Seed Res., Vol. 38(2): 121-126, 2010

Protogyny and Self-Incompatibility in Indian Mustard (*Brassica juncea* (L.) Czern & Coss)

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ABSTRACT: A protogynous line identified in Indian mustard was examined to study the interval of protogyny, stigma receptivity, pollen viability, fertility index and seed set. The results revealed that the protogynous interval was up to seven days in different plants, which showed stigma receptivity upto 3 days. The protogynous plants produced normal viable pollen but low seed set on selfing and high fertility index indicated self- incompatibility nature of the protogynous plants.

Key words: Fertility index, Self-incompatibility, Protogyny, Hybrid, Indian mustard

Rapeseed-Mustard (*Brassica juncea* (L.) Czem & Coss) is an important oilseed crop contributing 26.1 and 29.1% respectively, to the total oilseed area and production in India[1]. Continuous research efforts have been made in developing high yielding varieties mainly through individual plant selection in naturally occurring land races and progeny selection following hybridization. However, these efforts could not bring forth the desired level of yield which has reached a plateau. Significant yield improvement can be achieved by developing commercial hybrids.

Therefore, hybrid research is being undertaken for the last 50 years in *Brassica,* which were further intensified under the Indian Council of Agricultural Research(ICAR) project on "Promotion of Research and Development Efforts on Hybrids in Selected Crops," in 1989 .

A number of cytoplasmic male sterility (CMS) systems have been developed in the Indian mustard for exploitation of heterosis, to increase its yield level [2]. Major constraints for hybrid development in the available CMS systems are the absence of fertility restoration in most of the systems and unstable restoration or biological penalty associated with sterile cytoplasm. In addition, the nuclear background of these CMS systems will have to be diversified for use in commercial mustard hybrid production.

Diversification of reproductive systems is desired for hybrid development. Besides cytoplasmic genetic male sterility (CGMS) and self-incompatibility (SI) system, protogyny (Pg) systems could offer an option for easy hybrid development in *brassica*. Protogyny is the condition where stigmas are extruded from the florets prior to anther exsertion [3,4]. The period between stigma exsertion and initiation of anthesis is referred to as the protogynous interval. Al-Shehbaz reported that protogyny was associated with self incompatibility in *Brassica oleracea* and other members of cruciferae [5]. There are two possible explanations for the combinations of purported features favouring out crossing.

The duration of complete stigma emergence before anthesis (protogyny) in pearl millet heads varied among genotypes from 1-5 days [6]. *Zea mays* is normally a protandrous species, i.e. male flowering part dehisce before the female parts extrude, whereas *Zea diploperennis,* a wild relative of modern maize, is protogynous, silking well before the pollen is produced. This trait and transferred into cultivated *Zea may* [7]. All F_1 hybrid plants based on protogynous line exhibited precocious silking ranging from 9-14 days. The protogynous interval is a consistent trait, which is not influenced by different environmental conditions [8].

In a cross between two cultivars of Indian mustard, Agra Local and Varuna, protogynous plants

were observed in F_3 generation [9]. Hybrids involving protogynous line exhibited heterosis ranging from 19.5 percent to 56.4 percent, which may be attributed to differential heterosis in the component characters like number of siliqua on the main axis, number of seeds per siliqua and 1000 seed weight in certain specific crosses [10]. For Commercial use of the hybrids, maintenance and seed production requirements of the parental lines is essential. Keeping this in view the present study was undertaken with the objectives to i) study the nature of protogyny, stigma receptivity and pollen viability of protogynous system in *B. juncea* and ii) to examine the breeding behaviour of the protogynous line.

MATERIALS AND METHODS

The F_3 population derived from a cross between Agra local and Varuna was grown in separate block to study the nature of protogyny. The distance between the rows and plants were maintained at 45 cm and 15 cm, respectively and recommended package of practices was followed to grow a normal crop.

Determination of protogynous interval: To study the protogynous interval ten flower buds each on five plants were labeled . These were observed daily from the day of stigma exsertion till complete flower opening and anthers dehiscence. The protogynous interval was observed as the period between stigma exsertion and initiation of anthesis in a flower.

Breeding behavior of protogynous line: The following natural and artificial pollination treatments were carried out in twenty five freshly opened flower / buds selected at random in each of the protogynous plants at peak flowering period (9th January to 4th February).

1. Natural self pollination
2. Natural cross pollination
3. Bud pollination with self- pollen
4. Bud pollination with pollen of Agra Local
5. Selfing in freshly opened flowers
6. Crossing in freshly opened flowers

Pollination was carried out for seven days. For manual pollination pollens were transferred to the stigma by directly touching the dehiscing anthers of a freshly opened flower. The flowers were covered immediately after pollination with butter paper bags to prevent cross pollination.

Observations recorded

Fertility index: Fertility index (Fl) of the Pg line in relation to self/cross compatibility and self incompatibility was estimated [11, 12].

$$\text{Fertility Index (FI)} = \frac{\text{Average number of seeds per siliquae from natural cross pollination}}{\text{Average number of seeds per siliquae from self-pollination in freshly opened flowers}}$$

Plants having FI of more than 2.00 were classified as self-incompatible. Percent siliquae set and seed set were calculated based on the number of siliquae developed in each bud/flower labeled/pollinated and the numbers of mature seeds formed in the siliquae.

Stigma receptivity: The Pg plant was used as the female and Agra Local as pollen source to study the stigma receptivity of the Pg line. 12-15 buds in the Pg plant were selected at the same time in the bud stage one day before exsertion of stigma from flower bud and covered with butter paper bag to avoid contamination by undesired pollens. Following pollinations were made at different stages of stigma from flower bud (SR-1), one day after exserion of stigma (SR-2) and second day after exsertion of stigma (SR-3):

1. Self pollination with pollen from same Pg plant
2. Self pollination with mixed pollen from different Pg plants
3. Cross pollination with pollen from Agra Local

The pollination flowers were covered with butter paper bags to prevent any contamination. Percent siliquae set and seed set were calculated based on the number of siliquaes developed in each bud/flower labeled/pollinated and the numbers of mature seeds formed in the siliquaes.

Fertility and cross compatibility of pollens of protogynous line: Matured anthers collected randomly from Pg plants were smeared with 1 percent acetocarmine and examined under compound microscope. The

fully stained, round pollen grains were considered as fertile and remaining as sterile.

Pollens of Pg line were used for pollinating the emasculated flowers of three different inbred lines viz. Agra local, EC-308575 and IC-199715. Ten flowers each in four plants of the above three parents were pollinated. These were covered with butter paper bags. Percent siliquae set and seed set were calculated based on the number of siliquaes developed in each bud/flower labeled/ pollinated and the number of mature seeds formed in the siliquaes.

RESULTS

Nature of Protogyny: The protogynous interval i.e. the period between stigma exsertion from the flower bud and anther dehiscence was recorded seven days.

Percent siliquae set: The average percent siliquae set in natural self and cross pollination were lowest with 81 and 82 percent respectively, whereas it was significantly higher in all artificial methods of pollination (Table 1). The average percent siliquae set in various artificial pollination treatments ranged from 91-93 with highest value in artificial self pollination at bud stage and in artificial cross pollination in freshly opened flower.

Seeds per siliquae: The average number of seeds per siliquae ranged from 0.01 to 16.1 in different pollination treatments with the highest value obtained in artificial cross pollination at bud stage and lowest in natural self pollination (Table 1). In the artificial self pollination in freshly opened flower it was 0.018 and in artificial self pollination at bud stage it was 0.038. Among different cross-pollination treatments it was highest in artificial cross pollination at bud stage (16.1), followed by artificial cross pollination in freshly opened flower (8.5) and followed by natural cross pollination (6.9), respectively.

Even though the siliquae setting was recorded in all pollination treatments (both self and cross pollination), the average number of seeds per siliquae was negligible in self pollination treatments whereas, there was significantly higher seed number per siliquae in cross- pollination treatments. Therefore, the results indicated that the protogynous line is self-incompatible.

Table 1. Effect of various pollination methods on siliquae and seed set in protogynous line

Pollination treatments	Siliquae set (%)	No. of seeds per siliquae
NSP		
Range	77-85	0-0.05
Mean	81	0.01
NCP		
Range	79-86	5.7-8.3
Mean	82	6.9
ASPB		
Range	89-96	0-0.05
Mean	93	0.04
ASPF		
Range	88-95	0-0.04
Mean	91	0.02
ACPB		
Range	90-96	12.8-18.1
Mean	92	16.1
ACPF		
Range	92-96	7.7-9.7
Mean	93	8.5
CD(p=0.05)	1.24	2.6

NSP: natural self pollination; NCP: natural cross pollination; ASPB: artificial self- pollination at bud stage; ASPF: artificial self-pollination on open flower; ACPB: artificial cross pollination at bud stage; ACPF: artificial cross pollination on open flower.

Fertility index: The mean fertility indices for the pollination treatments was more than two and it ranged from of 140-infinity (Table 2). According to Watts the line would be self-incompatible if 'fertility index' value is more than 2.00 [11]. Therefore, the FI values confirm the self-incompatibility nature of the Pg line.

Stigma receptivity: The studies on stigma receptivity of the Pg line showed that percent siliquae set was 100% by using different pollen sources at different stages of stigma exsertion except when pollination was done with its own pollen one day before exertion of stigma (90%) (Table 3).

The average number of seeds per siliquae was 0.1 by selfing one day before exsertion of stigma (SR-0) and on the day of exsertion of stigma (SR-1). However, there was no seed set afterwards i.e., on

Table 2. Fertility index (F.I.) of protogynous line in relation to pollination methods

Date of pollination	Average No. of seeds/siliquae		F.I.	Average No. of seeds/siliquae		F.I.	Average No. of seeds/siliquae		F.I.
	NCP	NSP		ACFP	ASPF		ACPB	ASPB	
9 January	8	0	Inf.	9	0.04	225	12.8	0.05	256
11 January	7.15	0	Inf.	7.7	0	Inf.	13.9	0	Inf.
15 January	7	0	Inf.	7.95	0.03	265	17.1	0.05	342
22 January	7	0.05	140	7.7	0	Inf.	17.1	0.04	427.5
30 January	5.95	0	Inf.	7.9	0.03	263.3	17.5	0.05	350
1 February	5.75	0.03	191.7	9.65	0	Inf.	16.4	0.03	546.7
4 Febuary	6.85	0	Inf.	8.55	0.03	285	18.15	0.05	363

NSP: natural self-pollination; NCP: natural cross-pollination; ACPF: artificial cross pollination on open flower; ASPF: artificial self-pollination on open flower; ACPB: artificial cross pollination at bud stage; ASPB: artificial self-pollination at bud stage; Inf. -Infinity

Table 3. Stigma receptivity in protogynous line

Pollen source	Siliquae set(%) at				No. of seeds per siiquae at			
	SR-0	SR-1	SR-2	SR-3	SR-0	SR-1	SR-2	SR-3
Own Pollen	90	100	100	100	0.1±0.32	0.1±0.32	0.0	0.0
Mixed Pollen	100	100	100	100	0.2±0.42	0.2±0.42	0.0	0.0
Agra Local	100	100	100	100	13.6±1.71	12.5±2.17	11.6±1.84	9.8±1.62

SR-0: One day before exertion of stigma from flower bud; SR-1, SR-2, SR-3: First, Second and Third day exertion of stigma from flower bud, respectively.

SR-2 and SR-3 by selfing. Similarly, average seeds per siliquae at SR-0 and SR-1 by using mixed pollen (i.e., sibbing) were 0.2 but there was no seed set at SR-2 and SR-3 stages of stigma exsertion. There was sufficient seed set on protogynous line pollinated with pollens of Agra Local. Maximum receptivity of stigma was at the bud stage and on the day of exsertion of stigma from flower bud. It showed slow decline in seeds per siliquae with increasing number of days of stigma exsertion. The average seeds set per siliquae following cross pollination with Agra Local were in the range of 13.6 (at bud stage) to 9.8 (at SR-3 stage).

Pollen fertility and cross compatibility of protogynous line: Microscopic examination of pollen of Pg line showed that pollens were completely viable as they were fully stained, well filled and round in shape.

The percent siliquae set of Agra Local, EC-308575 and IC-199715 which were pollinated by the pollens of Pg plants was more the 90%. The number of seeds per siliquae ranged from l0.7 to 12.9 (Table 4) in three days of pollination, indicating pollen fertility and intra-specific cross compatibility of pollens of the protogynous line.

DISCUSSION

Protogyny is the condition where the stigmas are extruded from the florets prior to anther exsertion [3,4]. The protogynous interval i.e. the period between stigma exsertion and anther dehiscence in the Pg lines obtained from the F_3 population of

Table 4. Siliquae and seed set in varieties crossed with pollen of protogynous line

Female	Siliquae set (%)	No. of seeds per siliquae
Agra Local	100	11.56±2.7
EC-308575	100	12.0±2.4
IC-199715	90-100	11.4±3.49

derived from a cross between Varuna and Agra Local was found to be up to seven days. In Pearlmillet the duration of complete stigma emergence, the protogyny period before anthesis varied from 1 to 5 days in different genotypes. Existence of variation for protogynous interval within buffelgrass upto at least three days was reported [8]. An interval upto seven days gap in the emergence of female and male parts of a flower could be significant in restricting the inbreeding, thereby facilitating out crossing.

Though siliquae setting was recorded for all the pollination treatments including self and cross pollination, the average number of seeds per siliqua was negligible in self pollination treatments though there was significant seed set in cross pollination treatments. This indicated that the protogynous line is self incompatible. Seeds set per siliqua in different pollination treatments varied quantitatively. Such quantitative differences in seed set in various pollination treatments have also been reported earlier in a number of cole crops viz., Brussels sprouts [13], cabbage [14] and cauliflower [15]. Negligible seed set self-pollination treatments could be attributed to weather fluctuations during the pollination period, strong S-alleles and relatively reduced pollen fertility.

The number of seeds per siliqua was highest in artificial cross pollination at bud stage followed by artificial cross pollination in freshly opened flower and natural cross pollination. These results indicated that stigma was most receptive at bud stage with a subsequent decline. The higher seeds set in artificial cross pollination than in natural cross pollination could be attributed to the assured supply of sufficient pollens to the receptive stigma. Non attractiveness of flowers of the protogynous line to insect pollinators could be a factor for lower foraging resulting in lower number of seeds per siliquae.

The results of seed set obtained from different self and cross pollination treatments suggested the self incompatibility in the individuals of protogynous line, which was further confirmed by the fertility index. All plants belonging to the protogynous line were found self-incompatible, as their fertility indices were more than 2.00. The pronounced effect of inbreeding depression in broccoli led to the acquisition of SI, which is an out-breeding mechanism ensuring cross fertilization [16]. Hence, for the production of hybrid seed, these lines must maintain their incompatibility throughout flowering and pollination.

The studies on stigma receptivity of the Pg line showed that the siliquae setting was almost 100% by using different pollen sources at different stages of stigma exsertion which assured abundant supply of pollens. The average number of seeds formed per siliquae was negligible in case of selfing and sibbing treatments, whereas there was sufficient seed set on protogynous line pollinated with pollens of Agra Local. This again confirms the cross-compatible nature of Pg line. Maximum receptivity of stigma was recorded at bud stage and on the day of stigma exsertion from the bud. It showed a slow decline the in number of seeds per siliquae with the advancement of days of stigma exsertion. Stigma receptivity was recorded up to ten days in *Ogura, Moricandia* and *Erucoides* CMS systems of *Brassicas.* Peak stigma receptivity was observed upto three days after flower opening in case of *siifolia, Erucoides* and *Moricandia* [17]. Maximum stigma receptivity in *Brassica juncea* was reported to be one day before the opening of flower which declined after 3-5 days [18]. However, in another study, stigma of CMS lines of *Brassica juncea* remained receptive for 6-8 days after anthesis [19]. In the present study, however, the stigma receptivity was examined only up to 3 days after exsertion of stigma from flower bud.

Self incompatibility was first reported by Kolreuter [20] in the eighteenth century. The self-compatibility system is divided into two main groups: gametophytic and sporophytic. The ancestral *Brassica oleracea* was highly self incompatible; therefore, it is natural for the cole crops to be self-incompatible [21]. Self-incompatibility is an excellent mechanism to enforce outcrossing in angiosperms. Among the oilseed *Brassica* species, *Brassica rapa* vars. Brown sarson and Toria, *Brassica nigra* and *Brassica oleracea* are self-incompatible.

This is the first report of self incompatibility in Indian mustard derived from an intra-specific cross. Further studies will be needed to exploit its potential in hybrid development and commercial seed production.

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