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Multivariate Based Variability within Diverse Indian Mustard (*Brassica juncea* L.) Genotypes

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Abstract

Indian mustard (*Brassica juncea* L.) germplasm consisting 167 accessions including one check cultivar was evaluated for qualitative and quantitative traits. The present study was conducted to investigate genetic diversity and correlation among studied genotypes of *B. juncea* L. based on agro-morphological at NARC, Islamabad, Pakistan. To investigate the genetic diversity based on morphological characters, data was recorded on 20 quantitative and 12 qualitative traits. The calculated data was analyzed through two complementary methods, *i.e.* PCA (Principal Component Analysis) and cluster analysis. Among all the studied cultivars, significant diversity was recorded for different agro-morphological characters. Among all the parameters, maximum variance was recorded for pod shattering (427.2) followed by plant height (345.6), days to 100% flowering (336.2) and main raceme length (210.0). Among all the characters, the greatest and highly significant association (0.99) was found between days to maturity 50% and days to maturity 100% followed by correlation (0.86) among days to flowering 50% and days to flowering 100%, correlation value (0.71) was calculated among leaf length and leaf width. Using cluster analysis all the genotypes were divided into five major groups. It was observed that 7 out of 20 principal components with an Eigen value of ≥1.0 calculated for 73.92% of the total diversity observed between 167 accessions of Indian mustard (*B. juncea* L.). The contribution of first three PCs in the total PCs was 23.25, 12.87 and 11.24, respectively. Among all the investigated accessions two genotypes 26,813 and 26,817 showed great potential for seeds/silique, 1000-seed weight and seed yield/plant, respectively, so these genotypes are recommended for future breeding programs for achieving promising results.

Keywords

Genetic Diversity, Agro-Morphology, *Brassica juncea* L., PCA Analysis

1. Introduction

Brassica juncea L. commonly known as Indian mustard is globally used as oilseed, vegetable and condiments. The crop species is said to be one of the earliest domesticated species. *B. juncea* annually grows as wild and cultivated species over a large geographical range, across the Asia, Africa, Australia, America and Europe [1]. In the genus Brassicaceae, Indian mustard is one of the highly significant crop species. In Pakistan after cotton and rape seed, Indian mustard is the third highly important source of oil [2] [3].

So for the prosperity of the country, it is essential to increase the production of these crops and for maximum production, exploitation of genetic diversity of these crops is essential. Pakistan being an agricultural country but unfortunately it is deficient in the oilseed production and a large amount ($ 1054.7 million) is spent on the import about 77% of edible oil. After petroleum, Pakistan pays the second largest import bill for edible oil among food items [4].

Diversity can be determined by three different levels, first one is genetic diversity, in which the variation observed at gene and germplasm level, the second one is diversity of species, in which richness of species at a specific location and the third one is the Eco-system diversity, in which crop species interact with their environment. Diversity is potent for different crops to fulfill the gap between demand and production across the world [5]. Various morpho-biochemical and molecular methods are used to study genetic variability among local and exotic plant germplasm. The proper evaluation of important crop species helps in the identification and utilization of improved genotypes [6]-[11]. Therefore, the present study was performed to estimate and characterize the genetic variation and relationships among Indian mustard genotypes through agro-morphological traits to identify and select promising germplasm for traits of economic significance.

2. Materials and Methods

Plant material comprises 167 germplasm including 1 check cultivar of *B. juncea* L. for estimation of agro-morphological variation. The germplasm were provided by PGRI gene-bank, NARC, Islamabad, Pakistan. The present research work was conducted in the experimental area of NARC (33˚43'N and 73˚06'E) during the year 2012-2013 using augmented design. The average annual rainfall in this area ranges from 500 - 900 mm with 70% in summer and 30% in winter.

Row to row distance of 70 cm was maintained with one accession per row and the length of the row was 2.5 meter for each and every accession and genotype. During seed bed preparation the field was irrigated before sowing to provide conditions of optimum moisture while afterword no irrigations were given and the crop was grown under rain-fed conditions. The experimental germplasm were planted at a depth of 3 - 4 cm using hand drill. After germination to maintain proper plant population thinning was carried out by hand. After 30 days of germination weeding was carried out and because of no serious attack of insect pest no insecticides and pesticides were sprayed throughout the plant growth stage. The crop was harvested when almost more than 75% of plants were turned yellowish in color. Data were recorded for twenty quantitative traits such as Days to 50% flowering, Days to 100% flowering, Days to 50% maturity, Days to 100% maturity, Leaf length, Leaf width, Leaf width/leaf length ratio, Leaves per plant, Plant height, Primary branches per plant, Main raceme length, Silique per main raceme, Silique length, Silique width, Silique length and silique width ratio, Stem thickness, Seed per silique, 1000-seed weight, Seed yield per plant, Shattering percentage (%). The description of calculating different parameters is given in Table 1. The recorded morphological traits data were averaged and analyzed for simple statistics *i.e.* mean, variance, range, frequency distribution, coefficient of variance and standard deviation using computer software (MS-Excel 2007). Cluster and principal component analysis (PCA) was performed on the recorded data for quantitative traits. Before to cluster and PCA, mean of each parameter was standardized so that to avoiding scaling differences effects. For all the pairs of accessions Euclidean distance co-efficient were calculated. The Euclidean dissimilarity co-efficient matrices were utilized to estimate the association among the *B. juncea* germplasm with cluster analysis through complete linkage method (Statistica version 7.0 and NTSYS pc v 2.1).

3. Results

3.1. Correlation Study Based on Agronomic and Morphological Traits

During the present research work days to 50% flowering exhibited highly significant positive correlation with the parameter *i.e.* days to 100% flowering (0.86**), leaf length (0.28**) and leaves per plant (0.21**), similarly days to 50% flowering, had significant positive correlation with days to maturity 100% and leaf length/leaf width ratio (0.18*), plant height (0.17*), days to maturity 50%, leaf width and primary branches per plant (0.16*). While days to flowering 50% had significant negative correlation was observed with silique width (−0.18*). Days to 100% flowering had highly positive significant correlation with leaf length (0.37**), days to maturity 100% (0.29**), days to maturity 50%, leaves per plant and stem thickness (0.27**) and primary branches per plant (0.24**). While the trait like plant height (0.19*) and leaf width (0.18*) had found significant positive correlation with days to 100% flowering (Table 2).

Table 1. Detailed information for calculating 20 different quantitative traits of *B. juncea* L. genotypes.

Characters	Range	Description
Days to Flowering 50%	<80	Early
	80 - 90	Medium
	91 - 100	Average
	101 - 110	Late
	>110	Very Late
Days to Flowering 100%	<90	Early
	90 - 105	Medium
	106 - 115	Average
	116 - 130	Late
	>130	Very Late
Days to Maturity 50%	<140	Early
	140 - 150	Medium
	151 - 160	Average
	161 - 170	Late
	171 - 180	Very Late
Days to Maturity 100%	<150	Early
	150 - 165	Medium
	166 - 180	Average
	181 - 195	Late
	196 - 210	Extreme Late
Leaf Length (cm)	<5.0	Small
	5.1 - 15.0	Medium
	15.1 - 25.0	Large
	25.1 - 35.0	Extra-Large
Leaf Width (cm)	<5.0	Small
	5.1 - 10.0	Medium
	10.1 - 15.0	Average
	15.1 - 20.0	Large
Leaf Length/ Leaf Width Ratio	0.8 - 2.0	Small
	2.1 - 3.0	Medium
	3.1 - 4.0	Average
	>4.0	Large
Leaves Per Plant	9.0 - 12.0	Minimum
	12.1 - 16.0	Medium
	16.1 - 20.0	High
	20.1 - 24.0	Maximum
Stem Thickness (mm)	<15.0	Thin
	15.0 - 20.0	Average
	20.1 - 25.0	Thick
	>25.0	Highly thick

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Plant Height (cm)	<120.0	Dwarf
	120.0 - 140.0	Medium
	140.1 - 160	Average
	160.1 - 180.0	Tall
	>180.0	Taller
Primary Branches Per Plant	<5.0	Minimum
	5.0 - 10.0	Medium
	10.1 - 15.0	High
	>15.0	Maximum
Main Raceme Length (cm)	<20.0	Short
	20.0 - 40.0	Medium
	40.1 - 60.0	Large
	60.0 - 80.0	Very Large
Siliquae Per Main Raceme	<20	Less
	20.0 - 40.0	Less than Average
	40.1 - 60.0	Average
	60.1 - 80.0	More than Average
	>80.0	Highest
Silique Length (mm)	<35.0	Very Short
	35.0 - 45.0	Short
	45.1 - 55.0	Average
	>55.0	Maximum
Silique Width (mm)	<4.0	Thin
	4.0 - 5.0	Greater
	>5.0	Maximum Thickness
Silique Length/Silique Width Ratio	<10.0	Small
	10.0 - 12.0	Average
	>12.0	Large
Seed Per Silique	<15.0	Minimum
	15.0 - 20.0	Average
	>20.0	Maximum
1000-Seed Weight (g)	<4.0	Minimum
	4.0 - 8.0	Average
	>8.0	Maximum
Seed Yield Per Plant (g)	<5.0	Minimum
	5.0 - 10.0	Average
	10.1 - 15.0	More than Average
	>15.0	Maximum Yield
Shattering %	<20	High Resistant
	20.0 - 40.0	Low Resistance
	40.1 - 60.0	Medium Shattering
	60.1 - 80.0	High Shattering
	>80.0	Extreme Resistance

Table 2. Correlation coefficient among agro-morphological characters in *B. juncea* L. accessions.

Trait	DF50	DF100	DM50	DM100	LL	LW	LL/LW	L/P	ST	PH	PB/P	MRL	S/MR	SL	SW	SL/SW	S/S	TSW	SY/P	SH
DF50	1																			
DF100	0.86**	1																		
DM50	0.16*	0.27**	1																	
DM100	0.18*	0.29**	0.99**	1																
LL	0.28**	0.37*	0.30**	0.31**	1															
LW	0.16*	0.18*	0.12	0.12	0.71**	1														
LL/LW	0.18*	0.22**	0.21**	0.24**	0.27**	−0.41**	1													
L/P	0.21**	0.27**	0.20**	0.19*	0.49**	0.37**	0.15*	1												
ST	0.12	0.27**	0.42**	0.43**	0.29**	0.30**	−0.1	0.41**	1											
PH	0.17*	0.19*	0.06	0.05	0.29**	0.47**	−0.33**	0.36**	0.45**	1										
PB/P	0.16*	0.24**	0.30v	0.28**	0.44**	0.50**	−0.1	0.41**	0.43**	0.46**	1									
MRL	0.02	0.05	0.08	0.05	0.15*	0.18*	−0.17*	0.20**	0.14	0.38**	0.26**	1								
S/MR	0.11	0.14	0.40**	0.38**	0.40**	0.27**	0.05	0.30**	0.22**	0.32**	0.31**	0.59**	1							
SL	−0.06	−0.08	0.14	0.16*	−0.04	−0.08	0.03	−0.13	−0.09	−0.19*	0.07	−0.08	−0.09	1						
SW	−0.18*	−0.12	0.18*	0.19*	0.05	0.02	0.02	0.1	0.08	−0.1	0.06	0.08	0.15	0.16*	1					
SL/SW	0.13	0.05	−0.08	−0.08	−0.08	−0.07	−0	−0.18*	−0.12	−0.03	0	−0.12	−0.18*	0.50**	−0.75**	1				
S/S	−0.06	−0.07	0.29**	0.30**	0.20**	0.04	0.23**	0.03	−0.1	−0.14	0.01	−0.04	0.18*	0.02	0.26**	−0.25**	1			
TSW	0.03	0	0.18*	0.18*	0.03	−0.02	0.1	0	0.03	0.03	0.07	0.15	0.22**	−0.06	0.19*	−0.21**	0.16**	1		
SY/P	0.07	0.11	0.42**	0.41**	−0.02	−0.11	0.15*	0.1	0.20**	0	0.09	−0.07	0.05	0.08	0.06	0.02	0.1	0.06	1	
SH	−0.02	−0.08	−0.35**	−0.37**	−0.32**	−0.18*	−0.18*	−0.26**	−0.12	−0.17*	−0.21**	−0.23**	−0.43**	0	−0.1	0.09	−0.31**	−0.1	−0	1

Note: *significant at 0.05 (0.15 to 0.19 value); **highly significant at 0.20 & above probability level (more than 0.00 level); The green, yellow, white, red colors shows strong positive, weak positive, moderate positive, and strong negative correlations among different traits respectively.

3.2. Principal Component Analysis Based on Agronomic and Morphological Traits

Principal component analysis (PCA) was carried out based on twenty quantitative morphological characters. The 7 principal components account for 73.92% of the overall variability among the studied *B. juncea* L. accessions for the total phenotypic variations (**Table 3**, **Figures 1-6**). Among these seven principal components (PCs), the PCA-I was found to have 23.35% out of the total variability. Silique length (0.073), silique length/width ratio (0.224) and shattering percentage (0.506) contributed positively to first principal component. In contrast, days to flowering 50% and 100% (−0.385 and −0.494), days to maturity 50% and 100% (−0.680 and −0.679), leaf length (−0.711), leaf width (−0.569), leaf length/leaf width ratio (−0.112), leaves per plant (−0.616), stem thickness (−0.606), plant height (−0.513), primary branches per plant (−0.645), main raceme length (−0.391), siliqua per main raceme (−0.653), silique width (−0.193), seeds per silique (−0.230), 1000-seed weight (−0.203) and seed yield per plant (−0.251) contributed negatively.

The contribution of PC-II in total differences was 12.87% and was positively associated with days to maturity 50 and 100% (0.520 and 0.540), leaf length/width ratio (0.540), silique on main raceme (0.034), silique length (0.234), silique width (0.435), seed per silique (0.530), 1000-seed weight (0.270) and seed yield per plant (0.394), whereas days to flowering 50 and 100% (−0.176 and −0.133), leaf length (−0.149), leaf width (−0.506), leaves per plant (−0.187), stem thickness (−0.153), plant height (−0.580), primary branches per plant (−0.280), main raceme length (−0.243), silique length/width ratio (−0.245) and shattering percentage (−0.215) were negative associated with PC-II.

PC-III showed 11.24% of the total agro-morphological variation and positively associated with leaf width (0.161), leaves per plant (0.033), plant height (0.144), primary branches per plant (−0.015), main raceme length (0.365), siliqua per main raceme (0.266), seeds per silique (0.205), 1000-seed weight (0.232) and silique width (0.578), while negatively associated with days to flowering 50%

Table 3. Principal components (PCs) of agronomic traits among *B. juncea* L. genotypes.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Eigen value	4.67	2.57	2.25	1.61	1.38	1.27	1.03
Cumulative Eigen value	4.67	7.24	9.49	11.11	12.48	13.75	14.78
Percent Variance	23.35	12.87	11.24	8.07	6.89	6.35	5.15
Cumulative Variance	23.35	36.22	47.46	55.54	62.42	68.77	73.92
Traits				**Eigenvectors**			
Days to Flowering (50%)	−0.385	−0.176	−0.610	−0.463	−0.054	0.138	−0.309
Days to Flowering (100%)	−0.494	−0.133	−0.587	−0.443	−0.135	0.095	−0.251
Days to Maturity (50%)	−0.680	0.520	−0.183	0.273	−0.151	0.063	0.022
Days to Maturity (100%)	−0.679	0.540	−0.207	0.252	−0.147	0.037	0.016
Leaf Length	−0.711	−0.149	−0.066	−0.186	0.329	−0.396	0.004
Leaf Width	−0.569	−0.506	0.161	0.093	0.103	−0.417	−0.107
Leaf Length/Width Ratio	−0.112	0.540	−0.353	−0.423	0.274	−0.021	0.192
Leaves/Plant	−0.616	−0.187	0.033	−0.180	−0.026	−0.196	0.228
Stem Thickness	−0.606	−0.153	−0.029	0.193	−0.492	−0.025	0.165
Plant Height	−0.513	−0.580	0.144	0.133	−0.118	0.155	0.106
Primary Branches/Plant	−0.645	−0.280	0.015	0.243	−0.033	−0.160	−0.116
Main Raceme Length	−0.391	−0.243	0.365	0.095	0.246	0.563	−0.143
Siliqua/Main Raceme	−0.653	0.034	0.266	0.040	0.319	0.394	−0.030
Silique Length	0.073	0.234	−0.290	0.592	0.228	−0.224	−0.509
Silique Width	−0.193	0.435	0.578	−0.108	−0.253	−0.259	−0.414
Silique Length/Width Ratio	0.224	−0.245	−0.696	0.490	0.349	0.090	0.022
Seeds/Silique	−0.230	0.530	0.205	−0.103	0.332	−0.238	0.059
1000-Seed Weight	−0.203	0.270	0.232	−0.116	−0.007	0.378	−0.338
Seed Yield/Plant	−0.251	0.394	−0.248	0.236	−0.388	0.125	0.248
Shattering (%)	0.506	−0.215	−0.119	−0.069	−0.445	−0.069	−0.295

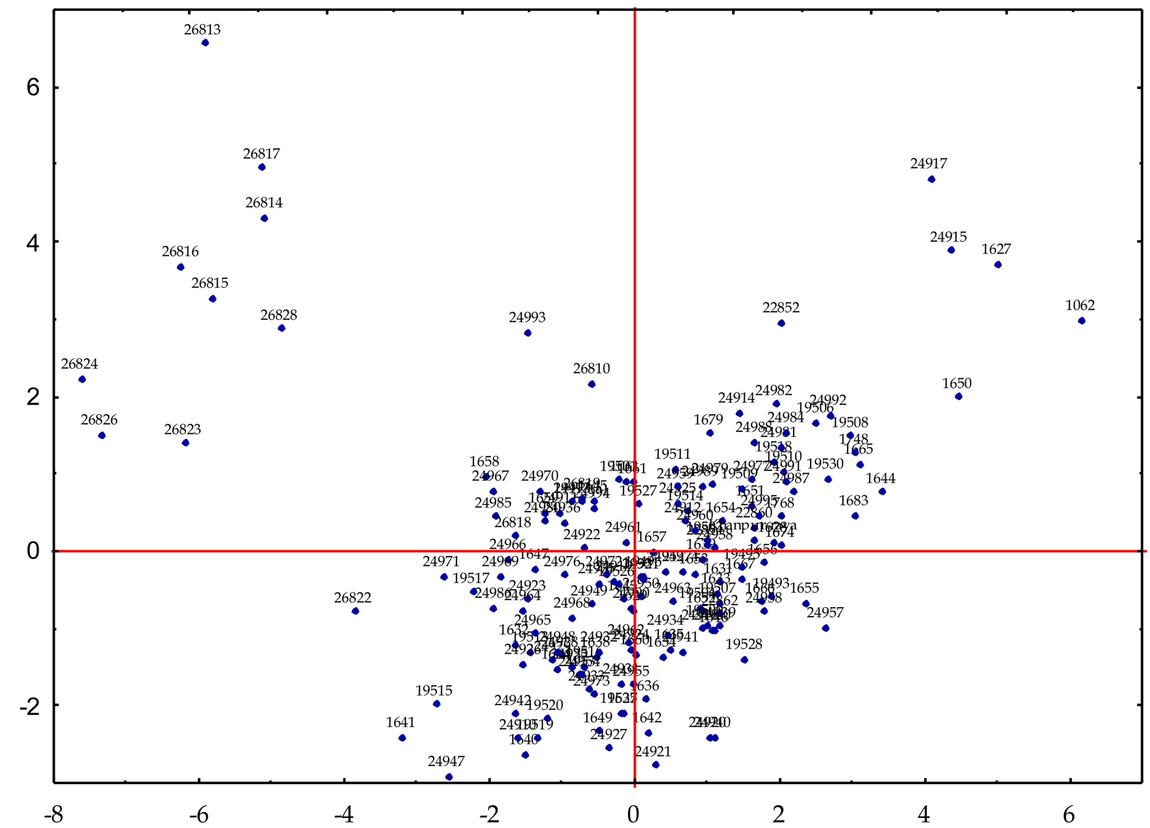

Figure 1. Scatter diagram og PC-I and II for agro-morphological traits.

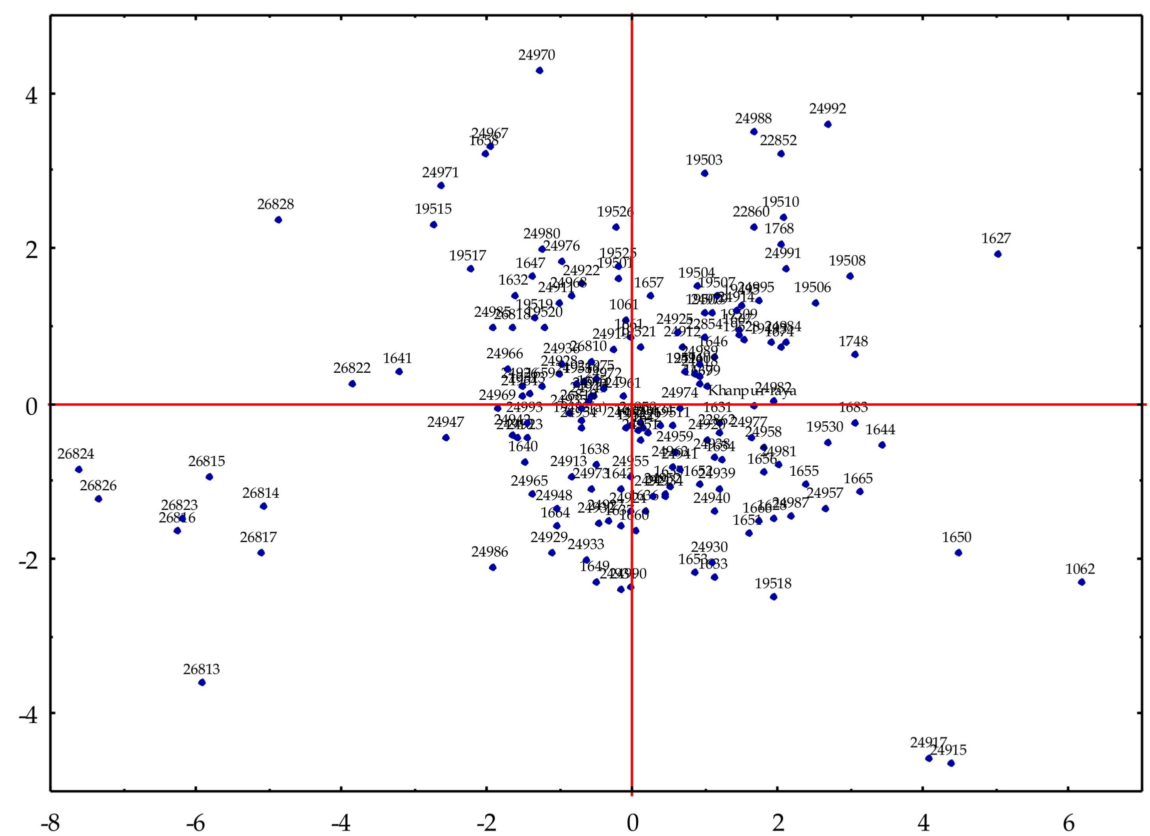

Figure 2. Scatter diagram of PC-I and III for agro-morphological traits.

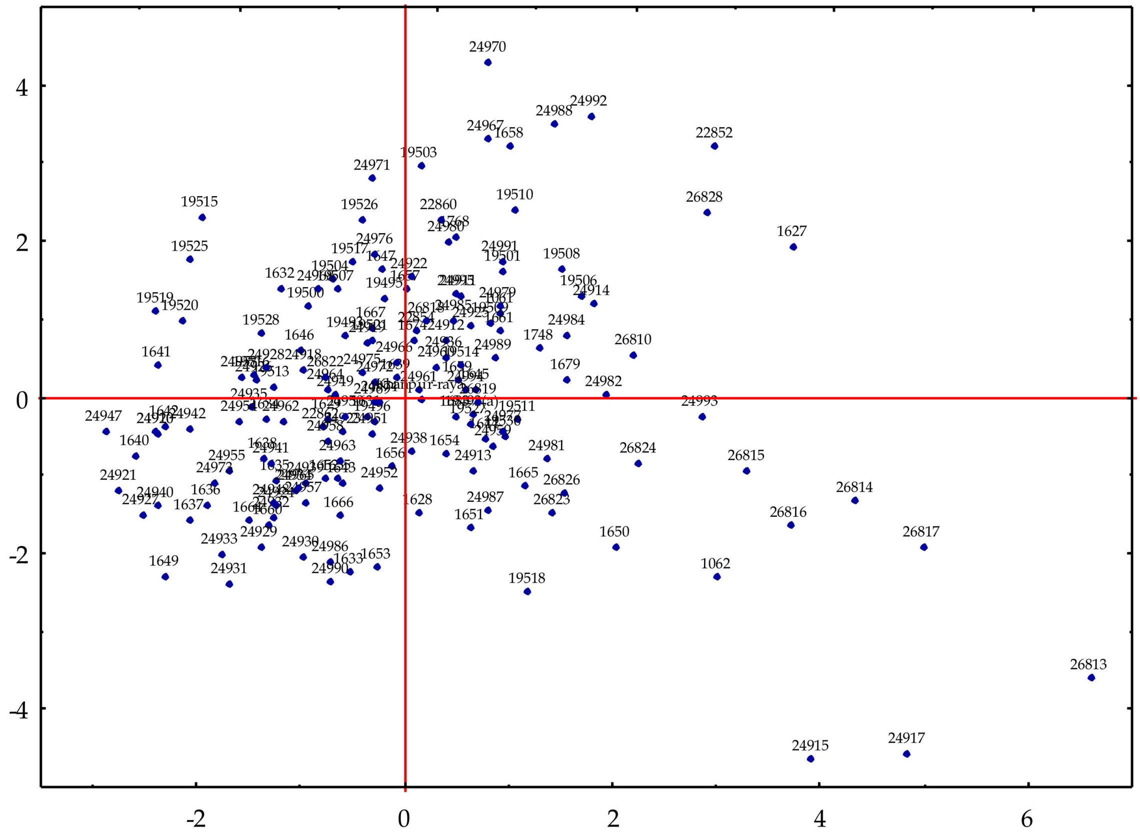

Figure 3. Scatter diagram of PC-II and III for agro-morphological traits.

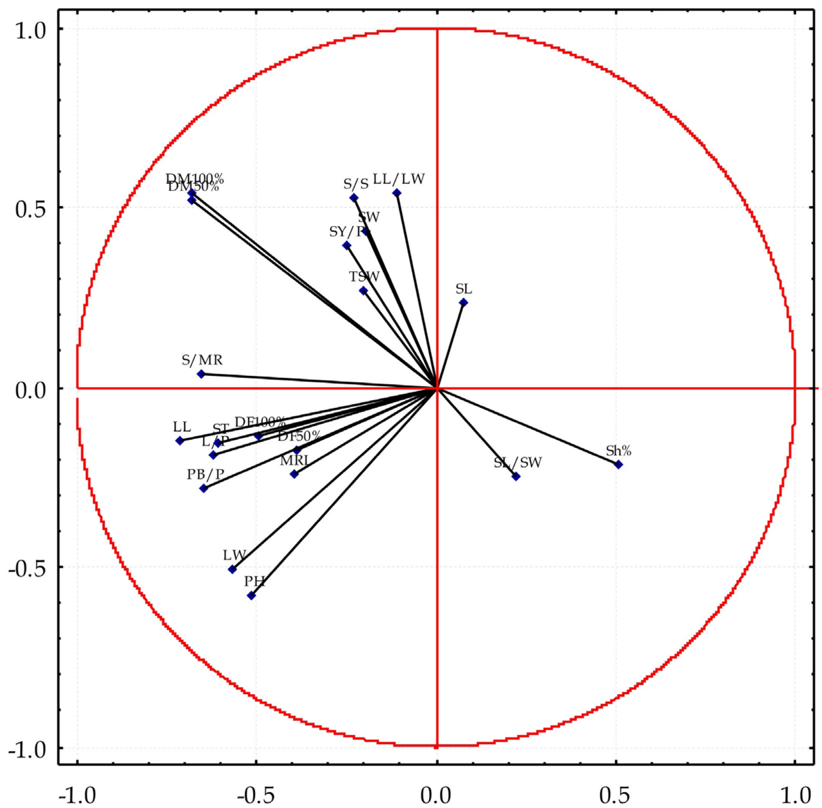

Figure 4. Contribution of morphological traits in the first two principal components.

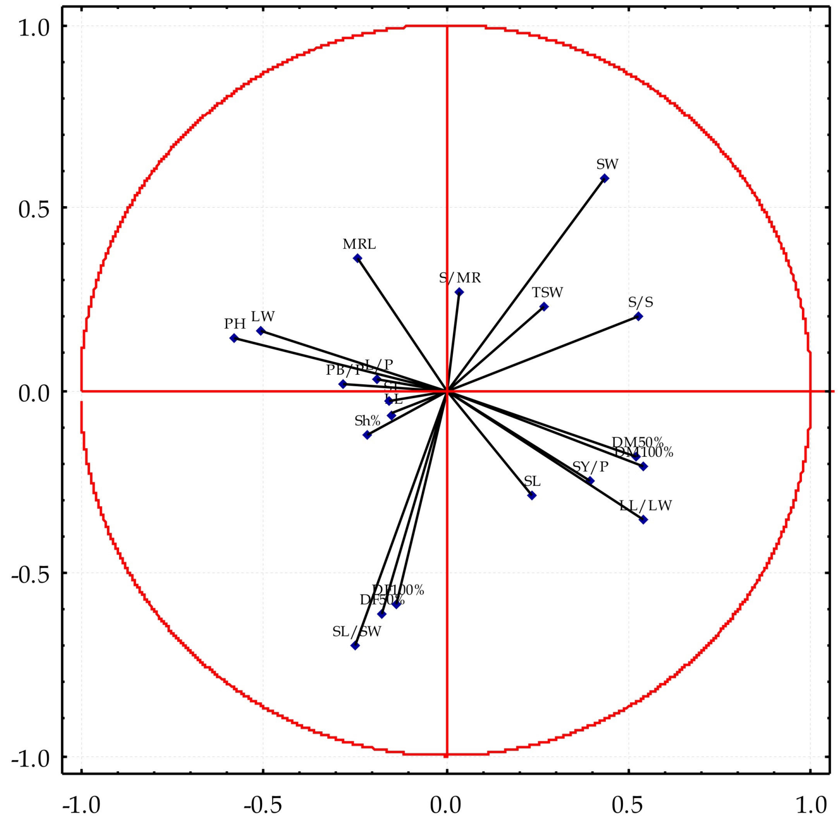

Figure 5. Contribution of morphological traits in the second and third principal components.

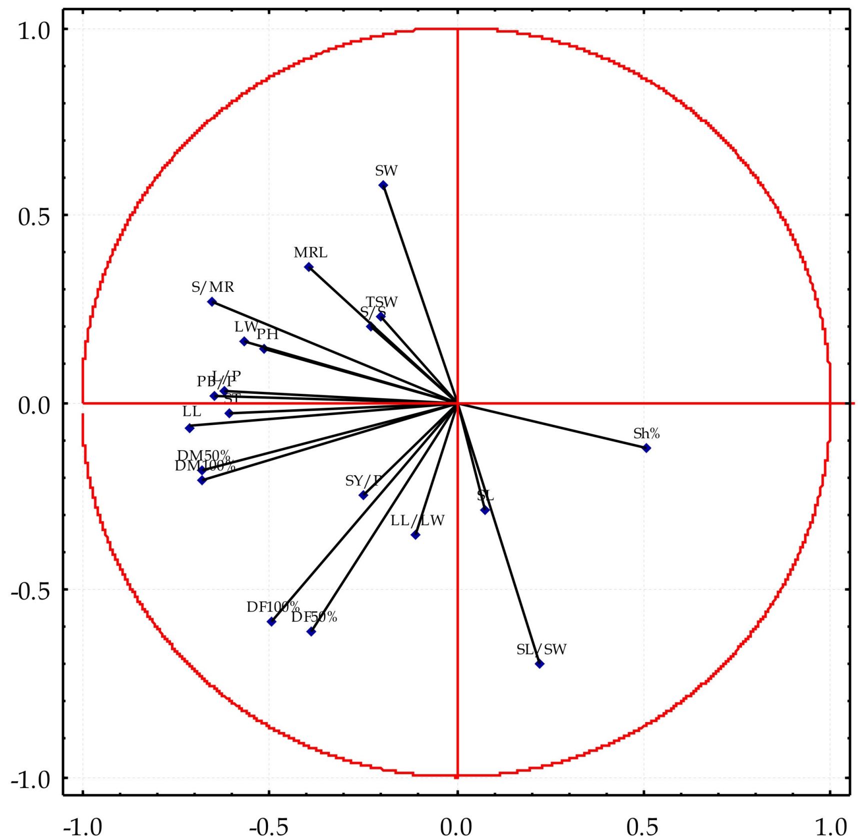

Figure 6. Contribution of morphological traits in the first and third principal components.

and 100% (−0.610 and −0.587), days to maturity 50% and 100% (−0.183 and −0.207), leaf length (−0.066), leaf length/leaf width ratio (−0.353), stem thickness (−0.029), silique length (−0.290), silique length/width ratio (−0.696), seed yield per plant (−0.248) and shattering percentage (−0.119).

The PC-IV contributed 8.07% in the total variability and were positively contributed to days to maturity 50% and 100% (0.273 and 0.252), leaf width (0.093), stem thickness (0.193), plant height (0.133), primary branches per plant (0.243), main raceme length (0.095), silique per main raceme (0.040), silique length (0.592), silique length/width ratio (0.490) and seed yield per plant (0.236). Similarly the traits *i.e.* days to flowering 50% and 100% (−0.463 and −0.443), leaf length (−0.186), leaf length/width ratio (−0.423), leaves per plant (−0.180), silique width (−0.108), seed per silique (−0.103), 1000-seed weight (−0.116) and shattering percentage (−0.069) contributed negatively.

In the total variability the contribution of PC-V was observed 6.89% and the parameter *i.e.* leaf length (0.329), leaf width (0.103), leaf length/width ratio (0.274), main raceme length (0.246), siliqua per main raceme (0.319), silique length (0.228), silique length/width ratio (0.349), seeds per silique (0.332) were positively contributed. Whereas the characters *i.e.* days to flowering 50 and 100% (−0.054 and −0.135), days to maturity 50% and 100% (−0.151 and −0.147), leaves per plant (−0.026), stem thickness (−0.492), plant height (−0.118), primary branches per plant (−0.033), silique width (−0.253), 1000-seed weight (−0.007), seed yield per plant and shattering percentage (−0.388 and −0.445) contributed negatively.

The PC-VI depicted proportion of variability as 6.35% and were positively associated with days to flowering 50% and 100% (0.138 and 0.095), days to maturity 50 and 100% (0.063 and 0.037), plant height (0.155), main raceme length (0.563), siliqua per main raceme (0.394), silique length/width ratio (0.090), 1000-seed weight (0.378), and seed yield per plant (0.125). Similarly the traits *i.e.* leaf length (−0.396), leaf width (−0.417), leaf length/width ratio (−0.021), leaves per plant (−0.196), stem thickness (−0.025), primary branches per plant (−0.160), silique length (−0.224), silique width (−0.259), seeds per silique (−0.238) and shattering percentage (−0.069) were negatively associated with PC-VI.

PC-VII accounted 5.15% of the total variation among agro-morphological traits. Days to maturity 50% and 100% (0.022 and 0.016), leaf length (0.004), leaf length/width ratio (0.192), leaves per plant (0.228), stem thickness (0.165), plant height (0.106), silique length/width ratio (0.022), seeds per silique (0.059) and seed yield per plant (0.248) were contributed positively to PC-VII. Whereas days to flowering 50% and 100% (−0.309 and −0.251), leaf width (−0.107), primary branches per plant (−0.116), main raceme length (−0.143), siliqua per main raceme length (−0.030), silique length (−0.509), silique width (−0.414), 1000-seed weight (−0.338) and shattering percentage (−0.295) depicted negative contribution.

4. Discussion

Maximum variation was calculated for shattering (427.2) followed by plant height (345.6), days to flowering 100% (336.2), main raceme length (210.0), days to flowering 50% (185.1), days to maturity 100% (122.9), days to maturity 50% (83.8). The results obtained from the present investigation were in closed agreement with that of [12] [13] [14] [15].

Our results were strengthened by many earlier plant breeders and scientists. The highly significant and positive correlation was observed between the parameters of days to flowering 50% and 100% and days to maturity 50% and 100% physiological maturity. These results were supported by the results of Nasim *et al.* [13], Alemayehu and Becker [16], by reporting highly significant and positive correlation among days to 50% and 100% flowering and days to 50% and 100% maturity. The results obtained by Zare [17] were also strongly supported our finding, who reported positive and highly significant correlation among important traits like days to flowering, days to maturity and plant height of *B. napus* L. accessions. Similarly Azadgoleh *et al.* [18] also reported positive and highly significant association among days to physiological maturity and plant height. Similarly the results of Rabbani *et al.* [19] also strengthen our findings. In the current investigation main raceme length was highly significant positive correlated with the trait of number of silique per main raceme. The results obtained by Rabbani *et al.* [19] and Nasim *et al.* [13] had also confirmed our findings. Plant height was also found positive and highly significant correlated to primary branches per plant, main raceme length and silique per main raceme which was also close agreement by previous research of Yasir *et al.* [20] who studied strong positive and significant association of plant height with primary branches per plant, main raceme length, pod on main raceme, pod length and seeds per pod.

Numerous plant breeders and plant scientist in the earlier era had practiced and got remarkable results of diversity in agro-morphological parameters for various Brassica individuals through two complementary methods *i.e.* cluster analysis and principal component analysis (PCA). These techniques were successfully observed in Indian mustard (*Brassica juncea* L.) germplasm by Gupta *et al.* [21], in Ethiopian mustard by Alemayehu and Becker [16] and in white head cabbage Balkaya *et al.* [22]. All the above mentioned plant breeders and plant scientist's results gives support to our present investigation that these 2 techniques are very supportive in estimated associations among individuals originated from different environments in a more clear approach. The classification of present studied *Brassica juncea* L. genotypes into various groups was not due to of its origination from diverse ecological zones of the country and world. Amurrio *et al.* [23], who investigated qualitative and quantitative traits of Iberian pea genotypes, were agreement with our results. During the present investigation among different accessions of *Brassica juncea* L. considerable level of genetic differences was observed for different agronomic and morphological characters. These studied parameters can play significant and interesting role in *Brassica juncea* L. cultivars and varieties development programs in future. In present study the seven groups were recorded and PC1 showed highest level of variability. The same findings were also recorded by Dias *et al.* [24] in diverse germplasm of *Brassica oleracea* L. originated from Portugal.

5. Conclusion

Among all the characters, valuable genetic diversity was observed through different agro-morphological traits *i.e.* days to maturity 50% and days to maturity 100%, days to 50% flowering and days to flowering 100%, leaf length and leaf width, main raceme length siliquae per main raceme, leaf width, primary branches per plant, silique length. Most of the traits contributed highly significant association for the said trait during the present investigation. These genotypes show that there is great potential in the studied accessions, which can be used in future breeding program as a productive genotypes.

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Conflict of Interest

The author's declares that there is no conflict of interests regarding the publication of this article.

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