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Short Communication

Peroxidase, polyphenol oxidase and acid phosphatase activities in the stigma-style tissue of *Camellia sinensis* (L) O. Kuntze following compatible and incompatible pollination

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Abstract

Changes in the activities of peroxidase, acid phosphatase and polyphenol oxidase in the stigma-style tissues of three clones of *Camellia sinensis* (L) O. Kuntze were investigated following legitimate and illegitimate pollination. The activity of polyphenol oxidase and peroxidase increases significantly while that of acid phosphatase and the amount of protein decrease after cross-pollination in all the clones studied when compared to self-pollination. The amount of increase or decrease in the activity of enzymes is clone-specific. The possible role of these enzymes in self-incompatibility mechanism in tea has been discussed.

Keywords: *Camellia sinensis*, compatible and incompatible pollination, enzymes.

1. Introduction

There are a few studies concerning changes in the activities of enzymes in stigma-style tissues following compatible and incompatible pollination [1–3]. Most of these were conducted in species characterized by gametophytic self-incompatibility [4–7]. Since legitimate and illegitimate pollination are affected by alternations in the metabolic status of stigma and stylar tissues [3], it would be imperative to study the changes in the enzyme activities of stigma-style tissues following compatible and incompatible pollination. Since the enzymes control biochemical reactions, and their synthesis is under the control of specific gene(s), any change in the activity of an enzyme would reflect in the pattern of gene expression and corresponding metabolic events in the cell. Hence, the enzymes can be used as tools to study the problem of self-incompatibility at the biochemical level. In the present investigation, changes in the activities of peroxidase, polyphenol oxidase and acid phosphatase along with total amount of protein have been studied in stigma-style tissues of three clones of *Camellia sinensis*, in an attempt to understand the biochemical aspects of self-incompatibility in tea.

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2. Materials and methods

The clones of *Camellia sinensis* (L) O. Kuntze, viz. TV1, TV19 and 19.29.2 used in the present investigation were obtained from the seed orchard of Beheating Tea Estate, Dibrugarh, Assam. Hand pollination was carried out among compatible clones during October–February of 1999, 2000, 2001 and 2002. Flower buds of the male parent were bagged with cloth bags at 'pre-balloon' stage, 18–24 h before pollination; the female parent was emasculated at 'balloon' stage and the pollen from the male parent dusted over the stigma and rebagged. Altogether, 8108 crosses were made during the study period. For self-pollination, the buds from each clone were bagged at 'balloon' stage (about to open). The bags in both the cases were removed 48 h after pollination by which time the stigmas had withered.

2.1. *Preparation of enzyme extract*

The styles with ovary were collected and frozen in 30% glycerol. Styles weighing about 450 mg were homogenized with 9 ml of cold 5% KCl (w/v) using a Potter–Elvehjem homogenizer fitted with Teflon pestle. The homogenate was centrifuged at 0 ± 2°C at $800 \times g$ for 10 min. The clear supernatant was used directly for the assay of enzyme activities and estimation of protein.

2.2. *Assay of peroxidase*

The activity of peroxidase was assayed as per Malik and Singh [8]. The assay mixture containing 2.5 ml of phosphate buffer (pH 6.5, 0.1 M), 0.2 ml of suitably diluted enzyme extract and 0.1 ml of *o*-dianisidine (1 mg/ml methanol) was incubated at 28°C in a water bath for 2 min. The reaction was started by adding 0.2 ml of H_2O_2. The change in absorbency was recorded at 430 nm using a stopwatch at 30 s interval for 5 min. The enzyme activity was expressed in terms of the rate of increase of absorbance per hour per mg protein.

2.3. *Assay of polyphenol oxidase*

The polyphenol oxidase activity was measured as per the method of Sarvesh and Reddy [9]. The assay mixture (3 ml) contained 2.0 ml of 2 M carbonate–bicarbonate buffer (pH 10), 0.15 M of *o*-catechol and 0.2 ml of suitably diluted enzyme extract. The assay mixture was incubated for 2 min at 25°C. The reaction was stopped by adding 0.5 ml of 5% (v/v) H_2SO_4. The change in absorbency was recorded at 420 nm for 2 min. The enzyme activity was expressed in terms of micromoles of quinone formed/h/mg protein.

2.4. *Assay of acid phosphatase*

The reaction mixture contained 0.5 ml of substrate solution (50 mg *p*-nitrophenyl phosphate in 10 ml water + 25 ml of acetate buffer, 0.1 M, pH 4.8) and 0.1 ml of suitably diluted enzyme extract. The mixture was incubated at 35°C for 30 min. The reaction was stopped by adding 2.4 ml of 0.1 N NaOH and the absorbency was recorded at 410 nm. The enzyme activity was expressed in terms of micromoles of *p*-nitrophenol released/h/mg protein [8].

2.5. *Protein*

The amount of protein was determined according to the method of Lowry *et al.* [10] using Bovine serum albumin as standard.

The data were collected from five–six sets of experiments and were statistically analyzed and the difference between the mean which had probability value (P) lower than 0.05 was considered as significant.

3. Results

The data in Table I show significantly higher activity of peroxidase in cross-pollinated styles when compared to self-pollinated styles of all the clones studied. The highest mean value was found in the cambod clone TV 19 (9.65), followed by TV 1 ($\bar{X}$ = 9.62) with a range of 7.82–10.81 and 9.13–10.42, respectively. Among the selfed styles, TV 1 exhibited the highest range as well as mean value for enzyme activity, which was followed by TV 19 and 19.29.2, respectively. Activities of polyphenol oxidase showed a considerable variation between self- and cross-pollinated combinations (Fig. 1). Very low or negligible enzyme activity was observed in all the clones studied, after self-pollination. In contrast, the crossed samples showed seven–ten fold increase in polyphenol oxidase activity over the selfed styles. The highest specific activity for this enzyme was recorded in the cross-pollinated samples of the cambod hybrid clone TV19 ($\bar{X}$ = 45.80), whereas the same clone revealed a mean value of 4.43, after self-pollination. These variations between selfed and crossed styles were found significant at $P_{0.01}$ level in all experimental clones (Table I).

The acid phosphatase activity was significantly higher ($P_{0.01}$) in the styles after self-pollination when compared with the crossed ones in all experimental clones. The highest enzyme activity was noticed in the self-pollinated samples of TV 19 ($\bar{X}$ = 30.54), followed by TV 1, whereas 19.29.2 clone showed the lowest activity both in selfed ($\bar{X}$ = 28.08) and crossed ($\bar{X}$ = 14.8) samples.

Table I
Level of activity of peroxidase, polyphenol oxidase and acid phosphates on protein contents after self- and cross-pollination in clones of *Camellia sinensis* (L) O. Kuntze

Clones	Type of pollination	Peroxidase (changes in absorbance/h/mg protein)		Polyphenol oxidase (*m*mole of quinone/h/mg protein)		Acid phosphatase (*m*mole *p*-nitrophenol/h/mg protein)		Total protein	
		Mean (± sd)	*t*-value	Mean (± sd)	*t*-value	Mean (± sd)	*t*-value	Mean (± sd)	*t*-value
TV 1	Self	4.98 ± 0.81	7.67	3.71 ± 0.21	47.6	29.83 ± 1.99	16.2	4.96 ± 0.20	0.038
	Cross TV 19	9.62 ± 1.02		35.79 ± 2.06		17.26 ± 1.02		4.97 ± 0.08	
TV 19	Self	4.19 ± 0.21	8.22	4.43 ± 0.38	33.89	30.54 ± 2.04	15.95	4.79 ± 0.52	1.09
	Cross TV 1	9.65 ± 1.9		45.80 ± 7.07		17.23 ± 1.44		5.20 ± 0.20	
19.29.2	Self	4.00 ± 0.59	11.21	3.84 ± 0.505	23.84	28.08 ± 0.76	21.76	4.80 ± 0.19	1.72
	Cross TV 1	9.29 ± 0.53		29.17 ± 5.14		14.81 ± 1.1		5.14 ± 0.18	

Values are mean ± sd of six sets of samples.

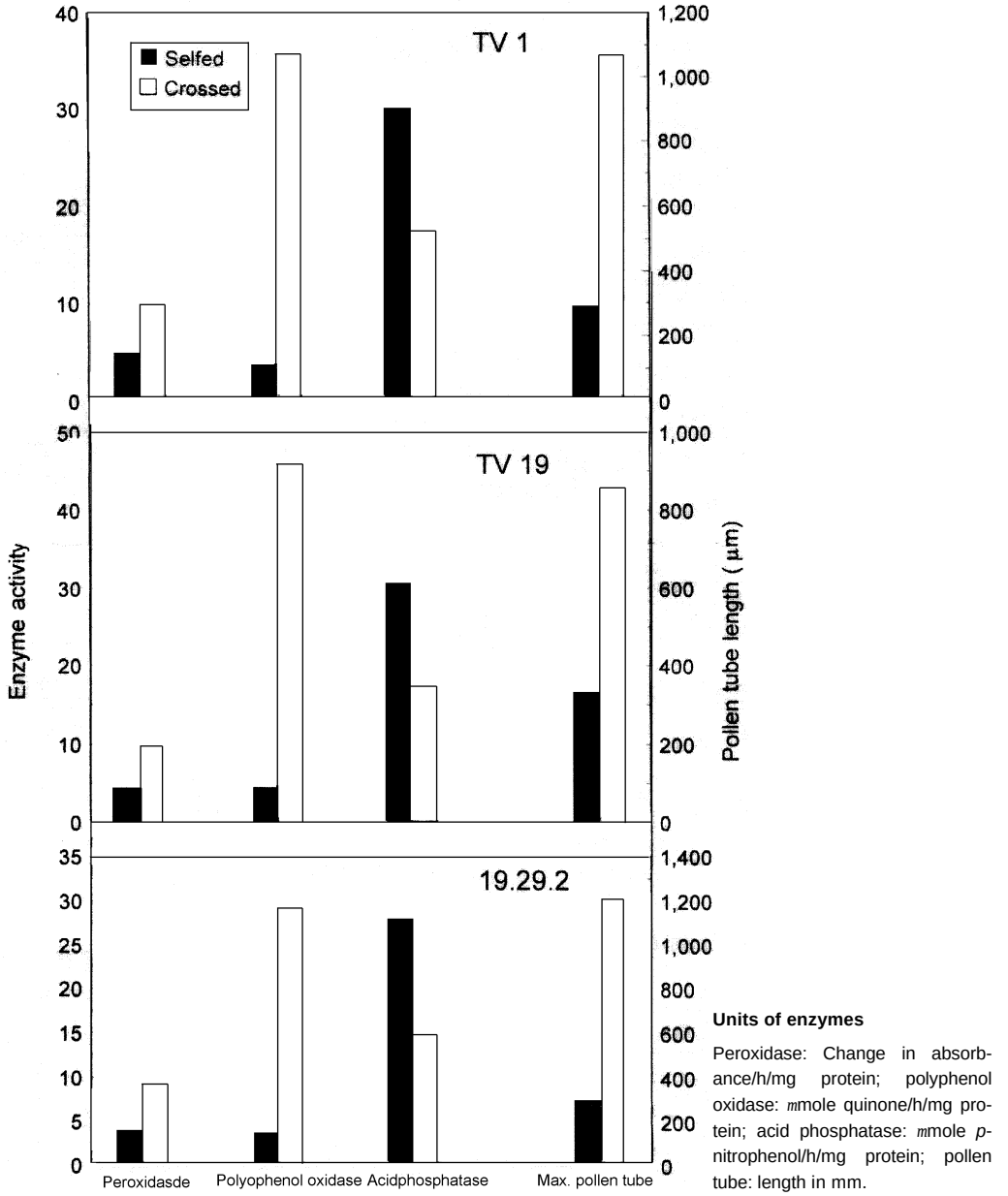

FIG. 1. Level of enzyme activities and pollen tube growth after self- and cross-pollination in the clones of *Camellia sinensis* (L) O. Kuntze.

The amount of total protein did not show any significant change between cross- and self-pollinated styles of all the clones studied. However, the amount of protein is slightly higher in cross-pollinated styles of all the clones studied (Table I).

4. Discussion

The enzyme may directly act to prevent the growth of selfed pollen tubes or sometimes may remain absent to disturb the growth of the pollen tube [11]. Higher level of peroxidase in cross-pollinated styles than in self-pollinated ones indicates its involvement in the regulation of pollen tube growth through styles. One cannot rule out the involvement of peroxidase, especially isozyme 10, as a nonspecific factor of the self-incompatibility reaction, but surely plays a significant part in this mechanism [1–3]. There are reports that this enzyme brings about changes in structural glycoproteins, other enzymes, pectin or cellulose present in the pollen tube wall [12, 13].

Elevated level of polyphenol oxidase in all the experimental clones following cross-pollination supports the complementary hypothesis [11] and the earlier assumption of Mayer and Harel [14] that the enzyme has the ability to produce quinone and selectively inhibit the activities of some other enzymes. Though the results of the present study do not confirm any direct relationship between this enzyme and the manifestation of self-incompatibility, it is possible that polyphenol oxidase acts as complementary factor along with other elements, like environment and genetic make up of the plant.

Higher level of acid phosphatase in self- than cross-pollination as observed in the present study is in agreement with the earlier finding of Dhaliwal and Malik [3] in stigma-style tissues of *Brassica compestris* var. Toria. It can be inferred that the inhibition of pollen tube growth is due to some factor(s) that could cause the production of acid phosphatase in higher quantity, as suggested by earlier workers [4, 11].

Self-incompatibility mechanism in angiospermic plant is widespread and has received considerable attention in recent years. Self-incompatibility genes have been identified, characterized, cloned and transferred to self-compatible lines in several species [15, 16].

Form the present study, it may be concluded that significant changes in the activities of certain key enzymes occur in the stigma and style tissues of *Camellia sinensis* following compatible and incompatible pollination. Histochemical evidence also showed higher percentage of pollen germination and longer pollen tube length on the stigmatic surface of cross-pollinated flowers than in self-pollinated ones [17]. Variations in the enzyme activities as observed in the present study show that the biochemical changes occur in the stigma-style tissues affected by self-incompatibility mechanism that in turn affects the metabolic status of these tissues. As the synthesis of these enzymes is controlled by their corresponding gene(s), their quantitative changes may be due to the changes in the regulation of expression of gene(s) and hence offer a basis to study the mechanism of self-incompatibility at molecular level.

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