INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 9, No. 3, p. 177-184, 2016

RESEARCH PAPER OPEN ACCESS

Genetic diversity analysis in f_5 population of *Brassica napus* L.

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Key words: Genetic diversity, F_5 population, *Brassica napus*

http://dx.doi.org/10.12692/ijb/9.3.177-184 Article published on September 30, 2016

Abstract

A field experiment was conducted with 66 F_5 genotypes of *Brassica napus* L. at the experimental field of Sher-e-Bangla Agricultural University, Dhaka to study the genetic diversity during November 2013 to February 2014. The genotypes were found significantly variable for most of the characters. The genotypes were grouped into six clusters. The highest inter cluster distance was observed between cluster IV and VI and the maximum intra cluster distance was found in cluster V. Considering group distance and other agronomic performance genotypes G12, G14, G15, G16, G17, G22 and G24 might be suggested for future hybridization program.

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Introduction

Brassica napus L is an important oil seed crop which belongs to family *Brassicaceae*. *Brassica* is a genus within the Brassicaceae (Cruciferae), commonly known as the mustard family. This family contains 375 genera and 3200 species includes crops, ornamental plants, and many weeds. The members of the genus are collectively known as cruciferous vegetables, cabbages, or mustards etc. Common types of brassica used for food include cabbage, cauliflower, broccoli, brussel sprouts, and some types of seeds (Wikipedia, 2013). It is ranked as the third most important oilseed crop after soybean and palm. Mustard and rapeseed seeds contain 42% oil, 25% protein (Khaleque, 1985). In addition, its meal has 38-40% protein that has a complete profile of amino acids including lysine, methionine and cystine.

The toxic content in *Brassica napus* are erucic acid and glucosinolate (Rashid, 2013). The oil is mainly used as edible product. Oil and fat are not only the source of energy (9-k.cal.g -1) but also contain fat-soluble vitamins A, D, E and K. Brassica vegetables are full of indole-3-carbinol, a compound which enhances DNA repair in cells and tissues and appears to block the growth regarding cancer tissues (BBC News, 2006 and Science Daily, 2010).

Both *Brassica napus* and *Brassica juncea* have remained one of the major sources of oil in the subcontinent for centuries. *Brassica napus* is sparingly grown for young leaves used as pot herb; more generally grown as forage for livestock feed, and as source of rapeseed oil. Rape oil used in food industry, as an illuminant and lubricant, and for soap manufacture. Residual rapeseed cake, though low in food value, used as livestock feed.

Rapeseed oil has potential market in detergent lubrication oils, emulsifying agents, polyamide fibers, and resins, and as a vegetable wax substitute.

According to Mondal *et al.*, 2001, oil crops produce 0.16 million tons of edible oil every year as against the total requirements of 0.5 million tons for a population of 130 million in Bangladesh.

The shortage of edible oil has become a chronic problem for the nation. To fulfill the requirement, the country has to import edible oils at the cost of huge amount of foreign exchange. The major activities of plant breeding are building up a gene pool of variable germplasm, selection of individual from the gene pool and utilization of selected individual to evolve a superior variety (Kempthorne, 1957).

Genetic diversity refers to sum total of genetic variations found in a species or population. It is a prerequisite for the development of improved cultivars with wider adaptability and broad genetic base. Diversity analysis greatly helps the breeder in identification and proper choice of parents for specific breeding objectives.

To realize heterosis, genetically divergent parents are generally considered to be useful. In such crosses more variability could be expected in the resulting segregating progenies (Joseph *et al.,* 1999). Precise information about the extent of genetic divergence on characters used for discrimination among the population is crucial in any crop improvement program, because selection of plants based on genetic divergence has become successful in several crops (Ananda and Rawat, 1984; De *et al.,* 1988).

Materials and methods

Location and experimental design

The experiment was conducted at the experimental field of Sher-e-Bangla Agricultural University, Dhaka – 1207 during November 2013 to February 2014. Field lay out was done after final land preparation. The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications.

Plant material

The healthy seeds of sixty six F_5 of *Brassica napus* collected from the Dept. of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University, which were used as experimental materials. The materials used in that experiment is shown in Table 1.

Table 1. Materials used for the experiment.

Gentypes	F5 Populations	Source
G1	Nap 108 × Nap 9901, P_1	SAU
G2	Nap 108 × Nap 9901, P_2	SAU
G3	Nap 108 × Nap 9901, P_3	SAU
G4	Nap 108 × Nap 9901, P_4	SAU
G5	Nap 9901× Nap 0130, P_1	SAU
G6	Nap 9901× Nap 0130, P_2	SAU
G7	Nap 9905 × Nap 108, P_1	SAU
G8	Nap 9905 × Nap 108, P_2	SAU
G9	Nap 9905 × Nap 108, P_3	SAU
G10	Nap 9905 × Nap 205, P_1	SAU
G11	Nap 9905 × Nap 205, P_2	SAU
G12	Nap 9906 × Nap 2066, P_1	SAU
G13	Nap 9906 × Nap 2066, P_2	SAU
G14	Nap 9906 × Nap 2066, P_3	SAU
G15	Nap 205× Nap 0130, P_1	SAU
G16	Nap 205× Nap 0130, P_2	SAU
G17	Nap 205× Nap 0130, P_3	SAU
G18	Nap 205× Nap 0130, P_4	SAU
G19	Nap 9905 × Nap 0130, P_1	SAU
G20	Nap 9905 × Nap 0130, P_2	SAU
G21	Nap 9908 × Nap 0130, P_1	SAU
G22	Nap 9908 × Nap 0130, P_2	SAU
G23	Nap 9908 × Nap 0130, P_3	SAU
G24	Nap 9908 × Nap 0130, P_4	SAU
G25	Nap 9908 × Nap 0130, P_5	SAU
G26	Nap 9905 × Nap 9908, P_1	SAU
G27	Nap 9905 × Nap 9906, P_1	SAU
G28	Nap 9905 × Nap 9906, P_2	SAU
G29	Nap 108 × Nap 2066, P_1	SAU
G30	Nap 108 × Nap 2066, P_2	SAU
G31	Nap 108 × Nap 2066, P_3	SAU
G32	Nap 108 × Nap 2066, P_4	SAU
G33	Nap 2066 × Nap 0130, P_1	SAU
G34	Nap 2066 × Nap 0130, P_2	SAU
G35	Nap 9906 × Nap 0130, P_1	SAU
G36	Nap 9906 × Nap 0130, P_2	SAU
G37	Nap 108 × Nap 0130, P_1	SAU
G38	Nap 108 × Nap 0130, P_2	SAU
G39	Nap 108 × Nap 0130, P_3	SAU
G40	Nap 9901 × Nap 205, P_1	SAU
G41	Nap 9901 × Nap 205, P_2	SAU
G42	Nap 9906 × Nap 9901, P_1	SAU
G43	Nap 9906 × Nap 9901, P_2	SAU
G44	Nap 9908 × Nap 2066, P_1	SAU
G45	Nap 9908 × Nap 2066, P_2	SAU
G46	Nap 9908 × Nap 9901, P_1	SAU
G47	Nap 9908 × Nap 9901, P_2	SAU
G48	Nap 9908 × Nap 9901, P_3	SAU
G49	Nap 9908 × Nap 9901, P_4	SAU
G50	Nap 9905 × Nap 9901, P_1	SAU
G51	Nap 9905 × Nap 9901, P_2	SAU
G52	Nap 9901 × Nap 2066, P_1	SAU
G53	Nap 9901 × Nap 2066, P_2	SAU
G54	Nap 9908 × Nap 9906, P_1	SAU
G55	Nap 9908 × Nap 9906, P_2	SAU
G56	Nap 9908 × Nap 9906, P_3	SAU
G57	Nap 9908 × Nap 9906, P_4	SAU
G58	Nap 9906 × Nap 205, P_1	SAU
G59	Nap 9906 × Nap 205, P_2	SAU
G60	Nap 9906 × Nap 205, P_3	SAU
G61	Nap 9906 × Nap 205, P_4	SAU
G62	Nap 2066 × Nap 205, P_1	SAU
G63	Nap 2066 × Nap 205, P_2	SAU
G64	Nap 2066 × Nap 205, P_3	SAU
G65	Nap 2066 × Nap 205, P_4	SAU
G66	Nap 108 × Nap 205, P_1	SAU

Agronomic management

The total area of the experiment was 56m×14m = 784m². Each replication size was 56m×3.5m, and the distance between replication to replication was 1m. The spacing between lines to line was 30cm. Seeds were sown in lines in the experimental plots on 11 November, 2013. The seeds were placed at about 1.5 cm depth in the soil. After sowing the seeds were covered with soil carefully so that no clods were on the seeds. The crop was fertilized at the rate of 10 tons of Cowdung, 250 kg Urea, 175 kg Triple Super Phosphate (TSP), 85 kg Muriate of Potash (MP), 250 kg Gypsum, 3 kg Zinc Oxide and Boron 1 kg per hectare. The half amount of urea, total amount of Cowdung, TSP, MP, Gypsum, Zinc Oxide and Boron was applied during final land preparation. The rest amount of urea was applied as top dressing after 25 days of sowing. All other agronomic management was done as and when necessary in optimum level to maximize the yield.

Data collection

The data were recorded on different characters such as days to 50% flowering, days to maturity, plant height (cm), no. of primary branches per plant, no. of secondary branches per plant, no. of siliqua per plant, no. of seeds per siliqua, siliqua length (cm) thousand seed weight (g) and seed yield per plant (g).

Statistical analysis

The data were analyzed statistically using MSTATC package. Multivariate analysis viz., Principal Component Analysis (PCA), Principal Coordinate Analysis (PCO), Cluster Analysis (CA) was done by using GENSTAT software.

Results and discussion

Principal Component Analysis (PCA)

Principal component analysis was carried out with 66 genotypes of *Brassica*. The computed eigen values for the 10 variables subjected to principal component analysis together with the corresponding proportion and cumulative explained variance are given in Table 1. Following the Proportion of Variance Criterion, three principal components were retained and these are the principal components whose cumulative explained variances were equal to or more than 99%. The principal component analysis resulted in the reduction of the 10 original variables to three independent linear combination, principal component of variables.

These three principal components account for 78.16% of the total variation. The first principal component accounted for 36.15 % of the total variation while principal components two and three accounted for 27.21 % and 14.8 %, respectively (Table 1). Zaman*et al.,* (2010) reported that first three axes accounted for 94.00% of the total variation whereas the first principal components accounted for 81.94%. But Khan (2010) reported that first three principal components accounted for 70.27% of the total variation where the first principal components accounted for 28.65%.

Table 1. Eigen values and yield percent contribution of 10 characters of 66 Genotypes of *Brassica napus* L.

Characters	Eigen values	Percent variation	Cumulative % of Percent variation
Day to 50% flowering	4.145	36.15	36.15
Day to maturity	3.119	27.21	63.36
Plant height (cm)	1.696	14.8	78.16
Number of primary branches per plant	0.987	8.61	86.77
Number of secondary branches per plant	0.757	6.61	93.38
Number of siliqua per plant	0.345	3.01	96.39
Siliqua length (cm)	0.196	1.71	98.1
Number of seed per silique	0.135	1.18	99.28
Thousand seed weight (g)	0.057	0.49	99.77
Seed yield per plant (g)	0.026	0.23	100

Sixty six *Brassica napus* genotypes were grouped into six different clusters non-hierarchical clustering (Table 2). These results confirmed the clustering pattern of the genotypes obtained through principal component analysis. Mahmud *et al.* (2008) reported four clusters; Rawhat and Anad (1981) reported seven clusters; Nath *et al.* (2003) five clusters in *Brassica* species and Begum *et al.* (2007) reported five clusters in linseed. Cluster II had the highest number of (19) genotypes followed by III and IV which had 13 and 12 genotypes, respectively. On the other hand, Cluster V, I and VI had 9, 8 and 5 genotypes respectively (Table 2).

Table 2. Distribution of genotypes in different clusters.

Cluster no.	No. of Genotypes	No. of population	Name of genotypes
I	G2, G3, G5, G42, G47, G48, G49, G55	8	Nap 108 × Nap 9901, P_2; Nap 108 × Nap 9901, P_3; Nap 9901× Nap 0130, P_1; Nap 9906 × Nap 9901, P_1; Nap 9908 × Nap 9901, P_2; Nap 9908 × Nap 9901, P_3; Nap 9908 × Nap 9901, P_4; Nap 9908 × Nap 9906, P_2
II	G6, G8, G10, G13, G16, G17, G19, G21, G23, G27, G28, G29, G30, G31, G36, G41, G43, G45, G60	19	Nap 9901× Nap 0130, P_2; Nap 9905 × Nap 108, P_2; Nap 9905 × Nap 205, P_1; Nap 9906 × Nap 2066, P_2; Nap 205× Nap 0130, P_2; Nap 205× Nap 0130, P_3; Nap 9905 × Nap 0130, P_1; Nap 9908 × Nap 0130, P_1, Nap 9908 × Nap 0130, P_3; Nap 9905 × Nap 9906, P_1; Nap 9905 × Nap 9906, P_2; Nap 108 × Nap 2066, P_1; Nap 108 × Nap 2066, P_2; Nap 108 × Nap 2066, P_3; Nap 9906 × Nap 0130, P_2; Nap 9901 × Nap 205, P_2; Nap 9906 × Nap 9901, P_2; Nap 9908 × Nap 2066, P_2; Nap 9906 × Nap 205, P_3.
III	G4, G9, G11, G20, G25, G26, G33, G34, G40, G44, G53, G59, G61	13	Nap 108 × Nap 9901, P_4; Nap 9905 × Nap 108, P_3; Nap 9905 × Nap 205, P_2; Nap 9905 × Nap 0130, P_2; Nap 9908 × Nap 0130, P_5; Nap 9905 × Nap 9908, P_1; Nap 2066 × Nap 0130, P_1; Nap 2066 × Nap 0130, P_2; Nap 9901 × Nap 205, P_1; Nap 9908 × Nap 2066, P_1; Nap 9901 × Nap 2066, P_2; Nap 9906 × Nap 205, P_2; Nap 9906 × Nap 205, P_4.
IV	G18, G22, G32, G35, G38, G39, G46, G58, G62, G63, G65, G66	12	Nap 205× Nap 0130, P_4; Nap 9908 × Nap 0130, P_2; Nap 108 × Nap 2066, P_4; Nap 9906 × Nap 0130, P_1; Nap 108 × Nap 0130, P_2; Nap 108 × Nap 0130, P_3; Nap 9908 × Nap 9901, P_1; Nap 9906 × Nap 205, P_1; Nap 2066 × Nap 205, P_1; Nap 2066 × Nap 205, P_2; Nap 2066 × Nap 205, P_4; Nap 108 × Nap 205, P_1.
V	G1, G7, G12, G15, G24, G37, G50, G51, G64	9	Nap 108 × Nap 9901, P_1; Nap 9905 × Nap 108, P_1; Nap 9906 × Nap 2066, P_1; Nap 205× Nap 0130, P_1; Nap 9908 × Nap 0130, P_4; Nap 108 × Nap 0130, P_1; Nap 9905 × Nap 9901, P_1; Nap 9905 × Nap 9901, P_2; Nap 2066 × Nap 205, P_3.
VI	G14, G52, G54, G56, G57	5	Nap 9906 × Nap 2066, P_3; Nap 9901 × Nap 2066, P_1; Nap 9908 × Nap 9906, P_1; Nap 9908 × Nap 9906, P_3; Nap 9908 × Nap 9906, P_4.
	Total	66	

The genotypes from cluster VI earned the highest cluster mean value for day to 50% flowering (43.10), days to maturity (69.20), number of siliqua per plant (184.47) and seed yield per plant (7.74), but the lowest cluster mean for number of seeds per siliqua (20.77) and 1000-seed weight (3.16 g), indicates that this cluster could be used as a parent for higher yield. On the other hand Cluster I produced the highest mean for number of primary branch (4.61) and 1000-seed weight (3.62 g), early flowering (40.63 days), short plant height (92.62 cm), short siliqua length (7.76) and number of seed per siliqua (20.77).

The genotypes included in cluster II were highest mean value for plant height (109.67 cm), and number of seed per siliqua (22.49).

Moreover, Cluster III had lower cluster mean for number of primary branch (3.23), number of secondary branch (1.51), siliqua length had higher cluster mean value (8.11), followed by cluster IV (36.67 days) suggested that this cluster composed of lowest number siliqua per plant (100.68) and seed yield (5.94). On the other hand, cluster V showed the early maturity plant (67.39), indicated the genotype of this cluster could be used for future hybridization program for early maturity plant. (Table 3).

Srivastav and Singh (2000) reported that cluster III had the highest number of primary, secondary branches and the highest mean seed yield per plant and cultivars in cluster V with 1000-grain weight.

Table 3. Cluster mean values of 10 different characters of 66 genotypes.

Characters	I	II	III	IV	V	VI
Days to 50% flowering	40.63	40.92	41.77	41.17	41.50	43.10
Days to maturity	68.75	68.42	68.92	68.21	67.39	69.20
Plant Height (cm)	92.62	109.67	109.49	101.81	97.21	108.36
Number of Primary Branches per plant	4.61	3.30	3.23	3.29	3.48	4.09
Number of secondary branches per plant	2.04	1.55	1.51	1.67	1.56	2.26
Number of Siliqua per plant	139.66	151.27	135.33	100.68	122.22	184.47
Siliqua length (cm)	7.76	8.23	8.11	7.82	7.94	7.89
Number of seed per silique	20.77	22.49	21.52	21.36	21.36	20.77
Thousand seed weight (g)	3.62	3.34	3.53	3.48	3.46	3.16
Seed yield per plant (g)	7.02	7.60	6.80	5.94	7.17	7.74

Canonical Variate Analysis (CVA)

Canonical Variate Analysis (CVA) was done to compute the inter-cluster distances. The intra and inter-cluster distance (D^2) values were shown in Table 4. In this experiment, the inter-cluster distances were higher than the intra-cluster distances thus indicating broader genetic diversity among the genotypes of different groups. Islam and Islam (2000) reported that the inter-cluster distances were larger than the intra-cluster distances. Uddin (1994) also reported similar result in mustard.

The highest inter-cluster distance was observed between clusters IV and VI (12.433), followed by between cluster V and VI (9.16), II and VI (7.555), III and VI (7.465), I and VI (7.059), I and VI (6.535), II and VI (5.07), II and V (4.655) and I and VI (4.124). In contrast, the lowest inter-cluster distance was observed between cluster I and VI (3.98), followed by IV and VI (3.49), I and VI (3.109), III and VI (2.775), and II and VI (2.424). However, the maximum inter-cluster distance was observed between the clusters IV and VI (12.433) indicating genotypes from these two clusters, if involved in hybridization may produce a wide spectrum of segregating population.

Dhillon *et al.* (1999) mentioned that maximum inter-cluster distance gave desirable segregants for the development of high yielding varieties with quality of oil for seed yield. On the other hand, the maximum intra-cluster distance was found in cluster V (1.0331), which contained of 9 genotypes, while the minimum distance was found in cluster IV (0.3786) that comprises 12 genotypes.

Table 4. Intra (Bold) and inter cluster distances (D^2) for 66 genotypes.

Cluster	I	II	III	IV	V	VI
I	0.6423	4.124	3.98	6.535	3.109	7.059
II		0.4299	2.424	7.555	4.655	5.07
III			0.5522	5.18	2.775	7.465
IV				0.3786	3.49	12.433
V					1.0331	9.16
VI						0.9163

The different multivariate analysis was superimposed in Fig. 1 from which it could be concluded that different multivariate techniques supplemented and confirmed one another. According to scatter diagram all the genotypes were apparently distributed into six clusters. It is assumed that the maximum amount of heterosis will be manifested in cross combination involving the genotypes belonging to most divergent clusters. Furthermore, for a practical plant breeder, the objective is to achieve high-level production in addition to high heterosis. In the present study the maximum distance existence between cluster IV and VI. Goswami*et al.* (2006) found moderate genetic diversity between parents had the good general combining ability effect and high specific combining ability as well as high mean values in F_2 in Indian mustard.

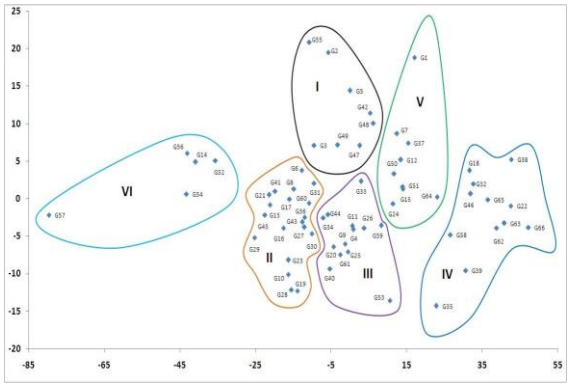

Fig. 1. Scatter diagram of 66 genotypes in *Brassica napus*.

Keeping this in view, it appears that the crosses between genotypes from cluster IV with cluster VI might produce high level of segregating population. The crosses between the genotypes belonging cluster II with cluster IV, cluster II with cluster V, cluster II with cluster VI, and cluster V with cluster VI might produce high heterosis in respect of earliness and yield. So the genotypes belonging to these genotypes have been selected for future hybridization program.

Contribution of traits towards divergence of the genotypes

The latent vectors (Z_1 and Z_2) obtained from principal component analysis (PCA). The important characters responsible for genetic divergence in the axis of differentiation in vector I (Z_1) were number of primary branch (0.2527), thousand seed weight (0.2454), siliqua length (0.0714) and number of seed per siliqua (0.0413). In vector II (Z_2), siliqua length (0.5112), seed yield per plant (0.1983), plant height (0.1728) and days to maturity (0.1139) (Table 4).

Table 4. Relative contributions of the ten characters of 66 varieties to the total Divergence.

Characters	Vector-1	Vector-2
Day to 50% flowering	0.0395	-0.0862
Day to maturity	-0.055	0.1139
Plant Height (cm)	0.0131	0.1728
Number of Primary Branches per plant	0.2527	-0.6676
Number of secondary branches per plant	0.0313	-0.0182
Number of Siliqua per plant	-0.1494	-0.0258
Siliqua length (cm)	0.0714	0.5112
Number of seed per silique	0.0413	-0.177
Thousand seed weight (g)	0.2454	-0.6723
Seed yield per plant (g)	-0.0586	0.1983

The role of plant height and siliqua length in both the vectors was important components for genetic divergence in these materials. On the other hand, the role of number of siliqua per plant had a minor role in the genetic divergence. Islam and Islam (2000) reported days to 50% flowering, plant height, primary branches per plant and number of siliqua per plant contribute maximum in divergence in rapeseed and mustard. Begum *et al.* (2007) reported that branches per plant and number of number of seeds siliquae contributed the maximum towards divergence in the existing linseed germplasm. Choudhary and Joshi (2001) concluded that plant height, secondary branches per plant, days to flowering and 1000-seed weight contributed the maximum towards genetic divergence.

Selection of parents for future hybridization

Selection of genetically diverse parents is the prime task for any plant breeding activities. Therefore, considering the magnitude of genetic distance, contribution of character towards divergence, magnitude of cluster mean and agronomic performance the genotypes G12 (Nap 9906 × Nap 2066, P_1), G14 (Nap 9906 × Nap 2066, P_3), G15 (Nap 205× Nap 0130, P_1), G16 (Nap 205× Nap 0130, P_2), G22 (Nap 9908 × Nap 0130, P_2), G24 (Nap 9908 × Nap 0130, P_4) for short duration and early maturity and G17(Nap 205× Nap 0130, P_3) for higher seed yield. Therefore, considering group distance and agronomic performance the inter-genotypic crosses between G16 and G12, G16 and G15, G16 and G24, G16 and G14, G12 and G14, G15 and G14, G24 and G14, G17 and G22 might be suggested for future hybridization program.

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