SHORT COMMUNICATION

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Stigmatic receptivity determines the seed set in Indian mustard, rice and wheat crops

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Stigmatic receptivity restricts the successful pollination in cereal crops. The present study deals with the biochemical test for enzymes producing in stigma of field crops such as Indian mustard, rice and wheat. The alcohol dehydrogenase and hydrogen peroxide assays revealed stigmatic receptivity as a violet color and oxygen bubbles released by the chemical reaction. Therefore, the 2 quick tests are in conformity to each other and supported the seed set data, which was utmost at blooming stage of flower ranged between 2–4 d All the 3 crops showed variation in stigmatic receptivity with respect to different time periods of blooming stages and hence, it may affects simultaneous pollen germination and tube growth, fertilization and seed set. The present finding suggests that the growth of pollen tube and stigma receptivity could be influenced by specific enzymes on stigma surface after 2–4 d of blooming stage, which contributes to proper seed set.

Introduction

In angiosperms, pollen-pistil interaction, leads to fertilization and seed set. The proteins and secretary fluids present at the stigma play a crucial role in pollen germination, pollen tube growth and successful fertilization.[1] Pollen-pistil interaction involves adhesion of pollen grain on stigma, pollen tube growth and entry of pollen tube into ovule, all these processes depends on stigma receptivity. Stigma receptivity is the ability of stigma to support the viable and compatible pollen to generate. In general at the time of anthesis, the stigma is receptive and may lose for one 2 or several days.[2-4] Successful pollination period is determined with age of ovule, pistil morphology and receptivity of stigma.[5] The proteins present at the pollen, stigma and extra cellular matrix of pistil play an important role in pollen-pistil communication. The time and duration of stigma receptivity should be accompanied for successful breeding of crops.[6] The receptivity of stigmas are characterized by assaying the activity of several enzymes such as peroxidase, esterase, alcohol dehydrogenase and acid phosphatase studied in different species.[7-10] In alcohol dehydrogenase test, the stigma give violet stain with Baker's solution, however the peroxidase enzyme of stigma releases the oxygen bubbles with hydrogen peroxide water treatment.[10,11] In the present study, the peroxidase and alcohol dehydrogenase tests was performed to examine the stigmatic receptivity at different stages of flower blooming and thereby healthy seed set in Indian mustard, wheat and rice crops.

Results and Discussion

All selected Indian mustard with protogynous flowers possessed dry type of stigma. The stigma receptivity was observed up to 7 d since flower opening; however the maximum stigmatic receptivity in Indian mustard crop was recorded on third and fourth day after commencing the blossom of flower (**Fig. 1A**). However, wheat and rice crops having dry stigma type revealed greatest dehydrogenase activity on second and third day of blooming stage of flower (**Fig. 1B and C**). In the hydrogen peroxide test, the number of bubbles recorded on the stigma of flowers up to 7 d after the commencement of bloom stage, which indicates the degree of receptivity of stigma. The maximum number of bubbles was observed on third and fourth day after flower opening in Indian mustard crop (**Table 1**), however wheat and rice crops showed highest number of bubbles on second and third day after floret opening (**Table 2**). The maximum 10 seeds per pod were observed on third day after opening of flowers in Indian mustard, however 7 and 6 seeds per inflorescence were recorded

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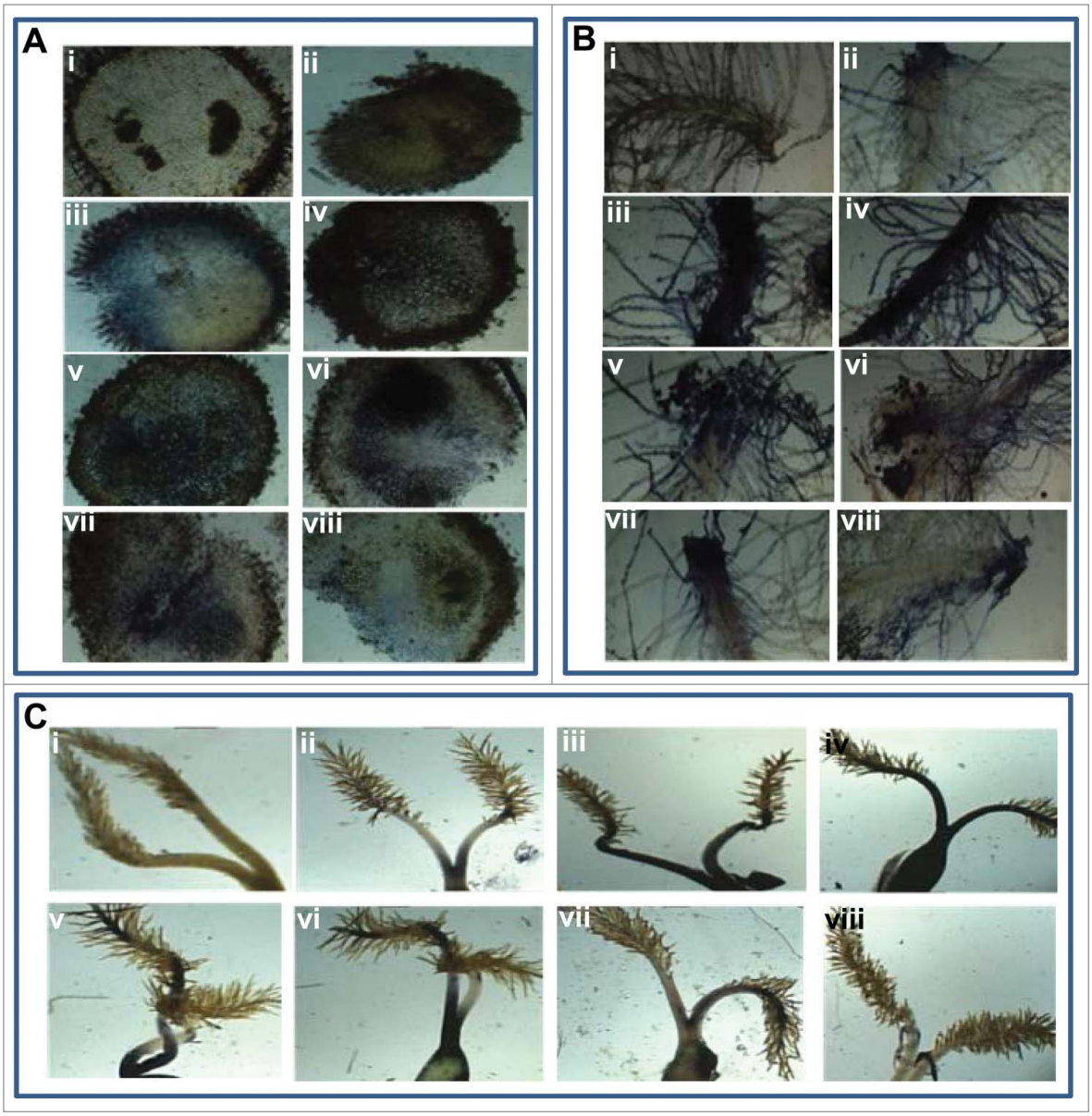

Figure 1. Evaluation of stigma receptivity of flowers of Indian mustard (**A**), wheat (**B**) and rice (**C**) in control (i) and after one (ii), 2 (iii), 3 (iv), 4 (v), 5 (vi), 6 (vii) and 7 (viii) days of commencement of bloom stage.

on the same age of group of flowers of wheat and rice, respectively (**Table 3**). Therefore, the 2 quick tests *viz.*, alcohol dehydrogenase and hydrogen peroxide are in conformity to suggest the potential role of receptivity of stigma in determining seed set. Owing to maximum receptivity in stigma, flowers showed dark violet stain and more number of bubbles as we have observed in all Indian mustard, wheat and rice crops. The finding presented herein might be useful for the breeder to make a cross in this stage and to bag flowers to prevent from contamination. These enzyme activities are positively correlated with stigmatic receptivity.[12] The present reports revealed that alcohol dehydrogenase and peroxidase on the stigma surface represent a peak receptivity after third day to fourth day in Indian mustard. The high activity of these enzymes indicates greatest receptivity of stigma and proper seed set. The alcohol dehydrogenase and peroxidase enzymes were used to evaluate stigmatic receptivity for successful fertilization in other crops.[13-14] The peroxidase on the surface of stigma facilitates communication between pollen and pistil by loosening the cell-wall components of the stigma which allow the entry of pollen tubes to fertilize ovule.[9] In the present investigation, stigma receptivity was maximum on third and fourth day after anthesis in Indian mustard, and on second and third day in rice and wheat. The synthesis of stigmatic peroxidase and alcohol dehydrogenase modulates stigma morphology, vigor, and receptivity and biochemical functions of papillae tissues. However, of level of these enzymes may vary among the species of plants. The extent and mode of localization of dehydrogenase and peroxidase enzymes in the stigma of Indian

Table 1. Stigma color, type and receptivity level using hydrogen peroxide (H_2O_2) procedure in Indian mustard at different blooming stages of flower including bud stage

Sl. No.	Flower stage (day)	Stigma color	Stigma type	Number of bubbles on stigma
1	Bud stage	Yellow green	wet	+
2	One	Yellow green	wet	+
3	Two	Yellow green	wet	+++
4	Three	Yellow green	wet	++++
5	Four	Yellow green	wet	+++
6	Five	Yellow green	wet	+
7	Six	Yellow green	wet	+
8	Seven	Yellow green	wet	+

+Weak positive response.
++Strong positive response.
++++Very strong positive response.

Table 3. Seed set determination by assessing the number of seeds present per pod/inflorescence in mustard, wheat and rice crops at defined conditions of flowering stages selected for stigmatic receptivity and pollination

Sl. No.	Flower stage (day)	Indian mustard (seed/pod)	Wheat (Seed/ inflorescence)	Rice (Seed/ inflorescence)
1	unopened	0.0	0.0	0.0
2	One	3.33	2.0	4.3
3	Two	5.33	5.0	4.3
4	Three	10.0	7.0	6.3
5	Four	8.0	3.3	3.0
6	Five	4.0	0.0	0.0
7	Six	2.33	0.0	0.0
8	Seven	0.0	0.0	0
	CD	0.819	0.682	0.625
	SE(m)	0.267	0.223	0.204
	SE(d)	0.378	0.315	0.289
	CV	11.222	17.804	15.713

mustard, rice and wheat showed positive correlation with peak receptive period of stigma that was evidenced as seed set at same stage of flower bloom. These two enzymes *i.e.*, peroxidase and alcohol dehydrogenase based study on stigmatic receptivity as a function of different stages of flower development showed that the stigmatic receptivity and seed set was highest over time periods ranged from 2 to 4 d after commencement of blooming of flower.

Materials and methods

The crops such as Indian mustard cv. Pusa Mahak, wheat cv HW2041 and rice cv. Pusa 6A were used to examine stigma receptivity. Indian mustard cv Pusa Mahak has protogynous flower. The wheat cv. HW2041 and rice cv Pusa 6A were CMS in nature. Freshly cut stigmas of protogynous flowers of Indian mustard, wheat and rice inflorescences were collected from the varieties grown in the field, Indian agricultural Research Institute, New Delhi, India. The protogynous flowers at different stage *viz.*, stage i, closed flower (control); stage ii, one day open flower; stage iii, 2 day open flower; stage iv, 3 day open flower; stage v, 4 day open flower; stage vi, 5 day open flower; stage vii, 6 day open flower and stage viii, 7 day open flower after commencement of bloom stage. Rice and wheat inflorescence studied up-to 7 d i.e., completely open flowers. All stigma examined were checked under a magnifier (X10) for any damage of surface, either of which may cause enzyme activity regardless of stigma receptivity.[15] For each species we have taken a total 10 stigmas of different stage of flower development to evaluate the receptivity. Samples and experiments were repeated 3 times.

Alcohol dehydrogenase based test known as Baker's procedure by Galen and Plowright (1987)[16] was performed with slight modification. The test solution consisted of 10 mL of 1M phosphate buffer (pH 7.4), diluted (1 part buffer to 2 part distilled water); 6 mg NBT (nitroblue tetrazolium); 5 mg NAD (nicotinamide adenine dinucleotide) and 1 mL ethanol. The fresh stigma were cut and put on large droplet of this test solution on slide and incubated at 8 °C in closed petri dish containing moist filter paper in the bottom. The stigma was inspected after 12–18 hrs under a microscope (X10) to locate the stained area and photographs taken using microscope Olympus Magnus (MLXi). A 6% of hydrogen peroxide (H_2O_2) solution was placed on the stigma and the appearance of bubbles was observed.[17]

Ten flowers were pollinated at different stages as mentioned above and were bagged with butter paper envelop and seed set in each pollinated flower was recorded and data was analyzed with OP Statistical package.

Table 2. Stigma color, type and receptivity level by means of hydrogen peroxide (H_2O_2) procedure of wheat and rice up to seven days after floret opening

Sl. No.	Flower stage (day)	Stigma color	Stigma type	Number of bubbles on stigma
1	unopened	white	dry	+
2	One	white	dry	++
3	Two	white	dry	++++
4	Three	white	dry	++++
5	Four	white	dry	++
6	Five	white	dry	+
7	Six	white	dry	+
8	Seven	white	dry	+

+Weak positive response.
++Strong positive response.
++++Very strong positive response.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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