrease in absorbance of hydrogen peroxide at 240 nm.

Statistical analysis: Three bushes represented each tea clone. All the experiments were carried out three times on three different days with triplicates. Since the values of biochemical estimations were compared with physiological characteristics, all enzyme estimations and physiological measurements were done on the same day to avoid the influence of external factors.

Results and discussion

Translocation: Significantly greater translocation of the recently fixed photosynthates from the mother leaves to the sink regions (growing axillary shoots) was observed in DT clones (Table 1) than in the DS ones. This observation substantiated the findings of Satyanarayana *et al.* (1992) who observed high yield in these clones (UPASI-1, UPASI-2, and UPASI-9) during drought season. Translocation of photosynthates from the mother leaves to the crop shoots was greater in UPASI-2 and smaller in UPASI-3. Negative correlation was observed between translocation and P_N ($r = -0.53$) which was significant at 5 % probability. This negative correlation could be due to the hypothesis that translocation of ^{14}C to the growing axillary shoot from the mother leaf would lead to lesser ^{14}C in the mother leaf. Storage of the fixed carbon in parts other than the growing axillary shoots could make a tea plant low yielding. Therefore a clone with greater translocation ability towards the growing axillary shoots should be selected for tea productivity.

Photoassimilation: P_G in DS clones was slightly higher than that of the DT clones and P_N was slightly higher in DT clones. It was because more fixed carbon in DS clones was utilised for photorespiration. Photorespiratory loss was the highest in UPASI-3 and the lowest in UPASI-2.

Table 1. Photoassimilatory characteristics of drought tolerant/susceptible tea clones. Experiments were carried out in the field between 08:00 and 10:00 h on three different days during the dry month, April 2002. Net photosynthetic rate, P_N and photorespiration rate, P_R are expressed as % ^{14}C assimilated (evolved), respectively, by maintenance leaf. $P_N + P_R = P_G$. Translocation is expressed as % ^{14}C translocated from maintenance leaf to growing axillary shoot. Harvests comprising three leaves and a bud were used for the extraction of enzymes. Enzymes were analysed during three different days during which physiological experiments were carried out. Ribulose-1,5-bisphosphate carboxylase (RuBPC) [µmol($^{14}CO_2$ fixed) s^{-1} kg^{-1}(protein)] and oxygenase (RuBPO) [µmol(O_2 consumed) s^{-1} kg^{-1}(protein)] activities and their ratio (C/O) and catalase activity [units per mg(protein)] were also determined.

	Clone	P_G	P_R	P_N	Transl.	RuBPC	RuBPO	C/O	Catalase
Drought tolerant	UPASI-1	82.90	1.07	81.83	16.03	144.30	59.8	2.41	2.97
	UPASI-2	78.60	1.01	77.59	20.39	123.50	62.4	1.98	2.57
	UPASI-9	80.80	1.03	79.77	18.17	135.10	56.9	2.38	3.34
	Mean	80.80	1.04	79.73	18.20	134.30	59.7	2.26	2.96
Drought susceptible	UPASI-3	82.90	1.97	80.93	15.13	136.80	65.6	2.03	2.11
	UPASI-8	81.70	1.82	79.88	16.48	145.60	75.9	1.92	2.03
	UPASI-17	79.90	1.78	78.12	18.32	157.60	89.2	1.77	2.01
	Mean	81.50	1.86	79.64	16.64	146.60	77.6	1.91	2.05
Critical difference	between clones	1.64	0.11	1.71	1.02	2.61	2.40	0.09	0.52
	between types (T×S)	1.34	0.09	1.40	0.84	2.13	1.96	0.07	0.43
	interaction	2.32	0.16	2.42	1.45	3.69	3.39	0.12	0.74
	C.V. [%]	1.48	5.77	1.57	4.30	0.38	0.71	3.00	15.30

RuBPCO activities varied significantly among the clones studied (Table 1). DT clones, UPASI-2 and UPASI-9, had very low activities of both RuBPC and RuBPO. On the other hand, DS clones UPASI-8 and UPASI-17 exhibited the highest RuBPC and RuBPO activities. This indicated that higher carboxylase activity alone will not result in increased productivity/drought tolerance because high carboxylase activity coupled with increased oxygenase activity resulted in high P_R in UPASI-17. Although all DT clones showed lesser RuBPC activity, their ratio of RuBPC/RuBPO was higher than that of DS clones. Our results coincided with the findings of Kuo *et al.* (1980). Hence the RuBPC/RuBPO ratio determines drought tolerance/productivity more than the quantitative concentrations of RuBPC or RuBPO.

Table 2. Linear relationship between the ratio of RuBP carboxylase/oxygenase (RuBPC/RuBPO) and rates of net photosynthesis (P_N) and photorespiration (P_R) or catalase activity. Regression formula Y = a + bX, where X is RuBPC/RuBPO and a and b are regression constants. *r* = correlation coefficient. $^{*}p<0.05$, $^{**}p<0.01$.

	RuBPC/RuBPO		
	r	a	b
P_N	0.481*	72.51	3.43
P_R	0.663**	3.81	–1.14
Catalase	0.765**	–1.31	1.83

Catalase: Plants with lower P_R had enhanced catalase activity (Table 1). This further supports the importance of testing the hypothesis that lesser P_R can be related to enhanced catalase activity. Catalase activity in the DT clones was higher than that of the DS clones. Its activity was the highest in UPASI-9 and the lowest in UPASI-17. High catalase activity in the DT clones could scavenge H_2O_2 formed in the photorespiratory pathway, and thereby minimise P_R. Low concentration of catalase induced in a barley mutant lethality under photorespiratory conditions (Kendall *et al.* 1983) indicating that hydrogen peroxide produced during photorespiration is toxic.

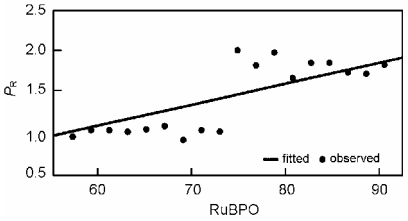

Fig. 1. Relation between RuBP oxygenase (X) [µmol(O_2 consumed) s^{-1} kg^{-1}(protein)] and photorespiration rate (P_R, Y) [%(^{14}C evolved)]. Y = a + bX (a = –0.393, b = 0.007). The equation is base on pooled values of different tea clones.

Relationship between enzymes and physiological characteristics: Significant positive correlations between RuBPC/RuBPO and P_N/catalase activity were found (Table 2). There was a significant negative correlation between RuBPC/RuBPO and P_R. These relationships indicated the importance of the specificity of RuBPCO to CO_2 and O_2. When the specificity of RuBPCO to CO_2 increases, there would be an increase in P_N besides an increase in RuBPC/RuBPO. Increased catalase activity could also have contributed to an increase in P_N by reducing P_R. Although the pathway of photorespiration is now well defined, its regulation towards increasing biomass productivity is less known. One approach would be to increase the CO_2/O_2 specificity of RuBPCO (Jordan

and Ogren 1981), but it is still uncertain whether this could be achieved.

There was no significant positive relationship of RuBPC activity between P_G (r = 0.272) and P_N (r = 0.139). Aoki (1990) reported that changes in the amount of proteins and RuBPC activity in tea had no correlation with the changes in P_N. However, we found a strong positive correlation between RuBPO activity and P_R (Fig. 1) and the correlation coefficient (r = 0.722) was highly significant at 1 % probability.

Significant negative relationship of catalase activity with photorespiration (r = –0.762) and RuBPO (r = –0.675) was established (Fig. 2*A*,*B*). These relationships supports the results of Zelitch (1990b,c, 1992) who performed this kind of experiments with tobacco plants. Cloning of the leaf catalase gene and the production of transgenic tea plants with enhanced catalase activity might help to confirm the biochemical linkage of these traits. Using a tobacco leaf catalase cDNA as a probe, Zelitch *et al.* (1991) found that leaves of a mutant had three fold greater steady-state level of catalase mRNA than the wild type. He also suggested that increased catalase activity does not result from constitutive over-expression of the catalase gene but from the conditions increasing P_N.

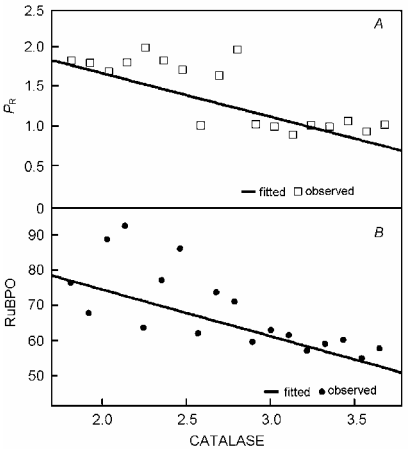

Fig. 2. Relation of catalase activity (X) [units per mg(protein)] with (*A*) photorespiration rate [%(^{14}C evolved)] and (*B*) RuBP oxygenase (RuBPO) activity [µmol(O_2 consumed) s^{-1} kg^{-1}(protein)]. Negative relationship of catalase with P_R and RuBPO was established based on pooled data of different tea clones. Y = a + bX; in *A* a = 2.81, b = –0.55, in *B* a = 361.8, b = –46.7.

Although the present investigation provides a significant insight into higher catalase activity and reduced P_R, it does not provide a direct relationship.

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