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Assessment of Genetic Diversity in Thirty-Five Genotypes of Oilseed *Brassica* Species using Principal Component Analysis

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A B S T R A C T

The principal component and factor analyses of 35 genotypes of different *Brassica* species was carried for pooled data of two years 2015-16 and 2016-17. The PCA indicated that first four principal components showed eigen values more than one and explained more than 80% of total variability in pooled analysis. Based on Varimax Rotation all fourteen characters were grouped in eight principal factors and siliquae per plant, plant height, main shoot length and siliquae on main shoot were the major contributing traits which accounted for 69.33% of total variation of 82.46%. The hierarchical cluster analysis divided 35 genotypes into six clusters. The cluster IV appeared as the largest cluster containing maximum numbers of genotypes 23 under pooled analysis. The mean performance of different clusters revealed wide range of differences among clusters. The genotypes V20 (GPM-O-1 X PT-303), V16 (PL-58 X BN-11), V27 (GPM-O-58), V5 (NGM-17 X T-42) and V15 (PL-58 X BN-10) in cluster IV showed very good performance for seed & oil yield per plant. While genotypes V11 (T-42 X NGM-17), V10 (T-42 X GPM-O-58), V19 (T-42 X PL-58) and V33 (T-42) in cluster III, IV and V exhibited very good performance for oil content.

Introduction

Rapeseed-mustard is the second most important edible oilseed crop in India after Soybean. It contributes about 23 % and 25 % in the total oilseed area and production, respectively. It is grown over an area of 6.5 million ha with production and productivity of 7.28 million tons and 1128 kg/ha, respectively

(Anonymous, 2015). Most of the mustard cultivars have very narrow genetic base which limits their further crop improvement. Genetic variability in respect to genetic diversity is the prerequisite for the crop improvement. Genetic diversity arises either due to geographical separation or due to genetic barriers to cross ability. The interspecific hybridization also could be one way to create

genetic variability and broaden the genetic base. The quantification of genetic diversity by biometrical approaches can help in choosing diverse parents for a successful breeding programme. The principal component and factor analysis is an important tool for the assessment of genetic divergence among the genotypes and to assess the relative contribution of particular trait to the total variability. It also helps in identifying most relevant characters by explaining the total variation in the original set of variables with as few of the components as possible and reduces the complexity or dimension of the problem (Zaman *et al.,* 2010). Thus, keeping all this in view, the present research work was planned to determine the importance of traits associated with seed and oil yield along with their inter-relationship and to cluster them using PCA analysis for all 35 genotypes of different oilseed *Brassica* species comprising 20 F_{2s}/F_{3s} populations (designated as V1 to V20) and 15 parents (designated as V21 to V35).

Materials and Methods

The experimental material comprised of 20 segregating populations (F_2s / F_3s) and 15 parents (Nine *B. juncea* lines, two *B. napus*lines, one line each of *B. rapa* var toria, *B. rapa* var. yellow sarson, *B. carinata* and *B. nigra*) Table 1. These genotypes represented a very wide range of diversity available in the respective species. The segregating populations were derived by attempting interspecific crosses during rabi 2013-14. F_1s thus produced were planted during 2014-15. Colchicine treatment was given to sterile interspecific F_1s. The F_1s were selfed to develop F_2 populations during rabi 2014-15. The F_2s were selfed to develop F_3s population. Twenty F_2s/ F_3s population along with fifteen parents were evaluated for two consecutive years Rabi 2015-16 and 2016-17 at research field, College of Agriculture Gwalior (MP)

India. The experiments were laid out in randomized block design with two replications at spacing of 45 X 15 cm in paired rows. Ten plants from parent and 40 plants from F_2s/ F_3s were selected randomly for recording of various observations. Data for different agronomic and qualitative traits *viz*. days to 50% flowering (DF), plant height (PH), nos. of primary branches per plant (PB), nos. of secondary branches per plant (SB), main shoot length (MSL), siliquae on main shoot (SOMS), siliquae per plant (SPP), siliqua length (SL), seeds per siliqua (SPS), test weight (TW), days to maturity (DM), seed yield per plant (SYPP), oil content (OC) and oil yield per plant (OYPP) were recorded from randomly selected plants.

Statistical analysis

Principal factor and cluster analyses were performed using SPSS 10.0. Principal factor analysis was carried out using principal component method for factor extraction. The principal components (PCs) with eigen roots more than one were retained. As the initial factor loadings were not clearly interpretable, the factor axes were rotated using Varimax rotation. The correlation values >0.5 between the traits and the principal components were considered for construing the relationship between the traits, and the principal Factor (PF). Principal factor scores were calculated using Anderson-Rubin method. Scatter plots were drawn using two main Principal factors in order to identify the most distinct and useful accessions with desirable traits in different clusters. Unweighted Pair-Group Method using Arithmetic Averages Method (UPGMA) of hierarchical cluster analysis was utilized with city block distances to classify all 35 genotypes

For studying different genetic parameters and inter-relationships, fourteen characters were taken into consideration from the randomly

selected 10 plants in parents and 40 plants in segregating population both years 2015-16 and 2016-17. Mean data of each character was estimated and pooled data of two years (2015-16 and 2016-17) was subjected to Principal Component Analysis (PCA).

Results and Discussion

Eigen values and percent variance

Principal component analysis indicated that only first four principal components (PCs) showed eigen values more than one and they cumulatively explained 82.46% of the total variability. The first PC (PC1) explained 36.69% of the total variation and the remaining three principal components explained 17.82, 14.83 and 13.13 variation, respectively (Table 2). The first one absorbed and accounted for maximum proportion of total variability in the set of all PCs and the remaining ones accounted for progressively lesser and lesser amount of variation.

Factor loadings of characters with respect to principal factors (Varimax rotation)

The analysis without rotation of axes could not load all the variables which indicated that it didn't offer much information regarding the idea of correlation between the variables and the principal components. The Varimax rotation, thus applied, resulted in loading of all the variables on different principal components. Factors' loadings of different variables are presented in Table 3. All fourteen variables showed high loadings on different principal factors, and none was left after rotation of the principal factor axes.

The first principal factor (PF-1) ascribed for number of siliquae per plant and it was designated as siliqua factor. The PF-2 had high loading for plant height and designated as height factor. Factor-3 had high loadings for

two variables i.e. main shoot length and number of siliquae on main shoot, this factor was designated as main shoot factor. The PF-4 was named as siliqua and seed factor as two variables *viz.* number of siliquae on main shoot and seeds per siliqua were loaded on this factor. Variables seed per siliqua, siliquae on main shoot and seed yield plant were loaded on the principle factor- 5, hence it was designated as seeds factor. PF-6 was designated as maturity factor as variables *viz.* days to 50%, flowering and days to maturity were loaded on this factor. Two variables *viz.* seed yield per plant and secondary branch were loaded on the principle factor- 7 hence it was designated as seed yield factor. The PF-8 had high loadings on variables secondary branches and seeds per siliqua and designated as branching factor.

Clustering pattern based on UPGMA method

Unweighted Pair-Group Method using Arithmetic Averages (UPGMA) of hierarchical cluster analysis was utilized with city block distances to classify the thirty-five genotypes into six clusters containing one to twenty-three genotypes.

The UPGMA method in hierarchical cluster analysis grouped 35 genotypes into six clusters (C), Table 4. Maximum number of genotypes i.e. 23 was grouped in Cluster IV (CIV). Four genotypes were grouped each in cluster I (CI) and cluster V (CV). Two genotypes were present in cluster III. Whereas, one genotype each was grouped in clusters CII and CVI.

Cluster means and general means of different characters

The cluster means and general means for various characters under pooled analysis have been presented in Table 5. The comparison of

cluster means revealed that Cluster IV had the highest mean values for eight characters *viz*., secondary branch (10.41), main shoot length (70.62), siliquae on main shoot (52.96), siliquae per plant (320.31), test weight (5.41), seed yield per plant (16.02), oil content (39.29) and oil yield per plant (6.28). This cluster was able to lead in respect of the highest cluster mean values for maximum characters. Among 14 characters, this cluster stood first for 8 characters.

The cluster II obtained the highest cluster mean value for six characters *viz*., days to 50% flowering (41.25), primary branch (6.45), secondary branch (12.35), siliqua on main shoot (72.60), siliquae per plant (327.72) and days to maturity (135.50 days). Cluster V also showed highest mean values for different 6 characters *viz*. days to 50% flowering (39.31 days), plant height (122.58 cm), primary branch (6.84), siliqua length (6.53 cm), seeds per siliqua (36.94), and oil content (43.52%).

The cluster I showed highest mean values for three characters *viz*. days to 50% flowering (36.94 days), plant height (111.27 cm) and days to maturity (131.56 days). Cluster III observed highest loading for three characters plant height (170.51 cm), siliqua length (5.59 cm) and seeds per siliqua (19.23) while cluster VI also showed highest loading for variables primary branch (9.93) and secondary branch (24.23).

Principal component analysis indicated that only first four principal components (PCs) showed eigen values more than one and they cumulatively explained more than 80% of the total variability under pooled study. The first principal component absorbed and accounted for maximum proportion of total variability in the set of all PCs and the remaining ones accounted for progressively lesser and lesser amount of variation. Similar results have also been reported earlier by Zada *et al.,* (2013),

Avtar *et al.,* (2014, 2017), Ray *et al.,* (2014), Neeru *et al.,* (2015) and Verma *et al.,* (2016). The Varimax Rotation was applied to estimate correlation between the variables and the principal components. This resulted in loading of all the variables on different principal components.

Based on similarities of variables all fourteen characters have been grouped in eight principal factors *viz.* siliquae per plant factor, height factor, main shoot factor, siliqua factor, seed per siliqua factor, maturity factor, seed yield factor and branching factor. Similar results were reported by Singh *et al.,* (2010), Zada *et al.,* (2013), Neeru *et al.,* (2015) and Avtar *et al.,* (2017). Such a grouping of similar type of variables having loaded on a common principal factor elaborates the successful transformation of fourteen interrelated variables into eight independent principal factors explaining 82.46% of the variability of the original set under pooled analysis.

It was observed from analysis that siliquae per plant, plant height, main shoot length and nos. of siliquae on main shoot were the major distinct variability contributing traits and accounted for 69.33% of the total variation. Thus, the successful transformation of fourteen morphological variables into four independent principal factors by means of grouping of similar type of variables on different principal factors elaborated and explained. These findings were in tune with those obtained by Neeru *et al.,* (2015) in Indian mustard. The UPGMA method with City Block distances in hierarchical cluster analysis has divided the thirty-five genotypes into six clusters (C). The cluster IV appeared as the largest cluster containing maximum numbers of genotypes 22 and 23 under pooled analysis. The numbers of genotypes in clusters I, II, III, V and VI were 4, 1, 2, 4 and 1, respectively.

Table.1 List of F_2s/F_3s population and parents used in research experiment

Genotype	Pedigree	Genomic constitution
V1	NGM-43 X PT-303	*B. juncea* x *B. rapa var toria*
V2	NGM-17 X PT-303	*B. juncea* x *B. rapa var toria*
V3	KM-11 X T-42	*B. juncea* x *B. rapa var yellow sarson*
V4	NGM-6 X T-42	*B. juncea* x *B. rapa var yellow sarson*
V5	NGM-17 X T-42	*B. juncea* x *B. rapa var yellow sarson*
V6	PL-58 X PT-303	*B. juncea* x *B. rapa var toria*
V7	PT-303 X GPM-O-5	*B. rapa var toria* x *B. juncea*
V8	(PT-303XGPM-O-5) X GPM-O-5	(*B. rapa var toria* x *B. juncea*) x *B. juncea*
V9	PT303 X GPM-O-5	*B. rapa var toria* x *B. juncea*
V10	T-42 X GPM-O-58	*B. rapa var yellow sarson* x *B. juncea*
V11	T-42 X NGM-17	*B. rapa var yellow sarson* x *B. juncea*
V12	PT-303 X *B. nigra*	*B. rapa var toria* x *B. nigra*
V13	PL-6 X BN-11	*B. juncea* x *B. napus*
V14	PL-6 X BN-10	*B. juncea* x *B. napus*
V15	PL-58 X BN-10	*B. juncea* x *B. napus*
V16	PL-58 X BN-11	*B. juncea* x *B. napus*
V17	BN-11 X PL-6	*B. napus* x *B. juncea*
V18	KM-11 X CRP-09	*B. juncea* x *B. carinata*
V19	T-42 X PL-58	*B. rapa var yellow sarson* x *B. juncea*
V20	GPM-O-1 X PT-303	*B. juncea* x *B. rapa var toria*
V21	NGM-43	*B. juncea*
V22	NGM-17	*B. juncea*
V23	KM-11	*B. juncea*
V24	NGM-6	*B. juncea*
V25	PL-58	*B. juncea*
V26	GPM-O-5	*B. juncea*
V27	GPM-O-58	*B. juncea*
V28	PL-6	*B. juncea*
V29	GPM-O-1	*B. juncea*
V30	BN-10	*B. napus*
V31	BN-11	*B. napus*
V32	PT-303	*B. rapa var toria*
V33	T-42	*B. rapa var yellow sarson*
V34	CRP-09	*B. carinata*
V35	Banarasi Rai	*B. nigra*

Table.2 Total variance explained by different principal component among 20 F_2s/F_3s populations and 15 parents of different *Brassica* species for pooled of years 2015-16 and 2016-17

Principal component	Eigen value	Per cent variance	Per cent cumulative variance
1	5.135	36.68	36.68
2	2.495	17.82	54.50
3	2.076	14.83	69.33
4	1.838	13.13	82.46
5	0.765	5.46	87.92
6	0.418	2.99	90.91
7	0.415	2.96	93.87
8	0.314	2.24	96.11
9	0.180	1.29	97.40
10	0.149	1.06	98.46
11	0.111	0.79	99.25
12	0.068	0.49	99.74
13	0.036	0.26	100.00
14	0.000	0.00	100.00

Percent variation explained by first four components = 82.46
First 4 principal component scores were used for clustering purpose

Table.3 Factor loadings of characters with respect to different principal factors (Varimax rotation) in 35 genotypes of different *Brassica* species

Trait	PF-1	PF-2	PF-3	PF-4	PF-5	PF-6	PF-7	PF-8
Days to 50%	0.004	0.093	-0.158	0.053	0.085	0.478	-0.462	0.126
Plant height	0.133	0.967*	-0.124	0.029	0.046	-0.157	0.006	-0.008
Primary branch	-0.000	-0.008	-0.062	-0.282	0.067	0.053	0.176	0.178
Secondary branch	0.023	0.022	-0.130	-0.122	-0.170	0.109	0.460	0.803*
Main shoot length	0.030	0.124	0.739*	0.529*	-0.282	0.229	-0.002	0.125
Siliquae on main	0.033	0.102	0.576*	-0.703	0.356	0.161	-0.012	0.034
Siliquae per plant	0.988*	-0.142	-0.205	0.018	0.021	-0.015	-0.036	0.004
Siliqua length	-0.004	-0.005	0.002	0.079	0.056	0.033	0.010	0.037
Seeds per siliqua	-0.041	-0.067	0.006	0.372	0.805*	-0.098	-0.108	0.310
Test weight	0.001	0.014	0.011	0.071	0.016	0.014	0.097	-0.076
Days to maturity	0.008	0.066	-0.238	0.026	0.078	0.778*	0.120	-0.173
Seed yield per	0.033	0.012	0.041	0.123	0.233	0.104	0.702*	-0.389
Oil content	-0.008	-0.003	0.047	0.167	0.181	-0.142	0.116	-0.065
Oil yield per plant	-0.006	-0.004	-0.009	0.109	0.078	0.085	0.02	0.041

Table.4 Clustering pattern of 20 F$_2$s/F$_3$s populations and 15 parents of different *Brassica* species during under pooled analysis

Cluster	Parents/population	Nos of lines
CI	**V3** (KM-11XT-42), **V18** (KM-11X *B. carinata*), **V23** (KM-11), **V32** (PT-303),	4
CII	**V35** (*B. nigra*)	1
CIII	**V28** (BN-10), **V30** (BN-11),	2
CIV	**V1** (NGM-43XPT-3-03), **V2** (NGM-17XPT-303), **V4** (NGM-6XT-42), **V5** (NGM-17XT-42), **V6** (PL-58XPT-303), **V7** (PT-303XGPM-O-5), **V8** ((PT-303XGPM-O-5) X GPM-O-5), **V9** (PT-303XGPM-O-5), **V12** (PT-303 X *B. nigra*), **V13** (PL-6XBN-11), **V14** (PL-6XBN-10), **V15** (PL-58XBN-10), **V16** (PL-58XBN-11), **V17** (BN-11XPL-6), **V20** (GPM-O-1-1XPT-303), **V21** (NGM-43), **V22** (NGM-17), **V24** (NGM-6), **V25** (PL-58), **V26** (GPM-O-5), **V27** (GPM-O-58), **V29** (PL-6), **V31** (GPM-O-1)	23
CV	**V10** (T-42XGPM-O-58), **V11** (T-42XNGM-17), **V19** (T-42XPL-58), **V33** (T-42)	4
CVI	**V34** (*B. carinata*)	1

Table.5 Cluster means vs general means for various characters in 20 F$_2$s/F$_3$s populations and 15 parents of different *Brassica* species for pooled of two years

Characters	Cluster-1	Cluster-2	Cluster-3	Cluster-4	Cluster-5	Cluster-6	General mean
Days to 50% flowering	36.94	41.25	51.25	44.13	39.31	55.00	43.39
Plant height	111.27	185.73	170.51	193.52	122.58	223.11	175.32
Nos of primary branches	5.24	6.45	4.12	5.23	6.84	9.93	5.52
Nos of secondary branches	9.41	12.35	4.10	10.41	3.61	24.23	9.61
Main shoot length	57.55	52.96	60.37	70.62	53.40	44.20	65.32
Siliquae on main shoot	40.71	72.60	43.85	52.96	40.54	37.25	49.73
Siliquae per plant	244.52	327.72	172.50	320.31	119.15	242.52	278.20
Siliqua length	4.98	1.25	5.59	4.91	6.53	4.98	5.04
Seeds per siliqua	15.16	4.82	19.23	14.80	36.94	12.52	17.28
Test weight	3.73	1.17	4.03	5.41	4.52	4.60	4.89
Days to maturity	131.56	135.50	137.38	136.39	132.62	150.00	135.83
Seed yield per plant	9.98	8.88	7.01	16.02	9.84	12.20	13.79
Oil content	36.46	33.83	38.86	39.29	43.52	35.50	39.16
Oil yield per plant	3.64	3.00	2.72	6.28	4.30	4.32	5.40

This analysis further showed that some of the genotypes belonging to various interspecific populations (F_3/F_2) and their parents were grouped into the same cluster, while many others fell into different clusters. This clustering pattern suggests that interspecific diversity does not necessarily represent genetic diversity; this might be due to free exchange of genetic material among different species and also due to natural and artificial selection forces resulting in perpetuation and stabilization of similar genotypes. These results were in agreement with the results reported earlier by Budhanwar *et al.,* (2010), Belete *et al.,* (2011), and Singh (2012), Doddabhimappa *et al.,* (2010), Singh (2012), Neeru *et al.,* (2015) and Avtar *et al.,* (2017).

In the present study, the mean performance of different clusters revealed wide range of differences among clusters (Table 5). The genotypes V20 (GPM-O-1 X PT-303), V16 (PL-58 X BN-11), V27 (GPM-O-58), V5 (NGM-17 X T-42) and V15 (PL-58 X BN-10) in cluster IV showed very good performance for seed and oil yield per plant due to possession of more numbers of siliquae per plant, long main shoot length, more siliquae on main shoot, more seeds per siliqua and higher test weight. While genotypes V11 (T-42 X NGM-17), V10 (T-42 X GPM-O-58) and V19 (T-42 X PL-58)in cluster III and IV& V33 (T-42)in cluster V, respectively exhibited very good performance for oil content and could be used as donor for the introgression of high oil content. Alemayehu and Becker (2002) found that both principal component and cluster analyses disclosed complex relationships among the Ethiopian mustard (*Brassica carinata* A. Braun) accessions and characters. Similar results were reported by Singh (2012), Zaman *et al.,* (2010), Singh *et al.,* (2010), Avtar *et al.,* (2017) and Nerru *et al.,* (2015).

The results of this study concluded that the sufficient variability was existed in the material. All the 35 genotypes (20 F_3s/F_2s population and 15 parents) have been successfully classified into six clusters and all the variables have been reduced to only eight principal factors. Based on mean performance of different clusters for different traits the genotypes V20 (GPM-O-1 X PT-303), V16 (PL-58 X BN-11), V27 (GPM-O-58), V5 (NGM-17 X T-42) and V15 (PL-58 X BN-10) were having high seed yield per plant and yield contributing components. The genotypes with superior oil content were V11 (T-42 X NGM-17), V10 (T-42 X GPM-O-58), V19 (T-42 X PL-58) and V33 (T-42) which can be utilized for evolving mustard varieties with high seed yield and oil content.

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