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Article

Comparative Evaluation of Yield and Fruit Physico-Chemical Characteristics of Five Commercial Cultivars of Pomegranate Grown in Southeastern Italy in Two Consecutive Years

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Abstract: This study, conducted over two consecutive seasons (2018–2019), evaluates the yield and fruit physico-chemical traits of five commercial cultivars ('Mollar', 'Dente di Cavallo', 'Acco', 'Jolly red' and 'Wonderful') grown in the orchard at Foggia (Puglia region, southeastern Italy). The results show significant variations among the investigated cultivars for many measured parameters; seasons also significantly affected some data. As an average of the years, both 'Dente di Cavallo' and 'Wonderful' significantly showed the highest numbers (16.8 and 15.2, respectively) and weights (9.3 Kg, respectively) of fruit per tree. 'Jolly red' had the highest percentage of edible portions (58.5%), the most intense red color in juice (a* value, 11.7), a light-red skin color (despite its name) (a* value, 31.1) and the smallest fruits (26.7% of diameter ≤ 80 mm). Total soluble solids of all cultivars were considerable above the minimum value (12 °Brix) required for commercial use. 'Wonderful' significantly presented the highest value of juice acidity (2.5 g citric acid 100 mL^{-1}) and the lowest MI value, and therefore it was classified as sour. Both 'Dente di Cavallo' and 'Wonderful' had significantly higher phenolic contents (591.0 and 519.1 mg 100 mL^{-1}, respectively) than the other cultivars, while 'Dente di Cavallo' significantly showed the highest antioxidant activity value (6.4 mmol TE 100 L^{-1}). The Stepwise Discriminant Analysis procedure allowed a significant differentiation among the cultivars. Correlation analysis showed a considerable relation among the different fruit traits. The present data are helpful in the selection of elite desirable pomegranate cultivars to be used for commercial production.

Keywords: pomegranate cultivars; yield; physico-chemical characteristic; phenol content antioxidant capacity; stepwise discriminant analysis; correlation

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1. Introduction

The pomegranate (*Punica granatum* L.) is precocious in fruit-bearing, compared to other fruit tree crops. [1]. Fruit with adequate quality with respect to size, color and flavor are produced 2–3 years after planting and commercial production levels are reached within 5–6 years [2]. In recent years, in Italy, as well as in many mild-temperate areas of European countries, an impressive production and consumption of this fruit has been observed. This is due to valid reasons, such as the interest for the farmers to diversify their agricultural production, the recognition of its functional properties, the presence of more attractive and productive cultivars, ameliorations in cultural practices, the spread of aril-ginning systems, which make consumption easier, and the development of numerous processed products [3,4]. Pomegranates are well adapted to areas with diverse pedoclimatic conditions, from hot to and dry areas, and successfully grow in Mediterranean countries [5,6]. Pomegranates are known to be fairly drought tolerant, although, under deficit irrigation regimes, they show a significant decrease in fruit yield and quality [7,8]. However, this response depends on the specific cultivar and characteristics of each pedoclimatic environment [9]. In Italy, this fruit tree is currently cultivated on 1420 ha [10], particularly in the southern regions, such as Sicily, Puglia, Basilicata and Calabria. The most widespread commercial cultivars currently grown in Italy are 'Wonderful' (selection from Florida), 'Acco' (selected in Israel) and 'Dente di Cavallo' (landrace from Italy), but other interesting foreign cultivars, including 'Mollar' (from Spain) and 'Jolly red' (native to Sicily, Italy), could also be introduced to cultivation in Italy. In pomegranates, the fruit's pomological characteristics and the chemical composition of the juice and the yield are also greatly affected by growing region, climate and cultural practices [11–14].

The fruit's physico-chemical parameters are important traits for producers, packaging factories, juice producers, marketers and finally consumers. In particular, the traits' characterizations are helpful for the identification of elite cultivars and necessary to develop a thorough understanding of the variability of the species for the selection of cultivars to be used in the fresh market or in the process industry [15]. Taking this aspect into account, little is known about the productive and qualitative responses of the newly introduced commercial cultivars in the different agricultural areas, and it is possible that, in the wake of new investments, they are used in new agricultural areas, although with unknown results.

Following this, the aim of this study is to evaluate and compare the yield and most important features of five commercial cultivars in a semi-arid and windy environment in the Puglia region, in order to provide relevant information to assist in pomegranate cultivar choice.

2. Materials and Methods

2.1. Site and Fruit Collection

A study was undertaken in 2018 and 2019 to evaluate the performance of five commercial cultivars ('Mollar', 'Dente di Cavallo', 'Acco', 'Jolly red' and 'Wonderful') grown in the same commercial orchard in the countryside of Foggia (Puglia region, southern Italy: 41° 27′ 08″ N, 15° 31′ 56″ E and altitude of 54 m a.s.l). At the beginning of the trial, the plants were 3 years old, spaced 5.5 × 3.0 m apart and trained into a vase shape. The experimental design was a randomized complete block design with three replicates per cultivar. Each plot had 3 rows with 8 trees in each row. The central tree in the middle row in each plot was used for data collection. A drip fertigation system and mulching with a plastic sheet along the tree rows were used. The drip irrigation system comprised a single pipe with drippers at 2 L h^{-1} flow rate and spaced every 40 cm. Irrigation was performed according to the values of the matric potential in the root zone. The seasonal irrigation volumes were 140 mm, with 12 watering events in 2018; and 395 mm, with 14 watering events in 2019. As for the fertilization, the doses per year applied in the experimental field were 115, 90 and 120 kg ha^{-1}, respectively, for N, P_2O 5 and K_2 O. The dose of nitrogen was divided into 3 from April to August. Phosphorus and potassium were administered annually in winter. Among the other operations that were performed was the thinning of small fruits during their development. The soil texture was clay loam [16], with an effective depth over 120 cm. The site of the research was in a typical semi-arid zone, characterized by a Mediterranean climate, which was classified as an accentuated thermos-Mediterranean climate [17], with temperatures that could fall below 0 °C in the winter and exceed 40 °C in the summer. Rainfall is unevenly distributed throughout the year and is mostly concentrated in the winter months with a long-term annual average of 559 mm [18]. The annual rainfall was 525.8 mm in 2018 and 452.5 mm in 2019, while the annual ETo (a representative climate evaporative parameter) were 1273.1 and 1352.7 mm in 2018 and 2019, respectively.

During each experimental season, from April to October, daily climate data were recorded at the nearest meteorological station, which was a few kilometers from the experimental area of the orchard.

2.2. Yield and Morpho-Pomological Analyses

In both years, the fruits were hand-picked from three plants per cultivar at the mature stage, according to Al-Maiman [19]. Fruit maturity was based on the total soluble solids content using a digital refractometer. The fruits were hand-picked when values of 14–15 °Brix were recorded for a sample of fruits from each variety. Total weight (kg per tree) and number of fruits (n) were measured for each tree at different dates, according to their ripening period: 'Acco' in early October; 'Mollar', 'Dente di Cavallo' and 'Jolly red' in mid-October; and 'Wonderful' in late October.

A representative sample of 12 fruits from each cultivar (four per plant) was used to determine the pomological and qualitative traits according to the International Union for the Protection of New Varieties of Plants [20]. They were randomly collected in plastic bags and stored in a portable ice box for transportation to the laboratory where measurements of the whole fruit, arils and physico-chemical analyses on the juice were conducted. Measurements of fruit, arils and juice were replicated three times.

The morpho-pomological fruit parameters were: total weight (g), equatorial diameter (mm), length (mm), percentage of fruit with a diameter of less than 80 mm (%), skin (peel) weight (g), number (n) and weight (g) of arils per fruit, aril edible portion (%), length (mm) and width (mm) of arils, fresh weight of 100 arils (g) and aril dry weight (%). The weight of the fruit, aril and tegument were determined by a precision digital balance Model S4202 (BEL Engineering s.r.l, Italy) with an accuracy of ±0.01 g. The size of the fruits and arils, including length (L) and diameter (D), were determined using a digital caliper (Maurer Corp, Munich, Germany) with an accuracy of ±0.01 mm.

2.3. Color of Fruit Skin, Arils and Juice Evaluation

Fruit skin, aril and juice color were recorded in the CIELab system, using a Minolta CR-400 colorimeter (Konica Minolta Sensing, Inc., Japan). In the CIELab color space, L* indicates the brightness, a* ranges from green (−a*) to red (+a*) and b* varies from blue (−b*) to yellow (+b*). The color characteristics of the pomegranate cultivars are potential indicators of the maturity index of the fruit for controlling the harvest and defining the commercial categories of the produce [21].

2.4. Physical and Chemical Analyses of Juice

The fruits were cut longitudinally and the arils, manually separated, were squeezed through a metal sieve to obtain the juice. The juice yield was determined in three replicates by manual extraction from 100 g of arils. Juice volume was determined by transferring juice into a measuring cylinder (cm^3 juice 100 g^{-1} of arils) and then separated in two aliquots. One was immediately frozen at −20 °C for high-performance liquid chromatography (HPLC) analysis of the phytochemical contents and antioxidant capacities. The other aliquot was used to evaluate the pH, TSS and total titratable acidity (TA). The TSS was determined by using a DBR35 digital refractometer instrument (XS Instruments, Carpi, MO, Italy) measuring the °Brix value. The TA was determined by using a PH-Burette 24 semi-automatic titrator (Mettler Toledo, Crison, Spain). Fresh juice (5 mL) was diluted with 50 mL of distilled water and titrated with 0.1 M NaOH to the phenolphthalein endpoint (pH 8.3). Titratable acidity values were expressed as percentage citric acid equivalents, as described by the Association of Official Analytical Cereal Chemists [22]. The Maturation Index (MI) was also calculated as the ratio of TSS:TA. The pH of the juice was measured with a Crison model Basic20 pH-m.

2.5. Total Polyphenols Determination

The determination of total phenol content (TPC) was performed by the Folin–Ciocalteu method, according to Difonzo et al. [23], with some modifications. In detail, 20 µL of juice was added to 980 µL of ddH_2O and 100 µL of Folin–Ciocalteu reagent. Following 3 min, 5% Na_2CO_3 solution was added, following incubation at room temperature for 60 min.

The absorbance was read at 750 nm using a Cary 60 spectrophotometer (Agilent, Cernusco, Milan). The TPC was expressed as gallic acid equivalents (GAEs) in mg L^{-1} juice.

2.6. Antioxidant Activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was performed by preparing a solution of DPPH 0.08 mM in ethanol, and the assay was conducted according to Ranieri et al. [24]. The ABTS-TEAC assay was performed as described by Re et al. [25], with the modifications reported by Ranieri et al. [24]. The results were expressed in mol Trolox equivalents (TEs) L^{-1}. Each sample was analyzed in triplicate.

2.7. Statistical Analysis

The dataset was tested according to the basic assumptions for the analysis of variance (ANOVA). The normal distribution of the experimental error and its common variance were verified through Shapiro–Wilk and Bartlett's tests, respectively. When required, Box–Cox transformations [26] were applied prior to analysis. For all experimental data among the cultivar and growing-season factors, a combined statistical analysis was performed. The differences in the means were determined using Tukey's honest significance difference post hoc tests at the 5% probability level. Furthermore, the morpho-qualitative variables were jointly considered in a multivariate approach and statistically processed applying a Stepwise Discriminant Analysis (SDA) with the experimental factor (cultivar) as a discriminating source [27–29]; ANOVA and SDA analyses were performed using the JMP software package, version 14.3 (SAS Institute Inc., Cary, NC, USA).

3. Results and Discussion

3.1. Weather Data

The weather conditions between the two seasons was quite different (Table 1). In fact, although the average temperatures of both seasons were similar, the months of June, July and August of 2019 were warmer (average temperature: 26.5 °C) than in 2018 (average temperature: 24.6 °C). Furthermore, the overall climate was windier and drier in 2019 (wind speed: 3.7 m s^{-1}, and total precipitation: 175.7 mm, respectively) than in 2018 (wind speed: 2.9 m s^{-1}, and total precipitation: 366.4 mm, respectively).

Table 1. Monthly mean maximum and minimum temperatures (T_{max}, T_{min}), relative air humidity (RH_{max} and RH_{min}), wind speed (W_s), radiation (Rad) and total precipitation (P) during 2018 and 2019 seasons.

Month	T_{max}	T_{min}	RH_{max}	RH_{min}	W_s	Rad	P
	(°C)	(°C)	(%)	(%)	(m s^{-1})	(Wm^{-2})	(mm)
				2018			
April	21.3	12.9	94.6	37.6	2.8	235.3	54.0
May	26.1	13.4	95.2	49.1	2.4	275.8	58.3
June	30.0	12.1	89.5	40.3	3.4	289.6	88.2
July	33.3	19.6	83.6	35.4	3.0	318.7	16.8
Aug	32.7	20.1	71.3	28.3	2.1	285.7	39.1
Sept	29.1	17.1	81.3	30.0	3.7	193.6	80.0
Mean	28.7	15.9	85.9	36.8	2.9	266.5	
Total							366.4
				2019			
April	20.6	8.2	94.4	51.0	3.7	190.2	40.3
May	21.3	10.2	95.3	56.3	4.0	232.9	86.7
June	33.2	17.5	85.9	35.1	3.7	252.2	9.2
July	33.7	19.5	84.0	33.9	3.7	258.8	30.0
Aug	34.8	20.3	79.9	33.9	3.6	225.6	5.7
Sept	29.5	16.8	88.7	42.6	3.6	175.5	3.8
Mean	28.8	15.4	88.0	42.5	3.7	222.5	
Total							175.7

3.2. Yield and Morpho-Pomological Characteristics

The average number of fruits per tree (n) and the fruit weight per tree (kg), according to total harvest were basically higher in 2019 than in 2018, because of the age of the plants

(Figure 1). In both years, the average number of fruits per tree showed non-significant differences among cultivars. However, as an average of the years, the highest values were noticed for 'Dente di Cavallo' (16.8), followed by 'Wonderful' (15.2) and the other cultivars (ranging from 12.0 to 14.7). The average yield (in the two years) was highest for 'Dente di Cavallo' (9.3 kg/tree), but not significantly different from 'Wonderful' (8.4 kg/tree), 'Acco' (6.6 kg/tree) and 'Mollar' (6.1 kg/tree), but statistically different from 'Jolly red' (5.2 kg/tree). The yield per plant was lower compared to that obtained in another study conducted using pots [30,31]. Our pomegranate plants were transplanted in a field in February 2017, therefore the plants were very young in the experimentation years of 2018 and 2019.

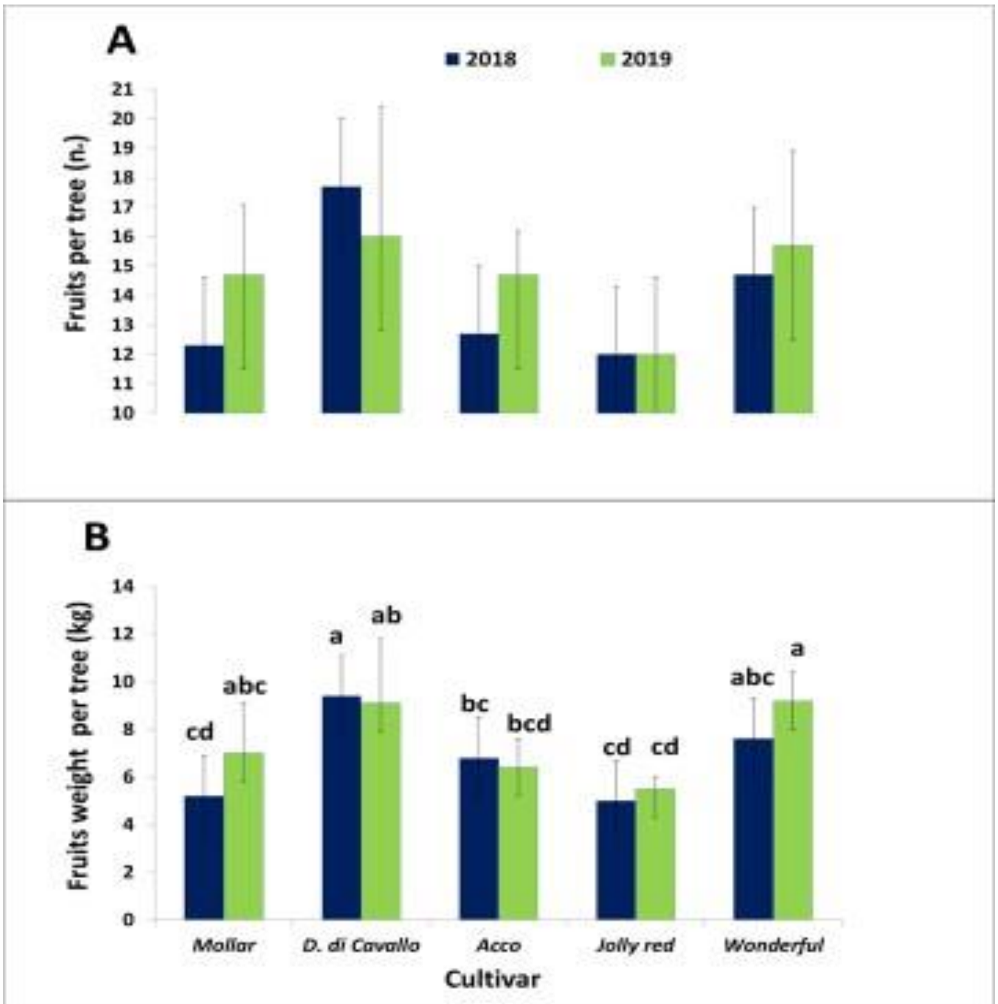

Figure 1. Number of fruits per tree (**A**) and fruit weight per tree (**B**) in different pomegranate cultivars. Average values ± std. dev. in 2018 and 2019 are shown. Different lower letters per year indicate significant differences at $p < 0.05$. Graph without letters means there were no significant differences between means.

In Table 2, the morphological fruit characteristics (weight, diameter, length and percent of small fruit) are shown. The average fruit weight did not differ between the two years, unless the 'Acco' resulted in a significantly heavier weight in 2018 (559.2 g) than in 2019 (332.8 g). As the average value between the years, the heaviest fruits were obtained for 'Dente di Cavallo' (520.1 g), which was not significantly different from the fruits of 'Wonderful' (473.8 g), 'Acco' (446.0 g) and 'Mollar' (444.4 g), but significantly different from 'Jolly red' (368.1 g). Therefore, when taking into account the fruit classification of the Codex Alimentarius Commission [32], it was determined that 'Dente di Cavallo' was classified in A (≥500 g); 'Mollar', 'Acco' and 'Wonderful' in B (401–500 g); and 'Jolly red' in C (301–400 g).

Table 2. Fruit characteristics of different pomegranate cultivars in 2018 and 2019 seasons.

Cultivar Parameter	Mollar	D. Cavallo	Acco	Jolly Red	Wonderful
Fruit weight (g)					
2018	415.1 ± 91.8 abc	542.1 ± 91.7 ab	559.2 ± 147.3 a	361.1 ± 88.4 bc	499.8 ± 78.9 abc
2019	473.7 ± 40.4 abc	498.2 ± 139.8 abc	332.8 ± 110.3 c	375.0 ± 45.3 abc	447.9 ± 122.1 abc
Fruit diameter (mm)					
2018	91.3 ± 5.5	97.3 ± 5.8	96.6 ± 5.1	87.0 ± 5.0	94.0 ± 5.5
2019	96.2 ± 9.1	92.4 ± 11.2	82.6 ± 3.9	90.0 ±18.8	96.02 ± 9.0
Fruit length (mm)					
2018	84.3 ± 5.5 a	88.6 ± 6.1 a	87.6 ± 8.7 a	77.0 ± 4.3 ab	85.5 ± 5.8 a
2019	81.5 ± 9.8 ab	81.2 ± 4.0 a	73.1 ± 4.9 b	78.8 ± 3.7 ab	84.2 ± 7.5 a
Fruit diameter ≤ 80 mm (%)					
2018	26.2 ± 4.9 a	13.7 ± 5.2 b	15.3 ± 2.3 b	30.8 ± 10.1 a	14.7 ± 2.2 b
2019	21.1 ± 5.2 ab	12.1 ± 6.2 b	19.1 ± 11.9 ab	22.6 ± 6.9 ab	13.6 ± 1.5 b

Average values ± SD followed by different letters between the years and cultivars were statistically different according to Tukey's test ($p < 0.05$).

Both the average fruit diameter (from 82.60 to 97.3 mm) and fruit length (from 77.8 to 85.6 mm) showed no significant differences either between cultivars or years, presenting in all cultivars an oblate shape. A survey conducted in Puglia on the different pomegranate genotypes reported similar values for fruit weight, diameter and length for 'Acco' in 2019, whereas our values were higher for 'Dente' and lower for 'Wonderful' for the same parameters [33]. Similar values have been reported in different geographical areas for 'Dente di Cavallo' and 'Wonderful' in the Campania region, Italy [34], 'Mollar' and 'Wonderful' in Spain [35] and 'Wonderful' and 'Acco' in Cyprus [36]. Conversely, our data were slightly higher than those reported in other studies conducted in the Sicily region (Italy) for 'Dente di Cavallo' [37], 'Acco' and 'Wonderful' [38]. The type of genetic material, age of the orchard, cultural practices and pedoclimatic conditions were all factors that may have contributed to these differences.

The percentage of small fruits (diameter ≤ 80 mm) significantly varied among the cultivars. The highest percentage, as an average of the two years, was observed for both 'Jolly red' (26.7%) and 'Mollar' (23.76%), which was significantly different from the other cultivars (ranging from 12.1 to 19.1%).

In Table 3, the aril characteristics, represented as the percentage of edible portion, length, width, weight of 100 arils and dry matter, are shown. The fruit edible portion was statistically different among cultivars, and it was also different between years only for 'Acco'. The highest values were found for 'Jolly red' (average 58.5%) followed by 'Wonderful' (average 51.9%), which were both significantly different from the remaining cultivars (ranging from 44.7 to 50.7%). These results are in line with those obtained by Fernandez et al. [35], but were slightly lower than those obtained by Usanmaz et al. [36]. Aril length and width showed no significant differences between the cultivars and years, and the average values were 9.2 and 8.0 mm, respectively. These values were similar to those recorded by Barone et al. [37], while they were slightly lower than those reported by Adiletta et al. [34] and Ferrara et al. [33]. The average fresh weight of 100 arils showed some differences between years and among cultivars. Significantly, the highest value was obtained for 'Jolly red' (41.9 g) in 2019, although it was not different from that of 'Mollar' (37.1 g) in the same year, but statistically different from all other cultivars (ranging from 30.3 and 33.8 g). Our results for 'Dente di Cavallo' are lower than those obtained by Barone et al. [37] and Adiletta et al. [34], while those for 'Wonderful' and 'Acco' are in accordance with the results obtained by Adiletta et al. [34] and Ferrara et al. [33], respectively.

Finally, the average dry weight values of arils in both years were significantly higher in both 'Acco' and 'Wonderful' (annual average: 23.0%) than the other compared cultivars (ranged between 19.2 and 21.6%).

Table 3. Aril characteristics of different pomegranate cultivars in 2018 and 2019 seasons.

Cultivar Parameter	Mollar	D. Cavallo	Acco	Jolly Red	Wonderful
Aril portion (%)					
2018	50.7 ± 0.7 b	47.9 ± 0.9 c	44.7 ± 4.5 c	58.7 ± 2.5 a	50.2 ± 1.2 b
2019	49.7 ± 1.0 b	49.3 ± 2.4 bc	50.2 ± 1.1 b	58.3 ± 2.7 a	53.7 ± 4.2 ab
Aril length (mm)					
2018	9.6 ± 0.5	9.5 ± 1.2	8.6 ± 0.8	9.1 ± 0.9	9.5 ± 1.8
2019	9.6 ± 0.8	9.2 ± 1.7	8.2 ± 0.7	9.0 ± 0.9	9.6 ± 1.4
Aril width (mm)					
2018	8.1 ± 1.0	8.1 ± 1.2	7.5 ± 0.6	7.9 ± 0.9	8.3 ± 0.7
2019	8.1 ± 1.1	8.1 ± 1.2	7.3 ± 0.6	7.9 ± 0.8	8.4 ± 0.8
Fresh weight of 100 arils (g)					
2018	31.7 ± 3.9 bc	30.3 ± 1.6 c	33.8 ± 2.5 bc	31.8 ± 5.1 bc	30.8 ± 3.1 c
2019	37.1 ± 2.8 ab	31.0 ± 1.5 c	31.5 ± 3.2 bc	41.9 ± 4.2 a	32.9 ± 3.0 bc
Aril dry weight (%)					
2018	20.4 ± 0.6 c	21.6 ± 1.1 bc	22.2 ± 0.9 ab	19.4 ± 1.0 c	22.6 ± 0.6 ab
2019	20.5 ± 0.6 c	19.2 ± 1.0 c	23.8 ± 1.2 ab	19.8 ± 1.4 c	23.3 ± 0.3 a

Average values ± SD followed by different letters between years and cultivars were statistically different according to Tukey's test ($p < 0.05$).

3.3. Color of Fruit's Skin, Arils and Juice

In Table 4, the mean values of the CIElab color (lightness, L*; redness a* and yellowness, b*) parameters of skin, aril and juice are reported. Differences among cultivars and/or between years are evident. Fruit skin and aril-color lightness was not significantly different between years, but different among cultivars, while juice lightness was significantly different both between years and among cultivars. Significantly higher skin-lightness values were recorded for 'Jolly red' (average: 55.2) and 'Mollar' (average: 52.1), with respect to the other compared cultivars (ranging from 39.5 to 43.6). Additionally, for arils, the highest lightness values were obtained for both 'Mollar' (average of years: 32.5) and 'Jolly red' (average of years: 32.4); although they were not significantly different from the remaining cultivars (values ranging from 24.4 to 25.0), they were less than 'Dente di Cavallo' in 2018, which showed the lowest value (19.6). As for juice lightness, the average values varied from 12.2 to 24.4, with the highest value being obtained for 'Jolly red' in 2018 and the lowest for 'Wonderful in 2019.

The redness parameters, which is a desirable quality attribute for processing and consumers [39], for the skin, aril and juice of almost all the cultivars presented higher values in 2019 in comparison to 2018. Probably, the warmest and windiest climate in 2019 led to a condition of higher stress in the plants, with a consequent increase in anthocyanins responsible for the red color [40]. When comparing the cultivars, the skin's redness was not significantly different among almost all of them (values between 35.0 and 43.8), but 'Jolly red' showed the lowest value (average: 31.1). The aril's redness was significantly higher in both 'Jolly red' and 'Acco' (averages: 23.0 and 20.8, respectively), while the highest juice-redness values were found in both 'Jolly red' and 'Mollar' (averages: 11.7 and 6.7, respectively). For the yellowness, both 'Mollar' and 'Jolly red' showed the highest average values for skin (31.8 and 30.6, respectively), arils (14.6 and 13.4, respectively) and juice (1.1 and 3.0, respectively).

Table 4. Characteristics of skin, aril and juice colors of different pomegranate cultivars in 2018 and 2019 seasons.

Cultivar Parameter	Mollar	D. Cavallo	Acco	Jolly Red	Wonderful
Skin colorL*					
2018	53.8 ± 4.5 a	40.0 ± 3.0 b	39.8 ± 3.5 b	54.8 ± 7.4 a	39.5 ± 4.3 b
2019	50.4 ± 2.6 a	41.0 ±2.6 b	42.0 ± 2.3 b	55.7 ± 3.5 a	43.6 ± 5.2 ab
Skin colora*					
2018	35.0 ± 9.4 ab	38.4 ± 3.1 ab	39.6 ± 3.3 ab	29.3 ± 0.3 b	34.4 ± 3.5 ab
2019	36.4 ± 5.0 ab	43.8 ± 4.3 a	40.4 ± 3.1 ab	33.0 ± 6.3 b	39.1 ± 4.6 ab
Skin colorb*					
2018	31.7 ± 5.3 ab	17.5 ± 1.7 c	17.5 ± 2.6 c	30.9 ± 5.6 ab	16.3 ± 4.1 c
2019	29.6 ± 1.7 ab	27.8 ± 1.9 ab	25.8 ± 2.2 b	32.7 ± 3.3 a	26.3 ± 3.0 b
Arilcolor L*					
2018	35.0 ± 5.8 a	19.6 ± 3.5 c	25.0 ± 2.7 bc	34.2 ± 8.5 ab	24.8 ± 3.2 bc
2019	30.0 ± 4.8 ab	24.4 ± 3.8 bc	24.4 ± 3.4 bc	30.7 ± 6.0 ab	24.8 ± 4.2 bc
Arilcolor a*					
2918	10.2 ± 2.3 c	13.3 ± 4.5 bc	18.2 ± 3.7 ab	22.3 ± 5.1 ab	18.0 ± 4.4 ab
2019	23.5 ± 4.5 a	25.5 ± 4.0 a	23.5 ± 3.0 a	23.7 ± 6.0 a	19.4 ± 7.0 ab
Aril colorb*					
2018	11.8 ± 1.2 cd	6.4 ± 1.8 e	7.9 ± 1.0 e	10.1 ± 1.7 cde	8.3 ± 1.4 e
2019	17.4 ± 2.2 a	12.9 ± 1.7 bc	11.9 ± 1.2 cd	16.7 ± 2.8 ab	9.7 ± 2.0 de
Juicecolor L*					
2018	13.5 ± 1.5 de	20.3 ± 0.3 b	20.8 ± 0.5 b	24.4 ± 2.1 a	20.5 ± 0.2 b
2019	20.9 ± 2.0 ab	16.5 ± 1.0 c	17.9 ± 2.2 bc	15.3 ± 1.9 cd	12.2 ± 2.0 e
Juicecolor a*					
2018	6.1 ± 0.8 a	1.3 ± 0.1 c	1.9 ± 0.7 bc	12.3 ± 6.1 a	1.4 ± 0.1 c
2019	7.4 ± 1.9 a	3.1 ± 0.8 b	3.6 ± 1.0 b	11.2 ± 5.0 a	5.1 ± 2.0 ab
Juice colorb*					
2018	1.0 ± 0.1 c	0.2 ± 0.1 d	0.2 ± 0.1 d	3.3 ± 2.7 ab	0.2 ± 0.1 d
2919	1.3 ± 0.2 bc	2.1 ± 0.2 b	1.8 ± 0.6 b	2.7 ± 1.5 ab	4.0 ± 1.4 a

Average values ± SD followed by different letters between years and cultivars were statistically different according to Tukey's test ($p < 0.05$). L* indicates lightness, a* is the red/green coordinate, and b* is the yellow/blue coordinate.

Aril- and juice-color parameters showed values lower than those corresponding to the skin. This is in agreement with the previous studies [1,38,41]. Moreover, the average values of the color parameter for 'Acco' and 'Wonderful' were consistent with those of the same cultivars described by Passafiume et al. [38] and Ferrara et al. [33].

Our data further showed that there is not always a clear correlation between pomegranate skin color and aril or juice color. This is in agreement with the previous studies [1,41].

3.4. Physical and Chemical Characteristics of Juice

The mean values of juice volume, TSS, pH, TA and TSS/TA in 2018 and 2019 of the examined pomegranate cultivars are shown in Table 5.

One of the most important parameters from a processing point of view is the juice yield, which nominally includes the juice volume obtained from 100 g of arils. These values were higher in 2018 (ranging from 74.5 to 85.6 cm^3 100^{-1} g) than in 2019 (ranging from 61 to 73.7 cm^3 100^{-1} g) for all the cultivars, with 'Mollar', 'Acco' and 'Jolly red' having the highest yields. Slight differences were noticed among cultivars for each year. Whilst our juice yield values for the analyzed cultivars were similar to those reported by Ferrara et al. [33,42], the data for 'Acco' (average of years: 74.1 cm^3 100 g^{-1}) and 'Wonderful' (average of years: 73.5 cm^3 100 g^{-1}) were higher than those reported for the same cultivars by Passafiume et al. [38] (67.3 and 38.4 cm^3 100 g^{-1}), respectively.

Table 5. Juice characteristics from different pomegranate cultivars in 2018 and 2019 seasons.

Cultivar Parameter	Mollar	D. Cavallo	Acco	Jolly Red	Wonderful
Juice yield (cm^3 100^{-1} g arils)					
2018	78.5 ± 13.4 ab	74.5 ± 10.8 ab	85.5 ± 6.4 a	85.6 ± 4.8 a	78.3 ± 3.4 ab
2019	61.0± 1.7 c	73.7 ± 3.2 b	69.0 ± 4.4 b	62.7 ± 4.9 bc	68.7 ± 4.5 b
TSS (°Brix)					
2018	14.7 ± 0.3 c	16.8 ± 0.2 b	16.2 ± 0.1 b	14.6 ± 0.4 c	17.7 ± 0.2 a
2019	16.1 ± 0.8 b	16.1 ± 0.7 b	16.6 ± 0.3 b	15.0 ± 0.4 bc	16.6 ± 0.5 b
pH					
2018	3.0 ± 0.1 c	2.8 ± 0.1 c	2.8 ± 0.1 c	3.6 ± 0.3 ab	3.3 ± 0.1 b
2019	3.7 ± 0.1 a	3.3 ± 0.1 b	3.3 ± 0.1 b	3.8 ± 0.1 a	3.2 ± 0.1 b
TA (g citric acid 100 mL^{-1})					
2018	0.6 ± 0.1 c	1.6 ± 0.1 b	1.7 ±0.2 b	0.6 ± 0.1 c	2.3 ± 0.2 a
2019	0.5 ± 0.1 c	1.2 ± 0.2 b	1.6 ± 0.1 b	0.5 ± 0.1 c	2.6 ± 0.2 a
TSS/TA (°Brix/%citric acid)					
2018	26.0 ± 4.2 ab	10.8 ± 0.5 c	9.4 ± 0.9 cd	23.4 ± 1.4 b	7.8 ± 1.0 d
2019	31.1 ± 4.5 a	13.4 ± 0.8 c	10.3 ± 0.1 c	30.8 ± 6.5 a	6.2 ± 0.9 d

Average values ± SD followed by different letters between years and cultivars were statistically different according to Tukey's test ($p < 0.05$).

For TSS, some significant differences among both the cultivars and between years were observed. As an average of the two years, 'Wonderful', 'Dente di Cavallo' and 'Acco' showed significant higher values (17.1, 16.4 and 16.4 °Brix, respectively) than both 'Mollar' and 'Jolly red' (15,4 and 14.8 °Brix, respectively). Moreover, in each year, the TSS varied from 14.6 °Brix for 'Jolly red' to 17.7 °Brix for 'Wonderful'. Therefore, all data for the analyzed cultivars were above the minimum value (12 °Brix) required for commercial use [43]. Primarily, our data are very similar to those reported in the other studies [33,37,38], but they are higher than those reported by Martínez et al. [44] for 'Dente di Cavallo' and' Wonderful', they were slightly lower than those obtained by Adiletta et al. [34].

The pH plays an important role in characterizing the fruit's sourness. The values of all compared cultivars were higher in 2019 (ranging from 3.3 to 3.8) than in 2018 (ranging between 2.8 and 3.6), but only for three cultivars ('Mollar', 'Dente di Cavallo' and 'Acco') were they significantly different between years. When comparing cultivars, the highest values were noted in both years for 'Jolly red' (average: 3.7) and in 2019 for 'Mollar' (3.7). Our range data were similar to that obtained in the other study [34,42,44,45], but the value for 'Wonderful' was slightly lower than that obtained by Fernandes et al. [35].

Concerning the TA, the highest values were noted for 'Wonderful' (average of years 2.5 g citric acid 100 mL^{-1}), which were statistically different from 'Acco' (average of years 1.6 g citric acid 100 mL^{-1}) and significantly higher than the other compared cultivars (ranging from 0.5 and 0.6 g citric acid 100 mL^{-1}).

The MI value has been reported to play an important role in evaluating the pomegranate fruit's taste, and is also an important quality criterion in the juice-processing industry for the formulation of food and beverage products [41]. It was found useful for the classification of many Spanish and Italian pomegranate cultivars [44,46] as sour (values 5–7), sour–sweet (17–24) and sweet (31–98). Our data for both years showed significantly higher values for both 'Mollar' (average: 28.5) and 'Jolly red' (average: 27.1), for which the flavors were considered between the sour–sweet and sweet. Significantly lower values were presented for both 'Dente di Cavallo' (13.4) and 'Acco' (10.3), which were slightly above the 'sour' limit, and, in particular, the lowest value (average: 7.0) was obtained for 'Wonderful' that can be identified as 'sour'. The average MI values obtained in our study were lower than those presented for the same pomegranate cultivars by Labbè et al. [47], Fernandes et al. [35], Adiletta et al. [34] and Passafiume et al. [38]. The MI values of 'Dente di Cavallo' and 'Acco' were much higher than those reported in Puglia, but similar for 'Wonderful' [33].

3.5. Total Phenol Content and Antioxidant Activity

The results show that total phenol content (TPC) and antioxidant activity (AA) tend to be higher in 2019 than 2018 (Figure 2). These differences may be explained by the driest climate that was recorded in 2019, which may have caused water stress in the plants and an increase in the above-mentioned juice traits. In this regard, Attanayake et al. [48] indicated that the accumulation of bioactive compounds was relatively higher in drier and warmer climates than in wetter and cooler conditions. As for the different cultivars, the TPC values were greater in 'Dente di Cavallo' (average of years 591.0 mg 100 mL^{-1}) followed by 'Wonderful' (average of years 519.1 mg 100 mL^{-1}), which were significantly higher than the remaining cultivars (ranging from 401.7 to 510 mg 100 mL^{-1}). The TPC range values of both 'Acco' and 'Wonderful' cultivars were similar to those reported for the same cultivars tested by Passafiume et al. [38]; whereas, for 'Dente di Cavallo' and 'Wonderful', the values were lower than those reported by Adiletta et al. [34].

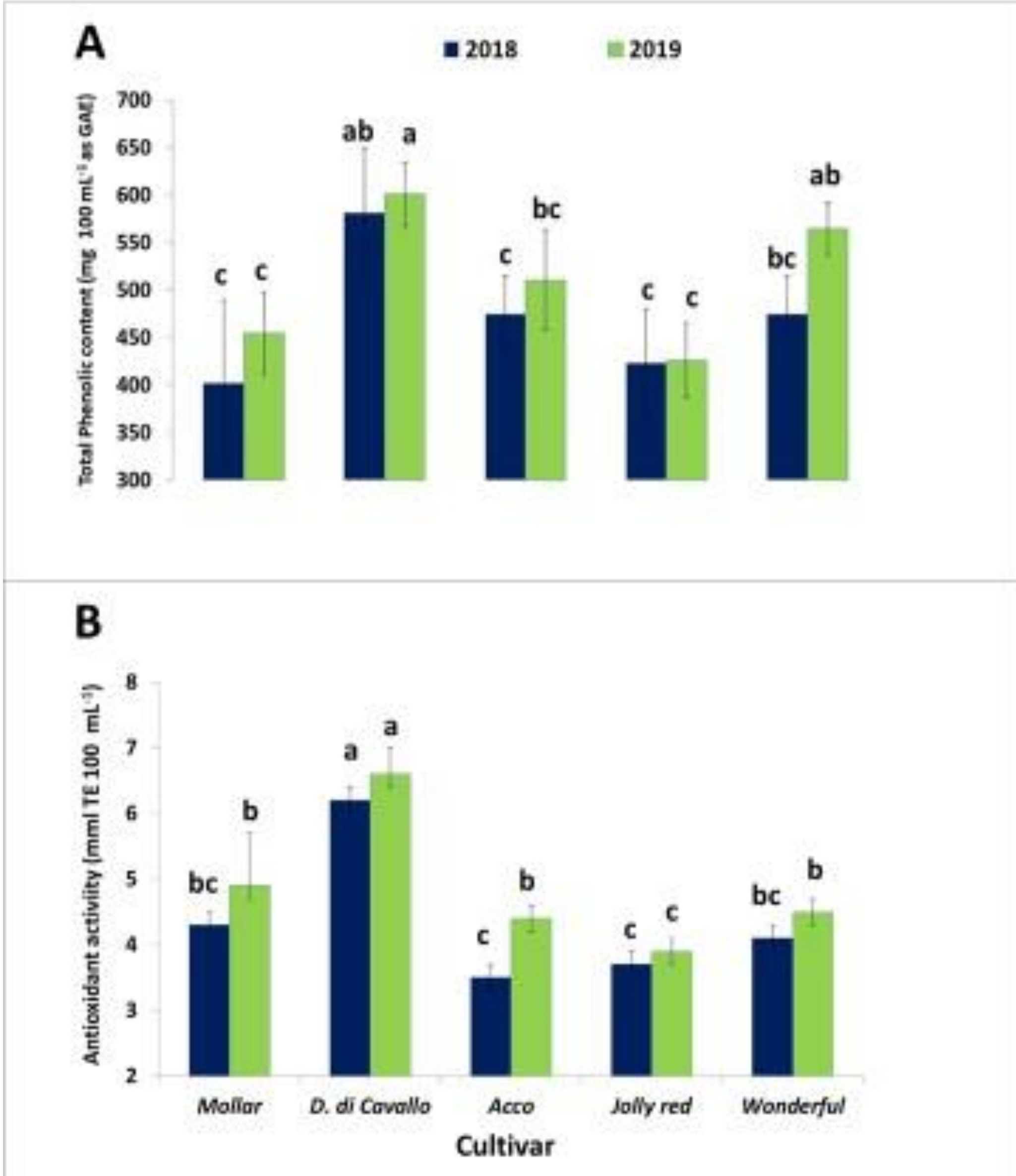

Figure 2. Total phenolic content (**A**) and antioxidant activity (**B**) in different pomegranate cultivars. Average values ± std. dev. in 2018 and 2019 are shown. Different letters per year indicate significant differences at $p < 0.05$.

Concerning the AA, the studied cultivars showed a variability according to their TPC. In particular, 'Dente di Cavallo' showed the highest value (average 6.4 mmol TE 100 L^{-1}), which was significantly higher than all other cultivars (average values ranging from 3.8 to 4.6 mmol TE 100 L^{-1}). The AA values of 'Acco' and 'Wonderful' were similar to those observed by Passafiume et al. [38]. Both TPC and AA were lower for 'Acco' and 'Wonderful', and more similar for 'Dente di Cavallo' with respect to the values reported by Ferrara et al. [33].

3.6. Multivariate Analysis on the Morpho-Qualitative Parameters

SDA selected eight morpho-qualitative parameters (aril diameter, aril dry weight, pH, TSS/TA ratio, antioxidant activity, juice color a*, fruit diameter $\leq$ 80 mm and juice yield) showing the best discrimination among the different cultivars. In particular, TSS/TA ratio and antioxidant activity were the first qualitative parameters added to the SDA procedure (F ratio = 18.28 and 33.99, respectively; $p < 0.0001$), while the last was the juice yield (F ratio = 3.41; $p < 0.035$). According to these results, the SDA procedure was performed only on the eight selected parameters(Appendix A).

Table 6 shows the standardized coefficients (scores) for the first two canonical axes ($CA_{1\text{-}2}$). Concerning the cultivar treatments, both the canonical axes were significantly affected (high absolute value of the standardized canonical coefficients and significant value of the correlation coefficients) by the eight selected parameters, with the exception of the aril diameter for CA_1, pH for CA_2 and juice color a* for CA_2. The CA_1 and CA_2 axes accounted for 73.45% and 22.45%, respectively.

Table 6. Standardized coefficients (scores) and Pearson's correlation coefficient for the first two canonical axes ($CA_{1\text{-}2}$), considering the different morpho-qualitative parameters as selected by Stepwise Discriminant Analysis (SDA). The corresponding percentages of accounted variation are also reported.

Parameters	Standardized Coefficients		Pearson's Correlation Coefficients	
Cultivar parameter	CA_1	CA_2	CA_1	CA_2
Aril diameter (mm)	0.38	−0.50	0.08 ns	−0.53 **
Aril dry weight (%)	0.77	−0.79	0.59 **	−0.70 ***
pH (-)	0.46	−0.91	0.51 **	−0.19 ns
TSS/TA ratio (°Brix/% citric acid)	1.62	0.34	0.88 ***	0.49 **
Antioxidant activity (mmol TE100 L^{-1})	−0.92	1.10	−0.61 ***	0.67 ***
Juice color a*	0.84	0.28	0.81 ***	0.14 ns
Fruit diameter ≤80 mm (%)	0.52	0.37	0.71 ***	0.36 *
Juice yield (cm^3 100 g arils)	0.95	−0.04	0.10 ns	−0.12 ns
% variance explained	73.45	22.45		

*: Correlation significant at the 0.05 level (*p*-value 0.05); **: correlation significant at the 0.01 level (*p*-value 0.01); ***: correlation significant at the 0.001 level (*p*-value 0.001); ns: not significant.

Similar considerations can be presented, considering the biplot graph and relative length and direction of the vectors (Figure 3). The results of the multivariate analysis applied to the cultivar treatment factor showed that the selected eight parameters by the SDA procedure allowed a significant differentiation among the cultivars. In particular, the 'Dente di Cavallo' cultivar was mostly characterized by the antioxidant activity, while 'Mollar' and 'Jolly red' by TSS/TA ratio.

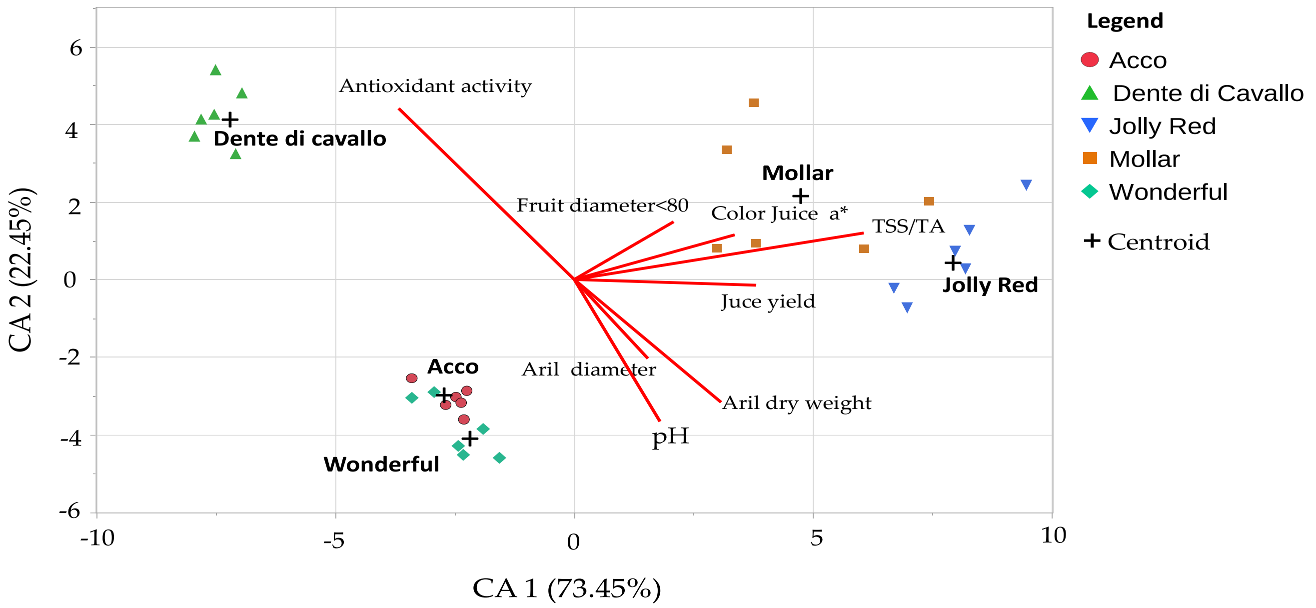

Figure 3. Stepwise discriminant analysis biplot of the morpho-quality parameters of considering the cultivar as the discriminant variables.

3.7. Correlation Analysis

The correlation matrix relating the different physical and chemical characteristics of pomegranate fruits is presented in Table 7. The results show that the fruit weight is strongly positively correlated with the fruit diameter (r2 = 0.66), and moderately with the fruit length (r2 = 0.44) and TSS (r2 = 0.37), and the fruit diameter is positively correlated with the fruit length (r2 = 0.55) and aril length (r2 = 0.51). Furthermore, the fruit length is positively correlated with aril length (r2 = 0.57) and aril diameter (r2 = 0.45). The data regarding the arils prove an existence of a very strong positive correlation between aril length and diameter (r2 = 0.78) and a positive correlation between aril portion and small fruit (diameter ≤80 mm) (r2 = 0.56). Therefore, an increase in fruit size and equatorial diameter does not appear to be a consequence of improving the edible portion of the fruit. In this regard, Opara et al. 39 obtained a high correlation for pomegranates between fruits with a pericarp and membrane weight. The fresh weight of 100 arils was negatively correlated with juice yield (r2 = −0.44), but positively correlated with pH (r2 = 0.44) and TSS/TA (r2 = 0.44). Aril dry weight was negatively correlated with both small fruit (diameter ≤80 mm) (r2 = −0.58) and TSS/TA (r2 = −0.67), but positively correlated with TSS (r2 = 0.59). The small fruit (diameter ≤ 80 mm) was negatively correlated with TSS (r2 = −0.58) and TPC (r2 = −0.39), but highly positive correlated with the TSS/TA (r2 = 0.66). TSS was negatively correlated with TSS/TA (r2 = −0.70), but positively correlated with TPC (r2 = 0.50). pH showed a weak negative relationship (r2 = −0.37) with TA and a positive one with TSS/TA (r2 = 0.36). Moreover, TSS/TA was negatively correlated with TPC (r2 = −0.52). Finally, as expected, a positive correlation was obtained between TPC and AA (r2 = 0.67).

Table 7. Coefficient of correlation (r) between physical and chemical properties of five pomegranate cultivars.

	Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	**Fruit weight**	1.00															
2	**Fruit diameter**	**0.66 ****	1.00														
3	**Fruit length**	**0.44 ***	**0.55 ***	1.00													
4	**Aril portion**	ns	ns	ns	1.00												
5	**Aril lenght**	ns	**0.51 ***	**0.57 ***	ns	1.00											

Table 7. *Cont.*

	Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
6	**Aril diameter**	ns	ns	**0.45 ***	ns	**0.78 ****	1.00										
7	**Fresh weight of 100 arils**	ns	ns	ns	ns	ns	ns	1.00									
8	**Aril dry weight**	ns	ns	ns	ns	ns	ns	ns	1.00								
9	**Fruit diameter ≤ 80 mm**	ns	ns	ns	**0.56 ***	ns	ns	ns	**−0.58 ***	1.00							
10	**Juice yield**	ns	ns	ns	ns	ns	ns	**−0.44 ***	ns	ns	1.00						
11	**TSS**	**0.37 ***	ns	ns	ns	ns	ns	ns	**0.59 ***	**−0.58 ***	ns	1.00					
12	pH	ns	ns	ns	ns	ns	ns	**0.40 ***	ns	ns	**−0.40 ***	ns	1.00				
13	**TA**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**−0.37 ***	1.00			
14	**TSS/TA**	ns	ns	ns	ns	ns	ns	**0.46 ***	**−0.67 ***	**0.66 ****	ns	**−0.70 ***	**0.36 ***	ns	1.00		
15	**TPC**	ns	ns	ns	ns	ns	ns	ns	ns	**−0.39 ***	ns	**0.50 ***	−0.12	ns	**−0.52 ***	1.00	
16	**AA**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**0.67 ***	1.00

Significant at $p \leq 0.05$ (*) or 0.01 (**); ns: non-significant.

4. Conclusions

A significant variability was observed for the physico-chemical traits among the analyzed cultivars and between years. According to the results, both 'Dente di Cavallo' and 'Wonderful' presented the highest yield, fruit weight and size.

Regarding the correlation among the fruit-quality traits, it was strongly positive between fruit weight and diameter and aril length and diameter, but it was moderately positive between aril portion and small fruit, TPC and AA.

The results of the multivariate analysis applied to the cultivar treatment factor show that 'Dente di Cavallo of the lo' cultivar was mostly characterized by antioxidant activity, while 'Mollar' and 'Jolly red' were characterized by TSS/TA ratio. Therefore, 'Mollar', 'Acco' and 'Jolly red' may be more appropriate for the fresh market because of the fruit size (smaller), qualitative traits and sweet taste, whereas 'Dente di Cavallo' and 'Wonderful' can be used for the production of juices because of their high phenolic composition and antioxidant activity, which have gained a high reputation for their health-benefit effects in recent years. The findings of this study can be helpful to assist pomegranate growers in choosing the most suitable cultivars to be used for commercial production according to the climatic conditions.

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Appendix A. Stepwise Discriminant Analysis (SDA)

The multivariate technique was used to explain the differences among different cultivars and to evaluate the main parameter(s) that played the most important role in determining those differences. The qualitative parameters were introduced stepwise as predictor variables into the discriminant analysis. Before performing the SDA, the values of each parameter were correctly standardized. According to the 'stepwise' procedure, the variables were included in the model one by one, choosing at each step the variable that made the most significant additional contribution to the discrimination (i.e., with the highest Fisher's test value, F ratio). Based on the selected variables, different discriminant functions (canonical axis, CAs) were computed by discriminant analysis.

The first two CAs accounted for the larger portion of the data variability, and they were considered for further data analysis. The selected CAs were represented graphically in a biplot, in which the canonical scores were plotted as symbols, whereas standardized canonical coefficients for each of the original variables were reported as vectors and used for interpretations.

The implemented multivariate approach (Stepwise Discriminant Analysis, SDA) allowed us to integrate the results of the analysis of variance previously reported, and to evaluate which of the morpho-qualitative parameters, simultaneously analyzed, mainly contributed to the separation of the experimental groups (cultivars). SDA was performed considering the cultivars as discriminating source. This experimental factor showed strong significant differences for all multivariate tests that were performed (Table A1). Therefore, it was necessary, through the stepwise procedure, to determine which of the morpho-qualitative parameter(s) played the most important role in causing the group differences. The selected parameters, showing a significant F-ratio value ($p \leq 0.05$), are reported in Table A2.

Table A1. Factors of multivariate tests to verify the significance between the different cultivars by discriminant analysis procedure.

Test	Value	Fisher's Test Value	DF †	Prob > F ‡
Wilks' Lambda	0.0006	13.77	32	<0.0001
Pillai's Trace	2.81	6.27	32	<0.0001
Hotelling–Lawley Trace	49.7	26.25	32	<0.0001
Roy's Largest Root	36.50	95.82	8	<0.0001

† DF: Degree Freedom; ‡ Prob: significance of Fisher's ratio.

Table A2. Group of morpho-qualitative parameters selected by stepwise discriminant analysis.

Parameter Selected	F-Ratio Value	Prob F †
Aril diameter (mm)	4.44	0.009
Aril dry weight (%)	6.28	0.0019
pH (-)	5.11	0.005
TSS/TA ratio (°Brix/% citric acid)	18.28	<0.0001
Antioxidant activity (mmol TE100 L^{-1})	33.99	<0.0001
Juice color a*	4.24	<0.012
Fruit diameter ≤80 mm (%)	3.53	0.025
Juice yield (cm^3 100 g arils)	3.41	0.035

† Prob F: F-ratio significance. a* is the red/green coordinate.

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