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Genetic Divergence of Quantitative Traits in *Brassica juncea* L. Genotypes Based on Multivariate Analysis

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Authors' contributions

This work was carried out in collaboration between all authors. Authors TB, TM and AR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ZH and MA managed the analyses of the study. Author MR managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Edible oil requirement of Pakistan is increasing every year due to growing population and per capita consumption. After cotton, rapeseed-mustard is the second most important source of edible oil in Pakistan, where it is cultivated under diverse agro-climatic regions. The present research was conducted at Oilseeds Research Institute, Faisalabad (Pakistan) to find out the genetic divergence among 10 genotypes of mustard (*Brassica juncea* L.) with three replications in RCBD design. Eight morphological characters were measured to find out superior genotype use as a donor parent in hybridization programs. The cluster analysis revealed to two clusters of genotypes on based similarity and difference in morphological trait. The first cluster comprises seven genotypes and second group consist of three genotypes. The result of dendrogram indicated that high genetic distance among the genotype in terms of studied traits. Day to maturity had significant highly positive correlation with plant height and days to flowering. Pod length had highly significant

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positive correlation with seeds per pods. Plant height had correlate significant positive with days of maturity and days to flowering that would have brought simultaneous improvement for these traits as a result of correlated response. The principal component analysis shows that PC1, PC2 and PC3 contribute 52.1%, 19.9% and 15.3% of total variation, respectively. Therefore, it is possible to utilize that genetic variation in selection and hybridization program to develop new or more productive *Brassica* varieties and to optimize donor parental lines.

Keywords: Brassica juncea L.; genetic variation; principal component analysis; cluster analysis.

1. INTRODUCTION

Edible oil requirement of Pakistan is increasing every year due to growing population and per capita consumption. After cotton, rapeseed-mustard is the second most important source of edible oil in Pakistan. The country is producing 220.3 thousand tons of rapeseed and mustard from an area of 238.9 thousand hectares [1]. The domestic edible oil consumption is much higher than production in the country. A huge amount of foreign exchange is being spent on the import of edible oil every year.

Rapeseed and Mustard is a rich source of oil and protein. Due to its high percentage of oil (40-42%), it is considered as a successful crop in dealing with oil problem in countries which are facing lack of oil. Plant genotype and environment interaction are among the factors which directly or indirectly affect the seed yield and the oil yield [2]. The oil derived from mustard canola is equally fit for human consumption and its meal is suitable for animals and birds especially for poultry.

Multivariate statistics such as cluster analysis and principal components analysis play an important role in the study of genetic divergence, suitable parents, and the genotype-environment relationship. In plant breeding programs, the genotypes which have more genetic distances used for crop improvement through hybridization. The distance between genotypes can be determined using cluster analysis. Principal components analysis used to group the studied genotypes in the two or three-dimensional space by summarizing yield (dependent component) into limited independent components [3].

Scientists have used principal component analysis method to speediness the genotype selection for desired traits and it is considered as useful for selection in early generations (Spranaij and Bos, [4]. Cheres and Knapp [5] used cluster method to assess genetic divergence of sunflower genotypes. Della Vega et al. [6] reported that principal component analysisis a potential method to determine the interaction properties of genotypes and environments as well as indirect selection for the seed yield.

Generally, the principal components analysis has been widely used in grouping different plant genotypes and evaluating various traits of plant populations [7,8]. Genetic divergence is essential to improve seed yield as it is a complex character which associated with other agro-morphological traits. Genetic distances between the genotypes of one species can be increases through hybridization among suitable parents and manipulation of heterosis for developing better genotypes [9]. Principal components analysis also helps in categorizing most appropriate traits by explaining the total pattern of variation in the original set of variables/components with few of the components as possible which reduces the complication or dimension of the problem.

Grouping of genotypes, based on genetic distance is effective only when numerous traits are studied at the same time in a crop breeding program. The purpose of this study is to assess genetic divergence of morphological traits, yield, and yield related components and also to group *Brassica juncea* L. genotypes, based on the genetic distances, so that superior genotypes could be identified as parents used in further plant breeding programs.

2. MATERIALS AND METHODS

The present research was carried out at Oilseeds Research Institute, Faisalabad (Pakistan) during the year 2015. The Ten genotypes of mustard (*Brassica juncea* L.) namely ZBJ-10020, ZBJ-10021, ZBJ-08047, ZBJ-09007, ZBJ-11030, ZBJ-11002, ZBJ-06012, ZBJ-08051 and Toriawere tested along Raya anmol as check. These genotypes were grown in a randomized complete block design (RCBD) with three replications. Each plot consists of 4 rows with 5m long. All the studied genotypes had diverse genetic background. These cultivars were sown with the help of a small seeded drill maintaining plot size of 5× 1.35 m^2. The distance between planting rows was 45 cm. Necessary measurements (agronomic field operations) were done during growing stages (i.e. number of days from planting to emergence, planting to stem elongation, planting to flowering, flowering period, planting to pod setting, pod setting, planting to physiologic maturity). Data were compared using Duncan's test at 5% probability level. Ten plants were designated randomly and marked them to record data on days to flowering, days to maturity, plant height (cm), branches plant^{-1}, pod length (cm), seeds pod^{-1}, 1000-seed weight (g), and yield (kg ha^{-1}).

To test divergence among the studied genotypes, the principal component analysis method was used and to group/cluster them Ward's method of cluster analysis was applied [10]. Principal components analysis was done based on the average of their recoded data of agronomic traits [11]. To perform the analysis, correlation matrix between them was estimated and accordingly components analysis was done using SAS and SPSS software.

3. RESULTS AND DISCUSSION

3.1 Cluster Analysis

In plant breeding the heterosis (hybrid vigor) depend upon the genetic distance between the parents. The hybridization between the extreme variant parents should result as superior F1. So plant breeders try to explore and grouped the different genotypes, in order to identify variation among these and utilize in the breeding program [12]. Cluster analysis is one of the mathematical approach that helps in grouping and selection of more effective parent [3]. It classifies the genotypes into different group based upon the difference and similarity. The genotypes that are more similar present in one group. These classified groups are called the cluster.

In this study, cluster analysis grouped the 10 genotypes into 2 clusters based on the phenotypic data (Table 1). Dendrogram in Fig. 1 showed that the first cluster is larger (contained 7 genotypes) than the second cluster (comprising of three genotypes).In cluster 1, genotype ZBJ-10020 has high similarity with ZBJ-09007 and different from the ZBJ-11030. As such in cluster 2, Toria and ZBJ-06012 had more similarity with ZBJ-08051. Among of all of these genotypes, ZBJ-08051 and ZBJ-10020 were recorded highest difference and located at the extreme of the dendrogram (Fig. 1). ZBJ-10021 was high yield, 1000 seed weight and good performance of other character. Performance of Toria was good due to short stature, early flowering and maturity, larger pod length and high number of seed per pods, but inferior due to low yield (Table 2).

Table 1. Grouping of genotypes into clusters

	Number of observations	Within cluster SS from centroid	Max. distance	Avg. distance from centroid
Cluster1	7	390604	165.541	425.072
Cluster2	3	106792	165.323	240.169

Table 2. Means of studied morphological parameters of eight genotypes

Sr. no.	Genotypes	Plant height (cm)	No. of branches	Yield (kg/ha)	Pod length (cm)	Seeds /pod	Days to flowering	Days to maturity	1000 seed weight
1	ZBJ-10020	213	9	2973	4.40	15	60	150	3.30
2	ZBJ-10021	213	8	3345	4.48	16	58	148	3.47
3	ZBJ-08047	209	8	2774	4.10	16	60	150	3.50
4	ZBJ-09007	214	10	2935	4.94	16	64	152	3.53
5	ZBJ-11030	209	9	2936	4.58	16	62	153	3.50
6	ZBJ-11002	205	8	2502	4.69	15	60	153	3.17
7	ZBJ-06012	199	7	2065	4.05	15	59	154	3.50
8	RAYA ANMOL	219	10	3025	4.66	16	60	151	3.47
9	TORIA	160	9	1869	5.69	18	52	132	3.53
10	ZBJ-08051	203	10	1607	4.19	15	59	149	3.57
	Mean	204.54	8.67	2602.90	4.58	15.80	59.03	148.53	3.46
	SD	22.25	1.42	743.78	0.59	1.86	3.11	6.48	0.12
	C.V (%)	10.88	16.39	28.58	12.97	11.78	5.27	4.37	3.39

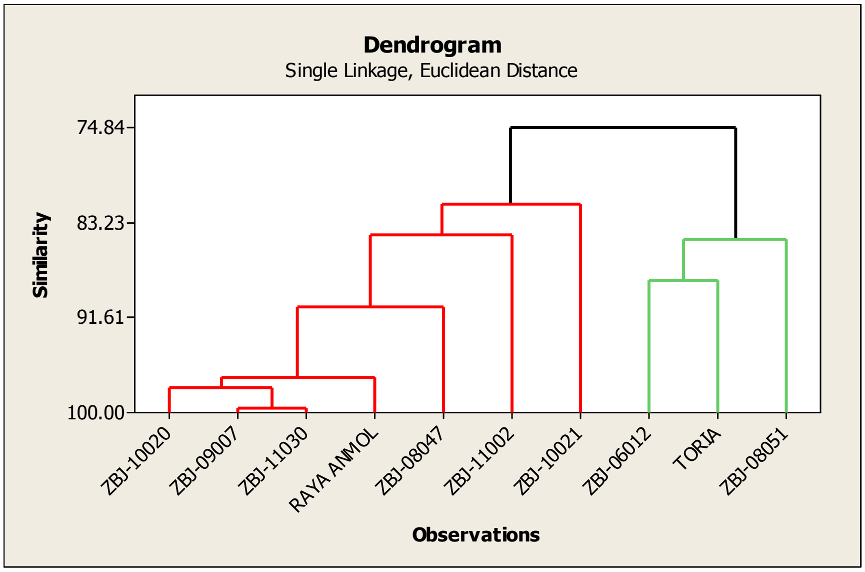

Fig. 1. Dendrogram of *Brassica juncea* L. genotypes

Hybridization among these genotypes could be used for the best combination of genes. Souza and Sorrells [13] explained that cluster analysis is the more effective approach to group the similar genotype and select the appropriated parents for breeding program. Group analysis is commonly used to study the genotypic variation in various species of *Brassica* [14,15].

3.2 Principal Component Analysis

Principal component analysis was done on eight characters of 10 *Brassica juncea* L. genotypes and identified the eight principle components, out of which three contributed 87.3% of total variation (Table 3). Each Eigen value of component contributes to total variation and selects those components which have Eigen value more than 1 [12]. Total variation of data is equal to the sum of all the Eigen value.

The first PC explained the 52.1% of the total variation for the studied traits, in which seed per pod, pod length, and 100 seed weight traits showed the negative contribution and other five traits showed a positive contribution in Table 3. The second component (PC2) showed 19.9% variation of the total variation. In this component, the highest positive co-efficient belongs to main yield contributing traits included branches, pod length, seed per pod and yield and single negatively coefficient is days to maturity. The third component (PC3) represents 15.3 % variation. The traits, i.e. yield, pod length, seed per pod and plant height were negative role, but 1000 seed weight, branches, days to flowering and days to maturity traits plays a positive role. Wide range of variation exists for day to maturity range from 132 days to 154 days, which may be able to be explained in accordance to the genetic makeup of genotype.

In PC 1 vs PC 2 score plot, varieties in quarter 1 had high variance for traits contributing positively to PC1 and PC 2, while the varieties of quarter 2 reflected much variance for traits contributing positively to PC 2 and traits having negative weights for PC 1. Varieties of quarter 3 exhibited much variance for traits contributing negatively for PC1 and for PC 2. Likewise, varieties in quarter 4 had much variance for traits contributing positively to PC 1 and for traits with negative weights for PC 2. Varieties placed at distant points of a quarter, represented the diversity from the other varieties of the same quarter and simultaneously, with other distant located varieties. Hence, genotypes ZBJ-09007, ZBJ-06012 and Toria were more diverse varieties than others in their respective quarter.

Barham studied genetic variation in 470 local wheat genotypes at west Iran [16]. He derived the 7 components that accounted 77.6 % of total variation and based on these components, 50 grouped of morphological were selected. Similarly, in studying phenotypical variations in local 43 wheat cultivar, Iglesias and Iglesias [17] specified the 3 group based on principal components analysis.

Correlation explains the relationship among two or more variables in definite manner. This statistical technique is use to find out the strength and direction of variables. The positive correlation between the traits show that changes in these traits are in same direction and negative correlation revealed that traits are opposite i.e. high value in one trait is related with decrease in other variable. This information is useful to predict the mutual relationship of complex characters which require in indirect breeding program.

The high variability for morphological traits has earlier been reported [18,19]. Day to maturity had highly significant and positive correlation with plant height and days to flowering while negative association with pods length and seed per pods traits, respectively. It showed that increase in plant height extends the vegetative growth period, which delays the plant ripening. Due to the short duration of the reproductive phase reduced pods length and seeds per pod. In Table 4, pod length and seeds per pods had highly significant positive correlation, which explained the increase in pod length, enhance the seeds per pod. But these two traits had negative and non-significant relation to yield. It revealed that increase in seeds per pods reduces the 1000 grain weight due to assimilates absorbs competition as a result yield is not affected. To enhance the yield through indirect breeding, we need to select those genotypes which are short stature, large pod length, high seed per pod and 1000 grain weight traits. The 1000-seed weight and number of seeds per pod are important yield contributing traits which showed moderate diversity hence it would be necessary to enhance the variability for these characters by hybridization with desirable donor parents [20]. Plant height had significant positive correlation with days of maturity and days to flowering that would have brought simultaneous improvement for these traits as a result of correlated response but significant negative correlation with seed per pod and pod length. It showed that with plant height extends vegetative growth that result delay plant flowering and ripening which reduced duration for pod length and number of seed formation in the pod.

Table 3. Eigen value, relative and cumulative variance of Eight components of morphological traits of *Brassica juncea* L.

Components	**First**	**Second**	**Third**	**Fourth**	**Fifth**	**Sixth**	**Seventh**	**Eighth**
Eigen value	4.171	1.591	1.226	0.683	0.264	0.049	0.017	0.000
Proportion of variance	0.521	0.199	0.153	0.085	0.033	0.006	0.002	0.000
Cumulative variance	0.521	0.720	0.873	0.959	0.992	0.998	1.000	1.000
Variable	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**
PH	0.458	0.214	-0.075	0.097	0.310	0.386	-0.540	0.438
Branches	0.019	0.647	0.322	-0.495	0.363	-0.137	0.282	0.039
Yield	0.237	0.407	-0.555	0.413	0.172	0.018	0.260	-0.450
Pod Length	-0.375	0.313	-0.292	-0.354	-0.433	0.581	-0.129	0.096
Seed Pod^{-1}	-0.402	0.325	-0.251	0.317	-0.152	-0.363	0.053	0.642
DF (50%)	0.427	0.274	0.061	-0.114	-0.595	-0.462	-0.363	-0.163
DM	0.475	-0.064	0.048	-0.003	-0.387	0.277	0.640	0.362
1000 SW	-0.152	0.294	0.654	0.580	-0.164	0.269	-0.030	-0.159

Table 4. Correlation of morphological traits of *Brassica juncea* L.

	Plant height	**Branches**	**yield**	**pod length**	**seed/pod**	**Days to flowering**	**Days to maturity**	**1000 seed wt**
Plant height	1							
Branches	0.1278	1						
Yield	0.6767*	0.0042 ns	1					
Pod length	-0.6345*	0.3522ns	-0.0895	1				
Seed/pod	-0.6618*	0.1873ns	-0.0429	0.8052**	1			
Days to Flowering	0.8446**	0.2014ns	0.4962	-0.4717	-0.5932	1		
Days to Maturity	0.8535**	-0.146	0.3785	-0.7426*	-0.8317**	0.8724**	1	
1000 seed wt	-0.2036	0.2955ns	-0.2483	0.0304	0.4206	-0.1183	-0.2676	1

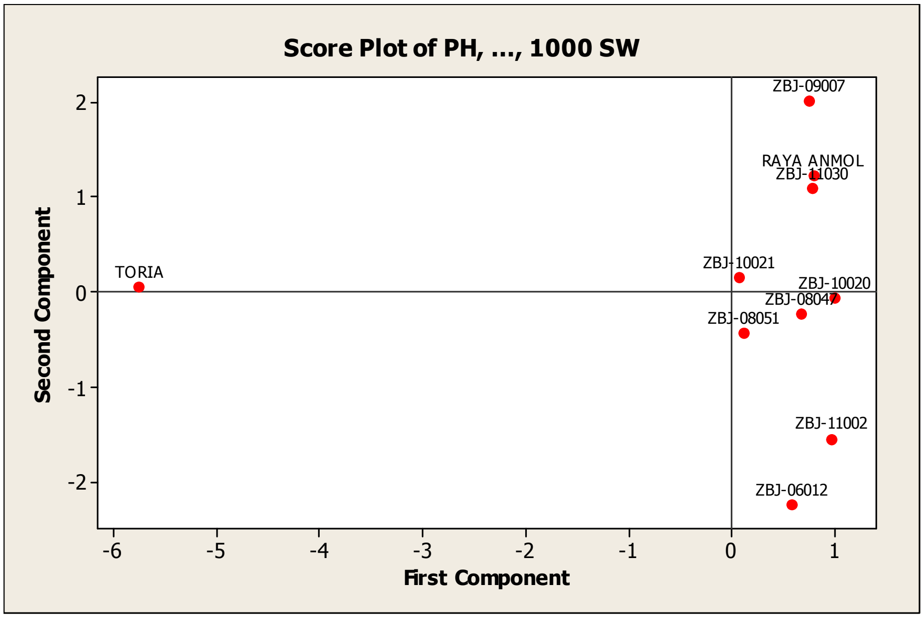

Fig. 2a. Score and loading Biplot of first two principal components in *Brassica juncea* L. genotypes

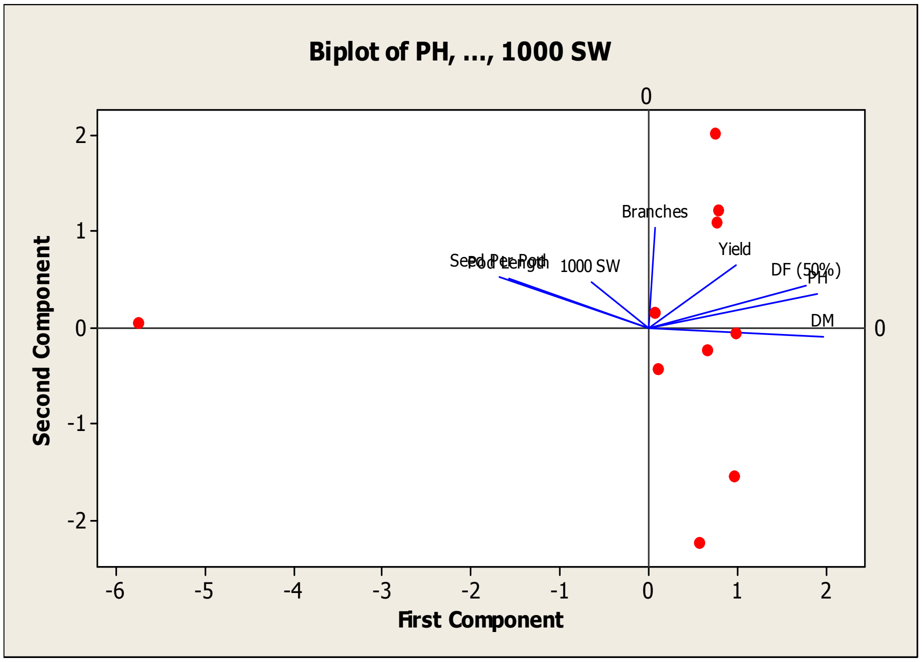

Fig. 2b. Score and loading Biplot of first two principal components in *Brassica juncea* L. genotypes

(PH= Plant Height, DF= Days to Flowering, DM= Days of Maturity and 1000SW= Thousand Seed Weight)

4. CONCLUSION

In this research it was observed that high genetic diversity of agro-morphological traits exists among studied accessions, therefore they can be tested for general and specific combining ability for their utilization in the breeding program. The principal component analysis show that PC1, PC2 and PC3 contribute 52.1%, 19.9% and 15.3% of total variation, respectively. The result of dendrogram indicated that high genetic distance among the genotype in terms of studied traits. Genetic diversity is essential to improve seed yield as it is a complex character which associated with other agro-morphological traits. Genetic distances between the genotypes of one species can be increases through hybridization among suitable parents and manipulation of heterosis for developing better genotypes. Therefore, it is possible to utilize that genetic variation in selection and hybridization program to develop new or more productive *Brassica* varieties and to optimize donor parental lines.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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