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Phenotypic, Productivity and Molecular Characterizations of Some Jojoba Genotypes Using Chloroplast DNA (cpDNA) Markers

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Abstract: This evaluation study was conducted during two successive seasons (2018 and 2019) on eight females of Jojoba genotypes (*Simmondsia Chinensis* (Link) Schneider) shrubs about 4 years old planted at Horticulture Research Institute- Giza governorate – Egypt. The evaluation includes vegetative growth (shoot length, leaf surface area, growth habit, growth vigor and leaves characters), flowering, seed production/plant, physical properties and seed oil percentage. Molecular studies of selected jojoba genotypes were conducted to determine their genetic diversity based on cpDNA techniques. It can be recommended from this study that F1, F5, F6 and F8 as the strongest genotypes, F7 and F8 as the most productive female as they recorded the maximum seeds yield. The top seed physical properties were shown with genotype (F7). Meanwhile, female shrub (F5) proved the superiority in seeds oil content. Total score for yield; oil; seed weight and fruit density (100 units). Present the general evaluation of different jojoba genotypes, genotypes (F7 and F8) seemed to be the superior genotypes it attained the uppermost score units (90.92 & 86.32 and 95.49 & 86.64 units) in two tested seasons, respectively. The trnL-F intergenic spacer and psbA-trnH intergenic region of cpDNA were amplified from extracted total genomic DNA. The results indicated that these regions served as a powerful tool for the identification of the tested genotypes of jojoba and their genetic relationships.

Key words: Jojoba · Genotypes · Phenotypic · Molecular · Chloroplast (cpDNA)

INTRODUCTION

Jojoba (*Simmondsia chinensis*), is a woody, evergreen, dioecious and perennial shrub. It is growing in desert and semi-desert areas, native to the southwestern United States and northern Mexico. Due to its high economic value, it has been introduced to Egypt in 1991 and cultivated by imported seeds from USA [1]. Jojoba is mainly grown for its seed oil, which represents about (40–60 %), this oil contains a valuable liquid wax that is very close to whale sperm [2]. The oil is being utilized by the cosmetic, pharmaceuticals and other industries for certain formulations [3]. The high demand for wax in the cosmetics industry is one of the factors that has been driving efforts to improve yields and hence profitability. Following a selection program for female cultivars producing large amounts of seeds, there has been a marked expansion of planted areas, particularly in dry lands [4]. Jojoba is considered one of the most practical and scientific solutions for desert plantation in Egypt. Hot summers, warm winters, desert soil and minimal water. Lesser possibilities for infection, lesser need for fertilizers and generous financial income, are certainly most encouraging to plant Jojoba in Egypt [5].

Jojoba can reveal some level of phenotypic variation, which is the ability to develop various phenotypes in response to environmental conditions [6]. Direct selection based on the identification of potential promising genotypes and their testing after vegetative propagation has made it possible to improve crops more quickly. The use of molecular markers is being considered an important step in the use of advanced techniques to

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identify genetic variation or diversity and to evaluate the relationships between and within populations [7]. Few comparative analysis studies at the molecular genetic level have been performed to detect genetic distances between jojoba genotypes occurring in different regions [8]. Even, to date, there are no studies performed using chloroplast DNA (cpDNA) characterization of jojoba genotypes. Chloroplast DNA (cpDNA) variations give higher resolution at the population level than nuclear markers, features which make cpDNA appropriate for comparative genomic studies [9]. The presence of polymorphisms at inter-and intra-specific levels has been revealed by the non-coding chloroplast DNA (cpDNA) region [10]. The trnL-F intergenic spacer and psbA-trnH intergenic region of cpDNA are non-coding characters and this region is more variable than the coding regions. Some research on the non-coding region exhibited high variations and mutations more frequently than that of coding regions [10].

In this paper, morphological, physiological and molecular studies of selected jojoba genotypes were conducted to determine their genetic diversity based on cpDNA techniques. The obtained results can be used to select the best shrubs to yield and the highest amount of seeds contained higher oil content.

MATERIALS AND METHODS

A field experiment was conducted at the Horticultural Research Institute, Agriculture Research Center, Giza- Egypt, during two successive seasons (2018, 2019). The aim was to evaluate eight females (F1 to F8) jojoba elite shrubs (4 years old) which were propagated by seeds and randomly collected from an open grown plantation located within the Horticultural Research Institute. The location is characterized by a semiarid Mediterranean climate of mild-rainy winters and hot-dry summers. These shrubs were growing in sandy soil at the distance of 3 × 1.5 meters and irrigated with drip irrigation system, irrigated twice weekly and received the same amount of water and subjected to the regularly recommended culture practices as well as free from pathogens and physiological disorders.

Soil chemical and physical characteristics and water chemical composition were determined by Soil, Water and Environmental Research Institute, Agric. Res. Center, according to the methods described by Jackson [11] (Tables 1 and 2).

Morphological characteristics, flowering, fruiting and yield as well as seed physical proprieties and genetic diversity based on cpDNA techniques were carried out as follows:

Vegetative growth characters of the shrubs:

- Circumference (m^3): was calculated from the measurements of the height of canopy (H) and it's two cross diameters (D1and D2) [12].

Twelve branches were selected from the mid-level and around of the plants, branches characters were calculated for detailed analysis. On each branch the following data were collected:

- Shoot length (cm)
- Growth habit and natural growth which is divided into: Straight, drooping and spreading according to Shaheen *et al.* [13].
- Growth vigor estimation; it is divided into the following categories: Strong, medium and weak according to Shaheen *et al.* [13].
- Leaves characters: Number of leaves/shoot was determined by number of leaves on the labeled branches and calculates per 1 meter. Leaf surface area (cm^2) was estimated from the following equation: Leaf surface area = (0.717×A) - 0.095, which (A) is the product of length by width [14].

Flowering Estimation: Start of flowering date: was recorded when 10% of flowers were opened for each shrub. Number of flowers/meter was estimated.

Fruit density (%): no. fruits/shoot length * 100
Yield/shrub (seed production kg/tree): at the harvesting date, the average yield for each selected genotype was calculated.

Seed physical proprieties and oil %: Seed length, width (cm) and seed weight (g). Seed oil content was extracted by Soxcelt apparatus according to the method described by Ayerza *et al*., [15].

Numerical Evaluation: Calculated on the basis of 100 units which were divided among the various fruit properties according to Hamed [16] with simple modification as 40 unite for the yield (g/shrub), 30 units, oil %, 15 units for seed weight (g) and 15 units for Fruit density (%) Each genotype that gave the best results in any character was given the full mark specified for this character, while each of the other tested strains took lower units to their qualities.

Table 1: Chemical and physical properties of soil

		Cations (meq/L)				Soluble anions (meq/ L)				
pH 2.5:1	E.C. ds/M (1:5)	Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3-	HCO_3^-	Cl^-	SO_4^-	$CaCo_3$
8.2	1.9	29.6	23.5	88.2	2.7	-	2.3	107.4	34.3	1.30

Carose sand %	Fine sand %	Silt %	Clay %
42	45.10	9.2	3.7

Table 2: The chemical composition of the irrigation water

		Soluble cations (meq/L)				Soluble anions (meq/ L)				
pH 2.5:1	E.C. ds/M (1:5)	Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3-	HCO_3^-	Cl^-	SO_4^-	S.A.R
7.12	1.406	1.8	0.7	2.5	0.2	-	0.29	4.51	0.4	2.23

Table 3: Sequences of the chloroplast trnL-trnF region with primer combinations of (C+D) and (E+F), respectively and trnH-psbA primer

Primer	Sequence 5'-3'
C	CGAAATCGGTAGACGCTACG
D	GGGGATAGAGGGACTTGAAC
E	GGTTCAAGTCCCTCTATCCC
F	ATTTGAACTGGTGACACGAG
trnH-psbA F	GTTATGCATGAACGTAATGCTC
trnH-psbA R	CGCGCATGGTGGATTCACAATCC

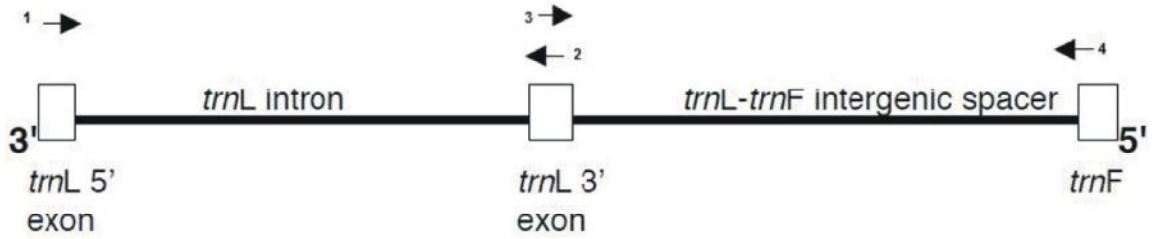

Fig. 1: Approximate location of *trnL-F* primers used in this study

DNA Extraction and Amplification: Total genomic DNA was extracted from young fresh leaves of eight females (F1 to F8) of jojoba strains using the DNeasy plant mini kit (QIAGEN, Germany) according to the manufacturer's instructions. The universal primer pairs for chloroplast trnL-F and trnH-psbA regions were amplified as recommended by Taberlet *et al.* [10] and Hamilton [17] (Table 3 and Fig. 1). PCR reactions were carried out in 25 µl reaction volumes and included 2 µl of genomic DNA, 3 µl of a 10X reaction buffer, 3 µl of 2 mM MgCl2, 3 µl of 1 mM dNTP solution, 3 pmol of each primer and 0.25 U RedTaqTM polymerase (Sigma-Aldrich, St. Louis, USA). The final volume was adjusted with deionized distilled water. The cycling parameters included an initial denaturation step at 96°C for 5 min followed by 35 cycles of 97°C for 45 s, 48°C for 30 s and 72°C for 90 s. A final extension step at 72°C for 5 min completed the reactions. Amplified products were observed on 1.5% agarose gel (1XTBE) with ethidium bromide staining.

Statistical Analysis: The experiment was arranged in a randomized complete blocks design and the obtained data were subjected to analysis of variance (ANOVA) and significant differences among means were determine according to Snedecor and Cochran [18]. In addition significant differences among means were distinguished according to the Duncan multiple test range Duncan [19] at probability of 5 %.

RESULTS AND DISCUSSION

Our results revealed extensive variability among eight female genotypes (probably reflecting the variability in their genetic backgrounds), which we were able to exploit for the identification of female shrubs with above-average reproductive traits.

Vegetative Growth Characters: Based on the analysis of variance of growth analysis characters (Table 4), the results revealed the presence of significant differences in

Table 4: Vegetative growth characters of the female Jojoba genotypes during 2018 and 2019 seasons

	Circumference (m^3)		Shoot length (cm)			
Gen.	1st season	2nd season	1st season	2nd season	Growth habit	Growth vigor
F1	2.99^{C}	5.10A	23.83^{B}	32.56^{C}	Straight	Strong
F2	2.44^{E}	3.50^{D}	18.86^{E}	32.78^{C}	Dropping	Medium
F3	2.72^{D}	3.20^{E}	16.46^{F}	31.33^{D}	Dropping	Medium
F4	3.35^{B}	3.90^{C}	24.85^{B}	27.78^{E}	Dropping	Medium
F5	1.16^{F}	4.50^{B}	30.78^{A}	39.56^{A}	Spread	Strong
F6	3.97^{A}	4.60^{B}	20.83^{D}	37.33^{B}	Spread	Strong
F7	2.82^{CD}	3.90^{C}	22.29^{C}	32.67^{C}	Spread	Medium
F8	3.91^{A}	5.00^{A}	16.35^{F}	39.56^{A}	Straight	Strong

Means followed by the same letter(s) within each column are not significantly different at P 0.05 level using Duncan's Multiple Range Test.

Table 5: Leaves characters of the female Jojoba genotypes during 2018 and 2019 seasons

	Leaves density %		No. leaves/shoot		LSA* (cm^2)	
Genotypes	1st season	2nd season	1st season	2nd season	1st season	2nd season
F1	79.26^{E}	77.94^{C}	18.33^{B}	25.34^{D}	4.67^{H}	4.64^{C}
F2	96.53^{B}	94.51^{B}	17.83^{C}	30.89^{A}	4.83^{G}	5.40^{B}
F3	103.5^{A}	96.82^{A}	16.94^{D}	30.33^{B}	4.99^{F}	6.00^{A}
F4	75.23^{F}	71.27^{D}	18.22^{B}	19.11^{F}	5.16^{E}	3.77^{E}
F5	65.13^{H}	61.23^{F}	19.00^{A}	23.78^{E}	5.33^{D}	4.69^{C}
F6	90.76^{C}	68.05^{E}	16.50^{E}	25.56^{D}	5.50^{C}	3.38^{F}
F7	82.98^{D}	93.36^{B}	17.89^{C}	30.89^{A}	5.67^{B}	4.71^{C}
F8	72.98^{G}	72.63^{D}	12.11^{F}	29.22^{C}	5.84^{A}	4.41^{D}

Means followed by the same letter(s) within each column are not significantly different at P 0.05 level using Duncan's Multiple Range Test. *Leaf surface area.

Fig. 2: Morphological characters of shoots and seeds of eight female jojoba genotypes

circumference (m^3) among eight female jojoba genotypes. Generally, the behavior of the genotypes differed from one to another grown under the same cultural conditions. In this concern, circumference ranged from 1.16 and 3.20 m^3 (F5, F3) to reach 3.97 and 5.10 m^3 (F6, F1) at both seasons, respectively. Regarding shoot length (Table 4 and Fig. 2) it's quite clear that shrubs (F5 in first season) and (F5 and F8 in the second season) had the longest shoot length (30.78, 39.56 and 39.56 cm), respectively. Conversely, the shortest shoots were observed in F3 and F8 genotypes in the first season and F4 in second season. Regarding the growth habit, genotypes F1 and F8 had a straight growth habit, while genotypes F5, F6 and F7 had a spread growth habit whereas genotypes F2, F3 and F4 was dropping growth. It's shown from the concerned data that genotypes F1, F5, F6 an F8 had strong growth, whereas genotypes F2, F3, F4 and F7 had medium growth.

Table 6: Date of flowering, number of flowers/shoot and fruit density (%) of the female Jojoba genotypes during 2018 and 2019 seasons

	Date of flowering		No. flowers/shoot		Fruit density %	
Genotypes	1st season	2nd season	1st season	2nd season	1st season	2nd season
F1	7/2	8/2	3.11^{A}	4.00^{A}	12.86^{B}	12.44^{A}
F2	20/2	18/2	1.69^{D}	3.33^{C}	9.69^{D}	10.22^{C}
F3	18/2	16/2	1.72^{D}	3.56^{B}	10.39^{C}	11.34^{B}
F4	2/2	1/2	2.44^{C}	2.44^{D}	10.49^{C}	9.41^{D}
F5	2/2	1/2	2.69^{B}	3.22^{CD}	9.60^{D}	8.09^{E}
F6	7/2	8/2	2.69^{B}	2.00^{F}	14.67^{A}	5.33^{F}
F7	15/2	14/2	1.78^{D}	3.11^{D}	8.36^{E}	9.57^{D}
F8	2/2	2/2	1.85^{D}	4.11^{A}	10.72^{C}	10.18^{C}

Means followed by the same letter(s) within each column are not significantly different at P 0.05 level using Duncan's Multiple Range Test

Leaves Characters: Data presented in Table 5 and Fig. 2 proved that leaves density (%) of genotype F3 was the highest (103.5 and 96.82 %) in both seasons. However, F5 expressed the lowest values. Regards number of leaves/shoot genotypes F5 (1st season) and F7 (2nd season) had the largest number of leaves/shoot (19.00 and 30.89), respectively. Whereas, F8 (1st season) and F4 (2nd season) were the lowest. The greatest values of leaf surface area were detected with genotype F8 in the first season (5.84 cm^2), while in the second one was obtained in genotype F3 (6 cm^2), whereas F1 and F6 produced leaves with the lowest leaf area.

Al-Soqeer [20] stated that, these significant variations in growth and physiological parameters among the genotypes might reflect partially their different genetic background. Variability in different morphological traits such as plant height, number of branches, number of internodes, leaf shape and area was demonstrated by Esmail *et al.* [21]. Genetic differences among genotypes in plant morphological characters have previously been reported Prat *et al.* [22]; El-Sayed [23] and Hammad *et al.* [24]. Benzioni *et al.* [25] found that, some clones exhibited excellent vegetative traits related to yield potential, such as, rapid growth and extensive branching on Jojoba evaluation. The characterization of growth habit and vigor can be coinciding with the bases of identification, this result in high variability in most characteristics between the different jojoba genotypes [23]. Botti *et al.* [26] and Prat *et al.* [22] asserted that, all leaf parameters showed differences among genotypes were due to genetic variations, could be explained by the natural growth habits and branching of genotypes.

Flowering Estimation: Among the studied 8 female genotypes (Table 6), the date of starting flowering, showed that three female shrubs (F4, F5 and F8) bloomed early (first week of February) and two female genotypes (F1 and F6) were middle bloomers (second week of February) and three strains (F2, F3 and F7) were late bloomed. Presented data also indicated that, number of flowers/shoot showed high variation between the selected female jojoba genotypes and ranged from (1.69) to (4.11) flowers/shoot. F1 in both seasons and F8 in 2nd season recorded the highest number of flowers/shoot. Regarding fruit density %, the 8 female genotypes studied in 2018 and 2019 showed wide variability in the number of fruits / 1m branch, genotypes F6 (1st season) and F1 (2nd season) had the greatest values (14.67 and 12.44). However, F7 (1st season) and F6 (2nd season) had the lowest records in this regard.

Seed Production and Seed Physical Properties: It can be noticed in Table (7) that, there were obvious significant differences in seed production among shrubs. Genotypes F7 and F8 had the potential to produce the highest seed yield/shrub (857.7, 1072.2 and 910.0, 1062.0 g/tree). Meanwhile, Genotype F3 produced the lowest yield (346.7 and 402.6 g/tree). Table (7) and Fig. (3) demonstrated the physical characterization of jojoba seeds of each genotype. It is obvious that genotype F7 surpassed other female genotypes in average seed length, diameter and weight. Moreover, F3 produced the shortest seeds. These data was clear in both seasons of study.

These results can be harmony with Botti *et al.* [26] on Jojoba. The genotypes were not coincident in the year of highest or lowest production, suggesting different responses of the genotype-environment interaction. Also, the average yield varied greatly between years and genotypes [27]. Al-Soqeer [28] noted that, jojoba seed weight ranged from 0.57 to 0.80 g, Ayerza *et al.* [15] reported that seed weight (100 seeds) ranged from 48 to 116 g. High-yielding plants with larger seeds appeal to jojoba plant selectors because larger seeds are easier to harvest and handle [29]. Plant growth, yield and seed oil content and demonstrated that yield per unit area showed high variation between the selected jojoba genotypes and seasons and stated that seed weight ranged from 0.56 to 1.17 g [30].

Table 7: Seed production and seed physical properties of the female Jojoba genotypes during 2018 and 2019 seasons

			Seed					
	Production g/tree		Length (cm)		Diameter (cm)		Weight (g)	
Genotypes	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
F1	411.7^{G}	500.00^{F}	1.41^{C}	1.62^{D}	1.14^{C}	1.06^{C}	1.30^{B}	1.21^{CD}
F2	455.0^{D}	558.6^{D}	1.32^{D}	1.38^{F}	1.17^{C}	1.18^{AB}	1.23^{C}	1.26^{C}
F3	346.7^{H}	402.6^{G}	1.30^{D}	1.32^{G}	1.17^{C}	1.19^{A}	1.11^{D}	1.17^{DE}
F4	416.0^{F}	496.0^{G}	1.64^{C}	1.65^{C}	1.15^{C}	1.01^{D}	1.29^{B}	1.08^{F}
F5	436.7^{E}	541.2^{E}	1.64^{C}	1.67^{C}	1.15^{C}	1.16^{B}	1.28^{B}	1.33^{B}
F6	520.3^{C}	628.0^{C}	1.72^{B}	1.73^{B}	1.21^{B}	1.18^{AB}	1.33^{B}	1.33^{B}
F7	857.7^{B}	1072.2^{A}	1.82^{A}	1.77^{A}	1.24^{A}	1.21^{A}	1.41^{A}	1.49^{A}
F8	910.0^{A}	1062.0^{B}	1.63^{C}	1.52^{E}	1.02^{D}	1.07^{C}	1.13^{D}	1.13^{EF}

Means followed by the same letter(s) within each column are not significantly different at P 0.05 level using Duncan's Multiple Range Test

Fig. 3: Seed physical properties of eight female jojoba genotypes

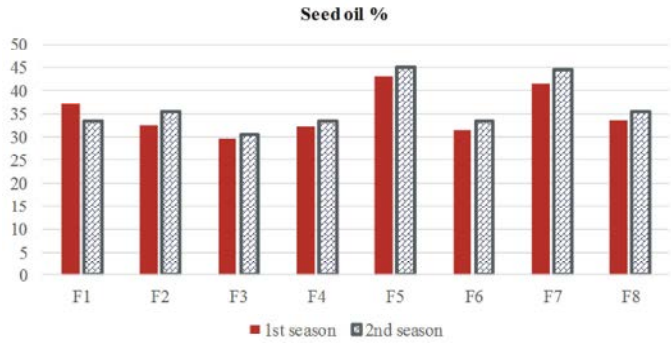

Fig. 4: Seed oil % of eight jojoba genotypes

Seed Oil % of Jojoba Genotypes: The results for seed oil percentage (liquid wax) (Fig. 4) revealed that, significant differences were seen among jojoba genotypes, the genotype F5 and F7 recorded the highest oil percent (43 and 45 %) and (41.5 and 44.5 %) in both seasons, respectively. On the other hand, F3 gave the lowest oil percent (29.70 and 30.5 %) in both seasons, respectively.

These results are in line with this report by Cappillino *et al*. [31]. However, there were discrepancies between the results of the oil content analysis of these genotypes and the results reported earlier by Ayerza *et al.* [15] and Botti *et al.* [26]. These differences in the annual variability in oil production due to the genetic-environment interaction have been reported in other arid zone species such as the olive and the moringa tree (Ayerza, [32] and (Ayerza [33] on jojoba.

Numerical Evaluation

Total Score for Yield and Fruit Quality (100 Unit): Data in Table (9) present the general evaluation of different jojoba genotypes, data cleared that, genotypes (F7 and F8) seemed to be the superior genotypes in yield (g/tree); oil %; seed weight (g) and fruit density (%) as it attained

Table 9: General score evaluation of the eight evaluated jojoba genotypes during 2018 and 2019 seasons

Parameters	Yield	Oil	Seed weight	Fruit density	Total Units
Units specified	40	30	15	15	100
First season					
F1	18.10	22.00	13.83	13.15	67.08
F2	20.00	23.33	13.09	9.91	66.33
F3	15.24	20.00	11.81	10.62	57.67
F4	18.29	22.00	13.72	10.73	64.74
F5	19.20	30.00	13.62	9.82	72.63
F6	22.87	21.33	14.15	15.00	73.35
F7	37.70	29.67	15.00	8.55	90.92
F8	40.00	23.33	12.02	10.96	86.32
Second season					
F1	18.83	25.95	12.18	15.00	71.97
F2	21.04	22.74	12.68	12.32	68.79
F3	15.16	20.72	11.78	12.32	59.99
F4	18.68	22.47	10.87	11.35	63.37
F5	20.38	30.00	13.39	9.75	73.53
F6	23.65	21.98	13.39	6.43	65.45
F7	40.00	28.95	15.00	11.54	95.49
F8	39.62	23.37	11.38	12.27	86.64

the uppermost score units (90.92 & 86.32 and 95.49, 86.64 units) in two tested seasons, respectively. On the other hand, the genotype (F7 and F8) produced higher yields, as well as the genotypes (F5 and F7) recorded higher oil (%) parameter, while the genotype (F7 and F6) surpassed in seed weight (g). While, the genotypes (F6 and F1) took the highest units in fruit density (%), in both evaluation seasons.

The tested genotypes could be arranged descending based on yield and oil % (70 units) as follows: F7 (67.37 and 68.95 unit), F8 (63.33 and 62.99 unit), F5 (49.20 and 50.38 unit), F6 (44.20 and 45.63 unit), F2 & F1 (43.33 and 44.79 unit), F4 and F2 (40.29 and 43.78 unit), F1 & F4 (40.10 and 41.15 unit) and F3 (35.24 and 35.88 unit) during two tested seasons, respectively. The present results are in harmony with those obtained by Hamed [16] and Ibrahim *et al.* [34] on date palm cultivars.

Amplification of DNA Coding for trnL-trnF and psbA-trnH Intergenic Region: The intron of the chloroplast trnL (UAA) gene and the trnL-trnF region were amplified with primer combinations of (C+D) and (E+F), respectively as recommended by Taberlet *et al.* [10] (Table 3 and Fig. 1). The PCR amplification of trnL-trnF region was successfully obtained for all selected genotypes; the samples had PCR products ranged from 450 to 500bp. Meanwhile, PCR amplification of the trnL intron was successfully obtained only for genotypes F1, F2, F5, F6 and F8 and amplification was not successful for the genotypes F3, F4 and F7. The length of the amplified fragments ranged from 700 to 750 bp. among the selected genotypes (Fig. 5).

The trnL-F intergenic spacer of cpDNA is non-coding characters and this region is more variable than the coding regions. The trnL intron and trnL-F spacer have been considered appropriate for investigations at various taxonomic levels. Analyses of non-coding chloroplast DNA sequences have revealed the existence of polymorphisms at the inter-and intra-specific levels [10, 35, 36]. According to our PCR experiments, the trnL intron was inferred to be present in five genotypes and absent in other three selected genotypes. The frequent loss of the intron reported in the parasitic plant Epifagus virginiana [37, 38]. The exact loss of intron from other chloroplast genes have been reported from other chloroplast genes [39, 40]. For instance, three insertions/deletions and three substitutions were observed between the trnL (3' exon) - trnF intergenic spacer region, of Acer pseudoplatanus and Acer platanoides [10]. One possible mechanism for the loss of trnL intron might be genomic deletion and in-frame intron deletion [41].

PCR amplification of psbA-trnH intergenic region was successfully obtained for five female jojoba strains (F4, F5, F6, F7 and F8). The samples had PCR products of about 280bp. Thus, the chloroplast psbA-trnH intergenic region gene is losing from the chloroplast genome of jojoba strains F1, F2 and F3. The failure of psbA-trnH intergenic region amplification in seedling plants may be due to nucleotide variations as single-nucleotide replacements that prevent PCR amplification [42]. The psbA-trnH intergenic region is one of the most variable regions in the angiosperm chloroplast genome. It is a common tool for plant population genetics and

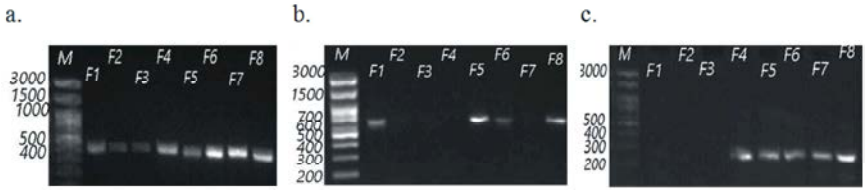

Fig. 5: Agrose gel showing amplified trnL intron (C-D) primer(a), trnL-trnF region (E+F) primer (b) and trnH-psb intergenic region (c) for selected eight jojoba genotypes., Lane M: 100 bp. Ladder.

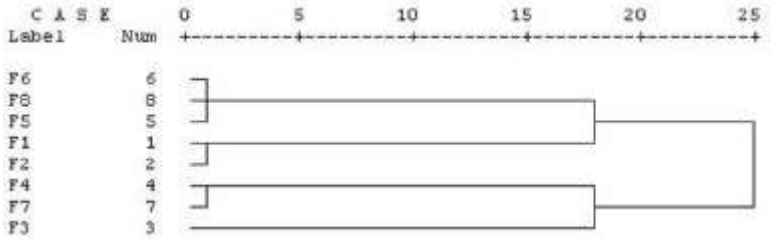

Fig. 6: Dendrogram using average linkage (between groups)

species-level phylogenetics [43]. The psbA gene encodes the D1 reaction center protein of photosystem II. The role of chloroplast psbA UTRs in the regulation of gene expression has been investigated intensively for more than twenty years [44].

Cluster Analysis: The UPGMA cluster analysis of genetic distances generated dendrogram (Fig. 6) which showed the genetic relationship among the selected genotypes (F1 to F8). The analysis illustrated that selected jojoba genotypes fell into two main clusters. The first main cluster was divided into two groups; the first one contains only F3 genotype. However, the second group involved F7 and F4 genotypes. Meanwhile, the second main cluster was divided into two groups: the first one grouped two females (F1) and (F2) and the second group involved F5, F8 and F6 genotypes.

The main hypothesis about how several species or other groups developed from a common ancestor and how they are related to each other is the analysis of a phylogenetic tree. The produced pattern indicated that female strains (F5, F8 and F6) have the same branch and very related to each other. However, strains (F1and F2) are related to the same cluster but with a different branch with the same branch point. The other cluster containing two branches, the first one grouped strains F4 and F7 which are closely related to each other but less related to the strain F3 in the second branch. These results are expected due to the selected jojoba genotypes were clone plants from the same cultivated area as indicated before. These results in agreement with Nahla *et al.* [45], revealed different relationships among the ten female and two male of jojoba genotypes using ISSR based dendrogram.

CONCLUSION

It could be concluded that the tested eight female Jojoba genotypes were significantly different in morphological characteristics, physiological aspects and genetic analysis. These variations are valuable in the breeding program and contribute to the germplasm collection and management of jojoba plants.

REFERENCES

1. Kumar, J., M. Heikrujam and V. Agrawa, 2016. Characterization of Male and Female Jojoba Plants Employing Antioxidants, Lipid Peroxidation and Physiological Studies. Journal of the American Oil Chemists' Society, pp: 1-11.
2. Hogan, I. and W.P. Bemis, 1983. Buffalo gourd and jojoba: Potential new crops for arid lands. Advances in Agronomy, 36: 317-349.
3. Hafeez, Z., S.A.H. Shah, M.I. Shahid and M.A. Bashir, 2020. Asexual propagation of most promising genotypes of jojoba *Simmondsia chinensis*. World Journal of Biology And Biotechnology, 5(2): 43-45.
4. Al-Obaidi, J.R., M.F. Halabi, N.S. Al-Khalifah, S. Asanar, A.A. Al-Soqeer and M.F. Attia, 2017. A review on plant importance, biotechnological aspects and cultivation challenges of jojoba plant. Biol. Res., 50(25): 1-9.
5. El-Mogy, N.S., 1999. Egyptian Experience in Planting Jojoba. Fourth International Water Technology Conference IWTC 99, Alexandria, Egypt, pp: 431-435.

6. Winn, A.A., 1996. Adaptation to fine-grained environmental variation: an analysis of within-individual leaf variation in an annual plant. Evolution, 76: 1569-1580.
7. Yanchuk, A., 2002. The role and implications of biotechnology in forestry. Forest Genetic Resources, 30: 18-22.
8. Bhardwaj, M., S. Uppal, S. Jain, P. Kharb, R. Dhillon and R.K. Jain, 2010. Comparative assessment of ISSR and RAPD marker assys for genetic diversity analysis in jojoba [*Simmondsia chinensis* (Link) Schneider]. J. Plant Bioche. Biotech., 19(2): 255-258.
9. Moon, J., J. Kim and C.S. Jang, 2016. "Development of multiplex PCR for species-specific identification of the Poaceae family based on chloroplast gene, rpoC2." Applied Biological Chemistry, 59(2): 201-207.
10. Taberlet, P., L. Gielly, G. Pautou and J. Bouvet, 1991. Universal primersfor amplification of three non coding regions of chloroplast DNA. Plant Mol. Biol., 17: 1105-1109.
11. Jackson, M.L., 1973. Soil Chemical Analysis", Constable Co., Itd., London, pp: 23-29.
12. International Jojoba Export Council, 2003: C/O sacks Tierrey 4250 North Civic Center B/V d. 4th floor Scottsdale, Arizona 85251-3900.
13. Shaheen, A.S., A.A. Aly and K.B. Eassa, 2010. Evaluation of some female jojoba" *Simmondsia Chinensis* Link" shrubs under south el-tahrir region conditions. J. Plant Prod., Mansoura Univ., 1(12): 1691-1705.
14. Kohorn, L.U., 1994. Shoot Morphology and Reproduction in jojoba: Advantages of Sexual dimorphism. Ecology, 75(8): 2384-2394.
15. Ayerza, R., 1996. Evaluation of eight Jojoba clones for rooting capacity, plant volume, seed yield and wax quality and quantity. Proceedings of the 9th International Conference on Jojoba and its Uses, Catamarca, Argentina, pp: 1-3.
16. Hamed, A.M., 2012. Evaluation of some Arabian date palm cultivars produced through tissue culture grown under Giza condition. Egypt. J. Appl. Sci., 27(4): 144-161.
17. Hamilton, M.B., 1999. Four primer pairs for the amplifiucation of chloroplast intergenic regions with intraspecific variation. Molec. Ecol., 8: 521-523.
18. Snedecor, G.W. and W.G. Cochran, 1972. Statistical methods. 7th ed. Iowa. State Univ. Press, Ames, Iowa, USA. 1980; 507.
19. Duncan, D.B., 1955. Multiple range and multiple F. tests. Biometrics, 11: 1-42.
20. Al-Soqeer, A., 2010. Establishment and early growth of some jojoba clones in Al-Qassim Region. J. Agronomy, 9: 157-162.
21. Esmail, A.E., A.K.G. Genaidy and I.A.A. Atteya, 2016. Increase the economic value of the jojoba (*Simmondsia chinensis)* yield using evaluation of distinctive clones grown under the Egyptian environmental conditions. J. Agri. Tech., 12(1): 145-165.
22. Prat, L., C. Botti and T. Fichet, 2008. Effect of plant growth regulators on floral differentiation and seed production in Jojoba (*Simmondsia chinensis* (Link) Schneider). Ind. Crops Prod., 27: 44-49.
23. El-Sayed, M.E.H., 2010. Studies of some local strains of Jojoba. Ph.D. Thesis, Faculty of Agric., Mansoura University, Egypt.
24. Hammad, A.A., Y.S.G. Abd El-Aziz and H.E. Afifi, 2017. Evaluation and selection of jojoba shrub strains under Ismailia governorate condition. Egypt. J. Appl. Sci., 32(12): 594-614.
25. Benzioni, A., E. Shiloh and M. Ventura, 1999. Yield parameters in young jojoba plants and their relation to actual yield in later years. Ind. Crops Prod., 10: 85-89.
26. Botti, C., L. Prat, D. Palzkill and L. Canaves, 1998. Evaluation of jojoba genotypes grown under water and alkalinity stresses in Chile. Ind. Crops Prod., 9: 39-45.
27. Osman, H.E. and A.A. Abo Hassan, 2013. Introducing jojoba in Arabian Desert: 1. Agronomic performance of nine jojoba clones selected in Makkah area in Northern and Western Saudi Arabia. International Journal of Theoretical and Applied Sciences, 5(1): 37-46.
28. Al-Soqeer, A., 2014. Evaluation of seven jojoba (*Simmondsia chinensis)* clones under Qassim region conditions in Saudi Arabia. Inter. J. Agric. Sci. Res., 3(10): 203-212.
29. Purcell, H.C., T.P. Abbott, R.A. Holser and B.S. Philips, 2000. Simmondsin wax ester levels in 100 high-yielding jojoba genotypes. Ind. Crop Prod., 12: 151-157.
30. Abd El-Mohsen, M.A. and A.M. Abd Allatif, 2015. Selection of some promising jojoba (*Simmondsia chinensis*) genotypes: Evaluation of tree growth and productivity. Egypt. J. Plant Breed., 19(6): 1953-1962.
31. Cappillino, P., R. Kleiman and C. Botti, 2003. Composition of Chilean jojoba seeds. Ind. Crops Prod., 17: 177-182.

32. Ayerza, R., 2011. Seed yield components, oil content and fatty acid composition of two cultivars of moringa (*Moringa oleifera* Lam.) growing in the Arid Chaco of Argentina. Industrial Crops and Products, 33: 389-394.
33. Ayerza, R., 2020. Variability in oil content in Argentine jojoba. Revista Mexicana Ciencias Agrícolas, 11(4): 841-852.
34. Ibrahim, S.G., A. M. Hamed and N.H. El-Gawad, 2014. Comparative study on production of some arabin and local date palm cultivars grown in the desert region at Giza governorate. Egypt. J. Agric. Res., 92(4): 1359-1375.
35. Xu, D.H., J. Abe, A. Kanazawa, J.Y. Gai and Y. Shimamoto, 2001. Identification of sequence variations by PCR RFLP and its application to the evaluation of cpDNA diversity in wild and cultivated soybeans. Theor. Appl. Genet., 102: 683-688.
36. Wang, W.P., C.Y. Hwang, T.P. Lin and S.Y. Hwang, 2003. Historical biogeography and Phylogenetic relationships of the genus Chamaecyparis (Cupressaceae) inferred from chloroplast DNA polymorphism. Plant Syst. Evol., 241: 13-28.
37. Wolfe, K.H., C.W. Modern and J.D. Palmer 1992. Function and evolution of a minimal plastid genome from a nonphotosynthetic parasitic plant. Proc. Natl. Acad. Sci. USA, 89: 10648-10652.
38. Simon, D., D. Fewer, T. Friedl and D. Bhattacharya, 2003. Phylogeny and self splicing ability of the plastid tRNA-Leu group I intron. J. Mol. Evol., 57: 710-720.
39. Jansen, R.K., M.F. Wojciechowski, E. Sanniyasi, S.B. Lee and H. Daniell, 2008. Complete plastid genome sequence of the chickpea (*Cicer arietinum*) and the phylogenetic distribution of rps12 and clpP intron losses among legumes (Leguminosae). Mol. Phylogenet Evol., 48: 1204-1217.
40. Downie, S.R. and J.D. Palmer, 1992. Use of chloroplast DNA rearrangements in reconstructing plant phylogeny. In: Soltis PS, Soltis DE, Doyle JJ (eds) Molecular systematics of plants. Chapman and Hall, New York.
41. Niu, D.K., W.R. Hou and S.W. Li, 2005. mRNA-mediated intron losses: Evidence from extraordinarily large exons. Mol. Biol. Evol., 22: 1475-1481.
42. Bru, D., F. Martin-Laurent and L. Philippot, 2008. Quantification of the detrimental effect of a single primer-template mismatch by realtime PCR using the 16S rRNA gene as an example. Appl. Environ. Microbiol., 74: 1660-1663.
43. Liu, Y., S.X. Yang, P.Z. Ji and L.Z. Gao, 2012. Phylogeography of Camellia taliensis (Theaceae) inferred from chloroplast and nuclear DNA: insights into evolutionary history and conservation. BMC Evol. Biol., 12: 92-105.
44. Zurawski, G., H.J. Bohnert, P.R. Whitfeld and W. Bottomley, 1982. Nucleotide sequence of the gene for the MR 32, 000 thylakoid membrane protein from Spinacia oleracea and Nicotiana debneyi predicts a totally conserved primary translation product of MR 38, 950. Proc. Natl. Acad. Sci., 79: 7699-7703.
45. Nahla, A., S. Adawy, M. Eliraq, A. Abo El-Khasab, Y. Abd El-Aziz, E. El-Bassel, A.S. Esmail, M. Attaia, S.H. Darwish, H. El-Ashry and M.S. Gawish, 2018. Selection and Agronomical Evaluation for Some Elite Genotypes of Jojoba. J. App. Life Sci. Inter., 18(1): 1-12.