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Phenotypic and Genotypic Characterization of a New Pomegranate (*Punica granatum* L.) Genotype Grown under Assiut Governorate Conditions in Egypt

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Abstract: The pomegranate (*Punica granatum* L.), a species of the *Lythraceae* family, is one of the most significant fruits in Mediterranean and subtropical regions. The key concern for the future of the pomegranate industry is therefore the development of new genotypes of pomegranates that are resistant and yield fruits with increased market demand. In this regard, a new promising pomegranate genotype was selected. This study aims to investigate the use of morphological, chemical, and molecular techniques to characterize a new genotype of pomegranate and evaluate it in comparison to the leading commercial pomegranate varieties in Egypt, Manfalouty and Assiuty. In addition to appreciating the genetic relationship between the new genotype and the two varieties present in Egypt. The studied genotypes displayed marked variability in the weight and shape of the fruits, as well as in the weight of the arils and juice yield. The new genotype (G) recorded the highest values in fruit weight, fruit yield kg/tree, juice percentage, TSS (%) content, total sugars, and anthocyanin in peel and juice. Our results confirm the effectiveness of SCoT and chloroplast cpDNA markers for assessing and identifying pomegranate genotypes. The obtained results showed that the chloroplast cpDNA marker had a relative higher level of polymorphism (72.6%) than that revealed by the SCoT marker (23.82%), and both markers are useful for identification and genetic variability analysis of pomegranate cultivars.

Key words: Pomegranate – New- Genotype- cpDNA - SCoT – Marker.

INTRODUCTION

The pomegranate (*Punica granatum* L.), a species of the *Lythraceae* family and one of the most important fruits in Mediterranean and subtropical regions. The northern Indian Himalayas and Iran are its native habitats. It was first cultivated in the Mediterranean regions of Asia, Africa, and Europe in ancient times. According to **Sarkhosh *et al.*, (2009)**, it is currently widely grown in Spain, Egypt, Russia, France, China, Japan, and the United States. The suitability of fruits for fresh

consumption depends on a variety of quality characteristics pertaining to their physio-chemical and mechanical features, such as the color of the peel, the absence of physical flaws, the amount of sugar, acidity, and flavor, among others. The arils, which are the edible part of the pomegranate fruit, are primarily consumed directly as a salad or table fruit or used in various industry products like juices and canned beverages, including alcoholic beverages, jellies, and jams, and they are also used for the flavoring, coloring, and medicinal effects of drinks (**Preece and Moersfelder, 2016**). Pomegranate export markets required larger fruits with big, juicy arils, red skin and arils, soft seeds, a balanced flavor, medium peel thickness, high levels of health benefits, long shelf life, and fruits without breaking or sunburn (**Holland and Bar-Ya'akov, 2009**). Due to unclear descriptions or phenotypic changes brought on by the environment, morphological traits frequently fail to clearly distinguish distinct cultivars (**Kumar, 1999**). Therefore, it is critical to use molecular markers for more accurate genotype and cultivar identification. For genetic study and characterization, a variety of marker systems have been used, including morphological, cytological, biochemical, and DNA markers (**Kumar, 1999; Gupta and Rustgi, 2004**). Different DNA markers have been used to examine the genetic variety of pomegranate cultivars, highlight how they relate to one another, and demonstrate genetic fingerprinting. These markers include RAPD (randomly amplified polymorphic DNA) (**Noormohammadi *et al.*, 2012**); inter simple sequence repeats (ISSR) (**Eldessoky *et al.*, 2017**); AFLP (amplified fragment length polymorphism) (**Sezai *et al.*, 2010**); simple sequence repeats (SSR), by **Patil *et al.* (2020)**; chloroplast, by **Chen *et al.* (2023)**, and Start Codon Targeted polymorphism marker (SCoT) by **Ahmed (2018)**. The SCoT technique has been successful in identifying cultivars and analyzing genetic diversity within and between plant species in many different plant species, including crop plants (**Collard & Mackill, 2009**), such as barley (**Dora *et al.*, 2017**), and potato (**Gorji *et al.*, 2011**), as well as fruit trees such as mango (**Luo *et al.*, 2010**), grapes (**Guo *et al.*, 2012**), and date palm (**Saboori *et al.*, 2020**). Many pomegranate varieties are well adapted and grown under Egyptian environmental conditions. Two of them are concentrated in the Assiut governorate and used in Egypt's production, and these variations vary somewhat in response to market and consumer demands. Despite Egypt's favorable environmental conditions for pomegranate production, exports are thought to be more limited than in other countries due to low fruit quality brought on by physiological defects like cracked fruit, sunburns, and a lack of internal coloring (**Gawish *et al.*, 2015**). The key concern for the future of the pomegranate industry is therefore the development of new genotypes of pomegranates that are resistant and yield fruits with increased market demand. In this study, a new promising pomegranate genotype was selected after a preliminary survey in the field between Manfalouty and Assiuty cultivars, which are considered among the most important pomegranate cultivars in Egypt. This new pomegranate genotype is successfully grown in Egypt and possesses desirable traits like resistance to cracking and sunburn, high-quality coloring in the peel and aril, softness of the seeds, and other crucial traits that will improve the quality of the fruits and prepare for their inclusion in the Egyptian pomegranate breeding and improvement program, which might encourage an increase in pomegranate production. The primary goal of this research is to investigate the use of an integrated morphological, chemical, and molecular technique to characterize the new genotype of pomegranate and evaluate it in comparison to the two cultivars, Manfalouty and Assiuty. In addition to appreciating the genetic relationship between the new genotype and the widely used varieties present in Egypt.

MATERIALS AND METHODS

The three genotypes of pomegranate (*Punica granatum* L.) used in this study during the 2022 and 2023 seasons were Manfalouty (Mn), Assiuty (As), which are the most widely used commercial cultivar in Assiut, Egypt, and the new promising genotype (G) (Fig. 1). The chosen trees were of the same age (15 years old), and they were cultivated in a private farm orchard on Assiut Road in Sahel Selim, Egypt, which is around 31 kilometres away from Assiut. Trees are spaced 5 × 5 m apart. The soil texture is heavy clay, and flood irrigation using Nile water was used. According to the Ministry of Agriculture's and Reclamation lands' guidelines, the trees were grown in the same orchard and exposed to the same common horticultural practices.

According to **Jackson (1967)**, the physical parameters of the experimental soil and the chemical characteristics of the irrigation water were determined and listed in Table (1). The three previously described cultivars were used in a completely randomized design over a period of two seasons, with nine trees per replication for each cultivar. The twenty-seven trees that were chosen for this investigation were strong, nearly uniform, and healthy. Each tree under study had twenty shoots labeled in various directions. The following attributes were examined:

Vegetative growth parameters: Tree height (m), canopy circumference (m), number of new shoots/ tree, shoot length (cm), shoot thickness (cm), number of leaves per shoot, and leaf area (cm^2).

Flowering: The date of the beginning of flowering and the date of full blooming were noted. At the bloom stage, the total number of flowers per tree, the number of perfect flowers per tree, and the number of male flowers were computed. The sex ratio was also calculated.

Fruiting and yield: The total number of fruits per tree was estimated. The number of sunburned and cracked fruits per tree and the ratio to the total number of fruits per tree were estimated. The average total yield per tree was then determined as kg/tree, and the marketable yield per tree was then determined.

Fruit quality characteristics: Randomly chosen samples of fruits (n=15) from each genotype were examined for the following physical and chemical characteristics:

Fruit physical properties: Fruit weight (g) was recorded along with measurements of the fruit's length and diameter (cm). Selected fruits were then manually peeled, their rind and capillary membranes (the non-edible part) were separated and weighed. A digital caliper was used to measure the peel thickness (cm) at the equator with 0.01 mm accuracy.

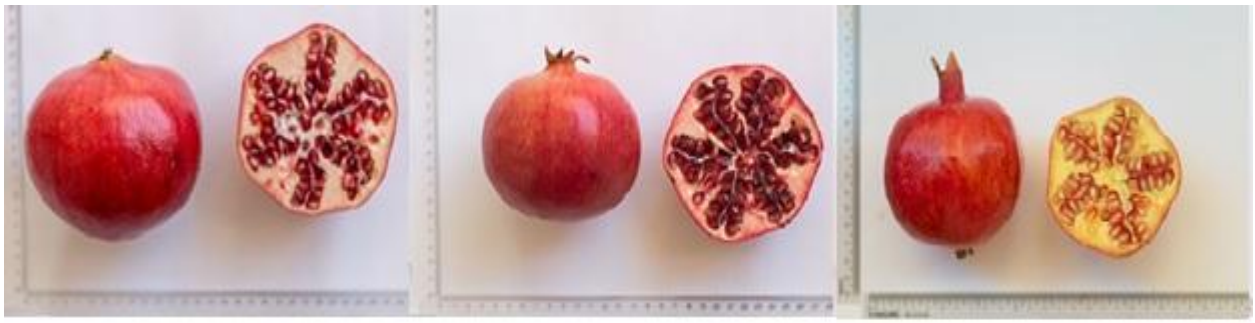
Biochemical composition of fruit juice: Total soluble solids percentage (T.S.S.%) and total titratable acidity (TTA%) were calculated using a hand refractometer and **A.O.A.C. (2005)**, respectively. The TSS/acidity ratio was computed by dividing TSS (%) by acidity (%). Total sugars (%) were determined utilizing the techniques outlined by **Dubois *et al.* (1956)**. The amount of total anthocyanin (mg/100 mL) was calculated in accordance with **Hsia *et al.* (1965)**. Fruit juice was evaluated for total tannins (%) using the Winton and Winton (1945) technique. The amount of vitamin (C) as ascorbic acid (mg) per 100 ml was calculated using **A.O.A.C. (2005).** Tannic acid was used as a standard in the measurement of the total phenolic (TP) content using a Folin-Ciocalteu reagent at 765 nm (**Singleton *et al.*, 1965**). According to **Hmid *et al*. (2013)**, the total flavonoid content of pomegranate fruit was assessed spectrophotometrically as a standard. The process is based on the combination of flavonoids with aluminium to generate a complex, and the amount of flavonoids was given as milligrams of rutin equivalent per liter of juice. The antioxidant activity was evaluated using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method in accordance with **Chen *et al.* (2008)** methods. The antioxidant activity was reported as a percentage drop in absorbance when compared to the control in order to represent the percentage of DPPH that was scavenged.

Statistical Analysis

All collected data were subjected to statistical analysis as described by **Snedecor and Cochran (1973)**. The significance among means was tested using LSD method according to **Walter and Duncan (1969).** Using the UPGMA clustering method and "IBM SPSS Statistics" for Windows (version 25), the generated similarity matrices were used to build a dendrogram tree among the cultivars under study.

Table (1). Physical and chemical soil properties of the experimental field

A- Physical analysis										
Sand (%)		Silt (%)		Clay (%)		Soil texture		F.C. (%)	W.P. (%)	A.W. (%)
17.7		29.1		53.2		Clay loamy		42.5	21.2	20.1
B- Chemical analysis										
Soluble anions (meq/l)				Soluble cations (meq/l)				PH 1:2.5	EC (ds/m)	SP
CO_3^-	HCO_3^-	Cl^-	SO_4^{-2}	Ca^{++}	Mg^{++}	Na^+	K^+			
-	3.65	4.0	2.56	2.97	2.73	4.20	0.31	7.75	1.0	67.5
C-Available nutrients (mg/kg)										
	N	P	K	Fe	Zn	Mn	Cu	E.C. ds/m	pH (1: 25)	$CaCO_3$
Total	677	340	452.5	3156	113	146	47	3.71	7.8	3.6
Avail.	63	13.7	61.2	21.1	5.7	16.6	2.6			



New genotype Manfalouty Assiuty

Fig. (1). Variation in fruit characteristics of the three genotypes: new genotype (G), Manfalouty (Mn), and Assiuty (As).

Isolation of genomic DNA and PCR amplification conditions

Fresh leaves were used to extract total genomic DNA using a modified version of **Doyle and Doyle's (1987)**, Cetyl trimethyl ammonium bromide (CTAB) technique. Eleven SCoT primers were applied to amplify regions that frame the ATG start codon in various pomegranate genotypes **(Collard and Mackill, 2009**), and amplified the universal primer pairs for the trnL-trnF region (C + D, E+F Primers) and psbA- trnH regions in accordance with **Taberlet *et al*. (1991)** and **Hamilton (1999)** recommendations (Table 2). The genomic DNA (20 ng), 0.5 U of Taq DNA polymerase, 2 X PCR buffer, 1.5 mM MgCl2, 0.2 mM of each dNTP, and 0.2 M of each primer were used in a 20µL polymerase chain reaction (PCR) for SCoT amplification. The following method was used for amplifying up the reactions in a Techne thermocycler (Bio-Rad, USA): 5 minutes at 95 °C, 40 cycles of 1 minute and 30 seconds each at 94 °C, 1 minute and 30 seconds at 48 -58 °C, and 2 minutes at 72 °C, with a final cycle of 12 minutes at 72 °C. All PCR products were visualized on a 2 % agarose gel. Under UV illumination, the DNA fragments were visualized and captured on camera using the gel documentation device.

Table (2). Characteristics of the tested primers

No.	Code primers	Sequence (5´- 3´)	GC%
1	SCoT1	ACG ACA TGG CGA CCA CGC	67
2	SCoT2	CAACAATGGCTACCACCC	56
3	SCoT3	ACG ACA TGG CGA CCC ACA	61
4	SCoT4	ACC ATG GCT ACC ACC GCA	61
5	SCOT5	CAACAATGGCTACCACGA	50
6	SCoT8	CAACAATGGCTACCACGT	50
7	SCoT10	ACAATGGCTACCACCAGC	50
8	SCoT11	AAGCAATGGCTACCACCA	50
9	SCoT12	ACGACATGGCGACCAACG	56
10	SCoT13	ACGACATGGCGACCATCG	61
11	SCoT14	ACGACATGGCGACCACGC	56
12	C	CGAAATCGGTAGACGCTACG	55
13	D	ATTTGAACTGGTGACACGAG	45
14	E	GGTTCAAGTCCCTCTATCCC	55
15	F	ATTTGAACTGGTGACACGAG	45
16	psbA-trnH F	GTTATGCATGAACGTAATGCTC	41
17	psbA-trnH R	CGCGCATGGTGGATTCACAATCC	56

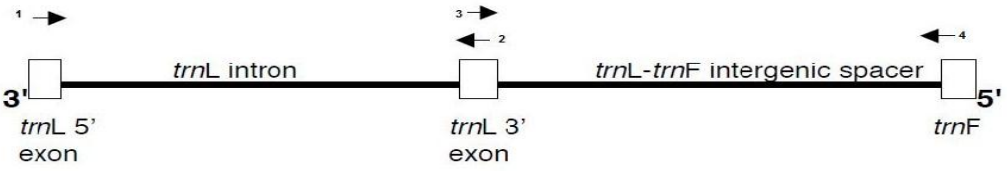


Fig. (2). Approximate location of trnL-F primers used in this study.

RESULTS AND DISCUSSION

Vegetative growth characteristics

The data in Table (3) manifested significant differences among genotypes in most measurements of vegetative growth. Data analysis also showed significant differences in the highest tree, which had a maximum in (G) and a minimum in (As) in the two seasons. Statistical analysis revealed that significant differences occurred in the trunk circumference and the number of shoots/tree among the studied genotypes, with (G) scoring the maximum value. The longest shoots were scored by (G), followed by (As) genotypes, while the lowest average of shoot length was noticed with (Mn) on both seasons. Data analysis also showed significant differences in shoot thickness among genotypes, with (G) being significantly superior for both seasons. Moreover, (Mn) exhibited the lowest average shoot thickness in both seasons. There was a variation noticed in the number of leaves/shoot, which was maximum in (G) in the two seasons and minimum in (Mn) in the two seasons. For the two seasons, the (G) genotype had the highest values for leaf area (8.34 and 8.93 cm2), whereas the (Mn) genotype had the lowest values (7.60 and 7.60 cm^2 for both seasons, respectively).

Table (3). Vegetative growth characters of the three pomegranate cultivars during two seasons (2022 & 2023)

Genotypes	Tree height (m)		Tree shade circumference (m)		No. of new shoot/tree		Shoot length (cm)		Shoot thickness (cm)		No. of leaves/shoot		leaf area (cm^2)	
	Season		Season		Season		Season		Season		Season		Season	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
G	3.70 a	3.82 a	5.73 a	6.03 a	35.33 a	38.33 a	56.67 a	56.67 a	0.91 a	0.93 a	83.70 a	93.70 a	8.34 a	8.93 a
Mn	3.33 b	3.48 b	5.23 b	5.50 b	25.00 c	30.33 b	44.67 c	44.67 c	0.61 c	0.66 b	60.00 c	76.33 c	7.60 c	7.60 c
As	2.88 c	3.05 c	4.33 c	4.60 c	30.00 b	37.67 a	51.67 b	51.67 b	0.82b	0.88 a	73.00 b	86.33 b	7.92b	8.33 b
L.S.D at 5 %	0.17	0.17	0.11	0.10	2.84	2.21	2.55	2.55	0.084	0.06	5.77	5.46	0.21	0.20

Means in the same column with the same letter have no significant difference (P<0.05)

Data presented in Table (4) display significant variations across the genotypes regarding the start of flowering, for the genotypes of pomegranates under study. It was found that genotype (As) started flowering the earliest for both seasons (20 and 25 of March, respectively), followed by genotype (G), which started flowering at (10 and 15 of April, respectively), during both seasons, and earlier than genotype (Mn), which started flowering at (25 and 28 of April, respectively), in the two studied seasons. The complete blooming data showed that during both of the examined seasons, genotype (As) was the earliest at 15 and 20 of April, respectively, followed by genotype (G) that had late blooming at 5 and 10 of May, respectively. **Patil *et al.* (2018)** reported that, to identify the floral form and its entire characteristics, initially, we need to be familiar with the flower bloom Period/Time and it is defined as a particular period in which a flower shows its floral developmental characteristics.

Table (4). The beginning of flowering and full blooming dates of the three studied pomegranate genotypes under Assiut governorate conditions among the two seasons (2022 & 2023)

Genotypes	The beginning of flowering		Full blooming	
	1st Season	2nd Season	1st Season	2nd Season
G	10/4/ 2022	15/4/ 2023	5/5/ 2022	10/5/ 2023
Mn	25/4/ 2022	28/4/ 2023	20/5/ 2022	25/5/ 2023
As	20/3/ 2022	25/3/ 2023	15/4/ 2022	20/4/ 2023

Flowering and fruit set percentage

The data in Table (5) showed significant differences among genotypes in most of the flowering characteristics of the three studied pomegranate genotypes. Meanwhile (Mn) achieved the highest number of male flowers per tree (425.0 and 386.7) in both seasons, respectively, while (As) gave the lowest range of male flowers per tree (323.3 and 290.0) during the two studied seasons. On the other hand, the variance in the means of perfect flower /tree among genotypes was significant, with (G) recording the largest one (424.7 and 459.7) and (As) giving the lowest value (325.7 and 360.0) in both seasons, respectively. Data analysis also showed significant differences in the number of flowers per tree among genotypes, with (Mn) being significantly superior (834 and 816) for both seasons. Moreover, (As) exhibited the lowest number of flowers per tree (649.0 and 650.0) in both seasons. The sex ratio percentage of (G) genotype had the highest sex ratio percentage with (0.533 and 0.575) in both seasons, respectively, while (Mn) exhibited the lower sex ratio percentage with (0.526) in second season.

Table (5). Flowering characteristics of the three studied pomegranate genotypes under Assiut governorate conditions among the two seasons (2022 & 2023)

Genotypes	No. of male flower/ tree		No. of perfect flower /tree		No. of flower /tree		Sex ratio (%)	
	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season
G	378.0b	340.0b	424.7a	459.7a	802.7b	799.7b	0.533a	0.575a
Mn	425.0a	386.7a	409.0b	429.3b	834.0a	816.0a	0.490b	0.526c
As	323.3c	290.0c	325.7c	360.0c	649.0c	650.0c	0.502b	0.554b
L.S.D at 5 %	8.288	8.481	6.686	6.885	12.07	12.25	0.0188	0.0215

Means in the same column with the same letter have no significant difference (P<0.05)

Pomegranates are distinguished by having both functionally male and hermaphroditic bisexual flowers on the same tree (**Madlen *et al.*, 2011**). Functional andromonoecy, as it is known, can lead to lower yields since male flowers are unable to produce fruit. The scientific literature on many key pomegranate development and physiological elements, including a thorough understanding of male and female flowering, is still lacking (**Hazel, 2011**). This is despite the crop having been produced as an agricultural crop since antiquity. The "fertile" and "bisexual" names for the hermaphroditic flowers relate to their well-formed male (filaments and anthers) and female (stigma, style, ovary) parts. Although somewhat inaccurate, hermaphroditic flowers are frequently referred to as "female" flowers because they are the variety that produces fruit. On closer inspection, the pistil of the male flowers reveals diminished female components but well-developed male parts. As a result, their function is more correctly portrayed as that of functionally male flowers (because flowers are not exclusively male but instead have degraded feminine elements). Male blooms usually fall off and don't produce fruit. The ratio of male and female flowers in an andromonoecious plant can vary depending on the season, plant age, location within the plant, and environment (Holland et al., 2009). Data in Table (6) declare the characteristics of mature fruits produced by the three studied genotypes. Statistical analysis revealed that significant differences were recorded in fruit weight and size. Genotype (G) recorded the highest values in fruit weight (440.7 and 456 g), while (As) recorded the lowest values (344.7 and 347.3 g) in both seasons, respectively. Regarding the fruit diameter, genotype (G) gave the highest value (9.24 and 9.36 cm) in the two seasons, respectively. The lowest value was recorded by (As) in both seasons and (Mn) in the second season. In respect to fruit length, significant differences were recorded between the three genotypes; the highest values of fruit length were recorded by genotype (G) (8.19 and 8.42 cm), while genotype (As) gave the lowest value for fruit length (7.82 and 7.58 cm) among the two seasons, respectively. On the other hand, the (As) gave the highest value for fruit peel weight percentage (37.10%) in the two seasons, but the lowest value was (29.77) in the (G) genotype. Moreover, (As) was higher on fruit peel thickness by (0.767 and 0.730 cm) than (G) and (Mn) genotypes among the two seasons, respectively.

The findings demonstrated an elevated relationship between fruit weight and both fruit dimension (length and diameter) and fruit components (peel total weight, aril total weight, hundred aril dry weight, and juice percentage). Thus demonstrating that selection for larger fruits will result in juicy fruits with greater total aril weight and vice versa. Fruits with thick peels may be able to withstand peel splits. The fruits are shielded from pests and viruses that enter the fruits through those cracks by a thick skin (peel) enclosing the edible arils (**Jalikop *et al.*, 2005**). The most important criterion employed throughout the sorting procedure is fruit weight. The typical weight of pomegranate fruit meant for export markets is between 275 and 325 g (**Najan, 2014**). High diversity in fruit size and rind was also discovered by **Nafees *et al*. (2015)**.

Table (6). Fruit physical characteristics of the three studied pomegranate genotypes, Promising (G), Manfalouty (Mn), and Assiuty (As), under Assiut governorate conditions during the two seasons (2022&2023)

Genotypes	Fruit weight (g)		Fruit diameter (cm)		Fruit length (cm)		Fruit peel weight (%)		Fruit peel thickness (cm)	
	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season
G	440.7a	456.3a	9.24a	9.36a	8.19a	8.42a	29.77c	29.96c	0.560b	0.593b
Mn	416.3b	423.7b	8.99c	9.09ab	8.03b	8.12b	31.13b	31.35b	0.597b	0.580b
As	344.7c	347.3c	8.47b	8.55b	7.82c	7.58c	37.10a	37.10a	0.767a	0.730a
L.S.D at 5 %	6.445	6.501	0.622	0.542	0.158	0.198	0.555	0.546	0.122	0.110

Means in the same column with the same letter have no significant difference (P<0.05)

As shown by the data in Table (7), genotype (G) produced the highest fruits per tree in the first and second seasons, respectively, with 409.7 and 441.3, followed by (Mn) with 373.3 and 402.7. While (As) produced the fewer fruits per tree (296.3 and 344.3 fruit per tree, respectively) in both seasons. The maximum marketable number of fruits per tree was (G) genotype, whose range was between 404.3 and 430.7, followed by (Mn) (313.0 and 346.0), and (As) produced the minimum fruit number per tree (246.7 and 295.9 in both seasons, respectively). The data show significant differences among the three genotypes in the marketable number of fruits per tree. The cracking fruit/tree's range was at the lowest level on (G), between 3.33 and 7.67. On the other side, the greatest number of cracking fruits per tree (35.00 and 40.67, respectively) were produced in (Mn) throughout both seasons. Genotype (G) exhibits the highest capacity for resisting sunburns on fruit and trees, which was the minimum (2.00 and 3.00) in both seasons, respectively. In contrast to (As), which has the greatest amount of sunburns on fruit and trees (22.67 and 20.00) in both seasons, respectively, and (Mn) was (25.33) in the first season only. The highest fruit yield kg/tree was scored by genotype (G) (180.5 and 201.4), while genotype (As) gave the lowest yield (102.2 and 118.6) in both seasons, respectively. Genotype (G) also produced the maximum marketable yield kg/tree (178.3 and 196.5), followed by (Mn) (130.3 and 146.6), while genotype (As) produced the lowest yield (85.5 and 102.7) in both seasons.

Table (7). Yield and fruit quality of the three studied pomegranate genotypes, Assiuty (As), Manfalouty (Mn), and a new genotype (G), under Assiut governorate conditions during the 2022 and 2023 seasons

Genotypes	No. fruit per tree (total fruit)		Marketable fruit / tree		Cracking fruit/tree		Sun burns fruit/tree		Yield kg /tree		Marketable yield kg/tree	
	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season
G	409.7 a	441.3 a	404.3 a	430.7 a	3.33 c	7.67 c	2.00 c	3.00 c	180.5 a	201.4 a	178.3 a	196.5 a
Mn	373.3 b	402.7 b	313.0 b	346.0 b	35.00 a	40.67 a	25.33 a	16.00 b	155.4 b	170.6 b	130.3 b	146.6 b
As	296.3 c	344.3 c	246.3 c	295.9 c	27.00 b	28.33 b	22.67 a	20.00 a	102.2 c	118.6 c	85.5 c	102.7 c
L.S.D at 5 %	9.168	8.746	8.009	8.086	3.481	3.702	3.319	3.351	4.655	4.697	4.564	4.570

Means in the same column with the same letter have no significant difference (P<0.05)

The findings in Fig. (3) showed that clear and significant differences were found between all genotypes of pomegranate used in the study during the two seasons. The greatest juice percentage across all genotypes was genotype (G) in the first and second seasons (49.9 and 49.35 %), respectively. While the lower average juice percentages for (Mn) and (As) in the first and second seasons were (45.67 and 46.04%) and (56.60 and 44.65%), respectively. To determine the juice's organoleptic quality, total soluble solids (TSS) must be determined. All of the varieties examined showed TSS values higher than the minimal threshold typically required for commercial usage (>12%). The maximum value of soluble solids TSS (%) content was obtained with G (19.13 and 20.37). While (As) recorded the minimum values (15.18 and 14.71), respectively, in the two seasons.

Our results were better than those found in earlier research on several Apulian (from 13.60 to 18.00), Spanish (from 15.10 to 17.70), and Californian (from 14.90 to 16.80) pomegranate genotypes (**Alcaraz-Mármol *et al*., 2017, Chater, *et al*., 2018**), but this can be a result of the weather and the harvesting period. These results are consistent with those **Martinez *et al.* (2006)** reported on various Spanish cultivars, whose TSS ranged from 12.36 to 16.32 °Brix. While **Akbarpour et al. (2009)** reported that TSS in 12 Iranian cultivars ranged from 15.17 to 22.03 °Brix.

In terms of total sugars, genotype (G) reported the highest values in both seasons (14.67 and 15.37 mg/100 ml juice, respectively). However, (As) genotype scored the lowest significant values during the two seasons (10.82 and 11.14 mg/100 ml juice), respectively. In respect to reducing sugars, genotype (G) gave the highest significant value during two seasons (13.85 and 14.51 mg/100 ml juice), respectively. Meantime, (As) genotype gave the lowest significant value during two seasons (10.02 and 10.39 mg/100 ml juice), respectively. The opposite was observed for non-reducing sugars; Mn recorded the highest significant value (0.877 and 0.967 mg/100 ml juice), respectively, during two seasons. (As) gave the lowest significant value during the second season.

According to data in Fig. 3 on acidity percentage, the (G) had the lowest significant percentage through the two seasons (0.92 and 0.90%, respectively), which fall under the category of "sweet cultivars" (acidity 0.9%), according to **Evreinoff (1957).** While the (As) cultivar had the highest percentage (1.56 and 1.52%, respectively) in the two seasons. Additionally, the results disclosed that (G) had fewer tannins (2.06 and 2.01) than the genotypes (As) and (Mn), which had, respectively, 2.49, 2.49, 2.48, and 2.42. Anthocyanins, which are phenolic compounds that give peel and juice their red color. In both seasons, (G) generated the highest levels of anthocyanin in peel and juice, while (As) in peel and juice had the lowest values in both seasons (0.173 and 0.180 & 0.397 and 0.387, respectively). In both seasons, phenols were higher in genotype (G) than in (As) and (Mn). In terms of the antioxidant value of juice, genotype (G) reported the highest values in both seasons. However, the (As) genotype scored the lowest values during the two seasons, respectively. Pomegranate juice is thought to be one of the best sources of antioxidants among fruit nectars (**Elfalleh *et al*., 2011**). According to **Melgarejo *et al.* (2011)**, pomegranate nectar is a reasonably good source of mineral elements. Due to epidemiological studies linking consumption of antioxidant-rich diets with lowered risks of cancer and heart disease, there is currently a lot of interest among consumers and researchers in phenolic and their free radical scavenging properties (**Pourreza, 2013**). The kind of cultivar has a significant impact on the pomegranate juice's antioxidant potential and phenolic component makeup. In order to develop fruit processing industries and choose the best desirable pomegranate genotypes for bringing into commercial cultivation, the results provide significant information about the composition of polyphenols and antioxidant capacity of pomegranate cultivars (**Hmid *et al*., 2017**).

The amount of vitamin C as mg ascorbic acid/100 ml of fruit juice exhibited a modest variation, reaching a maximum of 12.17 by (G) and (As) in the second season and (As) in the first season, while a minimum of 9.34 and 9.29 by (Mn) in both seasons, respectively. Regarding customer perception and the commercial angle, the quality qualities of pomegranate fruit are taken into consideration. Shape, size, skin color, aril color, water content, sugar content, and acidity are some characteristics that are more important in this regard (**Sarkhosh *et al*., 2020**). It was discovered that there are many variations in genotypes for these traits, which are mostly impacted by genetics, local environmental factors, and harvest timing. Additionally, the qualities that are most beneficial in establishing the optimal fruit consumption norms of these cultivars in processing sectors are fruit taste, aril color, and seed hardness (**Yuan *et al*., 2018**).

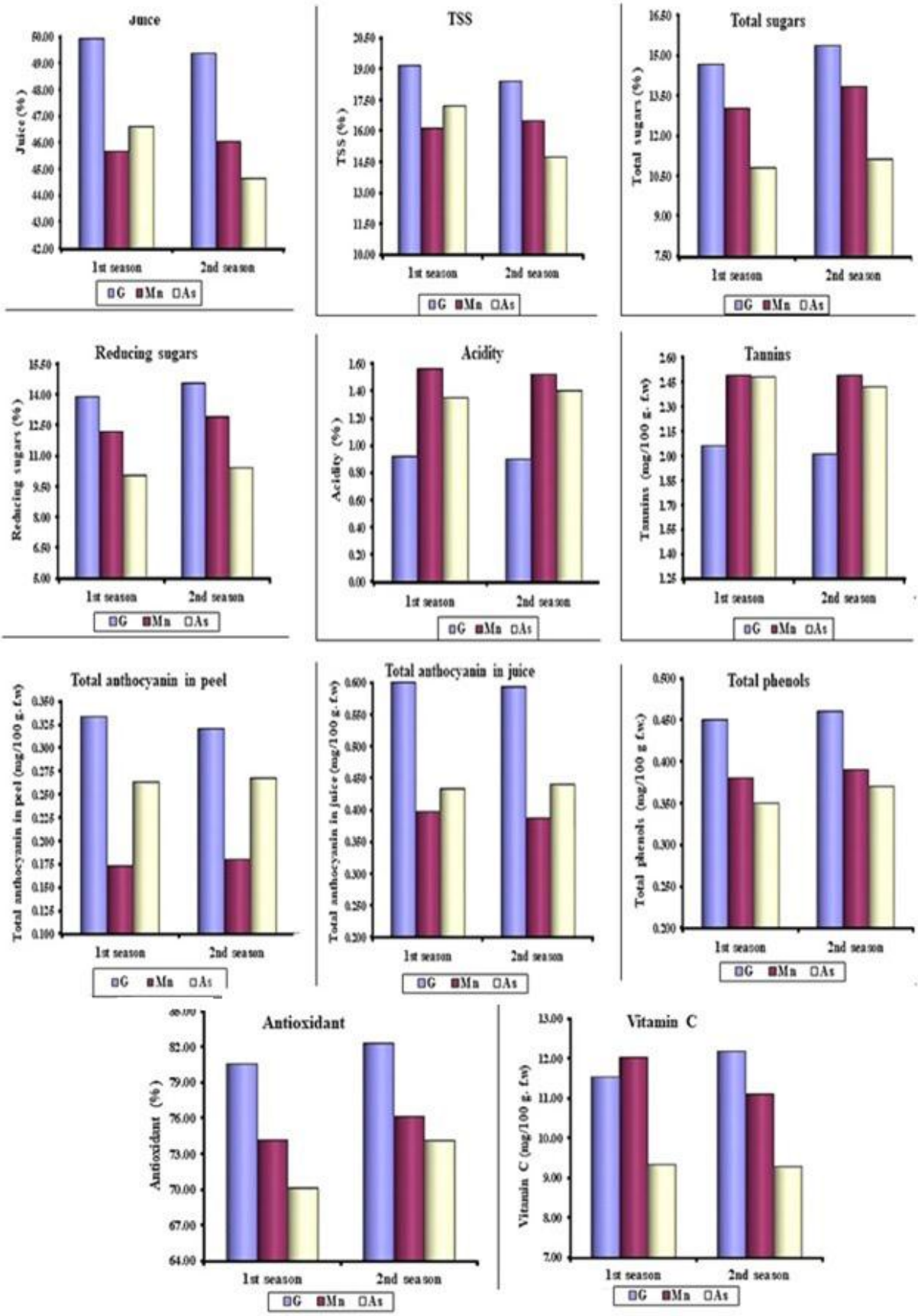


Fig. 3: Fruit juice chemical characteristics of the three studied pomegranate genotypes: promising genotype (G), Manfalouty (Mn), and Assiuty (As) under Assiut governorate conditions during the 2022 & 2023 seasons.

Molecular Analyses

The SCoT pattern analysis reveals that 11 of the 15 primers used produced repeatable and clear amplifications. A total of 118 bands were generated from the three studied pomegranate genotypes, including 24 polymorphic bands (20.3%), with an average of 2.2 bands per primer. The size of these bands ranged from 109 bp. for the SCoT2 primer to 4150 bp. for the SCoT12 primer. The number of bands per primer ranged from 5 for the SCoT10 primer to 17 for the SCoT1 primer, with an average of 10.72 bands per primer. The greatest number of polymorphism bands recorded by the SCoT 12 primer (5 bands) represented 41.6%, showing its greatest efficiency in the detection of polymorphism. While the SCoT2 primer seems to be, the less informative one since it generates 100% monomorphic bands and 0% polymorphic bands (Table 8). It is significant to note that all SCoT primers used except the SCoT2 primer generated unique bands (UNB) among the three tested genotypes, ranging from one unique band (SCoT5, SCoT10, and SCoT14) to the maximum number of (5) bands with the SCoT12 primer (Table 8).

Table (8). Summary of polymorphism generated by SCoT and chloroplast (cpDNA) primers and their amplification results among the three pomegranate genotypes

Primer	Size range (bp.)	TB	PB	MB	PPB	UNB
SCoT1	125-2190	17	3	14	17.6 %	3
SCoT2	109-2170	14	0	14	0%	0
SCoT3	145-2320	18	3	18	16.6%	3
SCoT4	330-2780	11	3	8	27%	3
SCOT5	240-1800	6	1	5	16.6%	1
SCoT8	530-3150	7	3	4	42.8%	3
SCoT10	230-1500	5	1	4	20%	1
SCoT11	350-3300	6	2	4	33%	2
SCoT12	290-4150	12	5	7	41.6%	5
SCoT13	238-2880	9	2	7	22.2%	2
SCoT14	253-2895	13	1	12	7.7%	1
C+D	950-1200	4	3	1	75%	3
E+F	420-800	2	2	0	100%	2
psbA -trnH	110- 1900	7	3	4	42.8%	3

TB: total number of bands, PB: number of polymorphic bands MB = number of monomorphic bands; PPB: percentage of polymorphic bands, UNB: number of unique bands.

For all genotypes under study, the trnL-trnF region was amplified using PCR, yielding repeatable and distinct amplifications. A sequencing issue in the entire trnL-trnF region demanded the use of two primer sets independently. The trnL intron was amplified by a C+D primer, whereas the partial trnL gene and the trnL-trnF regions were amplified by E+F primers (Fig. 2). For the three genotypes, the trnL intron's PCR amplification was effective, producing two bands of various molecular weights and a 75% polymorphism rate. The amplified trnL gene had lengths of 950 and 1200 bp. for G genotype, 960 and 1200 bp. for Mn genotype, and 970 and 1200 bp. for (As) genotype. The PCR amplification of the trnL-trnF region was successfully produced with repeatable and clear amplifications with different molecular weights among the three genotypes; thus, the (G) and (As) genotypes had similar amplified fragments at 420 bp., while the (Mn) cultivar had an amplified trnL-trnF region with 800 bp., which represented a maximum polymorphism rate (100%) (Table 8, Fig. 4). PCR amplification of the psbA -trnH intergenic region was successfully obtained with multiple loci among the studied genotypes, ranging in size from 110 to 1900 bp. where the new (G) genotype characterized by a unique band at 780bp. and the (As) genotype at 1900 bp.

The first step in genetic evaluation is to identify the appropriate molecular markers for genetic fingerprinting. The molecular markers' value in identifying genetic affinity and differentiating cultivars can be tested. In order to genetically fingerprint economically significant plant species, many molecular markers are employed and contrasted; the Punica plant is one such example. Many molecular markers were used to identify pomegranate cultivars, such as the RAPD marker (**Awad *et al.*, 2014**), SSR molecular markers (**Shahsavari *et al.*, 2021**), SCoT markers (**Ahmed, 2018**), and trnH-psbA and matK sequences (**Hajiahmadi *et al.*, 2013**). Data obtained from these investigations illustrate the molecular basis of a novel and promising pomegranate genotype (G) in comparison with two cultivars, Manfalouty and Assiuty, which are grown commercially in Egypt under Assiut governorate conditions.

The current research discussed the variations among the tested genotypes, and if these variations are also accompanied by significant agronomic traits, then plant breeders have a very good source of genetic material for enhancing that target plant (**Gorji *et al.*, 2011, Saboori *et al.*, 2021**). In line with our investigation using SCoT markers, the current study found that SCoT markers are highly effective for demonstrating genetic variability as well as for differentiating the analyzed pomegranate cultivars (**Ajal *et al.*, 2015**). Using SCoT markers, a prior study on twelve pomegranate cultivars grown in Egypt (**Ahmed, 2018**) revealed significant genetic diversity among the cultivars. There is a lot of potential for using these evaluated cultivars, which are commercially farmed in Egypt, in traditional breeding programs to enhance pomegranate qualities.

Due to their simplicity in isolation, purification, characterization, and cloning, cpDNA markers are frequently utilized in phylogenic investigations. Both the psbA-trnH intergenic region and the trnL-F intergenic spacer are non-coding regions of the cpDNA that are more changeable than the coding ones. Studies on the non-coding sections of cpDNA have revealed greater variances and more mutations than those found in the coding areas (**Baldwin *et al.*, 1995**). Therefore, the psbA-trnH intergenic area and the trnL-F intergenic spacer of the cpDNA are more appropriate parameters to examine evolution relationships in the lower taxa (**Bayer *et al.*, 2000** and **Skuza *et al.*, 2020**). According to our PCR experiments, the trnL-F intergenic spacer and psbA-trnH intergenic area were successfully produced with repeatable and clear amplifications with different molecular weights among the three genotypes. As a consequence, it provides a powerful complementary method for studying the genetic relationships and diversity of Punica species.

Phylogenetic relationships among pomegranate genotypes

According to the coefficient of genetic dissimilarity of Jaccard, the phylogenetic relationships among pomegranate genotypes based on each phenotypic and chemical character were determined using the UPGMA computer program. The highest distance (1.00) was recorded between the G and As genotypes, reflecting their genetic dissimilarity (Table 9a), while a low genetic distance (0.00) was estimated between the G and Mn genotypes, reflecting their genetic similarity. The obtained dendrograms divided the three studied genotypes into two groups. The first one grouped the G and Mn genotypes close to each other, while the other group included the As genotype (Fig. 5). The opposite was observed for the neighbor-joining phylogenetic tree based on SCoT, the chloroplast cpDNA dendrogram, and their combined analysis, in which a low genetic distance (0.00) was estimated between the G and As genotypes, reflecting their genetic similarity. The highest distance was recorded between the Mn and As genotypes, reflecting their genetic dissimilarity (Table 9b, Fig. 6). The obtained SCoT and chloroplast cpDNA dendrograms, or their combined data, divided the three studied genotypes into two groups. The first one grouped the G and As genotypes close to each other, while the other group included the Mn genotype (Fig. 6).

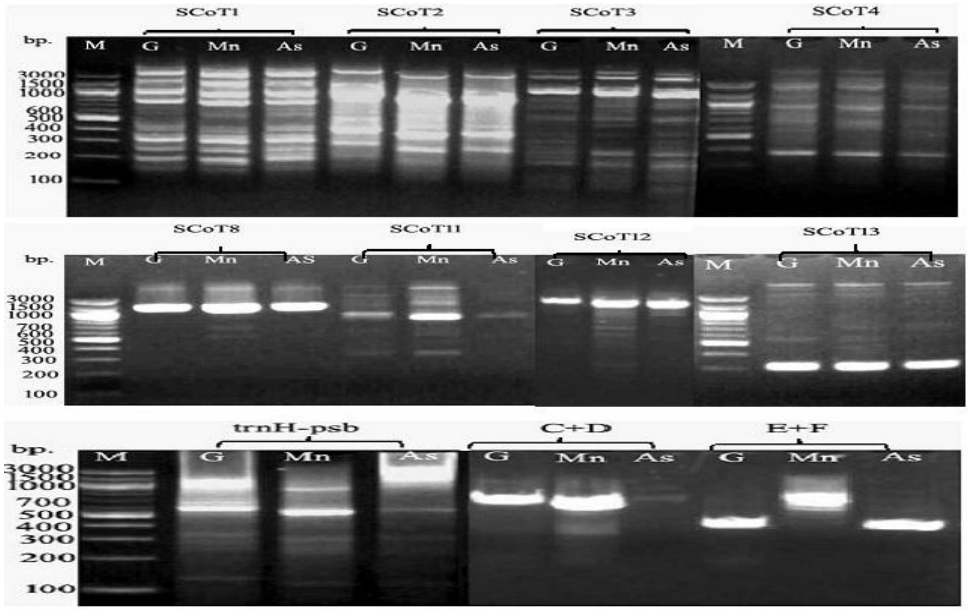


Fig. (4). The SCoT and chloroplast (cpDNA) patterns of the three genotype of pomegranate (*Punica granatum* L.), G: new genotype, Mn: Manfalouty, As: Assiuty; M: 100 bp DNA ladder marker.

It is evident that the clustering based on each phenotypic and chemical feature marker was different from that based on the SCoT, chloroplast cpDNA dendrogram, and their combined analysis. The phenotypic and fruit characteristics analyses allowed us to identify a specific differentiation pattern in which the (G) genotype was closely related to the (Mn) genotype, while the (As) genotype was apart from the group. The diversity pattern of morphological markers and molecular markers may differ. Only the female parent received the chloroplast gene, although both parents contributed to morphology and the environment had an impact on it (**Fitmawati, 2006**). Therefore, clustering analysis using the cpDNA of the studied pomegranate genotypes did not agree with the clusters of morphological markers as reported by **Kostermans and Bompard (1993)**. The phylogenetic tree based on SCoT, the chloroplast cpDNA dendrogram, and their combined analysis divided the three studied genotypes into two groups. The first one grouped the (G) and (As) genotypes close to each other in one cluster; this could be attributed to the closest genetic background, while the other group included the (Mn) genotype.

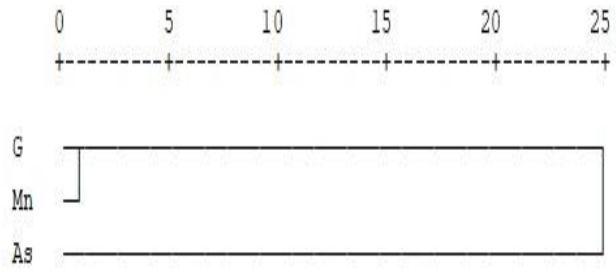


Fig. (5). Phylogenetic tree performed from the three genotypes based on each phenotypic and chemical character using UPGMA and similarity matrices computed according to Dice coefficient.

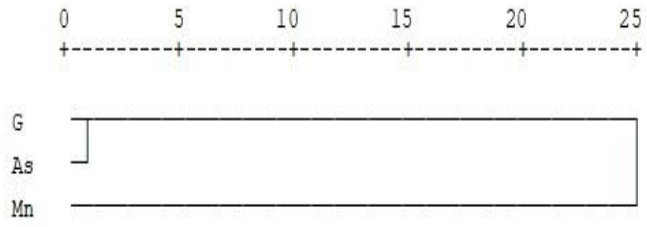


Fig. (6). Dendrogram for the three pomegranate genotypes constructed from the combined data analysis of SCoT and chloroplast DNA using UPGMA and similarity matrices computed according to Dice coefficient.

Table (9). Proximity matrix based on: (a) SCoT and chloroplast (cpDNA) patterns of combined analysis among the three pomegranate genotypes; (b) Phenotypic and chemical character of tested pomegranate genotypes

a

Genotypes	Matrix File Input		
	G	Mn	As
G	.000		
Mn	.000	.000	
As	1.000	.987	.000

b

Genotypes	Matrix File Input		
	G	Mn	As
G	.000		
Mn	.400	.000	
As	.000	1.000	.000

Conclusion

Many pomegranate varieties are well adapted and grown under Egyptian environmental conditions. Two of them are concentrated in the Assiut governorate and used in Egypt's production. Despite Egypt's favorable environmental conditions for pomegranate production, it is believed that exports are less plentiful than in other nations due to inferior fruit quality caused by physiological defects like cracked fruit, sunburns, and lakes of internal coloring. In this study, a new promising pomegranate genotype was selected after a preliminary survey in the field between Manfalouty and Assiuty cultivars, which is considered one of the most important pomegranate cultivars in Egypt. This new pomegranate genotype is successfully grown in Egypt and possesses desirable traits like resistance to cracking and sunburn, high-quality coloring in the peel and aril, soft seeds, and other crucial traits that will improve the quality of the fruits and the readiness to include them in the Egyptian pomegranate breeding and improvement program, which might encourage an increase in pomegranate production.

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