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Genetic diversity among indigenous pomegranate (*Punica Granatum*) accessions collected from Kashmir valley

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Abstract

The present study was aimed to determine the genetic variability in indigenous accessions of pomegranate collected from Kashmir Valley. On the basis of morphological and physico-chemical characteristics, 33 genotypes were identified by intensive survey in various districts of Kashmir valley during 2009-2010. Cluster analysis and PCA showed a significant phenotypic and genetic diversity among all the collected accessions. All the accessions could be grouped in to two major groups. Majority of them fall second group which was further clustered into two sub-groups. Accession No. 1 and 16 found unique and showed maximum dissimilarity from the rest of the accessions. The greater part of variance was accounted by traits such as yield/ tree, fruit length, fruit diameter, fruit weight, fruit volume, general appearance, total aril weight, fruit rind colour, fruit size and rind weight showed wide variability among the accessions which could be utilized for further and future breeding programme.

KEY WORDS: *Punica granatum*, diversity, Pomological, chemical, Kashmir valley

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Introduction

Pomegranate (*Punica granatum* L.) is highly acclimatized fruit crop growing under diverse climatic conditions ranging from temperate, subtropical and tropical. The pomegranate is thought to have originated in Iran, but became quite common in Mediterranean Regions, the Middle East, and Asia. The fruit has been a symbol of fertility since ancient times. Pomegranates have been recently used in various ways, mainly for different industrial usage fields, such as fruit juice,

conserve, vinegar (Kaya and Sözer, 2005; Maestre *et al*. 2000), and for medicinal purposes (Lansky and Newman, 2007; Neurath *et al*. 2005). This fact has consequently led to its prominent popularity in the world markets. Local varieties are numerous in each agro-ecological region. Few of them have been recognized as potential cultivars and growing in different parts of the country. Pomegranate has been considered, for a long time, as minor fruit crop. In recent years, its commercial cultivation increased considerably in many states of India like Maharashtra, Gujarat, Rajasthan, U.P., Haryana, Andhra Pradesh, Karnataka and Tamilnadu and to a limited extent in Jammu and Kashmir, Himachal and Uttarakhand. Total acreage is today about 107.30 (000 ha). The annual production is about 743.10 MT with an average productivity of 6.9 MT/ha (NHB 2011).

Genetic diversity is an important factor in any crop improvement programme for obtaining high yielding cultivars. Study of genetic divergence among the indigenous available plant genetic resource is a vital tool to the plant breeders for an efficient choice of parents for plant improvement. Genetically diverse parents are likely to contribute desirable segregants and or to produce high heterotic crosses. Parents identified on the basis of divergence for any breeding programme would be more promising (Arunachalam, 1981). Grouping or classification of genotypes based on suitable scale is quite imperative to understand the usable variability. Though significance of morphological traits and multivariate analysis for the characterization of pomegranate cultivars has been stressed in some studies (Mars and Marrakchi, 1999; Al-Said *et al*. 2009; Muradoglu *et al*.2006). Therefore, the objective of the present work was to characterize 33 indigenous pomegranate accessions using pomological and biochemical traits and analyze the contribution of different traits to the overall yield.

Materials and Methods

Survey for pomegranate trees was conducted in different districts of Kashmir valley during May-September 2009-2010. A total of 153 trees were initially labeled based on the interviews with local people and on the data from Directorate of horticulture of Jammu and Kashmir from three districts namely Srinagar, Ganderbal and Budgam. After first observations, many of these trees were excluded because they either showed heavy infestation of anar butterfly or symptoms of cracking or the average fruit weight was lower. Ultimately, 33 of them were selected to be studied further and individual trees were assigned a separate accession number named as table 1.

Every accession was evaluated for various morphological parameters of tree as per the standard procedures. Height of each plant was measured from ground level to the top of main branch or leader with the help of measuring tape and expressed in meters whereas, plant spread was measured in terms of the extent of canopy in two different directions i.e. North-South and East-West. Yield efficiency of tree was calculated as per the formula of Westwood and Robert (1970) and expressed in kg cm^{-2}. Fruits from selected trees were randomly taken for measuring physical attributes like weight, size, rind thickness, rind proportion, aril texture, aril colour, weight of arils, and juice content by following standard procedures. The total soluble solids were estimated by Atago hand refractometer and the values corrected at 20ºC with the help of temperature coefficient chart (AOAC, 2000). Titrable acidity, vitamin C, reducing sugar, total sugars and total anthocyanin content were determined as per Ranganna (2001).Sensory evaluation were carried by panel of 10 semi-trained judges for general appearance ,fruit shape, fruit rind colour, fruit size and aril colour by using pomegranate descriptor and the attributes were rated at a 4-point scale. The experimental methodology and data were analyzed as per the method suggested by Gomez and Gomez (1984). Clustering of genotypes into similarity groups was performed using the method tree procedure PROC CLUSTER based on Euclidean distance. In order to identify the patterns of morpho-physico-chemical variation and contribution of traits, principal component analysis (PCA) was conducted as PROC PRINCOP using SAS 9.2 software (SAS Institute, Cary, NC).

Result and Discussion

Table 1. Different indigenous accessions collected from Kashmir valley.

Code	Accession name	Code	Accession name	Code	Accession name
1	SKAU-Pg-Sr-001	12	SKAU-Pg-Sr-012	23	SKAU-Pg-Gb-006
2	SKAU-Pg-Sr -002	13	SKAU-Pg-Sr-013	24	SKAU-Pg-Gb-007
3	SKAU-Pg-Sr-003	14	SKAU-Pg-Sr-014	25	SKAU-Pg-Bd-001
4	SKAU-Pg-Sr-004	15	SKAU-Pg-Sr-015	26	SKAU-Pg-Bd-002
5	SKAU-Pg-Sr-005	16	SKAU-Pg-Sr-016	27	SKAU-Pg-Bd-003
6	SKAU-Pg-Sr-006	17	SKAU-Pg-Sr-017	28	SKAU-Pg-Bd-004
7	SKAU-Pg-Sr-007	18	SKAU-Pg-Gb-001	29	SKAU-Pg-Bd-005
8	SKAU-Pg-Sr-008	19	SKAU-Pg-Gb-002	30	SKAU-Pg-Bd-006
9	SKAU-Pg-Sr-009	20	SKAU-Pg-Gb-003	31	SKAU-Pg-Bd-007
10	SKAU-Pg-Sr-010	21	SKAU-Pg-Gb-004	32	SKAU-Pg-Bd-008
11	SKAU-Pg-Sr-011	22	SKAU-Pg-Gb-005	33	SKAU-Pg-Bd-009

Table 2: Indicators of variability in pomegranate morphological and yield traits studied

Character	Range		Mean±SE	SD	CV %	Skewness	Kurtosis	Bimodality
	Minimum	Maximum						
Plant height (m)	2.34	4.78	3.55±0.12	0.69	19.44	0.2098	-0.6949	0.4003
Plant spread (m)	1.23	2.65	1.69±0.07	0.39	23.07	0.7075	-0.3646	0.5106
Suckering capacity	4.00	42.00	18.15±1.76	10.08	55.54	0.4031	-0.4310	0.4047
No. of fruits/tree	47.00	245.00	157.50±10.40	59.60	37.84	0.8548	2.3385	0.3068
Yield/tree (kg)	7.20	59.02	35.04±2.62	15.06	42.98	1.4382	3.4818	0.4522
Yield efficiency (kg/cm^2)	0.20	2.21	0.85±0.09	0.52	61.18	1.1619	0.6746	0.5908
Leaf area (cm^2)	7.48	14.04	10.49±0.26	1.49	14.20	0.2175	-0.1233	0.3294
Fruit length (cm)	5.06	9.24	6.83±0.15	0.85	12.44	1.0161	2.6181	0.3433
Fruit diameter (cm)	6.16	9.84	7.56±0.13	0.77	10.18	0.9804	2.0212	0.3683
Fruit weight (g)	120.30	463.70	225±12.50	71.60	31.82	1.6421	3.9928	0.5067
Fruit volume	122.40	499.0	234.20±13.50	77.50	33.09	1.8051	4.5477	0.5424

(cm^3)								
Total aril weight (g)	58.00	250.00	133.25±7.63	43.80	32.87	0.5594	0.7628	0.3229
No. of arils/fruit	245.00	792.00	514.00±25.80	147.90	28.77	-0.0376	-0.8259	0.4042
Weight per aril (g)	0.18	0.33	0.26±0.01	0.04	15.38	-0.2683	-0.4052	0.3699
Rind thickness (mm)	1.50	3.64	2.45±0.10	0.55	22.45	0.3440	-0.3995	0.3851
Rind weight (g)	44.75	217.75	91.68±6.48	37.22	40.59	1.9068	4.2896	0.6106
Rind proportion (%)	27.76	65.11	41.16±1.45	8.33	20.24	0.7162	0.8164	0.3672
TSS (^{o}Brix)	11.50	16.00	13.87±0.24	1.39	10.02	0.3016	-1.1104	0.4975
Juice content (%)	25.59	62.37	43.05±1.63	9.34	21.70	0.3789	-0.5046	0.4086
Acidity (%)	0.30	0.57	0.41±0.01	0.07	17.07	0.7712	0.1631	0.4601
TSS/acid ratio	20.17	53.34	35.43±1.28	7.37	20.80	0.1856	-0.2358	0.3372
Ascorbic acid content (mg/100g of fruit)	7.96	20.68	14.70±0.57	3.30	22.45	-0.4400	-0.3921	0.4100
Reducing sugar (%)	6.00	10.12	7.91±0.20	1.12	14.16	0.0859	-0.9025	0.4196
Total sugar	7.24	12.92	9.35±0.22	1.29	13.79	0.5126	0.4431	0.3371

(%)								
Non-reducing sugar (%)	0.66	3.06	1.45±0.09	0.50	34.48	0.2654	-1.3905	0.5596
Anthocyanin content (mg/100 g of fruit)	9.14	19.30	13.60±0.60	3.46	25.44	-0.0439	-0.7373	0.3905
General appearance	1.14	4.00	2.63±0.13	0.73	27.76	-0.1501	-0.8938	0.4244
Fruit shape	2.00	3.77	2.83±0.09	0.53	18.73	-0.6110	-0.4190	0.4762
Fruit rind colour	1.00	3.52	2.37±0.12	0.71	29.96	1.4849	1.3797	0.6844
Fruit size	1.00	3.50	1.41±0.12	0.68	48.23	-0.2598	-1.2865	0.5293
Aril colour	1.50	4.00	3.01±0.14	0.82	27.24	1.0881	0.1348	0.6352
Cracking (%)	6.31	31.40	13.91±1.20	6.91	49.68	0.7072	0.0377	0.4490
Anar butterfly incidence (%)	9.42	38.62	22.20±1.24	7.10	31.98	0.2098	-0.6949	0.4003

Table 3.Eigen values and proportion of variance explained by 8 principal Components

Eigen value	8.87657	6.31913	3.14207	2.34867	2.0546	1.42284	1.16639	1.209
Proportion	0.2774	0.1975	0.0982	0.0734	0.0642	0.0445	0.0364	0.1384
Cumulative	0.2774	0.4749	0.5731	0.6465	0.7107	0.7551	0.7916	0.826

Table 4 Eigen/Latent vectors for thirty two traits of 33 indigenous pomegranate accessions

	PRIN1	**PRIN2**	**PRIN3**	**PRIN4**	**PRIN5**	**PRIN6**	**PRIN7**	**PRIN8**
Plant height	0.058176	0.106141	-.107615	0.278439	0.311825	0.096339	-.313652	0.172289
Plant spread	0.156172	0.047057	-.212378	0.266365	0.098540	0.048593	-.201781	0.237540
Suckering capacity	0.114195	-.011007	0.007452	0.216704	0.350072	-.244089	0.046942	0.260207
Number of fruits per/tree	0.090287	0.049495	-.463105	0.250646	-.013765	0.034727	0.019783	0.053535
Yield /tree	0.219916	0.005273	-.347166	0.186297	-.035795	58 .29520	0.052073	0.009892
Yield efficiency	0.180027	0.131118	-.174567	0.195563	0.026052	0.083298	0.239728	-.122826
Leaf area	-.013504	0.124475	-.204667	0.086686	-.370520	-.096959	0.408683	-.001184
Fruit length	0.299853	-.075161	0.095557	-.109757	-.060973	-.082045	-.065849	0.021425
Fruit diameter	0.311456	-.078763	0.041712	-.107315	0.009720	0.030117	0.043289	0.069168
Fruit weight	0.309363	-.101514	0.076724	-.102528	-.019789	0.091054	0.084434	0.077131
Fruit volume	0.312682	-.073196	0.056176	58 99552	-.026715	0.058608	0.100052	0.038402
Total aril weight	0.296895	-.049373	-.004944	-.220664	0.160229	-.047695	0.063971	0.030526
Number of /fruit	0.198931	-.051561	-.161870	-.374336	0.212980	0.127977	0.137564	0.153283
Weight per	0.19994	0.01042	0.20423	0.20418	0.03660	-	-	-

aril	1	0	0	5	2	.357860	.126847	.197693
Rind thickness	0.133423	-.176644	0.131041	0.336235	-.065441	-.306495	0.053279	-.049978
rind weight	0.252713	-.136315	0.151442	0.057422	-.225281	0.220233	0.082754	0.117412
Rind proportion	-.050473	-.069798	0.168374	0.302716	-.403823	0.310753	0.086750	0.170819
TSS	0.144342	0.252265	0.260702	0.009592	-.146566	-.014266	-.171753	0.137194
Juice content	-.012752	0.325634	-.091501	-.099684	0.129229	0.072927	-.033897	0.109659
Acidity	0.004862	-.282099	0.204230	0.185736	0.109198	-.075283	0.017597	0.144214
TSS/ acid ratio	0.061722	0.352487	-.017187	-.131065	-.118418	0.123390	-.105531	-.058292
Ascorbic acid content	0.085208	0.270125	-.085716	0.159207	-.095488	-.009434	0.086994	-.156386
Reducing sugar	0.127434	0.265579	0.284607	0.058062	-.035765	0.107357	-.191418	0.057113
Total sugar	0.101711	0.313775	0.175127	0.051062	-.055864	0.178553	-.198330	0.086946
Anthocyanin content	-.037240	0.305741	0.088058	-.010636	0.087844	-.132245	0.248471	0.272667
Gernal appearance	0.233938	0.017842	-.143860	-.155275	-.079828	-.175662	-.041567	-.060547
Fruit shape	0.125825	0.107050	-.017973	0.057415	0.205238	0.078078	-.054419	-.554661
Fruit rind colour	0.161209	0.067072	-.109213	-.132945	-.288590	-.193062	-.288359	-.195871

Fruit size	0.272171	-.103484	0.042600	0.078262	-.031880	0.120066	0.105435	-.030646
Aril colour	-.033016	0.253654	0.200704	-.036178	0.184332	-.004643	0.413377	0.115241
Cracking	0.016914	-.075363	0.195012	0.152625	0.283535	0.477483	0.143396	-.378557
Anar butterfly incidence	-.075592	-.240664	-.146329	-.099163	-.056061	0.292087	-.271414	0.186061

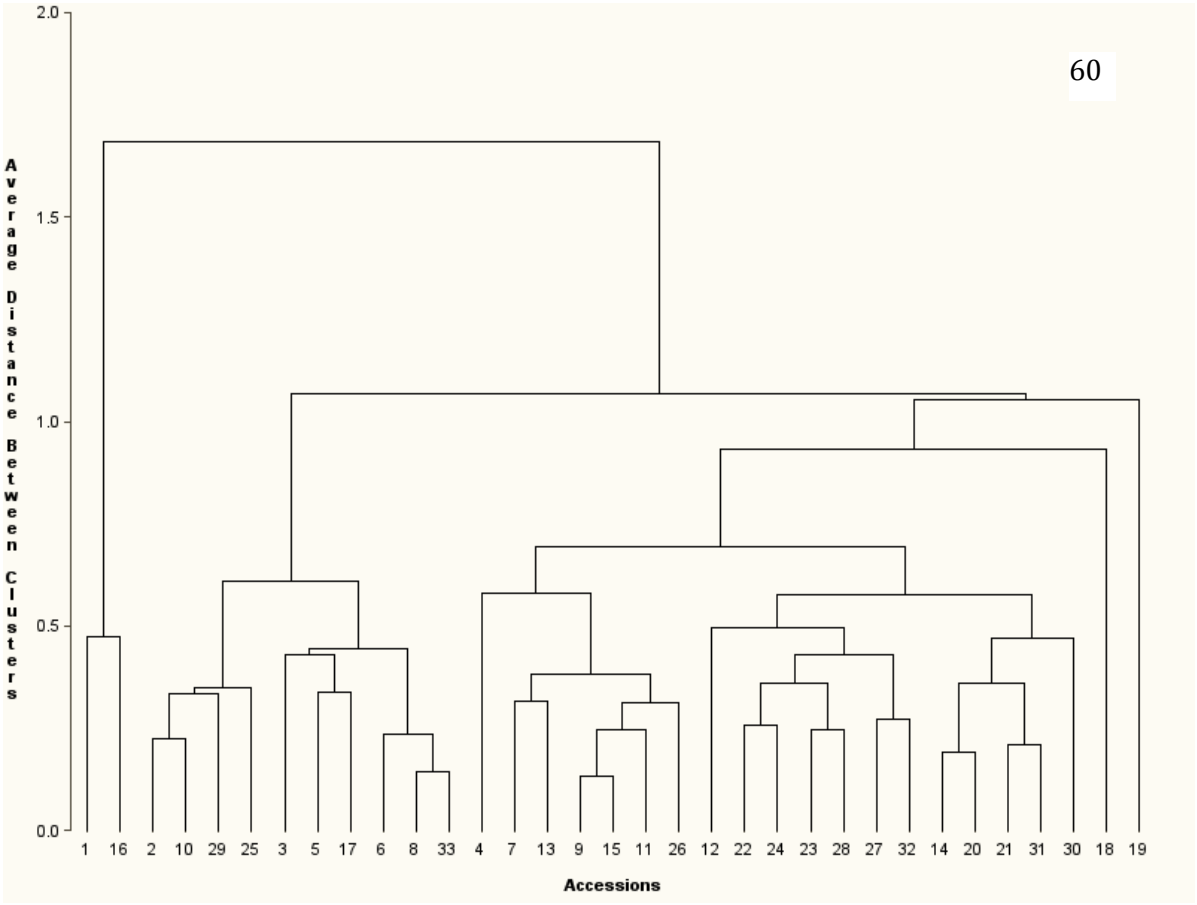

Fig.1: Dendrogram for the 33 indigenous pomegranate accessions produced by average distance cluster analysis; clusters based on pomological and biochemical traits (scale: Euclidean distances)

The data on morphological and physico-chemical variability are presented in Table 2. The lowest values of standard deviation were recorded in the case of number of weight per aril (0.04) followed by the titrable acidity (0.07). The highest standard deviation value was that for the number of arils per fruit (147) followed by the fruit volume (77). The coefficients of variation were the lowest for the TSS (10.02) followed by fruit diameter (10.18) however highest coefficient of variation value was for suckering capacity (55.54) followed by cracking % (49.68) and fruit size (48.23).

Skewness describes the symmetrical distribution pattern with respect to its dispersion from the mean. The skewness values showed that the data are normally skewed which are less than ± 2. However, positive skewness was recorded for plant height, plant spread, suckering capacity, number of fruit per tree, yield per tree, yield efficiency, leaf area, fruit length, fruit diameter, fruit weight, fruit volume, total aril weight, rind thickness, rind weight, rind proportion, TSS, juice content, acidity, TSS/acidity rato, reducing sugar, total sugar, non reducing sugar, fruit rind color, aril color, cracking percentage. Kurtosis tells the weight of the tails of a distribution. In the present set of data it was recorded platykurtic distribution pattern for number of fruits, yield /tree, yield efficiency, fruit length, fruit diameter, fruit volume, fruit weight, total aril weight, rind weight, rind proportion, acidity, total sugar, aril colour fruit cracking, however leptokurtic distribution for plant height, plant spread, suckering capacity, leaf area, number of arils /fruit, weight per aril, rind thickness, TSS, juice content, TSS/acid ratio, ascorbic acid content, reducing sugar, non reducing sugar, anthocyanin content, general appearance, fruit shape, fruit size, anar butterfly incidence. Bimodality of genetic admixture values provides evidence of strong isolation between two morphological and genetic clusters, supporting the existence of a sympatric genotypes pair within the gene pool. In the present study values are near to zero, explains the closeness among the genotypes for the traits under study. Similar results in pomegranate are also reported by earlier workers (Durgac *et al*.2008; Zaouay and Mars 2011)

The dendrogram based on Euclidean distance clustered accessions into two major groups (Fig. 1). The first group consisting of only two accessions (accession 1 and 16) detached at RMS distance of 1.68. They have no similarities with other accessions which were characterized by their higher fruit weight, number of arils and lower juice content. The second group having quite

heterozygous consisted of two sub-groups at EMS distance of 1.06.The first sub-group was further divided into two clusters at EMS distance of 0.933. The cluster first contains four accessions (accession 2, 10, 29 and 25) and their closeness is depicted from their lower plant spread, fruit weight, aril number and higher rind proportion. The cluster second contains six accessions (accession 3, 5, 6, 8, 17 and 33). They show similarities with respect to plant height, total aril weight and anthocyanin content. The second sub- group was also further grouped into two clusters at EMS distance of 1.05. The second cluster consists only one accession 19 and is popular with higher plant height, spread and rind thickness. The first cluster was further divided into two sub clusters. Among the two sub-clusters of the major second major first cluster, most of the accessions were closely related and grouped in the same branch with genetic dissimilarity ranging from 0.19to 0.57 indicating relatively lower diversity within the group. The second sub-cluster consists only accession 18.

The Eigen value obtained by PCA indicates that the first eight components provide a good summary of data explaining 82.6% the total variability (Table 3), similar as reported by Mars and Marrakchi (1999). The first component PCA1 had largest loading for yield/ tree, fruit length, fruit diameter, fruit weight, fruit volume, general appearance, total aril weight, fruit rind colour, fruit size and rind weight, represented 27.74% 0f the total variation. The PCA2 consists mainly juice content, TSS/acid ratio, ascorbic acid content, total sugars, anthocyanin content and aril colour contributed 19.75% of total variation. The PCA3 component had highest loadings for TSS, acidity, reducing sugars and constituted 9.82% of the total variation. The PCA4 component consists number of fruits per tree, weight per aril, rind thickness and shared 7.34% of total variation. The PCA5 had largest loadings for plant height, suckering capacity, fruit shape and represented 6.42% of the total variation. The PCA 6 had highest loadings for cracking, anar butterfly incidence, rind proportion and contributed 4.45% of total variation. The PCA7 consists mainly of yield efficiency, leaf area and represented 3.6% of total variation. The PCA8 had least loadings for all the variables and contributed only 1.38% of total variation (Table 4.). Our results confirmed those reported previously by several researchers on pomegranate germplasm (Mars and Marrakchi,1999; Drogoudi *et al*. 2005; Durgac *et al*. 2008).

Mars and Marrakchi (1999) found that the discriminating characters were fruit size, color, and juice characteristics. These analyses are very useful for its collection, management, and use in

future breeding programs. Nevertheless, morphological descriptors, which are environmentally influenced, are not enough to identify pomegranate cultivars because the differences among them are often ambiguous. Biochemical (Al-Said *et al*. 2009) as well as molecular (Jbir *et al*. 2008) markers are required to complete this study in order to evaluate and better estimate diversity among *Punica granatum* genetic resources.

Conclusions

Pomological and biochemical study of indigenous pomegranate accessions showed a existence of great diversity within the indigenous pomegranate germplasm. These analyses could be extremely useful for its collection, management, conservationa and utilization in future breeding programs. Nevertheless, morphological descriptors, which are environmentally influenced, are not enough to identify pomegranate cultivars because the differences among them are often ambiguous. Bio-chemical (isozymes) as well as molecular markers are required to complete this study in order to evaluate and better estimate diversity among *Punica granatum* genetic resources.

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