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Differential response of Germplasm to Inter-Specific Hybridization of Brassica spp.

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Abstract

Understanding the fact behind the incompatible reaction in inter specific crosses is important for attempting successful crosses. Inter-specific hybridization between *Brassica juncea* and *Brassica oleracea var botrytis* were developed using reciprocal crosses. Four varieties of Indian mustard namely Varuna, Kranti, Pusa Bold and Rajendra Suflam were crossed reciprocally with one Cauliflower variety Sabour Agrim. Several barriers were observed right from the pre-fertilization to post fertilization during embryo developmental stages. 78.26%, 64.96% and 63.68% siliqua was retained after 6 days, 10 days and 15 days of pollination respectively when *B. juncea* was used as female parent whereas 99.35%, 45.51% and 43.22% of siliqua were retained after 6 days, 10 days and 15 days of pollination respectively when *Brassica oleracea* var *bortytis* was used as female parent. Both pre and post fertilization barriers were predominantly found when *B. juncea* was used as female parent whereas post fertilization barriers were found in the reciprocal crosses. Differential varietal response to siliqua drops was observed in the reciprocal crosses. Maximum response in the success of crosses was obtained for variety Kranti in B. juncea X B oleracea cross whereas variety Rajendra suflam was highly efficient in reciprocal crosses.

Keywords

Distant hybridization; Cauliflower; Indian mustard; *B. juncea*; *B. oleracea*

Introduction

There are six cultivated species in Brassica genus among which *Brassica oleracea* is vegetable crop species adapted to cooler region. Whereas, *Brassica juncea* is amphidiploids species, covered 80% of the total areas under oilseed crop in India. Several important traits have been introgressed from diploid progenitors of Brassica to amphdiploids like self incompitability [1,2] and cabbage aphid (*Brevicoryne brassicae*) resistance from *B. oleracea* to *B. napus*. Re-synthesis of tetraploid Brassica species using diploid proginators was done decade before for better insight of their genomic relationships [3]. Several pre and post fertilization barrier was reported in this process and few techniques like ovary or embryo culture has got a level of success. In a cross between Brssica napus and B rapa; Janetta et al., [4] identified pollen tubes penetrates into the ovule after 48 hrs of pollination [5]. It has been reported that embryo development starts immediately after fertilization in Brassica sp. Peng and Yu [6] isolated multicelluar embryos at 5 DAP in the wide crosses between Chinese cabbage and Kale. Limited reports are available about the crosses between *Brassica juncea* and *Brassica oleracea var botrytis*. Success up to 25 percent and 9% were reported using embryo culture technique when *Brassica juncea* and *Brassica oleracea* respectively used as female parent [7]. Inter-specific gene transfer in Brassica sp. was carried out for several genes but transfer of genes between *Brassica juncea* and *Brassica olercea var botrytis* is very limited. Resistance to several diseases and pests are not available in C genome of Brassica that could be exploited through inter-specific hybridization. One of the limitations of such application is lack of understanding for crossability barriers between two species. That's why it is important to know the correct stage of barriers during fertilization process for increasing the efficiency of hybrid development between two species. Present investigation was carried out to find out the most commonly occurring hybridization barriers in two species.

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Materials and Methods

The experiment was conducted in protected conditions under poly house conditions at Bihar Agricultural University, Sabour, Bhagalpur in Complete Randomized Design with three replications. Sowing was done at two different dates for coinciding the flowering period. Four varieties of Indian mustard namely Varuna, Kranti, Pusa Bold, Rajendra Suflam were crossed reciprocally with the recently released tropical Indian cauliflower variety Sabour Agrim. Flower buds which would be opening in the next two days were bagged using butter paper bag and emasculated. Rest of the flower buds was removed. Similarly, the inflorescence of male plants was also bagged before flowering. At time of pollination, freshly opened flowers were collected and used for pollination to the female flowers. After pollination the inflorescence was bagged using butter paper bag. Data recording started at different days after pollination (DAP) as percent of siliqua retained after pollination. Flower bud retention after pollination at 3 days, 6 days, 10 days, 15 days and 20 days after pollination (DAP) were recorded. In the present study retention of flower buds on or before 3 DAP was considered as pre fertilization barriers and after that as post-fertilization barriers. Multi-cellular embryos were detected after 5 days of pollination by Peng and Yu [6].

Results and Discussion

A total of 546 reciprocal crosses were attempted among which 391 crosses were made for *Brassica juncea X B oleracea var botrytis* and 155 were for reciprocal crosses. Progress of flower buds for the development of embryos after pollination was taken as effective measure for studying the fertilization barriers. Significant differences were observed between the reciprocal crosses for percent retention of siliqua at different dates after pollination. Maximum success was obtained where *B. juncea* was used as female parent. No siliqua were shattered three days after pollination. Maximum reduction of siliqua was observed at 6 days after pollination. However, no significant differences were found beyond 15 days of pollination. 99.74% siliqua retained three days after pollination. Siliqua drop drastically increased at six days and 10 days after pollination where only 78.26% and 64.96% siliqua were retained at respective days after pollination (Figure 1). Barriers at fertilization or immediately after fertilization

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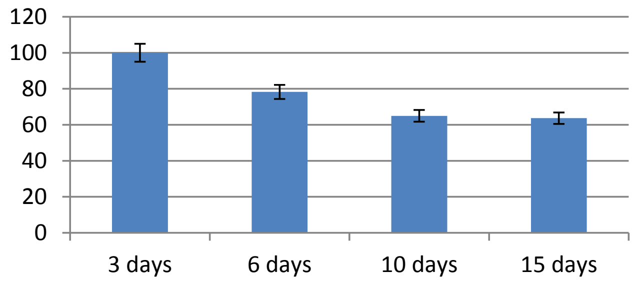

Figure 1: Percent retention of siliqua at different days after pollination (DAP) in *Brassica juncea X B. oleracea* cross.

at embryonic developmental stage were most prominent. *Sarmah and Sarla* [8] *reported pre-fertilization barriers in the crosses between Diplotaxis siettiana* and *Brassica juncea which could be overcome using irradiated pollen of Diplotaxis siettiana* by gamma rays where 5 day after pollination. Seventeen percent ovules showed entry of pollen tubes, ten days after pollination 27% ovules showed small globular embryos which did not grow further.

Peng and Yu [6] harvested multicellular embryos five days after pollination in the cross between Chinese cabbage and Kale, which showed maximum reduction due to lack of fertilization. Inter-specific crosses between *B. juncea* and *B. oleracea* were reported by Weerakoon et al. [7] observed success rate of pod formation by 25%. Success between tetraploid and diploid species is very less and to avoid crossibaility barriers it was suggested to go for ovule or embryo rescue techniques. Application of ovule culture technique was reported to increase the efficiency of BC_1 hybrids (*B napas* X *B. oleracea*//*B.oleracea*) 10 fold over the *in vivo* set [9].

In the reciprocal crosses, the drop of siliqua at three and six days after pollination was not observed. However, maximum drop was found at 10 DAP following which the rate of siliqua drop was markedly reduced. It clearly indicated the post fertilization barriers were prominent in such crosses. 99.36% siliqua were present at three as well as six days after pollination respectively but at 10 DAP and 15 DAP number of siliqua were retained 45.51% and 43.22% respectively (Figure 2). Peng and Yu [6] rescued embryo to develop inter-specific hybrids between *B. campestris* var *pekinensis* and *B. oleracea* var *acephale.*

Varietal interactions in reciprocal crosses were found significant. Maximum drop of siliqua was observed in the variety Pusa Bold at 6 DAP which was minimum in variety Kranti (Table 1). At 10 DAP the trend changed as maximum drop of siliqua was observed for variety Varuna (upto 30%) compared to minimum flower drop for Kranti and Pusa Bold (nearly 10%). It reflected that varietal response for fertilization barrier was different. Pre fertilization barrier was found higher in variety Pusa bold whereas post fertilization barrier is strong in variety Varuna. Among all the crosses maximum success was observed when variety Kranti used as female parent followed by using variety Rajendra Suflam. Hybrid seed was obtained in all the four crosses of *B. juncea* X *B. oleracea* var *botrytis*. However, the percentage of success was very low in all the four crosses. To increase the efficiency of hybrids produced in the crosses between *B. juncea* X *B. oleracea* var *botrytis*, embryo culture should be adopted. In the cross between *B. juncea* and *B. nigra,* Bhatt and Sarla [10] also reported both pre and post fertilization barrier in interspecific hybridization. Bud pollination and stump pollination along with ovary-ovule culture technique may be helpful in recovery of hybrids plants. The hybrid seed developed in the present study were sown and characterized morphologically along with their parents to confirm hybridity (Figure 3).

In the reciprocal crosses also varietal response towards the fertilization barrier was markedly differed. No significant difference was observed at three and six days after pollination (Table 2). However, drastic reduction of number of siliqua at 10 DAP was

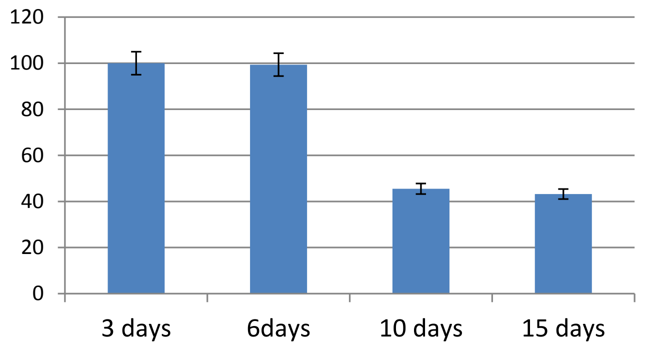

Figure 2: Percent retention of siliqua at different days after pollination (DAP) in *Brassica oleracea* var *botrytis X B. juncea* cross.

Table 1: Varietal responses for percent retention of siliqua in the cross of *B. juncea X B. oleracea.*

Varieties used as female parent	Total number of cross attempted	Number of siliqua retained/ percentage				Hybrid seed produced
		3DAP	6DAP	10DAP	15 DAP	
Varuna	98	98	84	54	54	2
Percentage (%)		100	85.71	55.10	55.10	0.02
Kranti	95	95	82	73	73	12
Percentage (%)		100	86.31	76.84	76.84	0.12
Pusa Bold	98	98	68	58	58	5
Percentage (%)		100	69.38	59.19	59.19	0.05
R. Suflam	100	100	78	65	64	14
Percentage (%)		100	78	65	64	0.14
CV (%)	1.32					
$CD_{0.05}$ (Variety X Days)	1.59					

Table 2: Varietal responses for percent retention of siliqua in the cross of *B. oleracea X B. juncea.*

Varieties used as male parent	Total number of cross attempted	Number of siliqua retained/ percentage			
		3DAP	6DAP	10DAP	15 DAP
Varuna	37	37	37	7	6
Percentage (%)		100	100	18.91	16.21
Kranti	38	38	38	18	18
Percentage (%)		100	100	48.64	48.64
Pusa Bold	40	40	40	22	22
Percentage (%)		100	100	55	55
R. Suflam	40	40	39	25	22
Percentage (%)		100	97.5	62.5	55.55
CV (%)	1.79				
$CD_{0.05}$ (Variety X Days)	1.94				

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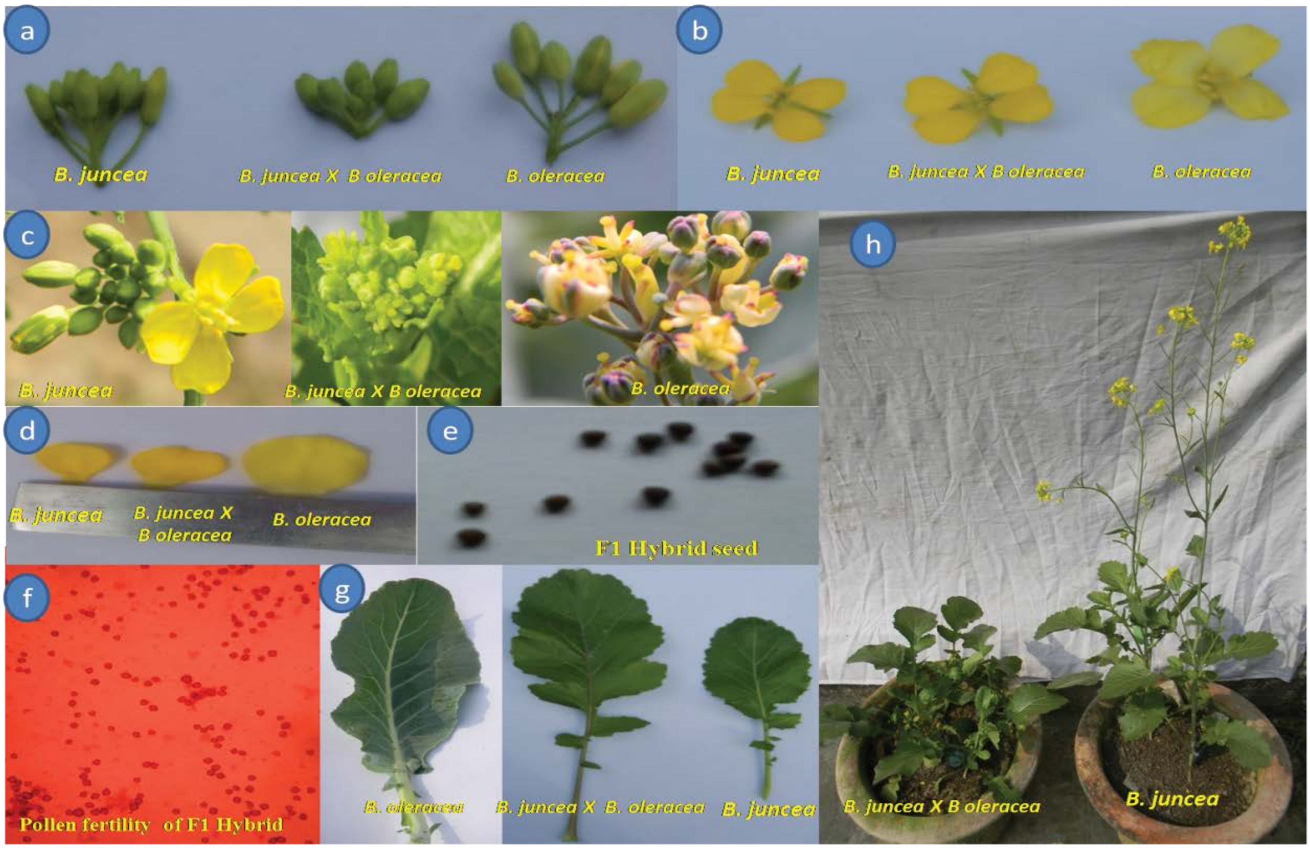

Figure 3: Comparison of F_1 hybrids (*B. juncea* cv. Rajendra Suflam L. X *B. oleracea* cv S. Agrim L.) with their parents for different characters. a. shape of flower bud; b. color and shape of petal; c. occurrence of deformities in flower buds; d. shape of flower petal; e. F_1 hybrid seed; f. F_1 pollen fertility status after colchicines treatment; g. leaf shape h. plant height during flowering.

observed in all the crosses followed by a mild reduction at 15 DAP pollination. At 10 DAP siliqua drops was recorded maximum in variety Varuna whereas in variety Rajendra suflam it was minimum. Much differences between the variety Pusa bold and Kranti was not observed. However, at 15 DAP variety Rajendra suflam followed by variety Varuna responded to flower drop to some extent. Although few crosses succeeded in almost all the crosses up to 20 DAP, none of them produced fully developed silqua no hybrid seed was obtained in any of the four crosses between *B. oleracea* var *botrytis* X *B. juncea*. It indicated only tetraploid species should be used as female parents to increase the efficiency of hybrids seed in interspecific hybridization.

Conclusion

In the present study, hybridization between *B. juncea* and *B. oleracea* var *botrytis,* both pre and post fertilization barriers commonly occurred using *B. juncea* as female parent. Whereas, post fertilization barriers were predominantly occurred using *B. oleracea* var *botrytis* as female parent. Varietal responses in reciprocal crosses was different as most efficient crosses were made using variety Kranti followed by Rajendra suflam as female parent. Though all the varieties were showing both pre and post fertilization barriers but Variety Pusa Bold was highly sensitive to pre-fertilization barriers while Varuna to Post fertilization barriers. In the reciprocal crosses Rajendra suflam was most efficient male parent for hybridization. To avoid pre and post fertilization barriers in brassica, ovule culture and embryo rescue technique may be utilized to increase efficiency. Variety Kranti as female parent could be useful for development of inter specific hybrids between *B. juncea* and *B. oleracea* var *botrytis.*

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