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Research Article

Multivariate analysis in Indian mustard genotypes for morphological and quality traits

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Abstract
Multivariate analysis was carried out with 20 morphological (including quantitative as well as qualitative) and five oil quality traits in 43 genotypes of Indian mustard. Principal factor analysis led to the identification of nine principal components (PCs) which explained about 77% variability. The first principal component (PC1) explained 16.65% 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 seed yield and component traits, leaf, oil and its quality factors. Based on PF scores, the genotypes *viz*., RH(OE)0801, EC597320, EC597341, EC597344, EC592579, EC592584 and JM6014(YS) have been identified superior for seed yield/plant, while the genotypes JM6009, JM6011, EC697334 and ZEM-1were found superior for oil content. Similarly, the genotypes JM6009, NUDBYJ-10, Pusa Mustard-21, RLC-2 and ZEM-2 showed superiority for erucic acid, whereas genotypes JM6004(YS), JM6026 and EC552583 exhibited superiority for glucosinolate content. These genotypes may further be utilized in breeding programmes for evolving mustard varieties having high seed yield and oil content; and with superior oil quality. Hierarchical cluster analysis resulted into eight clusters containing one to 16 genotypes. The results of cluster and principal factor analyses confirmed each other.

Key words
Indian mustard, cluster, principal factor, components and variability

Introduction

Indian mustard [*Brassica juncea* (L.) Czern & Coss.] is the premier oilseed *Brassica* which covers about 85-90% of the total area under cultivation of all these crops. At national level it is grown over an area of 5.79 million ha with production and productivity of 6.31 million tons and 1089 kg/ha, respectively (Anonymous, 2015). The major area under this crop exists in Rajasthan state followed by M.P., U.P., Haryana and West Bengal. The mustard oil is considered as the healthiest and nutritionally better vegetable oil. Moreover, it is main source of cooking medium in north- eastern parts of India. It consists of fatty acids like omega alpha-3 and omega alpha-6 which have beneficial properties.

The past experiences in mustard breeding indicate that there is an immense scope for increasing the seed yield to new levels by reshuffling the genes through hybridization in suitable parents and exploitation of heterosis may also play significant role for developing better genotypes. Therefore, the choice of suitable parents is a matter of great concern to the plant breeders. The multivariate 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. Thus, keeping all this in view, the present study was planned with the objectives of assessment of genetic divergence and principal component and factor analysis in 43 different Indian mustard genotypes.

Materials and methods

The experimental material for the present study comprised 43 different genotypes of Indian mustard which were grown during *rabi*, 2014-2015 in paired rows of 5 m length each with two replications under randomized block design at Oilseeds Research Area, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar. Recommended package of practices to raise a good crop was followed. Observations were recorded on five randomly selected plants in each genotype on15 morphological quantitative variables *viz.*, number of lobes/leaf, leaf length (cm), leaf width (cm), days to 50% flowering, days to maturity, plant height (cm), primary branches/plant, secondary branches/plant, main shoot length (cm), number of siliquae on main shoot, siliqua length (cm), number of seeds/siliqua, 1000-seed weight (g), seed yield/plant (g) and oil content (%). Observations were also recorded on five qualitative traits by giving scores in accordance with the standard DUS descriptor. These traits were leaf hairiness (1-absent, 3-sparse, 7-dense), leaf colour (1-light green, 2-medium green, 3-dark green, 4-purple green, 5-purple), dentation of leaf margin (1-entire, 3-auriculate, 5-lyrate, 7-pointed), siliqua angle with main shoot [3-appressed ($<21^0$),

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5-semi-appressed (21-30^0), 7-open (>30^0)] and seed colour (1-yellow, 2-dull grey, 3-reddish brown, 4-brown, 5-black). In addition to these, four fatty acids (oleic acid, linoleic acid, linolenic acid and erucic acid) (%) were estimated using the Gas Liquid Chromatography, whereas glucosinolate content **(**µmole/g of defatted seed meal) was estimated using Eliza Reader.

Principal factor and cluster analysis was carried out using SPSS 10.0 (Morrison, 1978). 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 43 genotypes 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 nine principal components (PCs) showed eigen values more than one and they cumulatively explained 77.16 % of the total variability. The first PC (PC1) explained 16.65 % of the total variation and the remaining eight principal components explained 11.87, 10.58, 8.89, 7.38, 6.70, 5.34, 4.96 and 4.75 % variation, respectively (Table 1). 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 Yousuf *et al.*(2011) in *Brassica campestris*, 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 presented in Table 2. 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 two important fatty acids along with plant height. Rest of the yield components were loaded on PF3. PF4 could be called as leaf factor as three leaf related traits like number of lobes/leaf, leaf length and leaf width were loaded on this factor. PF5 could be designated as oil factor along with seed boldness and siliqua length. The sixth principal factor was flowering and maturity factor and PF8 exhibited loading of only one quality trait i.e. glucosinolate content. Singh *et al*. (2010) in Ethiopian mustard, 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).

From the present analysis it was observed that number of primary branches/plant, number of secondary branches/plant, seed yield/plant, plant height, leaf color, number of siliqua on main shoot, main shoot length and siliqua angle with main shoot were the major distinct variability contributing traits which accounted for nearly half of the total variation in the set of 43 genotypes. Thus, the successful transformation of 20 morphological variables into nine independent principal factors by means of grouping of similar type of variables on different principal factors elaborated and explained 77 % of the variability of the original set. These findings are in tune with those obtained by Yousuf *et al.* (2011) in *Brassica campestris* and Neeru *et al*. (2015) in Indian mustard.

Using the principal factor scores (PF scores), three different graphs were plotted to represent the position of genotypes on X and Y-axis taking two most important factors at one time and to chalk out the breeding plan for further improvement by identifying superior parents for hybridization/ crossing programme. In Fig. 1, all the genotypes were plotted for PF1 (seed yield and its important components) and PF5 (oil content), in Fig. 2 the genotypes were plotted for PF5 (oil content) and PF2 (erucic acid content) and Fig. 3 represents plotting of all the genotypes taking PF5 (oil content) and PF8 (glucosinolate content). The perusal of Fig. 2 indicates that the genotypes EC597334, EC597341, JM6009 and RH(OE)0801 which were found superior for oil content, stood out towards the positive portion of PF5 axis in the plot, whereas the genotypes which had low erucic acid content clustered towards the negative side of PF2 axis. Such genotypes were JM6009, NUDBYJ-10, Pusa Mustard-21, RLC-2, ZEM-2 and EC552578-1. The genotypes which found place towards the positive end of PF5 and negative end of PF2 are supposed to be superior for both oil

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content and low erucic acid. On the basis of present investigation, genotypes JM6009, RLC-2 and ZEM-2 have been identified superior by taking both high oil content and low erucic acid collectively. 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; and with better oil quality as well or for obtaining transgressive segregants superior for all these traits of superiority in the segregating generations.

The UPGMA method with City Block distances in hierarchical cluster analysis divided the genotypes into eight clusters (C). Cluster membership of different genotypes is presented in table 3. Maximum number of genotypes *i.e.* 16 were grouped in Cluster IV (CIV) whereas, only one genotype each was grouped in clusters CIII and CVII. The clusters II, V, VI and VIII comprised 4, 12, 2, 4 and 3 genotypes, respectively. The relative association among different genotypes is presented in the form of dendrogram (Fig. 4) which was prepared using the rescaled distances. The resemblance coefficient between two genotypes is the value at which their branches join. The dendrogram elaborates the relative magnitude of resemblance among the genotypes as well as the clusters. This analysis further showed that the genotypes from different geographic regions were grouped together into the same cluster and vice-versa which suggested that geographical diversity does not necessarily represent genetic diversity and this may be due to free exchange of genetic material among different regions and due to operation of similar forces of natural and artificial selection resulting in perpetuation and stabilization of similar genotypes (Murty and Arunachalam, 1966). These results are in full agreement with those obtained earlier in Indian mustard by Sutariya *et al.* (2011), Singh (2012) and Shekhawat *et al.* (2014). Therefore, geographic diversity although important, was not the only factor responsible for determination of the genetic diversity among the genotypes.

Cluster-wise mean and general mean for all the traits studied are presented in table 4.The mean performance of different clusters revealed wide range of variation among these with respect to different traits. The perusal of the data reveals that CI comprised genotypes with more number of siliquae on main shoot, high oil and low glucosinolate content. Similarly, genotypes grouped in CII had high seed yield/plant with bold seeds. Cluster III which comprised only one genotype was characterized with long and broad leaves and open siliqua angle whereas, genotypes of CIV had more number of primary and secondary branches/plant and higher seed yield. Genotypes grouped in CV were earliest in 50% flowering and maturity with reduced height in comparison to genotypes of other clusters. Longest main shoot as well as siliqua were observed in the member genotypes of CVI whereas, CVII having only one genotype was characterized with more number of leaf lobes, dark green leaves with dense hairiness and pointed leaf margins. Three genotypes were grouped in CVIII which were tallest in height, had highest number of seeds/siliqua and desirable oil quality traits like high oleic acid and low erucic acid content.

As hybridization among diverse parents is likely to produce heterotic hybrids and desirable transgressive segregants in further generations, grouping genotypes 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 5. Inter-cluster distance was maximum between clusters I and VII (11788.81) followed by between CIII and CVII (9695.28) and CI and CVI (8582.15), whereas, the minimum inter-cluster distance was observed between CI and CV (1395.65). The crosses between the genotypes belonging to distantly located clusters are likely to produce good transgressive segregants and genotypes 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 IV (964.17) followed by in C VIII (831.61) and minimum in the CV (350.45). Intra-cluster distances were zero in CIII and CVII due to grouping of only one genotype in these clusters which were unique in characteristics. Results obtained in the present study are similar with those reported earlier by Lodhi *et al.* (2013), Avtar *et al.* (2014), Shekhawat *et al.* (2014), Mekonnen and Wakjira (2014) and Neeru *et al.* (2015) in different Brassica species. Based on the results of the present study, it is recommended to use the diverse genotypes, JM6014 (YS) and EC552584 (members of CIV) and JM6009 and JM6011 (members of CII) as one of the parents for improving seed yield, its important components and oil content. Similarly, for improving oil quality, the genotypes NUDBYJ-10, Pusa Mustard-21, ZEM-2 and JM6004 (YS) may be utilized as good source lines. 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.

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Table 1. Total variance explained by different principal components in Indian mustard

Principal component	Eigen value	Variability (%)	Cumulative variability (%)
1	4.16	16.65	16.65
2	2.96	11.87	28.53
3	2.64	10.58	39.11
4	2.22	8.89	48.01
5	1.84	7.38	55.40
6	1.67	6.70	62.10
7	1.33	5.34	67.45
8	1.24	4.96	72.41
9	1.18	4.75	77.16

Table 2. Factor loadings of traits with respect to different principal factors (Varimax Rotation) in Indian mustard

Trait/Principal factor	PF1	PF2	PF3	PF4	PF5	PF6	PF7	PF8	PF9
Secondary branches/plant	0.870*	-0.029	0.036	0.179	0.021	0.094	0.106	0.099	0.111
Primary branches/plant	0.835*	0.253	0.067	0.020	-0.135	0.026	-0.185	0.094	-0.026
Seed yield/plant (g)	0.790*	0.230	0.312	0.116	0.113	-0.041	0.079	-0.172	-0.126
Oleic acid (%)	-0.073	0.924*	0.041	0.058	-0.029	-0.113	-0.134	-0.134	-0.071
Erucic acid (%)	0.047	0.914*	0.195	-0.056	0.199	0.016	-0.106	0.029	0.004
Plant height (cm)	-0.306	0.681*	0.089	0.014	0.088	0.148	-0.183	0.240	0.025
Leaf colour	-0.220	0.068	0.727*	-0.002	0.080	-0.068	0.087	-0.163	0.131
Linolenic acid (%)	-0.174	-0.047	0.694*	-0.086	-0.169	0.177	0.001	0.105	-0.227
No. of siliqua on main shoot	0.060	0.364	0.626*	-0.005	-0.180	0.178	-0.045	-0.199	0.060
Main shoot length (cm)	-0.190	-0.309	0.491*	-0.204	0.233	-0.192	0.034	0.218	-0.453
Siliqua angle with main shoot	0.340	0.157	0.398*	0.010	0.379	-0.048	-0.222	-0.143	-0.291
No. of lobes/leaf	-0.008	-0.234	0.001	0.791*	-0.253	0.072	0.125	-0.030	-0.019
Leaf width (cm)	0.221	-0.138	0.168	0.768*	0.067	0.179	-0.030	-0.120	0.010
Leaf length (cm)	0.026	0.374	-0.180	0.727*	0.228	0.117	-0.046	0.082	0.065
Siliqua length (cm)	-0.096	0.071	-0.152	-0.134	0.781*	0.085	0.018	-0.031	0.300
Oil content (%)	0.057	0.000	-0.051	-0.227	0.665*	0.153	0.220	-0.213	0.130
1000- seed weight (g)	0.544	0.101	0.196	-0.101	0.645*	-0.060	0.068	-0.047	-0.125
Days to 50% flowering	0.129	0.052	0.081	0.221	0.002	0.904*	0.089	-0.094	0.020
Days to maturity	-0.084	0.004	-0.073	0.083	-0.061	0.890*	-0.076	-0.001	-0.125
Linoleic acid (%)	0.089	-0.032	-0.064	-0.047	-0.045	-0.019	0.848*	0.010	0.157
No. of seeds/ siliqua	0.558	-0.165	0.081	-0.142	-0.013	-0.070	0.676*	0.015	0.016
Seed colour	-0.002	0.198	0.020	0.208	-0.309	-0.117	0.575*	-0.462	-0.145
Leaf hairiness	0.086	0.368	0.304	-0.157	-0.033	0.293	0.429*	0.327	0.216
Glucosinolate content (µmole/g)	0.042	0.042	-0.008	-0.022	0.035	-0.106	-0.017	0.926*	-0.065
Dentation of leaf margin	-0.052	0.032	0.093	0.009	0.084	-0.133	0.123	-0.014	0.878*

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Table 3. Cluster membership and number of genotypes in each cluster of Indian mustard

Cluster No.	Genotypes	Number of genotypes
I	JM6004(YS) , JM6010, JM6026(YS) and EC552583	4
II	JM6009, JM6011, JM6018, EC597328, EC597320, EC597337, EC552581, RH (OE) 0801, EC597338 , NUDBYJ-10 , LES 47and ZEM-1	12
III	JM6012	1
IV	JM6014(YS), JM6015, EC597331, EC597333, EC597334, EC597335, EC597341, EC597344, EC597340, EC552573, EC552576, EC552578, EC552584, EC552579, Pusa Krishma and LES-46	16
V	EC597343 and RH (OE) 0902	2
VI	RH (OE) 0901, RH (OE) 0903, EC552578-1 and RLC-2	4
VII	EC597340	1
VIII	ZEM-2, Domo-4 and Pusa Mustard-21	3
Total		43

Table 4. Cluster means and general mean for different characters in Indian mustard

S. No.	Characters / Cluster No.	CI	CII	CIII	CIV	CV	CVI	CVII	CVIII	General Mean
1	No. of lobes/ leaf	8.1	8.2	8.0	8.2	8.5	7.7	8.8	8.3	8.2
2	Leaf length (cm)	48.3	50.8	57.8	51.9	48.8	48.1	53.2	46.9	50.7
3	Leaf width (cm)	19.6	21.2	22.4	20.2	19.3	19.6	19.2	20.7	20.3
4	Leaf hairiness	4.0	2.8	3.0	4.1	3.0	4.0	7.0	1.0	3.6
5	Leaf color	2.8	2.5	3.0	2.4	2.5	2.3	3.0	3.0	2.7
6	Dentation of leaf margin	5.0	3.8	3.0	3.5	5.0	3.5	7.0	3.7	4.3
7	Days to 50% flowering	49.3	49.6	48.0	49.7	45.0	46.8	47.0	48.3	47.9
8	Days to maturity	149.3	151.8	152.0	151.5	148.0	150.0	151.0	151.7	150.7
9	Plant height (cm)	212.3	222.0	204.3	219.8	193.5	242.9	244.0	256.4	224.4
10	Primary branches/plant	8.2	8.4	7.7	9.2	7.8	7.2	7.7	7.4	7.9
11	Secondary branches/plant	21.3	20.5	17.0	22.4	17.7	20.4	20.3	17.9	19.7
12	Main shoot length (cm)	65.3	77.3	67.7	74.5	72.5	91.9	59.7	81.2	73.8
13	No. of siliqua on main shoot	66.8	60.5	32.7	60.5	50.0	57.9	60.3	57.4	55.8
14	Siliqua length (cm)	3.9	4.0	3.8	3.9	3.6	4.1	3.7	3.9	3.9
15	Siliqua angle with main shoot	5.0	5.3	7.0	5.3	5.0	5.0	5.0	5.0	5.3
16	No. of seeds/siliqua	15.4	15.7	17.1	16.3	14.3	14.9	14.2	17.6	15.7
17	Seed yield/plant (g)	18.8	22.7	14.4	21.8	18.1	16.0	12.9	8.6	16.7
18	1000-seed weight (g)	3.5	4.4	4.0	4.0	3.6	3.9	3.8	3.4	3.8
19	Seed color	3.3	2.5	3.0	2.6	2.5	2.3	2.0	1.7	2.5
20	Oil content (%)	40.0	38.4	38.8	38.9	38.4	38.1	38.1	38.6	38.7
21	Oleic acid (%)	36.0	37.2	38.2	33.2	33.9	38.1	37.3	46.8	37.6
22	Linoleic acid (%)	29.1	27.4	25.3	27.3	27.8	27.7	30.7	23.2	27.3
23	Linolenic acid (%)	16.3	15.6	17.7	15.3	17.8	17.6	14.2	16.9	16.4
24	Erucic acid (%)	7.0	7.3	9.1	11.9	10.2	4.0	2.6	1.0	6.6
25	Glucosinolate content (µmole/g)	27.9	53.8	44.5	92.9	97.8	107.0	129.4	77.4	78.8

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Table 5. Inter and intra–cluster distances in Indian mustard

Cluster No.	CI	CII	CIII	CIV	CV	CVI	CVII	CVIII
CI	666.07							
CII	1726.53	819.73						
CIII	1996.55	1769.36	0.00					
CIV	5256.78	2445.36	4020.54	964.17				
CV	1395.65	3546.96	3607.28	1576.29	350.45			
CVI	8582.15	4212.89	7002.11	1841.63	3413.01	494.27		
CVII	11788.81	7055.58	9695.28	2738.21	4077.61	1854.00	0.00	
CVIII	5635.99	2823.13	5180.29	2774.98	5239.40	1922.55	3868.54	831.61

Diagonal – Intra-cluster distances Off-diagonal – Inter-cluster distances

Fig. 1. Distribution of Indian mustard genotypes based on Principal Factor 1 and 5

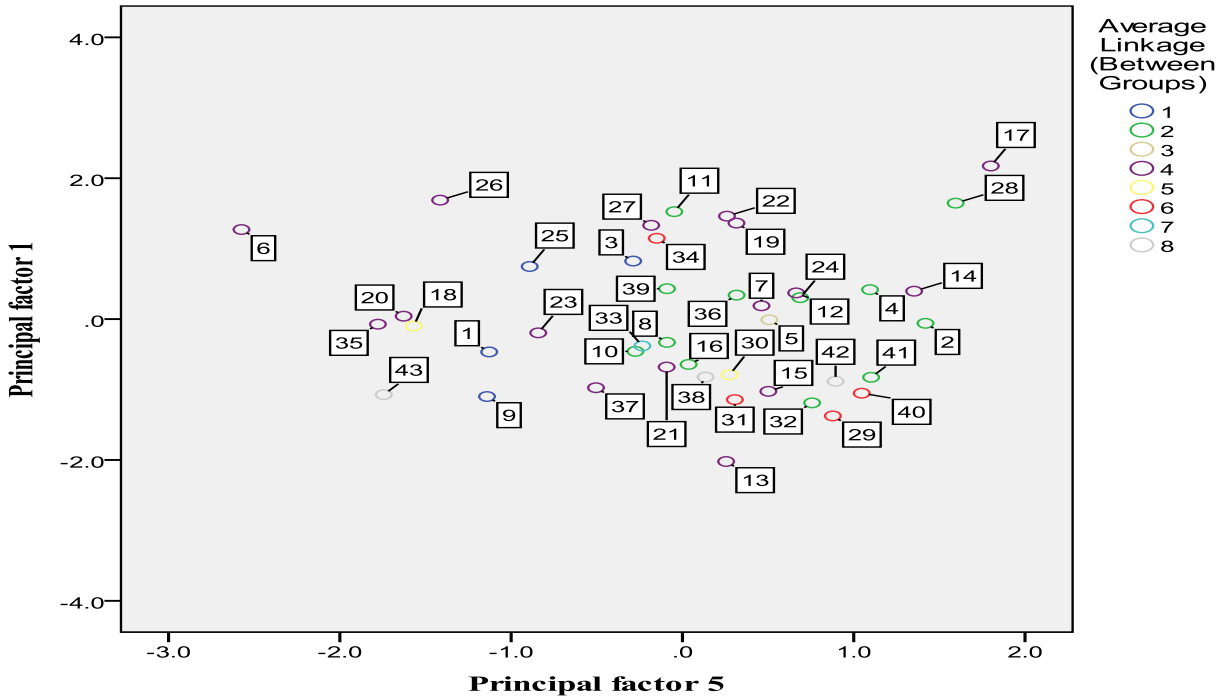

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Fig. 2. Distribution of Indian mustard genotypes based on Principal Factor 5 and 2

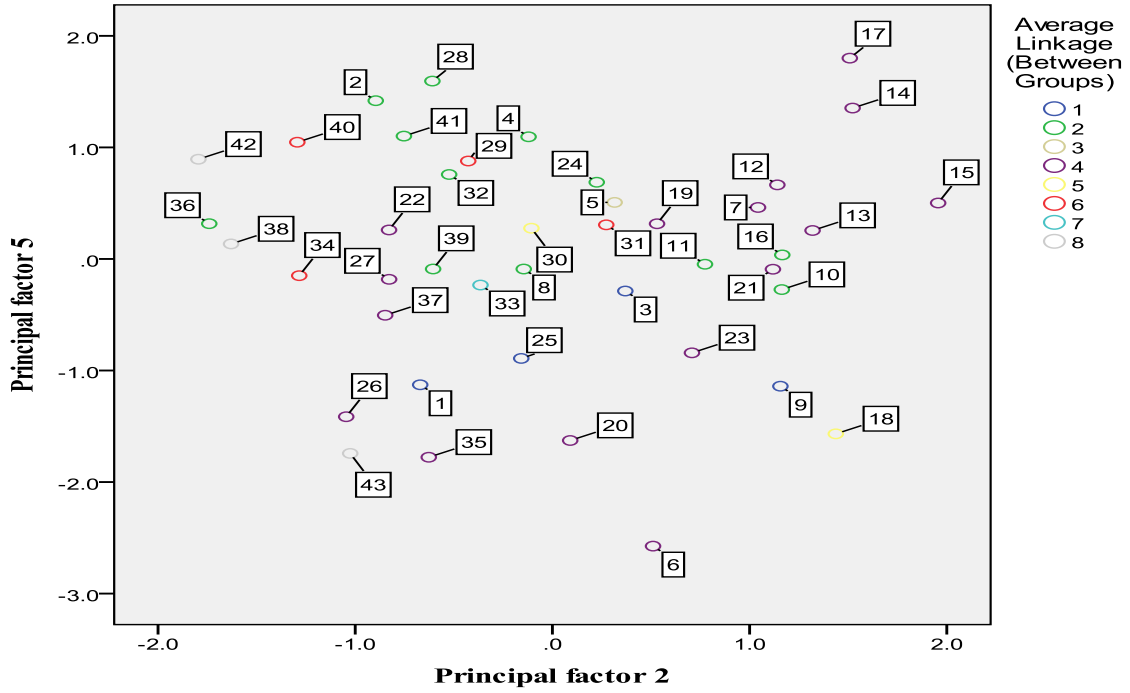

Fig. 3. Distribution of Indian mustard genotypes based on Principal Factor 5 and 8

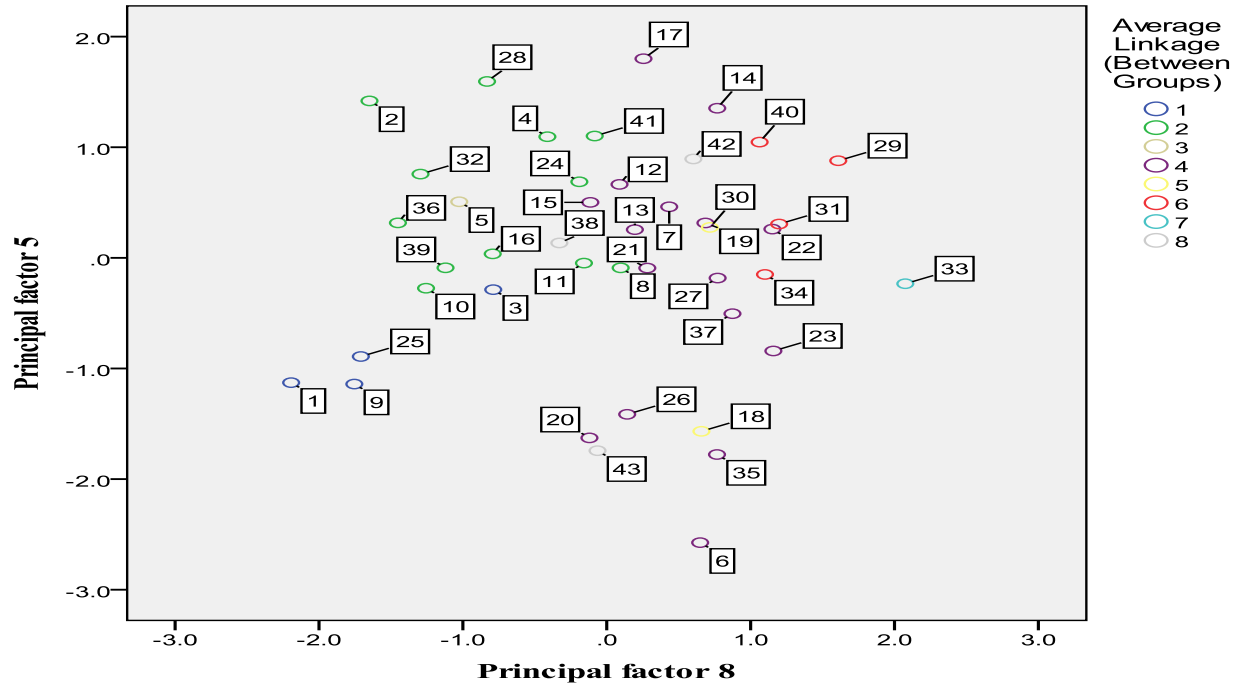

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Fig. 4. Dendrogram showing the clustering pattern of different Indian mustard genotypes