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Evaluation and classification of Indian mustard (*Brassica juncea* L.) genotypes using principal component analysis

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Abstract

Principal component and hierarchical cluster analyses were carried out with 25 quantitative and qualitative traits in 60 genotypes of Indian mustard (*Brassica juncea* L.). Principal factor analysis identified 11 principal components (PCs) which explained about 75% variability. PC1 had 13.19% of total variation in agro-morphological traits, PC2 depicted 10.07% of total morphological variability, and PC3 accounted for 8.56% of the total variation. Varimax rotation enabled loading of similar type of variables on a common principal factor permitting to designate them as seed yield, maturity, leaf and siliqua characters, and oil content factors. The genotypes JMM-937, RC-199, RH-0401(YS), Pusa Bold, Pusa Bahar, and KM-888 were found to be superior on the basis of principal factor scores with regard to seed yield, its main components, and oil content, when both the principal factors were considered together. These genotypes may further be utilized in breeding programmes for developing Indian mustard varieties with high seed yield and superior oil content. Hierarchical cluster analysis categorized all the 60 genotypes into 10 clusters containing one to 23 genotypes. Based on the inter-cluster distances, maximum genetic diversity was observed between clusters I and IV (221.4), followed between CII and IV clusters (200.5), C IV and C IX (191.8) and C IV and C X (181.5) indicating that genotypes from these clusters can usefully be hybridized for getting superior recombinants in segregating generations. The results of cluster and principal factor analyses confirmed each other.

Key words: Cluster, Indian mustard, principal factor, principal component and variability

Introduction

Indian mustard [*Brassica juncea* (L.) Czern & Coss.], an important species of the family brassicasae, is the second most important oilseed crop of India after groundnut. Inspite of many beneficial uses, it received adequate attention from the point of view of genetic improvement and management only after the inception of technological oilseeds mission in 1986. The requirement of edible oil is increasing at the rate of 4-5 per cent annually due to ever increasing population and improved standard of living. To meet out this increased demand, about 200 per cent increase in rapeseed mustard production is to be achieved within two decades. To further enhance the productivity, the past experiences in mustard breeding in India indicate that there is an immense potential for increasing the seed yield to new levels by reshuffling the genes through hybridization in suitable parents and exploitation of heterosis for developing better genotypes. Knowing genetic diversity of the existing genotypes and choice of suitable parents are essential before undertaking any crop improvement programme.

The multivariate analysis is an important tool for assessment of genetic divergence of the parents/ genotypes. Hamman (1972) suggested that the use of multivariate techniques could reduce several phenotypic measurements in large populations into fewer, more interpretable, and easily visualized dimensions. Initially, the importance of an individual variable is not known, and inclusion of various or all variables that have little or no connection with the problem, produces results that are very complicated.

Principal component analysis helps in identifying most relevant characters by explaining the total variation in the original set of variables with few of the components as possible, and reduces the complexity or dimension of the problem. Evaluation of germplasm is useful not only in selection of core collection, but also its utilization in breeding programmes. The present study, therefore, was planned with the objectives of evaluation and classification of sixty Indian mustard genotypes using principal component and factor analysis.

Material and Methods

The experimental material for the present investigation comprised of 60 Indian mustard genotypes which were grown during the *Rabi* 2011-2012 season at the research farm of the Oilseeds Section, Department of Genetics and Plant Breeding, CCS HAU, Hisar, India. Each genotype was grown in a plot size of 1.5m x 3m with a spacing of 30 and 10 cm row to row, and plant to plant, respectively. All the recommended package of practices was followed to raise a good crop. Observations on five randomly selected plants per plot were recorded for 16 quantitative traits including number of lobes per leaf, leaf length (cm), leaf width (cm), days to 50% flowering, days to maturity, plant height (cm), primary branches per plant, secondary branches per plant, main shoot length (cm), number of siliquae on main shoot, siliqua density on main shoot, siliqua length (cm), number of seeds per siliqua, 1000- seed weight (g), seed yield per plant (g), and oil content (%). In addition, observations were also recorded for eight morphological traits by giving scores according to DUS descriptor as given in the parentheses for 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], leaf growth habit (angle b/w stem & petiole) [1- erect (>85^0), 3- semi erect (66-85^0), 5-open (46-65^0), 7- semi-prostrate (31-45^0), 9- prostrate (<31^0)], petal colour [1- white, 2- cream, 3- light yellow, 4- yellow, 5- orange], siliqua surface texture [1- smooth, 2- intermediate, 3- constricted], beak length(cm), [3-short(<0.8cm), 5- medium(0.8-1.2cm), 7- long(>1.2cm)], siliqua angle with main shoot [3-appressed (<21^0), 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].

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 factors 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 60 genotypes.

Results and Discussion

Only the first 11 PCs showed eigen values more than one, and they cumulatively explained 75.26 % variability (Table 1). The first PC explained 13.19 % of the total variation, and the remaining 10 PCs explained 10.07, 8.56, 7.58, 6.99, 5.57, 5.22, 4.94, 4.69, 4.40 and 4.05 % variation, respectively.

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

Principal Component	Eigen Value	Per cent Variability	Cumulative %Variability
1	3.30	13.19	13.19
2	2.52	10.07	23.26
3	2.14	8.56	31.82
4	1.90	7.58	39.40
5	1.75	6.99	46.39
6	1.39	5.57	51.96
7	1.30	5.22	57.17
8	1.24	4.94	62.12
9	1.17	4.69	66.80
10	1.10	4.40	71.20
11	1.01	4.05	75.26

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

Characters / Principal Factors	PF1	PF2	PF3	PF4	PF5	PF 6	PF 7	PF 8	PF 9	PF 10	PF11
Leaf width(cm)	0.83*	0.07	0.15	-0.09	-0.12	-0.05	0.08	-0.06	-0.14	-0.15	0.01
Leaf length (cm)	0.80*	0.15	0.25	-0.08	-0.09	0.02	-0.08	-0.23	0.02	-0.06	-0.09
Days to 50% flowering	0.68*	0.08	-0.14	0.21	0.05	-0.08	0.02	0.28	0.19	0.29	-0.03
Days to maturity	0.63*	-0.08	-0.23	0.15	0.21	-0.03	-0.17	0.31	0.46	0.08	-0.07
Plant height (cm)	0.60*	0.07	-0.11	0.39	0.48	0.17	0.19	0.06	-0.02	0.17	-0.12
No. of siliquae on main shoot	0.16	0.91*	0.03	0.07	0.01	0.15	0.02	-0.06	0.05	-0.12	-0.03
Siliqua density on main shoot	-0.06	0.88*	0.08	0.05	-0.02	0.32	0.01	-0.07	0.01	-0.09	0.05
Oil content (%)	0.09	0.18	0.78*	-0.18	0.01	0.04	0.13	-0.04	0.00	-0.01	0.18
No. of lobes/ leaf	0.26	-0.23	0.68*	0.22	-0.11	-0.02	-0.18	0.01	0.06	0.01	-0.28
Beak length (cm)	-0.11	-0.28	0.61*	0.15	0.25	0.25	-0.01	-0.01	0.16	-0.09	-0.03
Siliqua surface texture	0.02	0.06	0.09	0.70*	0.09	0.04	-0.05	0.15	-0.09	-0.03	0.03
Siliqua angle with main shoot	0.00	-0.12	-0.07	0.70*	-0.39	-0.12	-0.07	-0.23	0.08	0.01	-0.05
Dentation of leaf margin	0.23	-0.11	0.01	0.11	0.77*	0.13	0.06	0.05	-0.14	-0.01	0.06
Main shoot length (cm)	0.26	-0.23	0.25	0.01	0.60*	-0.04	0.27	0.07	-0.08	0.02	0.31
Leaf colour	0.18	-0.04	0.06	0.20	0.01	0.78*	0.02	-0.31	0.09	-0.18	0.05
Petal colour	-0.27	-0.10	0.10	-0.24	-0.15	0.77*	-0.05	0.17	-0.09	0.11	0.04
Siliqua length (cm)	-0.08	0.17	0.04	0.31	-0.01	-0.05	0.71*	-0.01	0.14	0.08	0.00
Primary branches/ plant	-0.19	0.22	-0.02	0.18	0.16	-0.13	0.63*	0.30	0.01	-0.14	-0.41
Seed yield/plant (g)	-0.01	0.27	0.44	0.35	0.13	-0.02	0.60*	0.03	0.19	-0.10	-0.09
Seed colour	-0.03	0.00	-0.01	0.04	0.01	-0.09	0.03	0.81*	0.00	0.05	0.05
Secondary branches/plant	-0.01	0.01	-0.17	0.09	-0.10	-0.06	0.10	-0.04	0.83*	0.13	0.12
No. of seeds / siliqua	0.01	0.19	-0.01	0.08	-0.13	-0.17	0.45	-0.15	0.63*	0.30	0.21
Leaf growth habit	0.06	-0.06	-0.04	-0.06	0.01	0.01	-0.06	0.11	0.00	0.87*	0.05
Leaf hairiness	-0.20	0.04	-0.02	0.10	0.09	-0.17	-0.11	-0.46	-0.12	0.65*	-0.24
1000-seed weight (g)	-0.13	-0.04	-0.01	0.02	0.04	0.05	-0.07	0.10	-0.06	-0.03	0.89*

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 by Yousuf *et al*. (2011) in rapeseed, and Zada *et al*. (2013) in Ethiopian 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. The Varimax rotation thus, applied resulted in loading of all the variables on different principal components. Factors' loadings of different variables thus obtained are presented in Table 2. All twenty-five variables showed high loadings on different principal factors, and none was left after rotation of the principal factor axes. Moreover, it clearly grouped the similar type of variables by loading them together on a common principal factor. The principal factor (PF) 1, 4, 5 and 10 ascribed for 11 variables in total related to growth rates at different stages and vegetative parameters. All of these together can be designated as growth rate and leafiness factors. The principal factor 2, 7, 9 and 11 showed high loadings for eight variables i.e. no. of siliquae on main shoot, siliqua density on main shoot, siliqua length (cm), primary branches per plant, seed yield per plant (g), secondary branches per plant, no. of seeds per siliqua, and 1000-seed weight (g). All of these together can be designated as yield factor. The principal factor 6 and 8 can be designated as colour factor as it had high loadings for the variables i.e. leaf colour, petal colour and seed colour. The principal factor 3 showed high loadings for oil content (%), no. of lobes per leaf and beak length (cm). Similar results have also been reported by Singh *et al*. (2010) in Indian mustard, and by and Zada *et al*. (2013) in Ethiopian mustard.

Such a clear grouping of similar type of variables having loaded on a common principal factor elaborates the successful transformation of twenty five interrelated variables into eleven independent principal factors explaining 75.26% of the variability of the original set.

Further, all the genotypes were plotted on graph utilizing their principal factor scores based on two factors (Fig.1). The genotypes RC-12, RC-13, RC-14 and RC-18 which were found high yielding,

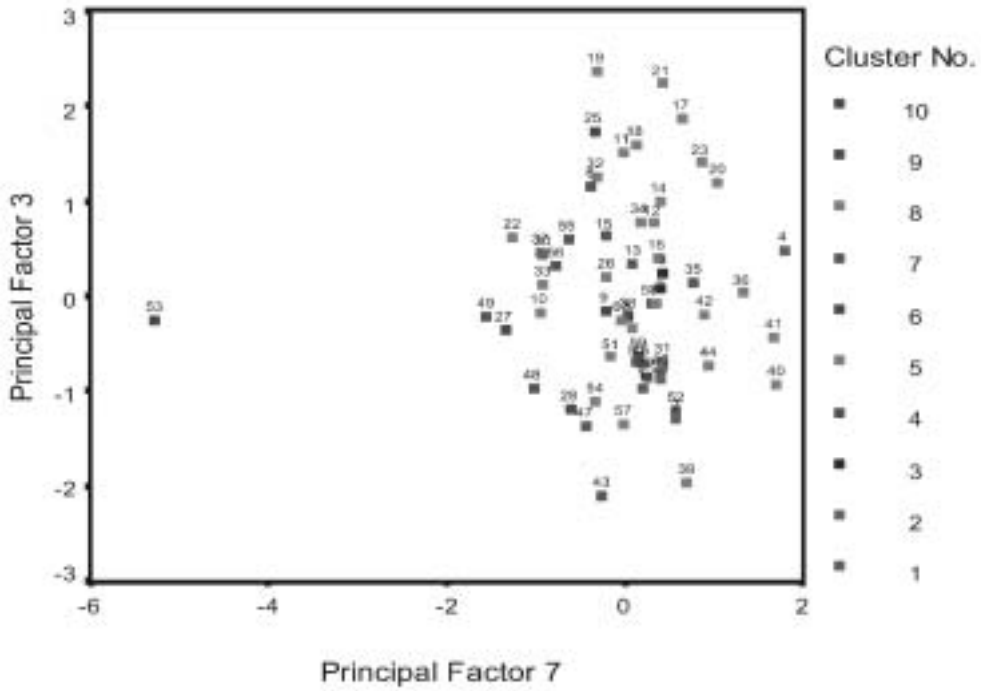

Fig. 1: Distribution of Indian mustard genotypes based on Principal Factor 3 and 7

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

Cluster No.	Genotypes	No. of genotypes
I	Purple mutant	1
II	RWH-1, RC-199, Pahari rai, ZEM-2	4
III	RC-781, UDN-69	2
IV	RH-0502	1
V	Varuna albino, RC-1425, Sarita, Kranti, T-6342, RH-9617, JMM-937, JMMWR-9348, Pusa Bahar, Pusa Bold, RC-29, RC-32, RC-60, KM-888, RH-7846, RH-0401(YS), RH-8912, RH-8701,RH-0749,RH-0406,RC-5, RC-13, RC-14	23
VI	Parkash, Shiva	2
VII	RH-0345, BIO-902, RC-2, RC-7, RC-15, RC-20, RC-21, RC-22, RC-23, RC-24, RC-25, RC-27, RC-30, RC-31, RC-33, RC-34	16
VIII	RAURD-25, RH-8814, RC-6, RC-8, RC-12, RC-18, RC-26	7
IX	EC-126743, EC-126745, ZEM-1	3
X	RC-28	1
	Total	60

aggregated towards the positive portion of PF 7 axis (seed yield factor) in the plot, whereas the genotypes with high oil content (KM-888, JMM-937, RH-0401(YS) and Pusa Bold) clustered towards the positive side of PF 3 axis (oil content factor). The genotypes placed towards the positive end of both the factors are supposed to be superior for both seed yield and oil content. The genotypes found superior for both seed yield and oil content factors hence were supposed to be superior for all the characters which define these two factors. The results of our present study showed that the genotypes JMM-937, RC-199, RH-0401(YS), Pusa Bold, Pusa Bahar, and KM-888 were better performers for both PF 3 and PF 7 factors, meaning that they were superior when seed yield per plant and oil content (%) were considered collectively. Therefore, it can be concluded that the six genotypes we identified may prove better parents in hybridization programme for improving both seed yield and oil content simultaneously.

Unweighted Pair-Group Method using Arithmetic Averages (UPGMA) of hierarchical cluster analysis was utilized with city block distances to classify the 60 genotypes into ten clusters containing one to twenty three genotypes (Table 3). The cluster V had the maximum number of genotypes i.e. 23, and the clusters I, IV and X each had only one genotype. The clusters II, VII, VIII and IX comprised of 4, 16, 7 and 3 genotypes, respectively, whereas, the clusters III and VI had two genotypes each.

This analysis further showed that some of the genotypes belonging to various regions were grouped into the same cluster, while many others fell into different clusters. This clustering pattern suggests that geographical diversity does not necessarily represent genetic diversity; this may be due to free exchange of genetic material among different regions, and also due to operation of natural and artificial selection forces resulting in perpetuation and stabilization of similar genotypes. These results are in agreement with the results reported earlier in Indian mustard by Kumar *et al*. (2007), Budhanwar *et al*. (2010), Belete *et al*. (2011), and Singh (2012). Therefore, geographic diversity although important, was not the only factor responsible for determination of the genetic diversity.

In the present study, the mean performance of different clusters calculated for different traits revealed wide range of differences among clusters with respect to these traits (Table 4). The cluster III (having two genotypes i.e. RC-781 and UDN-69) and cluster IV containing one genotype (RH-0502) showed very good performance for seed

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

Traits / Cluster No.	CI	CII	CIII	CIV	CV	CVI	CVII	CVIII	CIX	CX	Mean
No. of lobes/leaf	7.0	6.5	6.5	7.0	7.0	8.0	6.1	6.1	6.7	7.0	6.8
Leaf length (cm)	41.7	34.5	42.0	36.6	38.6	37.3	33.3	38.2	46.0	35.3	38.3
Leaf width (cm)	16.0	12.9	15.3	14.6	14.1	15.2	12.3	14.4	17.0	12.3	14.4
Days to 50% flowering	65.0	65.0	62.5	52.0	50.0	58.0	47.6	48.7	65.0	52.0	56.6
Days to maturity	165.0	159.5	152.5	154.0	152.9	152.0	152.3	152.9	167.0	159.0	156.7
Plant height (cm)	257.0	253.8	221.5	157.0	209.3	193.5	184.9	234.9	220.3	210.0	214.2
Primary branches/plant	6.0	7.8	5.5	9.0	6.5	6.0	6.4	7.7	5.7	5.0	6.6
Secondary branches/plant	11.0	16.0	11.5	12.0	12.0	11.0	13.6	11.3	13.3	13.0	12.5
Main shoot length (cm)	41.0	61.0	62.5	47.0	60.3	71.5	56.0	69.0	64.7	59.0	59.2
No. of siliquae on main shoot	31.0	45.3	60.5	80.0	38.6	29.0	36.6	50.0	41.0	47.0	45.9
Sliliqua density on main shoot	1.3	1.4	1.0	0.6	1.6	2.5	1.6	1.4	1.6	1.2	1.4
Sliliqua length (cm)	3.4	3.5	2.9	3.7	4.0	4.4	3.6	3.7	3.9	33.0	6.6
No. of seeds/siliqua	14.0	11.3	12.0	10.0	11.5	9.0	11.1	10.7	12.0	11.0	11.3
1000-seed weight (g)	3.4	3.3	3.8	4.8	4.4	4.5	4.8	3.5	3.3	3.4	3.9
Seed yield/plant (g)	19.0	19.5	26.0	23.0	19.7	10.0	13.6	18.0	12.3	8.0	16.9
Oil content (%)	34.4	37.2	37.2	38.9	37.7	37.4	35.7	36.0	35.8	34.3	36.5
Leaf hairiness	7.0	3.0	7.0	1.0	4.0	3.0	4.5	3.3	2.3	7.0	4.2
Leaf colour	3.0	3.0	1.5	3.0	2.4	3.0	1.8	2.7	2.3	1.0	2.4
Dentation of leaf margin	3.0	5.0	7.0	3.0	4.9	7.0	5.0	4.1	5.7	5.0	5.0
Leaf growth habit	5.0	5.5	4.0	3.0	4.2	5.0	4.4	3.3	3.7	5.0	4.3
Petal colour	1.0	3.3	3.5	3.0	3.5	4.0	3.6	3.6	3.7	3.0	3.2
Siliqua surface texture	3.0	2.8	3.0	2.0	2.5	2.5	2.2	2.4	2.0	3.0	2.5
Beak length (cm)	3.0	3.5	3.0	3.0	3.8	5.0	3.0	3.3	3.7	3.0	3.4
Siliqua angle with main shoot	7.0	5.5	6.0	3.0	5.2	6.0	4.9	5.0	5.0	7.0	5.5
Seed colour	4.0	2.8	3.5	2.0	3.1	4.0	3.6	3.7	1.0	3.0	3.1

yield per plant due to possession of more number of siliquae per plant; genotypes RC-781 and UDN-69 are disease tolerant and being used as parents for developing disease tolerant varieties at national level. Singh (2012) also reported that in Indian mustard, cluster means reflected appreciable variation for almost all the characters, especially seed yield potential, among different clusters. Grouping 33 *B. juncea* genotypes, Singh *et al.* (2010) also found different clusters which were among the most divergent clusters having both seed yield and high oil content performance. The clusters IV and VI genotypes were characterized for most seed attributing traits, while the remaining clusters were moderate performers for different characters. Zaman *et al*. (2010) reported highest cluster means for primary branches per plant and maximum seeds per siliqua, whereas the minimum seed yield per plant was obtained from cluster II; genotypes from cluster I had dwarf plant along with earliness in days to 50% flowering, days to maturity, and maximum number of primary branches per plant. On the other hand, Singh *et al.* (2010) reported genotypes from cluster V with shortest plant height along with earliest in days to 50% flowering and maturity, and cluster VIII with highest mean values for siliqua length, number of seed per siliqua, and seed yield along with high mean value for number of primary and secondary branches, main raceme length, and for oil content.

The intra and inter-cluster distances are presented in Table 5 .The maximum intra-cluster distance was observed in cluster VI (39.3) followed by cluster V (38.2) and minimum in cluster III (24.1). Intra-cluster distances were zero in clusters I, IV and X due to grouping of only one genotype in these clusters which were unique in characteristics.

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

Cluster No.	C I	C II	C III	C IV	C V	C VI	C VII	C VIII	C IX	C X
C I	**0.0**									
C II	91.6	**33.0**								
C III	128.8	104.2	**24.1**							
C IV	221.4	200.5	149.8	**0.0**						
C V	140.6	123.3	94.1	150.0	**38.2**					
C VI	167.5	149.3	125.9	171.1	94.3	**39.3**				
C VII	167.1	148.0	130.4	131.1	90.9	90.8	**32.8**			
C VIII	131.0	98.2	82.2	166.3	92.8	121.1	121.1	**33.7**		
C IX	116.5	102.3	90.5	191.8	103.5	111.8	130.0	104.8	**34.6**	
C X	163.9	138.9	115.7	181.5	105.2	129.4	110.8	119.5	114.9	**0.0**

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

Inter-cluster distance was maximum between clusters I and IV (221.4) followed between clusters II and IV (200.5), whereas, the minimum inter-cluster distance was observed between clusters VI and VII (90.8). 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. The cluster IV was superior for both seed yield and oil content. Based on the results of the present study it is recommended that genotype RH-0502 should be used as one of the parent for improving yield and component traits. However, for improvement of specific traits genotypes from cluster VI should be involved.

Results of this present study conclude that all 60 Indian mustard genotypes were successfully classified based on various qualitative and quantitative characters and all the variables have been reduced to only ten principal factors. This multivariate analysis used in the present study has enabled us in identifying superior genotypes for both seed yield and oil content, and genotypes promising for different combinations of characters. These

results will be useful in understanding the genetic diversity within a group of genotypes which can be put to a better use for evolving well defined approach for evaluation and characterization of genetic variation in raya crop.

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