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GENETIC DIVERGENCE IN *Brassica rapa* L.

S. NAZNIN[1], M. A. KAWOCHAR[2], S. SULTANA[3]
N. ZEBA[4] AND S. R. BHUIYAN[5]

Abstract

Different multivariate analysis techniques were used to classify 33 *Brassica rapa* L. genotypes. The genotypes were grouped into five clusters. Cluster I contained the maximum number of genotypes. Cluster III earned the highest cluster mean value for number of primary branches/plant, number of secondary branches/plant, number of siliquae/plant and seed yield/plant. Therefore, more emphasis can be given on cluster III for selecting genotypes as parents for the hybridization program. The highest intra-cluster distance (3.822) was found in cluster I and the lowest (0.000) in cluster V. The highest inter-cluster distance (15.705) was observed between clusters III and V showing wide diversity among the groups. Principal component analysis (PCA) showed that the first three principal components accounted for 99.38 % of the total variation observed. Analysis of the factor loading of the component character indicated that the characters number of siliquae/plant, plant height and days to maturity were found responsible for genetic divergence. The role of number of siliquae/plant in both the vectors was important components for genetic divergence in these materials. Among the possible 528 combinations, the highest inter-genotypic distance (1.5975) was observed between G-27 (BARI sarisha-9 x BARI sarisha-6 S-62) and G-31 (BARI sarisha-15). Considering group and inter-genotypic distance, cluster mean, contribution of different characters towards the total divergence and other agronomic performance the genotypes G-19 (BARI sarisha-6 x TORI-7 S-48), G-20 (F_6 x BARI sarisha-9 S-52), G-27 and G-30 (BARI sarisha-6 x TORI-7 S-37) from cluster III; G-26 (F_6 x BARI sarisha-9 S-15) and G-31 from cluster IV and G-33 (BARI sarisha-6) from cluster V would be considered as better parents for future hybridization program.

Keywords: Genetic diversity, principal component analysis, principal coordinate analysis, cluster analysis, *Brassica rapa* L.

Introduction

Brassica rapa L. commonly known as field mustard or turnip mustard belonging to the family Brassicaceae. The seeds of *Brassica rapa* L. contain 42% oil and 25% protein (Khaleque, 1985). It also serves as important source of raw material

[1-3]Scientific Officer (Plant Breeding), Tuber Crops Research Centre, Bangladesh Agricultural Research Institute, Gazipur-1701, [4&5]Professor, Department of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh.

for industrial use such as in making soaps, paints, hair oils, lubricants, textile auxiliaries, pharmaceuticals and so on. It is the third most important oil crop in the world accounting for over 16% of the world's edible oil supply (Anonymous, 2005). It also plays an important role to the total edible oil production in Bangladesh. Among the oil crops grown in Bangladesh *Brassica rapa* L. occupies the first position in respect of area and production. During 2010-11, about 0.252 million hectare of land was under *Brassica rapa* L. and mustard cultivation where produced about 0.246 million tons of seed, and national average yield was 0.977 ton/ha in this country. Total annual edible oil production was about 0.833 million tons which is very low against the requirement of Bangladesh. Bangladesh imported 89.97 thousand tons of edible oil of rapeseed in the year of 2010-11 to meet up the annul requirement, which costs Tk.3718.457 million (BBS, 2011). The main reasons behind these are the use of low yielding local indigenous cultivars, low management practices and reduction of cultivation area. The area for rapeseed and mustard was reduced from 0.3177 million hectare in 2000-01 to 0.2340 million hectare in 2008-09. There was 26.32% reduction in area for this crop (Bhuiyan, 2012). For increasing the production of *Brassica rapa* L., expansion of cultivated area and unit area production are needed. There is a limited scope for horizontal expansion of its cultivation. Thus, to increase the production of *Brassica rapa* L., production per unit area must be increased. Moreover the crop should fit in the cropping pattern. Therefore, high yielding and short duration *Brassica rapa* L. varieties should be developed to fit into the existing cropping pattern (T-amon-mustard-Boro).

Genetic diversity refers to sum total of genetic variations found in a species or population. Existence of genetic diversity is very essential to meet the present and future crop breeding challenges. It is a prerequisite for the development of improved cultivars with wider adaptability and broad genetic base. It can be estimated through biometrical procedures such as Mahalanobis's D^2-statistic and is possible to choose genetically diverse parents. Diversity analysis greatly helps the breeder in identification and proper choice of parents for specific breeding objectives. The selection of potential varieties in a breeding program is based on the knowledge of genetic diversity amongst them. 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). Therefore the present study was undertaken to collect information on

genetic divergence in the genotypes and selection of suitable diverse parents for the utilization in future hybridization program.

Materials and Method

The experiment was conducted at the field of Sher-e-Bangla Agricultural University farm, Dhaka, during rabi, 2011-12. Thirty-three genotypes were used in this experiment where three of them were used as checks. Among the genotypes, thirty were selected from F_9 segregating generation on the basis of their variation in different traits. The experiment was laid out in a Randomized Complete Block Design with three replications. Each plot was 3m long with two rows. The distance of 10 cm between plants, 30cm between rows and 1m between blocks were maintained. Seeds were sown in lines in the experimental plots on 29th October 2011. Recommended doses of fertilizers and standard cultural practices were applied for raising healthy crops. Data were recorded on randomly ten selected plants from each plot and plants were selected from middle to avoid border effect of the plot. Data were recorded on days to maturity, plant height (cm), number of primary branches/plant, number of secondary/branches plant, number of siliquae/plant, siliqua length (cm), number of seeds/siliqua, 1000 seed weight (g) and seed yield/plant (g). All the collected data of the study were subjected to statistical analysis. Multivariate analysis viz., Principal Component Analysis (PCA), Principal Coordinate Analysis (PCO), Canonical Variate Analysis (CVA) and Cluster Analysis were done by using GENSTAT 5 Release 4.1 (PC/Windos NT) software program (Copyright, 1997, Lawes Agricultural Trust, Rothamasted Experimental Station, UK).

Using standardized data, numerical measures of likeness/similarity were computed and distance matrix was constructed using Euclidean Distance Coefficients. Clustering by UPGMA (Unweighted Pair Group of Arithmetic Mean) method was executed (Siopongco *et al.*, 1999). Dendogram was constructed by using the SPSS.16 software. PCO is equivalent to PCA but it is used in calculating inter genotypic distance and intra cluster distance for all possible combination. When the clusters were formed, the average intra-cluster distances for each cluster was calculated by taking possible D^2 values within the member of a cluster obtained from the PCO. CVA was performed to get the inter cluster distances. Principal Components were computed from the correlation matrix (variance-covariance coefficient) and genotype scores were obtained from the first components. Two dimensional scatter diagram was prepared by using principal component score I in X-axis and II in Y-axis. Scree plot is a useful visual aid to determine an appropriate number of principal components and it was

constructed according to Johnson and Wichern, (2008). The factor loadings of characters were used from PCA for identifying the major characters responsible for maximum variability. Contribution of the different morphological characters towards divergence was discussed from the latent vectors of the first two principal components.

Results and Discussion

Cluster Analysis

Thirty three genotypes of *Brassica rapa* L. were grouped into 5 different clusters by applying non-hierarchical cluster using covariance matrix (Table 1). The results were more or less confirmatory with the cluster pattern of the genotypes obtained through Dendogram (Figure 1). Nath *et al.,* (2003) reported 5 clusters which support this result. Rameeh (2013) reported 4 clusters. Two dimensional scatter diagram was prepared by using principal component score I in X-axis and II in Y-axis. The scatter diagram also revealed that there were five apparent clusters. The genotypes were distantly located from each other (Figure 2). Cluster I contained the maximum number of nineteen genotypes followed by cluster II, cluster III and cluster IV having seven, four and two genotypes, respectively and cluster V having only one genotype (Table 1 and Figure 1). Mahmud *et al.,* (2008) and Dhillon *et al.,* (1999) recorded variable number of genotypes in different clusters. In the present study cluster I contained the genotypes G-1 (BARI sarisha-9 x BARI sarisha-6 S-73), G-3 (BARI sarisha-9 x BARI sarisha-6 S-51), G-4 (BARI sarisha-6 x TORI-7 S-19), G-6 (BARI sarisha-6 x TORI-7 S-62), G-7 (BARI sarisha- 9 x BARI sarisha-6 S-42), G-8 (F_6 x BARI sarisha-9 S-25), G-9 (BARI sarisha-6 x TORI-7 S-45), G-11 (BARI sarisha-9 x BARI sarisha-6 S-50), G-12 (BARI sarisha-6 x TORI-7 S-49), G-15 (BARI sarisha-6 x TORI-7 S-11), G-16 (BARI sarisha-9 x BARI sarisha-6 S-35), G-17 (F_6 x BARI sarisha-9 S-19), G-18 (BARI sarisha-6 x TORI-7 S-5), G-23 (BARI sarisha-6 x TORI-7 S-32), G-24 (F_6 x BARI sarisha-9 S-23), G-25 (F_6 x BARI sarisha-9 S-29), G-28 (BARI sarisha-9 x BARI sarisha-6 S-81), G-29 (BARI sarisha-9 x BARI sarisha-6 S-69) and G-32 (TORI-7). Cluster II was composed of the genotypes G-2 (F_6 x BARI sarisha-9 S-89), G-5 (BARI sarisha-9 x BARI sarisha-6 S-87), G-10 (BARI sarisha-6 x TORI-7 S-29), G-13 (F_6 x BARI sarisha-9 S-75), G-14 (F_6 x BARI sarisha-9 S-31), G-21 (F_6 x BARI sarisha-9 S-59) and G-22 (BARI sarisha-9 x BARI sarisha-6 S-92). Cluster III was constituted with G-19 (BARI sarisha-6 x TORI-7 S-48), G-20 (F6 x BARI sarisha-9 S-52), G-27 (BARI sarisha-9 x BARI sarisha-6 S-62) and G-30 (BARI sarisha-6 x TORI-7 S-37). On the other hand, cluster IV represented 2 genotypes

namely G-26 (F6 x BARI sarisha-9 S-15) andG-31 (BARI sarisha-15) and cluster V comprised only one genotype G-33 (BARI sarisha-6).

The genotypes from cluster III earned the highest cluster mean values for number of primary branches/plant (6.75), number of secondary branches/plant (5.00), number of siliquae/plant (172.58) and seed yield/plant (5.56 g) but the lowest cluster mean for siliqua length (5.08 cm), number of seeds/siliqua (16.58) and 1000-seed weight (2.87 g). On the other hand, Cluster V produced the highest mean values for plant height (113.33 cm), number of seeds/siliqua (26.67), days to maturity (99 days) and 1000-seed weight (3.40 g) but the lowest mean values for number of primary branches/plant (4.33), indicating late maturing and coarse seeded genotypes constituted this cluster. If parents from cluster III and V are involved in hybridization program then the highest heterosis in respect of yield, earliness, tallness, higher number of branches, seeds and siliquae/plant might be obtained. Srivastav and Singh (2000) reported that cluster III had the highest number of primary and secondary branches and the highest mean value for seed yield/plant and cultivars in cluster V with 1000-grain weight supported this result. Cluster II showed better performance in case of early maturity (82.42 days) and siliqua length (5.44 cm), On the other hand the genotypes included in cluster IV showed the lowest cluster mean for number of secondary branches/plant (1.50), the lowest siliqua length (5.03 cm) and also the lowest seed yield/plant (3.68 g) (Table 2).

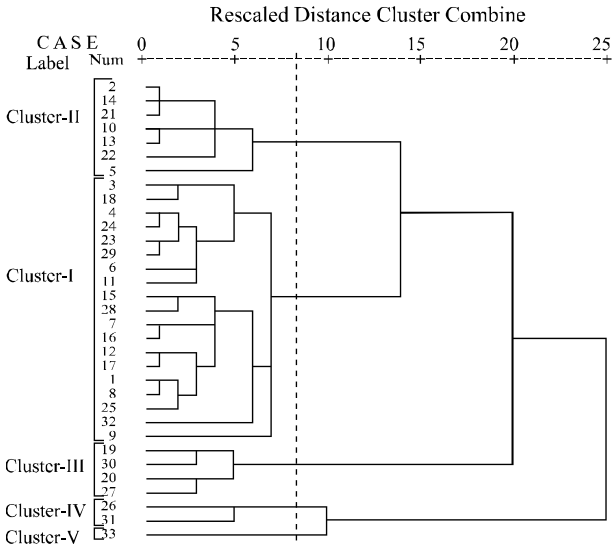

Figure 1. Dendogram of 33 genotypes of *Brassica rapa* L. by using morphological data and executed by UPGMA (Unweighted Pair Group of Arithmetic Mean)

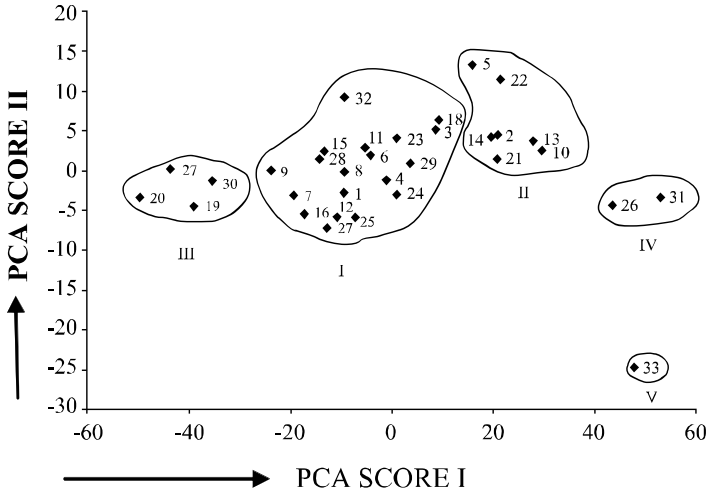

Figure 2. Scatter distribution of 33 genotypes of *Brassica rapa* L. based on their principal component scores superimposed with clustering

Table 1. Distribution of 33 genotypes of *Brassica rapa* L. in five clusters as per dendogram

Cluster	Number of genotypes	Name of Genotypes with code
I	19	G-1 (BARI sarisha-9 x BARI sarisha-6 S-73); G-3 (BARI sarisha-9 x BARI sarisha-6 S-51); G-4 (BARI sarisha-6 x TORI-7 S-19); G-6 (BARI sarisha-6 x TORI-7 S-62); G-7 (BARI sarisha- 9 x BARI sarisha-6 S-42); G-8 (F_6 x BARI sarisha-9 S-25); G-9 (BARI sarisha-6 x TORI-7 S-45); G-11 (BARI sarisha-9 x BARI sarisha-6 S-50); G-12 (BARI sarisha-6 x TORI-7 S-49); G-15 (BARI sarisha-6 x TORI-7 S-11); G-16 (BARI sarisha-9 x BARI sarisha-6 S-35); G-17 (F_6 x BARI sarisha-9 S-19); G-18 (BARI sarisha-6 x TORI-7 S-5); G-23 (BARI sarisha-6 x TORI-7 S-32); G-24 (F_6 x BARI sarisha-9 S-23); G-25 (F_6 x BARI sarisha-9 S-29); G-28 (BARI sarisha-9 x BARI sarisha-6 S-81); G-29 (BARI sarisha-9 x BARI sarisha-6 S-69) and G-32 (TORI-7)
II	7	G-2 (F_6 x BARI sarisha-9 S-89); G-5 (BARI sarisha-9 x BARI sarisha-6 S-87); G-10 (BARI sarisha-6 x TORI-7 S-29); G-13 (F_6 x BARI sarisha-9 S-75); G-14 (F_6 x BARI sarisha-9 S-31); G-21 (F_6 x BARI sarisha-9 S-59) and G-22 (BARI sarisha-9 x BARI sarisha-6 S-92)
III	4	G-19 (BARI sarisha-6 x TORI-7 S-48); G-20 (F_6 x BARI sarisha-9 S-52); G-27 (BARI sarisha-9x BARI sarisha-6 S-62) and G-30 (BARI sarisha-6 x TORI-7 S-37)
IV	2	G-26 (F_6 x BARI sarisha-9 S-15) and G-31 (BARI sarisha-15)
V	1	G-33 (BARI sarisha-6)

Table 2. Non-hierarchical cluster mean values for 9 characters of 33 genotypes of *Brassica rapa* L.

Character	Cluster				
	I	II	III	IV	V
Days to maturity	83.26	82.42	84.00	88.50	99.00
Plant height	93.83	88.35	95.06	96.28	113.33
Number of primary branches/plant	6.08	5.29	6.75	5.00	4.33
Number of secondary branches/plant	3.00	2.57	5.00	1.50	2.00
Number of siliquae/plant	137.75	108.38	172.58	82.84	84.00
siliqua length	5.30	5.44	5.08	5.03	5.21
Number of seeds/siliqua	17.35	16.95	16.58	20.66	26.67
1000 seed weight	2.89	3.08	2.87	2.93	3.40
Seed yield/plant	4.88	4.32	5.56	3.68	4.59

In many cases, the same cluster included genotypes from different eco-geographic region indicating the geographic distribution and genetic divergence did not follow the similar trend. This finding was in agreement with the findings of other researcher, Rawhat and Anad (1981), Gupta *et al.*, (1991) and Mitra and Saini (1998) reported the non-correspondence of genetic and geographic diversity.

Principal Coordinates Analysis (PCO)

Inter genotypic distance (D^2) were obtained from PCO for all possible combinations between pair of genotypes. Among the possible 528 combinations, the highest inter-genotypic distance (1.5975) was observed between G-27 (BARI sarisha-9 x BARI sarisha-6 S-62) and G-31 (BARI sarisha-15) followed by G-26 (F6 x BARI sarisha-9 S-15) and G-27 (BARI sarisha-9 x BARI sarisha-6 S-62) (1.5038); and G-27 (BARI sarisha-9 x BARI sarisha-6 S-62) and G-33 (BARI sarisha-6) (1.4781), while the lowest distance (0.1534) was observed between genotypes G-23 (BARI sarisha-6 x TORI 7 S-32) and G-29 (BARI sarisha-9 x BARI sarisha-6 S-69) (Table 3). The difference between the highest and the lowest inter genotypic distance indicated the presence of variability among the 33 genotypes of *Brassica rapa* L. studied.

The intra-cluster distance was computed by using the values of inter genotypic distance from distance matrix as per Singh and Chaudhary (1985). Cluster I showed highest intra-cluster distance (3.822) followed by cluster II (1.343), cluster III (0.747) and cluster IV (0.207). The cluster V showed zero intra-cluster distance due to containing only one genotype (Table 4).

Table 3. Ten of each higher and lower inter-genotypic distance (D^2) between pair of *Brassica rapa* L. genotypes

Genotypic Combination	Ten maximum (D^2) values	Genotypic Combination	Ten minimum (D^2)values
G-27 – G-31	1.5975	G-23 – G-29	0.1534
G-26 – G-27	1.5038	G-23 – G-24	0.1571
G-27 – G-33	1.4781	G-11 – G-12	0.1643
G-5 – G-27	1.407	G-8 – G-11	0.1757
G-15 – G-27	1.3257	G-9 – G-12	0.1779
G-30 – G-31	1.2914	G-7 – G-8	0.1864
G-20 – G-31	1.2842	G-17 – G-24	0.1912
G-3 – G-27	1.2362	G-10 – G-13	0.1916
G-20 – G-33	1.2134	G-12 – G-25	0.1952
G-18 – G-27	1.2127	G-4 – G-28	0.2123

Table 4. Average inter cluster distance and intra cluster distance (bold) for 33 genotypes of *Brassica rapa* L.

Cluster	I	II	III	IV	V
I	**3.822**	4.837	5.008	8.142	12.205
II		**1.343**	9.610	5.356	10.870
III			**0.747**	12.728	15.705
IV				**0.207**	6.674
V					**0.000**

Canonical Variate Analysis (CVA)

CVA was done to compute the inter-cluster distances. The values of inter-cluster distance (D2) are presented in Table 4. In this experiment, the inter-cluster distances were higher than the intra-cluster distances which indicated considerable range of genetic diversity among the genotypes of different groups. Mahmud *et al.,* (2011) and Zahan *et al.,* (2008) also reported similar result in *Brassica*. The highest inter-cluster distance was observed between cluster III and V (15.705), followed by those of between cluster III and IV (12.728), cluster I and V (12.205), cluster II and V (10.870), cluster II and III (9.610) and cluster I and IV (8.142). The maximum values of inter-cluster distance indicated that the genotypes belonging to cluster III were far away from those of cluster V. In contrast, the lowest inter-cluster distance was observed between cluster I and II (4.837) followed by those of cluster I and III (5.008), cluster II and IV (5.356) (Table 4). However, genotypes from clusters III and V if involved in hybridization may produce a wide spectrum of segregating population. Choudhary and Joshi (2001) reported that the derivatives selected from cross of diverse parents revealed greater diversity. Khan (2000), Dhillon *et al.,* (1999) and Harch *et al.,* (1999) also mentioned that maximum inter-cluster distance of

parents gave desirable segregants for the development of high yielding varieties with quality. The genotypes of cluster I and II were genetically closed.

Principal Component Analysis (PCA)

PCA is a statistical method which attempts to describe the total variation in multivariate sample using fewer variables than in the original data set (Bartolome *et al.*, 1999). In the end, the analysis results in the identification of the major attributes that are responsible for the observed variation within a given collection.

Principal component analysis was carried out with 33 genotypes of *Brassica rapa* L. The computed eigenvalues for the 9 variables subjected to principal component analysis, together with the corresponding proportion and cumulative explained variances are given in Table 5. The first principal component accounted for 91.36 % of the total variation while principal components two and three accounted for 6.39 % and 1.63 %, respectively (Table 5). 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%. However, 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 5. Eigenvalues and percentage of variation in respect of 9 principal components in 33 genotypes of *Brassica rapa* L.

Principal component axis	Eigenvalues	% of total variation accounted for	Cumulative of % Variation
1	20112	91.36	91.36
2	1406	6.39	97.75
3	358	1.63	99.38
4	72	0.32	99.7
5	32	0.15	99.85
6	24	0.11	99.96
7	7	0.03	99.99
8	3	0.01	<100
9	1	0.00	100

Scree plot is a useful visual aid to determine an appropriate number of principal components. The magnitude of an eigenvalues versus its number with the eigenvalues ordered from largest to smallest. To determine the appropriate number of components, we look for an elbow (bend) in the scree plot. The number of components is taken to be the point at which the remaining eigenvalues are relatively small and all about the same size (Johnson and Wichern, 2008). In this case, it appears without any other evidence, that three sample principal components effectively summarized the total sample variance (Figure-3). The first three principal axes accounted for 99.38% of the total variation among the characters describing 33 genotypes of *Brassica rapa* L.

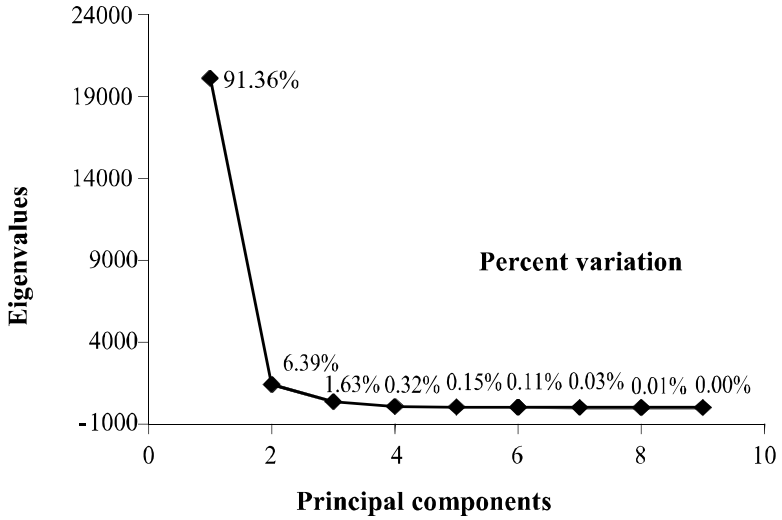

Figure 3. Scree plot constructed from eigenvalues vs number of principal components accounted for percent variations

Contribution of characters towards divergence of the genotypes

The factor loadings of characters from PCA retained three components identified the major characters responsible for maximum variability (Table 6). The first principal component (PC1) can be consider as the component of number of siliquae/plant (-0.99711). Principal component II (PC2) on the other hand indicated the importance of days to maturity (-0.50863), plant height (-0.83615) and number of seeds/siliqua (-0.20085). The characters associated with the principal component III (PC3) were siliqua length (-0.02179) and seed yield/plant (-0.01399) for high loadings. High loadings were also observed for days to maturity (-0.84123) and plant height (0.53200) but they were also important in PC2.

Table 6. Factors loadings for component character traits in principal component 1-3.

Characters	PC 1	PC 2	PC 3
Days to maturity	0.04343	-0.50863	-0.84123
Plant height	-0.00393	-0.83615	0.53200
Number of primary branches/plant	-0.01955	0.01539	-0.00478
Number of secondary branches/plant	-0.03383	0.00146	0.00042
Number of siliquae/plant	-0.99711	-0.02779	-0.04257
siliqua length	0.00089	0.00429	-0.02179
Number of seeds/siliqua	0.04479	-0.20085	-0.08176
1000 seed weight	0.00278	-0.01137	0.01079
Seed yield/plant	-0.01819	-0.02559	-0.01399

The latent vectors (Z_1 and Z_2) were also obtained from PCA (Table 7). The important characters responsible for genetic divergence in the axis of differentiation in vector I (Z_1) were plant height (0.1595), number of siliquae/plant (0.0652), number of seeds/siliqua (0.4366), days to maturity (0.3029) and 1000 seed weight (0.1287). In vector II (Z_2), number of siliquae/plant (0.1235) was important. The role of number of siliquae/plant in both the vectors was important components for genetic divergence in these materials. Afrin (2012) and Verma and Sachan (2000) reported that number of siliquae/plant in both the vectors were important components for genetic divergence. On the other hand, the role of primary branches number/plant, number of secondary branches/plant, siliqua length and yield/plant had a minor role in the genetic divergence. Dhillon *et al.*, (1997) reported that number of branches/plant showed minimum contribution to total divergence.

Table 7. Latent vectors for 9 morphological characters of 33 genotypes of *Brassica rapa* L.

Characters	Vectors 1	Vectors 2
Days to maturity	0.3029	-0.4296
Plant height	0.1595	-0.5943
Number of primary branches/plant	-0.3663	-0.1103
Number of secondary branches/plant	-0.3996	-0.2003
Number of siliquae/plant	**0.0652**	**0.1235**
siliqua length	-0.4881	-0.1761
Number of seeds/siliqua	0.4366	-0.2464
1000 seed weight	0.1287	-0.3393
Seed yield/plant	-0.3731	-0.4335

Selection of genotypes as parent for hybridization program

Selection of genetically diverge parents is the prime task for any plant breeding activities. Considering magnitude of genetic distance, contribution of different characters towards the total divergence, magnitude of cluster means for different characters and field performance the genotypes G-19 (BARI sarisha-6 x TORI-7 S-48), G-20 (F_6 x BARI sarisha-9 S-52), G-27 (BARI sarisha-9 x BARI sarisha-6 S-62) and G-30 (BARI sarisha-6 x TORI-7 S-37) from cluster III; G-26 (F_6 x BARI sarisha-9 S-15) and G-31 (BARI sarisha-15) from cluster IV and G-33 (BARI sarisha-6) from cluster V would be considered as better parents for future hybridization program. Singh and Gupta (1984) reported that out of 31 genotypes six genotypes were found to be suitable for use in one of their breeding program.

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