



## Evaluation and diversity analysis in Indian mustard [*Brassica juncea* (L.) Czern & Coss.] germplasm accessions on the basis of principal component analysis

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**Abstract:** Principal component analysis was carried out with 20 morphological traits (including quantitative as well as qualitative) among 96 germplasm lines of Indian mustard [*Brassica juncea* (L.) Czern & Coss.]. Principal factor analysis led to the identification of eight principal components (PCs) which explained about 70.41% variability. The first principal component (PC1) explained 16.21% of the total variation. The remaining PC's explained progressively lesser and lesser of the total variation. Varimax Rotation enabled loading of similar type of variables on a common principal factor (PF) permitting to designate them as yield factor, maturity factor and oil factor etc. Based on PF scores and cluster mean values the germplasm accessions viz., RC2, RC32 and RC51 (cluster I), RC95 and RC96 (cluster X) were found superior for seed yield/plant and yield related factors like primary and secondary branches/plant; while the accessions RC34, RC185 and RC195 (cluster III) and RC53 (cluster VIII) were found superior for oil content. These accessions may further be utilized in breeding programmes for evolving mustard varieties having high seed yield and oil content. Hierarchical cluster analysis resulted into ten clusters containing two to 26 accessions. The results of cluster and principal factor analyses were in confirmation of each other.

**Keywords:** Cluster, Diversity, Germplasm, Indian mustard, Principal component

### INTRODUCTION

*B. juncea* (L.) Czern & Coss., commonly known as Indian mustard is an amphidiploid species that originated through the interspecific hybridization of *Brassica rapa* and *Brassica nigra* (Nagaharu U, 1935). In most of the regions of the world, its cultivation has increased drastically during the last decades due to its inherent high yielding ability and relative tolerance to biotic and abiotic stresses and, by now, it is the third largest contributor of the world supply of vegetable oil. It is one of the most important oil and protein rich annual crops in the world. At national level it is grown over an area of 6.45 million ha with production and productivity of 7.28 million tons and 1128 kg/ha, respectively (Anonymous, 2015).

The utilization of a species into any crop improvement breeding programme depends not only on the degree of genetic diversity it holds, but also on the precise information regarding genetic divergence and relatedness among breeding materials. Agronomic characterization is a useful tool for the genotypic classification, varietal identification in seed production programmes through DUS traits and it also allows plant breeders to select valuable genetic resources to be strategically utilized

in different breeding programmes (Singh *et al.*, 2013). Therefore, the choice of suitable parents is a matter of great concern to the plant breeders. The principal component and factor analysis is an important tool for the assessment of genetic divergence among the parents/genotypes and also to assess the relative contribution of particular trait to the total variability. Principal component analysis 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 study was planned with the objectives of assessment of genetic divergence and principal component and factor analyses in 96 germplasm accessions of Indian mustard.

### MATERIALS AND METHODS

Ninety six germplasm accessions (Table 1) of Indian mustard (*Brassica juncea*) were grown in paired rows of 5 m length each at a spacing of 30 x 15 cm row to row and plant to plant at Oilseeds Research Area, Department of Genetics and Plant Breeding, CCS HAU, Hisar during *rabi*, 2015-16. Recommended package of practices to raise a good crop was followed. Observa-

tions were recorded on five randomly selected plants in each germplasm accession on 14 morphological quantitative variables *viz.*, plant height (cm), days to flowering, days to maturity, number of primary branches/plant, number of secondary branches/plant, seed yield/plant (g), seeds/siliqua, main shoot length (cm), siliqua length (cm), siliqua density, siliqua number on main shoot, number of lobes/leaf, 1000-seed weight (g) and oil content (%).

Data were also recorded on six qualitative traits by giving scores in accordance with the standard DUS descriptor. These traits were leaf angle [1-erect (>85°), 3-semi-erect (66-85°), 5-open (46-65°), 7-semi prostrate (31-45°) and 9-prostrate (<31°)], leaf hairiness (1-absent, 3-sparse, 7-dense), leaf colour (1-light green, 2-medium green, 3-dark green, 4-purple green, 5-purple), leaf dentations (1-entire, 3-auriculate, 5-lyrate, 7-pointed), siliqua angle with main shoot [3-appressed (<21°), 5-semi-appressed (21-30°), 7-open (>30°)] and seed colour (1-yellow, 2-dull grey, 3-reddish brown, 4-brown, 5-black).

Principal factor and cluster analyses were carried out 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 factors loading were not clearly interpretable, the factor axes were rotated using Varimax Rotation. The correlation values ≥0.5 between the traits and principal components were considered for constructing the relationship between the traits and that 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. UPGMA (Unweighted Pair-Group Method using Arithmetic Averages) method of Hierarchical Cluster analysis was utilized with city block distances to classify all the 96 germplasm accessions and dendrogram was prepared using the rescaled distances. Based on the method suggested by Romesburg (1990), the dendrogram was cut to form the clusters.

**RESULTS AND DISCUSSION**

Principal component analysis indicated that only the first eight principal components (PCs) showed eigen values more than one and they cumulatively explained 70.41% of the total variability. The first PC (PC1) explained 16.21% of the total variation and the remaining seven principal components explained 11.19, 9.36, 8.71, 7.07, 6.69, 6.01 and 5.13% 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. Similar results have also been reported earlier by Zada *et al.* (2013) in Ethiopian mustard; Avtar *et al.* (2014) in toria and Neeru *et al.* (2015) in Indian mustard.

The analysis without rotation of axes failed to load all the variables signifying that it could not offer much information regarding the idea of correlation between the variables and the principal components. Varimax Rotation was applied and this resulted in loading of all the variables on different principal components. Factors' loadings of different variables thus obtained are

**Table 1.** List of 96 germplasm accessions of Indian mustard.

Sr. No.	Accession No.	Sr. No.	Accession No.	Sr. No.	Accession No.	Sr. No.	Accession No.
1	RC2	25	RC34	49	RC91	73	RC152
2	RC5	26	RC35	50	RC93	74	RC154
3	RC6	27	RC36	51	RC95	75	RC161
4	RC7	28	RC37	52	RC96	76	RC162
5	RC8	29	RC38	53	RC99	77	RC163
6	RC12	30	RC46	54	RC102	78	RC164
7	RC13	31	RC47	55	RC104	79	RC165
8	RC14	32	RC48	56	RC105	80	RC166
9	RC15	33	RC49	57	RC106	81	RC171
10	RC18	34	RC50	58	RC107	82	RC174
11	RC20	35	RC51	59	RC108	83	RC175
12	RC21	36	RC52	60	RC110	84	RC185
13	RC22	37	RC53	61	RC111	85	RC195
14	RC23	38	RC54	62	RC112	86	RC260
15	RC24	39	RC57	63	RC114	87	RC261
16	RC25	40	RC61	64	RC116	88	RC263
17	RC26	41	RC74	65	RC118	89	RC264
18	RC27	42	RC77	66	RC127	90	RC265
19	RC28	43	RC78	67	RC129	91	RC268
20	RC29	44	RC81	68	RC134	92	RC270
21	RC30	45	RC85	69	RC135	93	RC273
22	RC31	46	RC86	70	RC142	94	RC275
23	RC32	47	RC87	71	RC148	95	RC280
24	RC33	48	RC89	72	RC150	96	RC283

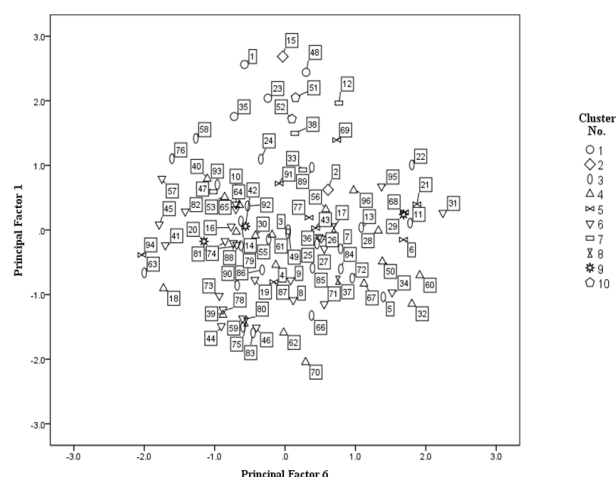
**Table 2.** Total variance explained by different Principal Components among Indian mustard accessions.

Principal component	Eigen value	Per cent variance	Per cent cumulative variance
1	3.24	16.216	16.216
2	2.23	11.193	27.409
3	1.87	9.364	36.773
4	1.74	8.716	45.488
5	1.41	7.077	52.565
6	1.34	6.699	59.264
7	1.20	6.015	65.280
8	1.02	5.130	70.410

presented in Table 3. The first principal factor (PF1) ascribed for three variables in total could be designated as yield factor as it enabled high loadings of seed yield with two of its most important component traits viz. primary and secondary branches/ plant. PF2 had high loadings of three variables i.e. days to flowering, plant height and days to maturity and could be designated as maturity factor. PF3 had high loadings of two variables i.e. seeds/siliqua and main shoot length. PF4 could be called as siliqua factor as three variables viz. siliqua length, siliqua density and siliqua number on main shoot were loaded on this factor. Three variables viz. number of lobes/leaf, leaf dentations and 1000-seed weight were loaded on the principle factor 5. PF6 could be designated as oil factor as only oil content variable is loaded on this factor. Three variables viz. leaf colour, seed colour and leaf hairiness were loaded on the principle factor 7 and could be designated as colour factor. PF8 had high loadings of two variables i.e. leaf angle and siliqua angle and could be designated as angle factor. Avtar *et al.* (2014) in toria and Singh *et al.* (2014) in Indian mustard also reported loading of similar type of variables on a common principal factor (PF).

**Table 3.** Factor loadings of different characters with respect to different Principal Factors (Varimax rotation) in Indian mustard accessions.

Traits	PF 1	PF 2	PF 3	PF 4	PF 5	PF 6	PF 7	PF 8
No. of secondary branches/plant	0.876*	0.077	-0.160	0.105	-0.097	0.050	0.000	0.125
No. of primary branches/plant	0.818*	0.193	-0.085	0.080	-0.136	0.037	-0.048	0.010
Seed yield/plant (g)	0.786*	-0.103	0.196	-0.122	0.233	-0.109	-0.164	-0.159
Days to flowering	0.130	0.887*	-0.140	0.096	0.052	-0.013	0.132	0.047
Plant height (cm)	-0.019	0.768*	0.255	0.214	0.035	-0.024	0.037	0.184
Days to maturity	-0.057	0.642*	0.136	0.136	0.030	-0.065	0.091	-0.046
Seeds/siliqua	0.067	-0.014	0.920*	0.242	0.048	0.061	0.117	-0.039
Main shoot length (cm)	-0.191	-0.098	0.876*	-0.317	0.044	-0.061	-0.084	0.035
Siliqua length (cm)	0.033	-0.016	0.029	0.837*	0.042	0.088	0.102	-0.052
Siliqua density	0.346	0.126	-0.018	0.702*	-0.036	0.145	0.259	-0.102
Siliqua no. main shoot	0.255	0.071	-0.088	0.485*	-0.294	0.422	0.175	-0.233
No. of lobes/leaf	0.019	0.029	-0.008	0.024	0.719*	0.026	0.346	0.158
Leaf dentations	0.086	0.496	0.013	0.005	0.625*	-0.041	-0.073	-0.311
1000-seed weight (g)	-0.323	-0.375	0.249	-0.102	0.574*	-0.069	-0.185	-0.145
Oil content (%)	-0.051	0.115	0.140	0.260	-0.006	0.808*	-0.123	-0.048
Leaf colour	-0.035	0.097	0.109	0.279	-0.020	-0.041	0.675*	-0.051
Seed colour	0.029	0.113	-0.052	-0.049	-0.072	-0.035	0.653*	-0.033
Leaf hairiness	-0.107	0.182	-0.027	-0.070	0.028	-0.067	0.644*	0.052
Leaf angle	-0.077	0.101	0.005	-0.038	0.364	0.329	-0.386	0.332*
Siliqua angle	0.034	0.165	-0.009	0.027	-0.015	-0.026	0.089	0.854*



**Fig. 1.** Distribution of Indian mustard accessions based on Principal Factors 1 and 6.

From the present analysis it was observed that number of primary branches/plant, number of secondary branches/plant, seed yield/plant, days to flowering, plant height, days to maturity, seeds/siliqua and main shoot length were the major distinct variability contributing traits which accounted for nearly half (36.77%) of the total variation (70.41%) in the set of 96 germplasm accessions. Thus, the successful transformation of 20 morphological variables into eight independent principal factors by means of grouping of similar type of variables on different principal factors elaborated and explained 70.41% of the variability of the original set. These findings are in tune with those obtained by Neeru *et al.* (2015) in Indian mustard.

The UPGMA method with City Block distances in hierarchical cluster analysis divided the accessions into ten clusters (C). Cluster membership of different accessions is presented in Table 4. Maximum number of

**Table 4.** Clustering pattern of 96 germplasm accessions of Indian mustard.

Cluster No.	Germplasm lines	No. of lines
CI	RC2, RC32, RC51, RC89	4
CII	RC5, RC24	2
CIII	RC6, RC8, RC13, RC20, RC22, RC23, RC31, RC33, RC34, RC91, RC104, RC107, RC108, RC114, RC127, RC150, RC162, RC165, RC175, RC185, RC195, RC260, RC264, RC265, RC270, RC273	26
CIV	RC7, RC27, RC37, RC46, RC48, RC57, RC61, RC93, RC99, RC102, RC105, RC110, RC111, RC112, RC116, RC129, RC142, RC283	18
CV	RC12, RC30, RC78, RC134, RC135, RC163, RC261, RC268, RC275	9
CVI	RC14, RC15, RC18, RC25, RC28, RC35, RC36, RC47, RC50, RC52, RC74, RC81, RC85, RC86, RC106, RC118, RC148, RC152, RC154, RC164, RC166, RC171, RC174, RC263, RC280	25
CVII	RC21, RC49, RC54, RC87	4
CVIII	RC26, RC53, RC161	3
CIX	RC29, RC38, RC77	3
CX	RC95, RC96	2

**Table 5.** Cluster means and general means for different characters in Indian mustard.

Traits/cluster number	CI	CII	CIII	CIV	CV	CVI	CVII	CVIII	CIX	CX	General Mean
No. of secondary branches/plant	28.55	23.50	18.82	16.12	20.11	17.32	21.48	18.67	23.40	28.10	21.61
No. of primary branches/plant	6.38	6.35	5.10	4.71	5.58	4.96	5.83	4.97	5.43	5.30	5.46
Seed yield/plant (g)	29.50	23.17	13.24	15.20	15.67	13.48	26.25	8.11	8.33	33.67	18.66
Days to flowering	51.25	47.50	47.77	44.78	52.33	48.56	45.75	55.00	44.67	46.00	48.36
Plant height (cm)	224.20	203.95	215.10	195.10	245.52	228.12	209.63	244.40	178.20	199.10	214.33
Days to maturity	150.75	144.50	145.08	145.28	146.44	146.96	147.25	150.33	144.00	144.00	146.46
Seeds/silique	13.05	14.10	12.58	12.87	12.08	11.85	13.40	13.63	12.40	10.80	12.68
Main shoot length (cm)	73.98	55.95	77.02	86.63	77.42	94.52	95.58	56.30	59.33	82.95	75.97
Silique length (cm)	3.30	3.50	3.28	3.51	3.34	3.37	3.63	3.27	3.40	2.85	3.35
Silique density	0.74	0.88	0.70	0.66	0.73	0.67	0.70	0.77	0.65	0.76	0.73
Silique no. main shoot	54.58	49.45	54.05	56.33	56.60	63.56	66.65	43.30	38.63	62.80	54.59
No. of lobes/leaf	5.50	4.00	5.35	5.33	5.44	4.64	5.25	4.00	4.00	4.00	4.75
Leaf dentations	4.50	3.00	4.46	4.78	2.78	4.28	5.50	3.00	4.33	4.00	4.06
1000-seed weight (g)	3.20	2.63	3.14	3.84	3.01	3.42	3.55	2.64	3.03	3.15	3.16
Oil content (%)	39.80	39.50	40.25	39.73	39.59	40.35	40.55	40.23	39.10	39.30	39.84
Leaf colour	1.75	2.00	1.96	1.94	2.33	1.92	2.25	2.33	1.67	2.00	2.02
Seed colour	3.00	3.00	2.77	3.11	3.33	2.88	3.50	3.00	3.67	3.00	3.13
Leaf hairness	2.50	2.00	2.77	3.22	3.00	3.00	2.50	7.00	3.00	3.00	3.20
Leaf angle	5.00	4.00	5.08	5.22	5.67	5.08	5.00	5.00	5.00	6.00	5.11
Silique angle	4.50	5.00	4.77	4.78	5.67	5.08	4.00	5.67	5.00	6.00	5.05

**Table 6.** Inter-and intra-cluster distances in Indian mustard accessions

Cluster	CI	CII	CIII	CIV	CV	CVI	CVII	CVIII	CIX	CX
CI	41.00									
CII	80.94	30.36								
CIII	75.71	78.27	52.17							
CIV	99.33	88.93	68.58	48.52						
CV	81.22	109.93	80.18	104.28	49.05					
CVI	89.33	115.75	71.02	82.51	75.94	47.04				
CVII	83.32	97.75	77.33	74.41	106.63	71.18	34.00			
CVIII	106.99	102.80	103.19	138.72	84.11	111.66	154.78	38.88		
CIX	129.16	81.26	100.03	94.5	139.93	141.89	138.51	114.89	24.12	
CX	96.41	88.85	82.43	77.26	107.09	94.97	66.88	157.77	114.41	26.20

accessions *i.e.* 26 were grouped in Cluster III (CIII) followed by 25 lines in cluster VI (CVI) whereas, only two accessions each were grouped in clusters CII and CX. The clusters I, IV, V, VII, VIII and IX comprised 4, 18, 9, 4, 3 and 3 lines, respectively. Similar results were obtained by Doddabhimappa *et al.* (2010), Singh (2012) and Neeru *et al.* (2015).

Using the principal factor scores (PF scores), graph was plotted to represent the position of genotypes on X and Y-axis by taking two most important factors *i.e.* yield factor and oil factor and to chalk out the breeding plan for further improvement by identifying superior parents for hybridization/ crossing programme. In Fig. 1, all the accessions were plotted for PF1 (seed yield

and its important components) and PF6 (oil content). Based on PF scores and cluster mean values (Table 5) the accessions *viz.* RC2, RC32 and RC51 (cluster I), RC95 and RC96 (cluster X) were found superior for seed yield/plant and yield related factors like primary and secondary branches/plant; while the accessions RC34, RC185 and RC195 (cluster III) and RC53 (cluster VIII) were found superior for oil content. From the foregoing discussion it can decisively be concluded that these accessions can be used as parents in hybridization programme for evolving Indian mustard varieties with high seed yield and oil content as well or for obtaining transgressive segregants superior for these traits of superiority in the segregating generations. Also, based on the PF scores and cluster mean values, the germplasm lines RC21, RC49 and RC54 were found superior for both yield and oil content. Similarly, 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.

The perusal of the data in Table 5, reveals that CI comprised germplasm accessions with more number of primary branches/plant, secondary branches/plant and seed yield. Similarly, genotypes grouped in CII had high siliqua density, semi-appressed siliqua angle and more seeds/siliqua. Cluster III was characterized with the accessions having high oil content and medium green foliage colour. Accessions of CIV had bold seeds and medium earliness in days to flowering. Lines grouped in CV were characterized with lesser leaf dentations, higher number of lobes/leaf and open type of leaf angle. High siliqua number on main shoot and high oil content were observed in the member accessions of CVI whereas, CVII was characterized with high siliqua number on main shoot, more siliqua length, high oil content and high seed yield/plant. The accessions grouped in CVIII were characterized with more leaf hairiness and high oil content. Cluster IX had lines with less plant height, medium earliness in flowering, semi-appressed siliqua angle and seed colour varied between red-brown to brown. Cluster X was having only two accessions which recorded the highest seed yield/plant.

As hybridization among diverse parents is likely to produce heterotic hybrids and desirable transgressive segregants in further generations, grouping germplasm lines in different clusters gives an opportunity for selecting them to serve the objectives in developing genotypes with specific characters. To assess the diversity inter and intra-cluster distances were calculated which are presented in Table 6. Inter-cluster distance was maximum between clusters VIII and X (157.77) followed by between CVII and CVIII (154.78) and CVI and CIX (141.89), whereas, the minimum inter-cluster distance was observed between CVII and CX (66.88)

followed by CIII and CIV (68.58). The crosses between the germplasm lines belonging to distantly located clusters are likely to produce good transgressive segregants and germplasm lines with better mean values can be selected among all the genotypes to suit the breeding programme. Maximum intra-cluster distance was observed in the cluster III (52.17) followed by in CV (49.05) and minimum in the CIX (24.12). Similarly, Pandey *et al.* (2013) evaluated 45 Indian mustard genotypes for the extent of diversity present and the maximum inter-cluster distance was found between clusters II and III indicating high genetic divergence among genotypes of these groups. Based on the results of the present study, it is recommended to use the diverse accessions RC2, RC32 and RC51 (cluster I), RC95 and RC96 (cluster X); and RC34, RC185 and RC195 (cluster III) and RC53 (cluster VIII) as one of the parents for improving seed yield, its important components along with oil content. These were the accessions which also got plotted on the better ends of scatter plot based on PF scores and hence, the results of cluster analysis and principal factor analysis confirmed each other.

## Conclusion

On the basis of present study, it can be concluded that the Indian mustard germplasm accessions having high seed yield/plant and yield contributing component traits *viz.*, RC2 (33.3 g), RC32 (30.7 g), RC51 (29.3 g), RC95 (35.0 g) and RC96 (32.3 g); and the accessions with superior oil content *viz.* RC34 (41.1%), RC53 (42.2%), RC185 (40.0%) and RC195 (40.7%) can be utilized in trait combination breeding programmes for evolving mustard varieties with high seed yield and oil content.

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