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RESEARCH ARTICLE

STUDIES ON ESTERASE AND PEROXIDASE ACTIVITY OF *Carissa carandas* Linn. IN RELATION TO STIGMA RECEPTIVITY

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ABSTRACT

The present investigation revealed the esterase and peroxidase activity in relation to stigma receptivity of *Carissa carandas* Linn. , a medicinally important plant belonging to the family Apocynaceae with a view to find out the stigma receptive period and correlation of stigma receptivity with the activity of esterase and peroxidase on stigma surface in order to provide information for fertilization as a basis for plant breeding programme. It Flowered during March to July and opened at 17:00 hrs to 22:00 hrs generally, but sporadic flower opening took place throughout the night. Anthers dehisced by longitudinal slit after flower opening. Single flower produced about 5160 pollen grains. Stigma was wet-papilate and above the anther level. The non-specific esterases and peroxidase were present densely all over the surface on the stigmatic head and in scattered manner just below the stigmatic head which may act in facilitating *in vivo* germination. Stigma showed maximum receptivity (66%) with mean pollen tube length 219µm after 3 hrs of anthesis, however stigma receptivity retained upto drooping stage to some extent. Prominent presence of esterase and peroxidase were observed during higher receptive period.

INTRODUCTION

Stigma receptivity refers to the ability of the stigma to support germination and tube growth of viable, compatible pollen (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).The receptive surface of stigma contains extracellular proteins either as a pellicle in the dry stigmas or as a component of the exudates in the wet stigmas (Heslop-Harrison and Shivanna,1977; Heslop-Harrison,1981; Shivanna and Johri, 1985). Esterase and peroxidase are the important components of the stigma surface proteins and its presence is related to stigma receptivity. So, stigma receptivity in terms of *In vivo* pollen germination of *Carissa carandas* Linn., belonging to the family Apocynaceae with reference to esterase and peroxidase activity at different time after anthesis, is of prime importance in the biology of sexual reproduction. It also aimed in making 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*., 2011).

MATERIALS AND METHODS

Plants of the same age and same species of *Carissa carandas* Linn, located at University campus, Santiniketan were selected. Stigma receptivity was examined by the method of Martin (1959) and Joshirao and Saoji (1989) first by fixing with acetic alcohol (1:1), softening with 4N NaOH and staining the stigmas with aniline blue. The method of Shivanna and Rangaswamy (1993) was followed for esterase location over stigma surface, by using alpha-naphthyl acetate as substrate, 0.15 M phosphate buffer (pH 6.8) and fast blue B Salt as coupling agent. The occurrence of bubbling action took place on the stigma as an indicator of peroxidase activity by using hydrogen peroxide (Kearns and Inouye, 1993). Microphotograph were taken by Zeiss (Axiostar plus) microscope at 20X magnification.

RESULTS AND DISCUSSION

Stigma of *Carissa carandas* Linn., was wet-papillate and the non-specific esterases were seen densely all over the surface on the stigmatic head and significant presence was observed within 3 hrs of anthesis (Fig.1). Stigma showed maximum receptivity (66%) with mean pollen tube length of 219µm after 3 hrs of anthesis and retained upto drooping stage. Prominent presence of peroxidase enzyme also observed (Fig. 2) within 3 hrs of flower opening (19 oxygen bubbles/minute by using hydrogen peroxide) during maximum receptive period of stigma and esterase activity. Presence of copious esterase over stigma surface and peroxidase coincided with its receptivity. When stigma receptivity became more, then the reaction product on the stigmas became intense due to resulting product, alpha-napthol which is colourless and forms a reddish insoluble complex with coupling agent, fast blue in case of esterase activity (Mattsson *et. al*, 1974; Ghosh and

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Table 1. Stigma receptivity (*in vivo* pollen germination) of *Carissa carandas* Linn

Time after flower opening	Bud condition	After 1hr.	After 3 hrs.	After 6 hrs.	Drooping stage
No. of stigma observed	10	10	10	10	10
Total No. of pollen over stigma	---	150	156	189	194
Total No. of germinating pollen over stigma	---	24	103	103	29
% of germinating pollen grains	---	16	66	54	15
Pollen Tube length (μm)	---	118	219	172	55
Esterase expression	++	+++	+++	++	+
Peroxidase activity	++	+++	+++	++	+

+++ : High. ++ : Moderate. + : Low. — : Absence

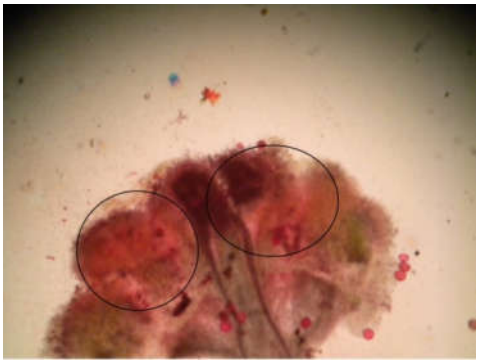

Fig.1: Presence of esterase over stigmatic surface.

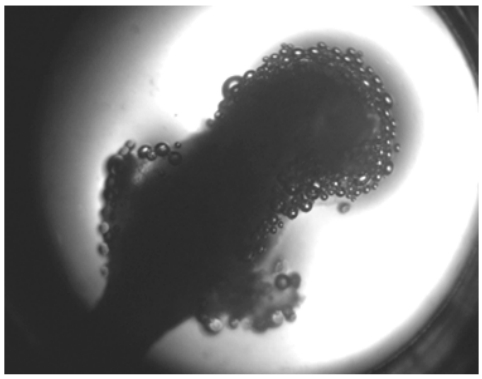

Fig. 2: Bubbles over stigma showing peroxidase activity

Shivanna,1984). Prominent presence of esterase and peroxidase enzymes were observed during higher receptive period of stigmas (Table 1). Any success in plant breeding programme depends on the timing and duration of the stigma receptivity. 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; Choudhury *et al*, 2008, 2011). High enzymatic activity in the stigmas in the form of esterase and peroxidase expression used as the indicator to assess the stigma receptivity and also for the detection of receptive part of the stigmatic surface. The release of stigmatic fluids including esterases and peroxidase have a dependence on stigma morphology, vigor of the stigma and its receptivity. It was observed that during high receptive period, esterase and peroxidase expression became significant in particular time of anthesis and suggested contribution of esterase and peroxidase towards stigma receptivity.

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