

Correlation between the morphological characters of pomegranate (*Punica granatum*) traits and their implications for breeding

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Abstract: Pomegranate is one of the most important horticultural crops in Iran. Selection of suitable genotypes resistant to unfavourable environmental and soil conditions and diseases is important for increasing the yield efficiency and acreage of this important crop. The aim of this research was to compare commercial pomegranate genotypes in Iran and to determine any correlations between morphological characteristics. Eight pomegranate types were used during the research. Twenty-six morphological characteristics were evaluated based on the pomegranate descriptor. Results from simple correlation analyses showed significant positive and negative correlations in certain important characteristics. Titratable acidity and vitamin C were in significant correlation with chlorophyll index and leaf weight. Factor analysis was used to determine the effective characteristics and the number of main factors. For each factor loading a value of more than 0.65 was judged as being significant. Effective characteristics were categorised into 5 main factors that contributed to 94.86% of the overall variance. Fruit characteristics were defined mainly by the first factor, contributing to 34.94% of the total variance. According to this study, leaf weight and chlorophyll index can be used for separation of sour from sweet cultivars in the juvenile phase.

Key words: Correlation, factor analysis, genotype, pomegranate, titratable acidity

1. Introduction

Pomegranate (*Punica granatum* L.), from the family Punicaceae, is an important and exportable fruit crop in Iran that has been cultivated for a long time. Iran is the centre of origin of pomegranate according to old documents and its cultivation has extended from Iran to other parts of the world (Levin, 1994). Today wild pomegranates grow in the northern and western forests, and in other districts of Iran. Estimation of the correlation between vegetative and reproductive characters in breeding programmes could provide useful information for breeders to determine the most efficient design for genotype evaluation (Tancred et al., 1995). Estimates of correlation coefficients allow comparison of indirect with direct selection, computation of correlated response in a second trait if selection pressure is applied to the first, and establishment of selection strategy (Falconer & Mackay, 1996). Correlation coefficients have been estimated in several fruits including pistachio and strawberry (Garcia et al., 2002; Karimi et al., 2009). Correlation coefficients for different parameters of pomegranate fruit were reported by Zamani et al. (2006). They reported that fruit characteristics such as peel thickness positively correlated with diameter of calyx and fruit weight with fresh and dry

aril weight. Sarkhosh et al. (2007) studied the relationships among fruit quantitative and qualitative characteristics of some Iranian pomegranate genotypes and reported that the anthocyanin content of arils negatively correlated with fruit size. They also postulated that fruit juice, aril, and seed characteristics are the main factors for separation of the pomegranate genotypes studied.

Modern objectives in plant breeding may be achieved by the evaluation of traits amongst genetic resources and eventually improve the tree parameters by collecting desirable characters in one cultivar. Although the use of molecular markers for genotype evaluation has proved useful, these methods are expensive. Morphological characters must be recorded for selection of parents and are the first choice used for describing and classifying the germplasm. Statistical methods including principle components or cluster analysis can be used for screening accessions. Additionally, some morphological characteristics have been used for evaluation of disease susceptibility, which could not be distinguished simply, and therefore may be useful as markers in breeding programmes (Karimi et al., 2009).

However, most correlations reported for pomegranate refer to fruit characteristics and there is no report about

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correlations between vegetative and reproductive traits. Therefore, the objective of this research was to study the correlations between vegetative and reproductive characters of pomegranate in order to determine their implications for breeding.

2. Materials and methods

Eight pomegranate types, each comprising 4 samples, were labelled to enable recording of their morphological specifications. The experiment was conducted in randomised block design (RBD) with 4 replications, each including 1 tree. The genotypes were described based on the pomegranate descriptor developed by the University of Florence, Italy. Twenty-six characteristics were identified for evaluating the chosen samples (Table 1). Twenty-five fully expanded leaves were removed from each tree to evaluate the characteristics of the leaves. The soluble solids content of arils was measured using a digital handheld refractometer and expressed as degrees Brix. Titrable acidity was determined by titration of 5 mL of fruit juice with 0.1 N NaOH and expressed as percentage of citric acid content (Zhang et al., 2010).

Analysis of variance, comparison of means, simple correlations, and factor and cluster analyses were carried out using SPSS and SAS software to reveal the relationships between the genotypes (Sheikh Akbari Mehr et al., 2012).

3. Results

3.1. Analysis of variance

Significant differences ($P \leq 0.05$) were detected among the cultivars for all the noted characteristics by analysis of variance (Table 2). Mean values of the studied morphological characteristics showed large variations between the genotypes for all of the measured traits. Mean values and the range of variability for the different characteristics of each genotype are presented in Table 3. Characteristics showing a greater quantitative range had higher coefficients of variation (CV), meaning increased possibilities for selection for those characteristics. Vitamin C of juice, titratable acidity, the ratio of edible part of fruit to total, aril fresh weight, and peel weight were the characteristics with the highest variation. Measures of fruit size showed a very large range from small to large, with the GSA genotype having the heaviest fruit and the largest fruit dimensions, while GSH had the lightest fruit (Table 1). The number of seeds in fruit was least amongst SHA and most in GAS. The greatest thickness of peel was found in the GSA genotype.

3.2. Correlations

The correlation between each pair of traits was calculated (Table 4). It was found that several leaf characteristics were in significant correlation with fruit characteristics. Fruit characteristics such as titratable acidity ($r = +0.89$) were positively correlated with chlorophyll index. Vitamin C

of juice was positively correlated with leaf fresh and dry weight. Peel weight was significantly correlated with calyx diameter ($r = +0.79$), fruit diameters ($r = +0.94$), and fruit length ($r = +0.93$). Number of seeds in fruit and seed firmness were in negative correlation with peel weight ($r = -0.78$) and pH of juice ($r = -0.72$).

Our findings showed that the number of seeds in fruit was correlated with fruit length ($r = +0.74$). Calyx diameter was in positive correlation with fruit weight ($r = +0.84$) and fruit diameter ($r = +0.81$).

3.3. Factor analysis

Factor analysis was used to determine the number of main factors in order to reduce the number of effective characteristics to discriminate between genotypes (Table 5). Based on factor analysis, the characteristics of fruits and seeds accounted for 34.94% of the variance as the first main factor. For each factor, a factor loading of more than 0.65 was considered as being significant. For the first factor, characteristics including fruit width, fruit diameter, calyx diameter, length of fruit, fruit length without calyx, peel weight, seed firmness, number of seeds in fruit, and edible part of fruit had a loading of more than 0.65 and defined 34.94% of the overall variance. The length and width of the leaf, leaf fresh and dry weight, seed dry weight, and vitamin C of juice were significant for the second factors with 20.76% of overall variance. The third factor with 15.26% of the overall variance contributed to characteristics such as chlorophyll index, pH of juice, and titratable acidity. The remaining factors were the number of leaves on node, shoot diameter (fourth factor), and peel thickness (fifth factor).

3.4. Cluster analysis

The pomegranate genotypes were grouped according to 5 factors. Cluster analysis divided the genotypes into 3 sub-clusters, each consisting of genotypes belonging to the cultivars SHA and ZAY; GDA, GSH, and ZGT; and SSH, GSA, and PKA. Based on the results, GDA, GSH, and ZGT were found to be in-between cultivars, but more resembled SHA and ZAY cultivars (Figure).

4. Discussion

Correlations between quantitative traits of pomegranate genotypes showed that leaf characteristics were in low correlation with fruit characteristics except for vitamin C and titratable acidity of juice, which were correlated with leaf weight and chlorophyll index. In a similar study, Mars and Marrakchi (1998) also reported that there were no correlations between fruit size and components of pomegranate. Titratable acidity of juice was in significant correlation with chlorophyll index. It was deduced that cultivars with green leaves are sourer and sweet cultivars have lighter leaves. Results of correlation analysis between characteristics showed that pH of juice was negatively

Table 1. Pomegranate genotypes used for morphological classification and means of their measured quantitative characteristics.

No.	Cultivar	Taste	Code	Fruit weight	Fruit diameter	Fruit length	Calyx diameter	Peel weight	Peel thickness	No. of seeds in fruit	Seed length	Seed diameter	Seed firmness
1	Gorch-e-dadashi	Sweet-Sour	GDA1	263.94	82.00	77.21	17.09	123.71	2.47	346	7.37	2.20	4.56
2	Gorch-e-dadashi	Sweet-Sour	GDA2	234.58	77.65	68.39	15.44	96.71	2.16	335	7.38	2.62	5.11
3	Gorch-e-dadashi	Sweet-Sour	GDA3	209.70	78.90	69.39	14.54	108.20	2.55	339	7.59	2.59	4.90
4	Gorch-e-dadashi	Sweet-Sour	GDA4	282.23	85.53	75.99	15.85	90.88	2.40	293	7.31	2.32	4.95
5	Gorch-e-shirini	Sweet	GSH1	143.85	72.30	63.08	12.72	73.91	2.04	277	8.66	2.68	5.13
6	Gorch-e-shirin	Sweet	GSH2	197.92	75.27	68.35	13.75	84.05	1.84	420	6.50	2.47	4.83
7	Gorch-e-shirin	Sweet	GSH3	186.99	74.34	66.31	13.17	83.51	2.40	352	7.96	2.86	4.54
8	Gorch-e-shirin	Sweet	GSH4	155.83	72.72	63.68	13.31	75.47	2.01	349	7.97	2.56	5.58
9	Shahvar	Sweet	SHA1	182.55	71.33	69.86	14.01	87.05	2.32	298	6.93	2.66	5.17
10	Shahvar	Sweet	SHA2	205.92	73.23	68.95	14.24	78.05	2.43	281	7.99	2.15	5.69
11	Shahvar	Sweet	SHA3	242.06	74.99	72.71	15.46	97.24	2.48	298	7.72	2.51	5.17
12	Shahvar	Sweet	SHA4	258.10	74.94	72.83	15.20	96.25	2.08	351	7.37	2.61	4.95
13	Zagh-e-yazdi	Sweet	ZAY1	293.29	83.09	77.53	16.40	126.91	2.54	379	7.73	2.05	4.70
14	Zagh-e-yazdi	Sweet	ZAY2	265.86	80.31	71.65	14.41	101.25	2.32	357	7.68	2.24	4.27
15	Zagh-e-yazdi	Sweet	ZAY3	259.18	80.17	72.63	13.78	107.23	2.49	289	7.66	2.34	4.85
16	Zagh-e-yazdi	Sweet	ZAY4	276.24	82.48	75.81	15.27	99.68	2.26	318	7.73	2.85	5.97
17	Zagh-e-gorch-e-torsh	Sour	ZGT1	303.37	85.58	74.69	18.56	124.18	2.58	449	7.13	2.50	5.56
18	Zagh-e-gorch-e-torsh	Sour	ZGT2	269.17	79.35	70.70	16.69	99.96	2.58	358	7.59	2.76	5.76
19	Zagh-e-gorch-e-torsh	Sour	ZGT3	232.95	78.57	69.43	19.98	102.90	2.70	413	6.95	2.59	4.99
20	Zagh-e-gorch-e-torsh	Sour	ZGT4	249.71	79.93	70.13	17.51	98.00	2.51	368	7.52	2.18	4.51
21	Shirin-e-shahvar	Sweet	SSH1	211.48	73.35	69.81	14.80	97.59	2.53	318	6.75	2.38	4.84
22	Shirin-e-shahvar	Sweet	SSH2	248.44	77.13	74.66	13.83	92.32	2.18	465	6.79	2.30	4.49
23	Shirin-e-shahvar	Sweet	SSH3	221.29	77.54	73.58	16.22	98.57	2.50	407	7.13	2.20	4.62
24	Shirin-e-shahvar	Sweet	SSH4	218.07	78.26	72.47	15.78	82.76	2.70	442	7.23	2.20	4.73
25	Gol sefid-e-ashkazar	Sweet	GSA1	409.46	92.07	95.63	18.98	191.78	2.67	635	7.51	2.73	4.46
26	Gol sefid-e-ashkazar	Sweet	GSA2	293.25	82.66	80.51	16.98	131.66	1.89	493	7.16	2.43	6.13
27	Gol sefid-e-ashkazar	Sweet	GSA3	330.57	86.63	86.19	19.78	163.57	2.35	511	7.69	2.75	3.62
28	Gol sefid-e-ashkazar	Sweet	GSA4	355.43	91.66	87.80	17.75	175.73	2.36	504	7.30	2.52	3.76
29	Poost ghermez-e-ali aghaei	Sour	PKA1	225.13	77.24	74.48	14.85	85.90	2.46	448	7.80	2.73	5.05
30	Poost ghermez-e-ali aghaei	Sour	PKA2	229.45	77.33	74.99	15.91	114.06	2.91	401	7.54	2.43	4.46
31	Poost ghermez-e-ali aghaei	Sour	PKA3	237.90	78.71	73.43	12.79	98.58	2.63	380	7.47	2.55	5.50
32	Poost ghermez-e-ali aghaei	Sour	PKA4	202.54	72.79	70.51	15.12	78.11	1.66	410	7.22	2.52	5.48

Table 2. Comparison of means of quantitative traits in pomegranate genotypes studied.

Genotypes	NLN	CHI	SHD	25LFW	25LDW	LEL	LEW	FRW	FRD	CAD	FLWC	FRL	TAFW
GDA	3.29 a	53.76 a	2.85 bc	8.46 cd	4.56 b	5.17 abc	1.56 b	247.61 bc	81.02 b	15.73 b	72.74 b	95.56 b	109.74 cd
GSH	2.89 ab	55.50 a	2.36 c	9.21 bc	4.72 ab	4.99 bcd	1.57 b	171.14 d	73.65 c	13.23 c	65.35 c	81.85 c	85.18 d
SHA	3.21 a	62.45 a	2.23 ab	9.39 bc	4.41 bc	5.18 abc	1.54 b	222.15 c	73.62 c	14.72 bc	71.08 b	91.02 b	125.78 bc
ZAY	2.85 ab	56.07 a	3.45 a	8.81 bc	4.30 bc	4.94 cd	1.49 b	273.64 b	81.51 b	14.96 bc	74.40 b	95.24 b	143.54 b
ZGT	2.92 ab	62.26 a	3.54 a	10.58 a	5.34 a	5.63 a	1.79 a	263.80 bc	80.85 b	18.18 a	71.23 b	91.14 b	132.90 bc
SSH	2.20 cd	57.93 a	2.37c	7.56 d	3.77 c	4.45 d	1.35 c	224.82 c	76.57 c	15.15 b	72.63 b	89.95 b	112.02 bcd
GSA	2.57 ab	60.86 a	2.71 bc	9.94 ab	4.73 ab	5.31 abc	1.58 b	347.17 a	88.25 a	18.37 a	87.53 a	109.54 a	172.88 a
PKA	2.07 d	62.07 a	2.49 c	8.98 bc	4.37 bc	5.57 ab	1.61 b	223.75 c	76.51 c	14.66 bc	73.35 b	95.27 b	131.48 bc
Genotypes	PEW	PET	TSEFW	TSS	PHJ	EPF	SDW	SNF	SEL	SED	SEF	VCJ	TIA
GDA	104.87 b	2.39 ab	19.30 b	15.22 abc	4.25 ab	90.44 bcd	10.15 c	328.68 cd	7.41 ab	2.43 ab	4.88 a	9.40 a	0.83 bc
GSH	79.23 c	2.07 b	14.35 b	16.45 a	5.11 a	70.82 d	8.58 c	349.62 bcd	7.77 a	2.64 a	5.02 a	13.91 a	0.62 c
SHA	89.64 bc	2.32 ab	14.25 b	16.02 ab	4.13 ab	11.53 abc	8.99 c	307.31 d	7.50 ab	2.48 ab	5.24 a	8.00 a	1.47 abc
ZAY	108.76 b	2.40 ab	14.56 b	15.20 abc	4.57 ab	128.97 a	8.78 c	335.93 bcd	7.70 a	2.37 ab	4.94 a	7.15 a	0.86 bc
ZGT	106.26 b	2.59 a	18.61 b	15.57 abc	3.9 b	114.29 ab	12.36 b	396.37 bc	7.30 ab	2.50 ab	5.20 a	11.88 a	1.50 ab
SSH	92.81 bc	2.47 ab	18.23 b	15.05 abc	5.16 a	93.79 bcd	13.09 b	408.43 b	6.97 b	2.27 b	4.67 a	4.12 a	1.05 abc
GSA	165.68 a	2.31 ab	49.23 a	14.74 bc	4.95 ab	123.64 a	17.79 a	536.25 a	7.41 ab	2.60 ab	4.49 a	13.42 a	1.20 abc
PKA	94.16 bc	2.41 ab	46.59 a	14.30 c	4.20 ab	84.88 cd	13.99 b	410.12 b	7.50 ab	2.55 ab	5.12 a	9.24 a	1.89 a

Same letters in each column are not significantly different at 5% level of probability using Duncan's multiple range test (DMRT).

Table 3. Pomegranate characteristics, range of variability, mean, and coefficient of variations for quantitative traits.

No.	Trait	Abbreviation	Unit	Mean	Max.	Min.	CV (%)
1	Number of leaves per node	NLN	-	2.75	3.64	1.78	10.45
2	Chlorophyll index	CHI	-	58.86	72.82	49.24	8.66
3	Shoot diameter	SHD	mm	2.88	3.96	1.96	10.90
4	25 Leaf fresh weight	25 LFW	g	9.12	11.98	6.21	8.16
5	25 Leaf dry weight	25 LDW	g	4.53	6.30	3.11	10.07
6	Leaf length	LEL	cm	5.16	6.13	4.06	6.48
7	Leaf width	LEW	cm	1.56	1.92	1.24	6.20
8	Fruit weight	FRW	g	246.76	409.46	143.85	10.93
9	Fruit diameter	FRD	mm	79.00	92.07	71.33	2.81
10	Calyx diameter	CAD	mm	15.63	19.98	12.72	6.52
11	Fruit length without calyx	FLWC	mm	73.54	95.63	63.08	4.28
12	Fruit length	FRL	mm	93.32	117.97	79.14	4.49
13	Total aril fresh weight	TAFW	g	126.69	206.16	67.96	14.76
14	Peel weight	PEW	g	105.18	191.78	73.91	12.65
15	peel thickness	PET	g	2.37	2.92	1.66	10.48
16	Total seed fresh weight	TSEFW	g	24.39	56.13	12.91	14.05
17	Total soluble solid	TSS	%	15.32	17.30	13.20	4.58
18	pH of juice	PHJ	-	4.54	5.88	3.13	14.04
19	Edible part of fruit	EPF	g	102.30	150.03	55.05	16.77
20	Seed dry weight	SDW	g	11.85	19.59	7.82	12.24
21	Number of seeds in fruit	NSF	-	384.09	635.50	277.00	12.12
22	Seed length	SEL	mm	7.45	8.66	6.50	4.65
23	Seed diameter	SED	mm	2.48	2.86	2.05	8.03
24	Seed firmness	SEF	kg	4.95	6.13	3.62	10.85
25	Vitamin C of juice	VCJ	mg/100 g fw	9.65	28.16	1.98	36.78
26	Titrateable acidity	TIA	%	1.18	3.10	0.47	22.88

CV, Coefficient of variation = (Standard error/Mean) × 100

Table 5. Eigen values and cumulative variance for 5 major factors obtained from factor analysis and the characteristics within each factor for pomegranate genotypes.

Factor	1	2	3	4	5
Cumulative variance (%)	34.94	55.72	70.98	85.77	94.86
Eigen value	9.08	5.04	3.96	3.87	2.33
NLN	-0.164	0.350	-0.449	0.694**	0.149
CHI	0.184	0.263	0.791**	-0.022	0.129
SHD	0.160	0.206	0.161	0.933**	-0.104
25 LFW	0.249	0.861**	0.022	0.243	-0.050
25 LDW	0.070	0.961**	0.022	0.243	-0.050
LEL	0.149	0.732**	0.592	0.138	0.101
LEW	0.145	0.893**	0.353	0.202	-0.057
FRW	0.969**	0.116	0.030	0.170	-0.130
FRD	0.921**	0.207	-0.149	0.083	-0.149
CAD	0.719**	0.438	0.095	0.141	-0.509
FLWC	0.978**	-0.018	0.083	-0.131	-0.002
FRL	0.963**	0.007	0.222	-0.050	0.048
TAFW	0.909**	0.044	0.353	0.169	0.025
PEW	0.971**	0.206	-0.07	0.054	-0.020
PET	0.220	-0.166	0.447	0.332	-0.754**
TSEFW	0.619	0.154	0.474	-0.561	0.138
TSS	-0.586	0.133	-0.494	0.297	0.184
PHJ	0.144	-0.308	0.651**	0.600	0.117
EPF	0.711**	-0.055	0.108	0.650	0.067
SDW	0.762**	0.100	0.288	-0.503	-0.269
NSF	0.744**	0.212	0.103	-0.571	-0.186
SEL	0.137	0.277	-0.093	0.223	0.885**
SED	0.039	0.773**	0.094	-0.318	0.553
SEF	-0.686**	0.279	0.478	0.449	0.139
VCJ	0.147	0.898**	-0.164	-0.199	0.323
TIA	0.080	0.126	0.966**	-0.049	0.153

**Significant factor loading (considered values above 0.65)

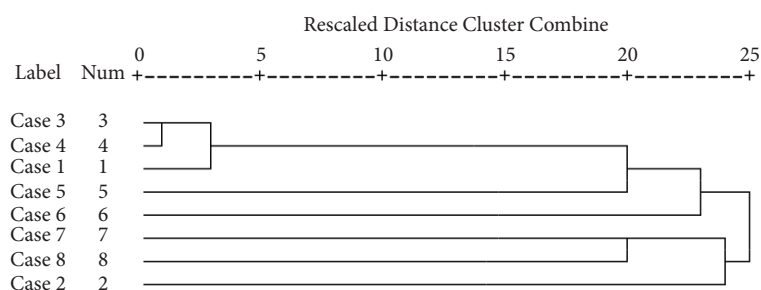


Figure. Dendrogram grouping the 8 pomegranate genotypes studied based on all main 5 factors and Ward's methods. 1: Gorch-e-dadashi, 2: Gorch-e-shirini, 3: Shahvar, 4: Zagh-e-yazdi, 5: Zagh-e-gorch-e-torsh, 6: Shirin-e-shahvar, 7: Gol sefid-e-ashkazar, 8: Poost ghermez-e-ali aghaei

correlated with dimensions of the leaves. This means that genotypes with small leaves have sourer juice than genotypes with larger leaves.

Factor analysis shows that the characteristics of the fruits provided the main factor, confirming 34% of the total variance, which must be taken into consideration when distinguishing between pomegranate genotypes. According to Zamani et al. (2006), fruit characteristics in pomegranate had the highest loading values for the first component in component analysis. Cluster analysis reveals a considerable variability that may be due mainly to recombination (resulting from out-crossing) combined with sexual and vegetative propagation for long-term and uncontrolled spread of plant material (Mars, 1996). Pomegranate is known to be at least partially cross-pollinated (Jalilop and Sampath-Kummar, 1990).

Some genotypes clustered together, including SHA and ZAY, GDA and ZGT, and SSH and GSA, and so it is possible to consider this group as multiclone varieties as reported for other fruits.

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In conclusion, according to this study, fruit characteristics such as fruit size showed the highest discriminating value and can be used for separation of pomegranate genotypes. The present study revealed a genetic relationship among pomegranate genotypes that can be used for selection of parents in breeding programmes. Moreover, it has been identified that titratable acidity ($r = +0.89$) was positively correlated with chlorophyll index and pH of juice was negatively correlated with the dimensions of the leaves. This means that genotypes with small and greener leaves have sourer juice than genotypes with larger and lighter leaves, and this can be used as an index for separation of sour from sweet cultivars in the juvenile phase.

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