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Evaluation of Various Planting Row Ratios of Parental Lines of *Brassica Napus* L. For Hybrid Seed Production.

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----------ABSTRACT----------
Research efforts on the use of genetic as well as cytoplasmic male sterile system have been intensified in recent past to produce hybrid seeds. Distance between pollinizer and seed plant rows as well as frequency of restorer line rows varied in different row ratio plots, which influenced cross pollination and yield of hybrid seeds significantly. Quality and quantity of seeds got changed with changing male to female line row ratios. Suitable row ratio is the most important factor. The present study revealed that for maximum percentage of pod set and more number of seeds set per pod, suitable male to female line row ratio was 2 : 4, followed by 1:4 and 1:8. As far as quality parameters of seeds like percentage of well formed seeds , oil content and average seed weight were concerned then row ratio 1:8 was the best, followed by 1:4 and 2: 4. As maximum seed setting on CMS plants is considered the most important factor, to evaluate various row ratios, so row ratio 2:4 was the best one but this study also showed that quality factors of seeds must also be taken into consideration by plant breeders while finding suitable row ratio.

Key Words *Brassica napus* , *Apis* species ,CMS system , Hybrid seeds , parental lines

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I. INTRODUCTION

Now-a-day there is an increasing demand for the hybrid seed production of various oilseed crops and this production has been facilitated with the development of various techniques like male sterility, self incompatibility and heterostyly etc. In order to favour cross fertilization, male sterility techniques are quite prevalent. These techniques are generally of two types : (a) genetic male sterility, in which pollen formation is failed because of one or more nuclear genes and (b) cytoplasmic male sterility (CMS) in which pollen formation is blocked or aborted because of a defect in cytoplasmic organelle (mitochondrion). Many successful hybridization schemes involve the use of CMS system. A specific mutation in the mitochondria which are situated in the cytoplasm is induced in proper nuclear background can lead to the failure of mature pollen formation. The male parent line carries the specific restorer genes which impart fertility to hybrid seed, usually designated a restorer(R) line. In case of rapeseed mustard a number of sources of cytoplasmic sterility are available. These include – Ogura, Tournefortii, Polyma, Carinata, Oxyrrhina and Siifolia. Despite a number of inherent problems associated with these systems, many of them are now on the thresholds of commercial exploitation. CMS systems are being experimentally used to produce hybrids in *B. napus* and *B. juncea* (Banga *et al*., 1997).

Discovery of CMS system in different crops by various workers (Jones and Emsweller, 1936; Jones and Clark, 1943; Stephens and Holland, 1954; Leclercq, 1969; Mayer, 1975; Erickson and Peterson, 1979a) has opened new avenues for F1 hybrid seed production. Soon after the discovery of male sterility and development of fertility restoring lines, a number of hybrids of many crops were released on commercial scale. Use of male sterility in hybrid seed production has become an important objective for producing economically cheaper seeds for farmers. Research efforts on the use of genetic as well as cytoplasmic male sterility system have been intensified in recent past for eliminating labour, intensive process of emasculation and subsequently pollination through honeybees. Hybrid seed production is feasible, only when pollen is transferred from one line to another. Success of hybrid seed production programme depends upon maximizing the seed set on female (CMS) line rows using various production techniques.

Accurate male to female row ratio is an important factor to maximize the hybrid seed production (Mishra and Kashyap, 1995). Various ratios of male-fertile to male-sterile rows have been worked out in case of *B. napus* by many workers (Mesquida and Renard, 1979b; Mesquida, 1983; Renard and Mesquida, 1987; Ohsawa and Nawal, 1988; Banga *et al*., 1995;1997), but more accurate and suitable information in this regard is required so that desired level of cross pollination may be obtained to maximize the seed set. Movement of *Apis* species between male and female rows are also affected by M:F row ratio. Bee foragers are more in number on male-sterile flowers which were closer to male-fertile rows as compared to those farther away.

Distance between rows of pollinizer (male) plants and seed (female) plants as well as the frequency of male fertile rows varies in various row ratio plots. Both of these factors influence cross- pollination and also the quantity and quality of hybrid seeds production. The male to female row ratio is strongly influenced by efficiency of pollinators and availability of viable pollen. It is also determined by cross return factor. Present study incorporated three male : female row combinations i.e 1:8, 1:4 and 2:4. Main aim of the study was to evaluate above mentioned row ratios on the basis of percentage of pod set, number of seeds set per pod, percentage of well formed seeds, average seed weight and oil content of seeds, so that suitable row ratio can be worked out.The expected outcome will help to enhance the knowledge to improve hybrid seed production techniques.

II. MATERIALS AND METHODS

To raise hybrid seed production plots of *Brassica napus*, seed of cytoplasmically male sterile (CMS) line (TCMS-PR-05) and restorer (R) line (TFR-91) of *B. napus* hybrid PGSH-51 were obtained from Department of plant breeding, Punjab Agricultural University, Ludhiana, Punjab, during each year of present study. Hybrid seed production plots were raised by following standard package of agricultural practices of PAU, Ludhiana. The male to female row ratios selected were 1:8, 1:4 and 2:4. In order to obtain synchronous flowering in two lines, early flowering male plants were detopped. Plots of various male : female row ratios were compared for percentage of pod set, number of seeds set per pod, percentage of well formed seeds, weight (g) of 1000 seeds and percentage of oil content in seeds, to find out suitable row ratio.The procedure of each parameter has been described below:

Percentage of pod set

In all the plots of different row ratios, 50 flowers were tagged daily in each female line, with threads of particular colour. The colour of thread represented the particular date. This experiment was done daily during flowering season. At time of reaping silique or pods having threads of particular colour in particular CMS row were collected separately from plots of various row ratios. The number of pods set out of 50 were recorded for each female line in different plots and percentage of pod set was calculated.

Number of seeds set per pod

The seeds recovered from the pods of above experiment, were counted separately. Thus average number of seeds per pod in particular CMS row was calculated.

Percentage of well formed seeds

In above mentioned seeds per pod, numbers of well formed seeds were counted and their percentage was recorded.

Seed Weight

Seeds collected from various CMS rows were counted and weighted separately with the help of electronic balance to calculate average weight/1000 seeds (g).

Percentage of oil content in seeds

Weighted seeds of different CMS rows of various plots were ground separately. Finely ground material was mixed with ether (Diethyl ether) and shaken vigorously by covering the container. Then this mixture was filtered. The filtrate was kept for evaporation of ether. In this way only oil was left in vials which was weighted with electronic balance. The percentage of oil content in seeds taken from various CMS rows of various plots of different row ratios was calculated.

Statistical analysis

Various data collected were consolidated, tabulated, transformed wherever felt necessary and then subjected to analysis of variance and significance was tested at 5 per cent level. To determine suitable male to female line row ratio, yielding parameter like percentage of pod set, number of seeds set per pod, percentage of healthy seeds, weight of 1000 seeds and percentage of oil content in seeds obtained from different CMS rows of different row ratio plots were compared

Results and Discussion

Results and discussion of various parameters regarding quality and quantity of hybrid seeds are given below:

Percentage of pod set

In plots of all the M:F row ratios, percentage of pod set was found to be maximum in CMS rows which were adjacent to R line rows and it declined gradually across various female rows towards CMS row which was at maximum distance from R line row. The value of percentage of pod set ranged from 36.448-55.378, 46.518-54.554 and 53.566-54.770 per cent in case of 1:8, 1:4 and 2:4 M:F row ratio plots, respectively (Table 1, 2 and 3).

These results, revealed that plots of different row ratios according to a declining order of percentage of pod set were as : 2:4>1:4>1:8. In plots of M:F row ratio 1:8, distance between male and female rows ranged from 30 cm (1st CMS row) to 240 cm (8th CMS row), while in plots of row ratio 1:4, this distance ranged from 30 cm (1st CMS row) to 120 cm (4th CMS row) and in case of the plots of planting ratio 2:4, distance from R to

CMS rows ranged from 30 to 60 cm. Thus the percentage of pod set on CMS rows decreased with increased distance from male fertile row because more distance between pollen donor and acceptor plants decreased the chances of pollination. Further, not only the distance form male to female row is important, the availability of enough male-fertile plants which contribute pollen for cross-pollination is also equally important. In M:F row ratio 2:4, maximum number of pollen contributor plants were available followed by 1:4 and 1:8 row ratio plots. Maximum pod setting was observed in planting ratio 2:4 followed by other row ratios, 1:4 and 1:8. Similar type of conclusions were drawn by Renard and Mesquida (1987) and Banga et al. (1993, 1997) in case of hybrid seed production plots of *B. napus*.

Number of seeds set per pod

Like the percentage of pod set, number of seeds per pod were also observed to be more on CMS rows nearest to the male rows and decreased gradually towards the farthest row in all the plots with different row ratios. Number of seed set/pod ranged from 5.916-10.360, 7.069-10.311 and 10.839-12.357 in case of M:F row ratio 1:8, 1:4 and 2:4 respectively (Table 1, 2 and 3). According to number of seeds set per pod, plots of various male-female row ratios may be arranged as : 2:4 > 1:4 > 1:8. Difference in average distance between male and female rows, percentage of pollinator plants and percentage of intersexual flower visits in plots of different M: F row ratios might be responsible for this variation. The same reasons were also thought to affect the percentage of pod set as discussed above.

Similar observations were recorded by Mesquida (1983) in hybrid seed production plots of *B. napus*. There was a marked pollination gradient across the female rows when two rows of male plants were sown with 7-10 rows of female plants on each side. Seed set was about 70 percent in rows next to male rows and decreased to 25 to 50 per cent in outer female rows.

Banga *et al*. (1993) have also demonstrated that the extent of hybrid seed set in the Indian mustard, *B. Juncea*, was maximum when male rows were sown in higher frequency i.e. 2:4 > 1:2 > 1:4. There was significant reduction when male-female row ratio was changed from 1:3 to 1:4. In another experiment, researchers (Banga *et al*., 1995) found that in the hybrid seed production plots of *B. napus*, comparatively more hybrid seed yield was achieved at 1M: 2F ratio than that at 1M : 3 F ratio. Ohsawa and Nawal (1988) reported that a pollen parent row ratio of 1:3 was suitable for hybrid seed production of *B. napus* with syrphid fly (*Eristalis cerealis*) as pollinator in cages.The results of present study are also in line with those of other workers on different crops(Moffett *et al*., 1976; Moffett *et al*., 1980; Satynarayana and Setharam, 1982;Drane *et al*., 1982; Loper and Davis, 1985; Loper,1987; Skinner;1988;Singh *et al*., 1988; ; Degrandi-Hoffman and Murales, 1989; Rodet *et al*., 1990; Nadre *et al*., 1996)

Percentage of well formed seeds

Percentage of well formed seeds was observed to be 57.053-68.017, 58.128-61.733 and 52.311 – 55.619 in case of plots with M:F row ratio 1:8, 1:4 and 2:4 respectively. The percentage of well formed or healthy seeds was more in CMS rows which were at maximum distance from the male rows and it decreased gradually across the CMS rows towards male row. Observations showed that various M:F row ratios in descending order of percentage of well formed seeds were : 1:8 > 1:4 > 2:4. This was probably due to the fact that number of pods per plants and number of seeds per pod were the lowest and thus more nutrients were available to developing seeds in 1st case (1:8), followed by 2nd (1:4) and 3rd (2:4) ones.

Average seed weight (g)

Seeds obtained from the CMS rows adjacent to R line rows showed less seed weight and it increased gradually with increasing distance from R rows in all the plots of various M:F row ratios. Weight in gram per 1000 seeds ranged from 1.862 – 3.439, 2.188 – 2.974 and 1.911 – 2.259 g in case of 1:8, 1:4 and 2:4 planting rows respectively (Table 20, 21 and 22)

The plots of various row ratios with respect to average weight per 1000 seed may be arranged as : 1:8 > 1:4 > 2:4. In case of 1:8 row ratio plots male row frequency was less and average distance between male and female line rows was more as compared to 1:4 and 2:4 row ratio plots. Thus percentage of pod set and number of seeds set per pod were relatively lower. Thus developing seeds got more nutrients and therefore, average seed weight was found to be higher in these plots, followed by 1:4 and 2:4 planting ratios.

Percentage of oil content

Range of oil content in seeds was recorded 35.705 – 41.891, 36.454- 40.300 and 34.320 – 36.422 per cent in case of plots with M:F row ratio 1:8, 1:4 and 2:4 respectively (Table 1, 2 and 3). It followed the same trend as was noticed in case of well formed seeds and average seed weight i.e. minimum oil content was observed in the seeds collected from CMS row nearest to the R line row and it increased gradually with distance and was found to be maximum in the seeds collected from CMS row situated farthest from the male line row.

From the results, it was obvious that on an average percentage of oil content was more in seeds of row ratio 1:8, followed by 1:4 and 2:4. This difference is probably due to fact that there was rich supply of nutrients to developing seeds in case of hybrid seed production plots of male-female row ratio 1:8 because in this case on

an average number of pods per plant and number of seeds per pod were lesser as compared to 1:4 and 2:4 row ratio plots.

III. CONCLUSION

From the above discussion it is clear that male –female row ratio 2:4 is the most suitable as far as percentage of pod set and number of seeds set per pod was concerned, followed 1:4 and 1:8 row ratios. But as far as percentage of well formed seeds, average seed weight (g) and percentage of oil content in the seeds was concerned, reverse was true i.e. seeds obtained from plots of male – female row ratio 1:8 were better for these quality parameters, followed by those of 1:4 and 2:4 row ratio plots. Plant breeders while evaluating various parental lines and their planting ratios, take total yield of hybrid seed on seed parent as one of the important parameters but present study showed that while finalizing the planting ratios, seed quality parameters should also be taken into account apart from the total seed yield. In fact if more CMS rows were planted (1:8) than good quality seeds were produced. The study will go in the long way to improve hybrid seed production practices and management technology in case of *B.napus* particularly and other crops in general.

Table 1: Crop yielding Parameters of CMS rows with M:F row ratio 1:8.

Yielding parameter	CMS rows							
	1	2	3	4	5	6	7	8
Percentage of pod set	55.378 (48.091)	51.713 (45.983)	45.167 (42.209)	44.977 (42.092)	44.949 (42.097)	39.240 (38.768)	38.561 (38.294)	36.498 (37.08)
	GM 41.827 SEM 1.960 $CD_{0.05}$ 5.946 CV 8.117							
No. of seed set/pod	10.360	8.482	7.687	6.716	6.457	5.735	5.579	5.196
	GM 7.026 SEM 0.432 $CD_{0.05}$ 1.311 CV 10.656							
Percentage of well formal seeds	57.053 (49.068)	59.181 (50.300)	59.438 (50.454)	60.673 (51.311)	60.811 (51.264)	66.170 (54.448)	67.340 (55.148)	68.017 (55.561)
	GM 52.194 SEM 1.929 $CD_{0.05}$ N.S.							
Weight per 1000 seeds (g)	1.862	2.404	2.407	2.647	3.025	3.094	3.439	3.273
	GM 2.769 SEM 0.155 $CD_{0.05}$ 0.471 CV 9.709							
Percentage of oil content	35.705 (36.546)	36.869 (37.383)	38.845 (38.552)	40.421 (39.450)	40.931 (39.774)	41.043 (39.837)	41.234 (39.947)	41.891 (40.323)
	GM 38.976 SEM 1.562 $CD_{0.05}$ N.S.							

Figures in parenthesis are arc sin transformations.

Table 2: Crop yielding Parameters of CMS rows with M:F row ratio 1:4.

Yielding parameter	CMS rows			
	1	2	3	4
Percentage of pod set	54.554 (47.614)	51.936 (46.110)	51.018 (45.584)	46.518 (42.996)
GM	45.576	SEM	0.630	
$CD_{0.05}$	2.015	CV	2.764	
No. of seed set/pod	10.311	9.623	9.038	7.069
GM	9.010	SEM	0.742	
$CD_{0.05}$	N.S.			
Percentage of well formal seeds	58.128 (49.750)	58.611 (49.985)	59.466 (50.575)	61.733 (52.070)
GM	50.595	SEM	1.414	
$CD_{0.05}$	N.S.			
Weight per 1000 seeds (g)	2.188	2.473	2.591	2.974
GM	2.559	SEM	0.563	
$CD_{0.05}$	N.S.			
Percentage of oil content	36.454 (37.127)	38.079 (38.048)	38.779 (38.470)	40.300 (39.377)
GM	38.255	SEM	1.901	
$CD_{0.05}$	N.S.			

Figures in parenthesis are arc sin transformations.

Table 3: Crop yielding Parameters of CMS rows with M:F row ratio 2:4.

Yielding parameter	CMS rows			
	1	2	3	4
Percentage of pod set	54.770 (47.741)	52.261 (46.300)	50.667 (45.386)	53.566 (47.046)
GM	46.6181	SEM	0.784	
$CD_{0.05}$	N.S.			
No. of seed set/pod	10.893	10.379	10.852	12.357
GM	11.107	SEM	0.446	
$CD_{0.05}$	N.S.			
Percentage of well formal seeds	52.311 (42.328)	53.257 (46.883)	55.619 (48.233)	53.653 (47.078)
GM	47.130	SEM	1.161	
$CD_{0.05}$	N.S.			
Weight per 1000 seeds (g)	2.234	2.236	2.259	1.911
GM	2.222	SEM	0.238	
$CD_{0.05}$	N.S.			
Percentage of oil content	34.320 (35.850)	35.634 (36.598)	36.422 (37.068)	35.929 (36.746)
GM	36.565	SEM	1.184	
$CD_{0.05}$	N.S.			

Figures in parenthesis are arc sin transformations.

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