



## Research Article

# Production of Restorer Lines from Segregating Progenies of *Brassica napus* L. Having Good Agronomic Value

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**Keywords:** Rapeseed; hybrid; CMS; restorer gene; testcross, pollen fertility; seed yield

### Abstract

Forty-four testcross progenies obtained from crossing between five CMS and forty-one candidate lines were evaluated both in field and laboratory condition to identify candidate restorer line(s). Nineteen testcross progenies were recorded as fully fertile with 100% plant fertility by counting the number of fertile plants from the total number of plants per lines. On the other hand, from pollen fertility test, 11 testcross progenies [206A × 001(6), 9905A × 030(2), 9905A × 027(6) (0.57), 206A × 37(1) (0.68), 9904A × 027(4) (0.83), 248A × 020(6) (1.08), 248A × 018 (1.12), 248A × 022 (1.13), 248A × 017 (1.58), 248A × 038(2) (1.96) and 248A × 001(6) (2.02)] were found with 0-2.02% pollen sterility that could be mentioned as fertile or restorer lines for making hybrids. Agronomic performances were also satisfactory for these selected test cross progenies. The genotype 248A × 017 took the shortest time (30.00) for first flowering as well as ripening followed by 248A × 007(1). The highest number of pods per plant was observed in the testcross progeny 248A × 022 (649.59) and the highest number of seeds per pod (30.33) in 248A × 020(6). For seed weight per plant, 206A × 001(6) was recorded with maximum value (0.08g) while the lowest (23.33) number of seeds per plant was found in this progeny. In case of seed yield per plant, 248A × 022 gave the highest yield (30.30). The seed yield of the progenies 248A × 017 (23.49), 9905A × 027(6) (20.39), 248A × 001(6) (17.26), 9904A × 027(4) (17.02) and 248A × 038(2) (16.53) were also in the highest level.

### Introduction

Oilseed crops and human civilization have a symbiotic relationship since ancient time. Rapeseed was mentioned in the Indian Sanskrit writings from 2000 B.C. (Hatje, 1989). However, oilseed rape has been grown since the 16<sup>th</sup> century in Europe. It has become a major world crop since 1960's (Kimber and McGregor, 1995). Oilseed rape is the second most important vegetable crop worldwide after soybean which having 14% of total oilseed production in

2000 (Weiss, 2000). It was predicted by Scarisbrick and Ferguson (1995) that brassica crops will play an increasing role to meet up the world's need for human and animal foodstuffs and industrial oils. Mustard and rapeseed are widely grown oilseed crops of Bangladesh. Both local and HYV were occupying 667242 acres of land with the yield of 311740 MT (metric ton) in 2018-19 (BBS, 2020). In Bangladesh, domestic need of edible oil is satisfied mainly

by mustard and sesame whereas 42% oil and 25% protein was obtained from Mustard and rapeseed seeds (Kaul, 2006). Additionally, complete profile of amino acids (38-40% protein) including lysine, methionine and cystine can also be fulfilled by its meal. Oil cake is a nutritious food item for cattle and fish as well as a good source of organic fertilizer. Among Brassicaceae crops, *B. napus* show hybrid vigor up to 200% of the parental lines in terms of seed yield (Fu et al. 1990, Jain et al. 1994). Thus, hybrid breeding is useful strategy to increase edible oil production. Due to the size and structure of flowers, it is quite difficult to develop commercial hybrid of *Brassica napus* and its seed production depending on manual emasculation and pollination. Self-incompatibility could be an effective system of hybrid development but as an amphidiploid species, *B. napus* do not express self-incompatibility (Hiroshi and Shripad, 2014). So here the only stable and applicable technique is cytoplasmic male sterility (CMS) to develop commercial hybrids. CMS is a maternally inherited trait encoded by a gene located in the mitochondria where pollen production is disturbed without affecting the female organ. Expression of a CMS gene can be masked by nuclear fertility restorer (Rf) genes that enable the plant to produce functional pollen. A cross between CMS induced female (A) and Rf gene bearer male (B) results to F<sub>1</sub> hybrid. In the F<sub>1</sub> plants, the Rf gene restores male fertility, and the combination of nuclear genomes from the CMS line and the restorer line produces hybrid vigor (Chen and Liu 2014). In Bangladesh, CMS that are used to develop hybrids are mainly imported from neighboring countries. It's very difficult to find out restorer of these CMS from the local cultivars. The segregating lines of exotic hybrids that are cultivated now as local cultivars by the farmers, need to be crossed with local cultivars to have the effective restorer lines or segregate them to resynthesize restorer line(s) from the segregating materials. So the objectives of the present study were: (i) to make testcrosses of CMS and segregating lines, (ii) to check the both the plant fertility and pollen fertility of testcross progenies, and (iii) to identify the effective restorer lines from the testcross progenies.

## Materials and Methods

The research work was carried out at the experimental farm, Department of Genetics and Plant Breeding, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur. The location of the experimental site was at the center of Madhupur Tract (24.05°N latitude and 90.25°E longitude) with an elevation of 8.4 meters from the sea level. The soil type of experimental field belongs to the Shallow Red Brown Terrace type under Salna series of Madhupur Tract (Brammer, 1971; Saheed, 1984) of Agro Ecological Zone (AEZ) 28. The land was prepared

thoroughly by 3-4 time ploughing and cross ploughing. Adequate soil fertility was ensured by applying additional quantities of N-P-K-S-Zn-B @ 120-35-65-20-5-1.5 kg/ha, respectively. Total TSP, MP and Gypsum, Zink Sulphate and Boric Acid were applied during final land preparation as basal dose (Halder et al., 2007). Cow dung was also applied during final land preparation @ 10 t/ha. Total urea was applied in three installments, at 15, 30 and 50 days after sowing (DAS).

### Developments of Testcross Progenies

Seeds of five CMS lines and forty-one selected lines synthesized from segregating materials of *Brassica napus* L hybrids were sowing in two rows for each of the materials. The seedlings were emerged out within four days after sowing. Stigma of the flower of CMS plants were opened by removing sepal and petal at the flowering time. Hand pollination was done by dusting fertile pollen of the selected plants from candidate restorer lines to obtain F<sub>1</sub> generation or testcross progenies. To avoid unwanted pollination, flowers were bagged for 3-4 days with proper tags. Seeds of 44 testcross progenies were collected after maturity. The seeds of F<sub>1</sub>'s and parents were stored in cold dry place for progeny evaluation in the following year.

### Identification of Restorer Lines

Seeds of 44 testcross progenies were sowing in two rows with row spacing of 30 cm and plant spacing of 15 cm within the row. Necessary intercultural operations were done during cropping period for proper growth and development of the plants. Irrigation was given at regular interval. Malathion 57 EC was applied three times as foliar spray at an interval of 10-15 days after seedling emergence to control aphid. Data were collected to identify the restorer lines as follows:

#### A. Percent fertile and sterile plant:

Total number of plants per progeny lines (F<sub>1</sub>) along with number of male fertile and male sterile plants was counted by visual observation of the flower morphology and presence or absence of pollen in the anther. The following formulae were used to calculate plant sterility and fertility (%).

$$\text{Plant sterility (\%)} = \frac{\text{No. of sterile plants}}{\text{Total no. of plants}} \times 100$$

$$\text{Plant fertility (\%)} = \frac{\text{No. of fertile plants}}{\text{Total no. of plants}} \times 100$$

The pollen grains of F<sub>1</sub> were classified in the Table 1 based on the percentage of pollen fertility as well as sterility (Virmani et al., 1997).

**Table 1:** Description of pollens

Pollen Sterility (%)	Pollen Fertility (%)	Category
100	0	CS (Completely sterile)
91-99	1-9	S (Sterile)
71-90	10-29	PS (Partially sterile)
31-70	30-69	PF (Partially fertile)
21-30	70-79	F (Fertile)
0-21	>80	FF (Fully fertile)

**B. Testing of pollen fertility:**

Anther content was stained with 1% acetocarmine to determine the pollen fertility. Round shaped and dark stained pollen grains were recorded as fertile (Vovk and Kudriavtseva, 1976)

$$\text{Pollen sterility (\%)} = \frac{\text{No. of sterile pollens}}{\text{Total no. of pollens}} \times 100$$

$$\text{Pollen fertility (\%)} = \frac{\text{No. of fertile pollens}}{\text{Total no. of pollens}} \times 100$$

**C. Agronomic performance of the testcross progenies:**

Ten competitive plants were selected randomly from each of the testcross progenies and were used for recording data on days to 1<sup>st</sup> flowering, days to 50% flowering, days to 100% flowering, days to first ripening, number of branches per plant, plant height (cm), number of pods per plant, pod length, number of seeds per pod, weight of seeds per pod (g), seed yield per plant (g). The recorded data for different characters were analyzed statistically using SAS (SAS, 2010).

**Results and Discussion****Analysis of Variance (ANOVA)**

A large range and significant variation were reported from the analysis of variance (Table 2) among 44 testcross progenies of *Brassica napus* for thirteen agronomic characters that indicates the presence of genetic variability.

**Identification of Restorer Lines**

The percentage of the male fertile plants ranged from 65-100% among the testcross progenies. Among 44 testcross progenies, one produced 68% male fertile plants within the line that described as partially fertile, five testcross progenies had 70-79% plant fertility and 38 had  $\geq 80\%$  plant fertility (Table 3). The total number of plants per line ranged from 2 [9905A  $\times$  030(2)] to 60 [206A  $\times$  001 (6)]. But as per the specific objective of the experiment to identify the restorer lines, 27 testcross progenies were considered whose fertility rate was above 95%. Among those 19 was reported 100% plant fertility among those were 206A  $\times$  001(6),

248A  $\times$  001(6), 248A  $\times$  004, 248A  $\times$  012(1), 248A  $\times$  013(6), 248A  $\times$  017, 248A  $\times$  018, 248A  $\times$  020(6), 248A  $\times$  022, 248A  $\times$  027, 248A  $\times$  030(2), 248A  $\times$  038(1), 248A  $\times$  038(2), 248A  $\times$  038(3), 9904A  $\times$  027(4), 9905A  $\times$  027(6), 9905A  $\times$  027(7), 9905A  $\times$  030(2), 9905A  $\times$  030(3)], six was revealed 98% fertility [206A  $\times$  013(6), 206A  $\times$  17 (1), 206A  $\times$  21(1), 206A  $\times$  21(3), 206A  $\times$  23(1), 206A  $\times$  37(1) and two [248A  $\times$  016(2) and 248A  $\times$  026] produced 96% fertile plants within the line (Table 3)

Twenty-seven progenies with more than 95% plant fertility showed different level of pollen fertility upon microscopic test (Table 3, Plate 1). As per the pollen fertility results plant can be referred to be the fertile, if the sterility percentage stands for 21-30 % and if it is ranged as 0-21%, it must be fully fertile (Virmani *et al.*, 1997). But in the present study, only those were considered with pollen sterility ranged from 0-2.02% as the objective of the experiment is to find out effective R lines. The testcross progenies 206A  $\times$  001(6) and 9905A  $\times$  030(2) gave 100% pollen fertility rate where as their total number of fertile pollen was 671 and 631 per focus, respectively. Nine test cross progenies i.e. 9905A  $\times$  027(6) (0.57), 206A  $\times$  37(1) (0.68), 9904A  $\times$  027(4) (0.83), 248A  $\times$  020(6) (1.08), 248A  $\times$  018 (1.12), 248A  $\times$  022 (1.13), 248A  $\times$  017(1.58), 248A  $\times$  038(2) (1.96) and 248A  $\times$  001(6) (2.02) were also be reported as heterozygous restorer line with lower pollen sterility rate (Table 3). Heterozygosity in the restorer lines were reported by Wang *et al.* (2013). Considering both plant and pollen fertility 11 testcross progenies were designated as the candidate source of restorer gene. One testcross progeny [248A  $\times$  007(1)] was found who gave 78% plant fertility but in case of pollen fertility it appeared as a partial sterile with 28.93% pollen fertility. This could be the source of maintainer gene and utilized in further experiment.

Testcross progenies were also evaluated for 12 agronomic traits including seed yield per plant to find out their yield potentiality as restorer in hybrid variety development. Agronomic performance of candidate restorer lines are described character wise in Table 2.

**Table 2:** Analysis of variance (ANOVA) for 13 yield contributing traits of 45 testcross progenies of *Brassica napus*

Source of variation	df	DFF	DFPF	DHPF	DEOF	DFR	NBPP	PH	PL	NSPP	WSPP	SYPP
Replication	2	12.38**	10.79**	30.02**	13.75**	6.29**	0.35	0.04*	0.24**	3.59	0.0002**	15.46*
Crosses	43	20.63**	26.04**	45.87**	203.73**	22.67**	0.74**	0.06**	1.73**	21.36**	0.001**	84.49**
Error	86	0.72	0.74	3.94	0.96	1.1	0.13	0.01	0.03	2.82	0	4.43

\* and \*\* indicate significance at 5% and 1% levels, respectively

DFF - Days to first flowering, DFPF - Days to 50% flowering, DHPF - Days to 100% flowering, DEOF – Days to end of the flowering, DFR - Days to first ripening, NBPP - Number of branches per plant, PH - Plant height (m), PL - Pod length (cm), NSPP - Number of seeds per pod, WSPP - Weight of seeds per pod (g), SYPP – Seed yield per plant (g)

**Table 3:** Fertility status of 44 testcross progenies of *Brassica napus*

Hybrids	Plant fertility					Pollen fertility				
	TNPPL	NFPPL	NSPPL	% F	Remark	NFP	NSP	% F	% S	Remark
206A × 001 (6)	60	60	0	100	FF	671	0	100	0	FF
206A × 013(6)	51	50	1	98	FF	794	88	90.02	9.98	FF
206A × 17 (1)	35	34	1	98	FF	714	93	88.47	11.53	FF
206A × 17(2)	11	8	3	73	PF	413	310	57.12	42.88	PF
206A × 20(3)	34	32	2	94	FF	454	464	49.45	50.55	PF
206A × 21(1)	13	12	1	98	FF	212	93	69	31	PF
206A × 21(3)	38	37	1	98	FF	515	131	79.72	20.28	F
206A × 22(1)	17	15	2	88	FF	665	128	83.85	16.15	FF
206A × 22(2)	25	23	2	92	FF	743	132	84.91	15.09	FF
206A × 23(1)	48	47	1	98	FF	632	102	86.1	13.9	FF
206A × 24(2)	33	29	4	87	FF	595	76	88.67	11.33	FF
206A × 25	38	36	2	94	FF	646	169	79.26	20.74	F
206A × 37 (1)	56	55	1	98	FF	733	5	99.32	0.68	FF
206A × 41(1)	0	0	0	0		0	0	0	0	
248A × 001 (6)	29	29	0	100	FF	778	16	97.98	2.02	FF
248A × 004	19	19	0	100	FF	665	80	89.26	10.74	FF
248A × 007(1)	28	22	6	78	F	193	474	28.93	71.67	PS
248A × 009 (4)	22	15	7	68	PF	892	82	91.58	8.42	FF
248A × 012(1)	40	40	0	100	F	656	137	82.72	17.82	FF
248A × 013(6)	31	31	0	100	FF	546	214	71.84	28.16	F
248A × 015(2)	29	27	2	93	FF	666	67	90.85	9.15	FF
248A × 016(2)	25	24	1	96	FF	530	210	71.62	28.38	F
248A × 017	18	18	0	100	FF	873	14	98.42	1.58	FF

**Table 3:** Fertility status of 44 testcross progenies of *Brassica napus*

Hybrids	Plant fertility					Pollen fertility				
	TNPPL	NFPPL	NSPPL	% F	Remark	NFP	NSP	% F	% S	Remark
248A × 018	9	9	0	100	FF	621	7	98.88	1.12	FF
248A × 019	7	5	2	71	F	327	327	50	50	PF
248A × 020(6)	25	25	0	100	FF	644	7	98.92	1.08	FF
248A × 022	9	9	0	100	FF	439	5	98.87	1.13	FF
248A × 023	9	7	2	78	F	803	266	75.11	24.89	F
248A × 025(1)R1	24	22	2	92	FF	931	253	78.63	21.36	F
248A × 025(2)R2	30	28	2	93	FF	781	224	77.71	22.29	F
248A × 025(3)R1	27	25	2	92	FF	960	72	93.02	6.98	FF
248A × 026	23	22	1	96	FF	587	59	90.86	9.14	FF
248A × 027	2	2	0	100	FF	395	27	93.6	6.4	FF
248A × 030(2)	6	6	0	100	FF	696	52	93	7	FF
248A × 038(1)	17	17	0	100	FF	500	287	63.53	36.47	PF
248A × 038(2)	26	26	0	100	FF	651	13	98.04	1.96	FF
248A × 038(3)	20	20	0	100	FF	616	43	93.47	6.53	FF
248A × 041(1)	34	28	6	82	FF	277	607	31.33	68.67	PF
248A × 044(1)	37	33	4	90	FF	376	511	42.29	57.71	PF
9904A × 027(4)	7	7	0	100	FF	42	12	99.17	0.83	FF
9905A × 027(6)	20	20	0	100	FF	869	5	99.43	0.57	FF
9905A × 027(7)	3	3	0	100	FF	214	178	54.59	45.41	PF
9905A × 030(1)	7	5	2	72	FF	1039	132	88.72	11.28	FF
9905A × 030(2)	2	2	0	100	FF	631	0	100	0	FF
9905A × 030(3)	19	19	0	100	FF	340	49	87.4	12.6	FF
9908A × 007(1)	51	43	8	84	FF	485	250	65.98	34.02	PF
9908A × 019(1)R1	39	39	0	100	FF	530	180	74.64	25.36	F

TNPPL - Total No. of plant per line, NFPPL - No. of fertile plant per line, NSPPL - No. of sterile plant per line, NFP - No. of fertile pollen, NSP - No. of sterile pollen,

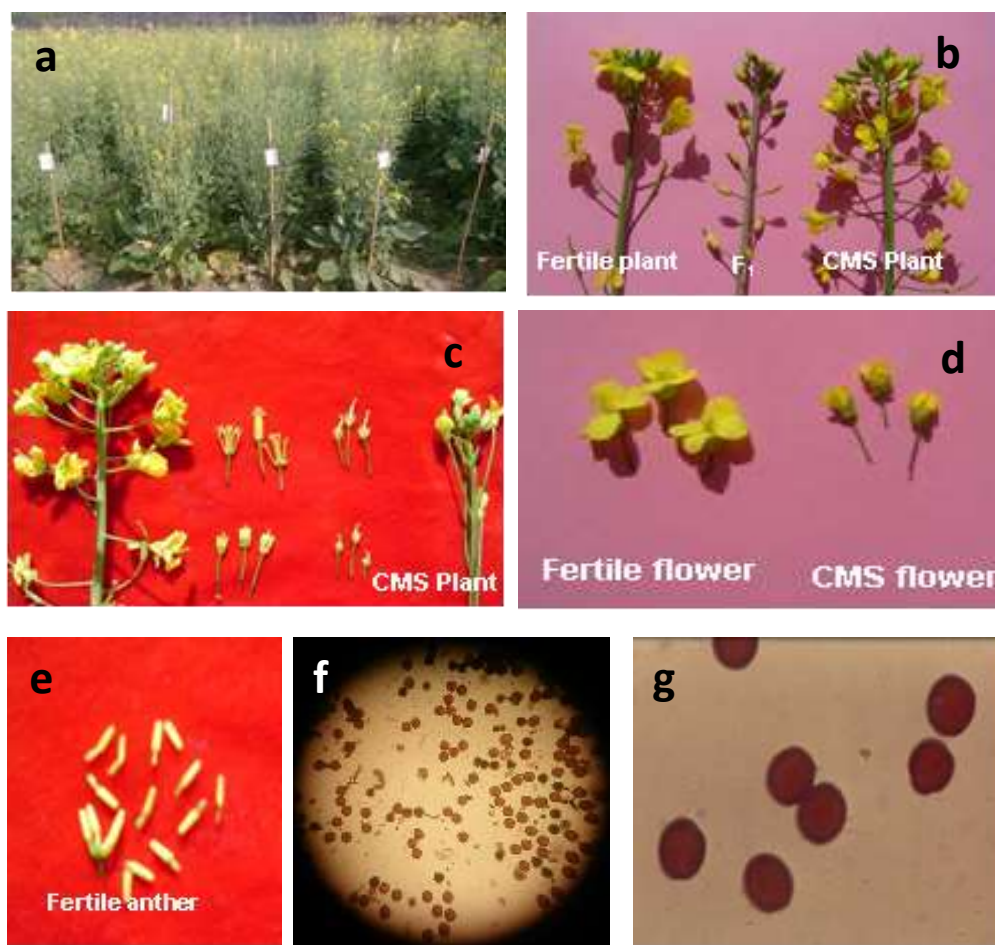
**Table 4:** Mean performance 44 testcross progenies of *Brassica napus* for 13 yield contributing traits

Crosses	DFF	DFPF	DHPF	DEOF	DFR	NBPP	PH	NPPP	PL	NSPP	WSPP	SYPP
206A × 001 (6)	32.67	41.33	50.00	91.33	92.00	3.00	1.31	109.39	6.66	23.33	0.08	8.43
206A × 013(6)	33.00	40.33	46.67	91.00	95.00	3.33	1.14	133.19	6.79	22.33	0.08	10.25
206A × 17 (1)	30.33	38.67	42.33	90.33	91.33	3.00	1.31	137.06	5.85	27.00	0.10	14.16
206A × 17(2)	31.33	37.33	40.33	80.67	92.67	3.44	1.43	259.27	7.92	26.67	0.07	18.15
206A × 20(3)	31.00	35.33	38.67	80.33	90.67	3.33	1.32	215.83	7.66	25.00	0.05	10.08
206A × 21(1)	36.67	40.67	47.67	82.33	89.00	3.00	1.38	336.51	7.77	25.00	0.06	19.12
206A × 21(3)	31.67	41.00	47.33	82.00	89.00	3.00	1.35	430.78	8.11	27.00	0.03	14.31
206A × 22(1)	32.67	37.00	39.33	78.33	92.33	3.22	1.18	260.72	6.47	28.00	0.06	14.83
206A × 22(2)	31.67	36.33	41.00	78.00	91.67	3.67	1.24	336.39	6.87	27.67	0.03	10.09
206A × 23(1)	36.67	39.67	34.67	75.00	91.33	4.11	1.15	199.67	7.37	27.67	0.04	7.35
206A × 24(2)	31.33	36.33	40.67	66.67	87.00	3.44	1.15	171.28	6.18	18.67	0.05	9.14
206A × 25	30.67	36.00	38.33	53.33	89.33	3.11	1.15	208.06	6.72	21.00	0.05	11.04
206A × 37(1)	33.67	40.00	44.33	92.00	90.67	3.00	1.31	130.67	7.40	26.33	0.05	6.95
248A × 001 (6)	30.33	35.67	41.33	88.33	89.33	4.00	1.37	271.22	6.81	27.00	0.06	17.26
248A × 004	31.33	34.33	40.00	65.33	87.00	4.11	1.09	232.44	7.22	30.67	0.04	10.01
248A × 007(1)	31.67	35.33	43.33	88.33	91.33	4.22	1.32	237.45	5.75	23.00	0.06	14.19
248A × 009(4)	30.33	34.33	44.67	83.33	92.67	3.33	1.30	172.00	6.98	25.33	0.05	8.60
248A × 012(1)	30.67	37.33	45.67	87.33	92.67	3.33	1.24	309.27	7.74	24.33	0.05	15.57
248A × 013(6)	32.00	36.00	40.33	83.00	92.67	3.44	1.23	236.11	7.36	27.33	0.06	13.38
248A × 015(2)	34.67	41.00	50.33	90.67	90.00	3.44	1.24	215.33	7.69	30.67	0.05	10.00
248A × 016(2)	31.33	36.33	41.67	81.00	93.33	3.44	1.20	241.83	7.02	30.00	0.07	16.02
248A × 017	30.00	32.67	38.33	67.67	86.00	4.22	1.23	469.86	7.28	26.33	0.05	23.49
248A × 018	33.33	37.00	39.00	78.33	92.00	4.44	1.35	376.39	7.97	28.33	0.03	9.84
248A × 019	30.67	30.33	39.00	76.33	91.67	3.96	1.27	343.67	7.22	28.67	0.05	16.07
248A × 020(6)	31.33	36.67	45.33	91.33	94.67	3.44	1.42	293.00	6.97	30.33	0.06	16.53
248A × 022	34.33	37.00	40.33	81.67	95.67	4.00	1.42	649.59	7.48	24.33	0.05	30.30
248A × 023	32.67	36.00	40.33	84.00	95.00	3.78	1.55	939.67	6.96	26.00	0.03	25.13

**Table 4:** Mean performance 44 testcross progenies of *Brassica napus* for 13 yield contributing traits

Crosses	DFF	DFPF	DHPF	DEOF	DFR	NBPP	PH	NPPP	PL	NSPP	WSPP	SYPP
248A × 025(1)R1	34.33	40.33	48.67	91.67	90.67	3.22	1.50	273.17	7.13	29.33	0.03	9.05
248A × 025(2)R2	33.33	39.00	47.33	93.00	93.00	3.22	1.37	264.22	6.43	26.33	0.03	7.93
248A × 025(3)R1	30.67	36.67	41.33	91.33	89.67	3.00	1.33	213.75	7.86	25.67	0.05	10.66
248A × 026	28.67	36.00	40.67	91.33	85.00	4.22	1.45	255.33	8.12	27.00	0.03	6.83
248A × 027	31.33	35.67	38.67	84.33	87.33	3.44	1.75	141.89	6.58	30.00	0.06	8.91
248A × 030(2)	32.00	35.33	38.33	86.33	93.67	3.55	1.63	406.39	9.38	30.33	0.03	12.19
248A × 038(1)	38.67	43.33	50.00	91.33	95.33	3.89	1.64	270.83	9.27	31.67	0.04	10.06
248A × 038(2)	38.67	42.33	48.33	87.67	90.00	3.00	1.41	275.16	7.63	28.00	0.06	16.53
248A × 038(3)	38.33	44.67	48.00	92.00	94.67	3.78	1.41	233.28	7.64	26.33	0.04	8.48
248A × 041(1)	30.33	39.67	47.00	87.00	90.67	3.77	1.42	184.84	7.38	29.00	0.05	8.68
248A × 044(1)	38.33	42.67	46.33	87.33	93.67	4.00	1.20	220.05	7.97	28.67	0.06	12.33
9904A × 027(4)	32.33	35.67	40.00	79.00	94.33	5.00	1.36	466.11	8.38	28.67	0.04	17.02
9905A × 027(6)	30.33	33.33	38.33	87.67	91.33	4.44	1.20	437.15	8.02	30.00	0.05	20.39
9905A × 027(7)	38.00	40.33	42.67	81.67	94.00	4.33	1.27	1125.11	7.13	27.33	0.02	22.50
9905A × 030(1)	30.33	36.67	40.33	79.33	93.33	3.78	1.25	307.55	7.94	30.00	0.04	13.44
9905A × 030(2)	36.00	39.00	40.67	76.33	90.67	3.44	1.30	318.39	8.79	27.00	0.04	11.50
9905A × 030(3)	32.33	36.33	39.33	78.67	90.67	3.00	1.44	201.94	7.60	25.00	0.06	11.42
9908A × 007(1)	31.67	36.00	42.33	88.33	98.33	3.00	1.44	207.72	7.76	26.33	0.06	11.79
9908A × 019(1)R1	33.00	35.33	40.67	87.00	95.33	3.00	1.44	220.94	7.63	26.67	0.02	4.42
Mean	32.75	37.57	42.61	83.25	91.69	3.58	1.34	303.05	7.41	26.99	0.05	13.14
SE	0.69	0.7	1.62	0.8	0.86	0.29	0.08	24.56	0.13	1.37	0.004	1.72
LSD (0.05)	1.37	1.39	3.22	1.59	1.71	0.58	0.16	48.87	0.26	2.73	0.01	3.42
CV (%)	2.59	2.29	4.66	1.18	1.14	10.05	7.23	9.93	2.19	6.22	11.06	16.01

DFF - Days to first flowering, DFPF - Days to 50% flowering, DHPF - Days to 100% flowering, DEOF - Days to end of the flowering, DFR - Days to first ripening, NBPP - Number of branches per plant, PH - Plant height (m), NPPP - Number of pods per plant, PL - Pod length (cm), NSPP - Number of seeds per pod, WSPP - Weight of seed per pod (g), SYPP - Seed yield per plant (g)



**Plate 1:** Showing a) testcross progeny lines, b) flowering branch of fertile and sterile plant c) flowers and anthers of fertile and CMS plant d) flowers from fertile testcross progeny and CMS plant e) anthers from pollen parent, f) focus of pollen under microscope from fertile testcross progeny, g) close view of fertile pollen from testcross progeny.

#### ***Days to Flowering***

The testcross progeny 248A × 017 took the shortest time (30.00) for first flowering as well as ended flowering and can be designated as early. On the other hand, the highest days was taken by 248A × 038(2) (38.67) and can be designated as late. The testcross progeny 206A × 37(1) took the longest period of time (92.00) to complete flowering and gave its first flower after 33.67 days of sowing (Table 4). The testcross progeny with highest sterility [248A × 007(1)] started flowering 31.67 days after sowing and ended 88.33 days after sowing.

#### ***Days to Ripening***

Ripening period was ranged from 86.00 days (248A × 017) to 95.67 days (248A × 022) among the testcross progenies (Table 4). The maximum progeny lines took the time for ripening near to the highest value except 248A × 001(6) (89.33). The progeny line 248A × 017 gave flowers early as well as started to be ripened early. The progeny line 248A × 007(1) produced first ripened pods at 31.33 days after sowing. Significant differences for days to ripening were also reported by Zare and Sharafzadeh (2012) and Hasan *et al.* (2014) in *Brassica napus* L.

#### ***Number of Branches Per Plant***

Number of branches per plant was ranged from 3.00 to 5.00 among the progeny lines (Table 4). The highest number of branches per plant was observed in the progeny line 9904A × 027(4) and was followed by 248A × 018 (4.44) and 9905A × 027(6) (4.44). The lowest number of branches per plant were observed in the progeny lines 206A × 001(6), 206A × 37(1) and 248A × 038(2) followed by 248A × 020(6) and 9905A × 030(2) (3.44). According to Sana *et al.* (2003), higher number of branches per plant that resulted from the genetic makeup of the crop and environmental conditions, plays a noticeable role towards the seed yield of the crop.

#### ***Plant Height***

Plant height was more or less similar for all the progeny lines and ranged from 1.20m in 9905A × 027(60) to 1.42m in 248A × 020(6) and 248A × 022. The progeny line 248A × 007(1) had plant with the height of 1.32m (Table 4). Significant differences for plant height in brassica genotypes were also reported by Azam *et al.* (2013) and Muhammad *et al.* (2014).



### Number of Pods Per Plant

The highest number of pods per plant was reported in the progeny line 248A × 022 (649.59) followed by 248A × 017 (469.86), 9904A × 027(4) (466.11) and 9905A × 027(6) (437.15). The progeny line 206A × 001(6) gave the lowest number of pods per plant (109.39) followed by 206A × 37 (1) (130.67). The progeny line 248A × 007(1) had 237.45 pods per plant. Khan and Khan (2003) and Sadat *et al.* (2010) also reported significant difference among rapeseed genotypes for silique per plant.

### Pod Length

The progeny line 9905A × 030(2) gave the longest pod (8.79cm) followed by 9904A × 027(4) (8.38cm) and 9905A × 027(6) (8.02cm). The shortest pod was observed in the progeny line 206A × 001(6) (6.66cm). Pod length of the progeny line 248A × 007(1) was recorded as 5.75cm. Aytac and Kinaci (2009) reported significant difference among rapeseed genotypes for pod length.

### Number of Seed Per Pods

The highest number of seeds per pod was found in the progeny line 248A × 020(6) (30.33) followed by 9905A × 027(6) (30.00), 9904A × 027(4) (28.67) and 248A × 038(2) (28.00). The lowest number seeds per pod were recorded in the progeny line 206A × 001(6) (23.33). Number of seeds per pod also showed significant differences in the experiment of Khan and Khan (2003).

### Weight of Seed Per Pod

The highest weight of seeds per pod was observed in progeny line 206A × 001(6) and recorded as 0.08g though its number of seeds per pod was less than other progeny lines. The results indicated that this progeny line produces bold seed. The progeny lines 248A × 001 (6), 248A × 020(6) and 248A × 038(2) had a value of 0.06g for seed weight per pod (Table 4). The lowest weight was recorded in the progeny line 248A × 018 (0.03g) that means it produces smaller size seeds.

### Seed Yield Per Plant

In case of seed yield per plant, the progeny line 248A × 022 gave the highest seed yield per plant (30.30g) followed by 248A × 017 (23.49g), 9905A × 027(6) (20.39g), 248A × 001(6) (17.26g), 9904A × 027(4) (17.02g) and 248A × 038(2) (16.53g) (Table 4). The lowest yield was recorded in the progeny line 206A × 37(1) (6.96g). Ali *et al.* (2003) and Aytac and Kinaci (2009) also reported similar results for seed yield per plant in rapeseed.

### Conclusion

From the plant and pollen fertility test, 11 testcross progenies were selected as the source of restorer genes and designated as restorer (R) lines. Among the progeny lines, 248A × 001(6), 248A × 017, 248A × 020(6), 248A × 022, 9904A × 027(4) and 9905A × 027(6) gave maximum benefits of selection for seed yield per plant as well as other agronomic traits. These lines could be utilized as restorer

(R) lines in the hybrid variety development in *Brassica napus* L.

### Author's Contribution

A.K.M. Aminul Islam designed the research plan; F.M. Era and N.K. Chowdhury performed experimental works, collected the required data & analysed the data; F.M. Era and N.K. Chowdhury prepared the manuscript. A.K.M. Aminul critical revised and finalized the manuscript. Final form of manuscript was approved by all authors.

### Conflict of Interest

The authors declare that there is no conflict of interest with present publication.

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