

Morphological and molecular characters of some S1 progenies of selfing, openpollination and F1 hybrid between some Egyptian pomegranate cultivars

Abstract

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Key words Pomegranate, *Punica granatum*, Open-pollinatio, Self-pollination, Polymorphism. The present study was carried out during 2018 and 2019 seasons . Twenty F1 and S1 progenies resulted from the combinations progenies of Manfalouty selfpollinated"MSP", Manfalouty X Nab El-Gamal "MN", Manfalouty open pollinated"MOP" and Tahrir open pollinated "TOP" were selected to measure several morphological quantitative and qualitative characters. Start Codon Targeted Polymorphism (SCoT) moleculer marker was used to assess the genetic variation. Results indicated that, MSP1 progeny gave the lowest shoot length and shoot diameter in both seasons, the same progeny gave the lowest values of number of leaves / shoot in both seasons. The MOP2 progeny is the earliest genotype for blooming for both seasons (15 and 14 of march, respectively). Regarding the fruit characters, TOP2 progeny gave the highest fruit diameter (99.67 and 96.00 mm), the highest fruit length (65.33 and 65.67 mm), the fruit weight (293 and 292.3 gm), arils weight (211 and 210.5 gm) and the highest juice volume (123.7 and 121.3 ml) in the first and second seasons, respectively. The progeny MSP2 gave the highest percent of TSS (14.93 and 15.43) in two seasons. The results of molecular analysis showed that, The ratio of polymorphism about of (48.38%), while he ratio monomorphism about of (51.62%). UPGMA-cluster analysis discriminated the three tested cultivars and four selected progenies based on data of SCoT at 0.723 similarity. Tahrir cultivar and MOP2 progeny which produced from Open pollination of Manfalouty showed the highest value of similarity (0.96). While, TOP2 progeny showed low similarity (0.72) with Nab-Gamal cultivar.

1. Introduction

Pomegranate (*Punica granatum* L.) is one of the most important oldest known edible fruits. It is a favourite table fruit of the tropical and subtropical regions in the world. It is grown in every humid tropical region. Pomegranate (*Punica granatum* L.) a member of family Punicaceae , which has only one genus (Punica) and two species *P. granatum* and *P. protopunica*, with chromosomal numbers of 2n = 16 and 18, respectively[6, 21]. In Egypt, the total cultivated area of pomegranate is about 79,893 fed which produced about 672,827 tons; among them about 100,000 tons are exported annually[22].

Most pomegranate cultivars known today are develop by human selection from naturally occurring varieties[11]. According to the result of the economic, medical and nutritional importance of pomegranate ; the global demand are growing for pomegranate fruits. There were many criteria for selecting pomegranate fruits, including high weight and percentage of aril, and volume of fruit, high red coloration of arils, attractive red color of peel, and high content of soluble solid with low of acidity, high content of vitamin C and anthocyanin and good yielding [14]

Breeding new varieties are being achieved by conventional breeding methods using seedling selection, hybridization and selection of superior genotypes. According to reports from Egypt, India, China , Israel, Turkmenistan, and Azerbaijan [16,19] variations have been developed by hybridization and selection. The hybrids had distinctive morphological characteristics.

The availability of genetic diversity and the origins of cultivars aid in the selection of parents for successful hybridization programmes. To determine genetic diversity among some pomegranate cultivars, molecular markers such as Randomly Amplified Polymorphic DNA (RAPD) [24], Amplified Fragment Length Polymorphism (AFLP) [17] and Simple Sequence Repeats Microsatellites (SSR) [5,11,24], Inter Simple Sequence Repeat (ISSR) [16] and Start Codon Targeted (SCoT) polymorphism [2,14] have been used to determine genetic diversity among some pomegranate

This study aimed to evaluated the phenotypic and genotypic characteristics and fingerprinting of fifteen S1 and five F1 progenies of Egyptian local varieties for selection new genotypes that can be used in future breeding programs to obtain new varieties that meet the criteria of productivity and more adaptable to Egyptian environmental conditions and climatic changes.

2. Materials and methods

This work was conducted in the pomegranate block of the farm of Shandaweel Island Research Station, Agricultural Research Centre, Sohag Governorate, Egypt during two growing seasons (2018 and 2019) and the laboratory of Fruit and Ornamentals Breeding Department and Biotechnology Research lab, Horticulture Research Institute, ARC, Gize, Egypt.

Evaluation of the pomegranate hybrids: In this part of the study evaluated pomegranate healthy progenies resulted from selfing pollination (S1) or hybridization (F1) between some of local pomegranate cultivars. At the farm of Shandaweel Island Research Station, ARC, Sohag Governorate, Egypt, all S1 and F1 seedling trees were planted at a distance of 3 x 4m. The soil texture is hard clay, and a flood irrigation system using Nile water was installed. Regular horticultural treatments were applied to all of the trees. Fifteen S1 progenies resulted from (open-pollinated of Manfalouty "MOP", self-pollinated of Manfalouty "MSP" and open -pollinated of Tahrir "TOP") as well as five F1 progenies resulted from Manfalouty \bigcirc X Nab El-Gamal \bigcirc "MN1-5" Five trees from each progeny were chosen. During the two seasons evaluated, the following parameter were recorded.

Vegetative growth: To examine vegetative growth and blooming characteristics, five shoots were labeled randomly on each selected tree in different orientations in March and April, Data of the vegetative characteristics included plant height (cm), plant thickness (cm), shoot length, Shoot diameter (mm), number of leaves/ shoot, and leaf area (cm²) where calculated by multiplying the length and width according to formula of Ahmed and Morsy (1999) [3]

Leaf area (cm2) = { (0.73) X length X width + 0.16 }.

Flowering and yield characters: During the flowering period, the following data was collected blooming dates, Fruit set percent, Fruit set period, perfect flowers ratio were recorded and Yield for each progeny (tree) was studied in the two seasons 2018 and 2019.

Fruit characters: At random, ten fruits at maturity stage were harvested. To determine fruit parameters, many traits were evaluated as follows: fruit weight (g), fruit size (cm), fruit diameter (cm), fruit length (cm), number of fruit chambers, fruit peel and aril were weighed by digital balance, percentage of arils was calculated relative to weight of fruit rind and capillary membrane (non-edible part), Number of arils in 100 g was recorded during two seasons, as well as soluble solid contenpt (°Brix) and Fruit juice volume (ml)

Statistical analysis : The experiment included in this study followed a completely randomized design. The obtained data were subjected to analysis of variance (ANOVA) according to Snedecor and Cochran (1972) [25]. Means were differentiated by Duncan's multiple range test at 5% level [4].

DNA Isolation Procedure: Fresh leaves tissues of pomegranate were collected separately from (Manfalouty, Tahrir and Nab El-Gamal) cultivars (as a parent plant) and from four selected progenies (MOP2, MOP1, MxN5, TOP2). The bulked DNA extraction was performed using DNeasy Mini Kit (QIAGEN). Isolation protocol of DNA according to Williams *et al.* (1990) [26].

Start Codon Targeted (SCoT) Polymorphism analysis procedure: PCR reactions were conducted using 7 SCoT primers, their names and sequences are shown in Table (1). The DNA amplifications were performed in an automated thermal cycle (model Techno 512) programmed for one cycle at 94° C for 4 min followed by 45 cycles of 1 min at 94° C, 1 min at 57° C, and 2 min at 72° C. The reaction was finally stored at 72° C for 10 min.

 Table (1): List of the primers names and their 18 nucleotide sequences used in the study for Start Codon Targeted (SCoT) Polymorphism procedure.

No	Primer codes	Sequence (5' to 3')
1	SCoT1	5` ACG ACA TGG CGA CCA CGC 3`
2	SCoT2	5° ACC ATG GCT ACC ACC GGC 3°
3	SCoT3	5° ACG ACA TGG CGA CCC ACA 3°
4	SCoT5	5' CAA TGG CTA CCA CTA GCG 3`
5	SCoT8	5' ACA ATG GCT ACC ACT GAG 3`
6	SCoT9	5' ACA ATG GCT ACC ACT GCC 3`
7	SCoT11	5' ACA ATG GCT ACC ACT ACC 3`

Data analysis: The similarity matrices were done using Gel works ID advanced software UVP-England Program. The relationships among genotypes as revealed by dendrograms were done using SPSS windows (Version 10) program. DICE computer package was used to calculate the pairwise difference matrix and plot the phenogram among cultivars[27].

3.RESULTS AND DISCUSSION

Evaluation of Vegetative characters: Data in **Table (2)** demonstrate the vegetative characteristics of the twenty pomegranate progenies investigated during the 2018 and 2019 seasons. Results of analysis of variance indicated that significant variations in the shoot length , shoot diameter , number of leaves/ shoot, and leaf area (cm) . Shoot length ranged from 40 cm to 90.67 cm in the different pomegranate progenies. The highest average of shoot length was 90.67 cm and 90 cm that achieved in progeny of MN2 at 2019 and 2018 season resulting from hybrid of Manfalouty \bigcirc X Nab El-Gamal \bigcirc respectively. while the lowest average of shoot length was 40 cm achieved with progenies of MSP1 resulting from Manfalouty self-pollinated in tow season study.

Data analysis showed also significant differences in the shoot diameter trait. Shoot diameter ranged from 5.53 mm to 8.30 mm in the different pomegranate progenies. The progeny MSP5 resulting from Manfalouty self pollinated produced the maximum shoot diameter(8.30 mm) and (8.17 mm) across the first season (2018) and the second season (2019), respectively. The progeny MSP1 gave the lowest value 5.70 mm at 2018 season, while MOP4 showed the lowest value (5.53 mm) at the second season 2019. Abou El-Khashab *et al.* (2005) [1] conducted studies on the vegetative growth of six native pomegranate cultivars in Egypt. According to their findings,

Montakhab cultivar had the fewest leaves per shoot and Manfalouty cultivar had the longest shoots and most internodes.

Table (2): Shoot length, shoot diameter and number of leaves/ shoot of twenty progenies of pomegranate used in the study during 2018 ,2019 seasons.

Progeny	Proge ny	shoot	length	shoot d	iameter		of leaves/ oot	Leaf (ci	area n²)
	code	2018	2019	2018	2019	2018	2019	2018	2019
Manfalouty	MOP1	69.67ab	68.67 ab	6.93ae	6.90 af	262.7 bc	261.7d	5.27 be	5.98*
open	MOP2	74.67 ab	77.00 ab	6.17 ^{cd}	6.10 df	212.0°	214.3 ef	3.86 🛤	3.721
pollinated	MOP3	72.67 ab	74.33 ab	5.83de	6.17 cf	305.3 bc	308.3 bf	5.78 ac	6.45 *
	MOP4	79.33 ab	79.33 ab	5.73 de	5.53f	318.7 bc	378.3 ad	4.51 %	4.25 f
	MPO5	72.00 ab	73.33 ab	7.37 ad	6.83 af	426.7 ab	423.3 ab	6.16 ª	6.21ª
Manfalouty	MSP1	40.00c	40.00°	5.70°	5.67 ef	198.7°	196.7 et	4.64 dg	4.73 e
self-	MSP2	57.00bc	56.67bc	7.87 ab	7.97 ab	322.7 bc	318.0 ^{bf}	6.06 ab	6.81
pollinated	MSP3	67.67 ab	60.00 bc	7.63 **	7.83 ≥	385.3 ac	390.0ac	4.85 df	5.290
	MSP4	66.67 ab	68.33 ab	6.20 ce	6.17 d	328.0 bc	326.7be	5.83 ac	6.51ª
	MSP5	75.00 ab	75.00 ab	8,30ª	8.17ª	434.7ab	428.0 ab	5.83 ac	5.99ª
Tahrir open	TOP1	69.00 ab	63.67	7.57 80	7.46 ad	294.7 bc	251.0 df	3.45h	3.85
pollinated	TOP2	64.33 ab	61.33 bc	6.73 ae	6.47 bf	289.3 bc	285.0 cf	4.39fg	5.13 d
	TOP3	74.67 ab	70.67 ab	7.73 ac	7.57 ad	532.0ª	463.3ª	5.13cf	5.340
	TOP4	76.00 ab	73.67 ab	7.10 **	6.97 af	325.3 bc	323.7 ^{bf}	6.03ab	6.23 *
	TOP5	65.67 ab	62.67 bc	7.50 *	7.20ªf	349.3 ac	365.0 ad	4.32 ^{fg}	4.42 f
Manfalouty	MN1	77.33 ab	75.00 ab	7.27 30	7.33 20	389.3≈	386.7 ac	6.19ª	6.78
♀ X Nab	MN2	90.00ª	90.67ª	6.63 be	6.70 af	378.7 *	321.3 bf	4.98h	3.95
El-Gamal	MN3	85.00ab	75.67 ab	7.20 ae	7.33 at	385.3 ac	319.03 ^{bf}	4.35fg	4.98
3	MN4	73.67 ab	76.33 ab	6.37 be	6.33 af	365.3 ≈	200.00f	5.40 ^{ad}	5.19
	MN5	71.67 ab	72.00 ab	6.47be	6.50 bf	448.0 ab	228.33 ef	Question Question	5.06d

* MOP: Manfaloty Open pollination. MSP : Manfaloty Selfing pollination. TOP: Tahrir Open pollination and MN: Hybrid Manfaloty X Nab El-Gamal

** The different letters (in the same column) represent statistically significant differences between progenies (p<0.05).

Considerable variation in number of leaves/ shoot was observed among progenies under study in Table (2). The highest values of number of leaves/ shoot were recorded for progenies TOP3 were 532 to 463 for the first 2018 and second season 2019, respectively. While, the lowest values were 198.7 and 196.7 leaves/ shoot were observed for progeny MSP1 in seasons, respectively. Statistical analysis revealed two significant differences in the leaf area for all progenies of different origins. In the first season 2018, the MN1 (progeny of the hybrid Manfalouty X Nab El Gamal) gave the highest value of leaf area 6.19 cm, while the TOP1 progeny of Tahrir open pollination gave the lowest value of leaf area (3.45 cm). In second season 2019, the MSP2 progeny resulting from (Manfalouty self-pollination) was recorded highest value of leaf area(6.81 cm), while the progenies MN2, TOP1 and MOP2 had the lowest value of leaf area ranging between(3.72 to 3.95 cm).

Evaluation of blooming characters: Data presented in **Tables (3)** described the flowering characteristics of the twenty studied pomegranate progenies during 2018 and 2019 seasons. It is obvious from the data that the results took similar trend during the two studied seasons.

Regarding to the date of flowering beginning of pomegranate progenies under study results showed that MOP2 progeny is the earliest one for both seasons (15 and 14 of March, respectively) followed by TOP3 and MN2 progeny (19 and 24 of march, respectively) during both seasons. While the other progeny were blooming later (4th week of march and even last week of April) on the other hand pomegranate progeny MSP5 recorded the last date of flowering during the two studied seasons (23 and 15 April, , respectively)

Progeny	Progen		Bloon	ning date		Flow	ering	Perfect	flowers
	y code	In	itial	End			d (day)	¢	10
		2018	2019	2018	2019	2018	2019	2018	2019
Manfalouty open	MOP1	Mar.30	Apr.1	May.20	May.19	51	48	53.33 ^{ac}	40.28 ^{bd}
pollinated	MOP2	Mar.15	Mar.14	May.5	May.8	51	51	41.11 ^{bc}	33.33cd
	MOP3	Mar.22	Apr .2	May.15	May.19	54	47	49.78ac	69.91ª
	MOP4	Mar.25	Mar.28	May.15	May.18	51	51	35.26°	46.53ad
	MPO5	Mar.25	Mar.28	May.12	May.17	53	50	47.98ac	46.82ad
Manfalouty	MSP1	Mar.20	Mar.25	May.5	May.14	46	50	70.30ª	68.26ab
self-	MSP2	Mar.21	Mar.25	May.7	May.16	47	52	57.14ªc	65.56ab
pollinated	MSP3	Mar.25	Apr.1	May.13	May.20	49	49	36.11°	33.59cd
	MSP4	Mar.22	Mar.26	May.8	May.18	47	53	49.54ªc	50.84ª0
	MSP5	Apr.23	Apr .15	jun.3	May.25	41	40	66.67 ^{ab}	49.29ad
Tahrir open	TOP1	Mar.25	Mar.27	May.8	May.8	44	46	40.45bc	27.14cd
pollinated	TOP2	Mar.23	Apr.3	May.10	May.15	48	42	55.56ªc	50.56ªc
	TOP3	Mar.19	Mar.23	May.12	May.13	54	51	44.19 ac	53.71ª
	TOP4	Mar.25	Mar.25	May.5	May.15	41	51	43.07ac	45.15ad
	TOP5	Mar.25	Mar.25	May.10	May.18	46	54	45.83ªc	34.79cd
Manfalouty	MN1	Mar.26	Apr.1	May.7	May.20	42	49	48.83ªc	48.41 20
Q X Nab El-	MN2	Mar.21	Mar.24	May.6	May.16	46	53	44.11ªc	21.43 d
Gamal 👌	MN3	Mar20	Mar.25	May.7	May.16	48	52	34.76 °	36.11 cd
	MN4	Mar.23	Apr.2	May.4	May.20	42	48	64.44ab	41.85 **
	MN5	Mar.20	Mar.25	May.15	May.20	56	56	44.02ac	64.92 al

Table (3) : Blooming date and flowering period of twenty progenies of pomegranate used in the study during 2018, 2019 seasons.

MOP: Manfaloty Open pollination. MSP : Manfaloty Selfing pollination. TOP: Tahrir Open pollination and MN: Hybrid Manfaloty X Nab El-Gamal

** The different letters (in the same column) represent statistically significant differences between progenies (p<0.05).

The reason for the delay in flowering of the pomegranate progenies resulting from cross- between Manfalouty \bigcirc X Nab El-Gamal \bigcirc to 3rd week of march and the 1st week of April) is due to the effect Nab El-Gamal cultivar in the hybrids, as indicated by *Ibrahim et al.*(1985) [13] found that Araby cultivar was earlier than Manfalouty and Nab El-Gamal under Assiut Governorate conditions.

As for the date of Flowering period (day) the date in **Table** (3)showed wide differences between pomegranate progenies under study. The flowering period typically lasted 40 days at the minimum and 56 days at the maximum. Data revealed that the period of Flowering was the shortest in the progeny of selfing of Manfalouty (MSP5) in the two seasons (41 and 40 days, respectively), while it was the longest for the progeny of hybrid (Manfalouty ×Nab-El Gamal (MN5) for both seasons (56days) in the two seasons.

Regarding the Initial fruit set date of pomegranate progenies under study, results in **Table (4)** indicated that. MOP2 and TOP2 progenies were earlier (at 29 march) during first season 2018 and (28march and 3 april) in the second season 2019 . While the other progeny were later (1st week to 4nd week of April) on the other hand pomegranate progeny MSP5 recorded the last date of fruit set (1May and 25 April) during the two studied seasons2018 and 2019 respectively.

Regarding to prefect flowers % resulting in **Table (3)** showed that the highest average of prefect flowers percentage in first season was (70.30) and that achieved in MSP1 progeny resulting from Manfaloty selfing pollination. In second season the highest average of prefect flowers percentage was (69.91%) and that recorded with MOP3 resulting from Manfaloty Open pollination.

Also results in **Table (3)** showed that the two progenies MN2 and MN3 resulting from hybrid between Manfaloty X Nab El-Gamal were revealed the lowest average of prefect flowers percentage (21.43%) at 2^{nd} season and (34.76%) at 1^{st} season, respectively.

Concerning the fruit set period, Data in Table (4) revealed that the period of fruit set was the shortest in the TOP4 progeny of open of Tahrir in the first seasons (40 days), while in the second season the progenies TOP1 and MSP5 were recoded the lowest average of fruit set period (41 day) on the other hand the period of fruit set was the longest for the open progeny of Manfalouty (MOP2) for both seasons (57 and 53 days) in the two seasons2018 and 2019, respectively.these results are in agreements with pervious findings in pomegranate Khalil et al (2014) [20], whom studied the vegetative growth of some S1 progenies and F1 hybrid between some Egyptian pomegranate cultivars and reported that the highest percentage of fruit set was recorded by selfed progeny of El-Tarir, on the other hand, open-pollination progeny of Nab El-Gamal exhibited the lowest percentage which reached to 17.15%, as regards yield/tree, the progenies resulted from self pollination of Nab-El Gamal and the hybrid between El-Tarir X Nab-El Gamal gave the highest yield/tree ranging between 21.75 and 22.50 Kg/tree.

 Table (4): Fruit set date and fruit set period of twenty progenies of pomegranate used in the study during 2018,2019 seasons.

Progeny	Progen		Fru	it set		Frui	t set	Fruit	set %
2.020	y code	In	itial	End	0	period	l (day)	10	
	235	2018	2019	2018	2019	2018	2019	2018	2019
Manfalouty	MOP1	Apr .13	Apr.11	May.29	May.29	46	48	37.78 ab	50.37*
open	MOP2	Mar.29	Mar .28	May.25	May.20	57	53	25.560	44.29*
pollinated	MOP3	Apr.2	Apr.12	May.25	May.29	53	47	42.01 ab	45.19
	MOP4	Apr .5	Apr.8	May.27	May.28	52	50	24.36 b	49.47
~	MPO5	Apr .5	Apr.8	May.22	May.28	47	50	31.06 ^b	53.33
Manfalouty	MSP1	Apr .1	Apr.5	May.15	May.24	44	49	61.95ª	57.63
self-	MSP2	Apr .1	Apr.5	May.14	May.26	43	49	45.87 ab	50.073
pollinated	MSP3	Apr .5	Apr.10	May.23	May.30	48	50	26.16 ^b	44.29
	MSP4	Apr .2	Apr.5	May.19	May.27	47	52	39.44 ab	47.62*
	MSP5	May.1	Apr.25	jun.15	jun.5	45	41	49.08 ab	57.94
Tahrir open	TOP1	Apr .4	Apr.7	May.18	May.18	44	41	30.38 b	41.11*
pollinated	TOP2	Apr .1	Apr.3	May.21	May.25	50	50	38.89 ab	48.52*
	TOP3	Mar.29	Apr.3	May.15	May.23	47	50	39.43 ab	45.19
	TOP4	Apr .6	Apr.3	May.16	May.25	40	52	33.49 ab	51.85*
	TOP5	Apr .5	Apr.5	May.20	May.28	45	53	33.33 ab	53.33*
Manfalouty	MN1	Apr .2	Apr.11	May.17	May.30	45	49	36.75 ab	46.82*
$\mathcal{Q} \mathbf{X} \mathbf{N} \mathbf{a} \mathbf{b}$	MN2	Apr .2	Apr.6	May.17	May.25	43	49	35.52 ab	49.61
El-Gamal	MN3	Apr .2	Apr.5	May.15	May.26	43	52	24.29 0	44.44*
6	MN4	Apr .4	Apr .10	May.16	May.30	42	50	48.89 ab	52.38 a
	MN5	Apr .2	Apr.5	May.19	May.27	47	52	35.90 ab	50.00 a

MOP: Manfaloty Open pollination. MSP : Manfaloty Selfing pollination. TOP: Tahrir Open pollination and MN: Hybrid Manfaloty X Nab El-Gamal

** The different letters (in the same column) represent statistically significant differences between progenies (p<0.05).

In addition results **in Table (4)**showed that the end of fruit set date for all F1 progenies ranged between 14 of May and 15 of Jun during the first season and from 18 of May to 5 of Jun in the second season

Regarding to fruit set percentage, the results in **Table** (4)showed that clear significant difference was found between progenies pomegranate that used in our study during the first growing season only. The highest values were observed for two progenies of "Manfaloty selfing pollination" MSP1, MSP5 at 61.95 %, 49.08 % respectively. Followed by MN4 progeny rustling from hybrid Manfalouty X Nab El-Gamal with (48.89%). While, the lowest percentage was recorded by MN3 (24.29 %) during the first season.

Also the data in **Table** (4) showed that no significant differences were found between all progenies pomegranate under study in fruit set during second season.

Evaluation of Fruit Characteristics: The data obtained from the evaluation of fruit characteristics of some progenies of pomegranate used in study during 2018 and 2019 seasons are presented in **Tables (5, 6, 7).** It is obvious from the data that the results took similar trend during the two studied seasons. The data showed significant variations in fruit weight (g), artil weight and peel weight (Table 5) and fruit length, fruit diameter, number of fruit chambers (**Table 6**) as well as percentage of arils (edible part), soluble solid content (T.S.S) and fruit juice volume during two seasons (**Table 7**).

It is clear from the data in **Table (5)** that the TOP progenies which obtained from Tahrir Open pollination and MN Hybrid which produced from hybrids between Manfaloty X Nab El-Gamal were recorded the highest average of fruit weight. Where TOP2 progeny recorded the highest value for fruit weigh was (293 and 292.3) in two season 2018 and 2019, respectively. While the MOP2 and MOP3 which produced from Manfaloty were showed the lowest level of fruit weight (110.0 and 134 & 125 and 141.7) in the first and second seasons, respectively

Aril weight ranged from 81.25 to 211gm of the two studied seasons, in the progenies of pomegranate used in study. During first season 2018 the highest aril weight (211 & 162.4 gm) was recorded on TOP2 progeny which produced from Tahrir Open pollination and MN3 progeny which produced from hybrids between Manfaloty X Nab El-Gamal, respectively, in the second season the progenies TOP2 (210.5) and TOP3 and TOP5 (159.1) were recorded the highest value of aril weight respectively. On the contrary, MOP2 and MOP3 progeny recorded the lowest value of aril weight (82.5 and 81.25 & 103.2 and 92.08) in the first and second seasons, respectively in both seasons, respectively.

Also results in **Table (5)** and **Fig. (1)** showed that MSP3 and MN2 progenies revealed the highest average of peel weight were (116.7 and 123.0 & 117.4 and 114.6) in the first and second seasons, respectively. while lowest average of the peel weight was (27.50 and 30.82 gm) in the first and second seasons, respectively and that obtained by MOP2 progeny.

Table (5): Fruit weight (g), artil weight (g) and peel weight(g) of twenty progenies of pomegranate used in the study during 2018,2019 seasons.

Progeny	Progen y code	Fruitw	eight (g)	Artil w	eight (g)	Peel weight (g)		
		2018	2019	2018	2019	2018	2019	
Manfalouty	MOP1	200.0ef	201.7fg	120.0 ef	121.0 ^{fh}	80.00 ef	80.67 df	
open	MOP2	110.0 ⁱ	134.0 ^h	82.50h	103.2 ^{ij}	27.50 ⁱ	30.82 ^j	
pollinated	MOP3	125.0 ^{hi}	141.7 ^h	81.25h	92.08j	43.75 ^h	49.58 ⁱ	
	MOP4	193.3 fg	230.0ce	125.7 df	151.8bd	67.67 fg	78.20 eg	
	MPO5	241.7 bd	239.3 ^{be}	140.2 ^{bf}	141.2ce	101.5 bc	98.13°	
Manfalouty	MSP1	155.7 学	178.3	93.40户	107.0 ^{hj}	62.27 #	71.33 fh	
self-	MSP2	187.0 fg	247.7be	115.9fg	153.6bc	71.06 eg	94.11°	
pollinated	MSP3	248.3 bc	247.7be	131.6 ^{cf}	131.3 %	116.7 ab	117.4ª	
	MSP4	220.3cf	218.7ef	116.8eg	116.8帥	103.6 bc	101.9 bc	
	MSP5	186.7 fg	190.0 #	119.5 ^{ef}	121.6 ^{fh}	67.20 fg	68.40 m	
Tahrir open	TOP1	203.3 df	202.3 fg	142.3be	141.6 ^{be}	61.00 #	60.70h	
pollinated	TOP2	293.0ª	292.3ª	211.0ª	210.5ª	82.04 ef	81.85 df	
	TOP3	260.3 ac	256.7 bc	161.4 ^b	159.1 ^b	98.93 cd	97.53°	
	TOP4	240.0be	237.7 be	156.0bc	154.5bc	84.00 de	83.18 de	
	TOP5	260.0 ac	256.7 bc	161.20	159.1 ^b	98.80 cd	97.53°	
Manfalouty	MN1	260.0 ac	261.0 0	150.8bd	150.8bd	109.2 ac	110.2 ab	
♀ X Nab	MN2	273.3 ab	254.7 bd	150.3bd	140.1 ^{cd}	123.0ª	114.6ª	
El-Gamal	MN3	270.7 ab	226.3 df	162.40	135.8df	106.9 ac	90.53 cd	
S	MN4	271.0 ab	266.0 b	154.5bc	151.6 ^{bd}	116.5 ab	114.4ª	
	MN5	237.7be	247.7 be	130.7cf	146.1 ^{be}	106.9ac	101.5 bc	

* MOP: Manfaloty Open pollination. MSP : Manfaloty Selfing pollination. TOP: Tahrir Open pollination and MN: Hybrid Manfaloty X Nab El-Gamal

****** The different letters (in the same column) represent statistically significant differences between progenies (p<0.05).

Data in **Table (6)** showed that, there were significant differences of fruit physical characters such as fruit length, fruit diameter and No. of fruit chambers among all progenies of pomegranate used in study during 2018 ,2019 seasons. Regarding to fruit length (mm) data indicated that, the Tahrir opened (TOP2) recorded the highest value (65.33 and 65.67 mm) in both seasons 2018 and 2019, respectively compared to other progenies. Meanwhile, the progeny of Manfalouty selfed (MSP5) recorded the lowest value of fruit length (49.67 and 49.00 mm) in both seasons 2018 and 2019, respectively. However, no significant differences were observed between progenies at the 1st season 2018 contrary the 2nd season 2019.

On the other hand statistical analysis in **Table (6)** revealed that Tahrir opened (TOP2) gave the highest fruit diameter (99.67 and 96.00 mm) in both seasons 2018and 2019, while the lowest fruit diameter was recorded by progeny of Manfalouty selfed (MSP5) (81.00 and 84.67 mm) for two seasons under study.

In addition, **Table (6)** and **Figs (1)** showed that the average number of fruit chambers ranged from 6.66 to 4.33 of progenies obtained from different cross combinations. The highest significant mean was (6.67) in both season , and that obtained by the MOP4 progeny which produced from Manfaloty Open pollination. On the other hand, the lowest significant mean in the first season was (4.33) that obtained by the MOP2 progeny, while in second season the lowest significant mean was (4.67) and was achieved with MN4 progeny which obtained from Hybrid Manfaloty X Nab El-Gamal and MOP2 produced from Manfaioty Open pollination.

According to **Ismail** *et al.* (2014) [15] research in Egypt, fruit weight for the Nab El-Gamal and Assuity cultivars ranged between 479.4 and 185 g, while fruit volume for the Nab El-Gamal and Wardi cultivars was highest at 416.6 cm3 and lowest (214.8 cm3), respectively.

Table (6) : No. of fruit chambers, Fruit length (mm) and peel weight Fruit diameter (mm) of twenty progenies of pomegranate used in the study during 2018,2019 seasons.

Progeny	Progen y code	Fruit ler	igth (mm)	Fruit dian	neter (mM)	No. of fruit chambers		
	Contraction of the	2018	2019	2018	2019	2018	2019	
Manfalouty	MOP1	56.33ª	52.33 ^m	87.33bd	83.00 bc	5.00bd	5.33 ac	
open	MOP2	54.00ª	52.33单	92.00 ad	88.33 ac	4.33 ^d	5.67 ℃	
pollinated	MOP3	56.00ª	54.67dg	91.33 ad	87.00 ac	5.00 bd	5.00bc	
-	MOP4	57.67ª	56.00 g	95.00 ab	91.33 ab	6.67ª	6.67ª	
	MPO5	61.33ª	57.67bc	94.00 ac	89.67 ac	5.33ad	5.33 ac	
Manfalouty	MSP1	56.67ª	54.33dg	94.00 ac	90.33 ac	5.33ad	5.00 bc	
self-	MSP2	60.67ª	58.00be	95.67 ab	91.33 ab	5.67 ad	5.67 ac	
pollinated	MSP3	58.00ª	57.33bg	96.33 ab	92.67 ab	5.00 bd	6.33 ab	
	MSP4	53.00ª	53.33 ^{eh}	99.00ª	94.67ª	4.67 ^{cd}	4.67°	
	MSP5	49.67ª	49.00h	84.67 ^d	81.00°	5.67 ^{ad}	5.67 ac	
Tahrir open	TOP1	54.33ª	52.67 ^m	85.33 cd	81.00°	5.67 ad	5.67 ac	
pollinated	TOP2	65.33ª	65.67ª	99.673	96.00ª	5.67 ad	5.67 ℃	
	TOP3	60.00ª	59.33bd	95.33 ab	91.00 ab	5.00bd	5.33 ac	
	TOP4	55.00ª	54.67dg	92.67ad	89.00 ac	5.67 ad	5.67 ac	
	TOP5	59.33ª	58.67bd	95.67 ab	91.33 ab	5.33 ad	5.33 ac	
Manfalouty	MN1	60.67ª	62.00 ^{ab}	95.00 ab	91.33 ab	5.00bd	4.67°	
♀ X Nab	MN2	59.67ª	58.00be	93.67 ad	89.33 ac	6.00 ac	5.00 bc	
El-Gamal	MN3	58.00ª	57.33bg	92.67 ad	89.00 ac	6.33 ab	6.00 ac	
5	MN4	58.67ª	57.00bg	98.33ª	94.00ª	6.00 ac	4.67°	
	MN5	57.00ª	57.67 bf	94.00 ac	90.33 ac	5.67 bd	5.67 ac	

* MO

P: Manfaloty Open pollination. MSP : Manfaloty Selfing pollination. TOP: Tahrir Open pollination and MN: Hybrid Manfaloty X Nab El-Gamal

** The different letters (in the same column) represent statistically significant differences between progenies (p<0.05).

The results in **Table** (7) and **Figs.(1)** showed that clear significant differences were found between in all progenies of pomegranate used in study during 2018 ,2019 seasons. The lowest average of number of Percentage of arils % (edible part) was (53.00%) in both season that achieved with MSP3 progeny which obtained from Manfaloty Selfing pollination compared to other progenies. while, the highest average of Percentage of arils % was (75.00% and 77.00%) in the first and second seasons, respectively that obtained by MOP2 progeny.

Significant differences existed for the percentages of T.S.S. in the two seasons 2018 and 2019. Regarding T.S.S ratio, the progeny of Manfalouty Selfing pollination (MSP2) gave the highest value in two seasons (14.93 and 15.43), respectively followed by the progeny of hybrid between Manfalouty X Nab El-Gamal (MN5) was (14.87 and 15.37), respectively. Manfalouty selfed progeny (MSP5) had the lowest value in the two seasons (11.50 and 12.00), respectively. Gowda *et al.* (2009) [12] observed that in additional investigations conducted in Egypt, Manfalouty cultivar fruits had the greatest significant total soluble solids (15.80°Brix), whereas fruits of the Araby cultivar yielded the lowest total soluble solids (14.63°Brix). Additionally, SSC content for the Hegazy cultivar ranged from 16.01°Brix to 12.55°Brix, according to Ismail *et al.* (2014) [15] in Egypt.

The Juice volume (ml/ 100 g) for all progenies is presented in **Table (7)**. Tahrir open pollinated progenies (TOP2 and TOP3) showed the highest Juice volume (123.7 and 126.0 & 121.3 and 123.7) in the first and second seasons, respectively. while lowest average of the Juice volume was (76.33 and 74.00 ml in the first and second seasons, respectively that obtained by MOP1 progeny

Table (7) :Percentage of arils % (edible part), T.S.S and Juice volume (ml/ 100 g) of twenty progenies of pomegranate used in the study during 2018,2019 seasons.

Progeny	Progen y code		Percentage of arils (%)(edible part)		S.S	Juice volume (ml/ 100 g		
	112	2018	2019	2018	2019	2018	2019	
Manfalouty	MOP1	60.00s	60.00h	13.17 ^{de}	13.67 ^{de}	76.33 ^f	74.00 ^f	
open	MOP2	75.00ª	77.00ª	14.33 ad	14.83 ad	104.3 b	102.0 ^b	
pollinated	MOP3	65.00 ^d	65.00°	14.00 ae	14.50 ae	95.67 bd	93.33 bd	
	MOP4	65.00 ^d	66.00 ^d	13.50be	14.00 be	103.0 b	100.7 ^b	
	MPO5	58.00 ^h	59.00 ⁱ	13.77 ae	14.27 ae	104.3 ^b	102.0 0	
Manfalouty	MSP1	60.00≞	60.00h	13.50 be	14.00 be	92.33 ce	90.00 ce	
self-	MSP2	62.00f	62.00 #	14.93ª	15.43ª	101.7 bc	99.33 bc	
pollinated	MSP3	53.00 ^k	53.00 ⁿ	13.67 ae	14.17 ae	84.33 ef	82.00 ef	
	MSP4	53.00 k	53.40m	13.67 ae	14.17 ae	79.67 ^f	77.33 ^f	
	MSP5	64.00°	64.00 ^f	11.50f	12.00 ^f	80.33 ^f	78.00 ^f	
Tahrir open	TOP1	70.00°	70.00¢	12.83 e	13.33 °	84.33 ef	82.00 ef	
pollinated	TOP2	72.00 ^b	72.00 %	14.87 ae	14.75 ae	123.7ª	121.3ª	
	TOP3	62.00f	62.00 #	14.77 ac	15.27 ac	126.0ª	123.7ª	
	TOP4	65.00 ^d	65.00°	13.00 de	13.50 de	91.33 de	89.00 de	
	TOP5	62.00 ^f	62.00 5	14.37 ad	14.87 ad	99.67 bc	97.33 bd	
Manfalouty	MN1	58.00 ^h	57.78j	13.37 ce	13.87 cd	92.00 de	89.67 de	
♀ X Nab	MN2	55.00j	55.00 ¹	13.23 de	13.60 de	85.33 ef	83.00 ef	
El-Gamal	MN3	60.00 E	60.00h	13.83 ae	14.33 ae	85.33 ef	83.00 ef	
3	MN4	57.00 ⁱ	57.00k	13.10 de	13.60 de	81.33 ^f	79.00 ^f	
	MN5	55.00j	5900 ¹	14.87 ab	15.37 ab	97.33 bd	95.00 bd	

* MOP: Manfaloty Open pollination. MSP : Manfaloty Selfing pollination. TOP: Tahrir Open pollination and MN: Hybrid Manfaloty X Nab El-Gamal

** The different letters (in the same column) represent statistically significant differences between progenies (p<0.05).

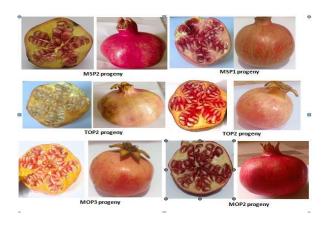


Figure (1) : Pomegranate fruits of some resulting progenies under study (shape, color, artil and no. of fruit chambers). MOP: Manfaloty Open pollination. MSP: Manfaloty Selfing pollination. TOP: Tahrir Open pollination.

Evaluation of Yield components Characteristics : The results in **Table (8)** showed that clear significant differences were found in the average of number of fruits per tree and yield (kg) per tree between different progenies of pomegranate used in the present study during 2018 ,2019 seasons. No. of fruit /tree ranged from 36 to 303. Progeny of TOP3 produced the highest number of fruits / tree 290 and 303 followed by TOP4 and TOP1 (250 and 248) in the first and second seasons, respectively. On the contrary, MSP3 progeny recorded the lowest fruit number / tree (50 and 36) in both seasons, respectively

The yield / tree varied from 7.65 to 78.98 kg. Progenies (TOP3, TOP4, TOP2, TOP1 and Top5) which produced from Tahrir Open pollination were recorded highest average of yield per (78.88 and 78.98 & 59.53 and 60,06 & 49.51 nd 50.77 & 47.79 and 48.50 & 47.58 and 48.08)) in the first and second seasons, respectively. While progenies (MSP3 and MSP5) which produced from Manfaloty Selfing pollination were recorded lowest average of yield per tree (8.934 and 14.51 & 7.650 and 12.03) in the first and second seasons, respectively. Progenies (MN2 ,MN4 and MN5) which obtained from hybrids between Manfaloty X Nab El-Gamal gave the intermediate yield in both experimental seasons. Generally, data indicated differences and significant variations in the yield of pomegranate progenies under study .These results are in line with some of investigators[1,7,8,12].

Table (8) Number of fruits/tree, yield kg /tree of twenty progenies of pomegranate used in this study during 2018, 2019 seasons.

Progeny	Progeny code	No. of	fruits/tree	Yield	kg /tree	
	2000000.0008	2018	2019	2018	2019	
Manfalouty open	MOP1	81.673	75.00 °	15.01 ^{sh}	16.079	
pollinated	MOP2	105.0 ⁱ	135.0 ¹	14.86 sh	18.72	
	MOP3	151.7 ^f	200.04	24.99f	29.23 8	
	MOP4	175.0°	184.0°	35.54 *	42.89 4	
	MPO5	100.0 ⁱ	49.00 2	11.84 si	15.12 9	
Manfalouty self-	MSP1	190.0 ^d	200.04	31.14 ef	36.59*	
pollinated	MSP2	95.67 ⁱ	87.33 m	16.34 #	24.27 5	
	MSP3	55.00	36.00*	8.93 %	14.51	
	MSP4	150.0f	160.0 #	35.26 **	35.86*	
	MSP5	50.00 ¹	41.00 9	7.651	12.03 j	
Tahrir open	TOP1	221.0°	235.0°	47.79°	48.50°	
pollinated	TOP2	161.0 ^f	169.0 ^f	49.51°	50.77°	
	TOP3	290.0ª	303.0ª	78.88ª	78.98ª	
	TOP4	250.05	248.0 5	59.53°	60.06 %	
	TOP5	116.3 ^h	183.0°	47.58°	48.08°	
Manfalouty Q X Nab	MN1	120.05	152.0 ^h	39.50 4	40.88 4	
El-Gamal o	MN2	103.0 ^{bi}	113.0 ×	30.87 ef	30.21 fa	
	MN3	61.67k	60.00°	16.24 5	15.21 9	
	MN4	84.67j	100.0 ¹	27.11 ^f	28.64 =	
	MN5	137.0g	125.0 ^j	29.73 ef	33.80 ed	

* MOP: Manfaloty Open pollination. MSP : Manfaloty Selfing pollination. TOP: Tahrir Open pollination and MN: Hybrid Manfaloty X Nab El-Gamal

** The different letters (in the same column) represent statistically significant differences between progenies (p<0.05).

Start Codon Targeted (SCoT) Polymorphism analysis: The SCoT analysis was performed on seventy seven DNA samples represent the three original cultivars of pomegranate (Manfalouty, Tahrir and Nab El-Gamal) and four selected progenies produced by S1 progenies between parental genotype using seven primers (SCoT1, SCoT2, SCoT3, SCoT5, SCoT8, SCoT9 and SCoT11). Table (9) and Fig. (2) showed the results where data revealed that total number of 31 SCoT amplified fragment with DNA molecular size ranged from 175 to 980 bp was recorded. The main number of generated fragments per primer was 4.43 fragments. The total number of recorded polymorphic bands was 15 bands this represents a level of polymorphism of (48.39%). While total number of monomorphic bands was 16 bands represents a level of (51.61%) monomorphism.

Table (9): SCoT fragments analysis with 7 primers of the DNA of three original cultivars of pomegranate (Manfalouty, Tahrir and Nab El-Gamal) and four selected progenies produced by F1 hybrids between those parental genotypes.

Primer	Total	Length	Monomorphic	Polymorphic	Unique	Polymorphie
Name	Band	range bp	Band	band	Band	%
SCoT 1	6	175 - 540	2	4	2	66.66%
SCoT 2	6	200 - 780	2	4	-	66.66%
SCoT 3	4	225-470	3	1	5	25%
SCoT 5	3	240-365	2	1	1	33.33%
SCoT 8	3	235 -360	3	2	-	
SCoT 9	4	270 - 500	3	1		25%
SCoT 11	5	250 - 980	1	4	1	80%
Total	31	175 - 980	16	15	4	48.38%

Similarity index (total primers): The similarity indices (Si) between the three cultivars of pomegranate (Manfalouty, Tahrir and Nab El-Gamal) and four selected progenies of F1 which produced by hybrids between parental genotype, based on the DNA fragment generated by using seven random primers SCoT1, SCoT2, SCoT3, SCoT5, SCoT8, SCoT9 and SCoT11 presented in **Table (10).** UPGMA cluster analysis (**Fig. 3**) showed that Tahrir cultivar and MOP2 progeny which produced from Open pollination of Manfalouty clustered together and showed high similarity (Si = 0.963).

Table (10): Similarity index as percentage (pairwise comparison) among the three cultivars of pomegranate (Manfalouty "M", Nab El-Gamal "N" and Tahrir "T") and four selected progenies (MOP2, MOP1, M x Ns, TOP2).

Similarity Matrix	м	N	Т	MOP2	MSP1	M x N5	TOP2
м	1.000				2		
N	0.926	1.000				-	
T	0.943	0.945	1.000				
MOP2	0.906	0.945	0.963	1.000			
MSP1	0.833	0.880	0.898	0.898	1.000		
M xN5	0.880	0.885	0.902	0.902	0.957	1.000	-
TOP2	0.756	0.723	0.739	0.739	0.780	0.837	1.000

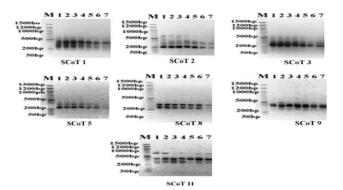


Figure (2): SCoT patterns of the 7 pomegranate genotypes revealed by 7 primers SCoT-1, SCoT-2, SCoT-3, SCoT-5, SCoT-8, SCoT-9, and SCoT-11. M: 100 bp DNA ladder marker, 1: Manfalouty, 2: Nab El-Gamal, 3: Tahrir, 4, 5,6 and 7 lanes for (MOP2, MSP1, MNP5 and TOP2 progenies)

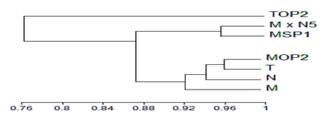


Figure (3): Dendrogram for . three cultivars of pomegranate (Manfalouty, Tahrir and Nab El-Gamal) and four selected progenies produced by F1 hybrids between parental genotype or their S1 selfing. Accessions constructed from the SCoT data using unweighted pair-group arithmetic (UPGMA) and similarity matrices computed according to Dice coefficient.

Regarding the genetic relationship between pomegranate cultivars under studies highest value of similarity index was (0.945) occurred between Tahrir and Nab El-Gamal cultivars while the lowest value of similarity index (0.926) occurred between Manfalouty and Nab El-Gamal cultivars. The similarity index Si= 0.943 was recorded between Manfalouty and Tahrir cultivars. Data in Table (10) and UPGMA cluster analysis in Fig. (2). showed that the MxN5 and MSP1 progenies of F1 nearly clustered together and showed similarity Si= 0.957. The TOP2 progeny which produced from open pollination of tahrir recorded the lowest percentage of similarity index with the parental genotypes with similarity index of (0.756, 0.723 and 0.739 for Manfalouty , Nab El-Gamal and Tahrir cultivars respectively) also the same progeny achieved the lowest value of similarity index with other genotypes so was separated in a single branch on the dendrogram. While The MOP2 progeny which produced from open pollination of Manfalouty recorded the highest percentage of similarity index with the parental genotypes with similarity index of (0.906, 0.945 and 0.963 for Manfalouty, Nab El-Gamal and Tahrir cultivars respectively).

4. Conclusion Finally we can conclude from our result that, The pomegranate progenies under the present study were widely differed in their growth, yield and physiochemical properties as well as the genetic structure which was obtained by SCoT analysis.Variations among these progenies could be mainly due to their genetically and adaptability differences.Additionally, there avialability for selecting the best hybrids from these programs with highly adapted strains to

Egyptian conditions with wide ranges of ripening dates to meet orders of local and international markets.

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