

## Comparing the number of Iranian pomegranate genotypes based on morphological and biochemical properties

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**Abstract:** *Punica granatum* L. is one of the oldest known edible fruits. Numerous chemical compounds have been isolated from pomegranate seeds, juice, and peels, which have beneficial effects on human health. This study aimed to perform the physicochemical and morphological properties of twenty-four pomegranate genotypes from various provinces of Iran. Fifteen fruits of each cultivar are collected at harvest maturity in the normal ripening period for the pomegranate from the Iranian pomegranate genetic collection in Yazd, Iran. Five fruits were randomly harvested from each of four orientations of the tree, and were immediately taken to the laboratory for analysis. Three replicates were maintained for each analysis. The results indicated the highest levels of anthocyanin was observed in S783 and R633, while polyphenols in Q529, the antioxidant capacity in N755 and the total soluble solids levels in R633 and the total acidity levels were found in K477 and E336. On the other hand, the fruit weight (in S948), fruit diameter (in SH1738), crown diameter (in R533), total weight of the seeds (in S948), peel thickness (in S716), peel colour (in S948), and red juice (in S783) are significantly affected by the genotype. At a similarity of 50%, the genotypes were divided into nine sub-clusters including A, B, C, D, E, F, G, H and I. These identified genotypes can be rolled out in future breeding programmes.

**Keywords:** anthocyanin; pomegranate; total acidity; total soluble solids

The pomegranate is a fruit-bearing plant from Punicaceae family which is considered native to Iran and its neighbouring countries. Iran is one of the largest producers and exporters of pomegranates in the world (Mirjalili 2016). The principal antioxidants, such as glutathione, catalase, glutathione peroxidase,  $\beta$ -carotene, glutathione reductase, phenolics, flavonoids, proanthocyanidins have been reported in the pomegranate juice, fruit peel, and seeds (Lansky & Newman 2007). Furthermore, pomegranates contain significant amounts of phenolic compounds which possess many therapeutic properties, including cancer prevention which is comparable to green tea due to the active biological compounds. The antimicrobial activity of phenols may be due to the damage to the structure and alteration of the permeability

mechanism of microorganisms, lysosomes, and cell walls. Although this type of activity is specific to some antibiotics, the general antimicrobial effects of many phenols are irreversible by dilution with water. Besides, bacteria cannot be immune to the initial inhibitory concentration of phenol. In the past, the root of this plant was used as a worm repellent in Iran (Mirjalili 2002). Pomegranate juice is also an important food and beverage product due to its phenolic compounds, such as anthocyanin, alginic acid and tannins. In traditional Greek medicine, pomegranate flowers were applied to treat diabetes (Saxena & Vikram 2004). This plant is used for allergy symptoms (Watanabe & Hatakoshi 2002), oral hygiene (Kim & Kim 2002), antimicrobial activity (Dahham et al. 2010), and inhibition of some tumour

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cells (Van Elswijk et al. 2004). Its seed oil is consumed as a supplement in the treatment of obesity and as a weight loss agent and used in the cosmetics industry as a moisturiser (Akpınar-Bayizit et al. 2012). Pomegranate extracts have been used since ancient times to treat several conditions, including parasitic and microbial infections, diarrhoea, ulcers, aphthae, haemorrhage, and respiratory complications. Modern applications include hormone replacement therapy and oral hygiene, as well as the treatment of immune suppression and cardiovascular complications. Moreover, other therapeutic properties such as antitumour, anti-inflammatory, antiviral, antibacterial, antidiarrheal, and anti-obesity are currently under investigation. Pomegranate compounds which could be beneficial for human health such as those in the seeds: ursolic acid and  $\alpha$ -tocopherol (apoptosis in cancer cells), sterols (inhibition of pro-inflammatory), punical acid (enhance B-cell function), hydroxybenzoic acids (inhibition and apoptotic death of human prostate cancer); in the juice and peel: hydroxycinnamic acids (strong inhibitor in cancer cells), proanthocyanidins and anthocyanidins (antiangiogenic, antioxidant and anticarcinogenic activities); in the peel: flavonols and flavones (anti-cancer) flavanone glycosides; in the leaf: apigenin human (inhibition of breast cancer); in the flower: maslinic acid (macrophages), Asiatic acid (control of prostate cancer cells (Lansky & Newman 2007)).

Moreover, the pomegranate is used in a fresh form, as a juice, is fermented, as dried seeds, frozen seeds, canned seeds, as a pomegranate paste, jelly, vinegar, and flavouring products. A review of the literature indicates different amounts of the total content of the composition of the secondary metabolites, such as phenolic compounds in the different pomegranate genotypes. However, the biochemical composition of the pomegranate genotypes is affected by various factors, such as the harvest time and test time, temperature, pH, light, oxygen, susceptibility to degradation by oxidising enzymes (Jaiswal et al. 2009), genotype, environmental conditions during maturation, and fruit ripening and cultivation conditions (Borochoy-Neori et al. 2009). The results of a previous study of four Iranian pomegranate cultivars showed that despite the similarities in some cultivars, there was a significant difference due to the chemical composition, making each cultivar suitable for a specific usage (Mousavinezhad et al. 2009). Despite the numerous pomegranate genotypes cultivated in different parts of Iran (about 800 genotypes), there is

limited data on the biochemical properties and the levels of the bioactive materials in these genotypes (Tehraniifar et al. 2010).

Therefore, this study was conducted to evaluate the quantitative and qualitative amount of the compounds in some pomegranate genotypes. The identified groups can be used as a kind of valuable genetic resource in the future breeding programmes.

## MATERIAL AND METHODS

**Plant material and experimental layout.** In October 2017, twenty-four pomegranate genotypes existing in the Iranian pomegranate genetic collection in Yazd, with different origins such as Markazi, Isfahan, Khorasan, Khuzestan, Fars, Kurdistan, Kerman, Golestan, and Kermanshah provinces, were harvested in three replicates (Table 1) (Figure 1).

**Observations recorded.** Laboratory-scale weighted morphological traits, such as the fruit weight and total seed weight of each cultivar was performed with an accuracy of 0.001. Digital callipers calculated the fruit diameter, peel thickness and crown diameter. The physicochemical properties were recorded using uncentrifuged specimens of the fruit. The samples were diluted with an aqueous pH 1.0, and 4.5 buffers, and the absorbance measurements were taken at the wavelength of the maximum absorbance of the pH 1.0 solution. The difference in the absorbance between two buffer solutions is due to the monomeric anthocyanin pigments (Giusti & Wrolstad 2001), the total phenol content of 0.1 mL of the sample/standard solution in 5% methanol was mixed with 1 mL of a Folin-Ciocalteu reagent diluted with water and 1 mL of 10%  $\text{Na}_2\text{CO}_3$  was added. The absolute absorbance was taken around 760 nm (Singelton et al. 1999), the assays, namely 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals scavenging tests, were

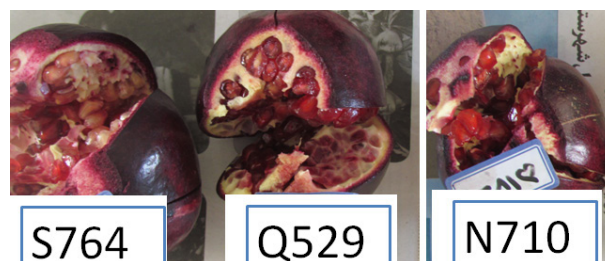


Figure 1. Some of the pomegranate genotypes  
S746 – Alakpoostghermez Saveh; Q529 – Shirinpoostghermez Sabzehvar; N710 – MalasNaalout Baneh

Table 1. List of the pomegranate genotypes

No.	Code	Genotype name	Origin	
			province	city
1	S716	Alakshirin Saveh	Markazi	Saveh
2	S733	Aghamohamadali Saveh	Markazi	Saveh
3	S742	Malasshirin Saveh	Markazi	Saveh
4	S764	Alakpoostghermez Saveh	Markazi	Saveh
5	S783	Tabestanishirin Saveh	Markazi	Saveh
6	S948	TorshMalas Saveh	Markazi	Saveh
7	E362	MalasShahvar Dastjerd	Isfahan	Dastjerd
8	E336	Malasshomareyek Dastjerd	Isfahan	Dastjerd
9	E696	Malaspoostnazok Zavareh	Isfahan	Zavareh
10	Q529	Shirinpoostghermez Sabzehvar	Khorasan	Sabzehvar
11	Q561	Malasaghdaai Torbatheidariyeh	Khorasan	Torbatheidariyeh
12	K161	Malasmarmar Ramhormoz	Khuzestan	Ramhormoz
13	K164	Malassouzok Ramhormoz	Khuzestan	Ramhormoz
14	K477	Malaspoostsorkh Ramhormoz	Khuzestan	Ramhormoz
15	F235	Malasporbarich Estahban	Fars	Estahban
16	F376	Atabakipoostghermezmalas	Fars	Shiraz
17	N710	MalasNaalout Baneh	Kurdistan	Baneh
18	N755	Malasnarpoostghermez Marivan	Kurdistan	Marivan
19	R327	Malassarjangan Bam	Kerman	Bam
20	R523	Malaspoostghermez Ravar	Kerman	Ravar
21	R533	Tough malasdarajeyek Ravar	Kerman	Ravar
22	R633	Malasdanehsefid Sirjan	Kerman	Sirjan
23	G858	Malastoughikoloukhi Gorgan	Golestan	Gorgan
24	SH1738	Malaspoostnazok Kermanshah	Kermanshah	Kermanshah

used to investigate the antioxidant potential of the pomegranate. In the DPPH test, a mixture of DPPH and the sample was prepared and was left for 30 min at room temperature. Then, the absorbance was read at 517 nm (Pokorny et al. 2001), the total soluble solids were evaluated by centrifuging the juices for 20 min at 2 000 rpm (Rotina 35R, Andreas Hettich GmbH & Co., Germany). The soluble solids content was measured with a refractometer (PAL- $\alpha$ , ATAGO, Japan). The data are expressed as Brix. A digital pH meter (AZ, Taiwan) was used to measure the pH (Mirdehghan & Rahemi 2007).

**Statistical analysis.** Fifteen fruits of each cultivar were collected at harvest maturity in the normal ripening period of the pomegranate. Five fruits were randomly harvested from each of the four orientations of the tree and were immediately taken to the laboratory for analysis. Three replicates were maintained for each analysis. To evaluate the nor-

mality of the distribution of the studied variables, the Kolmogorov-Smirnov test was used. The data were subjected to analyses of variance (ANOVA) using SAS (Ver. 7). For comparing the significance between the cultivars, Duncan's multiple range tests were used as a post hoc test.

## RESULTS

**Effect of genotype on biochemical traits.** Based on Table 2, the data showed statistical differences in the biochemical characteristics at the level of 1%. A change in the level of anthocyanin was observed from the lowest amount (2.3 mg/L) in E336 to the highest in S783 at 87.39 mg/L and R633 at 81.18 mg/L. Among the studied genotypes, Q529 had a higher polyphenol content. The antioxidant capacity varied from 933.353 mol/100 mL in N755 with the highest rate to 54.293 mol/100 mL in S733

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Table 2. Variance of the biochemical traits in the twenty-four pomegranate genotypes

	Mean square					
	DF	anthocyanin	polyphenol	antioxidant capacity	TSS	TA
Genotype	23	1 906/2**	73 923*	419**	7**	0/34**
Error	48	68/9	667 823	10	0/67	0/009
CV(%)	–	20	9	4	5	11

DF – degree of freedom; CV – coefficient of variation; \*, \*\*significant difference at a level of 5% and 1%; TSS – total soluble solids; TA – total acidity

and 54.35 mol/100 mL in S764. According to Table 3, the highest level of the total soluble solids (TSS) was in R633 (18.2667 °Bx) and Q529 (18.2660 °Bx), and the lowest level of the TSS was related to S733 (11.7333 °Bx). The maximum total acidity was seen in K477 (1.73%) and E336 (1.71%); the lowest level was related to S733 (0.51%). The genotypes studied were divided into two groups.

#### Effect of genotype on the morphological traits.

Different genotypes had a significant ( $P < 0.01$ ) impact on the total morphological properties (Table 4). According to the results, the highest fruit weight was related to S948 with 336.94 g and SH1738 with 330 g and the lowest fruit weight was related to R327 with 79.88 g. Per Table 5, the highest fruit diameter was in SH1738 with an average of 86.75 mm and S948

Table 3. A comparison of the genotypes for the biochemical properties of the pomegranate

Genotype code	Anthocyanin (mg/L)	Polyphenol (mg gallic acid/g)	Antioxidant capacity (mol/100 mL)	TSS (°Bx)	TA (%)
S716	32/453 <sup>efg</sup>	642/1 <sup>b</sup>	65/737 <sup>g</sup>	17/1667 <sup>abcde</sup>	0/84000 <sup>fghi</sup>
S733	49/120 <sup>cd</sup>	726/2 <sup>b</sup>	54/293 <sup>h</sup>	11/7333 <sup>k</sup>	0/51333 <sup>m</sup>
S742	60/550 <sup>bc</sup>	745/8 <sup>b</sup>	77/533 <sup>def</sup>	15/3333 <sup>ghij</sup>	0/58000 <sup>klm</sup>
S764	29/613 <sup>fgh</sup>	587/5 <sup>b</sup>	54/350 <sup>h</sup>	17/1000 <sup>abcde</sup>	0/75333 <sup>ghijk</sup>
S783	87/390 <sup>a</sup>	1011/4 <sup>b</sup>	80/233 <sup>cde</sup>	17/2333 <sup>abcde</sup>	0/67000 <sup>ijklm</sup>
S948	46/020 <sup>cde</sup>	527/5 <sup>b</sup>	87/780 <sup>b</sup>	16/5000 <sup>cdefg</sup>	0/64333 <sup>iklm</sup>
E362	46/323 <sup>cde</sup>	696/1 <sup>b</sup>	73/817 <sup>f</sup>	13/9667 <sup>i</sup>	0/83667 <sup>fghi</sup>
E696	3/573 <sup>i</sup>	574/5 <sup>b</sup>	82/263 <sup>bcd</sup>	16 <sup>efgh</sup>	0/70000 <sup>hijklm</sup>
E336	2/303 <sup>i</sup>	1099/8 <sup>b</sup>	67/617 <sup>g</sup>	17/6000 <sup>abcd</sup>	1/71667 <sup>a</sup>
Q529	45/430 <sup>cde</sup>	3030/5 <sup>a</sup>	55/350 <sup>h</sup>	18/2660 <sup>a</sup>	0/88000 <sup>efgh</sup>
Q561	72/943 <sup>ab</sup>	527/5 <sup>b</sup>	87/390 <sup>b</sup>	16/9333 <sup>abcdef</sup>	0/70667 <sup>hijkl</sup>
K161	15/540 <sup>hi</sup>	765/1 <sup>b</sup>	84/600 <sup>bc</sup>	16/2000 <sup>defg</sup>	1/37333 <sup>b</sup>
K164	42/600 <sup>def</sup>	1116/5 <sup>b</sup>	67/743 <sup>g</sup>	18/1667 <sup>ab</sup>	1/05333 <sup>de</sup>
K477	23/747 <sup>hg</sup>	1160/9 <sup>b</sup>	83/163 <sup>bcd</sup>	15/6667 <sup>efghi</sup>	1/73000 <sup>a</sup>
F235	7/850 <sup>i</sup>	883/1 <sup>b</sup>	68/017 <sup>g</sup>	14/5333 <sup>hij</sup>	1/09333 <sup>cd</sup>
F376	55/513 <sup>cd</sup>	1165/5 <sup>b</sup>	62/617 <sup>g</sup>	15/4000 <sup>fghij</sup>	0/92333 <sup>defg</sup>
N710	8/383 <sup>i</sup>	834/1 <sup>b</sup>	82/097 <sup>bcd</sup>	17/8667 <sup>abc</sup>	1/23333 <sup>bc</sup>
N755	30/960 <sup>efg</sup>	782/7 <sup>b</sup>	93/353 <sup>a</sup>	14/5667 <sup>hij</sup>	0/57667 <sup>klm</sup>
R327	45/523 <sup>cde</sup>	948/8 <sup>b</sup>	56/907 <sup>h</sup>	14/2333 <sup>ij</sup>	0/58667 <sup>klm</sup>
R523	57/360 <sup>cd</sup>	866/5 <sup>b</sup>	65/210 <sup>g</sup>	16/7667 <sup>abcdefg</sup>	0/55000 <sup>lm</sup>
R533	3/430 <sup>i</sup>	520/5 <sup>b</sup>	84/067 <sup>bc</sup>	15/8667 <sup>efgh</sup>	0/63333 <sup>iklm</sup>
R633	81/180 <sup>a</sup>	682/5 <sup>b</sup>	84/573 <sup>bc</sup>	18/2667 <sup>a</sup>	0/99333 <sup>def</sup>
G858	73/253 <sup>ab</sup>	989/1 <sup>b</sup>	74/757 <sup>ef</sup>	16/6333 <sup>bcdefg</sup>	0/69667 <sup>hijklm</sup>
SH1738	48/190 <sup>cd</sup>	807/5 <sup>b</sup>	82/603 <sup>bcd</sup>	17/1333 <sup>abcde</sup>	0/77333 <sup>ghij</sup>

The means within each column followed by the same letter are not different according to Duncan's test (at the probability level 1%); TSS – total soluble solids; TA – total acidity

Table 4. Variance of the morphological traits of the twenty-four pomegranate genotypes

	Mean square									
	DF	fruit weight	fruit diameter	crown diameter	total seed weight	peel thickness	seed softness	fruit peel colour	seed colour	juice colour
Genotype	23	1030**	276**	18/2**	5264**	7/61**	3/64**	32/6**	8/69**	11/9**
Error	48	1099	20/9	2/50	365	0/56	0	0	0	0
CV (%)	–	15	6	8	17	11	0	0	0	0

\*\*Significant difference at a level of 1%; DF – degree of freedom; CV – coefficient of variation

with an average of 86.68 mm, and the lowest fruit diameter was in the R327 genotype with an average of 40.46 mm. The results indicated the highest crown diameter in R533 with an average of 23.033 mm and the lowest diameter in the R327 genotype with an average of 12.4 mm. The highest average total weight of the seeds' gain in the S948 with 203.33 g, and SH1738 with 196.6 g and the lowest amount was

related to R327 with 32.06 g. The maximum peel thickness was found in S716 with a value of 10.46 mm and S733 with a value of 9.9 mm and the lowest peel thickness was found in R327 at 3.6 mm. E336, E362, K161, F235, S764, S783, R523, K164, R633, N710, R327, S742, S948 and S716 had the hardest seeds and the genotypes Q528, S731 and SH733 had the softest seeds. The rest of the genotypes fell between

Table 5. A comparison of the genotypes for the morphological properties of the pomegranate

Genotype code	Fruit weight (g)	Fruit diameter (mm)	Crown diameter (mm)	Total seed weight (g)	Peel thickness (mm)	Seed softness	Fruit peel colour	Seed colour	Juice colour
S716	207/45 <sup>bcdef</sup>	77/82 <sup>bcde</sup>	17/5 <sup>defg</sup>	53/33 <sup>hi</sup>	10/46 <sup>a</sup>	5 <sup>a</sup>	9 <sup>c</sup>	7 <sup>a</sup>	6 <sup>a</sup>
S733	244/22 <sup>bcd</sup>	76/37 <sup>bcdef</sup>	14/76 <sup>gh</sup>	93/33 <sup>efg</sup>	9/9 <sup>a</sup>	2 <sup>d</sup>	3 <sup>g</sup>	3 <sup>d</sup>	3 <sup>d</sup>
S742	212/6 <sup>bcde</sup>	76/48 <sup>bcdef</sup>	18/94 <sup>bcde</sup>	110 <sup>cdef</sup>	6/7 <sup>bcdef</sup>	5 <sup>a</sup>	9 <sup>c</sup>	3 <sup>d</sup>	3 <sup>d</sup>
S764	212 <sup>bcde</sup>	75/16 <sup>bcdef</sup>	20/24 <sup>abcd</sup>	108/3 <sup>cdef</sup>	7/86 <sup>b</sup>	5 <sup>a</sup>	3 <sup>g</sup>	3 <sup>d</sup>	3 <sup>d</sup>
S783	244/23 <sup>bcd</sup>	76/8 <sup>bcdef</sup>	20/96 <sup>abc</sup>	156/67 <sup>b</sup>	4/2 <sup>ij</sup>	5 <sup>a</sup>	2 <sup>h</sup>	7 <sup>a</sup>	6 <sup>a</sup>
S948	336/9 <sup>a</sup>	86/6 <sup>a</sup>	21/22 <sup>ab</sup>	203/3 <sup>a</sup>	7/67 <sup>bc</sup>	5 <sup>a</sup>	12 <sup>a</sup>	3 <sup>d</sup>	3 <sup>d</sup>
E362	247/51 <sup>bcd</sup>	79/28 <sup>abcd</sup>	18/51 <sup>bcde</sup>	70/4 <sup>gh</sup>	7/1 <sup>bcd</sup>	5 <sup>a</sup>	11 <sup>b</sup>	1 <sup>e</sup>	1 <sup>e</sup>
E696	207/22 <sup>bcdef</sup>	74/373 <sup>cdefg</sup>	21/160 <sup>ab</sup>	111/20 <sup>cdef</sup>	6/5 <sup>bcdef</sup>	4 <sup>b</sup>	5 <sup>e</sup>	3 <sup>d</sup>	3 <sup>d</sup>
E336	133/5 <sup>ghi</sup>	63/4 <sup>hi</sup>	14/8 <sup>gh</sup>	87 <sup>fgh</sup>	4/9 <sup>ghij</sup>	5 <sup>a</sup>	2 <sup>h</sup>	3 <sup>d</sup>	3 <sup>d</sup>
Q529	188/2 <sup>cdefg</sup>	70/6 <sup>efghi</sup>	19/1 <sup>bcde</sup>	93 <sup>efg</sup>	6/2 <sup>cdefg</sup>	2 <sup>d</sup>	11 <sup>b</sup>	3 <sup>d</sup>	3 <sup>d</sup>
Q561	267 <sup>bcd</sup>	78/4 <sup>abcde</sup>	19/1 <sup>bcde</sup>	160 <sup>b</sup>	7/26 <sup>bc</sup>	3 <sup>c</sup>	9 <sup>c</sup>	4 <sup>c</sup>	4 <sup>c</sup>
K161	196/9 <sup>bcdef</sup>	77/907 <sup>bcde</sup>	18 <sup>cdef</sup>	136/14 <sup>bcd</sup>	4/7 <sup>hij</sup>	5 <sup>a</sup>	5 <sup>e</sup>	7 <sup>a</sup>	6 <sup>a</sup>
K164	186/19 <sup>defg</sup>	69/467 <sup>efghi</sup>	16/733 <sup>efg</sup>	90/72 <sup>efg</sup>	6/43 <sup>bcdef</sup>	5 <sup>a</sup>	4 <sup>f</sup>	4 <sup>c</sup>	4 <sup>c</sup>
K477	176/2 <sup>efgh</sup>	68/5 <sup>fghi</sup>	20/8 <sup>abc</sup>	101/3 <sup>defg</sup>	6/4 <sup>bcdef</sup>	3 <sup>c</sup>	3 <sup>g</sup>	5 <sup>b</sup>	5 <sup>b</sup>
F235	236/51 <sup>bcde</sup>	77 <sup>bcdef</sup>	15/333 <sup>fg</sup>	115/8 <sup>cdef</sup>	5/6 <sup>defgh</sup>	5 <sup>a</sup>	5 <sup>e</sup>	4 <sup>c</sup>	3 <sup>d</sup>
F376	218/11 <sup>bcde</sup>	66/073 <sup>hig</sup>	17/567 <sup>defg</sup>	70/87 <sup>gh</sup>	5/3 <sup>fghi</sup>	4 <sup>b</sup>	5 <sup>e</sup>	4 <sup>c</sup>	5 <sup>b</sup>
N710	147/3 <sup>fgh</sup>	70/0 <sup>efghi</sup>	18/7 <sup>bcde</sup>	67/70 <sup>gh</sup>	7/02 <sup>bcd</sup>	5 <sup>a</sup>	6 <sup>d</sup>	3 <sup>d</sup>	3 <sup>d</sup>
N755	253/7 <sup>bc</sup>	82/3 <sup>abc</sup>	16/9 <sup>efg</sup>	140 <sup>bc</sup>	6/5 <sup>bcdef</sup>	4 <sup>b</sup>	9 <sup>c</sup>	7 <sup>a</sup>	6 <sup>a</sup>
R327	79/88 <sup>i</sup>	40/4 <sup>j</sup>	12/400 <sup>h</sup>	32/06 <sup>i</sup>	3/66 <sup>j</sup>	5 <sup>a</sup>	11 <sup>b</sup>	1 <sup>e</sup>	1 <sup>e</sup>
R523	122/8 <sup>hi</sup>	62/6 <sup>i</sup>	18/5 <sup>bcde</sup>	72/33 <sup>gh</sup>	7/43 <sup>bc</sup>	5 <sup>a</sup>	11 <sup>b</sup>	3 <sup>d</sup>	5 <sup>b</sup>
R533	250/2 <sup>bcd</sup>	83/8 <sup>ab</sup>	23 <sup>a</sup>	108/6 <sup>cdef</sup>	7/4 <sup>bc</sup>	3 <sup>c</sup>	4 <sup>f</sup>	3 <sup>d</sup>	3 <sup>d</sup>
R633	248 <sup>bcd</sup>	77/8 <sup>bcde</sup>	20/9 <sup>abc</sup>	127/4 <sup>bcde</sup>	6/9 <sup>bcde</sup>	5 <sup>a</sup>	4 <sup>f</sup>	1 <sup>e</sup>	1 <sup>e</sup>
G858	229/11 <sup>bcde</sup>	71/8 <sup>defgh</sup>	17/9 <sup>cdef</sup>	130 <sup>bcd</sup>	5/5 <sup>efghi</sup>	4 <sup>b</sup>	5 <sup>e</sup>	3 <sup>d</sup>	3 <sup>d</sup>
SH1738	330 <sup>a</sup>	86/3 <sup>a</sup>	19/4 <sup>bcde</sup>	196/67 <sup>a</sup>	4/9 <sup>ghij</sup>	2 <sup>d</sup>	9 <sup>c</sup>	4 <sup>c</sup>	4 <sup>d</sup>

The means within each column followed by the same letter are not different according to Duncan's test (at the probability level 1% and 5%)



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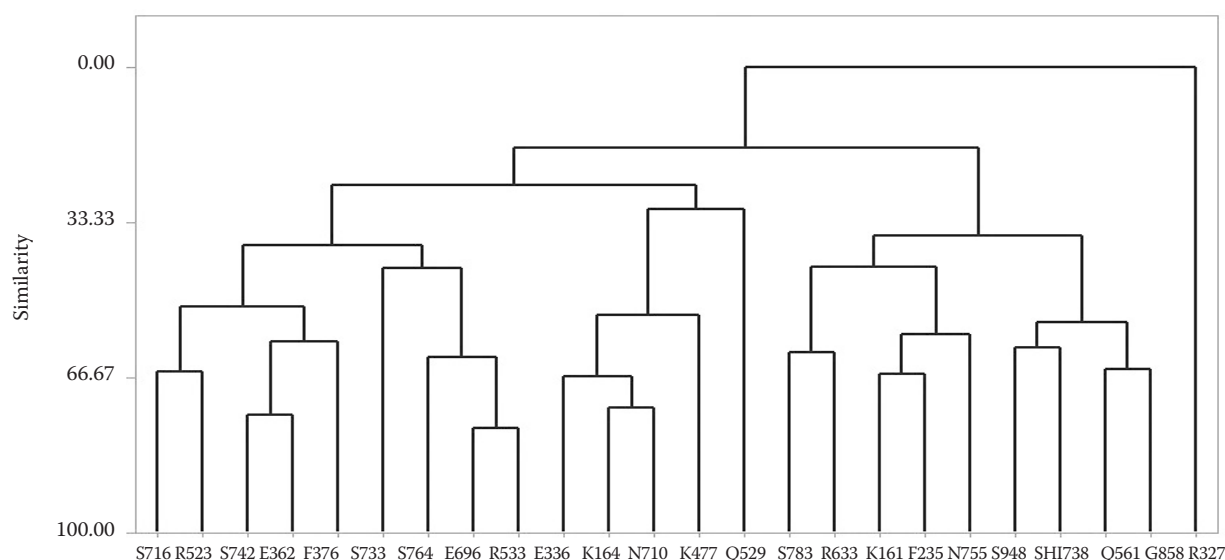


Figure 2. Dendrogram diagram of the biochemical and morphological properties of the pomegranate genotypes. The abbreviation of each genotype is recorded in Table 1

the mentioned genotypes. Based on Table 5, S948 had the darkest peel colour, and E336 and S783 have the lightest peel colour, which was yellowish. S783, N755, K161 and S716 had red seeds, and R633, E362 and R327 had cream-coloured seeds. S783, N755, K161 and S716 had red juice, while R633, E362 and R327 had cream-coloured juices.

**Dendrogram diagram.** The pomegranate genotypes were analysed to determine the similarity, and the dendrogram diagram shows these genotypes at a similarity level of 50% (Figure 2).

Based on the analysis results registered in the dendrogram at a 50% similarity level, the pomegranate genotypes were classified into the nine main groups of A, B, C, D, E, F, G, H and I. Group A consists of S716, R523, S742 and E362 are very similar in terms of the biochemical and morphological properties. Group B includes S733. The similarities were seen in group C S764, E696 and R533. Group D contains E366, K164, N710 and K477. Q529 belongs to group E. Group F is related to R633. Group G includes K161 and F235. In Group G, the only genotype is N755. Group H involved S948, SH1738, Q561 and G858. Group I has the singular R327 that is the most different from the genotypes of the other groups.

## DISCUSSION

When determining the total phenolic content (TPC) in the plant extracts, the presence of reducing interferants produces inaccurate estimations of the TPC

values. However, the phenol content and composition of the pomegranate juice are strongly influenced by the cultivar, agronomic and water conditions, harvest time, and pomegranate juice extraction method. The Folin-phenol reactant or gallic acid equivalence method is also called a mixture of phosphomolybdate and phospho-tungstate, which is used to measure the phenolic and polyphenolic antioxidants by the colorimetric method. This reagent is used for measuring the phenol and any reducing agents; therefore, the reduction capacity of the whole sample is measured, not just the phenolic compounds. On the other hand, the Folin-Ciocalteu test also has some drawbacks. First of all, the test is sensitive to the pH, temperature, and reaction time, and that is why it is necessary to accurately select the reaction state for coherent and reliable results. Secondly, the TPC overestimation is a major concern for the Folin-Ciocalteu test, owing to the contribution of the non-phenolic reducing agents present in the system when reducing the Folin-Ciocalteu reagent. Such examples of contaminants include reducing sugars and certain amino acids. Thus, the results of the TPC measurements may be overestimated by one size for comparison to the ones obtained by high-performance liquid chromatography (HPLC) methods. Moreover, the test is performed in aqueous systems, and its application for lipophilic phenols is limited, except for the case when modifications of the solvent system are applied (Blasco et al. 2005). Various methods are used to extract the natural antioxidants, most of which are inefficient. The pomegranate has

many phenolic compounds, and as a result has high antioxidant properties. There is a close relationship between the amount of the total phenol and antioxidant activity. Antioxidants in the free radical-scavenging the hydrogen from the hydroxyl group of the phenolic compounds. Therefore, a higher amount of the phenolic compound gives more hydrogen to the free radicals and prevents oxidation (Roginsky & Lissi 2005). The DPPH method is a simple way because it is a stable nitrogen radical and increases the synthesis of the antioxidant reaction with DPPH or even, in some cases, the reaction of some antioxidants with the free radical; this method is challenging. In addition, the reaction of the free radicals to the DPPH is reversible; which makes the antioxidant capacity of many antioxidants often underestimated. Other problems include the concentration D, sample volume, environmental conditions, solvent type, and reaction time (Prevec et al. 2013).

One of the important factors in the pomegranate's quality is the red colour of the seeds and their juice. Cyanidin derivatives cause red, delphinidin derivatives cause blue and purple, and pelargonidin derivatives cause the red-orange colour (Hernandez et al. 1999). The changes in anthocyanin content in the present study ranged from 2.3 mg/L in E336 to 87.390 mg/L in S783, which is consistent with the results of Mirjalili et al. (2019) and are close to the values obtained by Gil et al. (2000). The reason for the anthocyanin variation is due to the destruction and instability under the harvest and test times, location of the fruit, temperature, pH, light, and oxygen. Researchers believe that the diversity in the anthocyanin level could be due to genetic differences in the cultivars (Melgarejo et al. 2000).

Among the genotypes studied, Q529 had a higher polyphenol content than the other genotypes. The studies by (Tatari et al. 2011) and (Tehranifar et al. 2010) indicated a significant difference in the polyphenol levels in various genotypes.

The antioxidant capacity in the research by Mirjalili et al. (2019) varied, which is consistent with the results of the present study. (Akbarpour et al. 2009) showed a wide range of antioxidant activities in twelve Iranian pomegranate cultivars (Lamsari-Behshahr and Shishe-Kap). Mousavinejad et al. (2009) conducted the maximum antioxidant capacity in the Ostokhani Tabas genotype among eight Iranian pomegranate cultivars. The antioxidant level depends on the cultivar, environmental conditions during maturation and ripening of the fruit, the cultivation conditions, and extraction method (Borochoy-Neori et al. 2009). Due to the similarity of the planting and breeding conditions as well as

the extraction, the difference among the genotypes is due to their genetic origin.

Previous findings also reported the total soluble solids in the different genotypes range from 13.96 to 18.53 °Bx (Barzegar et al. 2004) which matches this study. The amount of the TSS in most Iranian commercial cultivars was higher than 17% which has made them desirable for export (JaliliMoghadam 2015). Feyzi et al. (2015) concluded that the cultivar and environmental conditions affect the percentage of the soluble solids.

The total acidity levels reported by Mirjalili et al. (2019) and Tatari et al. (2011) were 0.57–2.06% and 1.02–2.35%, respectively. Akbarpour et al. (2009) reported an acidity of 0.68% for Malas Yazdi and 1.53% for Malas Saveh. The acidity causes the sour taste of pomegranate juice. The main reason why soft-seeded pomegranates are popular in the market is that its taste and flavor. In Iran and India when preparing pomegranate products, especially pomegranate paste, sour varieties are more desirable (Mirjalili 2016). Depending on the type of application and demand, it is necessary to study and compare pomegranate genotypes in terms of the acidity.

## CONCLUSION

Twenty-four pomegranate genotypes belonging to different parts of Iran, which were bred in the collection of genetic reserves of Iranian pomegranate breeds in Yazd, were morphologically and biochemically studied, compared, and classified. All the studied genotypes were collected from the collection of genetic reserve and were grown under the same conditions in terms of the climate, temperature, and geography, nevertheless, the morphological and biochemical differences indicated a significant genetic impact. The grouping of all the existing genotypes in the mentioned collection, due to biochemical and morphological properties, should be considered a research priority.

## REFERENCES

- Akbarpour V., Hemmati K.H., Sharifani M. (2009): Physical and chemical properties of pomegranate (*Punica granatum* L.) fruit in maturation stage. American-Eurasian Journal of Agriculture and Environment Science, 6: 411–416.
- Akpınar-Bayizit A., Özcan-Yisay T., Yilmaz-Ersan L. (2012): The therapeutic potential of pomegranate and its products for prevention of cancer. In: Georgakilas A.G. (ed.): Cancer Prevention – from Mechanisms to Translational Benefits. IntechOpen.

<https://doi.org/10.17221/39/2021-CJGPB>

- Barzegar M., Fadavi A., Azizi T.M.H. (2004): Evaluation of physico-chemical composition of cultivated pomegranates in Yazd. Iranian Journal of Nutrition Science and Food Technology, 1: 9–14. (in Persian)
- Blasco A.J., Rogerio M.C., Gonzalez M.C., Escarpa A. (2005): Electrochemical index as a screening method to determine total polyphenolics in foods: A proposal. Analytica Chimica Acta, 539: 237–244.
- Borochoy-Neori H., Judeinstein S., Tripler E., Harari M., Greenberg A., Shomer I. (2009): Seasonal and cultivar variations in antioxidant and sensory quality of pomegranate (*Punica granatum* L.) fruit. Journal of Food Composition and Analysis, 22: 189–195.
- Dahham S.S., Ali M.N., Tabassum H., Khan M. (2010): Studies on antibacterial and antifungal activity of pomegranate (*Punica granatum* L.). Eurasian Journal of Agriculture and Environment Science, 9: 273–281.
- Feyzi F., Seifi E., Varasteh F., Hemmati K.H., Fereydooni H. (2015): Evaluation of some biochemical properties of two pomegranate cultivars in three different fruits during different cold storage conditions. Biomedical Research International, 5: 338–342.
- Gil M.I., Tomas-Barberan F.A., Hess-Pierce B., Holcroft D.M., Kader A.A. (2000): Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. Journal of Agricultural and Food Chemistry, 48: 4581–4589.
- Giusti M.M., Wrolstad R.E. (2001): Characterization and measurement of anthocyanins by UV-visible spectroscopy. In: Wrolstad R.E., Schwartz S.J. (eds.): Current Protocols in Food Analytical Chemistry. New York, John Wiley and Sons.
- Hernandez F., Melgarejo P., Tomas-Barberan F.A., Artes F. (1999): Evolution of juice anthocyanins during ripening of new selected pomegranate (*Punica granatum*) clones. European Food Research and Technology, 210: 39–42.
- Jaiswal V., Der Marderosian A., Porter J.R. (2009): Anthocyanins and polyphenol oxidase from dried arils of pomegranate (*Punica granatum* L.). Food Chemistry, 118: 11–16.
- Jalili Moghadam Z. (2015): A Comprehensive Guide for Pomegranate Cultivation. Tehran, Agricultural Education and Extension Publishing. (in Persian)
- Kim M.M., Kim K. (2002): Composition for improving oral hygiene containing *Punica granatum* L. extracts. Korean Patent 2002, KR 2002066042.
- Lansky E.P., Newman R.A. (2007): *Punica granatum* (pomegranate) and its potential for prevention and treatment of inflammation and cancer. Journal of Ethnology and Pharmacology, 109: 177–2060.
- Melgarejo P., Salazar D.M., Artes F. (2000): Organic acids and sugars composition of harvested pomegranate fruits. European Food Research and Technology, 211: 185–190.
- Mirdehghan S.H., Rahemi M. (2007): Seasonal changes of mineral nutrients and phenolics in pomegranate (*Punica granatum* L.) fruit. Journal of Scientia Horticulturae, 111: 120–127.
- Mirjalili S.A. (2002): Recognition of Pomegranate. Karaj, Agricultural Education Publishing.
- Mirjalili S.A. (2016): Pomegranate, biodiversity and genetic resources. Rostaniha, 17: 1–18. (in Persian)
- Mirjalili S.A., Ghabouli M., Poorazizi E., Aghajani M. (2019): Investigation of biochemical, phenolic and anthocyanins variation occurrence in juice of 25 genotypes of pomegranate (*Punica granatum* L.) malas cultivars. Ecophytochemistry of Medicinal Plants, 6: 1–13.
- Mousavinejad G., Emam-Djomeh Z., Rezaei K., Khodaparast M.H.H. (2009): Identification and quantification of phenolic compounds and their effects on antioxidant activity in pomegranate juices of eight Iranian cultivars. Food Chemistry, 115: 1274–1279.
- Pokorny J., Yanishlieva N., Gordon M. (2001): Antioxidants in food. Practical pomegranates grown in Turkey. Journal of Food Composition and Analysis, 15: 567–575.
- Prevec T., Šegatin N., PoklarUlrih N., Cigig B. (2013): DPPH assay of vegetable oils & model antioxidants in protic and aprotic solvents. Talanta, 109: 13–19.
- Roginsky V., Lissi E. (2005): Review of methods to determine chain-breaking antioxidant activity in food. Food Chemistry, 92: 235–254.
- Saxena A., Vikram N.K. (2004): Role of selected Indian plants in management of type 2 diabetes: A review. Journal of Alternative and Complementary Medicine, 10: 369–373.
- Singleton V.L., Orthofer R., Lamuela-Raventós R.M. (1999): Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In: Oxidants and Antioxidants Part A. Elsevier: 152–178.
- Tatari M., Ghazvini R.F., Ghasemnejad M., Mousavi S.A., Tabatabaie S.Z. (2011): Morphological and biochemical characteristics of fruit in some pomegranate cultivars in climatical conditions of Saveh. Seed and Plant Improvement Journal, 27: 69–87. (in Persian)
- Tehranifar A., Zarei M., Nemati Z., Esfandiyari B., Vazifeshenas M.R. (2010): Investigation of physico-chemical properties and antioxidant activity of twenty Iranian pomegranate (*Punica granatum* L.) cultivars. Scientia Horticulturae, 126: 180–185.
- Van Elswijk D.A., Schobel U.P., Lansky E.P., Irth Van der Greef H.J., Kiadis L.N. (2004): Rapid dereplication of estrogenic compounds in pomegranate (*Punica granatum*) using on-line biochemical detection coupled to mass spectrometry. Phytochemistry, 65: 233–238.
- Watanabe K., Hatakoshi M. (2002): *Punica granatum* leaf extracts for inactivation of allergen. Japanese Patent, JP 2002370996, A2 20021224.

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