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Assessment of Genetic Divergence among Diverse Local and Exotic *Brassica juncea* L. Germplasm for Yield Related Traits

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ABSTRACT

The study was conducted to estimate the genetic diversity among 331 diverse *Brassica juncea* L. genotypes using agro-morphological traits. Morphological evaluation helps in the identification of promising genotypes that directly or indirectly contribute to yield improvement. To estimate the genetic diversity data was recorded for 18 key traits for two successive years. The recorded data was analysed through principal component analysis (PCA). Valuable genetic variability was found for number of days to physiological maturity, days to flowering, plant height, and pod shattering percentage Among the 17 principal components, six PCs with eigenvalues ≥1 accounted for 68.58% of the total variation observed among the studied genotypes. The contribution of first three PCs to the overall diversity were 30.63%, 10.30%, and 8.92%, respectively. Several elite genotypes were identified that need further morpho-biochemical and molecular studies. These elite genotypes may be recommended for future breeding programs to achieve promising results.

Keywords: *B. juncea*, genetic diversity, principal component, elite genotype.

Key findings: A total of 331 local and exotic *B. juncea* were evaluated for important quantitative traits. Several elite local and exotic *B. juncea* genotypes were screened and identified, which will be valuable for further molecular breeding and crop improvement programs.

INTRODUCTION

In *Brassicaceae* family, the rapeseed and mustard have prominent position. There are multiple uses of *Brassica* species like vegetable, edible oil, condiment, and fodder. Rapeseed and mustard oil contain unsaturated fatty acids, so with every passing day the importance of these crops increases for vegetable oil (Ali et al., 2015). Rapeseed (mustard) is the second largest oil seed crop in Pakistan after cotton which nearly contributes 30% of total oil seeds and 27% to edible oil pool in the country (Saeed et al., 2013). Mustard *(Brassica juncea* L.*)*, generally known as Indian Mustard or Raya is worldwide cultivated as a condiment, a vegetable, and an oilseed crop. It is one of the two main species along with *Sinapis alba* (yellow mustard or white mustard) used for condiment mustard production worldwide. Seed colour is of much importance in mustard for either brown mustard (brown-seeded) or oriental mustard (with yellow seeds) in *Brassica juncea.* Seeds of brown mustard are grounded into flour which are used in European products to produce hot mustard, flour is also used in preparation of mayonnaise, sauces, and salad dressing (Skrypetz, 2003).

Multivariate analysis provides information about the key contributing traits in different component groups and help in the identification diverse genotypes. This method was used by previously researcher for *Brassica* species improvement like Zada et al. (2013) envisaged the agro-morphological based variation among Ethiopian mustard genotypes through multivariate analysis method. A total of 134 germplasm collected from different

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area of Pakistan and other countries were evaluated through cluster and principal component-based methods. The highest level of polymorphism was observed for yield/plant traits. The moderate level of variability was observed for other economical important characters like plant height, main raceme length, silique/main raceme etc. The PC1, 2 and 3 contributed 39.03% variability as compared to other principal groups. They reported four promising elite line (25939, 25942, 25994 and 26190) for future breeding programs. Hu et al. (2007) evaluated both European and Chinese *B. napus* genotypes for agronomically important morphological traits. Significant variations were observed in both countries' genotypes such as for plant height, primary branches/plant total plant yield, total number of siliques/terminal raceme and 1000-seed weight. The PC analysis data showed that PC1 contributed a total of 33.9% variability and PC2 accounted for 26% diversity. The PC1 was positively correlated with some important quantitative characters such as plant height, and number of silique/terminal raceme. While negative correlation was observed for 1000-seed, plant yield and primary branches/plant. The cluster analysis date showed separate groups for both Chinese and European accessions. However, the low and high erucic acid and glucosinolates genotypes were also clustered into one group. Also, the spring and winter genotypes were not separated from one each other. They recommended the European genotypes for the enriching the genetic background of Chinese rapeseed germplasm. The genotypes included in the present study had not previously been evaluated for genetic diversity. Therefore, this research was undertaken to assess the genetic variability among 331 local and exotic *B. juncea* genotypes using key morphological traits.

MATERIAL AND METHODS

Experimental design and plant material

The research work was performed under the fields of Plant Genetic Resources Institute (PGRI), National Agricultural Research Centre (NARC), Islamabad, Pakistan. Total 331 accessions of *Brassica juncea* L. were used as experimental material provided by PGRI, NARC, Islamabad Pakistan (Table 1). Germplasm was collected from diverse ecologies of the world.

Table 1. List of *B. juncea* accessions used during current study at PGRI, Islamabad

S. No.	Source of Collection	Accessions (No.)
1.	Canada	113
2.	Germany	17
3.	Pakistan	130
4.	Netherland	28
5.	USA	43
	Total	331

Germplasm was planted using augmented design. The inter genotype distance was 60 cm and row to row distance was 30 cm. The plot size consisted of two rows, each 5 meter in length. To ensure optimum performance and healthy plant growth, proper agronomic practices were followed from sowing to maturity. Seeds were hand drilled and proper thinning was carried out for obtaining the best plant population. After planting, weeds were removed by hand after 20-25 days. Five randomly selected plants were used for data scoring. All the genotypes of *Brassica juncea* L. were evaluated for 18 key traits (Table 2). The International plant genetic resource board (Williams, 1990) descriptor for *Brassica* was used for measurement and selection of traits.

Data analysis

To find out the genetic diversity among genotypes, Principal Component Analysis (PCA) was performed on mean data using STATISTICA 7 software (Sneath and Sokal, 1973). All morphological traits recorded during current study were analysed through multivariate analysis technique by using two complementary analyses known as principal component analysis (Sneath and Sokal, 1973).

To avoid the effects of scaling differences, means of every parameter was standardized before doing analysis. PCA was performed among 331 *Brassica juncea* L. genotypes based on 18 key morphological parameters using STATISTICA 7 software.

RESULTS AND DISCUSSION

Estimation of genetic diversity based on phenotypic data

In this study 331 genotypes of *Brassica juncea* L. including were assessed for different morphological parameters. For selection and measurement of morphological parameters the descriptors of IBPGR (International Board for Plant Genetic Resources) for *Raphanus* and *Brassicas* were used (Williams, 1990) with minor modifications. All genotypes were grown in field of PGRI, NARC, Islamabad. Genetic diversity among these genotypes was assessed for key quantitative traits. Among these genotypes significant differences were observed for different traits. The list of elite genotypes based on their better morphological performance are given in Table 2.

Table 2. Promising accessions identified for traits of interest in *B. juncea* L. germplasm

Traits of Interest	Range	Genotypes Identified
Disease resistance	Code-1	The majority of accessions showed stronger early growth & full stand without any disease due to timely sowing of crop
Flower initiation days (DFI)	≤50 days	Bj691, Bj933, Bj893, Bj914, Bj839, Bj865, Bj866, Bj889, Bj702, Bj714, Bj927, Bj935, Bj864, Bj936
Days to half flowering (50% DF)	≤55 days	Bj933, Bj691, Bj893, Bj914, Bj866, Bj839, Bj865, Bj927, Bj935, Bj714, Bj889
Flowering completion days (DFC)	≤60 days	Bj914, Bj933, Bj691, Bj865, Bj866, Bj893, Bj714, Bj927, Bj839, Bj935
No. of days to physiological maturity (DM)	≤160 days	Bj700, Bj701, Bj702, Bj772, Bj699, Bj705, Bj865, Bj914, Bj704, Bj714, Bj749, Bj870, Bj715, Bj603, Bj628, Bj696, Bj765, Bj839, Bj866, Bj868, Bj919, Bj921, Bj738, Bj933, Bj935, Bj616, Bj617, Bj624, Bj694, Bj709, Bj712, Bj731, Bj737, Bj739, Bj740, Bj741, Bj747, Bj748, Bj750, Bj754, Bj867, Bj889, Bj890, Bj893, Bj896, Bj604, Bj612, Bj625, Bj713, Bj722, Bj742, Bj745, Bj841
Pod shattering (%)	≤20%	Most of the studied genotypes were resistant to pod shattering
Plant height	≤160 cm	Bj921, Bj709, Bj708, Bj694, Bj781, Bj704, Bj705, Bj839, Bj936, Bj791, Bj928, Bj687, Bj789, Bj685, Bj742, Bj613, Bj795, Bj895, Bj849, Bj684, Bj606, Bj855, Bj602, Bj632, Bj861, Bj631, Bj914, Bj634, Bj865
No. of primary branches per plant (PB/P)	≥20 No.	Bj789, Bj769, Bj787, Bj868, Bj786, Bj790, Bj606, Bj781, Bj774, Bj860, Bj922, Bj799, Bj791
Main raceme length (MRL)	≥70 cm	Bj747, BJ754, Bj682, Bj748, Bj673, Bj662, Bj731, Bj646, Bj926, Bj874, Bj634, Bj705, Bj790, Bj654, Bj652, Bj648, Bj626, Bj745, Bj872, Bj657, Bj750, Bj935, Bj644, Bj719
Pods on main raceme (P/MR)	≥85 No.	Bj731, Bj798, Bj782, Bj902
Stem thickness (ST)	≥25 mm	Bj917, Bj782, Bj907, Bj753
Pod length (cm)	≥4.5 cm	Bj691, Bj689, Bj690, Bj920, Bj928, Bj933, Bj782, Bj781, Bj841, Bj908, Bj692, Bj930, Bj753, Bj936, Bj927, Bj688, Bj932, Bj931, Bj934
Pod width (mm)	≥4.5 mm	Bj874, Bj687, Bj743, Bj686, Bj782, Bj606, Bj604, Bj935, Bj781, Bj808, Bj611, Bj692
Leaf length (cm)	≥25 cm	The majority of accessions (202) exhibited desirable leaf length
Leaf width (cm)	≥10 cm	The majority of accessions (more than 250) exhibited desirable leaf width
No. of grains per pod (No.)	≥15 No.	Bj781, Bj935, Bj852, bj927, Bj782, Bj607, Bj689, Bj602, Bj865, Bj783, Bj911
Grain yield (g)	≥20 g	Bj796, Bj690, Bj688, Bj605, Bj689, Bj609, Bj692, Bj614, Bj691, Bj753, Bj623, Bj624, Bj603, Bj618, Bj783, Bj607, Bj610, Bj635, Bj928, Bj933
1000-grain wt. (g)	≥8 g	Bj659, Bj666, Bj645, Bj906, Bj864, Bj902, Bj658, Bj655, Bj853, Bj916

Principle component analysis based on phenotypic traits

All morphological traits recorded during current study were analysed through multivariate analysis technique by using two complementary analyses known as principal component analysis (Sneath and Sokal, 1973). To avoid the effects of scaling differences, means of every parameter was standardized before doing analysis. Principal component analysis (PCA) was performed among 331 *Brassica juncea* L. genotypes based on 18 quantitative morphological parameters. First 6 Principal components with an eigenvalue of ≥1 presenting 68.58% difference of the overall diversity found among the studied genotypes (Table 3).

Among these principal components the PC1 was found to have maximum 30.63% difference out of total diversity. The differences found in PC1 were mostly credited to length of main raceme (0.31) and grain yield (0.44), 1000-Seed weight, blackleg disease and pod length were also found to have positive weight on PC1 with relatively less in its magnitude. On the other hand, Flower initiation days & days to 50% flowering (-0.93), days to flower completion (-0.94), No. of days to physiological maturity (-0.88), pod Shattering (%) (-0.12), leaf length & no. of primary branches per plant (-0.68), leaf length (-0.55), plant height (-0.54), No. of primary branches per plant (-0.32), stem thickness (-0.38), pod width (-0.03) and No. of grains per pod (-0.01) were found to have negative weight on PC1 (Table 3). The contributions of different traits in PC2-PC6 are shown in Table 3 and Figures 1a and 1b.

Principal component analysis of *Brassica juncea* L. genotypes showed informative grouping pattern. The 1st 3 PCs were plotted to reveal the relationships between the genotypes. (Figures 2a-c) revealed relationship between the studied genotypes based on the first three PCs. The distribution of 331 accessions of *Brassica juncea* L. into the scatter plot clearly divided the studied genotypes into diverse groups. The separation of genotypes into distinct groups was clearly based on variations in morphological traits.

Table 3. Principal components of quantitative traits among *B. juncea* L. germplasm

Traits	PC1	PC2	PC3	PC4	PCS	PC6
Eigenvalue	5.51	1.85	1.55	1.24	1.15	1.04
Cumulative eigenvalue	30.63	10.30	8.61	6.88	6.40	5.77
Percent variance	5.51	7.37	8.92	10.15	11.31	12.34
Cumulative variance	30.63	40.93	49.54	56.42	62.81	68.58
Trait of Interest	Eigenvectors					
Blackleg disease	0.17	0.51	0.13	0.02	0.15	0.13
Days to flower initiation	-0.93	0.02	-0.28	-0.03	-0.08	0.00
Days to 50% flowering	-0.93	-0.01	-0.25	-0.02	-0.10	0.00
Days to flower completion	-0.94	-0.03	-0.24	0.00	-0.09	-0.02
Days to maturity	-0.88	0.07	-0.26	-0.06	-0.10	0.05
Pod shattering	-0.12	-0.12	0.39	-0.21	0.74	0.13
Leaf length	-0.68	0.18	0.51	0.09	-0.10	-0.12
Leaf width	-0.55	0.18	0.62	0.17	-0.12	-0.11
Plant height	-0.54	0.12	0.15	0.19	0.25	-0.14
Primary branches/plant	-0.68	-0.17	0.11	-0.23	0.09	0.15
Main raceme length	0.31	-0.31	0.41	0.09	-0.54	-0.08
Pods/main raceme	-0.32	-0.23	0.45	0.11	-0.10	0.20
Stern thickness	-0.38	0.57	0.07	0.18	-0.07	0.28
Pod length	0.16	0.72	0.04	-0.16	-0.01	0.10
Pod width	-0.03	0.06	0.19	-0.65	-0.16	-0.46
Seeds/pod	-0.01	0.20	0.08	-0.73	-0.17	0.23
Seed yield/plant	0.44	0.54	-0.07	0.13	-0.23	0.12
1000-Seed weight	0.02	-0.38	0.10	-0.11	-0.18	0.72

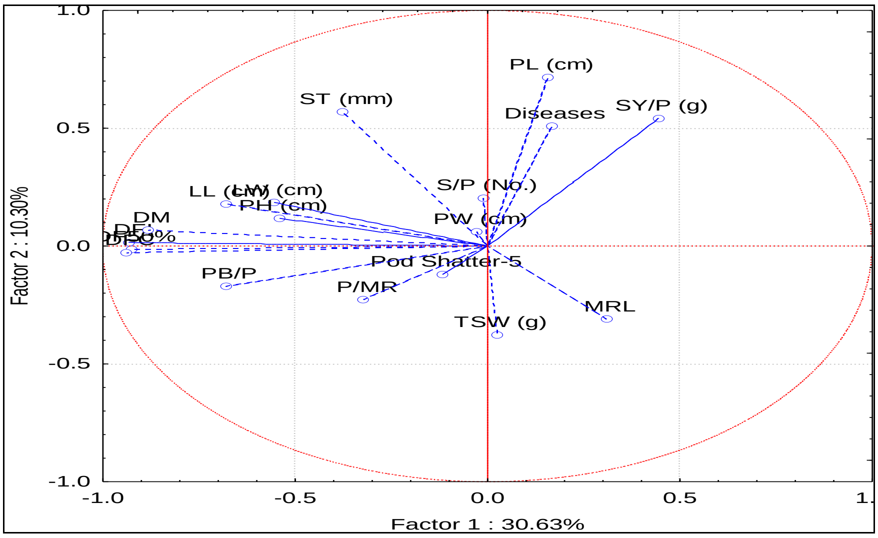

Figure 1a. Phenotypic traits contribution in PC1 and PC2

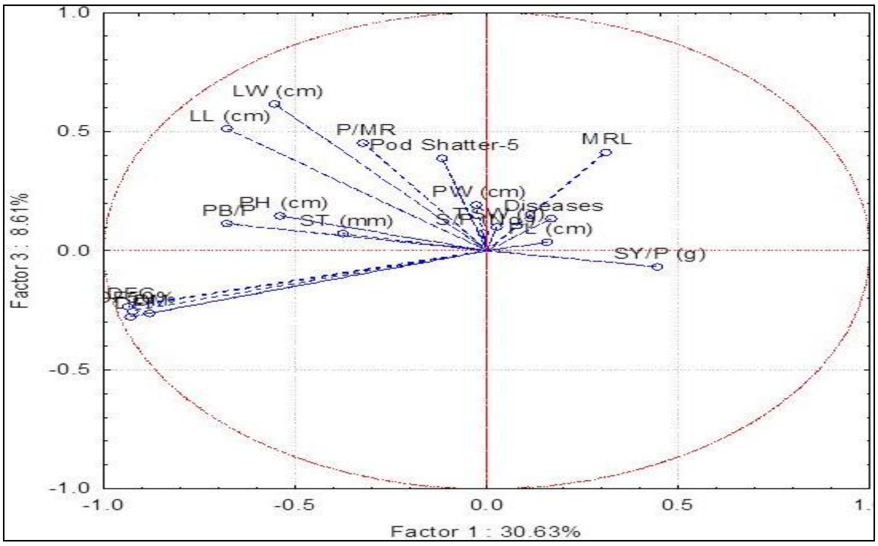

Figure 1b. Phenotypic traits contribution in PC1 and PC3

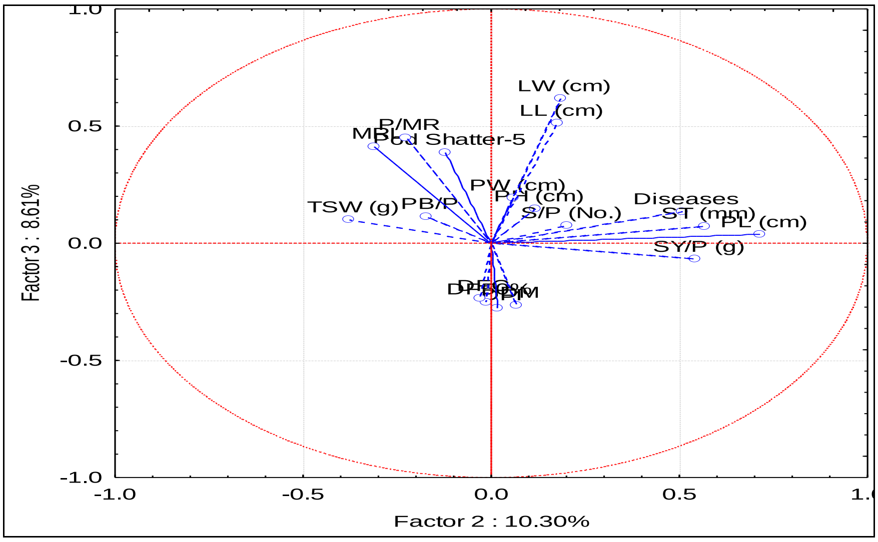

Figure 1c. Phenotypic traits contribution in PC2 and PC3

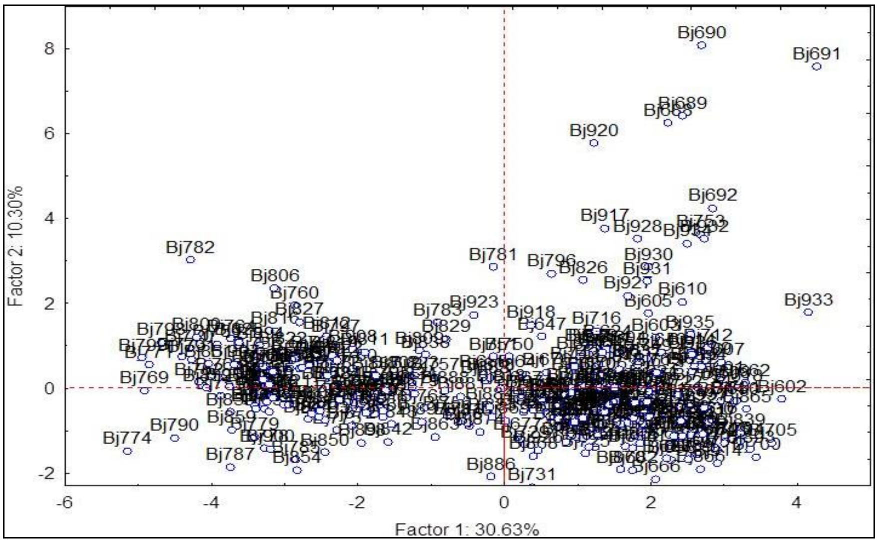

Figure 2a. Scatter Plot of PC1 & PC2 for phenotypic traits

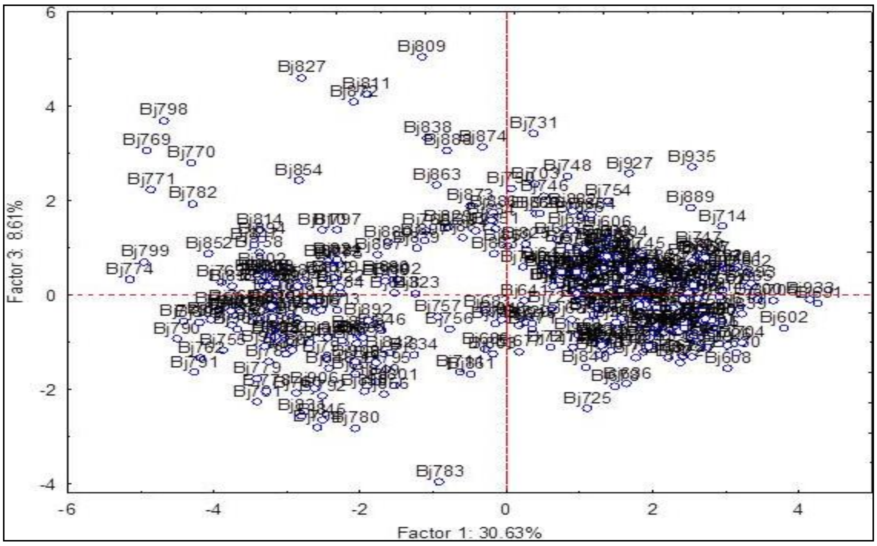

Figure 2b. Scatter Plot of PC1 & PC3 for phenotypic traits

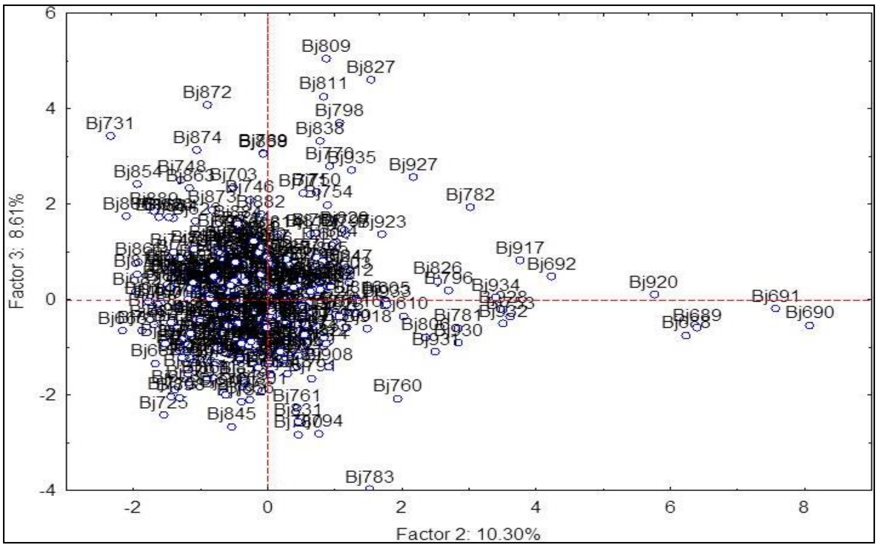

Figure 2c. Scatter Plot of PC2 & PC3 for phenotypic traits

Morphological based diversity helps in the identification of promising crop genotypes (Ibrahim et al., 2019). Accurate phenotyping is a basic step to categorize the different germplasm (Jan et al., 2017). Germplasm characterization through phenotyping is necessary for all plant breeders (Martins et al., 2006; Baig et al., 2017; Ibrar et al., 2018). So therefore, plant breeders must have to take the proper strategy for collection and evaluation of local germplasm to save them from extinction (Balkaya and Ergün, 2008). Genetic diversity in plant genetic resources provides an opportunity for plant breeders to develop new and improved varieties with desirable traits, including high yield, and resistance to pests and diseases (Govindaraj et al., 2015). The availability of genetic diversity carved in the breeding material play key role in breeding programme to develop a superior variety or hybrids. Generally, the genetically diverse parents are used to obtain a required recombinant in segregating generations. Multivariate analysis is a valuable tool for the assessment of genetic diversity (Singh et al., 2016). The order of the day is to investigate the genetic diversity of crops germplasm and explore each aspect of it and conserve it for future breeding programme (Iannetta et al., 2007). The conservation of germplasm is a key because after green revolution it is regularly declining that’s why maintenance of genetic diversity of certain useful crops germplasm through evaluation and characterization is badly needed. The crops germplasm is the main source of genetic divergence which are utilised in different breeding programmes throughout the world by plant breeders (Baranger et al., 2004).

Many scientists and plant breeders in the past conducted research on *Brassica* species and found interesting results regarding diversity for agro-morphological traits through two complementary techniques i.e., cluster analysis and principal component analysis. These techniques were successfully used by Khan et al. (2014) in *Brassica napus* L., and Zada et al. (2013) in *Brassica carinata* L. These techniques were also used by Balkaya et al. (2005) in white head cabbage. These findings are in line with our results. Our findings will be useful for future breeding programs of this important oilseed crop species.

During the current study 331 *Brassica juncea* L. genotypes were analysed through (PCA) based on phenotypic parameters. PCA grouped studied genotypes based on similarities and difference in phenotypic traits. The classification of genotypes into diverse groups exhibited genetic diversity among these genotypes. The genotypes were indicated on scattered diagram of PCA to show genetic diversity which can be used for improvement of desirable traits or new cultivar development. The findings of this study are supported by the work of Thakur et al. (2017) in *Brassica rapa* and Rabbani et al. (1998) in *Brassica juncea* L. genotypes. They reported that the classification of genotypes into distinct groups through PCA based on the difference of morphological traits level not due to their origin. Earlier the same results were also reported by Jan et al. (2018) in *B. carinata* and Ilyas et al. (2018) *in B. napus*. The diversity in these traits can play valuable and interesting role in the development of new and valuable *Brassica juncea* L. cultivars in the future breeding programme.

CONCLUSIONS

The appropriate assessment of economically important crops helps in the identification and utilization of superior-quality lines. In the present study, a notable and significant level of genetic divergence was observed for different quantitative traits. The contribution of traits in different PC groups was highly diverse. The classification of genotypes into diverse groups using PCA indicated substantial genetic diversity among the studied genotypes. In the current study of *Brassica juncea* L. genotypes, considerable genetic diversity was observed through agro-morphological traits, which can be utilized for the improvement and selection of existing genotypes or for the development of new cultivars.

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