Genetic Divergence in Advanced Lines of Oilseed Rape (*Brassica napus* ssp *oleifera* L.)

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Summary

A field experiment was conducted with 22 *Brassica napus* L. advanced lines at the experimental farm of Sher-e-Bangla Agricultural University, Dhaka from November 2008 to March 2009 to asses genetic diversity among advanced lines of *Brassica napus* L.. Different multivariate analysis techniques were used to classify 22 *Brassica napus* genotypes. The genotypes were grouped into four clusters. The highest inter-cluster distance was observed between clusters II and IV whereas the maximum intra-cluster distance was found in cluster II. Therefore, the genotypes belonging to cluster I and cluster II, cluster II and cluster III and cluster III and cluster IV have been selected for future hybridization program. The role of number of secondary branches per plant and number of siliqua per plant in both vectors were important components for genetic divergence in these materials. Considering group distance and other agronomic performance the inter-genotypic crosses between G1 and G2, G2 and G6; G6 and G7; G6 and G8 and G7 and G8 might be suggested for future hybridization program.

Key words

genetic, divergence, cluster, crosses, PCA, CVA, *Brassica napus*

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Received: June 6, 2011 | Accepted: December 4, 2011

Introduction

Oilseed rape (*Brassica napus* ssp *oleifera* L.) is third most important oil crop in the world. It is an important oil seed crop belonging to the family Cruciferae, out of which a total of six species play an important role in agriculture. Scientific interest in rapeseed and its economic importance has lately increased largely due to the use of the high-grade oil for food purposes and as a source for the production of biodiesel. In Bangladesh, rapeseed and mustard of *Brassica* is the major source of edible oil and the share of rapeseed and mustard was 2,53,640 tons, which comes to 52 percent of the total edible oil seed production. Among the oilseed cropped area 74% is covered by the mustard and rapeseed, 17% by sesame and 9% by groundnut (Anonymous, 2000). In Bangladesh, average yield per hectare is 733 kg whereas the world's average production is 1575 kg (Anonymous, 2005). Oilseed crops play a vital role in human diet but the consumption rate of oil in our country is far below than that of balanced diet (6 g oil per day per capita). The oil is mainly used as edible product. Oil and fat are not only the source of energy but they also contain fat-soluble vitamins A, D, E and K. They are more tolerant against *Alternaria* leaf blight and aphid attack than the varieties of *B. campestries* and *B. juncea*. High yield potential of *B. napus* is mainly due to elongate flower raceme with moderate number of large siliqua accommodating more number of bold seeds and also due to higher number of plants that can be accommodated per unit area. The genetic improvement of crops can be accelerated when broad range of genetic diversity is available in the genetic resources. Research on *Brassica* germplasm could enhance the edible oil production and nutritional benefits of these crops. The collection of these genetic resources and the assessment of genetic diversity within and between landraces should have priority for varietal improvement. Variability and genetic diversity are the fundamental law of plant breeding which is a major tool being used in parent selection for efficient hybridization program (Bhatt, 1973). Genetic diversity is one of the criteria of parent selection. It is a prerequisite for an efficient plant breeding program. It can be estimated through biometrical procedures such as Mahalanobis's D^2-statistic and it is possible to choose genetically diverse parents. Keeping the above hypothesis in view the proposed study with advanced lines of *Brassica napus* L. was undertaken.

Material and methods

The advanced lines of *Brassica napus* L. for the experiment were collected from the Department of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University (SAU) and three released varieties collected from Bangladesh Agricultural Research Institute (BARI) as presented in Table 1. The experiment was laid out in Randomized Complete Block Design (RCBD). The field was divided into three blocks; the blocks were sub-divided into 22 plots where genotypes were randomly assigned. The plot size was 3 m length with two rows. Row to row and plant to plant distances were 25 cm and 15 cm, respectively. Seeds were sown in lines in the experimental plots on 12 November, 2008. Adequate soil fertility was censured by applying of Urea, TSP, MP, Gypsum and Borax at 250-170-85-150-5 kg/ha, respectively. Urea was applied by two installments. Total amount of TSP, MP, gypsum and borax along with half of the urea were applied at the time of final land preparation as a basal dose during final land preparation. The second half of the urea was top-dressed at the time of flower initiation. Standard Intercultural operations (such as weeding, thinning, irrigation, pest management, etc.) were done uniformly in all the plots to ensure proper growth condition for the crop. Harvesting was started from February 20, 2009 depending upon the maturity. Observations were recorded on 10 randomly chosen plants from each plot. Data were collected on Days to 50% flowering, Days to 80% maturity, Plant height (cm), Number of primary branches per plant, Number of secondary branches per plant, Siliqua length (cm), Number of siliqua per plant, Number of seeds per siliqua, 1000 seeds weight (g) and Seed yield per plant (g). Multivariate analysis viz., Principal Component Analysis (PCA), Principal Coordinate Analysis (PCO), Cluster Analysis (CLU) were done by using GENSTAT 5 Release 4.1 (PC/Windos NT) software program (Digby et al., 1989).

Table 1. Sources of 22 genotypes of *Brassica napus* L.

Genotypes	Designation	Sources
G1	Nap 9904	SAU
G2	Nap 206	SAU
G3	Nap 0130	SAU
G4	Nap 205	SAU
G5	BS 8	BARI
G6	Nap 2037	SAU
G7	Nap 248	SAU
G8	Nap 179	SAU
G9	Nap 9905	SAU
G10	Nap 9908	SAU
G11	BS 7	BARI
G12	BS 13	BARI
G13	Nap 108	SAU
G14	Nap 2013	SAU
G15	Nap 94006	SAU
G16	Nap 9901	SAU
G17	Nap 2057	SAU
G18	Nap 9906	SAU
G19	Nap 2012	SAU
G20	Nap 2022	SAU
G21	Nap 2001	SAU
G22	Nap 2066	SAU

Results and discussion

Principal Component Analysis (PCA)

The PCA gives Eigen values of principal component axes of coordination of genotypes with the first axes totally accounted for the variation among the genotypes, whereas three of these Eigen values above unity accounted for 68.927% (Table 2). The first two principal axes accounted for 52.934% of the total variation among the characters describing 22 advanced lines of *Brassica napus* genotypes (Figure 1). Belete (2011) reported 91.4% variation contributed by first five principal components through principal component analysis in Ethiopian mustard. A two dimensional chart (Z_1-Z_2) of the genotypes was constructed based on two principal axes. The scatter diagram revealed that there were four apparent clusters. The genotypes were distantly located from each other (Figure 2).

Table 2. Eigen values and percentage of variation in respect of 10 characters in 22 *Brassica napus* L. genotypes

Principal component characters	Eigen values	Percentage of total variation accounted for	Cumulative percentage
Days to 50% flowering	2.870	28.695	28.695
Days to 80% maturity	2.424	24.239	52.934
Plant height (cm)	1.600	15.993	68.927
Number of primary branches per plant	0.992	9.923	78.850
Number of secondary branches per plant	0.716	7.162	86.012
Siliquae length (cm)	0.527	5.265	91.277
Number of seeds per siliqua	0.368	3.678	94.955
Number of siliqua per plant	0.263	2.634	97.589
1000-seed weight (g)	0.164	1.637	99.226
Seed yield per plant (g)	0.077	0.774	100.000

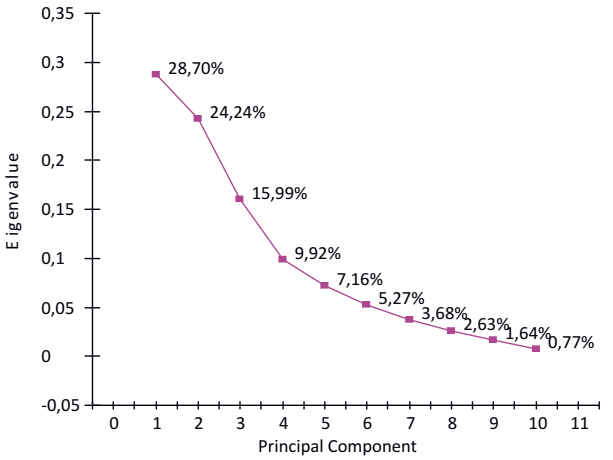

Figure 1. Scree plot constructed for eight principal component

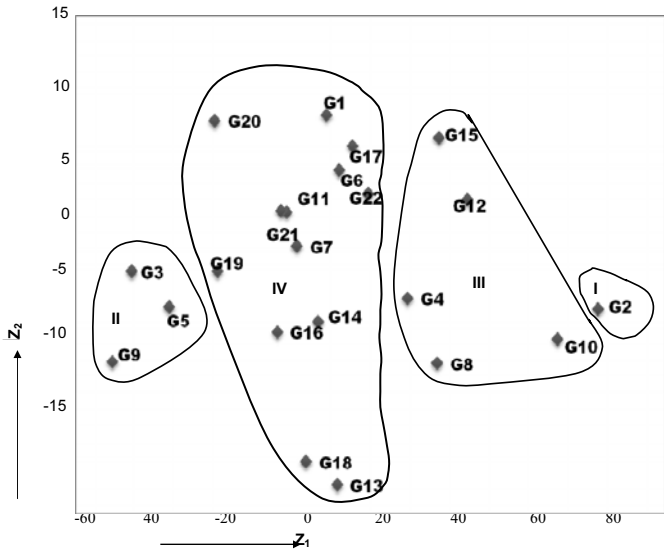

Figure 2. Scatter diagram of 22 *Brassica napus* genotypes based on their principal component scores superimposed with clustering

Non-Hierarchical Clustering

Twenty two *Brassica napus* genotypes were grouped into four different clusters of non-hierarchical clustering (Table 3). 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 IV had the highest number of (13) genotypes followed by III and II that had five and three genotypes, respectively. On the other hand, Cluster I had only one genotype (Table 3). Cluster I had only G2 (Nap 206) whereas Cluster II was composed of G3 (Nap 0130), G5 (BS 8) and G9 (Nap 9905). Furthermore, Cluster III constituted of G4 (Nap 205), G8 (Nap 179), G10 (Nap 9908), G12 (BS 13) and G15 (Nap 94006). Interestingly, cluster IV represents 13 genotypes namely G1 (Nap 9904), G6 (Nap 2037), G7 (Nap 248), G11 (BS 7), G13 (Nap 108), G14 (Nap 2013), G16 (Nap 9901), G17 (Nap 2057), G18 (Nap 9906), G19 (Nap 2012), G20 (Nap 2022), G21 (Nap 2001) and G22 (Nap 2066). According to the cluster means (Table 4), Cluster I showed better performance in case of early maturity, plant height, number of primary branches per plant, number of secondary branches per plant, siliqua length, number of seeds per siliqua, number of siliqua per plant and seed yield per plant. Thus indicates that genotype of this cluster could be used for parent in future hybridization program for higher yield as well as early maturity. The genotypes

Table 3. Distribution of 22 genotypes of *Brassica napus* L. in four clusters

Clusters	No. of genotypes	Description
I	1	G2(Nap 206)
II	3	G3(Nap 0130), G5(BS 8) and G9(Nap 9905)
III	5	G4(Nap 205), G8(Nap 179), G10(Nap 9908), G12(BS 13) and G15(Nap 94006)
IV	13	G1(Nap 9904), G6(Nap 2037), G7(Nap 248), G11(BS 7), G13(Nap 108), G14(Nap 2013), G16(Nap 9901), G17(Nap 2057), G18(Nap 9906), G19(Nap 2012), G20(Nap 2022), G21(Nap 2001) and G22(Nap 2066)

Table 4. Cluster means for 10 characters of 22 *Brassica napus* L. genotypes

Characters	Clusters			
	I	II	III	IV
Days to 50% flowering	38.00	37.22	38.73	37.79
Days to 80% maturity	101.00	102.56	102.40	103.36
Plant height (cm)	106.50	88.83	97.90	90.75
Number of primary branches per plant	6.50	3.70	5.01	4.64
Number of secondary branches per plant	20.60	15.17	14.37	13.02
Siliquae length (cm)	7.41	7.28	7.31	7.39
Number of seeds per siliqua	28.00	25.11	22.35	23.04
Number of siliqua per plant	285.93	176.77	254.30	216.50
1000-seed weight (g)	3.41	3.52	3.74	3.90
Seed yield per plant (g)	10.98	5.27	6.90	6.21

included in cluster II were early flowering, lowest number of primary branches per plant, lowest siliqua length, shortest plants and also lowest seed yield per plant. Moreover, Cluster III had the highest cluster mean for days to 50% flowering followed by Cluster I suggested that this cluster composed of late flowering genotypes. On the other hand, Cluster IV showed the highest cluster mean for days to maturity and 1000-seed weight while moderate cluster mean for siliqua length, number of seeds per siliqua indicating late maturing and coarse seeded genotypes constitute this cluster.

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 5. 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 higher than the intra-cluster distances. Uddin (1994) also reported similar result in mustard. The highest inter-cluster distance was observed between clusters II and IV (24.003), followed by the distance between clusters I and II (14.734), III and IV (13.833) and II and III (10.657). In contrast, the lowest inter-cluster distance was observed between cluster I and III (4.935), followed by I and IV (9.270) (Figure 3). However, the maximum inter-cluster distance was observed between the clusters II and IV (24.003) indicating that 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 II (1.140), which contained of three genotypes, while the minimum distance was found in cluster III (0.476), which was comprised of two genotypes. The different multivariate analyses were superimposed in Figure 3 from which it could be concluded that different multivariate techniques supplemented and confirmed one another.

A two-dimensional scatter diagram was constructed using component I as X-axis and component II as Y-axis, showing the relative position of the genotypes (Figure 2). According to scatter diagram all the genotypes were apparently distributed into four clusters. It is assumed that 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 was between clusters II and IV. 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. Main and Bahl (1989) reported that the parents separated by D^2 values of moderate magnitude generally showed higher heterosis. Keeping this in view, it appears that the crosses between the genotypes belonging to clusters I and II, clusters II and III, and clusters III and IV might produce high heterosis in respect of yield, earliness, tallness, and higher number of siliqua per plant. Also the crosses between genotypes from Cluster II with Cluster IV might produce high level of segregating population. So the genotypes belonging to clusters I and II, clusters II and III and clusters III and IV have been selected for future hybridization program.

Table 5. Average intra (Bold) and inter cluster distances (D^2) for 22 *Brassica napus* L. genotypes

Cluster	I	II	III	IV
I	0.544			
II	14.734	1.140		
III	4.935	10.657	0.476	
IV	9.270	24.003	13.833	0.697

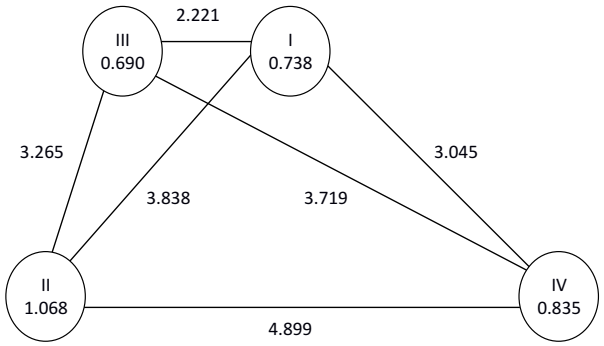

Figure 3. Diagram showing intra and inter cluster distances ($\sqrt{D^2}$) of 22 *Brassica napus* genotypes

Contribution of traits towards divergence of the genotypes

The latent vectors (Z_1 and Z_2) obtained from principal component analysis (PCA) are shown in Figure 4. The important characters responsible for genetic divergence in the axis of differentiation in vector I (Z_1) were days to 50% flowering (0.02385), plant height (0.16277), number of primary branches per plant (0.01645), number of secondary branches per plant (0.04513), number of siliqua per plant (0.98431), 1000-seed weight (0.00414), and seed yield per plant (0.02984). In vector II (Z_2), number of secondary branches per plant (0.22598), siliqua length (0.00110) and number of siliqua per plant (0.14651) were important. The role of number of secondary branches per plant and number of siliqua per plant in both vectors were important components for genetic divergence in these materials. Similar result was found by Dar et al. (2010) for number of siliqua per plant. On the other hand, the role of days to 80% maturity and number of seeds per siliqua had a minor role in the genetic divergence. Islam and Islam (2000) reported that days to 50% flowering, plant height, primary branches per plant, and number of siliqua per plant contributed maximum in divergence in rapeseed and mustard. Begum *et al.* (2007) reported that branches per plant and number of seeds per 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 maximum towards genetic divergence.

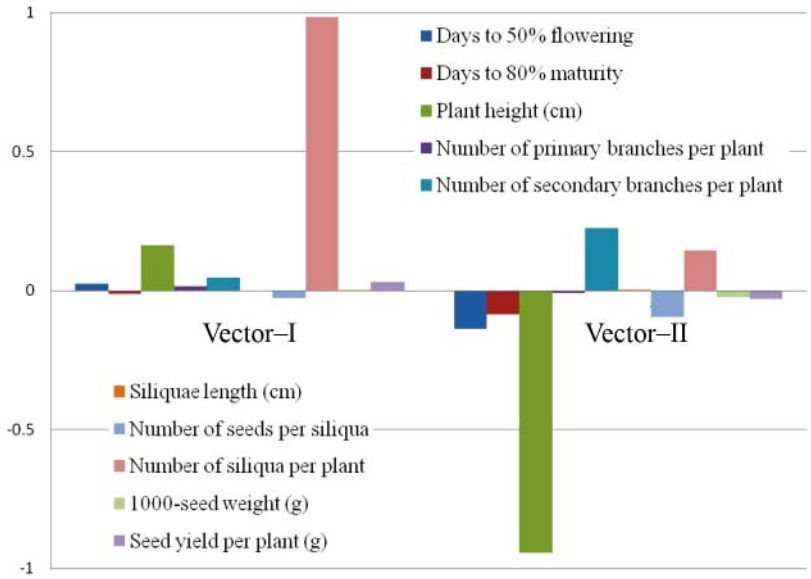

Figure 4. Latent vectors for 10 characters of 22 *Brassica napus* L. genotypes

Conclusion

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 following genotypes were promising: G1 for higher number of seeds per siliqua and long siliqua, G2 for higher number of secondary branches per plant, tallness, higher number of siliqua per plant and seed yield per plant, G6 for earliness, G7 for larger seeds, and G8 for higher yield per plant. Therefore considering group distance and other agronomic performance the inter-genotypic crosses between G1 and G2; G2 and G6; G6 and G7; G6 and G8, and G7 and G8 might be suggested for future hybridization program.

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