



Standardisation of hybrid seed production technology of first Indian mustard (*Brassica juncea*) hybrid NRCHB 506

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Received: 7 March 2012; Revised accepted: 25 June 2012

ABSTRACT

Field experiments were conducted in 2009–10 and 2010–11 to standardise the sowing time and planting ratio of the parental lines of the recently released Indian mustard (*Brassica juncea* L. Czern. & Coss) hybrid NRCHB 506. The results indicated that the optimum sowing time of parental lines of NRCHB 506 is the third week of October to achieve higher yield of good quality hybrid seed. Optimum planting ratio of female to male parental line for hybrid seed production was 8:2. Further increase in number of female rows per male row decreased the plant and plot yield of the hybrid seed production plot. The results pertaining to flowering phenology and characters, seed yield and its attributing traits indicated that delay in sowing beyond October reduces the value of those characters significantly leading to decline in seed yield from seed parent. The primary factor responsible for the reduction of values in the late sowing was the higher temperature during reproductive and seed feeling stage. Sowing in October and growing of plants in relatively high temperature extended the vegetative phase, i.e. delayed the flowering by four days as compared to that in November sowing which experienced a lower temperature during early growth stage. Delay in sowing reduced the flowering duration which had a direct impact on the number of flowers, siliqua set, seeds/siliqua and finally seed yield/plant.

Key words: Date of sowing, Hybrid seed production, Indian mustard, Phenology, Planting ratio, Seed yield

Rapeseed-mustard (genus *Brassica*) is an important group of edible oilseed crops, next only to groundnut, in India. Indian mustard (*Brassica juncea* L. Czern & Coss) alone occupies about 85% of the total area under these crops. It is a self-pollinated crop and is mainly pollinated by honeybees.

Commercial F1 hybrids based on *tour*, *ogu* and *polima* CMS systems have been developed and used in *B. napus* earlier. Restorer gene(s) was introgressed into *B. juncea* from *Trachystoma balli*, *Moricandia arvensis*, *Enarthrocarpus lyratus* to exploit commercial hybrid (Chauhan *et al.* 2011). Using *Moricandia* as CMS source and a suitable restorer in a heterotic combination, the hybrid NRCHB 506 was developed by Directorate of Rapeseed-Mustard Research, Bharatpur and released for commercial cultivation in 2008.

Development of a successful hybrid seed production technology in a crop, which is essential for the extension of hybrid technology to farmers precisely depends on the understanding of its pollination. Significant influence of

planting date exists for seed yield in mustard cultivars. Effective pollination leading to higher seed set depends on environmental factors, particularly temperature and relative humidity (Hall 2001). High temperatures coupled with the drying effects of low relative humidity affect female floral structures causing reductions in the duration of stigma receptivity, pollen germination on the stigmatic surface, and initial pollen tube growth (Prasad *et al.* 2001).

Standardization of planting ratio is an important aspect for economic hybrid seed production and it is more valid for a species, which is predominantly self-pollinated. A higher frequency of male rows in a planting ratio gave higher hybrid seed set in Indian mustard (Banga 1993). It was in the order of 2:4>1:2>1:3>1:4 (male: female). A significant reduction in hybrid seed set was recorded when the male: female ratio was changed from 1:3 to 1:4. This was associated with lower number of pods with few or no seeds in wider planting ratio. Cutts (1999) suggested that 1:1 planting ratio produced the highest hybridity over all environments, whereas low seed yield was reported by Starner *et al.* (1999) even with 1:3 (male to female) planting ratio (maximum of 417 kg/ha). Yadav *et al.* (2007) reported that female rows just adjacent the male row had the highest seed yield and it reduced gradually towards the central row positions (4th and

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5th row from the pollen parent row). This may be attributed to the attractiveness/preference of flowers of female parents by honeybees. In canola, the parental lines are planted in a ratio of 1:3 or 1:4 male to female plants depending on the preference (Clay 2009). Precise understanding of pollination requirements in terms of date of sowing and planting ratio is indispensable for hybrid seed production in Indian mustard. Therefore, studies were made to standardize the sowing time and planting ratio of the parental lines of the hybrid NRCHB 506 of Indian mustard to get maximum hybrid seed yield in the agro-climatic conditions of Delhi, India.

MATERIALS AND METHODS

The seed material of the parental lines of hybrid, NRCHB 506 was collected from Directorate of Rapeseed-Mustard Research, Bharatpur, Rajasthan, India. The female and male parental lines were MJA5 (A) and MJR1 (R) respectively. The MJA5 is a cytoplasmic male sterile (CMS) line and MJR1 is a self-fertile restorer line. Fields were chosen in isolation (at least 50 m apart, Tunwar and Singh 1988) in both the *rabi* seasons of 2009–10 and 2010–11 at field of Seed Production Unit, Centre for Protected Cultivation Technology (CPCT) of Indian Agricultural Research Institute, New Delhi. New Delhi is situated at 28° 35'N latitude and 77°12'E longitude and at an altitude of 228.6 m above mean sea level with a semi-arid and sub-tropical climate characterized by extreme hot summers and extreme cool winters. The soil is sandy loam in texture with slight salinity.

The female (A) and male (R) line seeds were sown in plots with adequate soil moisture, in three different dates, i.e. 1) 21 October, 30 October and 18 November following a planting ratio of 2:16 (R:A) in 2009–10. The row length was 5m with spacing between rows and between plants within a row (after thinning) as 50cm and 20cm respectively. The experiment was laid out in an RBD with three replications each with three sets of plots in 2:16 (R:A) planting ratio. Regular inspection of the plot to remove the off-types from the female lines starting at the onset of flowering till its termination was done. Examination of the pollen viability under microscope helped to detect the male sterile plant properly and to rogue out the pollen shedders. Flower size and morphology provided also a distinct identity between male sterile and fertile plant.

The female and male lines seeds sown in three isolated plots (by at least 50 m) each with a single planting ratio (male : female) among the three, i.e. 2:8, 2:12 and 2:16 in three replications (three sets in each replication) in the third week of October in 2010–11. From different row ratios, plot yield was calculated from the following formula

$$\text{Yield (tonnes/ha)} = \frac{(A \times m \times y)}{a \times (m + n) \times 10^6}$$

Where, A= plot area (m²), a= spacing (m²), m= female row number in row ratio, n= male row number in row ratio,

y = average plant yield (g).

To examine the rate of yield reduction due to every increase of female row per male row, average plant yield was calculated cumulatively for every increase in number of female rows starting from 4th row to 8th row separately in the 16:2 row ratio, like 8:2, 10:2, 12:2, 14:2 and 16:2 (Fig 1). It provided a rough estimate of average plant yield in 8:2, 10:2, 12:2, 14:2 and 16:2 (female to male) row ratio respectively. Then plot yield was calculated from above formula.

Daily weather parameters for the growing season were taken from the meteorological observatory of Division of Agricultural Physics, IARI, New Delhi, India and is presented in Fig 2. Observations were recorded on five randomly tagged plants in each row of female line for plant morphology such as plant height, number of primary branch etc., phenology such as first flowering, 50% flowering, flowering duration etc. and yield attributes such as number and length of siliqua in different branches, seed yield/plant and test weight. The hybrid seeds collected from the female plants in each date of sowing were sown with at least 400 plants in

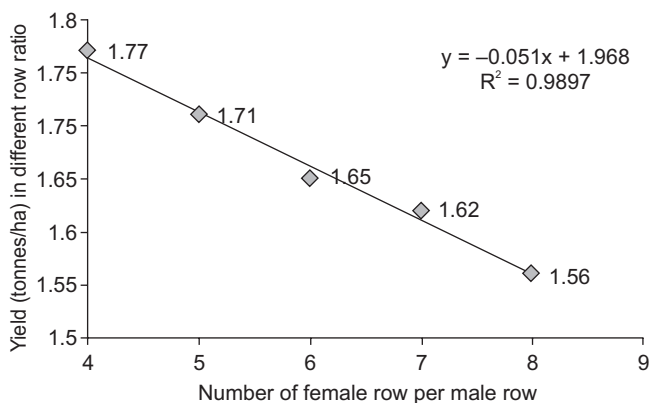


Fig 1 Relationship between number of female rows/male row and yield (tonnes/ha)

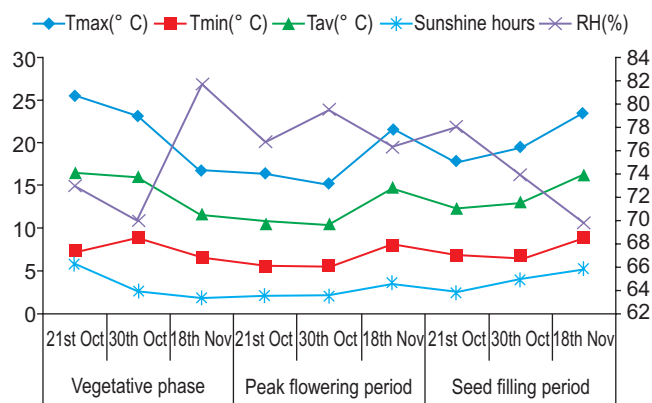


Fig 2 Weather parameter during crop growth period in three dates of sowing (primary vertical axis pertains to temperature and sunshine hours and secondary vertical axis pertains to relative humidity)

2010–11 for grow out test. A sample of authentic seeds of NRCHB 506 was grown for comparison. Plants with male fertility were considered as pure hybrid seed. Thousand seeds from each treatment were counted in all plots and weighed at 5–6% moisture content to measure the test weight. All the data were analysed using SAS software.

RESULTS AND DISCUSSION

Effect of dates of sowing on flowering and maturity

The days to 1st flowering, days to 50% flowering, flowering duration and maturity duration significantly decreased in female line in the 3rd date of sowing, i.e. 18 November (42.0, 52.0, 52.2 and 134.9 respectively) in comparison to those in earlier dates of sowing, i.e. 21 and 30 October, 2009 (Table 1). The maturity duration of the female parent was significantly higher in the first and second date of sowing (146 days) as compared to that in the last date of sowing (135 days). In case of male parent, days to first flowering and flowering duration in third date of sowing (37.5 and 55.4 respectively) were significantly different with first date of sowing but not with second date of sowing, i.e. 30 October. Days to 50% flowering didn't show any significant variation among the dates of sowing, though the highest value (51.7) was in first date of sowing (Table 1). In all the sowings the male parent flowered 3–5 days earlier to the female parent indicating no limitation of non-synchrony of flowering of parental lines for hybrid seed production in this particular hybrid. Panda *et al.* (2004) reported that delayed sowing after 16 October significantly reduced the days to flower initiation. Environmental stress as the most limiting factor besides genotype of the plants in deciding the optimum date of sowing has been reported by many researchers (Saidat *et al.* 2009).

Effect of dates of sowing on plant height and yield attributing characters

Plant height differed among the three dates of sowing with the highest value (187.5 cm) in first date of sowing (Table 2). Main stem length of female line in first date of sowing (80.2 cm) was significantly different from that in second and third date of sowing, but no significant difference between second and third date of sowing was recorded. Number of primary branches/plant, number of siliquae/

secondary branch, length of silique of main stem, number of seeds/silique of secondary branch and main stem in first date of sowing (7.2, 10.6, 4.4, 12.9 and 12.6, respectively) and second date of sowing (7.0, 9.1, 4.3, 12.3 and 11.8, respectively) showed significantly higher values compared to those in the third date of sowing, but no significant difference in these characters between first and second dates of sowing was recorded. Whereas number of siliquae/main stem, primary branch and number of seeds/silique of primary branch showed significant differences in all the three dates of sowing with the highest value (12.3) in the first date of sowing. Length of silique of primary branch in first date of sowing (4.6) was higher compared to second and third dates of sowing but no difference was recorded in between second and third dates of sowing. However, no significant difference in length of silique of secondary branch was recorded among the three dates of sowing, though the highest value (3.7) was in 1st date of sowing. Seed yield/plant drastically reduced in the third date of sowing while no significant difference in test weight of seed harvested from female plants grown in three dates of sowing was recorded (Table 2). Adamsen and Coffelt (2005) were not clear whether this resulted from lower pollination rates, which could affect reproductive efficiency, less favourable environmental conditions, or other unknown factors. Singh *et al.* (2002) reported that primary and secondary branches/plant were obtained higher when the crop was sown in between 10 and 30 October in India. Panda *et al.* (2004) observed that delay in sowing beyond 16 October reduced 1000-seed weight. This may be due to other factors of hybrid seed production such as pollinator abundance scarcity which can lead to poor seed setting resulting in lower yield (Abrol 2007), but no effect on seed weight. Khushu and Singh (2005) reported that seed yield decreased with delay in sowing after third week of October. Morshedi and Farahbakhsh (2009) reported that late sowing reduced total Nitrogen concentration in parts of the plants used for producing seed yield of canola.

Performance of hybrid seed lots produced under different dates of sowing

Hybrid seeds produced in different dates showed non-significant differences for flowering phenology, plant height and seed yield characteristics in comparison to those of the

Table 1 Phenology of parental lines in relation to date of sowing

Character	Date of sowing (2009–10)						CD (<i>P</i> =0.05) for female	CD (<i>P</i> =0.05) for male
	21 Oct		30 Oct		18 Nov			
	Female	Male	Female	Male	Female	Male		
Days to 1st flowering	46.2	43.0	45.0	40.1	42.0	37.5	1.5	4.08
Days to 50% flowering	59.9	51.7	58.1	49.8	52.0	46.2	2.5	NS
Flowering duration(days)	65.0	61.6	64.6	61.0	52.2	55.4	3.7	3.3
Maturity duration(days)	146.1	132.2	145.8	131.5	134.9	124.0	5.0	3.4

Table 2 Effect of date of sowing and planting ratio on seed yield and yield attributing parameters of female line

Parameter	Date of sowing			CD (<i>P</i> =0.05)	Planting ratio (female : male)			CD (<i>P</i> =0.05)
	21 Oct	30 Oct	18 Nov		8:2	12:2	16:2	
Plant height(cm)	187.5	181.8	164.8	0.7	187.5	186.5	185.5	NS
Main stem length (cm)	80.2	67.2	64.5	11.1	80.2	84.5	82.5	NS
No. of primary branch/plant	7.2	7.0	5.1	0.8	7.2	7	6.7	NS
No. of siliquae/main stem	30.4	26.7	21.1	1.1	36.5	33.7	29.3	0.9
No. of siliquae/primary branch	21.1	17.8	13.8	2.0	27.7	24.3	20.5	2.1
No. of siliquae/secondary branch	10.6	9.1	6.7	2.3	13.5	11.4	9.1	2.2
No. of seeds/siliqua of main stem	12.6	11.8	10.3	1.5	14.3	13.4	12.1	1.4
No. of seeds/siliqua of primary branch	12.3	12.1	10.1	0.5	13.1	12.6	10.7	0.8
No. of seeds/siliqua of secondary branch	12.9	12.3	10.9	1.3	12.3	12.0	10.7	0.8
Length of siliqua of main stem (cm)	4.4	4.3	3.9	0.4	5.0	4.5	4.0	0.4
Length of siliqua of primary branch(cm)	4.6	3.8	3.7	0.2	4.9	4.7	4.1	0.3
Length of siliqua of secondary branch (cm)	3.7	3.6	3.5	1.9	4.3	3.9	3.5	0.9
Seed yield (g)/plant	20.3	19.0	14.3	2.6	31.0	22.8	17.5	5.9
Test weight (g)	6.2	5.7	5.6	NS	5.5	6.2	6.4	0.6
Yield (tonnes/ha)					2.48	1.95	1.56	0.3

authentic hybrid seeds. However, the off-types (%) were significantly higher in hybrid seeds produced in first date of sowing (15.5) as compared to second (12.5) and third (9) dates of sowing.

Different sowing dates referring to different weather conditions had a large effect on the flowering phenology and yield attributing parameters of both the parents. Effective sunshine hours decreased towards later date of sowing during vegetative growth phase which lead to significant reduction in the floral and other plant morphological characters that lowered the seed yield. On the other hand, effective sunshine hours increased during seed filling period affecting the yield negatively. This is due to the physiological evolution and adaptability of the plant species and even varieties to sustain and benefit from different weather conditions during its different growth stages (Bhuiyan *et al.* 2008). Environmental stress as the most limiting factor besides genotype of the plants in deciding the optimum date of sowing has been reported by many researchers (Saidata and Hemawati 2009). It has been observed that mustard requires higher temperature for completion of the vegetative phase and cooler temperature and clear sky during reproductive phase for better realization of seed and oil yield. The cloudy and frosty weather during flowering is quite harmful, as it adversely affects honeybee activity. In our study, flowering characters deteriorated with increase in temperature and decrease in RH during flowering period especially when the sowing time is delayed beyond

October. Yield characters also followed the same pattern as the environmental conditions were altered for the entire crop growth period by changing the sowing date. Based on the findings from our study we can recommend third week of October is the optimum time for sowing of parental lines under Delhi conditions for higher hybrid seed yield of Indian mustard hybrid NRCHB 506. This phenomenon is strongly supported by a number of researchers but most of the studies were conducted on varieties (Singh *et al.* 2002 and Khichar *et al.* 2000). High temperature is the second most important stress next to drought, which can affect crop plants at any time and impose severe limitation on crop growth and development (Chauhan *et al.* 2011).

More number of off-types from hybrid seeds produced in first date of sowing indicated that the general recommended isolation distance is not sufficient to check outcrossing. Less off-type per cent from hybrid seeds produced in later planting dates may be due to short of time isolation. Keeping in mind the hybrid seed purity, the isolation distance recommended for Indian mustard seed production may be reviewed.

Effect of planting ratio

There was no significant difference in flowering phenology [days to first flowering (43 to 46 days), 50% flowering (52 to 59 days), flowering duration (61 to 65 days), maturity duration (133 to 146 days) etc.] of the parental lines (both male and female) among planting ratios. A non-

Table 3 Row position-wise seed yield (g)/plant in different planting ratios

Row position	Planting ratio (female: male)		
	8:2	12:2	16:2
1	34.9 ^a	26.6 ^a	25.4 ^a
2	31.5 ^a	25.4 ^a	24.6 ^a
3	25.8 ^a	23.5 ^a	20.7 ^b
4	31.8 ^a	23.2 ^a	17.8 ^b
5		21.8 ^b	14.2 ^c
6		16.4 ^c	13.0 ^{cd}
7			13.7 ^{cd}
8			10.7 ^d
Mean	31.0 ^a	22.8 ^b	17.5 ^b

Value followed by same letter are not significantly different

significant difference in different yield-attributing characters such as plant height, main stem length and number of primary branches was recorded in the planting ratios (Table 2). The number of siliquae/branch, number of seeds/siliqua and seed yield/plant decreased significantly with increase in proportion of female rows in a plot. In case of 8: 2 (A: R) planting ratio, the reduction in seed yield in different rows was non-significant (Table 3). In 12: 2 planting ratio, there was no significant seed yield reduction up to 4th row from the male row. But in 16: 2 female to male ratio, just beyond two female rows, adjacent to male row showed significant yield reduction. Plot yield (q/ha) had a strong negative correlation ($R^2 = 0.9897$) with increasing female to male ratio (Fig 1). The average test weight of seeds harvested in different planting ratios significantly increased with increase in female to male ratio (Table 2).

Non-significant difference in flowering and morphological characters among different planting ratios indicated no influence of female to male planting ratio on plant characters and phenology. But yield attributing characters showed significant differences among planting ratios. Contradictory results were reported by Selvakumar (2003) and Mankar (2000) in cauliflower and Indian mustard, respectively. When row wise seed yield was plotted in different planting ratios, it was observed that the reduction in seed yield became steeper with increase in female to male ratio. This suggests that higher hybrid seed yield is achievable in a narrow planting ratio in comparison to that in a wider planting ratio. Hence, 2:8 male to female planting ratio may be recommended for Delhi and adjoining areas to reap maximum hybrid seed yield. Our study is strongly supported by Yadav (2002) and Yadav *et al.* (2007) in sunflower and Indian mustard respectively.

ACKNOWLEDGEMENT

We are thankful to Dr K H Singh, Senior Scientist, Directorate of Rapeseed-Mustard Research, Rajasthan for

providing seeds of the parental lines and hybrid.

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