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## Studies on Cross Pollination between Manfaloty Pomegranate and Some Evaluated Import Cultivars

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### **Authors' contributions**

*This work was carried out in collaboration between three authors. Authors AAN, AAET and AAA designed the study, author AAN made the crosses. Authors AAET and AAA took data and collected samples from the field. Authors AAN and AAA performed the laboratory tests. Authors AAET and AAA made the statistical analysis. Author AAN wrote the manuscript. All authors read and approved the final manuscript.*

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### ABSTRACT

**Aims:** The present study was conducted in three successive seasons, to evaluate five pomegranate cultivars namely, Eversweet, Sweet, Aca, Wonderful and Blackberry in two seasons followed by one season to achieve crosses between the Manfaloty and these evaluated cultivars.

**Methodology:** Vegetative, flowering, physical and chemical parameters were measured for the new tested cultivars. Then hybridization was conducted between Manfaloty and the new evaluated cultivars using tested viable pollen grains and the resulted fruits from these crosses were physically and chemically evaluated.

**Results:** Sweet cultivar super passed the remaining cultivars in vegetative parameters (leaves number, shoot length and number of internodes). Meanwhile, Aca cultivar revealed the lowest value for vegetative growth parameters.

Eversweet cultivar showed the highest number of both hermaphrodite and male flowers. Moreover, Sweet cultivar showed earlier date for flowering; however, the Wonderful recorded lately date of flowering. Chemically, Blackberry cv. Super passed the rest of the evaluated cultivar in anthocyanin content followed by Aca, wonderful, Sweet and finally Eversweet. On the other hand, tannins was highest in Aca cv. followed by Blackberry, wonderful and both of Sweet and Eversweet. A vice versa trend for tannins was observed

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for total sugar. Total soluble solids / acidity ratio was highest in Sweet cultivar and low with Blackberry cultivars. Sweet cv. had a soft seeded, while semi soft seed was characterized by Eversweet cv.; however, Wonderful, Aca and Blackberry have hard, semi hard and very hard, respectively. Yield was highest within Aca and Wonderful cvs in both of the studied seasons. Genetically, Blackberry cultivar was far from the studied cultivars. Moreover, Wonderful cultivar showed a lower genetic similarity Aca, Sweet and Eversweet (0.28, 0.33 and 0.43, respectively).

Before achieving of crosses, viability of pollen grains of the five evaluated pomegranate were tested, it was differ from one cultivar to another, Wonderful cv. showed the highest pollen grains, followed by sweet cv. Aca and Eversweet; while, Blackberry occupy the last order in pollen grains viability.

**Conclusion:** A wide variation of pollen grains effect within physical and chemical fruit characteristics resulted from crosses between Manfaloty and these cultivars was observed.

*Keywords: Pomegranate; crosses; manfaloty; RAPD; evaluation.*

## 1. INTRODUCTION

Pomegranate (*Punica granatum*) is one of the oldest known edible fruits, which is cultivated in west Asia and the Middle-East countries since ancient times [1]. Pomegranate is mainly grown in tropical and subtropical regions of the world [2]. Pomegranate fruit, besides its fresh consumption, has different products in industry such as fruit juice, vinegar, jelly and especially its medicinal effects lead to its gaining popularity in the world markets [3] and [4].

Pomegranate juice has become very popular because with the discovery of its significant biological activity, the bioactivity has been attributed to the high level of antioxidant activity [5]. Phenolic acids, anthocyanin and ascorbic acid, either alone or in combination are responsible for antioxidant activity of pomegranate [6]. Due to the protective effect as an antioxidant,  $\beta$ -carotene has come into more true light in new discoveries that implies into more benefit and heavy commercial promotion [7]. Moreover, there is an increasing pomegranate demand, especially in outside markets. However, desired pomegranate characteristics such as, high and regular yield, large red and thin skinned fruit, high amount of red juice, aromatic, sweet, soft seeded arils and storage may change to both domestic and outside markets [8]. Therefore, breeding experiments should be conducted to get quality fruit. For this purpose crossing studies should be conducted by using selected standard pomegranate cultivars.

The aim of the present study is firstly, to evaluate new pomegranate cultivars known as (Aca, Wonderful, Eversweet, Sweet and Blackberry) for their qualitative and quantitative characteristics. Secondly, hybridize between local cultivar "Manfaloty" and the identified cultivars mentioned before.

## 2. MATERIALS AND METHODS

This work was conducted during three successive seasons (2011, 2012 and 2013) on healthy pomegranate trees cultivars (Aca, Wonderful, Eversweet, Sweet and Blackberry) planted at 3x3 m. These trees were planted in sandy soil at the desert way between Cairo and Alexandria at 65 km. and irrigated with drip irrigation. The studied trees were five years old and subjected to the horticulture practice. Ten trees were selected randomly for each

cultivar and growth performance, physical and chemical characteristics of fruits were observed during 2011 and 2012 seasons.

## **2.1 Growth Performance**

Plant height and trunk diameter were determined. Twenty shoots of one year sprout were tagged randomly in different sides in the plant to observe, number of leaves, shoot length and number of internodes.

## **2.2 Flowering and Yield**

The date of flowering was determined at the beginning of flowering, number of both male and hermaphrodite flowers were determined at the balloon stage and fruit setting% were also recorded/ tree on the twenty lagged shoots. Final fruitlet was counted and yield consequently calculated as average fruit weight x number of the resulted fruit in both of the studied seasons (2011 and 2012).

## **2.3 Physical Characteristics of the Fruit**

Three replicates of fruits, each one contain five fruits were used to determined physical and chemical characteristics (*i.e.* fruit weight, fruit volume, fruit length, fruit diameter, shape index, number of rooms, seed firmness, aril fruit weight (sarcotesta of fruit), aril fruit weight ratio (aril fruit weight / fruit weight), volume of juice and a taste panel) were recorded.

## **2.4 Chemical Constitutes of Fruits**

Total soluble solids (T.S.S) was determined by a hand refractometer, - Acidity of fruit juice was determined by titration with 0.1 normal sodium hydroxide with phenolphthalein as an indicator, according to [9], -Tannins content was determined as described by [10], - Total Anthocyanin was determined according to [11], -Total and reducing sugar were estimated using methodology described by [12] and non-reducing sugar was deduced from subtraction of reducing sugar from total sugar.

## **2.5 Crosses between Manfaloty and the Evaluated Cultivars**

This investigation was conducted during 2013 season. Pollen viability was tested firstly before crosses using 2,3,5-triphenyl tetrazolium chloride (TTC), staining solution, 1 part (10% TTC): 10 parts sucrose solution according to the methods described by [13], only the red colored grains were considered as a life and neglected any other colors. The tested types of pollination were hand-pollination using pollens of the abovementioned cultivars and open pollinations (labeling 10 flowers around the trees with five trees/ cultivar). For hand pollination, anthers were removed then pollens were directly applied to just starting to open flowers in which stigmas were sticky and receptive at (8 am) and the flowers were covered with parchment bags. The percentage of fruit set was calculated 10 days after pollination and percentage fruitlet were calculated.

Fruit resulted from all types of crosses were collected at the end of the season and examined for physical and chemical characteristics as described before.

## **2.6 DNA Fingerprint**

Total genomic DNA was extracted from young and fresh leaves of evaluated cultivars (Aca, Wonderful, Eversweet, Sweet and Blackberry) using modified CTAB protocol [14]. RAPD fragments were amplified according to [15]. Amplification reaction for RAPD consisted of 1.5mM 10X buffer, 1.5mM MgCl<sub>2</sub>, 100µM dNTPs (25µM each), 0.2 µM primer, 1U Taq polymerase, 50 ng of DNA and sterile water up to 25 µl. RAPD amplification was conducted as follow: an initial step at 94°C for 5 min. 40 cycles for each cycle 1 min. at 94°C, 1 min. at 37°C and 2min. at 72°C and final extension step at 72°C for 5 min.

The experiment included in this study followed a complete randomized design in factorial design. Data analyses were performed by MSTAT-C [16] computer statistical analysis program. The obtained data were subjected to analysis of variance (P=0.05) according to [17].

## **3. RESULTS AND DISCUSSION**

### **3.1 Phenotypic Characterization of Evaluated Pomegranate Cultivars**

Phenotypic characterization was estimated through some parameters illustrated in Table 1. Growth nature differ from one cultivar to another, it was upright in sweet and Wonderful, meanwhile, Eversweet, Aca and blackberry showed spreading shape according to describe of UPOV [18]. Plant height and trunk diameter always detected high value with Wonderful, Aca and Blackberry; however, Sweet and Eversweet recorded the lowest value except for trunk diameter in 2012.

It is obvious that sweet cultivar super passed the remaining cultivars in vegetative parameters (leaves number, shoot length and number of internodes). Meanwhile, Aca cultivar revealed the lowest value for vegetative growth parameters. On the other hand, Wonderful cultivar represented intermediate records regarding vegetative characteristics. The differences in vegetative growth habit of these new cultivars could be attributed to the efficiency to be adapted with the Egyptian environmental conditions.

As for flowering habits, Eversweet cultivar almost showed the highest significant number of both hermaphrodite and male flowers and this was consequently reflected on the sex ratio. Although, the blackberry cultivar detected intermediate trend for total number of hermaphrodite and male flowers, sex ratio was the highest significant for this cultivar; this could be interpreting by the decreasing of the number of male flowers. Aca and Sweet cultivars recorded the least significant value of both hermaphrodite and male flowers. The first date of flowering was observed and recorded (Table 2). Sweet cultivar showed earlier date for flowering; however, the Wonderful recorded lately date of flowering. In this respect, it was demonstrated that, flower life is one of the major qualitative parameter, which governs the pollination and ultimately the fruit set [2].

**Table 1. Morphological characterization of some pomegranate cultivars**

Cultivars	Growth nature	Plant height (m)		Trunk diameter (cm)		Av. no. of leaves /shoot		Av. Shoot length (cm)		Av. no. internodes/shoot	
		2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
Sweet	Upright	1.74	1.87	19.3	22.0	14.20	12.97	34.97	40.80	21.25	22.41
Ever sweet	Spreading	1.42	1.55	19.67	23.33	34.90	46.93	24.33	38.83	8.33	9.97
Wonderful	Upright	1.93	2.22	22.67	21.67	85.87	69.34	23.07	28.73	9.99	13.03
Blackberry	Spreading	2.06	2.20	31.67	33.67	66.30	97.07	27.30	39.67	10.90	13.30
Aca	Spreading	2.05	2.20	28.00	31.00	26.50	40.10	14.57	21.37	6.00	10.07
L.S.D 5%		0.41	0.38	6.12	10.54	14.34	12.32	4.63	5.80	1.66	1.17

**Table 2. Flowering characterization, no. of fruits and yield of some pomegranate cultivars**

Cultivars	Total of har./tree		Male flowers/tree		Sex ratio		Flowering date		Fruit %		No. of fruits/ tree		Yield/ tree	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
Sweet	11.07	16.30	5.23	9.67	67.91	62.76	7/4	9/4	28.75	22.34	18.63	21.77	8.81	7.62
Ever sweet	44.23	56.90	17.47	16.67	71.68	77.34	10/4	9/4	28.65	26.87	23.00	30.57	6.68	5.05
Wonderful	23.63	34.93	17.60	9.43	55.96	78.74	13/4	15/4	34.98	35.12	26.00	29.63	10.12	9.97
Black berry	25.57	28.23	8.70	6.13	74.61	82.16	12/4	9/4	24.10	27.81	18.20	21.43	6.22	5.66
Aca	10.43	15.23	10.43	6.63	50.00	69.67	12/4	10/4	36.32	35.92	39.57	24.00	16.82	13.87
L.S.D 5%	12.29	13.97	5.01	5.19	7.75	7.11			2.10	1.21	6.12	5.08	2.10	1.05

It was clear from the data that cv. Wonderful and Aca were superior in respect of fruit set percentage compared to the remaining cultivars (Table 2). Otherwise, number of fruit per plant was detected by cv. Aca in the first season and cv. Wonderful and Eversweet in the second season. However, cv. Sweet and Blackberry showed the lowest number of fruit per plant.

Aca and Wonderful showed the highest yield/ tree in both of the studied seasons. It is obvious that cvs. Eversweet and Blackberry almost have a constancy trend in respect of yielding. The yield factor attributed to number of fruits/plant and size of the fruit which various due to genetic makeup of the cultivar. Apart of genetic makeup, environmental factors and age of plants affects the number and size of the fruits which in turn is responsible for overall yield efficiency [2].

### **3.2 Physical Characteristics of Fruit in Some Pomegranate Cultivars**

Physical parameters of the evaluated cultivars during 2012 and 2013 are cleared in Table 3. Wonderful and Aca cultivars were super passed the other cultivars in respect of average fruit weight. Meanwhile, no significant differences were observed in average fruit weight with Blackberry, Eversweet and Sweet in the first season. Aca cultivar exhibit a superior fruit weight in the second season; however, Eversweet cultivar showed the least value of average fruit weight. At the same time fruit volume follow the same trend of fruit weight.

Results showed that fruit length was highest with Aca and Sweet cvs. in the first season; however, these cultivars introduce an intermediate value of fruit length in the second season. Moreover, the least value was observed by Eversweet and Wonderful in the first and second seasons, respectively. On the other hand, fruit diameter revealed significant differences among the tested cultivars; e.g. Sweet cultivar detected the highest value in the first season. No significant differences were observed between Blackberry and Wonderful in the first season; Blackberry and Eversweet in the second season. The ratio between fruit length and fruit diameter was calculated to determine the differences among the tested cultivars in shape. This ratio was ranged from 0.93 to 1.08 in the first season and 0.72 to 1.02 in the second season. It is appearing that fruit shape was influenced by this ratio, e.g. Blackberry, Sweet and Eversweet takes almost a roundish shape, while Aca and Wonderful showed an ovate shape (Fig. 1).

In Wonderful cultivar, the number of rooms inside the fruit revealed the least value during the two growing seasons; while, no differences were observed within Blackberry, Aca and Sweet during the studied seasons (Table 4). Aril fruit weight detected significant highest value in Aca cultivar in both seasons; this consequently affected on aril fruit ratio, it was highest within Aca in the first season and Blackberry in the second season. As for juice volume, the analysis of variance revealed that Eversweet cultivar recorded significantly the maximum volume of juice, while Blackberry showed the minimum content of fruit juice. On the other hand, aril fruit weight was minimum in cv. Blackberry and Eversweet in the first and second seasons, respectively, which was significantly less in comparison to the remaining cultivars under study. Seed firmness was also determined through the taste panel estimation, it could be concluded that the Sweet cv. Had a soft seeded, while semi soft seed was characterized by Eversweet cv.; however, Wonderful, Aca and Blackberry have hard, semi hard and very hard, respectively.

Bases of evaluation of the aforementioned pomegranate were related to [4,19,20,21] and [22].



**Fig. 1. Fruits of five pomegranate cultivars (Blackberry, Wonderful, Aca, Eversweet and Sweet)**

### 3.3 Chemical Constitutes of Fruits

Data of the qualitative fruit traits obtained from juice of pomegranate cultivars are given in Table 5. Blackberry cv. Super passed the rest of the evaluated cultivar in anthocyanin content followed by Aca, wonderful, Sweet and finally Eversweet. It is well known that Anthocyanin is a member of phenolic compounds that contribute to the red, blue or purple colors of many fruits, including pomegranate juice and also known by their antioxidant activity [4] and [23].

Peel color and Aril color were affected by anthocyanin content (Fig.1); it was noticed that peel color ranged from yellow (Eversweet) to black (Blackberry), while aril color ranged from white-pink (Sweet and Eversweet) to red (Blackberry).

On the other hand, tannins was highest in Aca cv. Followed by Blackberry, wonderful and both of Sweet and Eversweet. A vice versa trend for tannins was observed for total sugar; Wonderful represented the highest value of total sugar. Meantime, Eversweet revealed a least significant value of total sugar. Almost the same analogous of results were detected by non-reducing sugar. Non significance variant was revealed between Wonderful and Blackberry for total soluble solid content in the first season; while, no significant differences were obtained among all of the evaluated cultivars except for Sweet in the second season. Acidity exhibited a highest significant percent with (Sweet and Aca) and (Aca and Woderful) in the first and second seasons, respectively. Total soluble solids / acidity ratio was highest in Sweet cultivar and low with Blackberry cultivars, this factor (TSS/ acidity ratio) is an efficient index used to define the fruit quality which appear in the taste panel test according to personals. The score of the taste panel were between zero to ten. The taste panel ranged from 6 (Blackberry) which could be estimated as sour taste to nine (Sweet) estimated as sweet taste. However, Wonderful and Aca revealed a sour-sweet taste with a scorable taste panel (7) for both of them. Significant variations in soluble solid content and acidity of pomegranate juice have been reported by [24] and [25]. Moreover, it was stated that, the maturity index (TSS/ acidity) also appeared to be good indicator of fruit maturity as it increased significantly during fruit ripening [4].

**Table 3. Fruit physical characteristics of some pomegranate cultivars**

Cultivars	Fruit weight (g.)		Fruit volume (cm <sup>3</sup> )		Fruit length (cm)		Fruit diameter (cm)		Shape index	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
Sweet	338.72	349.90	363.33	335.00	10.51	7.77	10.58	8.60	0.99	0.90
Ever sweet	290.53	210.53	316.67	213.33	7.80	9.43	8.48	10.63	0.93	0.89
Wonderful	542.99	336.54	555.00	351.25	9.79	6.47	9.10	8.97	1.08	0.72
Black berry	341.70	263.90	364.33	308.33	9.54	9.43	8.99	10.63	1.06	0.89
Aca	425.10	453.69	448.33	503.75	10.31	7.60	9.78	7.43	1.06	1.02
L.S.D 5%	26.05	15.24	19.29	13.28	0.47	0.24	0.45	0.26	0.06	0.06

**Table 4. Number of rooms, seed weight, seed firmness and aril fruit weight of some pomegranate cultivars**

Cultivars	No. of rooms/ fruit		Seed firmness		Aril fruit weight (g.)		Aril fruit weight ratio		Juice volume (cm <sup>3</sup> )	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
Sweet	6.00	6.00	soft	soft	259.67	218.54	1.38	0.34	270.85	278.90
Ever sweet	6.33	5.67	Semi soft	Semi soft	171.00	106.89	1.22	0.74	289.62	305.50
Wonderful	5.33	5.33	Hard	Hard	149.33	122.89	1.01	0.57	226.30	251.40
Black berry	6.00	6.00	Very hard	Very hard	148.67	178.33	1.33	0.87	183.22	190.10
Aca	6.00	6.00	Semi hard	Semi hard	306.00	238.67	1.84	0.73	202.65	203.70
L.S.D 5%	0.21	0.19	--	--	1.57	0.81	0.32	0.25	0.02	0.77

**Table 5. Chemical constituents of five pomegranate cultivar**

Cultivar	Anthocyanin mg/100ml		Tanins mg/100ml		T. sugars mg/100ml		Non R. sugar mg/100ml		R. Sugar mg/100ml		TSS%		Acidity%		TSS/acidity ratio	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
Sweet	0.91	0.95	1.67	1.00	28.40	28.90	6.28	6.88	22.12	22.02	16.80	16.17	2.20	2.33	7.65	6.96
Ever sweet	0.82	0.87	1.19	1.16	24.02	24.55	3.62	4.21	20.40	20.34	16.80	16.00	2.10	2.05	8.00	7.82
Wonderful	0.99	1.09	1.82	1.75	33.31	33.51	9.92	10.29	23.39	23.21	15.90	15.33	2.17	2.70	7.34	5.07
Aca	2.52	2.56	2.56	2.49	26.35	26.47	1.84	1.83	24.51	24.64	16.90	15.50	2.20	3.10	7.68	5.00
Black berry	4.26	4.31	2.06	2.08	27.18	27.03	0.92	3.88	26.26	23.15	15.00	16.33	2.47	2.23	6.15	7.32
L.S.D 5%	0.07	0.11	0.12	0.06	0.017	0.02	0.02	0.01	0.02	0.33	0.23	0.23	0.09	0.39	0.21	0.19



### 3.4 DNA Fingerprint as Detected by RAPD Marker

#### 3.4.1 Polymorphism detected by RAPD marker

Five RAPD primers were tested with the DNA of five pomegranate cultivars (Sweet, Eversweet, Wonderful, Aca and Blackberry). These primers produced multiple band profile which ranged from 18 to 29 amplicon (Fig.2). Total number of amplicons amplified by the five primers was 115 with an average 23 amplicon/ primer (Table 6). The number of polymorphic bands ranged from 16 (OPO06) to 28 (OPA20 and OPZ12), representing percentage of polymorphism ranged from 88.89% (OP06) to 100% (OPA20). The size of the amplified bands varied according to the used primers, it was ranged from 115bp to 755bp.

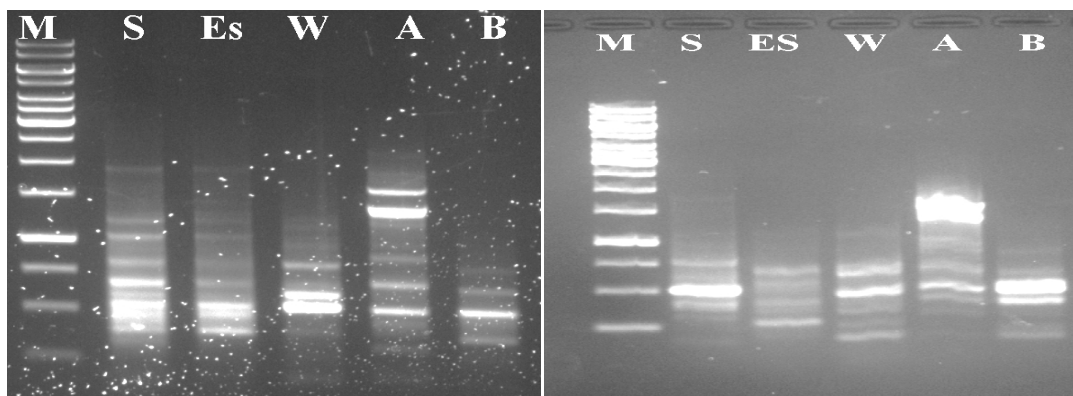


Fig. 2. Polymorphism detected by RAPD marker with Sweet(S), Eversweet(Es), Wonderful(W), Aca(A) and Blackberry(B). M: Ladder molecular weight marker

Table 6. Polymorphism and its percentage as detected by RAPD marker

Primer	Total no. of amplicons	Monomorphic amplicons	Polymorphic amplicons	Percentage of polymorphism
OPC15	19	1	18	94.73
OPA20	28	0	28	100.0
OPZ12	29	1	28	96.55
OPO17	21	4	17	80.95
OPO06	18	2	16	88.89
Total	115	8	107	
Average	23	1.6	21.4	

Genetic diversity for some Iranian pomegranate cultivars was evaluated using AFLP marker and obtained a total 112 polymorphic bands from 237 fragments, ranging in size from 50 to 800 bp, which generated from seven primer combinations. The percentage of polymorphic DNA bands ranged from 40% to 58.06% with an average of 47.26% [26].

### 3.5 Cluster Analysis

Dendrogram obtained from UPGMA cluster analysis of genetic distances (Fig.3) revealed that, all of Aca, Sweet and Eversweet genotypes were separated in one cluster. Moreover, this cluster was divided into two groups, one group included both of Sweet and Eversweet

and second one contain only Aca cultivar. However, the Blackberry cultivar was separated in the second. Meantime, the third cluster contains Wonderful cultivar. Authors showing that the clustering of the cultivars is not related to the geographical distance [27], [28] and [29].

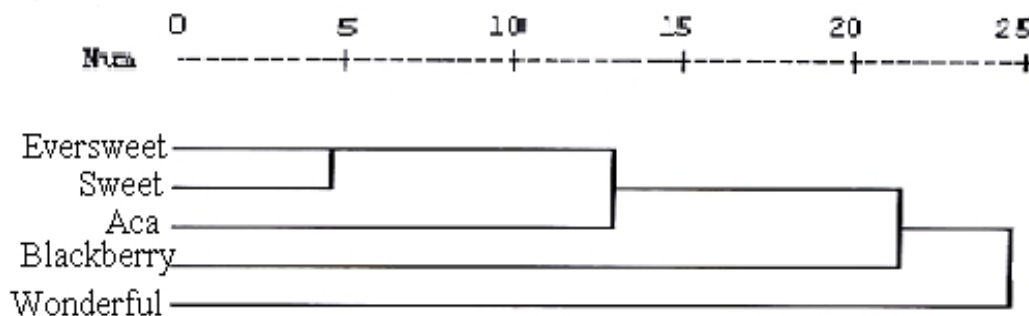


Fig. 3. Dendrogram using average linkage (between groups)

### 3.6 Genetic Similarity

The genetic similarity ranged from 0.18 (Blackberry and Eversweet) to 0.81 (between two genotypes of Sweet and Eversweet). A high value of genetic similarity was observed between Aca cultivar and both of Sweet and Eversweet cultivars, it was 0.73, and 0.75, respectively (Table 7).

On the other hand, it was clear that, Blackberry cultivar was far from the studied cultivars within genetic similarity; e.g. it was recorded genetic similarity 0.26 with Sweet, 0.40 with Wonderful and 0.45 with Aca. Moreover, Wonderful cultivar showed a lower genetic similarity Aca, Sweet and Eversweet (0.28, 0.33 and 0.43, respectively). It could be stated that Sweet, Eversweet and Aca cultivars have share with a common genetic background. The vice versa was true for both Wonderful and Blackberry cultivars. The range of the genetic similarity in pomegranate cultivars was estimated by several authors [30,31] and [32].

Table 7. Genetic similarity as detected RAPD marker

	ES	S	W	A	B
ES					
S	0.81				
W	0.43	0.33			
A	0.75	0.73	0.28		
B	0.18	0.26	0.40	0.45	1.00

### 3.7 Hybridization between Manfaloty (Mother Plant) and the Evaluated Pomegranate cvs (Parent Plant)

Before the assignment of hand pollination, viability of pollen grains was analyzed (Table 8). Wonderful cv. showed the highest viability of pollen grains, followed by sweet cv., Aca and Eversweet; while, Blackberry occupy the last order in pollen grains viability. However, no statistical differences in pollen viability between Wonderful, Aca, Sweet and Eversweet. Pollen viability, pollen germination and production capacity of different type of pomegranate flowers varied according to cultivars and flower type [33].

The percentage of fruit set resulted from hybridized flowers ranged from 7.69% (Manfaloty x Blackberry) to 35.71 (Manfaloty x Wonderful). Percentage of fruitlet zero (Manfaloty x Blackberry) to 100% (Manfaloty x Aca) while, open pollination introduce six fruit set flowers out of eighteen open pollinated flowers representing 33.3% of resulted flowers and 50% of fruitlet.

**Table 8. Pollen viability, fruit set% and fruitlet% as affected by pomegranate cultivars**

Cultivar	Pollen grain viability%	Type of cross	Fruit set %	Fruitlet %
Eversweet	89.72	Manfaloty x Eversweet	21.05	25.00
Sweet	93.84	Manfaloty x Sweet	50.00	75.00
Aca	90.51	Manfaloty x Aca	13.04	100.0
Blackberry	68.23	Manfaloty x Blackberry	7.69	0.00
Wonderful	95.10	Manfaloty x Wonderful	35.71	80.0
		Open	33.33	50.00
L.S.D at 5 %	11.25		1.0	0.02

### 3.8 Effect of Pollen Grains Type on Fruit Characteristics

Quantitative and qualitative fruit characteristics were affected by the source of pollen grain used in the pollination (xenia effect). It is clear that fruit weight, fruit length, fruit diameter, fruit shape and fruit volume were decreased with all of the used pollen grain types in comparison with open pollination (Table 9). Whereas, pollen grains of Sweet cv. increased the value of these characteristics mentioned before comparing with the remaining crosses type. On the other hand, pollen grain of Wonderful recorded the least volume for fruit weight, fruit length, fruit diameter, shape index, fruit volume and juice volume. No significant differences was observed in the number of room when open pollination, Sweet and Wonderful pollen grains were used. Meanwhile, seed weight was increased when pollen grains of Sweet cultivar used in comparable with control followed by open pollination, Aca, Eversweet and Wonderful. Moreover, aril weight and aril/ fruit weight ratio was superior with Aca pollen grains, while, open pollination and pollen grains of Sweet cv. raised the lowest ratio/ fruit weight.

**Table 9. Physical characteristics affected by pollen grains of different pomegranate cultivars (2013)**

Treatments	F. weight gm.	Mean F. length cm	mean F. diameter cm	Shape index	F. volume cm <sup>3</sup>	No. of rooms fruit	Juice volume cm <sup>3</sup>	Seed weight gm.	Ariel weight F. g gm.	Ariel/ fruit weight ratio
Manfaloty x Eversweet	186.56	6.01	7.20	0.84	140.00	5.00	197.34	336.00	248.11	1.44
Manfaloty x Sweet	313.02	7.99	8.55	0.94	276.67	6.00	332.37	680.00	414.80	1.35
Manfaloty x Aca	183.86	6.91	7.10	0.97	146.33	6.00	195.63	538.32	463.30	2.61
Manfaloty x Wonderful	119.37	5.73	6.34	0.90	93.33	5.67	106.14	191.81	191.81	1.63
Open pollination	372.92	9.96	9.34	1.07	376.67	6.00	345.80	650.00	440.00	1.18
L.S.D 5%	27.91	0.40	0.36	0.08	28.6	0.23	0.15	12.41	14.13	0.24

**Table 10. Chemical characteristics affected by pollen grains of different pomegranate cultivars (2013)**

Treatments	Anthocyanin mg/100ml	Tannins mg/100ml	T. Sugar mg/100ml	Non R. sugar mg/100ml	R. sugar mg/100ml	TSS %	Acidity %	TSS/acidity ratio
Manfaloty x Eversweet	0.64	1.05	26.78	3.78	23.00	16.23	2.01	8.07
Manfaloty x Sweet	0.66	1.34	32.67	9.12	23.55	15.94	2.10	7.59
Manfaloty x Aca	0.87	1.91	30.31	3.34	26.97	16.76	2.23	7.52
Manfaloty x Wonderful	0.86	1.90	28.65	10.11	18.54	14.72	2.17	6.78
Open	0.82	2.01	20.31	6.98	13.33	14.76	2.22	6.65
L.S.D 5%	0.05	0.08	1.02	0.91	0.23	0.51	0.09	0.18

Data of the chemical fruit traits obtained from different pomegranate crosses are given in Table 10. There were significant differences between Manfaloty x Sweet, Manfaloty x Eversweet and remaining cross types in anthocyanin content. On the other hand, tannins was highest in fruits resulted from using both of Aca and wonderful as a parent; while, Sweet and Eversweet showed low content of tannins when used as a parent in compare to open pollination. Total sugar was highest in fruits of Manfaloty x Sweet; meantime, Eversweet revealed a least significant value of total sugar when used as a pollen parent. Non-reducing sugar was highest with Wonderful as parent followed by Sweet. All of the tested types crosses recorded a high value of TSS content in comparison to the control (open pollination) with the exception of 'Manfaloty x Wonderful. Almost the same trend was observed regarding acidity except for Aca parent. Total soluble solids / acidity ratio was highest in Eversweet as a parent and low with Wonderful as a parent. These results are in accordance with [34].

#### 4. CONCLUSIONS

Statistically significant differences were observed among the evaluated cultivars. The assessment of these cultivars implies a great potential for using them in the breeding program, one cultivar could be used to improve coloring (Blackberry), other one to increase yielding (Aca and Wonderful) and sweaty taste (Sweet). However, obtained results from different crosses type lead to recommended Sweet cultivar as pollinator; it improves physical and chemical fruit characteristics.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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