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Differential Activity of four Selected Enzymes in the Pistils of two Members of the Family Bignoniaceae

Gulshan Chaudhary*, Prem Kumar Dantu*# and Arvind M. Kayastha*,#**

Abstract: *In the present study two members of the family Bignoniaceae, Tacoma stans, a fruit bearing plant, and Pyrostegia venusta, a twine that does not bear fruits were chosen to study the activity of four enzymes, namely, peroxidase, acid phosphatase, polyphenol oxidase and esterase. The aim of the study was to understand if pollination with viable and non-viable pollen grains has any influence on the activity of the four selected enzymes in post pollinated pistils. T. stans produces viable pollen grains while P. venusta produces non-viable pollen grains. Seven developmental stages starting from anthesis till 12 hours of flower opening were identified for both the plants. Controlled pollinations were carried out in the two plants. Stigma of T. stans was pollinated with self pollen grains that were viable while P. venusta stigma received pollen grains that were non-viable. In T. stans the four enzymes peroxidase, acid phosphatase, polyphenol oxidase and esterase showed normal activity though peroxidase activity was comparatively subdued. In P. venusta, however, peroxidase showed hyper-activity while the other three enzymes, acid phosphatase, polyphenol oxidase and esterase were more subdued. The results have been discussed in the light of the available literature.*

Keywords: *Peroxidase, Acid phosphatase, Polyphenol oxidase, Esterase, Tacoma stans, Pyrostegia venusta, Bignoniaceae.*

I INTRODUCTION

Development of fruits with healthy seeds is the culmination of the natural process of pollination and fertilization. Seeds are important for the plant for propagation of the progeny and for a breeder to assess the results of breeding while developing new cultivators. Members of Bignoniaceae are important garden plants because of their colourful showy flowers. The plants of this family are shrubs to small trees to woody climbers. Many of them such as *Tecoma stans* produce copious fruits and seeds through which they are propagated, while quite a few as *Pyrostegia venusta* do not bear fruits and so produce no seeds (Anonymous, 1976, 1988). Incidentally *Pyrostegia* is amongst the more beautiful genera and will be a breeders delight to produce new colours. Of the several reasons for not bearing fruits one important factor is that the pollination time and stigma receptivity may not be matching.

In the present study two genera of Bignoniaceae, *Tacoma stans* and *Pyrostegia venusta* have been selected with respect to their fruit and seed bearing ability. *T. stans*, a bushy shrub has yellow flowers producing viable pollen grains and bears copious fruits and seeds. However, *P. venusta* is a woody climber orange flowers producing non-viable pollen grains and does not bear any fruits or seeds at Agra. Several studies (Shivanna and Sastri, 1981; Neog et al., 2004; McInnis et al., 2006; Page et al., 2006) have shown a close correlation in stigma receptivity and some key enzymes. However, it is to be seen whether non-viable pollen grains elicit similar enzyme activity in the pistils as viable pollen grains do. Thus, in the present study activity of certain enzymes *viz.*, peroxidase, acid phosphatase, polyphenol oxidase and esterase in the post pollinated styles was compared in the two plants *Tacoma stans* and *Pyrostegia venusta*, former a fruit bearer and the latter a non-fruit bearer.

II MATERIALS AND METHODS

Studies on *Tacoma stans* and *Pyrostegia venusta* were carried out during November to February 2006-2007 in the Botanical Garden of the Dayalbagh Educational Institute, Dayalbagh, Agra.

Defining Stages of Floral Development

Seven developmental stages starting from anthesis till 12 hours of flower opening were identified for both the plants. These seven stages are:

* Corresponding author: Prem Kumar Dantu, Plant Biotechnology Lab, Department of Botany, Faculty of Science, Dayalbagh Educational Institute (Deemed University), Dayalbagh, Agra 282110, India. E-mail: premdantu@gmail.com

*,**# Arvind M. Kayastha, School of Biotechnology, Faculty of Science, Banaras Hindu University, Varanasi 221005, India. Tel: +91-542-2368331, Fax: +91-542-2368693, E-mail: kayasthabhu@gmail.com

(1) Stage 0 = anthesis

(2) Stage 1 = 1 h after pollination

(3) Stage 2 = 2 h after pollination

(4) Stage 4 = 4 h after pollination

(5) Stage 6 = 6 h after pollination

(6) Stage 8 = 8 h after pollination

(7) Stage 12 = 12 h after pollination

Controlled Pollination

Emasculation and controlled pollination was carried out in both the plants, *T. stans* and *P. venusta*. Early in the morning, a day before controlled pollination, mature buds of the two plants were emasculated and bagged. Mature anthers were collected and stored for use the following day. The following day after anthesis the stigma were pollinated with the pollen from the stored anthers at seven selected periods: at anthesis, 1 h after anthesis, 2 h after anthesis, 4 h after anthesis, 6 h after anthesis, 8 h after anthesis, 12 h after anthesis.

Preparation of Enzyme Extract

Only pollinated pistils that showed pollen grains were collected for enzyme extraction except for those at Stage 0, which obviously did not receive any pollen grains. Before extracting enzyme, the presence of pollen on stigma of both the plants was confirmed by observing under a stereozoom. The four enzymes selected for the present study were, peroxidase, acid phosphatase, polyphenol oxidase and esterase. Pistils from the two plants were collected and stored in ice till extraction of the enzymes. Pistils (200 mg) without ovaries were homogenized in cold buffer (buffers varied with enzyme) using a pre-chilled mortar and pastel. The homogenate was centrifuged at 4 ^{o}C at 10, 000 rpm for 10 min. The clear supernatant was used directly for assay of enzyme activity (Jorgensen and Vejlby, 1953; Ching and Metzger, 1987; Neog et al., 2004; McInnis et al., 2006).

Peroxidase was extracted in 0.1 M potassium phosphate buffer of pH 7; acid phosphatase was extracted in 0.1 M sodium acetate buffer of pH 4.8; polyphenol oxidase was extracted in 2 M carbonate–bicarbonate buffer of pH 10; esterase was extracted in 20 mM phosphate buffer of pH 10.

Enzyme Assays

Peroxidase

Peroxidase activity was assayed by guaiacol method (Malik and Singh 1980; Neog et al., 2004; McInnis et al., 2006). The assay mixture contained 2.5 ml potassium phosphate buffer (pH 7.0, 0.1 M), 0.2 ml of suitably diluted enzyme extract and 0.05 ml of guaiacol. The reaction was started by the addition of 0.05 ml of H_2O_2. Change in absorbance was recorded at 436 nm at 20 s intervals for 3 min. The enzyme activity was recorded in terms of rate of increase in absorbance/sec and expressed as µmoles per min.

Acid Phosphatase

Acid phosphatase activity was assayed by the *p*-nitrophenyl phosphate method (Neog et al., 2004; Ching and Metzger, 1987). The reaction mixture contained 0.5 ml of the substrate *p*-nitrophenyl phosphate, 2 ml of 0.1 M acetate buffer of pH 4.8 and 0.1 ml of suitably diluted enzyme extract. The absorbance was recorded at 410 nm and enzyme activity expressed in µmoles per min.

Polyphenol Oxidase

Polyphenol oxidase activity was assayed by the *o*-catechol method (Sarvesh and Reddy, 1988; Jorgensen and Vejlby, 1953). The assay mixture contained 2.0 ml of 2 M carbonate-bicarbonate buffer of pH 10, 0.15 M of *o*-catechol and 0.2 ml of suitably diluted enzyme extract. Change in absorbance was recorded for 3 min at 420 nm and enzyme activity expressed in µmoles per min.

Esterase

Esterase activity was assayed by napthol-acetate method (Sawhney et al., 1981). The assay mixture contained 2.0 ml of 20 mM phosphate buffer of pH 10, 0.1 M of 1-napthol acetate and 0.2 ml of suitably diluted enzyme extract. Change in absorbance was recorded at 420 nm for 3 min and enzyme activity expressed in µmoles per min.

Statistical Analysis

All the experiments were repeated thrice. The data of enzyme activity was subjected to analysis of variance (ANOVA) by Duncan's multiple variance test at the 5% level of significance (P = 0.05) using SPSS 14.

III RESULTS

Selection of Stages

The different stages of the flower from anthesis have been chosen randomly. The flowers in both *T. stans* and *P. venusta* are tubular with four fertile stamens and one sterile staminode. The pistil is about 3 cm long in *T. stans* and 5-6 cm in *P. venusta* with a bifid stigma (Fig. 1 A-D). In *P. venusta* the petal colour faded in 8 h and petal tube gets detached 12 h after pollination. While in *T. stans* the petal tube detached only 24 h after pollination.

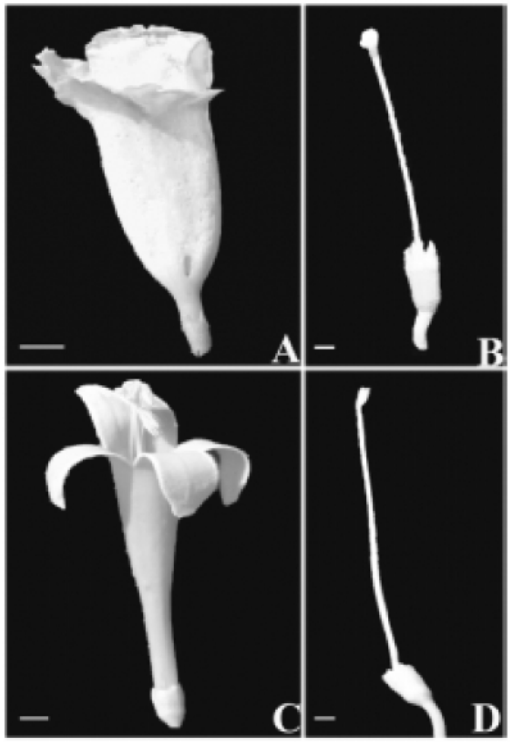

Figure 1: Flower and Pistils of *Tecoma stans* (*A* & *B*) and *Pyrostegia venusta* (*C* & *D*) (Fig. A bar = 0.68 cm; Fig. B bar = 0.53 cm; Fig. C bar = 0.85 cm; Fig. D bar = 0.72 cm).

Enzyme Activity

In *T. stans* and *P. venusta* the four enzymes studied showed a burst in activity after anthesis and pollination. A high peroxidase activity was recorded at anthesis in the two plants. In *T. stans* the enzyme showed a sudden acceleration in activity of almost 700% at Stage 4 and 6 than at Stage 0 and Stage 2. The peroxidase activity dropped as suddenly by Stage 8 reaching a minimum by Stage 12. Interestingly, the activity of peroxidase was much higher in *P. venusta* than in *T. stans* (Table I, Fig. 2A). In *P. venusta* peroxidase activity increased several folds after an initial lag in activity. Stage II onwards peroxidase activity accelerated to almost 7200% at Stages 4 and 6 and then declined by Stages 8 and 12 (Table II, Fig. 2A).

The other three enzymes acid phosphatase, polyphenol oxidase and esterase behaved very differently in the two plants. Overall activity of these three enzymes was much higher in *T. stans* than in *P. venusta* (Fig. 2B-D). Acid phosphatase activity increased at Stage 1 (122 µmoles per min) in *T. stans* and then dropped at Stage 2 (61 µmoles per min), thereafter there was a steady increase till the peak activity reached at Stage 8 (179 µmoles per min). In *P. venusta* though acid phosphatase activity showed slight increase but was almost five times less than that in *T. stans* (Tables I & II, Fig. 2B). The increase in polyphenol oxidase activity at Stage 1 was sharp in both the plants (from 1110 at Stage 0 to 3328 µmoles per min at Stage 1 in *T. stans*; from 503 at Stage 0 to 2135 µmoles per min at Stage 2 in *P. venusta*). Thereafter, the PPO activity dropped between Stages 2 and 8 in the two plants. In *P. venusta* PPO activity further dropped reaching minimal activity at Stage 12 (330 µmoles per min) while at this stage in *T. stans* the enzyme showed a sudden acceleration in activity (about 1530 µmole per min) (Tables I & II, Fig. 2C).

Table I: Activity of Peroxidase, Polyphenol Oxidase, Acid Phosphatase and Esterase in the Pistils of *Tecoma stans* at Different Time Periods after Pollination. Values followed by the same letter(s) in each Column are not Significantly Different (*P* = 0.05)

Hours after pollination	*Enzyme Activity (µmoles min⁻¹)*			
	Peroxidase	*Polyphenol oxidase*	*Acid Phosphatase*	*Esterase*
0	3090.5^{a}	1110.5^{a}	51^{a}	351^{ab}
1	3734^{a}	3328.5^{b}	122.5^{ab}	503^{ab}
2	17104^{b}	2132.5^{ab}	61.5^{ab}	225^{ab}
4	20955.5^{b}	1629.5^{ab}	142.5^{ab}	1544^{b}
6	18543^{b}	1822^{ab}	130^{ab}	180^{ab}
8	2807^{a}	717^{a}	179.5^{b}	167.5^{ab}
12	333.5^{a}	1529.5^{ab}	32.5^{a}	53.5^{a}

The initial burst in the activity of esterase was much higher in *P. venusta* at Stages 1 and 2 (781 and 726 µmoles per min, respectively) then onwards the activity decreased rapidly reaching a minimum at Stage 8 (14.5 µmoles per min). In *T. stans* the activity of esterase actually dropped at Stage 2 (225 µmoles per min) after an initial increase at Stage 1 (503 µmole per min) (Fig. 2D) thereafter, a sharp acceleration in activity could be seen at Stage 4 (1544 µmoles per min) eventually decreasing to a minimum at Stage 12 (53.5 µmoles per min) (Tables I & II, Fig. 2D).

Table II: Activity of Peroxidase, Polyphenol Oxidase, Acid Phosphatase and Esterase in the Pistils of *Pyrostegia venusta* at Different Time Periods after Pollination. Values followed by the same letter(s) in each column are not Significantly Different (*P* = 0.05)

Hours after pollination	*Enzyme Activity (µmoles min⁻¹)*			
	Peroxidase	*Polyphenol oxidase*	*Acid Phosphatase*	*Esterase*
0	2839.5^{a}	503.5^{ab}	10.5^{a}	239.5^{c}
1	926.5^{a}	1925^{ab}	26.5^{ab}	781^{f}
2	625.5^{a}	2135^{b}	10^{a}	726^{e}
4	45294^{b}	1646.5^{ab}	6.66^{a}	376.5^{d}
6	43495.5^{b}	1870^{ab}	14.55^{a}	77^{b}
8	1920^{a}	1080^{ab}	45^{b}	14.5^{a}
12	582.5^{a}	381^{a}	8.9^{ab}	22^{a}

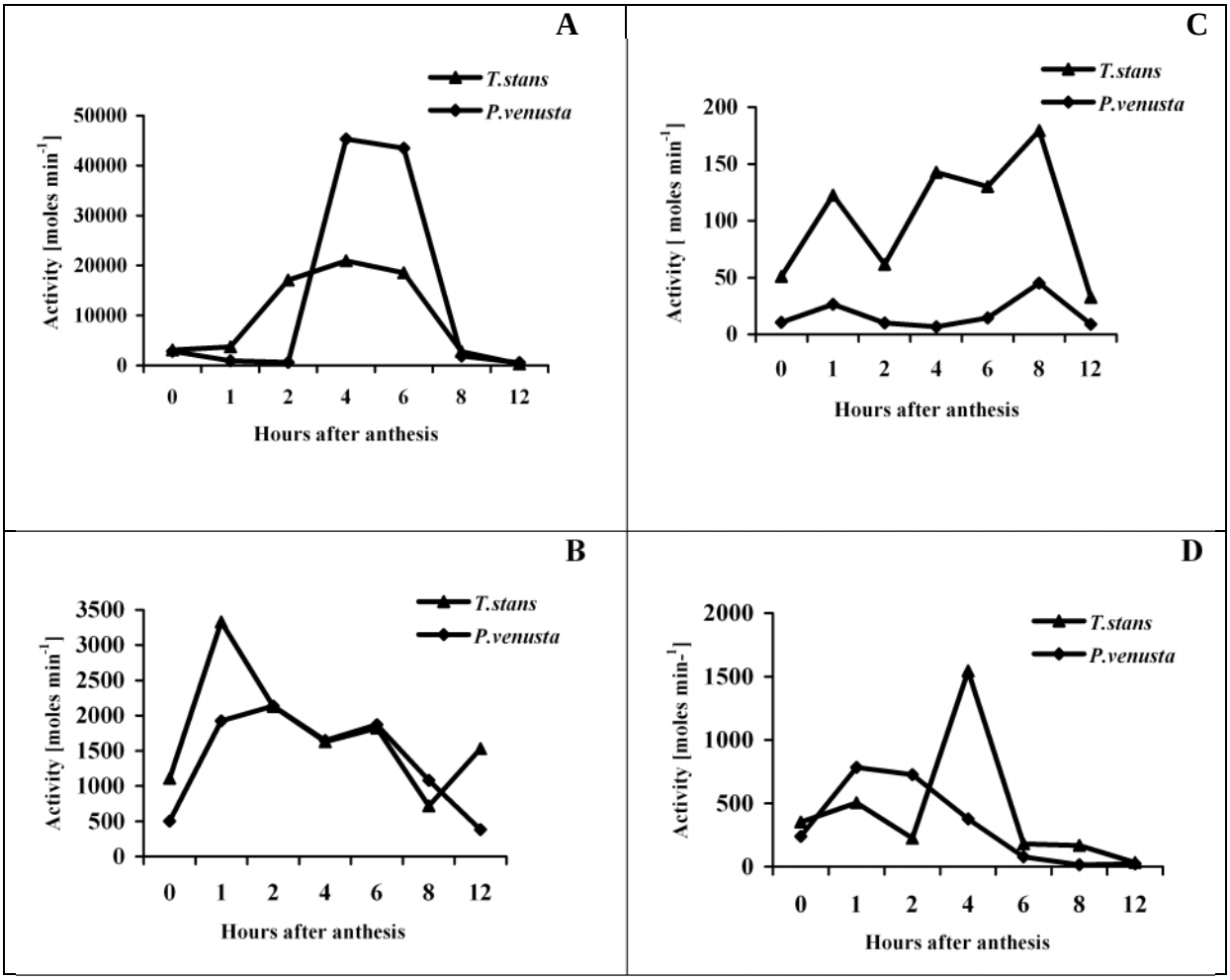

Figure 2: Activity of the Enzymes, Peroxidase (*A*), Acid Phosphatase (*B*), Polyphenol Oxidase (*C*) and Esterase (*D*) in the Pistils of *Tecoma stans* and *Pyrostegia venusta* at Different Stages after Pollination (0 = at Anthesis; 1 = at Pollination; 2 = 2 h after Pollination; 4 = 4 h after Pollination; 6 = 6 h after Pollination; 8 = 8 h after Pollination; 12 = 12 h after Pollination).

IV DISCUSSION

Activity of the four enzymes studied namely peroxidase, acid phosphatase, polyphenol oxidase and esterase in the two plants was informative. In *P. venusta*, which produces non-viable pollen grains and does not bear fruits, peroxidase activity was observed to be much higher than in *T. stans*, which has viable pollen grains and bears fruits. The other three enzymes *viz.*, acid phosphatase, polyphenol oxidase and esterase showed much less activity in *P. venusta* in comparison to *T. stans*.

The activity of enzymes in the pistil was studied to ascertain if the stigma and style behaviour was normal. Peroxidase has been implicated as an indicator of stigma receptivity (Galen and Plowright, 1987; Dupius and Dumas 1990; Daffni and Mote Maues, 1998; Stpiczynska, 2003) and mere adherence of pollen grains to stigma has been shown to increase peroxidase activity independent of the penetration of pollen tube (Galen and Plowright, 1987; Bredmeijer, 1982). The burst in peroxidase activity in *P. venusta* in the present study indicates that mere landing of even non-viable pollen is sufficient to trigger peroxidase activity, which appears to be a classical example of a defense response (Welinder, 1992; Penel et al., 2003; McInnis et al., 2006). Peroxidase enzyme which is known to be a defense induced enzyme (Cheong et al., 2002; Delannoy et al., 2003; Do et al., 2003; McInnis et al., 2006) showed greater activity in *P. venusta* than in *T. stans*. It is possible that pollen grains landing on stigma set in signals which activate peroxidase as a defense strategy. The activity of this enzyme is modified by germinating pollen grains of *T. stans* (Bredemeijer, 1984) while the non-germinating pollen grains of *P. venusta* are perceived as intruders and the peroxidase activity increases tremendously.

The other three enzymes *viz*., acid phosphatase, polyphenol oxidase and esterase behave differently in the two plants. Their activity appeared to be more of a response to the growth of pollen tube since the activity of these enzymes was much higher in *T. stans*, which bears fruits, than in *P. venusta*, which does not bear fruits.

The elevated levels of acid phosphatase in *T. stans* in comparison to the low levels in *P. venusta* clearly indicate a role for this enzyme in pollen tube growth in the style. This enzyme is probably produced in response to the penetrating pollen tube for providing inorganic phosphates through hydrolysis of phosphate esters (Ching et al., 1987).

Polyphenol oxidase activity in both the plants was comparable, except at Stage 1 when its activity was much higher in *T. stans*. The consistently enhanced activity of polyphenol oxidase in the two plants is in line with its possible role in the defense mechanism (Mayer and Harel, 1979; Bashan et al., 1987; Tyagi et al., 2000).

In wet and dry stigmas of a wide range of plants the time of stigma receptivity has been correlated with esterase activity (Heslop-Harrison, 1975; Heslop-Harrison and Shivanna, 1977; Kohn and Waser, 1985; Lavithis and Bhalla, 1995). A moderate level of esterase activity is maintained at all the Stages in *P. venusta* with a slight burst in activity at Stage 1 and 2 indicating probably that stigma was receptive during this period. However, in *T. stans* esterase showed high activity at Stage 4 which probably could be a response to the germinating pollen and esterase must be facilitating in the penetration of the pollen tube into the stigma by hydrolyzing the cutin layer (Heslop-Harrison 1975; Lavithis and Bhalla, 1995; Hiscock et al., 2002).

In conclusion, the enhanced activity of peroxidase and polyphenol oxidase in *P. venusta* indicates their role mainly in the defense mechanism than anything to do with stigma receptivity. Esterase activity in *T. stans* could have been triggered by the germinating pollen and is in line with its role in hydrolyzing the cutin of stigma. Acid phosphatase in *T. stans* could be involved in supporting the pollen tube growth by providing phosphates. The activity of these two enzymes is minimal in *P. venusta* where the pollen are not viable and do not germinate in the style.

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