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Assessment of genetic divergence using Mahalanobis D^2 and principal component analysis of qualitative and quantitative characters in pomegranate genotypes under sub-tropics

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ABSTRACT

Studies were carried out to assess the genetic divergence among 30 pomegranate genotypes using Mahalanobis D^2 and Principal Component Analysis of 14 quantitative characters. Thirty genotypes were grouped into eight clusters. Maximum (10) genotypes were included in cluster I and minimum (1) in clusters VII and VIII. The maximum inter-cluster distance of 72.74 was observed among genotypes of the cluster V and VII and minimum (23.85) between the clusters III and IV; and II and VIII. The principal component analysis showed more than 82 per cent of the variability for qualitative and quantitative characters in different genotypes. The genotypes of cluster VIII was observed with highest character mean for number of hermaphrodite flowers (467), number of fruits per tree (84.2), juice per cent (67.3) followed by cluster V with the highest yielding (17.6 kg/tree) genotypes with fruit weight (262 g), aril weight (24.2 g), TSS (12.9%) and TSS: acid ratio (29.2). Fruit yield, fruit length, leaf length, TSS: acid ratio were observed significant variables components and the genotypes Ganesh, Mridula, Amlidana and P-26 were found with maximum values corresponding to these four variables. Selecting genotypes from divergent clusters and utilizing them in hybridization programme is likely to produce desirable recombinants, and may lead to improvement in pomegranate for yield and quality traits.

Key words: Pomegranate, genetic divergence, quantitative characters, principal component analysis.

INTRODUCTION

Pomegranate (*Punica granatum* L.) is an economically important commercial fruit species belonging to the family Lytheraceae is a favourite table fruit in tropical and sub-tropical regions of the world. In India, pomegranate grows well under semi-arid conditions and thrives best under hot dry summer and cold winter provided irrigation facilities are available. Pomegranate fruit is consumed directly as fresh as well as fresh juice which can also be used in beverages for jellies, jam and paste and for flavouring and colouring agents (Fadavi *et al.*, 3). Pomegranate aril juice (100 ml) provides 16 per cent of an adult's daily vitamin C requirement and is a good source of vitamin B5 (pantothenic acid), potassium and antioxidant polyphenols. Concentrated juice and other extracts of the pomegranate bear properties such as antioxidant, anti-inflammatory, and anti-atherosclerotic against some diseases (osteoarthritis, prostate cancer, heart disease, HIV-1) (Malik *et al*., 5). The nutritional and medicinal properties of fruit increase its economic importance and hence fetch foreign exchange for the country. To boost pomegranate production in India, both for domestic and export purposes, development of improved varieties is required which bear fruits having attractive rind and bold and soft seeds with dark red and sweet aril. The basic step for crop improvement relies on characterization and identification of cultivars. Multivariate analysis such as D^2 cluster and principal component analysis have been proved to be useful tools in selecting genotypes for improvement. Mahalanobis (4) D^2 analysis has been successfully used in measuring the variability. Principal component analysis is a useful device for representing a set of variables by a much smaller set of composite variables that account for much of the variance among the set of original variables. It allows visualization of the differences among the individuals, identification of possible groups and relationships among individuals and variables. An understanding of nature and magnitude of variability among the existing pomegranate germplasm is a pre-requisite for its improvement. Precise information on the nature and degree of variability helps the plant breeder in choosing the diverse parents for purposeful hybridization. Since a limited identification and characterization of various genotypes has been documented till date in sub-tropical pomegranate. Therefore, the present study was carried out with 30 pomegranate genotypes to understand the genetic diversity its genetic improvement by D^2 and principal component analysis.

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MATERIALS AND METHODS

Thirty pomegranate genotypes are being maintained at Punjab Agricultural University, Ludhiana are planted during 2002-03 and received uniform cultural practices during the course of studies. The data on parameters such as leaf size (length and breadth), number of hermaphrodite flowers per plant, number of fruits per plant and yield (kg/ plant) were recorded by taking the tree as a unit and replicated twice by following the randomized block design. The fruits were harvested and samples were transferred to the laboratory for measuring the physical and chemical parameters of fruit such as fruit size (length and breadth), fruit weight, 100-aril weight, peel weight, juice per cent, total soluble solids, acidity of juice and TSS/ acid ratio. Genetic variability was studied following Mahalanobis's generalized distance (D^2) extended by Rao (12). Clustering of genotypes was done according to Tocher's method (Rao, 12) and principal component analysis was worked out using JMP 9 software (SAS Institute, Inc). An average intra-cluster distance was calculated by the following formula suggested by Singh and Chaudhary (14).

$$Dp^2 = \sum_{i}^{p} \sum_{i}^{p} 1\ W^{ij} (X_{i1} - X_{i2}) (X_{j1} - X_{j2})$$

Where, W_{ij}= variance-covariance matrix W^{ij} is the reciprocal of (W_{ij}), (i, j=1, 2.....p), X_{i1}= sample mean for i^{th} character for first sample, X_{ij}= sample mean for i^{th} character for sample.

RESULTS AND DISCUSSION

Thirty genotypes were classified into eight clusters on the basis of D^2 values (Table 1). Cluster I was the largest with ten genotypes, followed by cluster II (5 genotypes) and cluster III (5 genotypes). Cluster IV, V and VI included four, two and two genotypes, respectively, whereas, cluster VII and VIII were having one genotype each. Thus, formation of cluster with different genotypes indicate diversity among genotypes. The grouping of genotypes into different constellations did not follow any specific pattern and was found independent of their geographic region. There was no correlation between geographical and genetic diversity. The diverse grouping of genotypes in same cluster with different origins might be due to unidirectional selection pressure practiced by the breeders in couture the promising genotypes. As far, such kinds of results were reported by Rahaman and Munsur (11) in lime, and Manchekar *et al.* (6) in Alphonso mango.

Table 1. Clustering pattern obtained by Mahalanobis D^2 analysis for pomegranate genotypes.

Cluster No.	No. of genotype(s)	Genotype(s)
I	10	Anar-Shirin-Mohamad-Ali, Chawla-I, Mridula, G-137, P-26, Khog, Jhodpur white, Co-1,Russian Seedling and Panipat Selection
II	5	Ps-75-K3, Shirin Anar, Moga Local, Kandhari-Ganga Nagari and Achakdana
III	5	Anar-Mohereb-Shirin, Amlidana, Bhota-I, Bhota-II and Mallas
IV	4	Jyoti, Assam Local, Chawla-II and Kali Shirin
V	2	Ganesh and Kandhari
VI	2	Bhota-III and Anardana Selection-I
VII	1	Anardana Selection-II
VIII	1	Anar Shirin

The range of intra-cluster distance was from minimum of 0.0 in seventh and eighth cluster to maximum of 20.9 in the fifth cluster (Table 2). This apparently indicates that cluster V have genotypes that are relatively distant from each other than the other clusters which have lower D^2 distances except cluster VII and VIII, which had only one

Table 2. Average intra and inter Mahalanobis D^2 cluster distance matrix of pomegranate genotypes.

Cluster	I	II	III	IV	V	VI	VII	VIII
I	20.67	25.99	32.50	25.77	30.48	40.03	43.58	43.41
II		19.46	27.80	29.42	32.01	33.85	28.20	23.85
III			13.38	23.85	38.30	24.85	34.23	59.39
IV				15.01	33.33	35.53	32.76	48.75
V					20.91	45.30	72.74	67.07
VI						13.19	31.83	56.29
VII							0.00	40.66
VIII								0.00

genotype. The maximum inter-cluster distance of 72.7 was observed among the fifth and seventh cluster indicating large genetic differences among genotypes of these two clusters. Minimum inter-cluster distance of 23.85 was observed between the III and IV clusters, cluster II and VIII indicating significantly lesser genetic differences among the genotypes of these four clusters. Maximum inter-cluster distance is indicative that genotypes falling in these clusters had wide diversity and can be used for hybridization programme to get better recombinants in the segregating generations. Low level of intra-cluster distances was indicative of narrow genetic variation within the cluster. Genotypes of same cluster would not yield desirable recombinants. Different intra- and inter-cluster distances were recorded previously for various fruit crops like pomegranate, walnut, almond, and pecan cultivars (Akbarpour *et al.*, 1). The character means were also worked out for the genotypes falling in these eight clusters (Table 3). Cluster VIII was found to be the cluster consisting of genotypes of high leaf length (8.68 cm), number of hermaphrodite flowers (467), number of fruits per tree (84.2), juice content (67.3%) and low acidity (0.5%). Similarly, cluster V with the highest yielding (17.6 kg/ tree) genotypes having highest fruit weight (262 g), aril weight (24.2 g), TSS (12.9%) and TSS/acid ratio (29.2). Cluster VII was characterised with maximum fruit length (6.27 cm), fruit breadth (6.90 cm) and minimum peel weight (40 g). Maximum leaf breadth (2.42 cm), acidity (2.91%) and TSS/acid ratio (27.6) were found in clusters VI and III, respectively. The least mean value for aril weight (12.22 g) and TSS (11%) was represented by cluster VIII, whereas cluster IV was found to be the cluster consisting of genotypes of minimum leaf length (6.34 cm), leaf breadth (1.94 cm), fruit length (5.52 cm) and fruit breadth (5.62 cm). Minimum mean number of hermaphrodite flowers (355) and juice per cent (34.8) was observed in cluster VI and V, respectively. Similarly, cluster VI with the genotypes of lower mean values for number of fruits per tree (63.8). The analytic observations concluded that cluster V appeared to be the most promising cluster to get the high yielding genotypes with maximum quality traits (aril weight and TSS). Also, the genotypes of cluster VIII were considered for better performance in quantitative (number of hermaphrodite flowers and number of fruits per tree) and quality traits (juice per cent and low acidity). Such character mean based clustering have been reported by Sharma *et al.* (13) in Persian walnut. Cluster based mean estimations are very useful in targeting the genotypes for breeding programme, as they prevent the tedious efforts of screening the inferior germplasm lines. Hence, genotypes from desirable clusters could be directly used for final field evaluation in advanced breeding experiments.

The eigens value was found more than 82 per cent by the first four components, *i.e.* PC1, PC2, PC3 and PC4 (Table 4), which accounted for 33.27, 20.45, 16.92 and 11.75 per cent, respectively, of the total variability of PCA. Similar results were reported by Noormohammadi *et al.* (10) who revealed that PCA

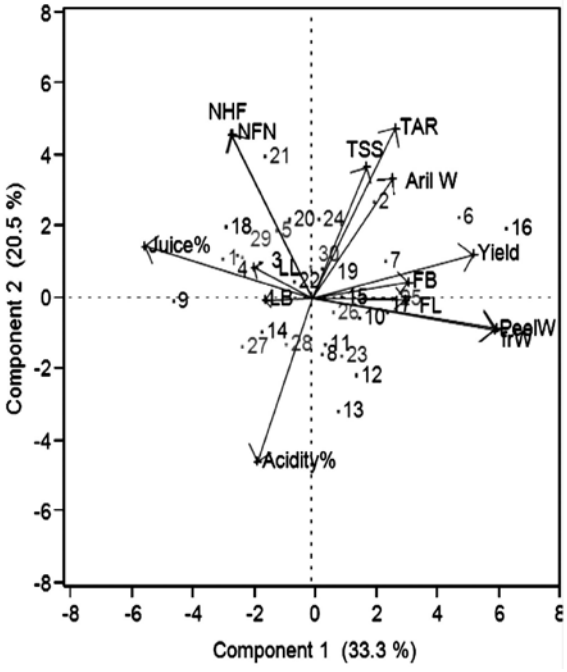
Table 3. Mean value of quantitative characters in eight clusters of pomegranate genotypes.

Characters	Cluster							
	I	II	III	IV	V	VI	VII	VIII
Leaf length (cm)	7.07	8.02	7.03	6.34	7.75	7.64	6.48	8.68
Leaf breadth (cm)	2.01	2.15	2.14	1.94	2.05	2.42	2.10	2.41
No. of hermaphrodite flowers	417	441	431	429	371	355	393	467
No. of fruits per tree	74.9	79.5	77.7	77.3	67.0	63.8	70.3	84.2
Yield (kg/tree)	12.4	12.8	13.7	14.2	17.6	13.1	8.27	10.5
Fruit length (cm)	5.93	5.65	5.70	5.52	6.01	6.26	6.27	6.17
Fruit breadth (cm)	6.42	6.06	5.81	5.62	6.71	6.70	6.90	6.14
Fruit weight (g)	167	159	179	184	262	206	119	123
Aril weight (g)	17.3	15.2	15.3	16.6	24.2	14.7	16.1	12.2
Peel weight (g)	55.7	54.7	59.9	61.9	90.2	68.6	40.0	41.1
Juice (%)	53.0	55.8	47.1	49.5	34.8	43.1	65.0	67.3
TSS (%)	12.6	12.3	12.4	12.1	12.9	11.4	12.4	11.0
Acidity (%)	0.49	1.61	2.91	0.53	0.46	2.75	2.82	0.50
TSS/ acid ratio	27.6	12.7	4.45	23.7	29.2	4.75	4.41	22.2

Table 4. Eigen values and proportion of total variability for quantitative characters of pomegranate genotypes as explained by principal components.

Principal component	Eigen value	Per cent variability	Cumulative variability
1	4.65	33.27	33.27
2	2.86	20.45	53.73
3	2.36	16.92	70.65
4	1.64	11.75	82.41
5	0.92	6.57	88.99
6	0.67	4.79	93.78
7	0.46	3.34	97.13
8	0.18	1.30	98.44
9	0.12	0.90	99.34
10	0.05	0.37	99.72
11	0.03	0.24	99.96
12	0.00	0.02	99.98
13	0.00	0.00	99.99
14	0.00	0.00	100.00

coordination based on two first components (factors) confirmed cluster analysis using combined data when eigen value for first and second components were 20.88 and 9.45 per cent, respectively in Iranian pomegranate germplasm. Yilmaz *et al.* (15) revealed that eigen values of the first 3 components were able to represent 32.67 per cent of total variance in PCA and further, eigen value of pomological PCA analysis was able to represent 73 per cent of total variance. The first component PC1 correspond to the genotypes with high yield, fruit weight and peel weight (Fig. 1; Table 5), includes the genotypes; Ganesh, PS-75-K3 and Mridula with maximum values for these characters. Likewise, PC2 values were also characterised by higher number of fruits per tree, number of hermaphrodite flowers, aril weight, TSS and TSS: acid ratio and genotypes corresponding to three characters with higher values were found to be Mridula, P-26, PS-75-K3 and Mallas (Table 5; Fig. 1). The component PC3 showed characters with higher values of leaf breadth, fruit length and fruit breadth and grouped the genotypes like Bhota-III, Amlidana, Anardana Selection-I and II corresponding to these characters. The characters leaf length with maximum values found in PC4 and genotypes characterised within this group were Moga Local, Assam Local and Anar Shirin.The highest negative values for PC1 indicate the genotypes with minimum juice per cent, lower number of fruits per tree and number of hermaphrodite flowers and the genotypes included were Mridula, Ganesh, Jhodpur White, Anardana Selection-I, Anardana Selection-II,



1	Anar Shirin	16	Ganesh
2	Ps-75-K3	17	Kandhari
3	Kandhari-GangaNagari	18	Moga Local
4	Anar-Shirin-Mohamad-Ali	19	Mallas
5	Chawla-I	20	G-137
6	Mridula	21	P-26
7	Jyoti	22	Khog
8	Anar-Mohereb-Shirin	23	Jhodpur White
9	Amlidana	24	Co1
10	Bhota-I	25	Russian seedling
11	Bhota-II	26	Panipat Selection
12	Bhota-III	27	Shirin Anar
13	Anardana-Selection-I	28	Achikdana
14	Anardana-Selection-II	29	Chawla-II
15	Assam Local	30	Kali Shirin

Fig. 1. Quantitative characters variability in pomegranate germplasm. LL = leaf length, LB = length breadth, NHF = number of hermaphrodite flowers per tree, NFN = number of fruits per tree, Yd = yield, FL = fruit length, FB = fruit breadth, FW = fruit weight, AW = aril weight, PW = peel weight and TAR = TSS/acid ratio.

Table 5. Component loading for leaf and fruit physical characters of pomegranate genotypes.

Character	PC1 (33.27%)	PC2 (20.45%)	PC3 (16.92%)	PC4 (11.75%)
Leaf length (cm)	-0.29	0.13	0.51	0.63
Leaf breadth (cm)	-0.24	-0.01	0.74	0.51
No. of hermaphrodite flowers per tree	-0.41	0.71	-0.24	0.38
No. of fruits per tree	-0.41	0.71	-0.24	0.40
Yield (kg/ tree)	0.82	0.19	-0.28	0.42
Fruit length (cm)	0.47	-0.00	0.75	-0.11
Fruit breadth (cm)	0.49	0.06	0.70	-0.18
Fruit weight (g)	0.94	-0.13	-0.15	0.25
Aril weight (g)	0.41	0.52	0.36	-0.13
Peel weight (g)	0.93	-0.13	-0.15	0.25
Juice (%)	-0.85	0.22	0.24	-0.29
TSS (%)	0.27	0.57	-0.18	-0.31
Acidity (%)	-0.28	-0.71	-0.07	0.21
TSS/ acid ratio	0.42	0.74	0.09	-0.22

Bhota-I and II. The genotypes Mridula, Ganesh and Ps-75-k3, which had the lowest PC2 value stands out especially due to the low values for acidity (Fig. 1). The highest negative value of PC3 was obtained for character yield and the genotypes clubbed with the least value for yield were Shirin Anar, Amlidana and Anardana Selection-II. Likewise, TSS was found to have maximum negative value for component PC4 with the genotypes Amalidana, Anardana Selection-II and I. PCA had been used to evaluate germplasm of different species, *viz.*, mango (Benevides *et al*., 2), pomegranate (Mars and Marrakchi, 7), and guava (Nogueira *et al*., 9). PC analysis helped to select a set of genotypes with better fruit quality (Mratinic *et al.*, 8).

Genetic divergence of 30 pomegranate genotypes assessed with Mahalanobis D^2 revealed that the inter-cluster distances were larger than the intra- cluster distances indicating a wider genetic diversity between genotypes of cluster with respect to trait considered. PCA analysis found to reduce the data of 14 qualitative and quantitative characters in four most significant variables or components, which showed maximum and stable variability among all the characters under this study. The four most significant variables observed were yield, TSS:acid ratio, fruit length, fruit weight and leaf length. The genotypes corresponding to these variables with maximum values would be taken into consideration for selection of diverse genotypes for future breeding programmes and also it is sufficient to take one or more genotypes from each of these groups for use in future breeding programmes. The most promising among them were Ganesh, Mridula, Amlidana and P-26, which were characterized by maximum yield, fruit length, leaf length and TSS/acid ratio.

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