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Assessment of genetic diversity in different genotypes of mustard (*Brassica juncea* L.)

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Abstract

The present study is about the estimation of genetic diversity among oilseed cultivars based on morphological characterization to fill the gap between production and consumption of edible oil. The research was conducted during 2017-2018 in Randomized Complete Block Design with six genotypes like juncea Group, Mirpur bithoro-I, Samara road-II, UCD-5/12, P-53-126/40, Rainbow and JS-13 (check) repeated in triplicate at Oilseeds Research Section Tandojam to determine the mean performance and genetic diversity on different genotypes of mustard (*Brassica juncea* L.). The results showed that all the genotypes had dissimilarity and significant differences ($P \leq 0.01$) for growth and yield performance for all parameters. Mean performance indicated that among the cultivars UCD-5/12 surpassed all genotypes for days to 75% flowering (58.33), branches plant^{-1} (7.53) and yield (1133.9 kg ha^{-1}). P-53-126/40 and Rainbow showed highest values in plant population plot^{-1} (159.00) and day to 90% maturity (127.65). More number of pods plant^{-1} (544.8) and yield plant^{-1} (25.68 g) were recorded in Samara road-II. Mirpur bithoro-I and JS-13 indicated maximum plant height (162.27cm), seed index (1000 grain weight, g) (4.62) and oil content (36.54%) respectively. Considering the genetic distance, out of 15 pair of comparisons, 3 pairs showed the highest genetic distance which can extensively be utilized in heterosis breeding; however, 12 pairs of comparisons revealed narrow genetic distance, which may be proved useful in backcross breeding programs. In total, first three principal component accounted 80.1% variability, which is considered very high and could be utilized for further breeding programs in mustard.

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Introduction

Mustard belongs to the family, Brassicaceae originated primarily from near Himalaya region and secondarily from European-Mediterranean area and Asia (Neeru *et al.,* 2015). Major producing regions of this crop are China, Canada, Northern Europe and India (Kumar *et al.,* 2016). It is mainly self-pollinating although up to 30% out crossing also arises under natural field conditions, counting upon wind and bee activities (Rakow and Woods, 1987). Oil and fat are essential items in human diet as they provide energy, improve taste and palatability of food. Mustard seed in general, contains 30-33% oil, 17-25% proteins, 8-10% fibers, 6-10% moisture and 10-12% extractable substances (Pandey *et al.,* 2013).

Mustard (*Brassica juncea* L.) is one of the most vital oil seed crops of Pakistan. Pakistan is chronically deficient in the production of edible oil and this is continuously growing due to the rapid increment in population. In Pakistan, *Brassica juncea* is mostly cultivated for the purpose of edible oil (Mustafa *et al.,* 2017). Local production of edible oil meets only 14% requirement of the country and remaining 86% is imported. During the year 2015-16, contribution of the local oil seed crops was 0.556 million tons while 2.967 was imported from other countries to meet the requirement of the country (Iqbal *et al.,* 2016).

The study of genetic divergence is essential to develop cultivars with increased yields, wider adaptation, desirable quantities, and pest and disease resistance. Inclusion of more diverse parents in hybridization programme increases the chances of obtaining a good genetic variation and gives a broad spectrum of variability in segregating generations. For the choice of diverse parents, the information on genetic relationships and diversity among the available genotypes are helpful in the formulation of crop improvement programme.

The main reason of low production is a little attention on oilseed development and genetic improvement work. At the same time the demand is increasing due to rapid growing population and increasing per capita consumption (18 kg) of edible oil (Iqbal *et al.,* 2016). Hence, the goal of this study was to estimate the genetic diversity among some oilseed cultivars based on morphological characterization to fill the gap between production and consumption of edible oil which is increasing year by year.

Materials and methods

Experimental design

The experiment was conducted during the year 2017-2018 at the field of Oilseeds Section, Agriculture Research Institute, Tandojam Sindh, Pakistan, to assess the genetic diversity in different mustard (*Brassica juncea* L.) genotypes in Randomized Complete Block Design with three replications having plot size of 5 X 1.35 meters. Six *Brassicajuncea* varieties were sown in three rows keeping 45 cm a part from each other. The parental lines were Mirpur bithoro-I, Samaro road-II, UCD-5/12, P-53-126/40, Rainbow and JS-13 (Check).

Following characters were recorded with brief methodology;

Days to flowering (75%): Number of days were counted from seeding to 75% flowering.

Days to maturity (90%): Count number of days from seeding to physiological maturity (90% seeds in pods change color from green to brown or yellow at main branches of the plants).

Plant population plot^{-1}: The number of plants germinated (m^{-2}) was counted in all plots of each treatment and averages were worked out.

Plant height (cm): The plant height (cm) was measured at maturity of the crop by measuring top to bottom to tip of plant in labelled in each treatment.

No. of Branches plant^{-1} : The number of branches plant^{-1} was counted at maturity of the crop in labelled plants in each treatment and mean was worked out.No. of Pods plant^{-1} : The total number of pods plant^{-1} was counted at maturity of the crop in labelled plants in each treatment and average pods plant^{-1}

were calculated.

Yield plant^{-1} (g): The total yield of labelled plants was weighted and divided with total number of plants examined to obtain weight of seeds plant^{-1} in each treatment.

Yield (kg ha^{-1}): The yield (kg ha^{-1}) was worked out by using the following formula:

$$\text{Seed yield (kg per ha)} = \frac{\text{Yield pe}}{\text{Plot a}} \quad (1)$$

Seed index (1000 grain weight, g): Seed index value was observed on the basis of 1000 seeds manually separated by counting in each treatment and were weighted to record seed index in gram.

Oil content (%): For oil content percent in brassica the seed from each treatment were crushed to obtain oil by soxhelate method (on weight loss basis). The oil content percentage was calculated according to following formula.

$$\text{Oil content (\%)} = \frac{\text{Oil weight of sample}}{\text{seed weight of sample}} \times 100$$

Statistical analysis

Analysis of variance was applied to the data according to the method given by Gomez and Gomez (1984). The means of the genotypes for all the traits were compared by using the least significant difference (LSD) at 5% probability level. The recorded data was analyzed through two computer softwares such as Statistix 8.1 which was used to compute mean performance and principle component analysis (PCA) and SPSS v.21 that was used to assess the genetic distance.

Results

Analysis of variance

The ANOVA was laid out for ten attributes which were collected from six genotypes of mustard given in Table 1 and 2. The obtained results of mean square revealed that *Brassica* genotypes performed significantly at $P \leq 0.01$ probability level for all the attributes.

Mean performance

Mean performance for parents is shown in Table 3 and 4. The results expressed that the maximum days to 75% flowering was taken by the genotype UCD-5/12 (58.33) followed by Rainbow (58.00).

Table 1. Mean squares for different morphological traits of mustard.

Source of variance	D. F	Days to75% flowering	Days to90% maturity	Plant population plot^{-1}	Plant height (cm)	Branches plant^{-1}
Replications	2	2.38	5.55	0.22	1.82	0.01
Genotypes	5	102.22**	12.62**	630.32**	198.92**	0.45**
Error	10	2.12	0.15	1.68	1.63	0.04
Total	17	-	-	-	-	-

** = Highly significant at $P \leq 0.01$ probability level.

The results recorded for days to 90% maturity indicated that maximum days to 90% maturity was exhibited by Rainbow (127.65), followed by Mirpur bithoro-I (127.00). The maximum value for plant population plot^{-1} was displayed by P-53-126/40 (159.00) followed by UCD-5/12 (157.00). While, Mirpur bithoro-I (162.27 cm) demonstrated the highest value for plant height followed by P-53-126/40 (154.23 cm). Regarding branches plant^{-1}, the genotype UCD-5/12 (7.53) manifested the highest estimate for the character branches plant^{-1} followed by JS-13 (Check) (7.43). However, the maximum pods plant^{-1} were counted in Samaro road-II (544.87) followed by Mirpur bithoro-I (519.33). For yield plant^{-1}, Samaro road-II (25.68) obtained the highest yield plant^{-1} followed by Mirpur bithoro-I (24.25). However, the maximum yield (kg ha^{-1}) was articulated by UCD-5/12 (1133.9) followed by Mirpur bithoro-I (1083.9). In terms of 1000-grain weight (g), JS-13 (Check) (4.62) achieved the most value followed by

Mirpur bithoro-II (4.19), whereas among genotypes, the most value for oil content was exposed by the genotype JS-13 (Check) (36.54) followed by Samaro road-II (36.53).

Genetic distance/ Coefficient of percentage

The dissimilarity matrix of tested genotypes was found on the basis of Euclidean genetic distance calculations. Genetic dissimilarity values for all 15 pair wise compressions of the total 6 mustard genotypes are presented in Table 5.

The estimated genetic distance values ranged between 37.61 and 251.12.

Table 2. Mean squares for different morphological traits of mustard.

Source of variance	D. F	Pods plant^{-1}	Yield plant^{-1} (g)	Yield(kg ha^{-1})	1000 grain weight (g)	Oil content (%)
Replications	2	9.6	0.75	675.39	0.00	0.09
Genotypes	5	28253.3**	41.28**	8714.36**	0.11**	26.70**
Error	10	35.7	1.04	467.31	0.00	0.03
Total	17	-	-	-	-	-

** = Highly significant at $P \leq 0.01$ probability level.

Table 3. Mean performance of parents and different crosses for yield and its traits in mustard.

Genotypes	Days to 75% flowering	Days to90% maturity	Plant population plot^{-1}	Plant height (cm)	Branches plant^{-1}
Mirpur bithoro-I	55.33	127.00	120.67	162.27	6.90
Samaro road-II	46.00	122.67	135.00	143.17	6.53
UCD-5/12	58.33	123.67	157.00	151.60	7.53
P-53-126/40	45.66	125.00	159.00	154.23	6.93
Rainbow	58.00	127.67	144.00	142.47	7.36
JS-13 (check)	56.00	123.33	136.67	142.27	7.43
LSD	2.65	0.71	2.36	2.32	0.40

The narrow genetic distance of 37.61 was observed between Mirpur bithoro-I/Samaro road-II, followed by P-53–126/40/Rainbow (58.99), Rainbow/JS-13(Check) (62.43),UCD-5/12/JS-13(Check) (91.62), P-53–126/40/JS-13(Check) (116.29), UCD-5/12/Rainbow(125.15), Mirpur bithoro-I/JS-13(Check) (130.51), Samaro road-II/JS-13(Check) (152.67), UCD-5/12/P-53-126/40 (153.10), Mirpur bithoro-I/Rainbow (177.62), Mirpur bithoro-I/UCD-5/12 (190.49) and Samaro road-II/Rainbow (196.89). However, the broad genetic distance of 251.12 was articulated between Samaro road-II/P-53-126/40, followed by Mirpur bithoro-I/P-53-126/40 (232.35) and Samaro road-II/UCD-5/12 (214.45).

Table 4. Mean performance of parents and different crosses for yield and its traits in mustard.

Genotypes	Pods plant^{-1}	Yield plant^{-1} (g)	Yield(kg ha^{-1})	1000 grain weight (g)	Oil content (%)
Mirpur bithoro-I	519.33	24.25	1083.9	4.36	29.65
Samaro road-II	544.87	25.68	1077.8	4.04	36.53
UCD-5/12	339.73	18.65	1133.9	4.19	36.27
P-53-126/40	313.67	20.30	983.7	4.22	31.60
Rainbow	360.20	20.50	1011.6	4.23	35.65
JS-13 (check)	393.57	15.50	1063.3	4.62	36.54
LSD	10.86	1.86	39.32	0.16	0.33

Principal component analysis

Principal component analysis (PCA) was carried out on the basis of correlation for yield and its associated traits. Ten components were extracted from the ten studied traits (Table 6 and 7) by PCA analysis. The first four components revealed more than one Eigen value while the rest of three components disclosed lower Eigen values than one.

Table 5. Genetic distance / coefficient of percentage analysis in mustard on the basis of morphological traits.

Genotypes	Euclidean distance				
	Mirpur bithoro-I	Samaro road-II	UCD-5/12	P-53-126/40	Rainbow
Mirpur bithoro-I	**				
Samaro road-II	37.61	**			
UCD-5/12	190.49	214.45	**		
P-53-126/40	232.35	251.12	153.10	**	
Rainbow	177.62	196.89	125.15	58.99	**
JS-13(Check)	130.51	152.67	91.62	116.29	62.43

Dissimilarity matrix.

The first, second and third principal component accounted for 37.2%, 23.0% and 19.8% of total variation, respectively. The cumulative percent of variance was accounted for 80.1% in the first three components. In the first PC, branches plant^{-1}, days to 75% flowering, plant population plot^{-1}, yield (kg ha^{-1}), 1000-grain weight (g) and oil content (%) expressed positive loadings, but the remaining traits revealed negative loadings. Correspondingly, plant population plot^{-1}, pods plant^{-1},yield plant^{-1}(g), yield (kg ha^{-1}) and oil content (%) presented greater positive loading, but the rest of the traits exposed negative loading in the second PC. The third PC had high positive components loading with days to 75% flowering, branches plant^{-1}, pods plant^{-1}, yield plant^{-1}(g), yield (kg ha^{-1}), 1000-grain weight (g), oil content (%), while the other characteristics demonstrated negative loading. In the fourth PC, days to 90% maturity, pods plant^{-1}, 1000-grain weight (g) and oil content (%) showed positive loadings, however the traits to go revealed negative loading. The greater positive loading was observed in the fifth PC with plant population plot^{-1}, plant height (cm), yield (kg ha^{-1}), 1000-grain weight (g) and the rest of the traits revealed negative loading.

Table 6. Vector loadings and explained percentage variance by 10 PCs.

Characters	Eigenvectors				
	PC1	PC2	PC3	PC4	PC5
Days to 75% flowering	0.31	- 0.31	0.35	- 0.15	- 0.37
Days to 90% maturity	- 0.05	- 0.52	- 0.16	0.12	- 0.57
Plant population plot^{-1}	0.27	0.21	- 0.48	- 0.37	0.03
Plant height (cm)	- 0.22	- 0.44	- 0.08	- 0.48	0.33
Branches plant^{-1}	0.48	- 0.17	0.09	- 0.14	- 0.11
Pods plant^{-1}	- 0.40	0.06	0.42	0.15	- 0.02
Yield plant^{-1} (g)	- 0.48	0.05	0.01	- 0.11	- 0.30
Yield (kg ha^{-1})	0.01	0.08	0.55	- 0.57	0.09
Seed index	0.24	- 0.32	0.23	0.44	0.47
Oil content (%)	0.27	0.48	0.22	0.08	- 0.26
Eigen values	3.71	2.30	1.98	1.08	0.90
Percent of variance	37.2	23.0	19.8	10.9	9.1
Cumulative percent of variance	37.2	60.2	80.1	90.9	100.0

Discussion

Genetic diversity of plants determines their potential for improved efficiency and hence their use play a great role for breeding.

To overcome the menace of this uniformity, it is essential that genetic variability, present in both the cultivated and wide species, is systematically exploited and used to generate new gene complexes for higher grain yield and tolerance to biotic and abiotic stresses.

Analysis of variance

The analysis of variance revealed that all the varieties performed significantly different for all the attributes which were studied suggesting that studied materials possess fruitful genetic resources for a variety of traits, thus can extensively be used for upcoming breeding programs. Almost similar results for the analysis of variance have also been reported by Sandhu *et al.*, 2017; Chandra *et al.*, 2018; Tudu *et al.*, 2018 as these researchers found the significant genetic variance for all the traits.

Table 7. Vector loadings and explained percentage variance by 10 PCs.

Characters	Eigenvectors				
	PC6	PC7	PC8	PC9	PC10
Days to 75%flowering	- 0.08	0.63	- 0.21	- 0.23	- 0.02
Days to 90%maturity	0.41	- 0.21	0.18	0.29	0.14
Plant population plot^{-1}	- 0.02	0.42	0.29	0.42	0.24
Plant height (cm)	0.14	0.03	0.43	- 0.35	- 0.25
Branches plant^{-1}	- 0.62	- 0.47	0.27	0.00	0.01
Pods plant^{-1}	- 0.19	0.17	0.50	- 0.00	0.54
Yield plant^{-1} (g)	- 0.40	0.12	0.02	0.38	- 0.56
Yield (kg ha^{-1})	0.27	- 0.23	- 0.19	0.40	0.09
Seed index	0.04	0.20	0.16	0.46	- 0.25
Oil content (%)	0.36	- 0.01	0.49	- 0.15	- 0.39
Eigen values	1.35	1.17	4.25	- 2.00	- 2.48
Percent of variance	0.0	0.0	0.0	0.0	0.0
Cumulative percent of variance	100.0	100.0	100.0	100.0	100.0

As for as mean performance is concerned, the JS-13 (Check) not only revealed better performance, but also the rest of the parents performed brilliantly for various traits among the varieties. These results are in agreement with the research of Mustafa *et al.* (2017) who also obtained the same results accordingly.

Genetic distance between the pairs

With regards to genetic distance, it ranged between 37.61 and 251.12. Out of 15 pair comparisons from genetic distance, only 3 pairs of comparisons exposed greater genetic distance (above than 214.45). Our findings are comparable to Kumari and Kumari (2018) for 1000-grain weight and days to 75% flowering genetic divergence. The higher genetic distance between parents, the higher heterosis in progeny can be observed (Chandra *et al.*, 2018). Of the particular note, these 3 pairs can further be utilized in heterosis breeding programs in mustard crop because these pairs contain a wide range of genes for various traits. Tudu *el al.* (2018) also observed the similar results for plant height, branches plant^{-1} in terms of genetic distance. Khan *et al.* (2013) reported higher values for yield plant^{-1} and days to 90% maturity. On the other hand, 12 pairs of comparisons estimated narrow genetic distance (below than 196.89). Consequently, these pairs of genotypes could be proved trustworthy breeding materials for backcross breeding.

PCA is an influential tool to obtain partial lines for successful breeding programs (Avtar *et al.*, 2017). Principal component analysis revealed that out of total ten, first four principal components were extracted having Eigen value more than one. Bibi *et al.* (2017) got good results for some of the traits which were used this research for principal component analysis. Yet, first three principal components (PCs) contributed 80.1% of the total variability amongst the mustard genotypes, which is significantly high and can be exploited for further breeding programs in mustard genotypes. Superior results for principle component analysis in days to 75% flowering, days to 90% maturity, yield plant^{-1}, oil content and 1000-grain weight along with higher loading values were also contributed by Verma *et al.* (2016). However, the remaining seven principal components contributed 20.0% towards the total diversity for this lot of mustard genotypes.

Conclusion

The results of the current study showed the presence of genetic diversity among the mustard genotypes. Of special note, the genotype P-53-126/40 articulated its superiority due to larger genetic distance, thus can further be evaluated for its adaptability and yield in different surroundings so can be exploited as a reliable breeding material for further experiments. Diverse parents of this experiment can be used for hybridization in order to get useful recombinants in the segregating generations.

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