

D²-ANALYSIS SUGGESTS WIDER GENETIC DIVERGENCE IN POMEGRANATE GENOTYPES

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ABSTRACT

Pomegranate (*Punica granatum* L.) is a nutraceutically important commercial fruit crop grown around the world. Despite its commercial importance, the study on breeding aspect particularly genetic improvement of available germplasm is lacking. The present study on the D²-based divergence analyses using Euclidian and Tocher's methods indicated the presence of appreciable amount of genetic diversity in the twenty genotypes of pomegranate. Amongst the five clusters, cluster-1 had maximum number of genotypes (8), while the cluster-4 and 5 contained the least (1). The intra-cluster distances ranged from 0.00 to 3775261.500 indicating that the genotypes in clusters have dissimilarity for morphological features and performance. The members of cluster 4 and 5 exhibited maximum divergence (inter-cluster distance 273946208.000) followed by the members of cluster 2 and 5 (inter-cluster distance 168996736.000) and cluster 3 and 4 (inter-cluster distance 134498224.000). 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. The genotypes Crab and Hakubotan identified in the present study will contribute in future breeding efforts aimed at genetic improvement in pomegranate genotypes for commercial success of production.

INTRODUCTION

Pomegranate (*Punica granatum* L.) is an important fruit of tropical and subtropical regions of the world, and is grown largely in the area of Mediterranean basin, Afganistan, Iran, Pakistan and India, North and South American country. In India, Maharastra, Gujarat, Rajasthan, U.P., Haryana, Andhra Pradesh, Karnataka and Tamilnadu and to limited extent in Jammu and Kashmir, Himachal and Uttarakhand are the major pomegranate growing states. Total acreage today is about 130.8 (000 ha). The annual production is about 1345.7 MT with an average productivity of 10.3 MT/ha (Anonymous, 2015). Two types of pomegranate are found in India, cultivated (*Punica granatum* L.) and wild types (*Punica protopunica*). Pomegranates are used as dessert fruit and spices purpose also because it is rich sugar content, vitamin and minerals (Ray, et al., 2014).

Genetic diversity is a basic demand for pomegranate crop improvement programme because genetically diverse parents when crossed can bring together diversity of gene combinations either to cultivate heterosis or to obtain superior recombinants. Germplasm is the rich source of genetic diversity, which is often utilize to develop many ameliorated varieties of crops (Hazara et al., 2002). Although a wide range of genetic diversity is accessible in genotypes of pomegranate (Patil and Sanghavi, 1980), the improvement in the pomegranate is narrowed. The genetic divergence between populations can be quantified by using convenient statistical

methods, of which multivariate analysis has been found to be most appropriate (Joshi and Dhawan, 1966 and Kumar, 2015).

The aim of present investigation is to assess the genetic divergence among germplasm of pomegranate, to spot the most diverged parents for use in hybridization programme. In this study various physical, chemical and nutraceutical properties of pomegranate accessions grown in mid-hills of Himachal Pradesh were carry out to identify the existence of any relationships between the physical, chemical and nutraceutical traits of the fruit. Therefore, an initiation was made to evaluate the magnitude of genetic diversity among twenty genotypes of pomegranate for average fruit quality contributing characters.

MATERIALS AND METHODS

The present investigations were carried out in the Department of Fruit Science, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauli, Solan (H.P.) during 2012-13. The experimental area is located at Nauli (at an altitude of 1220 m above mean sea level between 31°N latitude and 77 °E longitude). The experimental area falls in the mid hill zone of Himachal Pradesh. This particular district is also famous for the production of pomegranate. Among various district, Solan is particularly known for the quality fruits, vegetables and seed production. Annual precipitation is 3.50-184.30 mm during that year. Twenty genotypes of pomegranate evaluated in the present study (Table 1a). The recommended package of

practices was followed to have quality fruits with respect to pruning, fertilizer irrigation requirement and plant protection. Attention on twenty four characters viz., average fruit weight, seed content, weight of 100 arils, juice content, total soluble solids, acidity, total sugars, anthocyanins and phenols content were observed on three random plants in each replication (Table 1b). The analysis of genetic divergence using Mahalanobis's (1936) D^2 statistics was carried out as described by (Rao, 1952). On the basis of magnitude of generalized statistical distance D ($D = \sqrt{D^2}$) values, the genotypes were grouped into different clusters as suggested by Tocher (Rao, 1952).

RESULTS AND DISCUSSION

Extensive research work concerning the traits of pomegranate cultivar variability regarding genotype, growing region, climate, maturity and cultural practices prevalent in many pomegranate producing regions were reported (Ferrara *et al.*, 2014).. In this respect, various methods have been employed in the analysis of genetic variation in many crop species. The value of D^2 -statistic has been demonstrated in choosing parental stocks for cross-breeding.

On the basis of D^2 -analysis, all the twenty genotypes were grouped into five clusters. The intra-cluster distances ranged from 0.00 to 3775261.500 (Table 2) indicating that the genotypes in clusters have dissimilarity for morphological features and performance. The members of cluster 4 and 5 exhibited maximum divergence (inter-cluster distance 273946208.000) followed by the members of cluster 2 and 5 (inter-cluster distance 168996736.000) and cluster 3 and 4 (inter-cluster distance 134498224.000). The members of cluster 2 and 4 were least divergent (inter-cluster 14612848.000). The inter-cluster distances were larger than the intra-cluster distances indicating a wider genetic diversity between genotypes of clusters with respect to trait considered. 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 levels of intra-cluster distances were indicative of narrow genetic variation within the cluster. Hence, selection of parents from this cluster is to be avoided (Rai and Mishra, 2005). Genotype of same cluster would not yield desirable recombinants. It is concluded that genotypes of pomegranate with wide genetic variation accompanied with useful characteristics could be effectively employed in intra specific crosses with the hope that this would lead to the transmission of higher genetic gain for different putative traits major being yield related traits from practical utility point of view. On the basis of the performance of different genotypes and the cluster analysis, the pomegranate genotypes have been identified for different characters could serve as an outstanding genetic basis and a source of germplasm for pomegranate breeding programmes. The dendrogram of 20 pomegranate genotypes was constructed based on morphological data in order to examine the genetic diversity by Tocher method (Fig.1). Dendrogram showed in cluster 1, the genotypes, the cluster analysis grouped the genotypes into five major clusters, i.e. 1

to 5. Amongst the all, cluster-1 had large genotypes (8), cluster-2 and 3 had 5 genotypes and cluster-4 and 5 had only 1 genotype respectively. The resultant five clusters showed the genetic diversity. The clustering pattern shows the influence of environment, pedigree and center of origin. Similar work on pomegranate reported by Verma *et al.*, 2012 in which they found that 'Kashmiri Local' was maximum dissimilarity among different collections from temperate, subtropical and tropical conditions.

As per Euclidean Distance shown in (Fig. 2), the maximum intra cluster distance was 3775262 and minimum was 1778978. Highest inter cluster distance (273946200) was

Table 1a: List of genotypes used in the present study

| S.N. | Name of genotypes | Description |
|------|-------------------------|---|
| 1 | Eve | Genotypes introduced from USDA-ARS, national clonal germplasm repository, university of California, Davis USA |
| 2 | Orange | |
| 3 | Sogdiana | |
| 4 | Alk Pust Ghermez Saveh, | |
| 5 | Gulyalek | |
| 6 | Parfyanets | |
| 7 | Ovadan | |
| 8 | Cloud | |
| 9 | Dewey | |
| 10 | Al-Sirin-Nar | |
| 11 | Saharnyi | |
| 12 | Podarok | |
| 13 | Kaim-anar | |
| 14 | Loulou | |
| 15 | Green Globe | |
| 16 | Purple Heart | |
| 17 | Crab | |
| 18 | Nusai | |
| 19 | Hakubotan | |
| 20 | 20090265 | |

Table 1b: Characters of pomegranate considered in the present study

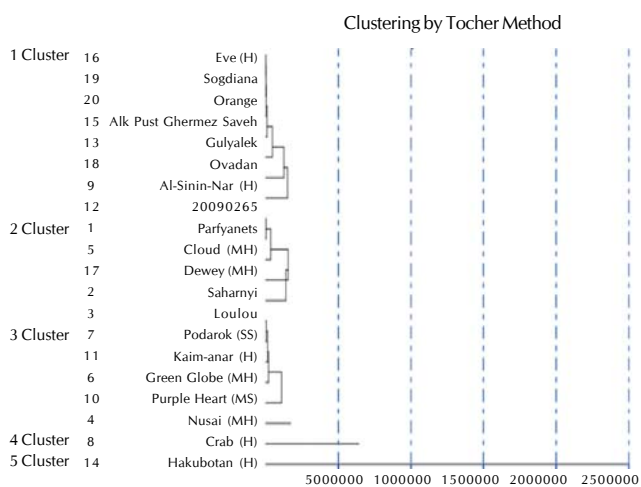
| S.N. | Characters |
|------|------------------------------|
| 1 | Plant height (m) |
| 2 | N-S plant spread (m) |
| 3 | E-W plant spread (m) |
| 4 | Average stem girth (mm) |
| 5 | Length of internodes (cm) |
| 6 | No. of stems |
| 7 | No. of suckers |
| 8 | Thorn length (mm) |
| 9 | Leaf area (cm ²) |
| 10 | Fruit weight (g) |
| 11 | Fruit length (mm) |
| 12 | Yield (kg/ plant) |
| 13 | Fruit breadth (mm) |
| 14 | Rind thickness (mm) |
| 15 | Aril content (%) |
| 16 | Aril : rind ratio |
| 17 | 100 aril weight(g) |
| 18 | Juice percentage |
| 19 | TSS (B) |
| 20 | Acidity (%) |
| 21 | Reducing sugars (%) |
| 22 | Non-reducing sugars (%) |
| 23 | Anthocyanins (mg/ 100g) |
| 24 | Phenols (mg/ 100g) |

Table 2: Inter & Intra Cluster Distances (Tocher method)

| Clusters | Cluster 1 | Cluster 2 | Cluster 3 | Cluster 4 | Cluster 5 |
|-----------|--------------|---------------|---------------|---------------|---------------|
| Cluster 1 | 2302952.250 | 18829416.000 | 17037648.000 | 59621376.000 | 80451144.000 |
| Cluster 2 | 18829416.000 | 3775261.500 | 65427920.000 | 14612848.000 | 168996736.000 |
| Cluster 3 | 17037648.000 | 65427920.000 | 1778977.750 | 134498224.000 | 25817416.000 |
| Cluster 4 | 59621376.000 | 14612848.000 | 134498224.000 | 0.000 | 273946208.000 |
| Cluster 5 | 80451144.000 | 168996736.000 | 25817416.000 | 273946208.000 | 0.000 |

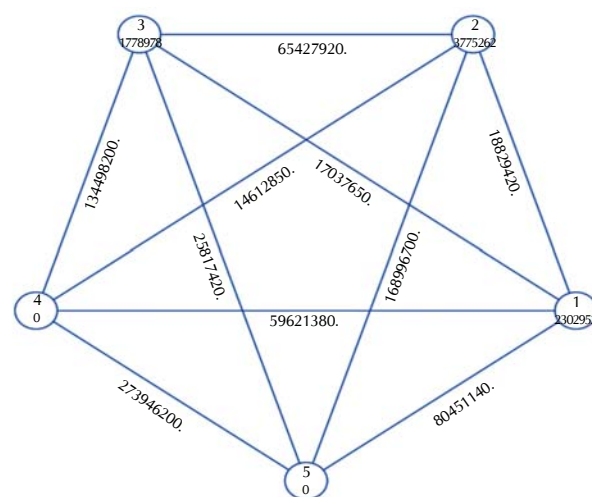
Table 3: Mean inter and intra cluster distance among four clusters in omegrante genotypes on the basis of D² Statistics by Euclidian method (A-E) and (Tocher's Method (I-V)).

| Cluster (Euclidian method) | Number of genotypes | Name of genotypes | Cluster (Tocher method) | Number of genotypes | Name of genotypes |
|----------------------------|---------------------|---|-------------------------|---------------------|---|
| A | 5 | Green Globe, Podarok, Purple Heart, Kaim-anar, Hakubotan | I | 8 | Eve, Sogdiana, Orange, Alk Pust Ghermez Saveh, Gulyalek, Ovadan, Al-Sirin-Nar, 20090265 |
| B | 3 | Saharnyi, Loulou, Crab, | II | 5 | Parfyanets, Cloud, Dewey, Saharnyi, Loulou |
| C | 3 | Parfyanets, Cloud, Dewey | III | 5 | Podarok, Kaim-anar, Green Globe, Purple Heart, Nusai |
| D | 2 | Al-Sirin-Nar, 20090265 | IV | 1 | Crab |
| E | 7 | Nusai, Gulyalek, AlkPust Ghermez Saveh, Eve, Ovadan, Sogdiana, Orange | V | 1 | Hakubotan |

**Figure 1: Clustering of genotypes by Tocher's method**

recorded between cluster 4 and 5 followed by (168996700) between cluster 2 and 5 and (134498200) between cluster 3 and 4 while lowest (14612850) was observed between cluster 2 and 4. Such diverse genotypes characterize by maximum inter-cluster distance will differ in phenotypic performance and therefore will have genes with different magnitude of effects. In such cases chance to obtain favorable transgressive segregants are more on the basis of results obtained.

On the basis of relative magnitude of D² values, the test genotypes were grouped into five clusters (Table 3) based on different quantitative traits using Euclidian (A-E) and Tocher's (I-V) method with variable number of entries in each cluster indicating the presence of genetic diversity in the genotypes of present study. Cluster-E included highest number of genotypes (7) comprising (Nusai, Gulyalek, Alk Pust Ghermez Saveh, Eve, Ovadan, Sogdiana and Orange) followed by cluster-A (5) comprising (Green Globe, Podarok, Purple Heart,

**Figure 2: Mahalanobis Euclidean Distance (Not to scale)**

Kaim-anar, Hakubotan) while lowest number of genotypes included in cluster-D (2) comprising (Al-Sirin-Nar, 20090265). Cluster-I had maximum number of genotypes (8) comprising (Eve, Sogdiana, Orange, Alk Pust Ghermez Saveh, Gulyalek, Ovadan, Al-Sirin-Nar, 20090265) followed by cluster-II and III (5) comprising (Parfyanets, Cloud, Dewey, Saharnyi, Loulou) and (Podarok, Kaim-anar, Green Globe, Purple Heart, Nusai) whereas, cluster-IV and V had minimum number of genotypes (1) comprising (Crab) and (Hakubotan) respectively.

The formation of different clusters with variable number of entries in each cluster indicates diversity among genotypes. The genotypes from different countries or agro-ecological zones were found to scattered in different clusters. This suggests that a pattern of clustering of accessions was independent of their geographic origin. No parallelism was found between genetic and geographic diversity. This mixed grouping of genotypes from different origins in same cluster could be due

Table 4: Cluster means for different characters of pomegranate among 20 genotypes by Euclidian and Tocher methods

| Characters | Clusters [A-E (Euclidian)/I-V (Tocher's method)] | | | | |
|------------------------------|--|------------------|------------------|------------------|------------------|
| | A/I | B/II | C/III | D/IV | E/V |
| Plant height (m) | 2.16 1.87 | 2.49 2.48 | 2.36 2.18 | 1.60 2.15 | 1.99 2.10 |
| N-S plant spread (m) | 1.35 1.42 | 2.20 1.74 | 1.26 1.34 | 1.38 1.68 | 1.45 1.61 |
| E-W plant spread (m) | 1.23 1.27 | 1.91 1.63 | 1.35 1.30 | 0.87 1.69 | 1.41 1.26 |
| Average stem girth (mm) | 40.40 37.92 | 79.54 69.60 | 65.84 37.57 | 42.53 88.17 | 38.49 65.31 |
| Length of internodes (cm) | 3.23 2.88 | 3.13 2.98 | 2.91 3.22 | 2.78 3.24 | 2.96 3.34 |
| No. of stems | 2.00 1.88 | 2.55 2.47 | 2.33 1.60 | 1.00 2.33 | 2.04 3.33 |
| No. of suckers | 0.80 5.58 | 5.88 4.33 | 3.22 0.87 | 5.33 5.67 | 5.00 0.67 |
| Thorn length (mm) | 5.99 6.48 | 7.45 7.67 | 7.50 6.55 | 7.41 6.52 | 6.71 7.18 |
| Leaf area (cm ²) | 18.92 11.44 | 19.97 17.18 | 15.37 18.07 | 6.84 20.14 | 13.23 19.10 |
| Fruit weight (g) | 220.20 197.30 | 165.67 200.48 | 218.49 224.05 | 210.20 150.12 | 197.38 204.45 |
| Fruit length (mm) | 69.66 61.25 | 64.92 65.69 | 65.13 71.24 | 60.90 61.76 | 62.80 63.52 |
| Yield (kg/ plant) | 4.54 2.38 | 6.02 5.17 | 3.88 5.08 | 1.69 3.87 | 3.03 2.86 |
| Fruit breadth (mm) | 82.49 69.58 | 71.05 72.91 | 73.63 79.95 | 71.48 69.53 | 68.75 80.30 |
| Rind thickness (mm) | 3.04 2.37 | 2.57 2.53 | 2.36 2.90 | 2.16 2.17 | 2.49 3.56 |
| Aril content (%) | 55.55 59.31 | 56.69 58.20 | 56.41 56.29 | 65.63 48.32 | 57.28 54.11 |
| Aril : rind ratio | 1.43 1.74 | 1.88 1.88 | 1.78 1.50 | 2.47 1.61 | 1.51 1.32 |
| 100 aril weight (g) | 27.55 28.95 | 26.75 29.56 | 28.48 31.05 | 22.09 17.91 | 31.05 12.51 |
| Juice percentage | 53.35 43.23 | 45.13 49.81 | 49.42 57.54 | 46.59 34.60 | 44.17 35.70 |
| TSS (°B) | 15.04 12.78 | 14.11 14.04 | 14.10 14.52 | 13.58 14.43 | 12.57 15.57 |
| Acidity (%) | 1.23 1.65 | 1.49 1.19 | 1.06 1.11 | 1.61 1.72 | 1.65 2.15 |
| Reducing sugars (%) | 9.76 7.20 | 7.77 8.84 | 9.19 8.85 | 7.38 6.73 | 7.16 6.84 |
| Non-reducing sugars (%) | 1.58 1.52 | 1.78 1.87 | 1.78 1.76 | 1.35 1.34 | 1.62 1.04 |
| Anthocyanins (mg/ 100g) | 12.95 16.69 | 24.49 13.33 | 7.15 14.61 | 17.85 28.31 | 15.61 3.23 |
| Phenols (mg/ 100g) | 587.80 614.33 | 692.61 540.69 | 514.09 641.10 | 844.22 916.68 | 592.03 651.57 |

to extensive utilization of few donor species to generate pomegranate genotypes across world or due to unidirectional selection pressure practiced by the breeders in tailoring the promising cultivars.

The cluster mean value for twenty four characters is presented in Table 4. The perusal of data indicated considerable differences for all the characters among clusters. It can be seen from the cluster means that each cluster has its uniqueness that separated it from other characters. Cluster A was characterized by maximum length of internodes (3.23 cm), fruit weight (220.20 g), fruit length (69.66 mm), fruit breadth (82.49 mm), rind thickness (3.04 mm), juice percentage (53.35

%), TSS (15.04 °B), reducing sugars (9.76 %) and low mean value for number of suckers (0.80), thorn length (5.99 mm), aril content (55.55 %) and aril : rind ratio (1.43). Maximum mean value for plant height (2.49 m), N-S plant spread (2.20 m), E-W plant spread (1.91 m), average stem girth (79.54 mm), number of stems (2.55), number of suckers (5.88), leaf area (19.97 cm²), yield (6.02 kg/plant), non-reducing sugars (1.78 %) and anthocyanins (24.49 mg/ 100g) whereas minimum mean value for fruit weight (165.57 g) were represented by cluster B. cluster C was characterized by highest mean value for thorn length (7.50 mm), non-reducing sugars (1.78 %) and least value for acidity (1.06 %), anthocyanins (7.15 mg/100g)

and phenols (514.09 mg/ 100g). Cluster D was characterized by maximum mean value for aril percentage (65.63), aril: rind ratio (2.47), phenol (844.22 mg/100g) and least value for plant height (1.60 m), E-W plant spread (0.87 m), length of internodes (2.78 cm), number of stems (1.00), leaf area (6.84 cm²), fruit length (60.90 mm), yield (1.69 kg/plant), rind thickness (2.16 mm), 100 aril weight (22.09 g) and non-reducing sugars (1.35 %). Cluster E was characterized by maximum mean value for 100 aril weight (31.05 g) and acidity (1.65 %) whereas minimum mean value for average stem girth (38.49 mm), fruit breadth (69.75 mm), juice percentage (44.17 %), TSS (12.5 °B) and reducing sugars (7.16 %).

Maximum mean was recorded in cluster-I for aril content (59.31 %) however minimum mean value for plant height (1.87 m), length of internodes (2.88cm), thorn length (6.48 mm), leaf area (11.44 cm²), yield (2.38 kg/plant) and TSS (12.78 °B). cluster-II had maximum mean value for plant height (2.48 m), N-S plant spread (1.74 m), thorn length (7.67mm), yield (5.17 kg/plant), aril:rind ratio (1.88) and non-reducing sugars (1.87%) while minimum mean value was observed in phenols (540.69 mg/100g). In cluster-III maximum mean value observed for the characters fruit weight (224.05 g), fruit length (71.24 mm), 100 aril weight (31.05 g), juice percentage (57.54 %) and reducing-sugars (9.85) whereas minimum mean value observed for the character N-S plant spread (1.34m), average stem girth (37.57 mm), number of stems (1.60), number of suckers (0.87) and acidity (1.11 %). Cluster-IV was characterized by maximum mean value for E-W plant spread (1.69 m), average stem girth (88.17 mm), number of suckers (5.67), leaf area (20.14 cm²), phenols (916.68 mg/100g) and least value for fruit weight (150.12 g), fruit breadth (69.53 mm), rind thickness (2.17 mm), aril content (48.32 %), juice percentage (34.60 %) and reducing sugars (6.73 %). Cluster-V was characterized by maximum length of internodes (3.34 cm), number of stems (3.33), fruit breadth (80.30 mm), rind thickness (3.56 mm), TSS (15.57 °B), acidity (2.15%) and low mean value for E-W plant spread (1.26 m), aril : rind ratio (1.32), 100 aril weight (12.51 g), non-reducing sugars (1.04 %) and anthocyanins (3.23 mg/100g).

In conclusion, On the basis of both Tocher as well as Euclidian methods the genotypes Crab and Hakubotan identified for contribute in future breeding efforts aimed at genetic

improvement in pomegranate genotypes for commercial success of production.

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