Studies on Esterase and Peroxidase activity of *Lens culinaris* Medik. in relation to Stigma Receptivity

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ABSTRACT:

The current study determined the esterase and peroxidase receptivity of *Lens culinaris* Medik. an economically important pulse crop from the Fabaceae family, in order to determine the stigma receptive period and the correlation of stigma receptivity with the activity of esterase and peroxidase on the stigma surface in order to provide information for fertilization as a basis for plant breeding. It bloomed from December to February and normally opened between 18:00 and 19:00 hours. After flower opening, anthers dehisced through a longitudinal slit. A single flower produced approximately 7000 pollen grains. The stigma was wet-papilate. Nonspecific esterases and peroxidase were found in dense concentrations all over the surface of the stigmatic head and in a dispersed pattern just below the stigmatic head.

Key Words: Stigma receptivity, stigma receptivity, wet-papilate, nonspecific esterases.

INTRODUCTION:

Susceptibility to stigma can be measured by in vivo germination, esterase and peroxidase activity. Interactions between pollen and pistils play an important role in sexual reproduction in flowering plants. Pollen grain production and dispersal have biological and genetic effects on high-quality fruit and seed production.

Stigma receptivity is a key factor for successful completion of post-pollination events. Generally highest immediately after flowering, the duration of receptivity varies among species and is affected by temperature and humidity (Shivanna and Johri, 1989). The stigma generally aids in hydration and germination of pollen from its own species and closely related taxa (Heslop-Harrison, 1981).

The receptive surface of stigma contains extracellular proteins, either as membranes in dry stigma or as part of the exudates in moist stigma (Harrison and Shivanna, 1977; Heslop-Harrison, 1981; Shivanna and Johri , 1985). Esterase and peroxidase are important

The constituents of stigma surface proteins and their presence are associated with stigma sensitivity. Therefore, the stigma receptivity for *in vivo* pollen germination of *Lens culinaris* Medik.

The legume family in terms of esterase and peroxidase activity at various times after flowering is of paramount importance for the biology of sexual reproduction.

We also aimed to establish a link between esterase and peroxidase activity and susceptibility to stigma (Stone *et al*., 1995; Lavithis and Bhalla, 1995; Choudhury *et al*., 2012).

MATERIALS AND METHODS:

Stigma receptivity is an important stage in flower maturation and can significantly affect pollination rate and pollination success at different stages of the flower life cycle. Susceptibility to stigma was observed using the standard method of Joshirao and Saoji (1989). For this purpose, stigma were first fixed with alcohol acetate (1:1) and softened with 4N NaOH until the tissue was soft. They were then washed and mounted in 0.05% destained aniline blue in 0.5M NaH2PO4. The tissue was flattened with light pressure and removed for microscopic observation.

The stigma surface was studied according to the esterase method proposed by Shivanna and Rangaswamy (1992). Two solutions (solution A and solution B) were prepared. Solution A contained sucrose (10%), Fast Blue B salts, 0.15 M phosphate buffer (pH 6.8), and α-naphthyl acetate as a substrate for the esterase, solution B excluding the substrate. Contains the same ingredients as solution A. The two solutions were dispensed into separate Petri dishes with 1% agar plates and freshly cut stigmas were dipped into the agar plates, leaving the entire stigma in the solution. Selected stages of excised pistils were immersed separately in solutions 'A' and 'B' and incubated at 250° C. for 22 minutes in a humid chamber. After the indicated incubation time (10–20 minutes), stigmas were removed and washed with phosphate buffer (pH 6.8). Stigma surface esterase activity was assayed in bulk stigmas treated in 50% glycerol and observed by light microscopy. In addition, stigma surface details were examined (Dafni and Maues, 1998). Blister development was measured using hydrogen peroxide (H_2O_2) on the stigma surface as an index of peroxidase activity (Dafni, 1992).

RESULT AND DISCUSION:

The stigmas of *Lens culinaris* Medik. is wet and Non-specific esterases were found densely over the entire stigma surface and significant development was observed within 6 hours after flowering (Table -1, Fig.- 1). The stigma showed maximum receptivity (65%) with an average pollen tube length of 285 µm 6 hours after anthesis and persisted until the drooping stage (Table- 1, Figure -3). A distinct presence of the enzyme peroxidase was observed within 6 h of flower opening (21 oxygen bubbles per minute using hydrogen peroxide) and also during the period of maximal uptake of stigmas and esters (Fig.- 2).). Abundance of esterases and peroxidases on the stigma surface was consistent with receptivity. As stigma sensitivity increased, the reaction product on the stigma became stronger with the resulting product, alpha-naphthol. α-Naphthol has a reddish insoluble complex with the coupling agent and is almost blue in the case of esterase activity (Mattsson *et al.* 1974, Gauche and Sivanna (1984). A clear occurrence of the enzymes esterase and peroxidase was observed during the longer absorption times of stigmas (Table -1, Figures 1, 2). The success of plant breeding programs depends on the timing and duration of susceptibility to stigma.

Fuss and Sedgley (1991) studied the timing of stigma sensitivity, pollen tube growth, and self-incompatibility in relation to fertility in Banksia coccinea. Bhattacharya and Mandal (2004) studied pollination, pollen germination and stigma receptivity in Moringa oleifera. Ghosh and Shivanna (1984) studied Zephyranthes stigma structure and cytochemistry and pollen-pistil interaction. The cytochemical localization of non-specific esterases and peroxidases are important constituents of stigma surface proteins and their presence is associated with stigma sensitivity (Shivanna and Rangaswamy, 1993; Kearns and Inouye, 1993). Wet stigma secretions contain lipids, phenolic compounds, proteins, carbohydrates, lectins, amino acids, and phosphatases, including esterases and peroxidases (Vasil, 1974; Dafni and Maues, 1998; Vaughton *et al.*, 2010). . Joshirao and Saoji (1989), Tandon et al. (2001), Bhattacharya and Mandal (2003), Bhattacharya *et al*. (2004), Sreekala *et al.* (2008), Choudhury *et al.* (2008, 2012), Kulloli *et al*. (2010), Allen *et al.* (2011), Kukade and Tidke (2013), Biswas and Mohammad (2016) observed that the expression of esterases and peroxidases became prominent during the receptive stage, especially during the flowering stage, suggesting that esterases and peroxidases are associated with stigma sensitivity.. Peroxidase and esterase activity is being used as indicator to assess the stigma receptivity for maximum pollen germination *in vivo* condition.

Table –1. *In vivo* pollen germination of *Lens culinaris Medik.*

Stigma observation time	Bud condition	After -3hrs	After -6hrs.	After-9hrs.	During Drooping stage
Total number of stigmas observed	10	10	10	10	10
Total number of pollen retained on the stigma	-	143	240	112	61
Mean number of	-	15	65	9	3

germinated pollen					
Percentage of germinated pollen(%)	-	10	27	9	4
Mean tube length (µm)	-	110	285	132	30
Esterase activity	+	++	+++	++	+
Peroxidise activity	+	++	+++	++	+

CONCLUSION:

Esterase and peroxidase expression becomes prominent at some point during the hyper-receptive stage. This suggests that esterases and peroxidases contribute to the stigma receptivity of *Lens culinaris* Medik. During the peak period of acceptance, Stigma showed maximum pollen germination (*in vivo*). Thus, stigma susceptibility to pollen germination *in vivo* in terms of esterase and peroxidase activities at different time points after flower opening may be determined by specific plant species, as the success of a breeding program depends on the timing and duration of stigma sensitivity. It is important for successful breeding of flowering plants.

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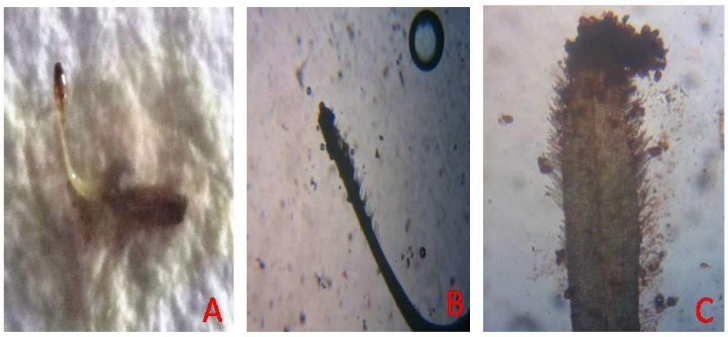

Fig. -1. Presence of esterase over stigmatic surface.

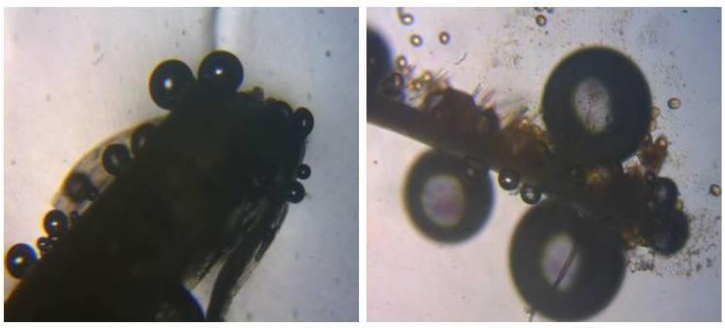

Fig. -2. Bubbles over stigma showing peroxidase activity

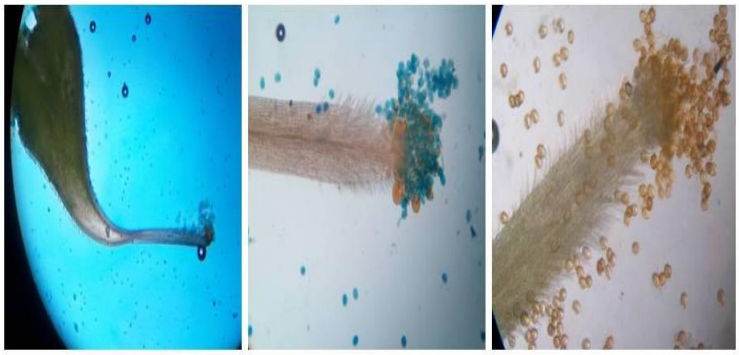

Fig-3. *In-Vivo* Pollen Germination

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