Studies on Stigma Receptivity of *Grewia asiatica* L. with Reference to Esterase and Peroxidase Activity

Krishnendu Dey[1], Subrata Mondal[2], Sudhendu Mandal[3*]

[1,2,3]Department of Botany, Visva-Bharati, Santiniketan-731235, West-Bengal, India.

Abstract— *The present paper deals with the stigma receptivity in terms of in vivo pollen germination of Grewia asiatica L. (Tiliaceae), an economically fruit yielding plant, with a view to find out the stigma receptive period and correlation of stigma receptivity with the activity of esterase and peroxidase on stigmatic surface in order to provide information about reproduction as a basis for fertilization and plant breeding programme. Flowers open at 7.00 hrs to 8.30 hrs. Anther dehisces by longitudinal slits just before flower opening. The non-specific esterase and peroxidase are present densely all over the stigmatic head. Stigma showed maximum receptivity (91.5%) with mean pollen tube of 338µm after 3 hrs of anthesis. Conspicuous presence of esterase and peroxidase was observed during higher receptive period. Prominent presence of peroxidase enzyme was also observed after 3hrs of flower opening (45 oxygen bubbles/minute by using hydrogen peroxide) during maximum receptive period of stigma.*

***Keywords— Stigma receptivity, esterase, peroxidise activity, Grewia asiatica* L.**

I. INTRODUCTION

Receptivity of the stigma is a critical factor for successful completion of the post-pollination events (Joshirao and Saoji 1989). Physiological, cytochemical, biochemical and structural features of the stigma are of prime importance in the sexual life of a plant leading to the effective post-pollination events which produce mature fruits and seeds for future generation and other uses. Stigma receptivity refers to the ability of the stigma to support pollen germination and tube growth of viable, compatible pollen grain (Shivanna 1998). Stigma receptivity is a critical stage in maturation of the flower that may greatly influence the success of pollination at different stages in the life cycle of the flower (Barrett, 2002).

Generally receptivity is maximum soon after anthesis, it varies among species and depends upon temperature and humidity (Shivanna and Johri, 1985). Joshirao and Saoji(1989) also stated that the period of receptivity may vary from species to species. The receptive surface of the stigma contains extracellular proteins either as an extracuticular layer called pellicle in dry stigma or as a component of the exudates in wet stigma (Heslop-Harison and Shivanna, 1977; Heslop-Harrison, 1981; Shivanna and Johri, 1985). Esterase and peroxidase are the important components of the stigma surface proteins and their presence is related to stigma receptivity .Therefore, stigma receptivity in terms of *in vivo* pollen germination of *Grewia asiatica* L. belonging to the family Tiliaceae with reference to esterase and peroxidise activity at different times after anthesis is of prime importance in the reproductive biology. These observations were further correlated between esterase and peroxidase activity with stigma receptivity (Stone *et .al.* 1995; Lavithis and Bhalla, 1995; Bhattacharya *et. al* 2004; Choudhury *et. al*. 2008, 2011, 2012).

II. MATERIALS AND METHODS

Plants of *Grewia asiatica* L. growing in and around Visva-Bharati university campus at Santiniketan were selected (Fig. 1). Stigma receptivity was observed by the method of Martin (1959) and Joshirao and Saoji (1989); first by fixing with acetic alcohol (1:1), softening with 4N NaOH until the tissue become soft. These were then washed and mounted in 0.05% decolorized aniline blue in 0.5 M NaH_2PO_4.The tissue got flattened by applying gentle pressure and was taken for observation under microscope.

Cytochemical localization of non-specific esterases is based on hydrolysis of the substrate, alpha –napthyl acetate. The resulting product, alpha –napthol, is colourless, and forms a reddish insoluble complex with the coupling reagent, fast blue B (Ghosh and Shivanna 1984). Unpollinated but fully developed flowers were collected. For this experiment two types of solutions (A and B) were prepared out of which solution A contained fast blue B, sucrose (10% w/v), phosphate buffer (0.15M,pH 6.8) and alpha-napthyl acetate as a substrate for esterase, whereas solution B lacked the substrate and was used as control. A few drops of both the types of solution were taken on a separate grooved slide and excised stigmas were dipped into solutions A and B separately. The slides were then incubated at 25º C in a humidity chamber (Shivanna and Rangaswamy, 1993) for 22 minutes. After incubation period, the stigmas were removed and rinsed thoroughly with

phosphate buffer (pH 6.8).The stigmas were mounted with 50% glycerine, observed under light microscope at low magnification (10%) and details of the stigmatic surfaces were studied.

The occurrence of bubbling action on the stigma surface as an indicator of peroxidase activity was determined by using hydrogen peroxide (H_2O_2) (Kearns and Inouye 1993) Microphotographs were taken by Zeiss (Axiostar plus) microscope at 20X magnification.

III. RESULTS AND DISCUSSION

Stigma of *Grawia asiatica* L. is wet-papillate and the non—specific esterases are seen densely all over the surface on the stigmatic head and significant presence (reddish colour) is observed within 3hrs of anthesis. Stigma showed maximum receptivity (91.5%) with mean pollen tube length 338 µm after 3 hrs of anthesis (Table-1, Fig.-2). Prominent presence of peroxidase enzyme was also observed within 3 hrs of flower opening (45 oxygen bubbles /minute by using hydrogen peroxide H_2O_2) during maximum receptive period of stigma and esterase activity (Table-1, Figs.- 3, 4). Presence of copious esterase over stigma surface and peroxidase coincided with its receptivity. When stigma receptivity becomes more, 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 agent, fast blue (Mattsson *et. al.*, 1974; Ghosh and Shivanna, 1984) in case of esterase activity. Prominent presence of esterase and peroxidase enzymes was observed during higher receptive period of stigma.

TABLE 1
STIGMA RECEPTIVITY (IN *VIVO* POLLEN GERMINATION) OF *GREWIA ASIATICA* L.

Time after flower opening	Bud condition	After 1 Hour	After 3 Hours	After 6 Hours	Dropping Stage
No. of stigma observed	10	10	10	10	10
Total No. of pollen over stigma	32	80	95	105	112
No. of germinating pollen over stigma	-	60	87	82	78
% of germinating pollen grains	-	75	91.5	78	69.5
Pollen Tube Length (µm)	-	143	338	273	156
Esterase Expression	+	++	+++	+++	++
Peroxidase Activity	+	++	+++	++	++
Bubbles/Minute by using H_2O_2	25	35	45	40	15

+: Low, ++: Moderate, +++: High, -: Absence

Successful breeding programme depends on the timing and duration of the stigma receptivity. However, in protogynous and protandrous species the stigma becomes receptive one or a few days before or after anthesis (Lloyd and Webb, 1986; Williams *et al.,* 1991; Sedgley and Hardard, 1993). The duration for which the stigma remains receptive is also variable; it may last just for a day, or may remain receptive for many days. Generally unpollinated stigma remains receptive for a longer period (Ascher and Peloquin, 1966; Shivanna and Rangaswamy, 1969). The only effective method is through controlled pollinations and subsequent studies on fruit and seed set. As this takes a long time, pollen germination and pollen tube growth following pollination can also be used to assess stigma receptivity.

The most effective technique to study pollen germination and pollen tube growth in pistil is through aniline blue fluorescence (Shivanna and Rangaswamy, 1993). Aniline blue preferentially stains callose. Since pollen tubes invariably contain callose as a layer of the cell wall and as plugs, pollen tubes show bright fluorescence and can be clearly distinguished from the pistillate tissue. The wet-type stigma secrets exudates containing lipids, phenolic compounds, proteins, carbohydrates, lectins, amino acids, phosphatase including esterase and peroxidase (Lavithis and Bhalla, 1995; Bhattacharya *et.al.* 2004; Chuodhury *et a*l. 2008, 2011, 2012, Kulloli and Sreekala 2009).

High enzymatic activity in the stigma in the form of esterase and peroxidase expression was used as indicators to assess stigma receptivity and also for the detection of receptive part of stigmatic surface. The release of stigmatic fluids including esterase and peroxidase has a dependence on stigma morphology, vigour of the stigma and its receptivity. It was observed that

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. Choudhury *et al*. (2011) studied stigma receptivity of *Rauvolfia serpentina* (L.) Benth. ex. Murz with reference to erterase and peroxidase activity and pointed out that stigma showed maximum receptivity (69%) with mean pollen tube length of 169 µm after 1 hr of anthesis and prominent presence of esterase and peroxidase was observed during higher receptive period. Bhattacharya and Mandal (2004) studied pollination, pollen germination and stigma receptivity in *Moringa oleifera* Lamk. and observed that maximum receptivity (40%) with *in vivo* germinating pollen (48.47%) was recorded in third day after flower anthesis in natural population and the delayed receptive period indicates cross pollination strategy. Choudhury *et. al*. (2012) also studied on esterase and peroxidase activity of *Carissa carandas* L. in relation to stigma receptivity and pointed out that stigma showed maximum receptivity (66%) with mean pollen tube length 219 µm after 3 hrs of anthesis which gets support from the present investigation.

FIGURE- 1. *GREWIA ASIATICA* L. PLANT

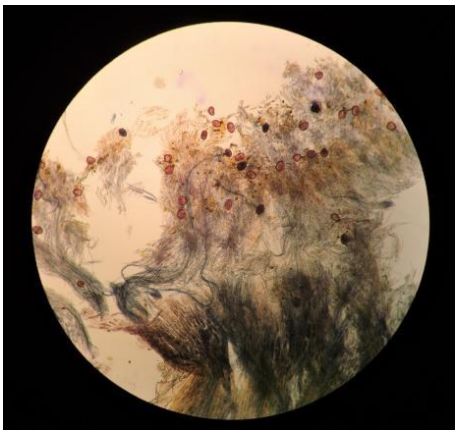

FIGURE-2. *IN VIVO* POLLEN GERMINATION

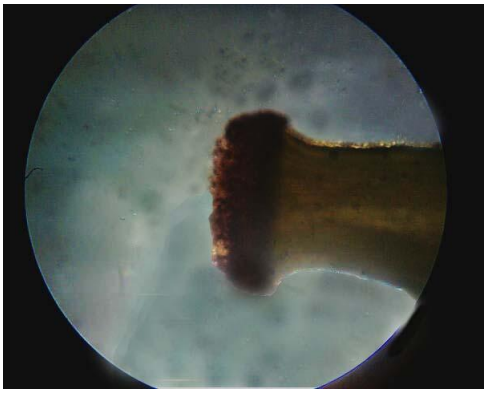

FIGURE-3. PRESENCE OF ESTERASE (REDDISH COLOUR) OVER STIGMATIC SURFACE.

FIGURE-4. PEROXIDASE ACTIVITY INDICATED BUBBLES ON RECEPTIVE STIGMA.

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