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Physical and Chemical Characteristics of Pomegranates

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Abstract. **Twenty pomegranate (*Punica granatum* L.) accessions were collected from different regions in northern Greece and evaluated under uniform conditions for leaf dimensions, frost resistance, and fruit physical and chemical characteristics, such as the juice antioxidant activity [ascorbate equivalent antioxidant activity (AEAC)], using the radical DPPH, ascorbic acid, total phenolic and total anthocyanin contents. Considerable variation in the characteristics studied was found and valuable pomological traits were exhibited. Cluster analysis produced cases of homonymy between some accessions. Principal component analyses showed that the component explaining the greatest variability positively correlated with percent juice, fruit chroma, AEAC, total anthocyanins, and soluble solids content (SSC), but negatively correlated with fruit fresh weight (FW), fruit and seed hue angle (less red color), total acidity, and leaf dimensions. Fruit size was positively correlated with acidity, while acidity was negatively correlated with SSC. Skin thickness and FW were correlated with fruit hue angle and chroma, suggesting that red color may better develop in thick-skinned and/or small-sized pomegranates. Total anthocyanin content was negatively correlated with FW and fruit hue angle. AEAC was positively correlated with total anthocyanin and ascorbic acid contents, the latter one constituted a 15% contribution to AEAC. The associations found among physical and chemical traits suggest that consumers interested in a healthier produce should be directed to small and red pomegranates.**

The pomegranate (*Punica granatum* L.) fruit deserves special attention by consumers interested in consuming nutritional food with excellent taste. Dietary supplementation with pomegranate is believed to relate with cancer prevention (Afaq et al., 2003), LDL oxidation and atherosclerosis (Aviram, 2002), and improvement of menopausal syndromes (Mori-Okamoto et al., 2004). Its beneficial effects may be related with its high antioxidant activity, resulting from the presence of a variety of biologically active compounds (Aviram, 2002; Halvorsen et al., 2002). Studies have not been done to determine whether great variability exists in the antioxidant activity among different pomegranate genotypes. This would allow breeders to select and breed genotypes with higher levels of compounds and also provide a way of increasing the dietary intake of antioxidant compounds. Information on the possible relationships between fruit characteristics, such as color, fresh weight, skin thickness etc, with juice chemical characteristics, such as the antioxidant activity, would provide useful information for addressing consumer choices for healthier produce.

Evaluations in pomegranate physical and chemical properties in local material have been made in Italy (Barone et al., 2001), Tunisia (Mars and Marrakchi, 1999), and Turkey (Ozkan, 2003), where genotypes selected from Greece were also evaluated (Ozguven et al., 1997). In Greece, pomegranate is spread mainly as a minor fruit, and traditional plantations use local varieties. Recently, its culture increased considerably and is of significant importance to evaluate and conserve local genetic material.

The present study describes the variation among 20 pomegranate accessions selected from different areas in northern Greece and grown under uniform conditions. The objectives of the present study were to a) determine the variability in the juice antioxidant activity and other chemical properties and find whether associations exist between fruit physical and chemical properties, b) determine the overall degree of polymorphism in the characteristics studied, and c) describe and detect similarities in the local material.

Materials and Methods

Plant material. Twenty pomegranate accessions were collected from various areas in northern Greece and maintained in a collection at the Pomology Institute, Naoussa, Greece. The trees were 5 years old and planted at a 5 ×1.5 m distance in a randomized block design of six trees per genotype in two replicate trees per block.

Physical characteristics. Leaf and pedicel length and leaf width of 50 fully expanded leaves randomly chosen from the tree were measured. Fruit were harvested when most of their color was red and transferred to the laboratory and sorted for size and uniformity of shape. Fruit were hand cracked and the visual appearance of fruit and seed (aril) were evaluated by a 15-member untrained panel. The 10 best accessions were scored on a 10-point hedonic scale (1 = poor, 10 = best) and remaining ten accessions were scored zero. Thickness of skin at equatorial area, fruit fresh weight (FW), and seed and tegmen weight of six-fruit replications were measured. The fruit and seed color L*, a*, and b* dimensions were measured using a chromatometer (Minolta, Ramsey, N.J.), and the hue angle (0° = red-purple; 90° = yellow) and chroma (departure from gray toward pure chromatic color) parameters were calculated according to McGuire (1992).

A severe frost took place on 8 Apr. 2003 (with temperature reaching –7 °C) and provided the opportunity to assess the ability of genotypes to withstand low temperatures. The percentage of damaged stems was assessed.

Chemical analyses. At harvest, soluble solid content (SSC) and total acidity (TA) were determined in juice extracted using a food processor from a three fruit samples per accession. SSC was measured using a digital refractometer (model PR-1, Atago, Japan) and TA by titration to pH 8.2 with 0.1 N NaOH and expressed as citric acid content (g/100 mL).

Upon harvest, six fruit per genotype were frozen at –20 °C and chemical analyses were performed within a 3-month period. Frozen fruit were divided into two halves using a hammer, and intact arils were hand separated and used for analyses or where required juice was extracted using a pestle and mortar.

Chemicals were purchased from Sigma Chemical Co. (St. Louis, Mo.). Assays were performed using an automated UV-visible spectrophotometer.

Antioxidant activity assay. Antioxidant activity in readily extracted juice from frozen fruit was determined using the stable 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical (Blios, 1958), which has an intense violet color but turns colorless as unpaired electrons are sequestered by antioxidants. Reaction mixtures containing 0 or 20 µL diluted juice (1:5 in MeOH) and 2980 µL of 106.5 µM DPPH in MeOH were vortexed and then held at room temperature for 4 h. The absorbance of the reaction mixtures was measured at 517 nm, and AEAC (mM) was extrapolated from a standard curve prepared using 0 to 2.7 mM ascorbate (ASC) ($y = (x + 5.836)/15.816$; $r^2 = 0.999$; $P < 0.001$).

Total anthocyanin assay. In total, 200 µL of readily extracted juice from frozen fruit, was diluted in 5 mL 99 MeOH : 1 HCL and stored for 24 h in the dark, when absorbance was measured at 530 nm. Results were expressed as mmoles of cyanidine-3-glucoside per 100 mL juice, using a molar extinction coefficient of 29.600.

Total phenolic assay. Total soluble pheno-

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Table 1. Mean values of % frost damage, leaf length, visual scoring and fruit physical properties of 20 pomegranate accessions.

Accession no.	Frost damage (%)	Leaf length (mm)	Visual scoring	Fruit fresh wt (g)	Seed wt (g)	Tegmen wt (g)	Skin thickness (mm)	Juice (%)	Fruit hue°	Fruit chroma	Seed hue°	Seed chroma
11002	83.3	5.0	0.3	267.2	0.304	0.064	3.5	33.0	77.6	41.7	29.7	19.4
11003	100.0	6.4	0.2	284.5	0.180	0.068	4.0	36.6	60.5	40.2	27.6	22.2
11005	75.0	5.0	6.3	333.3	0.208	0.056	3.0	29.8	65.6	44.1	32.5	24.8
11006	79.0	6.9	15.9	445.1	0.332	0.088	4.0	26.8	68.8	42.2	48.8	21.5
11009	43.8	6.1	11.5	271.3	0.208	0.064	6.3	31.9	36.8	52.6	30.1	17.1
11010	33.3	5.1	9.9	245.5	0.268	0.060	7.0	32.1	33.8	49.6	37.2	22.6
11011	83.3	6.6	7.3	394.2	0.292	0.064	4.0	30.6	65.0	45.4	29.7	18.9
11012	100.0	3.7	1.7	293.2	0.308	0.068	7.0	35.7	42.4	50.2	31.7	15.6
11014	98.3	4.0	0.7	310.8	0.292	0.052	6.0	32.6	32.0	48.4	25.3	17.2
11015	84.0	3.7	7.2	273.0	0.324	0.072	7.0	31.4	34.2	48.7	31.3	15.0
11016	52.1	4.3	4.6	344.2	0.260	0.048	4.0	35.8	40.2	44.9	26.7	15.5
11018	90.3	4.9	0.9	303.5	0.371	0.090	4.2	33.7	60.2	46.1	28.5	12.0
11019	100.0	5.0	1.7	301.7	0.360	0.064	7.0	34.1	61.8	47.7	29.7	16.0
11020	87.5	4.6	1.4	315.7	0.344	0.072	5.5	34.5	30.4	49.0	30.0	20.7
11021	75.0	5.1	4.9	351.6	0.360	0.092	5.0	30.5	88.8	43.0	28.1	17.4
11022	82.5	5.6	2.1	386.3	0.192	0.048	5.0	30.6	67.3	40.4	29.2	16.1
11025	59.6	5.5	7.3	342.8	0.228	0.060	3.0	31.0	54.9	42.2	34.2	25.0
11026	50.7	3.2	4.4	350.8	0.375	0.095	4.6	34.4	57.9	48.8	25.9	18.8
11029	62.5	5.4	3.2	351.5	0.320	0.090	4.5	32.3	86.8	43.4	31.0	21.0
11041	33.8	4.4	6.9	402.6	0.300	0.070	4.4	29.3	64.2	42.3	32.3	25.3
P	23.0[z]	<0.001	<0.001	0.020	<0.001	<0.001	<0.001	0.375[z]	<0.001	0.001	0.060	0.485
LSD	---	0.2	4.3	39.2	0.06	0.01	0.6	---	25.0	5.8	---	---

[z]LSD calculated for arcsine transformed values.

Table 2. Mean values of antioxidant activity [ascorbate equivalent antioxidant capacity (AEAC)] and ascorbic acid, total anthocyanin, total phenolic, soluble solid (SSC), and total acid (TA) contents in 20 pomegranate accessions.

Accession no.	AEAC (mM)	Ascorbic acid (mg/100 mL)	Total anthocyanins (mmol/100 mL)	Total phenolics (mg/100 mL)	SSC (%)	TA (g/100 mL)
11002	19.7	4.3	62.3	44.5	15.0	0.349
11003	16.5	2.6	65.4	47.7	16.5	0.314
11005	16.2	4.8	61.6	44.8	15.3	0.314
11006	12.1	1.7	49.9	44.2	14.7	2.050
11009	15.9	2.7	63.0	48.0	15.8	0.406
11010	17.0	2.5	64.8	55.1	14.8	0.467
11011	15.4	1.3	58.3	47.4	15.0	2.001
11012	18.5	2.8	55.1	49.0	16.7	0.338
11014	17.5	1.9	67.4	53.6	15.9	0.378
11015	17.4	2.1	66.4	41.3	15.9	0.360
11016	18.8	2.3	62.6	43.7	16.8	0.417
11018	16.0	3.8	66.3	49.5	16.1	0.330
11019	18.5	3.1	68.5	69.7	16.5	0.341
11020	17.3	3.1	72.4	64.8	16.9	0.361
11021	14.6	1.7	63.4	52.7	15.8	0.525
11022	17.8	1.6	50.7	42.2	15.2	1.104
11025	17.4	2.6	61.4	42.4	14.8	2.168
11026	25.0	5.2	64.0	40.7	17.0	0.401
11029	10.0	2.0	42.7	22.5	14.4	0.212
11041	16.8	2.0	53.4	27.3	14.8	2.392
P	<0.001	0.001	<0.001	0.110	0.495	<0.001
LSD	2.10	1.40	7.50	---	---	0.62

Table 3. Variables selected with factor loadings and explained cumulated proportion of variation for the first three eigenvectors.

Characteristic	Factor 1 (37.3)	Factor 2 (50.3)	Factor 3 (61.2)
Percent frost damage	0.304	0.416	0.290
Leaf length	–0.739	0.553	0.019
Leaf blade length	–0.724	0.583	0.023
Leaf blade width	–0.692	0.433	0.247
Fruit fresh weight	–0.689	–0.309	0.206
Seed weight	0.260	–0.365	0.821
Tegmen weight	–0.056	–0.406	0.758
Skin thickness	0.546	0.369	0.286
Percent juice	0.738	0.122	–0.103
Fruit hue°	–0.593	–0.346	0.278
Fruit chroma	0.669	0.197	0.167
Seed hue°	–0.616	0.160	0.227
Seed chroma	–0.534	–0.155	–0.437
Ascorbate equivalent antioxidant activity	0.670	–0.306	–0.290
Ascorbic acid	0.455	–0.387	–0.151
Total anthocyanins	0.719	0.260	–0.064
Total phenolics	0.502	0.620	0.223
Soluble solids	0.817	0.058	0.132
Total acids	–0.710	–0.120	–0.075

lics were determined with the Folin-Ciocalteu reagent and results were expressed as mg gallic acid/100 mL.

Ascorbic acid assay. Seeds (about 0.8 g) were extracted in 1 mL of ice-cold 6% (w/v) metaphosphoric acid containing 0.2 mM DTPA. The tegmens were removed, towel dried, and weighed. The extract was centrifuged at 10,000 g_n for 4 min at 4 °C and the supernatant recovered. ASC was measured using the spectrophotometric method described by Takahama and Oniki (1992). The reaction mixture contained a 50-µL aliquot of extract in 60 mM potassium phosphate buffer (pH 6.3), and the difference in absorbance at 265 nm was measured before and 2 min after the addition of ascorbate oxidase (1 U/mL). Results were expressed as mg ASC/100 mL.

Statistical analysis. Analysis of variance, correlation analysis, principal component

Table 4. Correlation matrix of pomegranate physical and chemical characteristics. Absolute linear correlation ≥ |0.50| are marked in bold.

Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. Leaf length	1															
2. Fruit fresh weight	NS	1														
3. Seed weight	NS	NS	1													
4. Tegmen weight	NS	NS	**0.702**	1												
5. Skin thickness	NS	–0.487	NS	NS	1											
6. Percent juice	NS	**–0.590**	NS	NS	NS	1										
7. Fruit hue	NS	0.468	NS	NS	**–0.543**	NS	1									
8. Fruit chroma	NS	**-0.509**	NS	NS	**0.730**	NS	**–0.713**	1								
9. Seed hue	0.448	NS	NS	NS	NS	**–0.633**	NS	NS	1							
10. Seed chroma	NS	NS	NS	NS	–0.445	NS	NS	NS	NS	1						
11. Ascorbate equivalent antioxidant activity	**–0.613**	NS	NS	NS	NS	0.463	NS	NS	–0.459	NS	1					
12. Ascorbic acid	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**0.577**	1				
13. Total anthocyanin	NS	**–0.599**	NS	NS	NS	0.450	**–0.539**	0.444	NS	NS	**0.513**	NS	1			
14. Total phenolics	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**0.742**	1		
15. Soluble solids content	**–0.503**	NS	NS	NS	NS	**0.762**	–0.445	0.450	**–0.539**	**–0.511**	**0.586**	NS	**0.618**	**0.511**	1	
16. Total acids	NS	**0.717**	NS	NS	NS	**–0.649**	NS	NS	0.493	0.465	NS	NS	NS	NS	**–0.548**	1

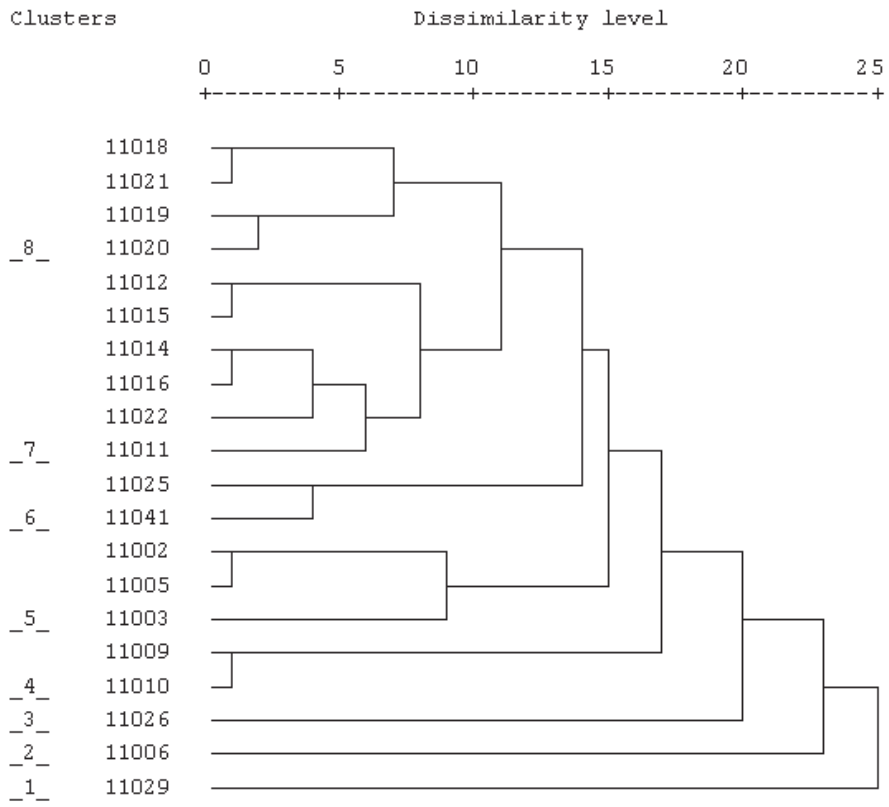

Fig. 1. Cluster dendrogram of 20 Greek pomegranate accessions.

analysis (PCA), and between-group linkage cluster analysis using the method of squared Euclidean distance, were performed using SPSS (Chicago, Ill.). Percentage data were arcsine transformed before analysis. LSD values were calculated in cases that significance at $P \leq 0.05$ variance was found among accessions.

Results and Discussion

Great variation in the percentage frost damage, leaf characteristics, and fruit physical and chemical traits of the pomegranate accessions was found (Tables 1 and 2). AEAC varied 2-fold, with accessions 11002 and 11026 containing the greatest amounts of antioxidant compounds, which is useful information for breeders and nutritionists. Total phenolics varied between 22.5 and 69.7 mg/100 mL and anthocyanins between 42.7 and 72.4 mmol/100 mL. Ascorbic acid content was between 1.3 and 5.2 mg/100 mL, values slightly greater than 0.4 to 2.2 mg/100 mL reported by Al-Khatani (1992), probably attributed to genotypic differences and/or different analyses methods used, particularly since ascorbic acid oxidation is fast in readily extracted juice (Marti et al., 2001).

Differences in frost resistance were found, the percentage stem injury ranging between 33.3% and 100.0%, suggesting that some accessions (11009, 11010, 11016, 11025, and 11041) could be suitable for growing in frost susceptible areas. From the results of fruit and seed visual evaluation, popular accessions were 11009, 11010, and 11015 due to a very intense red color. Large pomegranate accessions (11006, 11011, 11025, and 11041) were also popular but they were all sour varieties (TA > 1.8%), according to Evreinoff classification (1957).

PCA was applied to mean values of measured traits for detecting the most important factors of variability and to describe the relationship between variables and observations (Table 3). The interdependence of the variables was investigated by analysis of correlation (Table 4). PCA produced three components accounted for a cumulative 61.2% of variation; the most important variables integrated by the first component (37.3% of variance) were percent juice, fruit chroma, AEAC, total anthocyanins, and SSC, while negative correlation had leaf dimensions, FW, seed hue angle, and TA. The second component (13.0% of variance) was mainly correlated with percent frost damage, leaf dimensions, and total phenolics, while negative correlation had tegmen weight. The seed and tegmen weights were the most important variables of the third component (10.9% of variance) and were highly correlated ($r = 0.702$). Most measured traits had high discriminating values, while it is interesting that some of the consumers' most perceivable fruit characters such as fruit and seed hue color and FW had an opposite relationship with juice antioxidant properties. Traits related to fruit size and color, and juice (color, SSC, TA, and pH) characteristics, but no sepal number and calyx dimensions, had similarly high discriminating values when 30 Tunisian pomegranate accessions were evaluated (Mars and Marrakchi, 1999).

Although large pomegranates are more appealing to the consumer, these are more likely to be acidic. This was suggested by the positive correlation between fruit weight and TA ($r = 0.717$), while the fruit and seed visual evaluation showed that the most popular were large but also acidic pomegranates. SSC was negatively correlated with TA ($r = –0.632$), and similarly Melgarejo et al. (2000) found that sour pomegranate contain more TA and less SSC than sour–sweet and sweet pomegranates.

Skin thickness and FW were correlated with fruit hue ($r = –0.543$ and 0.468, respectively) and fruit chroma ($r = 0.730$ and $r = –0.509$, respectively) suggesting that red color (low hue° and high chroma values) may better develop in thick-skinned and/or small

pomegranates. Moreover, FW was negatively correlated with percent juice (r = –0.590), suggesting that small fruit may be better for juice purposes. Similarly, Jalikop and Kumar (1998) reported that small fruit are juicier as well as soft-seeded. No significant correlations were found among fruit and seed color parameters, suggesting that external color does not reflect internal color.

Regarding the juice antioxidant characteristics, total anthocyanin content was negatively correlated with FW (r = –0.599) and fruit-hue (r = –0.539), suggesting that small and /or red fruit contain more anthocyanins in juice. AEAC was positively correlated with SSC (r = 0.586) and negatively with leaf length (r = –0.613). Total phenolic and anthocyanin content were positively correlated (r = 0.742), while AEAC was correlated with total anthocyanin (r = 0.513) but not total phenolic content. Gil et al. (2000) found that the phenolic punicalagin is the most potent antioxidant found in pomegranate juice, but that anthocyanins also contributed to total antioxidant capacity of the juice. AEAC was also significantly correlated with ASC (r = 0.577), a potent antioxidant, but constitutes a relatively low (15%) contribution to AEAC in pomegranate, being similar to values reported for other fruit (Wang et al., 1996).

Cluster analysis produced eight clusters showing cases of homonymy (low dissimilarity levels) between accessions 11018 and 11021, 11019 and 11020, 11012 and 11015, 11014 and 11016, 11002 and 11005, and 11009 and 11010, indicating that a close relationship, or synonymy, between those accessions exist (Fig. 1). High dissimilarity level was found in accessions 11026, 11006, and 11029, being highly heterogeneous among the rest studied accessions. The studied accessions were only selected from the northern part of Greece and probably constitute a portion of the indigenous germplasm. Further evaluations as well as hybridization within accessions with favorable traits from an agronomic and nutritional perspective would be necessary for evolving new varieties.

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