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Comparative study of floral biology using detergent and confirm self-incompatibility system in protogynous lines of Indian mustard (*Brassica juncea*)

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ABSTRACT

Seven genotypes of mustard [*Brassica juncea* (L.) Czernj and Coss.], protogynous lines (F_{3-5} generation), cytoplasmic male sterility (CMS) line and different concentrations (3, 5 and 8% (w/v)) of detergent (synthetic detergent powder was used in present investigation. There is effect of detergent which induces more prominent male sterility expression in Pusa Bold followed by Ashirwad genotypes and more vigorous during floral bud initiation, resist to scorching effect and delayed in flowering. Furthermore, the floral attributes, viz. anther length (mm), no. of pollen grains/bagged flower, pollen fertility, stylar length (mm), stigma receptivity, no. of ovules and seed set (%) were highly significant and which induced 100 % male sterility. Similarly, the pollen-ovule ratio was found to be non-significant and the yield0/plant significantly declined with increase in detergent concentration. The PG line showed more prominent stigma exertion, extended protogynous interval (9-10 days) and stigma receptivity (3-4 days) from its protrusion compared to induced male sterile line. The expression of male sterility in PG and CMS lines showed the partial sterility (30-50%) and absolute (100%) respectively. Further, the PG line had low seed set on selfing even it produces normal pollen (pollen viability test) but it was high seed setting occurs with out-crossing hence, it was confirmed presence of self- incompatibility system in the protogynous plants.

Key words: *Brassica juncea*, Detergent, Induced male sterile, Protogynous line (PG), Pusa Bold, Self-incompatibility

The developments of hybrids using male sterility sources are routine in several field crops including Indian mustard [*Brassica juncea* (L.) Czernj and Coss.]. The cytoplasmic male sterility (CMS) system in brassica has many limitations, viz. non-availability of restorer lines, genetic non-uniformity, vulnerability to pest and diseases, laborious maintenance of A, B and R lines, non-synchronization of flowering between A and R lines, unstable sterility over environments and incomplete fertility restoration (Singh *et al.* 2017, Sinha *et al.* 2016).

Systematic and co-ordinated efforts for developing hybrids in rapeseed-mustard was initiated in 1989 under the ICAR sponsored project on Promotion of Research and Development Efforts on Hybrids in Crops using two CMS systems in *B. juncea*, i.e. ogu and tour and another one was reported in *B. napus*, i.e. polima (Levania and Chauhan 2006). The major emphasis was given to the simultaneous development of CMS-fertility restorer systems. The approach led to the development of seven new CMS systems, viz. *siifolia*, *oxyrrhina*, *muralis*, *catholica*, *nigra*, *moricandia* and *trachystoma* till 1995. These systems also have several limitations, particularly non-availability of full fertility restoration; hence it could not be utilized for hybrid development (Singh and Singh 1992). The development of genetically engineered system of male sterility, i.e. barnase-barstar system was one of the milestone by utilizing the hybrid vigor in rapeseed-mustard (Jagannath *et al.* 2002, Singh *et al.* 2017). Rawat and Anand (1979) reported that the male sterility in Indian mustard but, it could not be utilized due to incomplete fertility restoration.

Due to the failure of conventional breeding methods in identifying the restorer gene(s) in *B. juncea*, it was felt to find out restorer gene(s) in the same species from which cytoplasm had been intro-gressed. The concerted efforts were made at National Research Centre for Plant Biotechnology (NRCPB), New Delhi for the development of fertility restorer through protoplast fusion between the wild species

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and *B. juncea*. These efforts resulted in the development of fertility restorer for mori and trachy CMS systems. Two more CMS systems, viz. lyratus and canariense have also been reported. The Mori CMS and fertility restorer were found to be associated with severe chlorosis and retarded growth. Similarly in trachy CMS system, the lot of segregation for fertility/sterility coupled with crooked siliqua formation was observed. Therefore breeders are looking alternative sources such as chemical hybridizing system (CHA), protogynous system (PG), and naturally available source, i.e. self-incompatibility (SI) system to exploit heterosis in several field crops including *Brassica* sp.

A plenty of chemicals are available in market, which are capable of selective suppression of the pollen development are known as chemical hybridizing agents (CHAs). The chemical hybridizing agents were used for large scale commercial production of most hybrid seeds in different crops (Cross and Schulz 1997). These CHAs causes range of effects, viz. feminization of male florets, abnormal development of reproductive organs, inhibition of early anther development, abnormal tapetal behavior and suppression of microspore development (Chauhan and Kinoshita 1982). Chauhan and Singh (2002) reported the changes in floral biology, pollen fertility, seed-set and total yield after spraying with various concentrations of detergent a commonly used in *B. juncea* plants.

The protogynous line was derived from the cross between Agra local × Varuna by Singh (2008). The floral morphology of PG lines is complex and it is not worth to conclude that the failure of seed setting in these lines is attributed to self-incompatibility alone. So to reveal such mechanisms and to avoid self-fertilization, promotion of outcrossing using different selfing/pollination/cutting the stigma thereafter pollination was attempted in the present investigation.

In view of above background, the present investigation was framed to study the comparative floral biology in chemically induced male sterile line, CMS line, protogynous line and to confirm the presence of self-incompatibility in PG lines of Indian mustard.

MATERIALS AND METHODS

The agronomical good performing seven genotypes of mustard (Table 1) were screened based on doses of chemical sprayed and best combination of genotypes and concentrations were considered for the next successive years to undertake further floral biology study.

The protogynous line (F_3 generation) was procured from Department of Seed Science and Technology, Indian Agricultural Research Institute, New Delhi. This line was covered with nylon net to avoid out-crossing and allowed crossing among them. The 5-6 inflorescences from each row was covered with polybag and subjected to pollen fertility and stigma receptivity tests at different intervals. The data on important floral traits also recorded and compared with the induced male sterility lines as well as CMS lines (CMS used only to compare the pollen fertility).

Table 1 List of mustard varieties and their important attributes

Varieties	Maturity (Days)	Oil content (%)	Average yield (q/ha)
Pusa Bold	110-145	42	18.00
Ashirwad	125-135	31-41	14.50-23.58
Pusa Jagnnath	125	37-43	16.09-19.75
Pusa Agrani	110	39-40	17.50
Pusa Mahak	118	40	17.50
Pusa Tarak	121	40	18-19.25
Pusa Mustard-24	140	36.55	19.5-20.21

Materials procured from IARI, New Delhi.

A CMS line was procured from Department of Seed Science and Technology, IARI, New Delhi.

The field experiment was conducted during 2012-13, 2013-14 and 2014-15 at ICAR-Indian Institute of Seed Science, Mau, Uttar Pradesh, India. All genotypes of mustard were scrutinized the unsurpassed genotype using detergent with various concentrations (3, 5 and 8% (w/v)) to induce male sterility. The seeds of seven genotypes, CMS and protogynous lines were sown in a randomized row design with distance between rows and plants were maintained at 45 cm and 15 cm respectively. 25 plants in each row was selected and sprayed with different concentration of detergent only once (a week prior to floral bud initiation, i.e. 21 days after sowing) and rest of 25 plants were sprayed with distilled water. Each treatment was replicated thrice. Similarly, two rows of 25 plants were left as untreated and rest of plant rows were treated. The inflorescences of treated and untreated plants were bagged before preceding to pollen fertility test.

Observations on floral and floral traits were recorded, viz. open/closed flower, initiation of flowering, open of flower, size of anther and style, time of stigma receptivity, time of pollen viability, number of pollen grains and ovules per flower, pollen–ovule ratio, seed-setting, yield/plant and mode of pollination from the 6–7 flowers at the base of an inflorescence at the time of anthesis was collected nearly 20 days after spray from 75 plants of each treatment.

Three different concentrations (3, 5 and 8% (w/v)) of detergent (synthetic detergent powder (surf-excel), were used to induce male sterility in mustard crop. The chemically inducing male sterile methodology was followed by Chauhan and Singh (2002).

The pollen was collected from the flowers during anthesis and dusted on glass slide, then a drop of carmine acetic acid (CAA) stain was added. The specimen was covered with a cover slip then viewed under a light microscope and the pollen grains were categorized in two types, i.e. normal shaped/properly stained was counted as viable pollen and unstained considered as non-viable (Shivanna and Rangaswami 1992).

The peroxidase enzyme activity test was used to test the stigma receptivity in protogynous lines flower and this test

also detects the presence of alcohol dehydrogenase enzyme (Dafni 1992). The test solution encompasses 10 ml of 1 M phosphate buffer (pH 7.3–7.5), diluted (1 part buffer with 2 parts distilled water); 5–10 mg nitroblue-tetrazolium to give a slight yellow colour; 6 mg of nicotinamide adenine dinucleotide and 1 ml of ethanol (95%). The fresh stigma was cut with sharp blade in field and immediately transferred the stigma on the droplet of this test solution present on slide. All the slides kept in closed petri dish and incubated at room temperature. After 20-40 minutes the stigma was inspected under a magnifier (10×) microscope.

A total of seven treatments (S_0 to S_6)/different methods of selfing/pollination control methods were attempted in three plants. The individual plant with well bloomed floral branch was selected for each method of pollination/selfing. The number of flowers selected based on availability up to peak flowering period and feasibility of pollination. The number of flowers pollinated and also number of seed/ siliqua setting at the time of harvesting were recorded and per cent of siliqua set/seed setting was calculated.

The total of three plants and three floral branches/plant were selected to attempt pollination techniques. The selected flower stigma was cut with blade carefully without damaging rest of floral parts and immediately pollinated. In case P_1, randomly selected pollens were used, where as in case of P_2 freshly dehisced pollen were used and P_3 the opened flower pollens were used (older pollens). The numbers of flowers pollinated and number of seeds/siliqua setting were recorded and calculated per cent of siliqua set/seed setting.

PG lines of Pusa Mahak genetic background segregating materials (F_3) were used as protogyny-self-incompatible line as female and pollen donor as RJN-145 NPG) in C_1. The Pusa Bold and RJN-145 were used as female and pollen donor as PG line in C_2. The appropriate two floral branches were selected from PG lines, then emasculated during evening hours and pollinated with pollens of RJN-145 in the next day morning (C_1). Similar procedure also followed in C_2 treatment but, fresh and older pollens were used for crossing. The no. of flowers pollinated, no. of siliqua set and no. of seeds/siliqua were recorded.

RESULTS AND DISCUSSION

Comparative study of floral biology in mustard was carried out during initial year (2012-13) using seven genotypes of mustard. It was observed that the male sterility expression was more prominent in Pusa Bold and Ashirwad and both the genotypes showed more vigorous during floral bud initiation and also resist scorching effects with detergent spray (3, 5 and 8% (w/v)) than the remaining genotypes (Fig 1 a and b). Therefore, amidst genotypes, Pusa Bold was selected for further investigation.

The chemically induced male sterility (Table 2) and its floral physio-morphological differences compared with protogynous lines and CMS lines during *rabi* 2013-14 and *rabi* 2014-15. The investigation revealed that the flowering in the plants treated with 3, 5 and 8% detergent were delayed flowering by 38, 40, and 42 days respectively as compared to untreated plants (35 days). The anther length showed significantly reduction with concentration of detergent, i.e. 1.9, 1.7 and 1.5 mm compared with control (2.1 mm). The no. of pollen grains/bagged flower were significantly reduced with concentration of detergent (28593, 16923 and 13900 pollen grains/ flower with 3, 5 and 8% respectively) compared to normal plant (31055 pollen grains/ flower).

Furthermore, the pollen fertility was tested at regular intervals, which indicated that a plant sprayed with 2, 3 and 8% detergent were highly significant to create complete pollen sterility even after 25 days of treatment. Therefore treatments with detergents were found to be quite effective in inducing 100% pollen sterility (8%). The pollen fertility was tested again with bagged flowers; it was unsuccessful to produce any seeds which indicate complete sterility. In

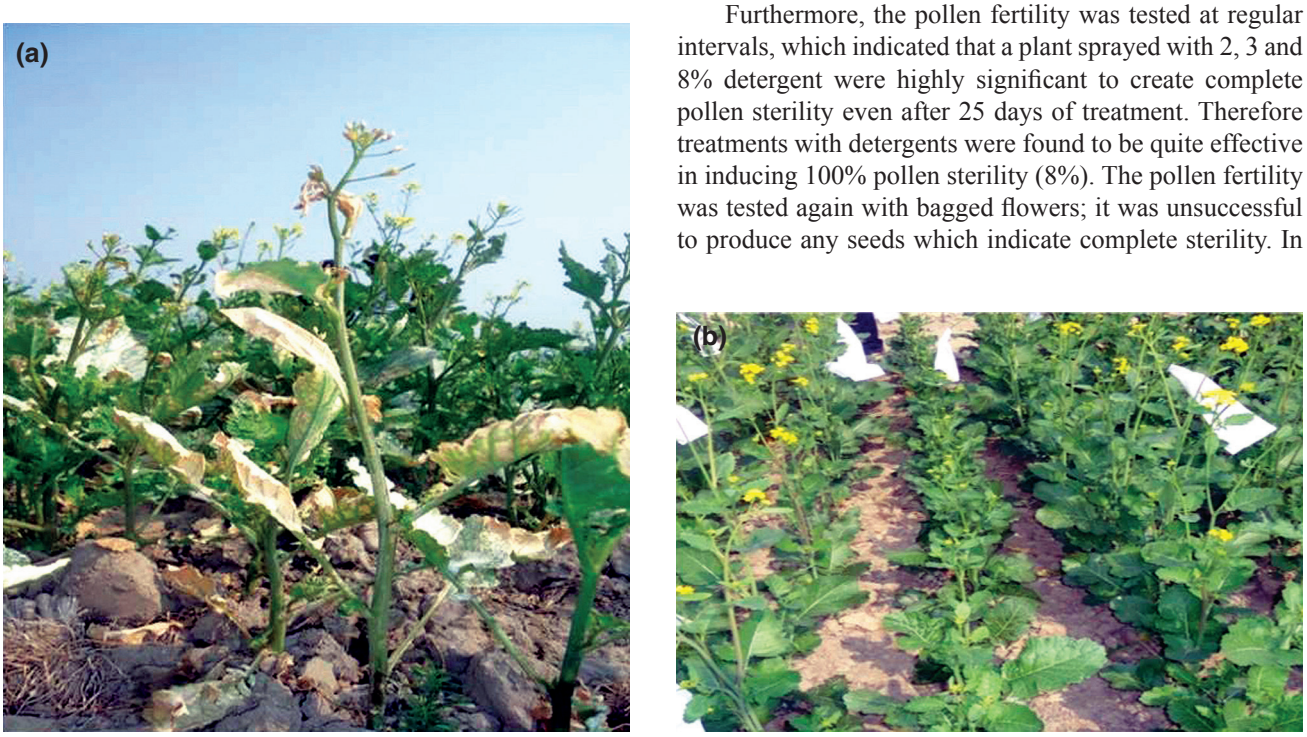

Fig 1 Effect of detergent treatment on floral morphological traits. (a) Scorching effect after detergent spray, (b) Field view of floral bud initiation in detergent sprayed plants (3-6 days delay).

Table 2 Comparative floral morphology of treated and untreated plants**

Traits	Plants treated with detergent (%)				CD (P = 0.05)
	Control	3 %	5 %	8 %	
Floral bud initiation (days)	35	38	40	42	0.11
Anther length (mm)	2.1	1.9*	1.7*	1.5*	
No. pollen grains/bagged flower	31055	28593*	16923*	13998*	152.81
Pollen fertility (%)					
1st day after flowering	90.33***	0.00	0.00	0.00	
10th		0.00	0.00	0.00	
15th		4.67	0.00	0.00	
20th		15.33	2.17	0.00	
25th		18.00	5.43	0.00	
Stylar length (mm)	2.0	2.2*	2.6*	3.1*	0.1
Time of stigma receptivity	8.00 am- 10.00 am	7.30 am- 10.30 am, (receptive upto 2-3 days)			
No. ovules/flower	18	17*	14*	12*	0.99
Pollen – ovule ratio	1709:1	1666:1	1238:1	1150:1	
Seed set (%)	90.50	92.83*	92.17*	89.93	1.05
Total yield/plant (g)	27.62	22.53*	17.09*	5.21*	1.00
Mode of pollination	Self+ Cross	Out-crossing			

*Significantly different from control at 5% level, **Data from six flowers selected randomly among the 75 untreated plants and 75 plants treated with each concentration, ***Mean value from control plants throughout flowering period.

the same way, in case of stylar length in the buds of treated plants, it was found that, length of style was significantly increased (2.2, 2.6 and 3.1 mm with 3, 5 and 8% respectively) compared with control (2.0 mm) and also increased the duration of stigma receptivity up to one hour.

Variations may be due to encompass of a surface-active agents and it is a builders (phosphates) and fillers in detergent. The same detergent also act as an additives, e.g., anti-deposition agents, optical brighteners, bluing agent, bleaching agent, foam regulators, organic sequestering agent and enzymes etc. Alkalines, e.g. sodium carbonate (soda ash) and sodium borate are commonly added to neutralize the acid constituents of dirt (Kumar *et al.* 2017a, Kumar *et al.* 2017b). It seems that the presence of sodium carbonate in the detergent; which causes male sterility in mustard plants. Similarly some other detergents such as Nirma and Soda ash also containing the similar constituents as detergent; because having these detergents also induce the male sterility to a considerable extent in rice (Singh 1999, Singh and Singh 1992). In view of above fact, still the basic research has to be framed to explore the actual mechanism of inducing

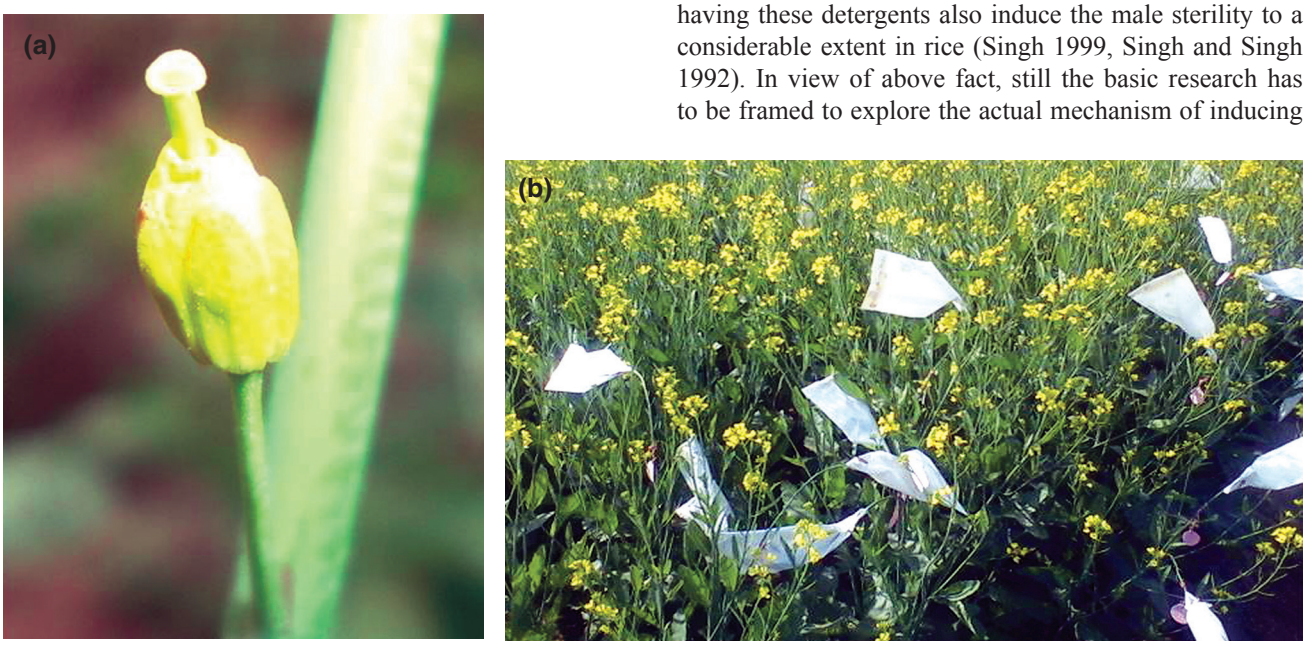

Fig 2 (a) Elongation of style/exertion of stigma in detergent treated plants, (b) Different methods of selfing/pollination imposed on PG lines.

male sterility at molecular level. It was also noticed that style length in the buds of treated plant was significantly increased as compared to control (Fig 2 a and b) hence, the increase in size of style; which helps the stigma to protrude out of all the buds of an inflorescence.

Chauhan and Singh (2002) reported that the possible reason for elongation of the style is due to the presence of some enzymes in detergent. The stigma of such floral buds was slightly longer receptive and which showed the presence of 50–60 pollen grains per stigma compared to control plant (100–150 pollen grains/stigma). Similarly, there was significant reduction in the number of ovules/ flower (may be due to detergent effect on female floral organ) and pollen–ovule ratio. On contrary, there was a significant increase in the seed-set percentage in treated plants with lower concentrations (3 and 5% detergent, which increases seed set 92.83 and 92.17%, respectively) compared to control (90.5%) hence, this increase in the seed-set percentage may be attributed to the fertilization of all the ovules due to out-crossing by honey bees (Singh and Singh 1992). While as the concentration of detergent increases this causes reduction of the seed set % compared with control plants. In case of yield, the significant decline was observed, i.e. 22.53 g, 17.09 g and 5.21 g compared with control (27.62 g) and the reduction in total yield is due to reduction of seed size, seed weight and delay in recovery of plant growth after scorching effect with detergent (Vinutha *et al.* 2014a, b; Agarwal and Kumar 2016).

The present investigation was agreement with Chauhan *et al.* (2011) have also effectively induced male sterility using a noval ester azetidine 3-corboxylate in *Brassica juncea*. Similarly, Cross and Ladyman (1991) and Kofoid (1991) have also used same chemical for effectively induced male sterility in wheat. Furthermore, the various chemicals like benzotrizole, surf excel, ethrel, GA_3, nirma, pendimethylin, maleic hydrazide, arsenic trioxide and sodium arsenate have also used to induce complete male sterility in *Brassica juncea* (Singh and Chauhan 2001, Chauhan and Singh 2002, Singh and Chauhan 2003, Singh and Chauhan 2004, Lavania and Chauhan 2006, Chauhan *et al*. 2007, 2010, Kumar *et al.* 2015, Kumar *et al.* 2016)

Comparison studies between PG lines, induced male sterility and CMS line

In case of protogynous lines (F_{3-5} generation), it was found that the stigma exertion is more prominent, protogynous interval extended up to 8-10 days and stigma remained receptive up to 3-4 days from its protrusion compared to induced male sterile line. Furthermore, the expression of male sterility was the partial male sterility (25-50%) and closed type flowers (indehiscent in nature) noticed in PG lines compared to induced male sterile line where it was 100% sterility with 8 % detergent and in CMS lines it was found absolute, i.e. 100% male sterility. The present finding was in agreement with Chakrabarty *et al.* (2007) and they reported that the maximum stigma receptivity up to three days after flower opening in three different CMS system such as Siifolia, Erucoides and Moricandia. Likewise, Mankar *et al.* (2007) reported that the stigma remain receptive for 6 to 8 days after anthesis in CMS lines of *Brassica juncea*. Furthermore, Shafer (2000) reported that the existence of variation for protogynous interval of at least three days in buffel grass, while as Lavana and Banga **(**1984) reported that the maximum stigma receptivity in *B. juncea* to be initially one day prior to opening of flower and the stigma receptivity declined after 3-5 days

Different methods of selfing to confirm self-incompatibility in PG lines

In this investigation different selfing/pollination methods were attempted with six different treatments, viz. S_0, S_1, S_2, S_3, S_4, S_5 and S_6 and the results were presented in the Table 3 and Fig 2b. It was found that, there was no seed setting (0 %) upon selfing (S_1 and S_2) because the morphology of the flower (covering of male reproductive structure by petals for longer period) acts as barrier for selfing. Similarly, the treatment S_3 (open the flowers and remove the pollens after emergence of stigma then cover it) was employed, but there was no significant seed setting indicates and there was no such cleistogamous nature mechanism exists in this system. However, very low and negligible seed setting was observed (less than 1 %). In case of treatments S_5 and S_6 which showed hardly 2 % seed setting hence, it may be attributed to pollen contamination or experimental error. The treatments S_0 and S_4 the seed setting showed 93% in both the cases because of it was allowed out crossing. Therefore the failure of seed setting upon selfing in these PG lines was not because of floral morphology and protogynous nature; rather it is mainly due to the existence of self-incompatibility mechanism.

Different methods of pollination to break self-incompatibility

For identification of self-incompatibility in PG lines the stigma was cut followed by pollination. The result revealed that, there was no significant seed setting in cut stigma and after pollination in P_1 (Table 4). However, fresh and older pollens were used for pollination even low per cent of seed setting was noticed in P_2 (2.9%) and P_3 (1%) hence; it is indicated that stigma is not an inhibition site; rather the self-incompatibility in these lines.

This may be because of pollen-style or ovule-pollen interaction. Therefore, the self-incompatibility may be attributed to the gamatophytic self-incompatibility.

Direct and reciprocal crosses between PG and non-PG lines

The direct cross generated using PG (Pusa Mahak genetic background as female) and NPG (pollen donor as RJN-145) lines and it was revealed that high seed setting (94-95%) was observed in C_1 (Table 5), hence it indicated that PG line had no seed set on selfing but, seed set occurs with out-crossing hence, PG lines have associated with self-incompatible system. Similarly, the reciprocal cross from NPG (RJN-145 and Pusa bold) × PG line, it was found that

Table 3 Number of siliqua and number of seed set per siliqua in response to different pollination/selfing methods

Treatment	Plant 1			Plant 2			Plant 3			Mean		
	No. of flowers pollinated	No. of siliqua set	No. of seeds/ siliqua	No. of flowers pollinated	No. of siliqua set	No. of seeds/ siliqua	No. of flowers pollinated	No. of siliqua set	No. of seeds/ siliqua	No. of flowers pollinated	No. of siliqua set	No. of seeds/ siliqua
S_0	50	50	6	45	35	6	45	45	5.8-6	46.66	43.33 (93%)	5.86
S_1	50	0	0	50	0	0	50	0	0	50	0	0
S_2	40	0	0	30	0	0	40	0	0	36.66	0	0
S_3	25	1	3	20	0	0	30	0	0	25	0.33 (1%)	3
S_4	45	45	6.4	46	42	6.4	35	30	6.6	42	39 (93%)	6.46
S_5	40	2	1	25	0	2	15	0	0	26.66	0.66 (2%)	1.5
S_6	55	1	2	40	0	0	20	1	1	38.33	0.66 (2%)	1.5

S_0 – Control: open floral branch, S_1 – cover the floral branch using butter paper bags to avoid out-crossing, S_2- open the petals and then cover with the butter paper bags, S_3- open the flowers and manual pollination (once), S_4- open the flowers and allow for natural pollination, S_5- open the flowers and remove the pollens after emergence of stigma then cover it, S_6- open the flower and manual pollination (multiple pollinations- three days).

Table 4 Number of siliqua and number of seed set per siliqua in different pollination techniques

Treatment	Plant 1			Plant 2			Plant 3			Mean		
	No. of flowers pollinated	No. of siliqua set	No. of seeds/ siliqua	No. of flowers pollinated	No. of siliqua set	No. of seeds/ siliqua	No. of flowers pollinated	No. of siliqua set	No. of seeds/ siliqua	No. of flowers pollinated	No. of siliqua set	No. of seeds/ siliqua
P_1	30	0	0	25	0	0	32	0	0	29	0	0
P_2	25	1	1	18	1	1	25	0	0	22.66	0.66 (2.9%)	0.66
P_3	30	0	0	25	1	1	20	0	0	25	0.33 (1%)	0.33

P_1-Cut the stigma and pollination, P_2-cut the stigma and pollination with fresh pollens, P_3- cut the stigma and pollination with older pollens

Table 5 C_1- Cross between PG (Pusa Mahak back ground) × NPG (RJN-145)

Treatment	Plant 1			Plant 2			Mean		
	No. of flowers pollinated	No. of siliqua set	No. of seeds/ siliqua	No. of flowers pollinated	No. of siliqua set	No. of seeds/ siliqua	No. of flowers pollinated	No. of siliqua set	No. of seeds/ siliqua
Branch 1	30	28	9	30	29	10	30	28.5 (95%)	9.5-10
Branch 2	40	37	10	28	27	11	34	32 (94%)	10.5-11

low seed setting (15% and 27% with old and fresh pollens) in case of C_2 (Table 6). Therefore this variation may be attributed to the pollen defect in the PG lines hence, it was also confirmed through pollen viability test (microscopic study) and concluded that PG lines produce normal and viable pollen but, it had low seed set on selfing and high seed set occurs out crossing. Based on direct and reciprocal crosses and seed setting has been confirmed that the self-incompatibility were closely associated with PG lines. The present investigation was in agreement with Watts (1968) reported that the pronounced effect of inbreeding depression in broccoli crops which led to the acquisition of self-incompatibility, which is an out-breeding mechanism ensuring out-crossing. Al-Shehbaz and Ihsan (1977) was emphasized that the protogyny is associated with self-incompatibility in *Brassica oleracea* and other members of

Table 6 C_2- Cross between NPG (RJN-145 and Pusa bold) × PG (Pusa Mahak back ground)

Treatment	Plant 1 (Pusa Mahak)			Plant 2 (RJN-145)			Plant 3 (Pusa Bold)			Mean		
	No. of flowers pollinated	No. of siliqua set	No. of seeds/ siliqua	No. of flower pollinated	No. of siliqua set	No. of seeds/ siliqua	No. of flower pollinated	No. of siliqua set	No. of seeds/ siliqua	No. of flower pollinated	No. of siliqua set	No. of seeds/ siliqua
Older pollen	22	4	4	7	1	1	10	1	10	39	6 (15%)	5
Fresh pollen	20	4	4	14	3	8	18	7	8	52	14 (27%)	7

cruciferae family. Hence, incompatibility has to maintain in these lines throughout flowering and pollination and explore these lines for hybrid seed production in mustard and other members of cruciferae. Chakrabarty *et al.* (2011) reported that the protogynous interval and stigma receptivity were up to 13 days and 3 days respectively in different mustard plants. They also emphasise on pollen viability test of protogynous plants, i.e. normal viable pollen produce by protogynous plants but, low seed set on selfing and high seed set on out crossing and confined presence of self-incompatibility system. Shen *et al*. (2008) suggested that the line with CMS + SI had combined advantages and which helps to overcome the disadvantages of both CMS and SI systems.

Conclusion

The alternate way to increase the yield of mustard crops is to explore the hybrid technology for enhancing the yield and to meet the future projected demand. The present investigation found that, the detergent treatments were quite effective in inducing male sterility compared to PG line which showed the partial sterility (30-50%) and the CMS line showed absolute sterility (100%). Furthermore, it was also confirmed that self- incompatibility was highly associated with PG plants. Therefore, the identified results must be revalidated in extensive larger scale in larger area with fine tuning of agronomic standards (planting ratio/ isolation distance etc.) and to explore its potentiality to generate hybrid in mustard crops.

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