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STUDIES ON ESTERASE AND PEROXIDASE ACTIVITY OF *CLERODENDRUM INERME* (LINN.) GAERTN. IN RELATION TO STIGMA RECEPTIVITY

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ABSTRACT
The present investigation deals with the stigma receptivity of *Clerodendrum inerme* (Linn.) Gaertn. (Verbenaceae) in terms of *in vivo* pollen germination with a view to find out the optimum stigma receptive period and correlation of stigma receptivity with the activity of esterase and peroxidase on stigmatic surface in order to provide information about fertilization as a basis for successful plant breeding programme. The plant flowers during August to December. Flowers open at 15:30 h to 17:45 h. Anthers dehisced by longitudinal slit after flower opening. Non-specific esterase and peroxidase are present densely all over the stigmatic surface and remain in scattered on the bi-fid stigma which may act in facilitating *in vivo* pollen germination. Stigma showed maximum 53% receptivity with mean 253±112.15 µm pollen tube length after 24 h of flower anthesis. Intense amount of esterase and peroxidase are present on the stigma during high receptive period.

KEY WORDS: Stigma receptivity, esterase, peroxidase, *Clerodendrum inerme* (Linn.) Gaertn.

INTRODUCTION

The stigma is the localized part of the pistil and the pollen grains are trapped on the stigmatic surface during pollination for successful fertilization. A successful post pollination event largely depends on the receptivity of stigma (Joshirao and Saoji, 1989). Stigma receptivity refers to the ability of the stigma to support germination and tube growth of viable pollen (Shivanna and Owens, 1989). Stigma receptivity is an important stage towards the maturation of flower which may greatly influence the success of post-pollination events at different stages in the life cycle of the flower (Barrett, 2002). Though receptivity of the stigma may vary from species to species and depends upon temperature as well as humidity (Joshirao and Saoji, 1989). But it becomes optimum soon after anthesis (Shivanna and Johri, 1985). The receptive surface of stigma contains some extracellular proteins, which remain either as a pellicle in the dry stigmas or as a component of the exudate in the wet stigmas (Heslop-Harrison and Shivanna, 1977; Heslop-Harrison, 1981; Shivanna and Johri, 1985), but significant exudation appears during high receptive period. Esterases and peroxidase are the important components of the stigmatic exudation and its presence is related to stigma receptivity. Thus, stigma receptivity of *Clerodendrum inerme* (Linn.) Gaertn. belonging to the family Verbenaceae has prime importance in the biology of sexual reproduction and assayed in terms of *in vivo* pollen germination with reference to esterase and peroxidase activity at different time after anthesis. Objective of this study is to find out a correlation between esterase and peroxidase activity with stigma receptivity (Stone *et. al*., 1995; Lavithis and Bhalla, 1995; Bhattacharya *et. al*., 2004; Choudhury *et al*., 2012; Dey *et al*., 2016).

MATERIALS & METHODS

Plants of same age and same species of *Clerodendrum inerme* (Linn.) Gaertn. growing at Hooghly district (22°53´ N latitude and 87°50´ E longitude) of West Bengal, India were selected. Stigma receptivity was examined by the method of Martin (1959) and Joshirao and Saoji (1989). For this purpose 20 pollinated stigmas with some part of style were fixing with acetic alcohol (1:1) and softening with 4N NaOH. Finally softening stigmas were stained with aniline blue (water soluble aniline blue 0.05% in 0.05 M Na_2HPO_4). The method of Shivanna and Rangaswamy (1993) was followed for esterase location over stigma surface by using alpha-naphthyl acetate as a substrate, 0.15 M phosphate buffer (pH 6.8), and fast blue B Salt. The occurrence of bubbling action on the stigma as an indicator of peroxidase activity was determined by using 4% aqueous solution of Hydrogen peroxide (Kearns and Inouye 1993). Microphotograph were taken by Olympus Trinocular Microscope (Model: CH20i B1ME, No: iIID 195) at low magnification (10X×10X) as well as high magnification (10X×40X). For SEM, fresh stigmas were collected during their high receptive period and washed with 0.015 M sodium phosphate buffer (pH 7.2) and fixed in 2% glutaraldehyde for 4 h and were dehydrated in an ethanol series of 50%, 70%, 80%, 90% v/v and absolute for 10 min in each grade. The dehydrated stigmas were passed through a mixture of ethanol and amylacetate at 1:1, 1:2 and 1:3 ratio respectively for 5 min in each grade. Stigmas were preserved in pure amylacetate solution. After critical point drying and gold coating (IB2-ion Coater), photograph were taken in Scanning Electron Microscope (Hitachi S-530 SEM, Japan) to examine the surface pattern and described following the terminology of Heslop-

Harrison (1992) and Heslop-Harrison and Shivanna (1977).

RESULTS

Stigma of *Clerodendrum inerme* (Linn.) Gaertn. is wet as well as non-papillate and the non-specific esterases are seen densely all over the bi-fid stigma. Significant presence of esterase is observed within 24 h of flower anthesis (2nd day flower during afternoon). Maximum 53% stigma showed *in vivo* pollen germination along with mean 253±112.15 µm pollen tube length over the stigmatic surface towards its receptivity on the second day flower during afternoon (Table 1 and Fig. 1). Prominent presence of peroxidase enzyme was observed within 24 h of flower opening (9±0.94 oxygen bubbles/2 min by using hydrogen peroxide) during high receptive period of stigma and esterase activity (Table 1 and Fig. 1). Presence of copious esterase over stigma surface and peroxidase coincided with its receptivity. Prominent presence of esterase and peroxidase enzymes was observed during higher receptive period of stigmas (Table 1). In case of esterase activity, when stigma becomes more receptive, then the reaction product on the stigmas becomes intense due to resulting product, alpha-napthol, which is colourless and forms a reddish insoluble complex with coupling agent, fast blue (Mattsson *et. al*., 1974, Ghosh and Shivanna, 1984).

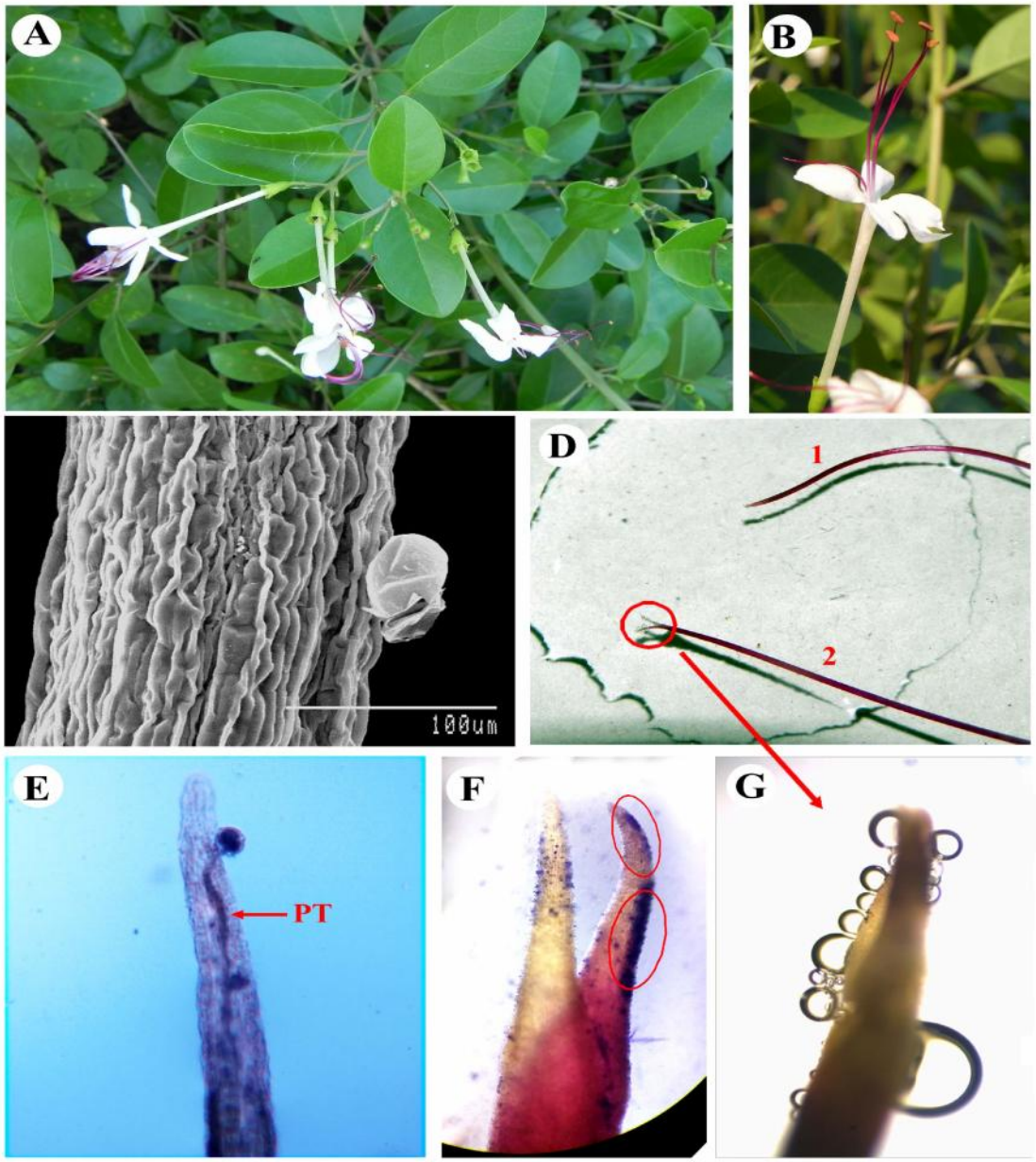

FIGURE 1: *Clerodendrum inerme* A. Plant habit, B. Single flower, C. SEM of stigma showing *in vivo* pollen germination (×500), D. Peroxidase activity (1= 1st day stigma and 2= 2nd day stigma), E. *In vivo* pollen germination (LM 10X), F. Esterase activity (LM 10X) and G. Peroxidase activity of 2nd day stigma (LM 10X)

TABLE 1: Stigma receptivity of *Clerodendrum inerme*

Period after flower opening	Day of flower anthesis			
	1st day	2nd day (Morning)	2rd day (afternoon)	Drooping stage
Total number of stigmas observed	20	20	20	20
Total number of pollen retained on the stigma	0.40	0.60	0.75	
Average number of germinated pollen	0.10	0.25	0.40	-
Percentage of germinated pollen	25	42	53	-
Mean pollen tube length(µm)	77±14.18 (R 70-100, N=10)	216 ±97.43 (R 130-400, N=10)	253±112.15 (R 150-420, N=10)	88±24.40 (R 50-130, N=10)
Number of evolved bubbles/ 2 min in H_2O_2 solution	-	2.60±0.51 (R 2-3, N=10)	9±0.94 (R 8-10, N=10)	1.50±0.53, (R 1-2, N=10)
Intensity of stigma receptivity (Peroxidase activity)	+	++	+++	+
Intensity of stigma receptivity (Esterase activity)	+	++	+++	+

± Standard Deviation, N= Number of observation, R= Range. + = Low, ++= Moderate, +++=High and - =Absent

DISCUSSION

Stigma is the ideal place for post pollination event after successful placement of pollen. Success in plant breeding programme depends on the timing and duration of the stigma receptivity. Duration of stigma receptivity is variable; sometimes it may last for a day or may remain receptive for many days. Moreover, stigma receptivity is varied from species to specie. In a condition of protogyny and protandry, stigma remains receptive for one or a few days before or after anthesis (Lloyd and Webb, 1986; Williams *et al*., 1991). The wet-type stigma secrets exudates which contain lipids, phenolic compounds, proteins, carbohydrates, lectins, amino acids, phosphatase including esterase and peroxidase (Lavithis and Bhalla, 1995; Bhattacharya *et. al*., 2004; Chuodhury *et. al*., 2008; Kulloli and Sreekala, 2009). High enzymatic activity on the stigmas in the form of esterase and peroxidase expression is used as indicators to assess the stigma receptivity and also for the detection of receptive part of the stigmatic surface. Though release of stigmatic fluids including esterases and peroxidase has a dependence on stigma morphology, vigour of the stigma and its receptivity. Present finding of stigma receptivity is collaborated with the work of Bhattacharya and Mandal (2004), Choudhury *et al*. (2012) and Dey *et al*. (2016) in *Moringa oleifera* Lamk., *Carissa carandas* Linn. and *Grewia asiatica* Linn. respectively.

CONCLUSION

During high receptive period, esterase and peroxidase expression becomes significant in particular time of anthesis and suggests contribution of esterase and peroxidase towards stigma receptivity of *Clerodendrum inerme* (Linn.) Gaertn. During high receptive period stigma showed maximum pollen germination (*in vivo*). So, stigma receptivity in terms of *in vivo* pollen germination with reference to esterase and peroxidase activity at different time after flower anthesis is important for reproductive success of a particular plant species, because any success in breeding programme depends on the timing and duration of the stigma receptivity.

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