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RESYNTHESIS OF NEW R LINES IN *Brassica napus* L.

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Abstract

Identification of male fertility restorer genotypes for rapeseed CMS lines towards hybrid development in spring habit rapeseed (*Brassica napus* L.) adapted for short day winter season was studied. The experiment was conducted at the experimental farm and laboratory of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Salna, Gazipur during October, 2008 to March, 2011. An exotic CMS-based F_1 hybrid of rapeseed was selfed to get F_2 generation with a view to resynthesizing restorer line. As a result a restorer line for Nap248A Z_1 and Nap248A Z_2 cytoplasmic male sterile lines was identified in the F_3 generation of the exotic F_1 rapeseed hybrid which appears as the first case so far reported as achievement in Bangladesh in this regard. Genetic analysis further revealed fertility restoration for Nap248A Z_1 and Nap248A Z_2 cytoplasmic male sterility was controlled by a single dominant nuclear gene as a simple genetic phenomenon.

Keywords: Fertility restorer, *Brassica napus* L., dominant gene.

Introduction

Rapeseed (*Brassica napus* L.) is a major source of edible or industrial oil. *B. napus* varieties have high seed and oil productivity with bold seeds. High yield potential of *B. napus* is mainly due to elongate flower raceme with moderate number of large silique accommodating more number of bold seeds and also due to higher number of plants that can be accommodated per unit area. The yield of rapeseed and mustard is generally low in Bangladesh as compared with the world average. The main problems for this low yield are the use of low yielding local indigenous cultivars, unavailability of locally developed hybrids and low management practices. The present yield of mustard in Bangladesh is 867 kg/ha which is far below the level attained in the developed countries (1907 kg/ha) of the world (FAO, 2011).

Cytoplasmic male sterility is a maternally controlled mechanism which causes failure in pollen formation. Several CMS systems such as pol- (Fu, 1981), ogu- (Ogura, 1968), nap- (Thompson, 1972; Shiga and Baba, 1973) and tour-CMS (Rawat and Anand, 1979; Sodhi *et al*., 1994; Daniell *et al*., 2004) have been developed in rapeseed. CMS-based hybrid rapeseed cultivars have been grown

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on a commercial scale in China. But very few research works have been done in Bangladesh to increase yield and production through exploitation of heterosis in rapeseed. Utilization of heterosis in developing hybrid cultivars could lead to increased productivity of this crop. However, some rapeseed CMS systems have drawbacks such as sensitivity of sterility to low or high temperature, inconsistency of hybridity, difficulty in finding restorer genes and poor restoration of fertility.

A- and B-lines of *B. napus* were available in the department of Genetics and Plant Breeding, Bangabandhu Sheikh Mujipur Rahman Agricultural University. But no complete restorer (R-) lines of aforesaid A-lines have been developed in Bangladesh. So, identification of restorer lines may open the scope of hybrid variety development of rapeseed in Bangladesh. Identification of restorer lines having different cytosources is a prerequisite of hybrid variety development program. Investigations leading to identification of restorer lines and development of new restorer lines in *B. napus* have a bright scope for development of commercial hybrid varieties. Considering the scope of work in this line, a new programme of hybrid rapeseed breeding was initiated in the 2008. The present investigation was undertaken with the following objectives: 1) To identify restorer (R) lines for *napus* CMS systems (Z_1 and Z_2), 2) To develop new restorer (R) lines from Chinese *B. napus* hybrid and 3) To determine the inheritance of fertility restorer gene.

Materials and Method

The research work was conducted at the experimental farm, Department of Genetics and Plant Breeding, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Salna, Gazipur during the period from October 2008 to March, 2011. The seed of CMS-based rapeseed hybrid were collected from China. The CMS line Nap248A Z_1, Nap248A Z_2 and its maintainer Nap248B were received from the Department of Genetics and Plant Breeding, BSMRAU, Salna, Gazipur. The seeds of CMS-based rapeseed hybrid as a source of restorer gene, CMS line Nap248A Z_1 and Nap248A Z_2 and their maintainer were sown in October 2008 to November 2008 in the experimental field. Ten rows of 3m each constituted an experimental unit. The plant spacing was 50 cm × 15 cm. Recommended doses of manures and fertilizers were applied. The exotic hybrid seed of *B. napus* was sown in separate plot in the experimental field on 22 October, 2008 and the seed of the line Nap248A Z_1 and Nap248A Z_2 and their maintainer were sown on 5th November, 2008 in separate plots of the same experimental field. Twenty plants of F_1 hybrid were selected for selfing to develop F_2 generation. The CMS line Nap248A Z_1 and Nap248A Z_2 were pollinated with pollen from CMS-based F_1 hybrid which were known as backcrosses. Removal of sepal and petal from the upper portion of buds of both

Brassica genotypes was done in the evening to expose stigma for pollination and bagged to prevent out-crossing. Hand pollination was carried out in the following morning by dusting pollen from the same F_1 hybrid plant (known as bud selfing). On the other hand, for crossing (Nap248A $Z_{1 \text{ or } 2} \times F_1$), pollen of F_1 was dusted to the stigma of a single plant of Nap248A Z_1 and Nap248A Z_2 each. In all cases, the pollinated flowers were paper bagged for three days to prevent unwanted pollination and tagged carefully. The self (F_2) and cross seeds (Nap248A $Z_1 \times F_1$ and Nap248A $Z_2 \times F_1$) were collected after physiological maturity followed by threshing and drying and preserved in cold storage for next year experimentation. Number of viable pollen grains in ten fields per slide was counted. A drop of 2% aceto-carmine with ferric iron was put on a slide (Yan *et al*., 2009). The anthers were cut into halves and the pollen grains squeezed out and stained for 1 min (Liu *et al*., 2005). Slides were then observed under a light microscope at a magnification of 400×. Viable pollen grains were stained red while the non-viable ones remained pale yellow. The pollen grains of various types were counted and pollen stainability expressed as a percentage. Two anthers from each of three flowers per plant were used for analysis and average number of viable pollen grains per microscopic field was calculated for each plant. Data on five plants were collected and average number of viable pollen grains per microscopic field per plant was calculated. Number of nonviable pollen grains in ten fields per slide was counted. F_2 generation of 20 plants and backcross generation of Nap248A $Z_1 \times F_1$, Nap248A $Z_2 \times F_1$ were grown during November 2009 to March 2010 in the experimental field, Department of Genetics and Plant Breeding, BSMRAU. The seeds of F_2, Nap248A $Z_1 \times F_1$, Nap248A $Z_2 \times F_1$ and Nap248B obtained from previous year experiment were used as plant materials. The 20 F_2 families were sown in 20 non-replicated plots. Each plot consisted of three rows of 3m long each. The row to row and plant to plant distances were 30 and 15 cm, respectively. The two BC populations were grown in separate plots along with their maintainer. Each plot consisted of two rows of 3 m length. The row and plant spacing were 30 and 10 cm, respectively. Seeds of different entries were sown in separate plots in the experimental field on 8th November, 2009. The seedlings emerged out within four days after sowing. Four F_2 families out of thirteen were selected. One plant from each of four F_2 families was selected on the basis of male fertility for self- and cross-pollination. The selected plants of F_2 families were bud-selfed to develop F_3 generation. The CMS line Nap248A Z_1 and Nap248A Z_2 were pollinated with pollen from selected F_2 plants (progeny of CMS-based F_1 hybrid). The resultant progeny were designated as F_1' (Nap248A $Z_1 \times P_1$), F_1' (Nap248A $Z_1 \times P_2$), F_1' (Nap248A $Z_1 \times P_3$), F_1' (Nap248A $Z_1 \times P_4$), F_1' (Nap248A $Z_2 \times P_1$), F_1' (Nap248A $Z_2 \times P_2$), F_1' (Nap248A $Z_2 \times P_3$) and F_1' (Nap248A $Z_2 \times P_4$). Removal of sepal and petal from the upper portion of buds of both *Brassica*

genotypes was done in the evening to expose stigma for pollination and bagged to prevent out-crossing. Hand pollination was carried out in the following morning through dusting pollen of selected F_2 plant (known as bud pollination). On the other hand, for crossing (Nap248A $Z_{1 \text{ or } 2} \times F_2$), pollen of F_2 plant was dusted on the stigma of Nap248A Z_1 and Nap248A Z_2. In all cases, the pollinated flowers were paper bagged for three days to prevent unwanted pollination and tagged carefully. The self (F_3) and cross (Nap248A $Z_1 \times F_2$ and Nap248A $Z_2 \times F_2$) seeds were collected after physiological maturity followed by threshing and drying and preserved in cold storage for next year experimentation. Data were collected from each of 20 F_2 families on whole plot basis on the following aspects: counting of total number of plants in each plot, counting of fertile plant, counting of sterile plant and counting of viable and non-viable pollen. The recorded data for different characters were analyzed statistically using Microsoft Excel worksheet functions. χ^2-tests were performed according to the formula described by Zaman *et al*. (1982). Eight F_1' and four F_3 families were grown during November 2010 to March 2011 in the experimental field, Dept. of Genetics and Plant Breeding, BSMRAU. Seeds of eight F_1' (Spring × Winter) and four F_3 families obtained from previous year experiment were used as plant materials. The four F_3 families were sown on four non-replicated plots. Each plot consisted of two rows of 3m long each. The row to row and plant to plant distances were 50 and 15 cm, respectively. The eight hybrids F_1' (Nap248A $Z_1 \times P_1$), F_1' (Nap248A $Z_1 \times P_2$), F_1' (Nap248A $Z_1 \times P_3$), F_1' (Nap248A $Z_1 \times P_4$), F_1' (Nap248A $Z_2 \times P_1$), F_1' (Nap248A $Z_2 \times P_2$), F_1' (Nap248A $Z_2 \times P_3$) and F_1' (Nap248A $Z_2 \times P_4$) were grown in separate plots. Each plot consisted of two rows. The row and plant spacing were 50 and 15 cm, respectively. Seeds of different entries were sown in separate plots in the experimental field on 5 November, 2010. The seedlings emerged out within four days after sowing. Data were collected from each of eight hybrids (F_1') populations on whole plot basis on the following aspects: counting of total number of plants in each plot, counting of fertile plants and counting of sterile plants. The recorded data for different characters were analyzed statistically using Microsoft Excel worksheet functions. χ^2-tests were performed following Bagdonavicius (2011).

Results and Discussion

The detailed results of this study have been presented in tables and figures and discussed as follows: The different populations used in this experiment were divided into two groups on the basis male sterility and male fertility. Ten floral traits were studied for determination of male fertility and male sterility. The male sterile plants produced crinkled petals and short stamens bearing small conical anthers of pale green colour. The pollen grains of these plants were shriveled

and did not stain with aceto-carmine (Fig. 1a and Fig. 1b). Singh (2006) also reported narrow petals add non-viable pollen grains in CMS *B. juncea*. Whereas the fertile plants produced normal petals and stamens bearing yellow coloured normal anthers. The pollen grains of these plants were round and stained with aceto-carmine (Fig. 1.c, Fig. 1.d and Fig. 1.e). Mean data on floral traits of fertile and sterile genotypes found in different populations are presented in Tables 1 and 2, respectively and described below:

Petal width (mm), petal length (mm), long filament length (mm), short filament length mm), style length (mm), stigma diameter (mm), anther length (mm), anther width (mm), total number of pollen/field, viable pollen and non-viable pollen in sterile CMS genotypes were lower than male fertile genotypes found in F_2 segregating and backcross population (Saha *et al*., 2011). Petal width ranged from 3.31mm and 4.95mm. The highest petal width was observed in Nap248A $Z_1 \times F_1R$ (sterile) and the lowest in F_2 (sterile) (Table 1). The highest petal length exhibited by Nap248A $Z_1 \times F_1R$ (sterile) (9.05mm) and the lowest in F_2 (sterile) (7.65mm). The highest long filament length was the highest in Nap248A $Z_2 \times P_4R$ (sterile) (2.78mm) followed by Nap248A $Z_2 \times P_3R$ (sterile) and the lowest in F_2 (sterile) (1.52mm). Nap248A $Z_2 \times F_1R$ (sterile) showed the highest short filament length (1.22mm) followed by Nap248A $Z_2 \times P_3R$ (sterile) and F_2 population showed the lowest (0.73mm) for this trait. Style length was the highest in Nap248A $Z_2 \times P_3R$ (sterile) (10.10mm) followed by F_2 (sterile) and it was the lowest in Nap248 A Z_1 (7.02mm). The genotype Nap248A $Z_2 \times F_1R$ (sterile) exhibited the highest stigma diameter (1.13mm) followed by Nap248A $Z_1 \times F_1R$ (sterile) and Nap248 A Z_1 the lowest. Anther length ranged from 1.70 to 1.94mm. The highest value for anther length was showed by the genotype Nap248A $Z_2 \times P_3R$ (sterile) followed by Nap248A Z_2 and the lowest by Nap248 A Z_1. Anther width ranged from 0.73 to 0.86mm. Saha *et al*. (2011) also reported low anther length and breadth in $CMSZ_1$ and $CMSZ_2$ lines of *B. napus*. Total number of pollen per microscopic field at 400× magnification ranged from 4.0 to 5.3. The highest % of viable pollen was observed in Nap248A $Z_1 \times F_1R$ (sterile) (60.38%) followed by Nap248A $Z_1 \times P_4R$ (sterile) and the lowest value for this trait was 0.00% showed by the genotype F_2 (sterile). F_2 (sterile) population showed the highest % of nonviable pollen (100%) followed by Nap248A $Z_2 \times P_3R$ (sterile). However, Nap248A Z_1 showed the lowest % of nonviable pollen (30.95%). The average petal width (mm), petal length (mm), long filament length (mm), short filament length (mm), style length (mm), stigma diameter (mm), anther length (mm), anther width (mm), total number of pollen/field, % viable pollen and non-viable pollen in CMS genotypes were found as 4.43, 8.26, 2.15, 1.08, 8.72, 1.11, 1.84, 0.77, 4.4, 43.99% and 56.01%, respectively.

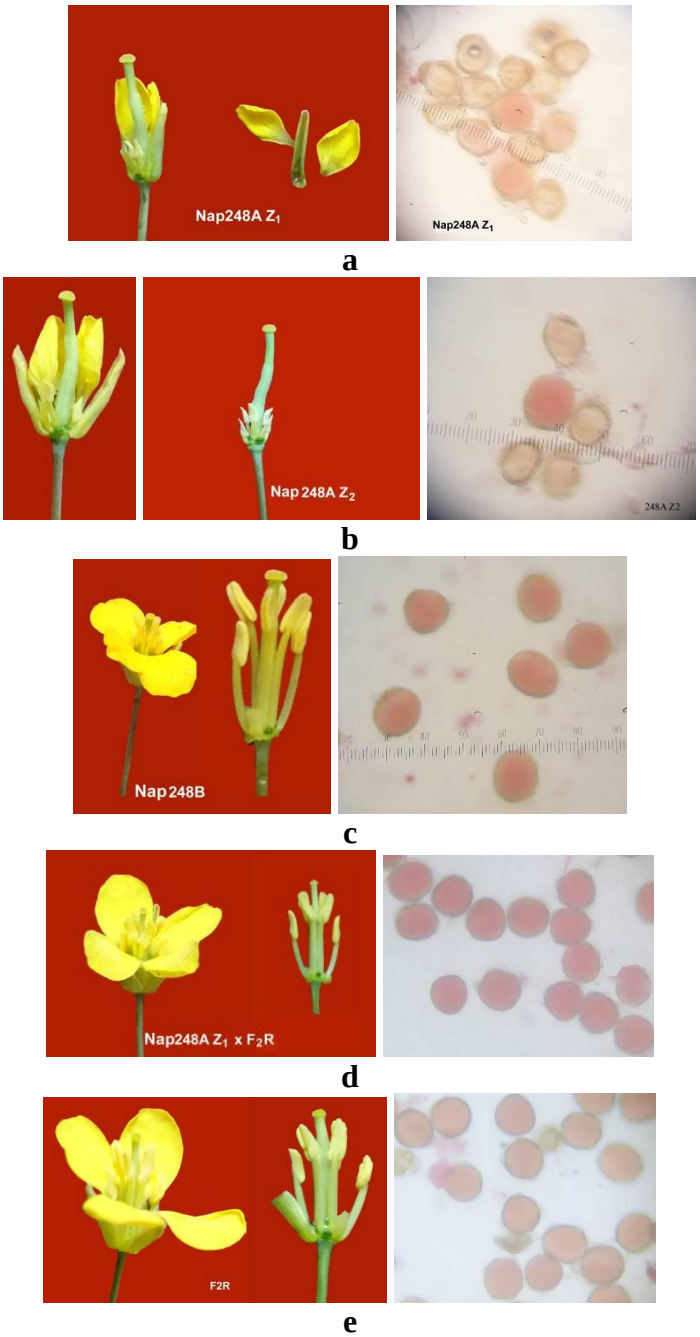

Fig. 1. (a-e) Stamen and anther morphology and pollen grain

a) Nap248A Z_1 (*B. napus* L.), b) Nap248A Z_2 (*B. napus* L.), c) Nap248B (*B. napus* L.), d) Nap248A $Z_1 \times F_2R$ (*B. napus* L.) e) F_2R (*B. napus* L.)

The performance of male fertile genotypes was found extremely high compared to that of sterile ones for all studied floral traits except % nonviable pollen. It might be due to the presence of "Rf" genes in the nucleus which suppressed the activity of extranuclear gene present in fertility restored genotypes. The genotype F_2 (fertile), a product of self-fertilization in exotic F_1s, was found to produce the widest (8.78mm) petal whereas the narrowest (7.25mm) petal was found in the genotype Nap248A $Z_2 \times F_1R$ (fertile) (Table 2). The minimum petal length was produced by the genotype Nap248A $Z_2 \times F_1R$ (fertile) (12.7mm) and maximum petal length was produced by F_1R (fertile) (14.65mm). The hybrid population F_1R (fertile) produced the longest (14.65mm) petal followed by F_2 (fertile) and Nap248A $Z_2 \times F_1R$ (fertile) produced the shortest (12.7mm) petal. Similar trend was observed in case of long filament length and short filament length. The long filament length ranged from 5.50 to 10.80mm while the short filament length ranged from 4.40 to 7.55mm. The genotype Nap248A $Z_2 \times F_1R$ (fertile) produced the longest (13.35mm) style followed by Nap248A $Z_2 \times P_1R$ (fertile) and the genotype Nap248A $Z_1 \times F_1R$ (fertile) produced the shortest style. The highest (1.77mm) stigma diameter was found in the genotype Nap248A $Z_2 \times F_1R$ (fertile) and the lowest (1.61mm) was found in Nap248A $Z_1 \times F_1R$ (fertile). The longest (4.90mm) anther was produced by the hybrid F_1R (fertile) followed by F_2 (fertile) and the shortest (4.58mm) anther was produced by Nap248A $Z_2 \times P_3R$ (fertile). Anther width ranged from 1.50 to 1.60mm. The highest (34.30) total number of pollen grain per microscopic field at 400× magnification was observed in case of genotype F_1R (fertile) followed by the plants obtained in segregating F_2 (fertile) population and the lowest (22.10) was in the genotype Nap248A $Z_2 \times P_1R$ (fertile) and this trend was similar to the trait short filament length. Nearly similar trend was observed in case of % viable pollen grain and % nonviable pollen grain. However, % of viable pollen grain was found the highest (89.13%) in Nap248A $Z_1 \times F_1R$ and Nap248A $Z_1 \times P_3R$ (fertile) and it was found the lowest (79.88%) in the hybrid F_1R (fertile). The % of nonviable pollen grain ranged from 10.87 to 20.12%.

The mean values for petal width indicated that the petal width of fertile genotypes were 70.65% higher than sterile genotypes (Tables 1 and 2). Likewise, all other floral traits studied in this investigation showed higher per cent increase in fertile genotypes over sterile genotypes except % of nonviable pollen. The fertile genotypes showed the highest per cent (486.36%) increase over sterile genotypes for total number of pollen grain per microscopic field followed by the short filament length (366.67%) and the long filament length (200.93%) and the lowest per cent increase (48.85%) over sterile genotype was observed for the style length. However, % nonviable pollen grain decreased by -75.98% in the fertile genotypes over the sterile ones.

Table 1. Floral traits of sterile rapeseed genotypes of different populations/ generations

Genotype	Petal width (mm)	Petal length (mm)	Long filament length (mm)	Short filament length (mm)	Style length (mm)	Stigma diameter (mm)	Anther length (mm)	Anther width (mm)	Total number of pollen /field	% viable pollen	% nonviable pollen	Growing season	Remarks
Nap248 A Z_1	4.82	8.91	1.86	1.13	7.02	1.08	1.7	0.75	4.2	69.05	30.95	Rabi, 2008-09	Sterile
Nap248A Z_2	4.68	8.04	2.68	1.19	10.08	1.1	1.92	0.75	4	35.00	65.00	Rabi, 2008-09	,,
F_2	3.31	7.65	1.52	0.73	10.1	1.13	1.9	0.86	4.5	0.00	100.00	Rabi, 2009-10	,,
Nap248A Z_1 × F_1R	4.95	9.05	1.85	1.2	7.55	1.13	1.8	0.81	5.3	60.38	39.62	Rabi, 2010-11	,,
Nap248A Z_2 × F_1R	4.7	8.14	2.67	1.22	9.53	1.13	1.86	0.74	5	48.00	52.00	Rabi, 2010-11	,,
Nap248A Z_1 × P_4R	4.85	8.95	1.82	1.16	7.12	1.13	1.82	0.81	4.6	69.57	30.43	Rabi, 2010-11	,,
Nap248A Z_2 × P_3R	4.68	8.11	2.69	1.2	10.1	1.12	1.94	0.73	4.2	33.33	66.67	Rabi, 2010-11	,,
Nap248A Z_2 × P_4R	4.61	7.88	2.78	1.15	9.95	1.1	1.91	0.75	4.1	36.59	63.41	Rabi, 2010-11	,,
Minimum	3.31	7.65	1.52	0.73	7.02	1.08	1.7	0.73	4	0.00	30.43		
Maximum	4.95	9.05	2.78	1.22	10.1	1.13	1.94	0.86	5.3	69.57	100.00		
Mean	4.58	8.34	2.23	1.12	8.93	1.12	1.86	0.78	4.49	43.99	56.01		

Table 2. Floral traits of fertile rapeseed genotypes of different populations/ generations

Genotype	Petal width (mm)	Petal length (mm)	Long filament length (mm)	Short filament length (mm)	Style length (mm)	Stigma diameter (mm)	Anther length (mm)	Anther width (mm)	Total number of pollen /field	%Viable pollen	%Non-viable pollen	Growing season	Remarks
F_1R	8.8	14.65	10.8	7.55	12.9	1.76	4.9	1.56	34.3	79.88	20.12	Rabi, 2008-09	Fertile
F_2	8.78	14.48	10.5	7.48	12.8	1.75	4.88	1.55	33.2	80.42	19.58	Rabi, 2009-10	,,
Nap248A Z_1 × F_1R	7.28	12.9	5.65	4.4	12.2	1.61	4.63	1.5	23	89.13	10.87	Rabi, 2010-11	,,
Nap248A Z_2 × F_1R	7.25	12.7	5.58	4.5	13.35	1.77	4.6	1.6	24.8	87.90	12.10	Rabi, 2010-11	,,
Nap248A Z_1 × P_1R	7.31	12.9	5.51	4.48	13.1	1.71	4.68	1.55	25.8	87.21	12.79	Rabi, 2010-11	,,
Nap248A Z_1 × P_2R	7.3	12.89	5.52	4.49	13.15	1.74	4.65	1.57	24.7	87.85	12.15	Rabi, 2010-11	,,
Nap248A Z_1 × P_3R	7.25	12.78	5.5	4.43	13.06	1.68	4.65	1.52	23	89.13	10.87	Rabi, 2010-11	,,
Nap248A Z_2 × P_1R	7.29	12.85	5.51	4.52	13.2	1.73	4.61	1.55	22.1	88.24	11.76	Rabi, 2010-11	,,
Nap248A Z_2 × P_2R	7.3	12.81	5.53	4.49	13.1	1.74	4.68	1.55	24.8	86.69	13.31	Rabi, 2010-11	,,
Nap248A Z_2 × P_3R	7.32	12.82	5.5	4.53	13	1.77	4.58	1.6	24.3	88.89	11.11	Rabi, 2010-11	,,
Nap248A Z_2 × P_4R	7.3	12.86	5.54	4.52	12.9	1.73	4.65	1.54	24	86.67	13.33	Rabi, 2010-11	,,
Minimum	7.25	12.7	5.5	4.4	12.2	1.61	4.58	1.5	22.1	79.88	10.87		
Maximum	8.8	14.65	10.8	7.55	13.35	1.77	4.9	1.6	34.3	89.13	20.12		
Mean	7.56	13.15	6.47	5.04	12.98	1.73	4.68	1.55	25.8	86.55	13.45		
% increase over sterile genotype	70.65	59.20	200.93	366.67	48.85	55.86	154.35	101.30	486.36	96.75	-75.98		

Table 3. Segregation pattern of fertility and sterility of F_1, F_2, BC and F_1' generations of rapeseed

Generation	Combination	Plants observed			Expected ratio	χ^2	P	Growing season
		Total	Fertile	Sterile				
F_1R	Unknown	100	100	0	1:0	-	-	Rabi, 2008-09
F_1R	unknown	75	75	0	1:0	-	-	Rabi, 2008-09
F_2R	$F_1R \times F_1R$	578	430	148	3:1	0.11	0.74	Rabi, 2009-10
BC_1	Nap248A $Z_1 \times F_1R$	45	24	21	1:1	0.20	0.65	Rabi, 2010-11
BC_1	Nap248A $Z_2 \times F_1R$	42	22	20	1:1	0.10	0.76	Rabi, 2010-11
F_1' (S × W)	Nap248A $Z_1 \times P_1R$	35	35	0	1:0	-	-	Rabi, 2010-11
F_1' (S × W)	Nap248A $Z_1 \times P_2R$	33	33	0	1:0	-	-	Rabi, 2010-11
F_1' (S × W)	Nap248A $Z_1 \times P_3R$	34	18	16	1:1	0.12	0.73	Rabi, 2010-11
F_1' (S × W)	Nap248A $Z_1 \times P_4R$	35	17	18	1:1	0.03	0.87	Rabi, 2010-11
F_1' (S × W)	Nap248A $Z_2 \times P_1R$	38	38	0	1:0	-	-	Rabi, 2010-11
F_1' (S × W)	Nap248A $Z_2 \times P_2R$	36	36	0	1:0	-	-	Rabi, 2010-11
F_1' (S × W)	Nap248A $Z_2 \times P_3R$	36	19	17	1:1	0.11	0.74	Rabi, 2010-11
F_1' (S × W)	Nap248A $Z_2 \times P_4R^2$	39	21	18	1:1	0.23	0.63	Rabi, 2010-11

P_1, P_2, P_3 and P_4 stand for a selected male fertile plant in four different F_2 families, respectively. One plant was taken from each family.

F_1' = Hybrid between spring and winter type rapeseed. S= Spring, W = Winter

The F_1 plants grown from exotic seed were examined for male fertility and male sterility. The floral traits clearly indicated that all the plants were male fertile (Table 2). It was confirmed from the pollen viability test of the F_1 plants where the ratios of male fertile to male sterile plants were 1:0 (Table 3). Four hundred thirty plants were found male fertile out of a total of 578 plants in F_2 generation. The backcross Nap248A $Z_1 \times F_1R$ showed 24 male fertile and 21 male sterile plants out of 45 plants. Likewise Nap248A $Z_2 \times F_1R$ showed 22 male fertile and 20 male sterile out of 42 plants. The male fertile plants showed floral features typical of Nap248B, the maintainer of Nap248A Z_1 and Nap248A Z_2 (Fig. 1.c and Fig. 1.e). The pollen grains were not shriveled and normally stained with aceto-carmine while the male sterile plants showed floral features typical of Nap248A Z_1 and Nap248A Z_2 i.e. crinkled petals and short stamens bearing small conical anthers. The pollen grains were shriveled and did not stain with aceto-carmine (Fig. 1.a and Fig. 1.b). Nap248A $Z_1 \times P_1R$, Nap248A $Z_1 \times P_2R$, Nap248A $Z_2 \times P_1R$ and Nap248A $Z_2 \times P_2R$ hybrid populations produces all male fertile plants while Nap248A $Z_1 \times P_3R$, Nap248A $Z_1 \times P_4R$, Nap248A $Z_2 \times P_3R$ and Nap248A $Z_2 \times P_4R$ populations produces a mixture of male fertile and male sterile plants (Table 3).

The ratios of male fertile to male sterile plants in all F_1 generations were tested with χ^2 statistic. The values of P for χ^2 test of fertile:sterile ratio in case of F_2 generation, backcross population and F_1 generation indicated that the segregation of male fertility trait follows Mendelian monogenic inheritance and also the plant number 1 (designated as P_1R) and the plant number 2 (designated as P_2R) were homozygous dominant for male fertility restorer gene (Rf). Thus the selfed progeny of P_1R (the F_3 generation) and P_2R (the F_3 generation) were identified as new male fertility restorer lines. These findings are in accordance with that of Liu *et al*. (2005) that fertility restoration for 681A cytoplasmic male sterility was controlled by a single dominant nuclear gene which might originate from *B. juncea*. Verma *et al*. (2000) stated that restorer gene for polima CMS in *B. campestris* was controlled by a single dominant gene.

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