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Studies on stigma-surface esterases in four RET *Impatiens* of Western Ghats

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Abstract

The present study deals with the qualitative test for cytochemical localization of non-specific esterases on stigma surface of four endemic balsams *viz., Impatiens elegans* Beddome, *I. floribunda* Wight, *I. maculata* Wight and *I. tangachee* Wight. The style is short, solid, wet and non-papillate in all four candidate species. Stigma lobes are star shaped, when it shows maximum receptivity in all cases. In all the selected *Impatiens* species, stigma surface esterases could be detected as a thin layer on the stigma of flowers after the anther shedding. The esterases are present copiously on stigmatic lobes and slightly on the stigmatic head. The *Impatiens* species showed variation in stigma receptivity period and hence it did not support contemporary pollen germination and tube growth. The growth of pollen tube and stigma receptivity were influence by the presence of specific esterases on stigma surface.

Keywords : esterases, *Impatiens elegans, I. floribunda, I. maculata and I. tangachee,* RET (Rare Endangered and Threatened), stigma receptivity

INTRODUCTION

One of the important events in the sexual reproduction of flowering plants is the interaction of male gametophyte with mass sporophytic tissues of pistils. The major step that takes place during the pollen-pistil interaction is the recognition and acceptance or rejection of pollen grains by the pistil. Pollen-pistil interaction deals with a series of sequential events in the pistil from the time of pollen grain adhesion on the stigma, until the pollen tube enters into the ovule. The receptive stigma surface contains extra cellular proteins either in the form of pellicle or as a component of exudates (Heslope-Harrison and Shivanna, 1997; Heslope-Harrison, 1981; Shivanna and Johri, 1985). Stigma-surface protein play a crucial role in pollen germination, pollen tube entry into the stigma and probably incompatibility responses (Heslop-Harisson and Heslop-Harisson 1975; Knox *et al.*, 1976; Shivanna, 1979) and it is covered with an exudate containing lipids, phenolic compounds, carbohydrates, proteins, phosphates, lectins, and amino acids including esterases (Baker *et al.*, 1974; Vasil, 1974). Esterases are important component of the stigma surface protein and its presence is related to stigma receptivity (Bhattacharya and Mandal, 2003).

Impatiens belongs to the family Balsaminaceae that is comprised of more than 1,000 species. In India, the genus is represented by 204 species, that are mainly distributed in montane and highland areas of shola forests and pasture. There are 92 species available in Peninsular India, out of which more than 80 are endemic, including *Impatiens elegans, I. floribunda, I. maculata* and *I. tangachee* and are confined to Western Ghats (Nair, 1991). These populations are rapidly declining due to various factors, especially by reproductive constraints (Sreekala *et al.*, 2007). Studies on the esterases in stigmas are indicative to assess the stigma receptivity (Levithis and Bhalla, 1995; Tandon *et al.*, 2001) and reproductive capacity. Against this background, the present investigation has been carried out to know the location of esterases qualitatively and cytochemically on the stigma surfaces at different time intervals, after anthesis on *Impatiens elegans, I. floribunda, I. maculata* and *I. tangachee*, in order to assess the receptivity of the stigma in these species.

MATERIALS AND METHODS

The candidate species of *Impatiens* were collected from forest areas of Rajamala, Pettimudi, Neymakkad gap, Kanthaloor of Munnar, Idukki district, Kerala state, South India and were maintained in garden for further detailed studies. Fifty matured flowers for both, before and after anthesis were selected. A total of 5 flowers at every time were collected from each plant of ten samples. The anthesis time was observed periodically and morphology of the stigma was thoroughly studied under light microscope (Leica-DME, Germany). Flowers of different stages (first day, second day, third day and fourth day after anthesis) were brought to the laboratory and the pistils were excised and maintained in the petri dishes with moist filter paper. Stigma receptivity was studied by the method suggested by Dafni (1992).

To study the surface-stigma esterases, we followed the method suggested by Shivanna and Rangaswamy (1992). Two types of solutions namely 'A' containing

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fast blue B, sucrose (10% w / v), phosphate buffer (0.15M, pH 6.8) and á-napthyl acetate) and 'B' containing all constituents in the solution 'A' except á-napthyl acetate were prepared for the location of esterases on the stigma surface. The excised pistils of selected stages were dipped in solution 'A' and solution 'B' separately and incubated at 25^0C in a humidity chamber for 22min. After the specific period of incubation (10-20min), the stigmas were removed and washed with phosphate buffer (pH 6.8). The stigma surface esterases activity were studied in whole mount of treated stigmas in 50% glycerine and observed under light microscopic field (Lieca DME, Germany).

RESULTS AND DISCUSSION

Morphology of stigma was studied at different times after anthesis and it has been observed that, all selected *Impatiens* posses wet and non-papillate type of stigma. Non-specific esterases occurred throughout the stigma, mainly on the stigmatic lobes and slightly on the stigmatic head in all the species of *Impatiens* except *Impatiens elegans* (Table. 1). In *I. floribunda,* the flowers open during 0500-0800hr, the stigma is wet and non-papillate type and the esterase has been located slightly on the stigmatic head (fig 1c-d). The presence of esterases was significant on the third day and fourth days after anthesis (Table 2). *Impatiens maculata* bloom at early morning around 0400-0800hr. The stigma type of *Impatiens maculata* is wet and non-papillate and the location of esterases has been mostly on the stigmatic lobe and slightly on the stigmatic head (fig 1e-f). Significant presence of enzyme was observed on the third day after anthesis. In *Impatiens elegans,* the flowers open between 0300-0500hr. The stigma-surface esterases were found only on the stigmatic lobes after 3 days of anthesis (fig 1a-b). The percentage of stigma receptivity is found more (up to 75%) in *Impatiens elegans* (Table 3). In *Impatiens tangachee* the flowers are bright rose in colour and bloom at morning (0700-0900 hr). The type of stigma is wet and non-papillate. The receptivity and esterases activities were seen on the stigmatic lobes and slightly on the stigmatic head (fig 1g-h). Significant presence of esterases was observed only on the third day of anthesis (Table 2). The present study indicates that, presence of adequate esterases over stigma surface coincides with its receptivity.

In *Impatiens,* the flowers are always zygomorphic and although the flower size and shape varies considerably between species, the breeding system is remarkably uniform in almost all the species (Grey-Wilson, 1980). Generally the dithecal, tetrasporangiate anthers lie as a cap above the gynoecium and release pollen grains. The stigma becomes receptive only after the pollens were released and coupled with this the subsequent shedding of the androecium unit, ensures cross pollination. The coherent stigma spread and expose the star shaped receptive surface. Significant increase of esterases was observed on the third day of anthesis. There was no presence of esterases seen on 2^{nd} day before and 5^{th} day after anthesis in all the selected plants. The stigmas did not show any esterases activity when treated with solution B. Stigmas were also considered receptive when the lobes assumed star shape.

Productivity of flowering plants depends mainly upon the pollen viability and stigma receptivity. Any blockage or shortage on these factors may affect the pollen-pistil interaction. Pollen-pistil interaction is unique to the flowering plants and it is prerequisite for effective fertilization and subsequent fruit and viable seed set. The maximal esterases activity period of these *Impatiens* species varied from third day to fourth day (Table 3). The maturity of the flower, time of anthesis and availability of exudates may influence receptivity (Bhattacharya *et al.,* 2004).

Enzyme activity was found to be correlated with stigmatic receptivity (Levithis and Bhalla, 1995). The present investigation showed the presence of esterases on the stigmas during its high receptivity period (3^{rd} day to 4^{th} day) in four *Impatiens* species and it might be considered that intense presence of esterases is one of the associated factors for stigma receptivity. The presence of esterases becomes more in a particular time after anthesis when the stigmas become more receptive (Stone *et al.,* 1995; Bhattacharya and Mandal, 1997; 2003). Our present investigation is in accordance with other findings of Baker *et al.* (1974) and Ghosh and Shivanna (1984), whose conclusions showed that, there is a correlation between the location of esterases and stigma receptivity. Almost all *Impatiens* are cross-pollinated, as field observation and pollination events of the *Impatiens* revealed that they might favour cross-pollination. Malik and Dhaliwal (1986) reported that, cross-pollinated stigmas have more esterases activity than self pollinated ones. Self pollinated species produce low amount of proteins in the exudates (Barret, 2002). It has been well established that esterases are the chief constituents of the stigma-surface proteins (Shivanna and Rangaswamy, 1992). In the present investigation, the stigmas become receptive after shedding of androceium but at the same time, its pollen viability reduced. The mechanism which is avoiding the self pollen grains and preferring the pollens from other flowers favours cross-pollination. The cytochemical localization of non-specific esterases on *Impatiens elegans, I. floribunda, I. maculata* and *I. tangachee* vary each other and show correlation with intense presence of esterases and peak receptive period of stigmas.

Table 1. Cytochemical localization of esterases on stigma of ***Impatiens***

Name of taxa	Type of stigma	Esterases location
Impatiens elegans	Wet, non-papillate	Stigmatic lobes
Impatiens floribunda	Wet, non-papillate	Stigmatic lobes and stigmatic head
Impatiens maculata	Wet, non-papillate	Stigmatic lobes stigmatic head
Impatiens tangachee	Wet, non-papillate	Stigmatic lobes stigmatic head

Table 2. Results of esterases activity at different time intervals on stigma of ***Impatiens***

Name of taxa	Time of anthesis (Hour)	Days after anthesis			
		I day	II day	III day	IV day
Impatiens elegans	0300-0500 hr	—-	—-	++	+
Impatiens floribunda	0500-0800 hr	—-	—-	++	+
Impatiens maculata	0400-0800 hr	—-	—-	++	+
Impatiens tangachee	0700-0900 hr	—-	—-	++	+

++- Significant presence +-Insignificant presence

Table 3. Percentage of stigma receptivity in ***Impatiens***

Name of taxa	Days after anthesis			
	I	II	III	IV
Impatiens elegans	—	—	75	14
Impatiens floribunda	—	—	60	40
Impatiens maculata	—	—	50	30
Impatiens tangachee	—	—	55	30

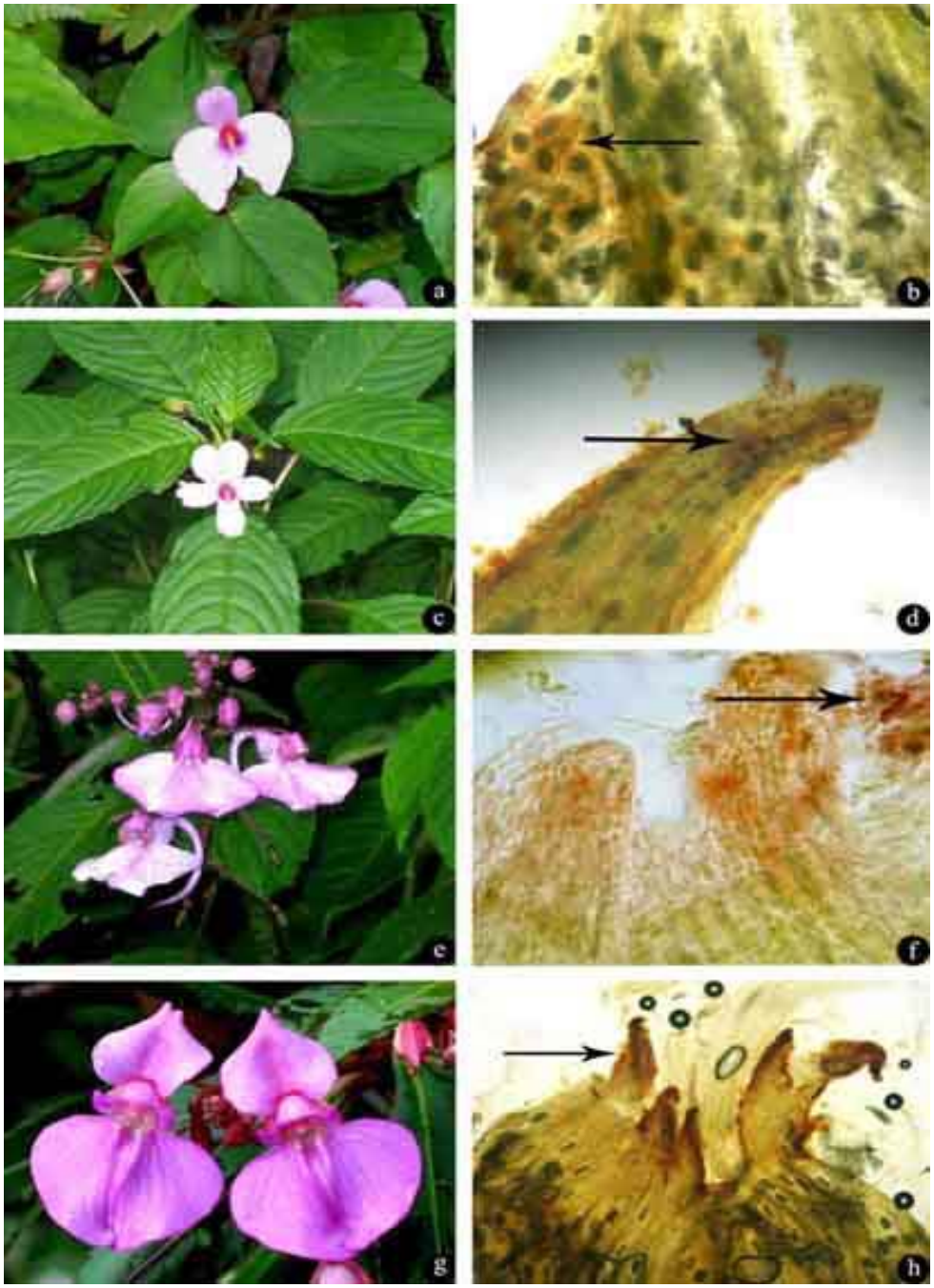

Figure 1. Studies on Surface-Stigma esterases in four *Impatiens* species

a) *Impatiens elegans* - Flower,

b) *Impatiens elegans*- stigma surface-esterases at the tip

c) *Impatiens floribunda*-Flower

d) *Impatiens floribunda*-stigma surface-esterases

e) *Impatiens maculata*- Flower,

f) *Impatiens maculata*- stigma surface esterases,

g) *Impatiens tangachee*-flower,

h) *Impatiens tangachee*-stigma-surface esterases.

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