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Comparative Study of Some Male and Female Jojoba Genotypes

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Abstract: This investigation was conducted in the experimental orchard of Horticulture Research Institute, Agricultural Research Center, Giza, Egypt. Two experiments was set up to differentiate between male and female Jojoba .The first experiment (I): Identification of the difference between male and female nursery seedlings by using morphological traits. Seeds of jojoba were planted in 2016 and 2017 seasons. The second experiment (II), was carried out during 2019 and 2020 seasons, to study the in morphological and chemical difference between five genotypes of male and female shrubs was studied the vegetative growth, flowering number/m, seed production/plant, oil production/plant for female shrubs were also studied. Sub terminal stem cutting of the evaluated male and female shrubs was planted to study their rooting ability and fresh weight of roots, Leaves chemical analysis and molecular genetic identification between individuals of five males and five females by using RAPD and SCoT-PCR analysis. The results of growing the jojoba seedlings the percentage of male plants was (63.30% & 65.83%) higher than the female (36.7% & 43.16%) in the two seasons, respectively the ratio of male-to-female was nearly of (2:1). The results of comparing adult jojoba male and female characters indicated that the male's plants were superior than females in most of the tested vegetative characters. Moreover vegetative propagation by males stem cuttings achieved higher rooting percentage (46&49%) than females (26 & 29 %) and fresh weight of roots on males was greater than females. The superior male genotype G5 scored highest values of inflorescences/ meter (20.75 & 19.88) in the two studied seasons, female genotype G4 were superior than others in oil production (326.6 & 337.6g) in the two studied seasons . In this study we attempted to characterize molecular genetic identification and detection sex determination between individuals of five males and five females from Jojoba cultivar using two RAPD primers were used to amplify male and female individuals, the two primers were found to have sex specificity in analysis. However, three SCoT primers (SCoT 3, SCoT 7 and SCoT11) were found to have sex specificity for male in analysis. While, Primers (SCoT 2 and SCoT 5) were found to have sex specificity for female individual analysis.

Key words: Jojoba (*Simmondsia chinensis*) · Male · Female · RAPD · SCoT-PCR analysis · Sex determination markers

INTRODUCTION

Jojoba (*Simmondsia chinensis* L.) is a dioecious, perennial shrub that is cultivated for its unique liquid wax, which is similar to sperm whale oil. Jojoba oil maintains viscosity at very high temperatures. Furthermore, it is commonly used as a lubricant and an ingredient for cosmetics, toiletries and livestock feed and pharmaceutical preparations. Jojoba also can be used for afforestation, abatement of desert creep [1]. Being dioecious, a seeded plantation has high seed variability [2]. Males and females differ from one another in morphological, physiological and biochemical traits between sexes; this may be due to differential development [3]. Many previous studies indicated that males and females of some dioecious species differed in their leaf chemical components [4-6]. These physiological differences have been reported in dioecious plants, which showed that males are more tolerant than females under certain conditions such as drought and salinity which alters the ratio between male and female plants. Most studies have focused on mature individuals and

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very little work is reported on cuttings [7, 8]. Similarly, there are no existing morphological trait methods for distinguishing sex at an early age in jojoba [9].

Molecular markers play a significant role in the protection of biodiversity, identification of promising cultivars and quantitative trait loci (QTL) mapping, etc. [10]. DNA markers, which are not prone to environmental influence, can accurately identify sex in dioecious plants, thereby overcoming the need to wait till flowering for sex determination. There are many methods to determine the sex of a plant, including restriction fragment length polymorphisms (RFLP) in Asparagus [11] and random amplified polymorphic DNA (RAPD), with or without sequence characterized amplified regions (SCAR), which has been used extensively in Salix viminalis, microsatellite (GATA) based banding patterns in *Carica papaya* and sex-linked amplified fragment length polymorphism (AFLP) [12-14]. This experiment was set up with an objective to distinguish the differences between jojoba sexes (shrubs and rooted cuttings). RAPD and SCoT markers were employed to analyze genetic diversity in jojoba genotypes belonging to ten different genotypes for sex differences.

MATERIALS AND METHODS

Site Description: This investigation was conducted in the experimental orchard of Horticulture Research Institute, Agricultural Research Center, Giza, Egypt. Two experiments was set up to differentiation between male and female Jojoba traits as follow:

The First Experiment (I): Identification of the Difference Between Male and Female Nursery Seedlings by Using Morphological Traits: Seeds of jojoba were germinated in 2016 and 2017 seasons and treated with 0.50 % Rizolex-T50WP fungicide then were dipping at a depth of 2 cm in plastic bags (16 x 20 cm) filled with a mixture of peat moss and sand (1:4) in a greenhouse at Horticulture Research Institute. The bags were irrigated regularly, about 250 seeds were germinated in August 2016 without any dormancy-breaking, the percentage of germination was (92 %) achieved within a month. So 230 plants were obtained, the same number of seeds were germinated in the second season, the percentage of germination was (90%), hence 225 plants were obtained. About 120 of the nursery seedlings were randomly selected, to identify the percentage of male to female in In both seasons.

The Second Experiment (II): Identification of the Difference Between Male and Female Shrubs by Using Morphological, Vegetative and Chemical Characteristics, Vegetative Propagation and Molecular Diversity Assessment: Five genotypes (G1, G2, G3, G4 and G5) male and Female of each genotype on seven years old that produced from seedy propagation and grown in the experimental orchard in Horticulture Research Institute grown at the distance of 3 × 1.5 m under drip irrigation system and received the same agricultural practices according to the recommendation of Ministry of Agriculture, Egypt, were chosen to compare the difference in morphological and chemical traits qualities between them, vegetative propagation and Molecular diversity assessment of male and female shrubs during 2019 and 2020 seasons as follow. Soil chemical and physical characteristics and water chemical composition were determined by Soil, Water and Environmental Research Institute, Agric. Res. Center (Tables 1 and 2, respectively) according to the methods described by Jackson [15].

The Following Characteristics Were Recorded:

Vegetative Growth and Flowering Characteristics of Jojoba Male and Female Shrubs: In 2019 and 2020 seasons, both selected sexes were evaluated as follows:

Circumference: circumference (m) = $\pi \times 1/2$ (DI ×D2)

D1 and D2 are the two cross diameters of the canopy, we measured shrub geometry using a modification of methods outlined by Mudrak *et al*. [16].

- Shrub height: measured by meter.
- Twelve branches were selected from around the adult shrubs (4 shoots represents a replicate) for estimation of the following data:
- Average shoot length (cm).
- Average number of leaves per meter was counted on the labeled branches. Samples of 30 leaves (The 4^{th} and 5^{th} leaves from the top of the branch) were collected from different directions of genotypes [6].
- Average leaf length "L" (cm), width "W" (cm) and L/W ratio were measured [6].
- Average flowering density: average number of flowers per meter of females and average number of inflorescences per meter of males were estimated at the end of February in 2019 and 2020 seasons.

Table 1: Physical and chemical properties of the soil under study

Property	Value	Property	Value
Sand (%)	27.48	Available micronutrients (mg kg^{-1})	
Silt (%)	34.22	Fe	6.71
Clay (%)	38.30	Mn	6.52
Texture	Clay loam	Zn	4.68
$CaCO_3$ $gkg^{-1}{}_3$	45.6	Soluble ions (meq/L)	
EC (dS m^{-1})	2.92	Ca_{++}	13.8
pH (1:2.5) susp.	7.88	Mg_{++}	10.5
Organic matter (%)	2.29	Na^+	4.6
Available macronutrients (mg kg^{-1})		K^+	0.70
N	33.30	HCO_3-	5.8
P	5.50	Cl^-	8.0
K	360	SO—$_4$	15.8

Table 2: The chemical analyses of the tested water sample (Nile water) collected from the experimental area

		Cations (meq/L)				Anions (Meq/L)			
E.C (dS/m)	pH	Ca^{++}	Mg^{++}	Na^+	K^+	HCO_3-	Cl-	SO_4--	SAR
0.55	7.84	1.50	1.53	1.32	0.18	1.40	1.40	1.73	1.07
		Some macro micro nutrients (ppm)							
N	P	K	Fe	Mn	Zn	Cu	Pb	Ni	B
1.36	0.54	7.02	0.02	0.04	0	0.04	0.01	0.01	0.07

Yield and Oil Percentage and Yield of Female Shrubs:

- Seed yield (g): was recorded at the end of July for female shrubs.
- Seed physical properties: seed weight (g), length (cm) and width (cm) were measured (10 seeds per each replicate).
- Oil content percentage was determined by extracting the oil from the dried and crushed seed samples (three replicates for each genotype) by Soxhlet apparatus using petroleum ether (60-80°C) as a solvent for 16 hours, according to the method described by Juan [17] and Ayerza *et al*. [18]. Oil yield (g) was estimated using the following formula:

Oil yield (g) = [(seed sample before extracting – seed sample after extracting) / seed sample before extracting] × seed yield.

Vegetative Propagation of Male and Female Plants: Mother plants (of both sexes) were propagated by 60 leafy stem cuttings of each plant, which prepared from sub-terminal developing shoots. Cuttings of 10-12 cm and about 0.5 cm in diameter with four pairs of leaf buds were collected and dipped for 10 seconds in indole butyric acid (IBA) solution at 3500 ppm and planted in a mixture of peat moss and sand (1:4) under mist during July. After 3 months the cuttings were removed and percentages of rooting, fresh weight of roots were recorded by Bashir *et al*. [19].

Chemical Analysis: In 2019 and 2020 seasons leaf samples were collected from the 3rd to the 5th node taken from jojoba male and female genotypes for the chemical analysis.

- Photosynthetic pigments (chlorophyll a, b and carotenoids) were measured in jojoba leaf using the method of Lichtenthaler [20].
- Phenolic content was assayed, as described by Danil and George [21].
- Free proline content was determined following the method of Bates *et al*. [22].
- Crude protein: Total organic nitrogen (N) was determined in jojoba leaves according to the method of Kjeldahl A.O.A.C. [23] for dry material. Crude protein content was obtained by multiplying the nitrogen (N) value by 6.25.

Molecular Diversity Assessment

DNA Isolation: Genomic DNA of each jojoba genotype accession was extracted according to modified CTAB (Cetyl Trimethyl Ammonium Bromide) method Wang, *et al*. [24]. The quantity of RNase-treated DNA of all the plant accessions was determined on agarose gel (0.8%), compared with a standard lambda DNA marker (Amersham Biosciences, Piscataway, NJ, USA).

RAPD and SCoT-PCR Amplification: Two RAPD and eight SCoT primers (Integrated DNA Technologies, Inc, USA) were randomly selected and tested on isolated

genomic DNA of jojoba genotype accessions. The PCR reactions were carried out in a 25 uL volume containing Taq polymerase (1 unit), genomic DNA (50 ng), ISSR/SCoT primers (0.80 mM), dNTP (0.1 mM), 10X PCR reaction buffer (2.5 uL), Triton X (1%) and MgCl2 (3 mM). PCR amplifications were carried out with a preliminary cycle of 120 s at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 57°C, 60 s at 72°C and a final extension of 7 min at 72°C. The amplification products were resolved on 1.5 % agarose gels.

DNA banding pattern photos were photographed using Bio-1D Gel Documentation system and were analyzed by Gel Analyzer 3 software which scored clear amplicons as present (1) or absent (0) for each primer and entered in the form of a binary data matrix.

Statistical Analysis: The obtained data were subjected to analysis of variance (ANOVA) according to Snedecor and Cochran [25] and the means of male and female genotypes were compared by Duncan multiple range test at 5% property [26].

RESULTS AND DISCUSSION

Two experiments were set up to identify the differentiation between male and female Jojoba traits as follow:

The First Experiment (I): Identification of the Difference Between Male and Female Nursery Seedlings by Using Morphological Traits: Studying jojoba plant clear that takes a long period to identify the differentiation between male and female plants. Because, it was difficult to distinguish between them in the young ages. After three years from planting the seed, About 120 of the nursery seedlings were randomly selected to differentiate between male and female plants during flowering.

Regarding the presented data in Table (3) it was observed that, from a the total of (120) plants was grown up the number of female plants was (44 & 41). While, the number of male plants was (76 & 79) in the first second season, respectively. In addition, the sex percentage of female was (36.67% & 43.17%) and the sex percentage of male was (63.33% & 65.83%) in two studied season has a ratio of male-to-female was nearly of 2:1. The superiority of male plants than female plants were in agree with that was found by Li *et al*. [27] and Randriamanana *et al*. [28] who found that female plants can display sensitivity to environmental stress than males and with Ince *et al*. [29] who reported that Jojoba has a ratio of 5:1 male-to-female ratio which cause low seed production.

Table 3: Number and percentage of male and female jojoba plants in 2019 and 2020 season

	Number			Percentage		
	Female	Male	Total	Female	Male	Total
2019	44.00B	76.00A	120	36.67B	63.33A	100
2020	41.00B	79.00A	120	34.17B	65.83 A	100

Vegetative growth and flowering characteristics of Jojoba male and female shrubs:

Tree Circumference (m): Based on the analysis of variance of vegetative growth characters in Table (4), the results revealed significant differences in tree circumference among female and male jojoba genotypes. In the first season, females mean value (4.73 m) was higher than males (4.38 m), while the male value (5.71m) was higher than females (5.65 m) in the second season. Data also indicated that jojoba genotype G3 significantly gave the highest values of tree circumference (5.18 & 6.59 m), while genotype G1 gave the lowest values (3.96 & 4.93 m), in the first and second season, respectively. The highest combinations between sexes and genotypes in respect to tree circumference were obtained for female of genotype G4 in the first season and for female and male of genotype G3, in the second season (with the same value). These result can be coincide with Shaheen, *et al.* [41].

Tree Height (m): Data in Table (4) revealed that, tree height of male trees appeared higher values (1.76 & 2.27m) as compared to females (1.33 & 1.62 m), in the first and second season, respectively. genotypeG4 appeared the highest value of tree height, while G2 appeared the lowest value, in the first and second season, respectively. The highest interaction between the two factors was obtained under male trees of genotype G3, while the lowest interaction was obtained under female trees of genotypes G5 in both seasons. Previous studies on growth characteristics between males and females of dioecious plants have shown that females are smaller than the males and the females grow more slowly [30-31].

Shoot Length (cm): Data presented in Table (5) and Fig. (1) showed that shoot length for male genotypes (40.60 & 42.33 cm) were significantly higher than female genotypes (39.00 & 37.40 cm), in the first and second seasons respectively. Genotype G3 was superior in the 1st season, while genotype G4 was superior in the 2nd season. The highest interactions between the two factors regarding to shoot length were scored by male genotypes of G1 & G3 in the 1st season and by male genotype G4 in the 2nd season, while, the lowest values were expressed by

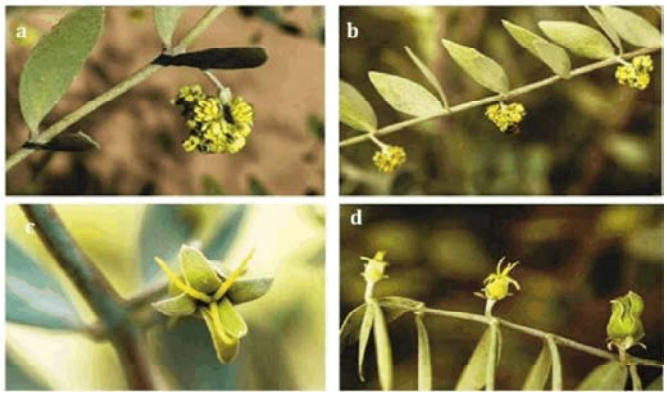

Fig. 1: Jojoba female and male flower:
a- Close-up of male buds. b- Terminal branch of jojoba male.
c- Close-up of mature female flower. d- Terminal branch of jojoba female.

Table 4: Vegetative growth characters of the female and male Jojoba genotypes in 2019 and 2020 seasons

	Tree circumference (m)			Tree height (m)		
Genotype	Female	Male	Mean	Female	Male	Mean
			First season; 2019			
G1	3.77h	4.14f	3.96E	1.40e	1.55d	1.48C
G2	4.24f	4.40e	4.32D	1.31f	1.23fg	1.27D
G3	5.34b	5.02c	5.18A	1.23fg	2.15a	1.69B
G4	5.50a	3.93g	4.72B	1.50d	2.05b	1.78A
G5	4.80d	4.40e	4.60C	1.22g	1.80c	1.51C
Mean	4.73A	4.38B		1.33B	1.76A	
			Second season; 2020			
G1	4.71f	5.15e	4.93E	1.60de	2.05c	1.83B
G2	5.34d	5.34d	5.34D	1.65d	1.60de	1.63C
G3	6.59a	6.59a	6.59A	1.67d	2.80a	2.24B
G4	6.28b	5.34d	5.81B	1.70d	2.60b	2.15A
G5	5.34d	6.12c	5.73C	1.50e	2.30bc	1.90B
Mean	5.65B	5.71A		1.62B	2.27A	

Means within a column or row having the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level.

Vegetative characteristics and flowering of adult female and male jojoba genotypes:

female genotype G4 in 1st season and by female genotypes G2 in 2nd season. El-Sayed [32] mentioned that different shrubs of jojoba varied greatly in their growth rate that appeared clearly from length of their shoots and number of internodes dependent on genotypes.

Number of Leaves/M: Data in Table (5) showed that mean value of male genotypes were significantly higher in number of leaves/meter (66.40 & 67.28) than female genotypes (59.99 & 63.70) in the first and second seasons, respectively. Genotype G5 was superior in the two studied seasons. The highest values of number of leaves/meter were scored by male genotypes G5 in the two studied seasons, while, the lowest values were expressed by female genotype G5 in the two studied seasons, the lowest values were expressed by male genotype G4 in 1st season and by male genotypes G3 in 2nd season. In another study by Inoti *et al*., [2], male Jojoba seedlings showed a higher number of leaves. These results were consistent with studies on *Schiedea salicaria* which showed evidence of sexual dimorphism, where males had higher mass based photosynthetic rate and specific leaf area than females [33].

Number of Female Flowers and Male Inflorescences/M: The mean number of inflorescences/meter for the male genotypes (15.36 & 15.18) was significantly higher than the number of flowers on female genotypes (12.00 & 13.32) in the first and second seasons, respectively. Moreover, the mean of genotype G2 was superior in the first season in respect to number of flowers and inflorescences/ meter, while genotype G5 was superior in second seasons. The highest values of number of inflorescences/meter were scored by male genotypes G5 (20.75 & 19.88) in the two studied seasons, while the lowest values of flowers/meter expressed by female genotype G5 in the first season and by female genotype G1 in the second season. These results are in line with El-Sayed [32], who found that the number of flowers was dependent on genotypes of dioecious plants. These differences are thought to exist because females allocate more resources to reproduction than males and therefore should have fewer resources for vegetative growth [34].

Leaf Length (Cm), Leaf Width (Cm) and Leaf Shape Index of Female and Male Jojoba Genotypes in 2019 and 2020 Seasons:

Leaf Length: Table (6) revealed that leaf length of jojoba male genotypes (3.91 & 4.11 cm) were significantly higher compared to female ones (3.22 & 3.05 cm) in the first and

Table 5: Flowering and vegetative characteristics of adult female and male jojoba genotypes in 2019 and 2020 seasons

	Shoot length (cm)			No. of leaves/m			No. of flowers and inflorescences/m		
Genotype	Female	Male	Mean	Female	Male	Mean	Female	Male	Mean
					First season; 2019				
G1	39.00e	44.00a	41.50B	58.13e	51.52h	54.83E	12.82g	9.84i	11.33E
G2	38.33f	37.67g	38.00C	58.26e	73.45c	65.86B	13.04f	16.80b	14.92A
G3	41.67c	44.00a	42.84A	54.40f	63.64d	59.02D	11.21h	15.91c	13.56D
G4	34.50h	42.00b	38.25C	75.36b	50.00i	62.68C	14.09d	13.50e	13.80C
G5	41.50d	35.33g	38.42C	53.81g	93.41a	73.61A	8.84j	20.75a	14.80B
Mean	39.00B	40.60A		59.99B	66.40A		12.00B	15.36A	
					Second season: 2020				
G1	41.33d	42.00c	41.67B	69.69b	63.50d	66.60B	11.30i	16.67b	13.99C
G2	33.67j	44.67b	39.17C	69.21c	59.70e	64.46C	14.85d	11.93h	13.39D
G3	37.67g	36.00h	36.83E	63.71d	55.56i	59.64D	14.15e	12.03h	13.09E
G4	39.50f	48.33a	43.92A	58.39g	59.32f	58.86E	13.49f	15.40c	14.45B
G5	34.83i	40.67e	37.48D	57.50h	98.33a	77.92A	12.80g	19.88a	16.34A
Mean	37.40B	42.33A		63.70B	67.28A		13.32B	15.18A	

Means within a column or row having the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level.

Table 6: Leaf length (cm), Leaf width (cm) and Leaf shape index of female and male jojoba genotypes in 2019 and 2020 seasons

	Leaf length (cm)			Leaf width (cm)			Leaf shape index (L/W)		
Genotype	Female	Male	Mean	Female	Male	Mean	Female	Male	Mean
					First season; 2019				
G1	3.83d	4.03b	3.93A	1.70a	1.57b	1.64A	2.25e	2.57c	2.41B
G2	3.67f	3.90c	3.79B	1.53bc	1.53bc	1.53B	2.40d	2.55c	2.47B
G3	2.57i	4.10a	3.34D	1.47c	1.37d	1.42C	1.75g	2.99b	2.37C
G4	3.37g	3.77e	3.57C	1.73a	1.13e	1.43C	1.95f	3.34a	2.64A
G5	2.67h	3.77e	3.22E	1.17e	1.47c	1.32D	2.28e	2.56c	2.42B
Mean	3.22B	3.91A		1.52A	1.41B		2.13B	2.80A	
					Second season: 2020				
G1	2.53h	3.67e	3.10E	1.33e	1.43d	1.38E	1.90e	2.57c	2.23C
G2	3.37f	3.77d	3.57C	1.47d	1.47d	1.47D	2.29d	2.56c	2.43A
G3	2.57h	4.17b	3.37D	1.73b	1.30e	1.52C	1.49g	3.21a	2.35B
G4	2.80g	4.70a	3.75B	1.73b	1.47d	1.60B	1.62f	3.20a	2.41A
G5	3.97c	4.23b	4.10A	1.80a	1.53c	1.67A	2.21d	2.76b	2.49A
Mean	3.05B	4.11A		1.61A	1.44B		1.90B	2.86A	

Means within a column or row having the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level

second seasons, respectively. Similarly, the genotype G1 recorded (3.93 cm) as the highest leaf length in the 1st season, whereas, the genotype G5 attained the highest value (4.10 cm) in the 2nd season. The highest interactions of leaf length were scored by male genotypes G3 & G4 in the first and second seasons respectively, while the lowest values were recorded by female genotype G3 in the two studied seasons.

Leaf Width: Data presented in Table (6) demonstrate that the leaf width of jojoba female genotypes (1.52 & 1.61 cm) was higher than male ones (1.41 & 1.44 cm), in first and second seasons, respectively. Additionally, the mean of genotype G1 recorded the highest value of leaf width in the 1st season (1.64 cm), while mean genotype G5 recorded the highest value in the 2nd season (1.67 cm). The highest values of leaf width were scored by female genotypes G4 & G5 in the first and second season respectively, while the lowest value was recorded by male genotype G4 in the first season and genotype G3 in the second season. Inoti, *et al*. [2] found that single leaf area was found to be significantly superior for the males as compared to the females hence used to determine sex in Jojoba seedlings.

Leaf L/W Ratio of Female and Male Jojoba Shrubs: Data in Table (6) showed that, the genotypes of male shrubs were significantly superior in leaf shape index (2.80 & 2.86) than the female ones (2.13 and 1.90) in the first and second seasons, respectively. Moreover, the

mean genotype G4 was superior in the first and second seasons. The highest interaction between the two factors was obtained in male G4 which was significantly superior in the first and second season. Otherwise, the female G3 was the lowest in the first and second season. Generally, leaf shape index of male leaves were higher than female leaves through the two studied seasons. These results are in harmony with Hassan [6] and Khattab *et al.,* [35] who reported that differences have been reported in dioecious species between male and female plants in growth and vegetative characters.

Yield and Seeds Physical Properties of Female Jojoba Genotypes: The presented data of yield of female genotypes and the seeds physical properties (Table 7) revealed that , the genotype G4 had significantly higher yield values (787.0 & 776.0 g) in the first and second seasons, respectively, while, the lowest values were obtained by genotype G1 (405.7 g) and genotype G5 (501.3 g), in the first and second seasons, respectively. As regard to the seed physical properties, it could be noticed that, the seeds of genotype G5 attained the highest weight (1.41 & 1.49 g) and length (1.82 & 1.77 cm), while the lowest values of seed weight were achieved by genotype G2 in the first season and by genotype G3 in the second one . Moreover, the lowest seed length was acquired by genotype G2 genotype in the first and second seasons. Similarly, a narrow variation was observed in seed width in the two studied seasons.

These results are nearly similar to those obtained by Abu El-Khashab *et al.* [36] and El-Sayed [32]. Also, El-Torky *et al.,* [37] found clear differences between clones. Furthermore, Yermanos [38] indicates variation in yield and seed characteristics from one year to the next due to genotype and environmental factors such as temperature. The highest or lowest production, suggesting different responses of the genotype-environment interaction. Also, the average yield varied greatly between years and genotypes [39-40].

Oil Content (%) and Oil Production G/Tree in the Seeds of Female Jojoba Genotypes: Data in Table (8) revealed that, seeds of female genotypes G2 appeared the maximum oil percentages (43.0 & 44.2 %) while genotypes G1 Appeared the minimum, through the two studied seasons, respectively. Regarding the oil production/tree, it appeared that females of genotype G4 were superior than others in oil production (326.6 & 337.6) through the two studied seasons, respectively. While the Genotype G1 produced the lowest oil production (130.6 & 179.3) through the two studied seasons. These results were in harmony with Shaheen *et al*. [41].

Vegetative Propagations Characters

Roots Weight: The means of root weight for male and female Jojoba cuttings that cleared in Table (9) illustrated the higher root weight of male genotypes in the 2019 season (2.17 g) than female ones (2.01g). While in the 2020 season, no significant difference was observed between the means of male and female genotypes. Similarly, the maximum root weight acquired by genotype G2 (2.55 & 2.69 g), in the first and second seasons, respectively. While genotype G3 shows the minimum values in the two seasons. For the effect of interaction, the male genotype G2 has the greatest value of root weight (2.73 & 2.67 g) at first and second seasons shared with female of genotype G2 that exhibit the greatest values of (2.70 g) at 2nd season. Whereas, the lowest values were obtained by the male of G3 in both seasons.

Rooting %: The means of rooting percentage of stem cuttings of female and male jojoba shrubs as shown in Table (9) and Fig. (2) give a clear evidence that stem cuttings from mean male jojoba genotypes in the two studied seasons has significantly higher rooting percentage (46 & 49%) as compared to female jojoba genotypes (26 & 29 %), in the first and second seasons, respectively. Moreover, Mean genotype G2 recorded the highest rooting value (50 & 55%), in the first and second seasons, shared with G3 in the first season. While genotype G5 Shows the lowest values in the two studied seasons. The effect of interaction appears in male of genotype G2 which demonstrated the highest rooting percentage (60 & 65 %) in the first and second seasons, respectively. Otherwise, male G1 shows the lowest values in the two studied seasons. These results were agreed with Inoti *et al.* [42] who reported that rooted cuttings of jojoba showed that males demonstrated higher foliage growth compared with the females. Moreover, Bala *et al*. [43] reported that the highest rooting was in male stem cutting but in female stem cutting were quite lower observed with use of IBA.

Similar results were observed by Correia and Diaz [7] who reported that despite the higher leaf control of water loss by females, they reduce the water potential to the same values as male plants, probably due to specific characteristics of the root system or of the conducting xylem. They suggest that the ecological advantage of male plants in older communities is due to a higher competition for water uptake.

Table 7: The yield and seeds physical properties of female jojoba genotypes in 2019 and 2020 seasons

	Yield (g)		Seed weight (g)		Seed length (cm)		Seed width (cm)	
Genotype	2019	2020	2019	2020	2019	2020	2019	2020
G1	405.7E	522.7D	1.23C	1.27B	1.32D	1.37C	1.17B	1.18A
G2	741.0B	743.3C	1.11D	1.16C	1.30D	1.32C	1.17B	1.19A
G3	733.3C	750.3B	1.29BC	1.08D	1.64C	1.65B	1.15B	1.01B
G4	787.0A	776.0A	1.33B	1.33B	1.72B	1.73A	1.24A	1.21A
G5	445.0D	501.3E	1.41A	1.49A	1.82A	1.77A	1.21AB	1.18A

Means within a column having the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level.

Table 8: Oil content (%) and oil production g/tree in seeds of female jojoba genotypes in 2019 and 2020 seasons

	Oil content (%)		Oil production g/tree	
Genotype	2019	2020	2019	2020
G1	32.20D	34.30E	130.6E	179.3E
G2	43.00A	44.20A	318.6B	328.5B
G3	31.50D	35.20D	231.0C	264.1C
G4	41.50B	43.50B	326.6A	337.6A
G5	33.50C	36.50C	149.1D	183.0D

Means within a column having the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level.

Table 9: Roots weight and rooting % of female and male cuttings jojoba genotypes in 2019 and 2020 seasons

	Roots weight (g)			Rooting (%)		
Genotype	Female	Male	Mean	Female	Male	Mean
			First season; 2019			
G1	2.04d	1.92e	1.98C	20.00c	10.00d	15.00D
G2	2.36c	2.73a	2.55A	40.00b	60.00a	50.00A
G3	1.88e	1.67f	1.78E	40.00b	60.00a	50.00A
G4	1.91e	1.89e	1.90D	10.00d	60.00a	35.00B
G5	1.88e	2.62b	2.25B	20.00c	40.00b	30.00C
Mean	2.01B	2.17A		26.00B	46.00A	
			Second season; 2020			
G1	2.10b	1.84d	1.97C	20.00g	12.00i	16.00E
G2	2.70a	2.67a	2.69A	45.00d	65.00a	55.00A
G3	1.91c	1.46e	1.69E	42.00e	60.00c	51.00B
G4	1.92c	1.80d	1.86D	13.00h	63.00b	38.00C
G5	1.83d	2.71a	2.27B	25.00f	45.00d	35.00D
Mean	2.09A	2.10A		29.00B	49.00A	

Means within a column or row having the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level

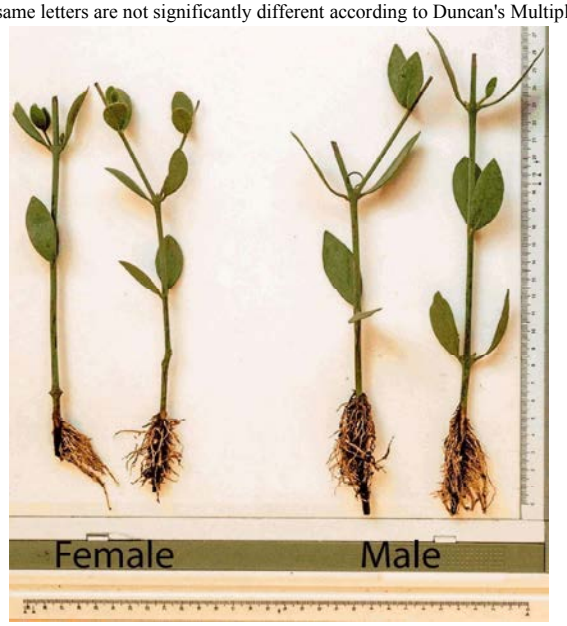

Fig. 2: Stem cuttings of female and male jojoba shrubs

Table 10: Chlorophyll A and B, carotene in leaves of jojoba female and male jojoba genotypes in 2019 and 2020 seasons

	Chlorophyll a (mg/g)			Chlorophyll b (mg/g)			Carotene (mg/g)		
Genotype	Female	Male	Mean	Female	Male	Mean	Female	Male	Mean
					First season; 2019				
G1	0.470ab	0.444c	0.457A	0.338d	0.286e	0.312D	0.315b-d	0.301c-e	0.308B
G2	0.487a	0.423d	0.455A	0.280e	0.363bc	0.322C	0.348a	0.267fg	0.308B
G3	0.400ef	0.473ab	0.437B	0.421a	0.411a	0.416A	0.305c-e	0.329a-b	0.317A
G4	0.440c	0.385f	0.413C	0.267e	0.377b	0.322C	0.323bc	0.247g	0.285C
G5	0.411de	0.463b	0.437B	0.344cd	0.334d	0.339B	0.295de	0.283ef	0.289C
Mean	0.442A	0.438A		0.330B	0.354A		0.317A	0.286B	
					Second season: 2020				
G1	0-.454b	0.480a	0.467A	0.328d	0.200f	0.264D	0.313bc	0.311bc	0.312B
G2	0.425c	0.489a	0.457A	0.301e	0.375bc	0.338C	0.453a	0.258d	0.356A
G3	0.471ab	0.358e	0.415C	0.408a	0.406a	0.407A	0.312bc	0.324b	0.318B
G4	0.400d	0.454b	0.427B	0.312de	0.397ab	0.355B	0.303bc	0.258d	0.281D
G5	0.457b	0.403d	0.430B	0.358c	0.354c	0.356B	0.306bc	0.299c	0.303C
Mean	0.441A	0.437A		0.341A	0.347A		0.337A	0.290B	

Means within a column or row having the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level

Chemical Analysis

Chlorophyll A and B, Carotene in Leaves of Jojoba Female and Male Jojoba Genotypes

Chlorophyll A Content: As shown the chlorophyll A content in Table (10), it was noticed that no significant differences were observed in chlorophyll a content between mean of male and female genotypes through the two studied seasons. Furthermore, the means of genotype G1 & G2 were significantly higher than others in the two studied seasons. As for the effect of interaction, it was differ from the first and second season.

Chlorophyll B Content: The current study in Table (10) showed that, the chlorophyll B acquired a higher content in the men of males than females in the first season, while in the second season no significant differences were observed. However, the mean of genotype G3 had higher chlorophyll b in the two studied seasons. While, the lowest values were obtained by G1. According to the interaction effect, G3 in female and male genotypes had the highest value in the two studied seasons. While, the male G1 in both seasons shared with the female genotypes G2 & G4 in the first season in appearing the lowest value.

Carotene Content: Data of Carotene content in Table (10) showed the superiority of female genotypes (0.317 & 0.337 mg/g) than male genotypes (0.286 & 0.258 mg/g) in the two studied seasons, respectively. Moreover, the mean of genotype G3 in 2019 and genotype G2 in 2020 gave the highest values. Otherwise, the G4 followed by G5 had the lowest value in the two studied seasons. Female genotype G2 has the highest genotype carotene content (0.348 & 0.453 mg/g) in two studied seasons, respectively. While, the least values were recorded in male genotype G4 in the first season and genotype male G2 & G4 in the second season, the rest genotypes had in between.

Photosynthesis is a process particularly sensitive to drought stress. Decreases in chlorophyll and carotene contents take place under water deficit conditions, however carotenoids are less sensitive. Carotene serve as important photo protectors and suppress lipid peroxidation [44]. The earlier studies report higher chlorophyll and carotene concentrations in males than females under drought stressed conditions, Xu *et al.* [45] and Chen *et al.* [46]. Similarly, the chlorophyll a and b were evaluated in leaves of male and female Jojoba genotypes, differences between genders were found which was attributed to higher drought tolerance of males than females [3]. On the other side, Leigh *et al*. [4] indicated that no difference in leaf chlorophyll content between males and females could be detected.

Total Phenols, Proline and Crude Protein in Leaves of Jojoba Female and Male Jojoba Genotypes

Total Phenols: Table (11) clears that leaf total phenols content was significantly higher in the means of female (0.358 & 0.364%) than male (0.306 & 0.24%) in both seasons, respectively. Moreover, the mean genotype G4 was significantly superior in the first and second season, shared with G5 in the first season and G3 in the second one. While, the lowest value in the two studied seasons was in genotype G1. As for the interaction effect, the female genotype G4 & G5 showed higher total phenol

Table 11: Total phenols, proline and crude protein in leaves of jojoba female and male jojoba genotypes in 2019 and 2020 seasons

	Total phenols mg/g			Proline (mg/100g)			Crude protein (%)		
Genotype	Female	Male	Mean	Female	Male	Mean	Female	Male	Mean
				First season; 2019					
G1	0.292de	0.295de	0.294D	44.40f	61.80c	53.10D	19.47e	21.13b	20.30A
G2	0.290de	0.285e	0.323C	67.90h	54.65e	61.28B	19.94d	20.65c	20.30A
G3	0.355b	0.321c	0.338B	65.28b	72.31a	68.80A	20.50c	20.03d	20.27A
G4	0.395a	0.320c	0.358A	44.56f	65.20b	54.88C	17.76g	18.67f	18.22B
G5	0.389a	0.310cd	0.350A	42.62g	59.51d	51.07E	14.29h	22.14a	18.22B
Mean	0.358A	0.306B		46.95B	62.69A		18.39B	20.52A	
				Second season: 2020					
G1	0.303de	0.301e	0.302D	45.56h	62.45d	54.01B	20.09d	21.74b	20.92A
G2	0.350b	0.312de	0.331C	37.99j	55.64f	46.82D	20.48c	20.46c	20.47C
G3	0.395b	0.331c	0.363A	63.12c	72.97a	68.05A	20.63c	20.73c	20.68B
G4	0.383a	0.357b	0.370A	46.47g	65.33b	55.90B	17.84e	15.99f	16.92E
G5	0.389a	0.320cd	0.355B	42.79i	59.90e	51.35C	14.36g	22.99a	18.68D
Mean	0.364A	0.324B		47.18B	63.25A		18.68B	20.38A	

Means within a column or row having the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level

content in both seasons. While, the lowest values were obtained from G1 & G2 in female and male genotypes during the two studied seasons. The above result was in harmony with Khattab *et al.,* [47] who recorded that, females of jojoba have a marginally significant higher concentration of total phenols than males.

Proline Content: As regard to the proline content in Table (11), it could be noticed that the male of Jojoba genotypes showed significantly higher proline content (62.69 & 63.25) compared to female genotypes (46.95 & 47.18) in both seasons, respectively. Furthermore, the mean of genotype G3 was the highest in both studied seasons. While, the lowest value was obtained by genotype G5 in the first season and genotype G2 in the second one. For the interaction effect, the male of G3 gave the highest value in both studied seasons, while the female genotype G5 recorded the lowest values in the first season and genotype G2 in second season. these results were agree with Hassan [6] who reported that, proline content was found to be higher in male Jojoba plants than female plants and plays a major role in osmotic adjustment, plasma membrane integrity protection, scavenging hydroxyl radicals and prevention of denaturation of proteins, Bartels and Sunkar, [48]. However, based on Hamayl et al., [49] it can be advocated that on-farm investigation should be conducted in real field conditions of saline prone areas and sprayed with proline to confirm the performance of male.

Protein Content: Significant differences were recorded in protein content in leaves among male and female genotypes (Table 11). The male genotypes showed significantly higher protein content (20.52 & 20.38%) as compared to female genotypes (18.39 & 18.68 %) in the first and second seasons, respectively. The mean of genotype G1 showed highest protein content in both seasons, shared with G2 & G3 in the first season, while, mean of genotype G4 & G5 recorded the lowest value in both seasons . The highest interaction effect appeared in male genotype G5 (22.14 and 22.99 %), in the first and second seasons, respectively. While female genotype G5 recorded the lowest values in both seasons.

These results were in agreement with many previous studies on dioecious plants [4, 50, 5].

Molecular Genetic Identification and Sex Determination Between Individual's Male and Female Jojoba (*Simmondsia chinensis*) Species: In such plants, gender influences economic values, breeding schemes and opportunities for commercial harvest. The development of molecular strategies for early sex identification of dioecious taxa has been a priority in breeding programs for their greater economic potentials. Moreover, studies on marker technology regarding dioecy in general would render a better understanding of the developmental as well as the evolutionary pathways of dimorphism and Sex-specific markers in dioecious taxa which could be generated through DNA analysis using PCR technology have been proved to be a reliable strategy, as such markers for sex prediction can be analyzed at any developmental stage of growth.

RAPD-PCR Analysis: The RAPD technique, Williams *et al*. [51] is a simple identifier of polymorphism and has been used to screen markers of sex determination

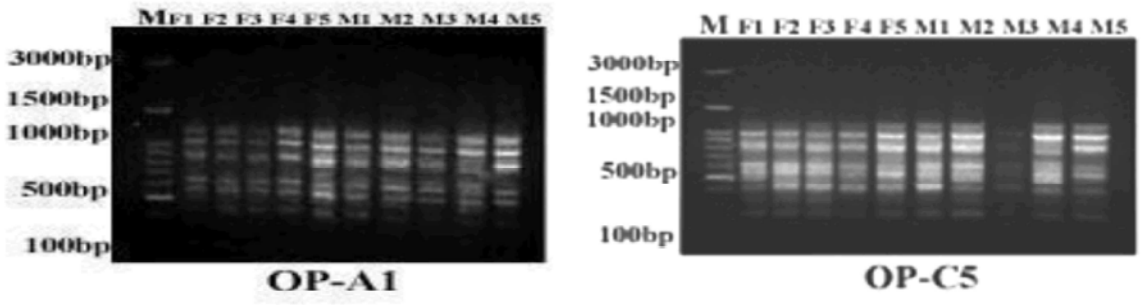

Fig. 4: RAPD -PCR Profile for five males and five females from Jojoba (*Simmondsia chinensis*) amplified with two primers

Table 13: Molecular genetic data produced from amplified banding patterns of RAPD analysis

						Unique Band		
Primer Name	Sequence 5`---------- 3`	Total Band	Monomorphic Band	Polymorphic Band	Male	Female	Polymorphic %
OP-A1	CAG GCC CTT C	7	5	2	-	f1, f2, f3, f4(785bp)	28.57%
OP-C5	GAT GAC CGC C	9	3	6	m1, m2, m3m4(485bp)	-	66.66%
Total	16	8	8	1	1	50%	

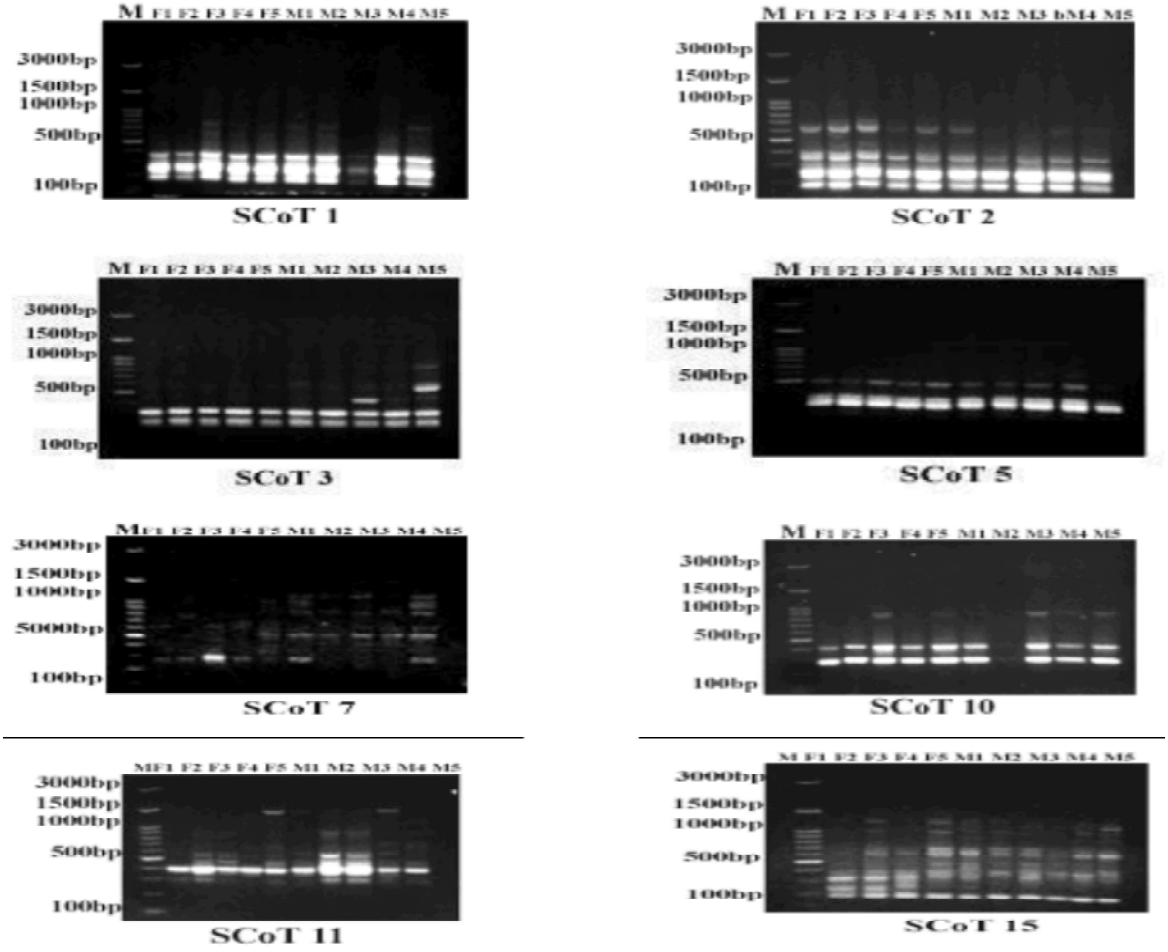

Fig. 5: SCoT -PCR Profile for five males and five females from Jojoba (*Simmondsia chinensis*) with eight primers

Table 14: Molecular genetic data produced from amplified banding patterns of SCoT analysis

Primer Name	Sequence 5`---------- 3`	Total Band	Monomorphic Band	Polymorphic Band	Unique Band		Polymorphic %
					Male	Female	
ScoT 1	ACG ACA TGG CGA CCA CGC	5	3	2	-	-	66.66%
SCoT 2	ACG ACA TGG CGA CCA CGC	5	3	2	-	f1, f2, f3(390bp)	40%
SCoT 3	ACG ACA TGG CGA CCC ACA	5	2	3	m1, m2m3m4m5m (840bp)		60%
SCoT 5	CAA TGG CTA CCA CTA GCG	4	4	-	-	-	-
SCoT 7	ACA ATG GCT ACC ACT GAC	6	-	6	m1, m2, m3, m5(1080bp	-	100%
SCoT 10	ACA ATG GCT ACC ACC AGC	3	2	1	-	-	66.66%
SCoT 11	ACA ATG GCT ACC ACT ACC	7	2	5	M4, m5(730bp)	-	71.42%
SCoT 15	CCA TGG CTA CCA CCG GCT	10	2	8	-	F1, f2 (240bp)	80%
Total	45	18	27	3	2	60%	

in several plants, i.e. *Salix viminalis* L. Alstrom-Rapaport *et al*. [12], *Cannabis sativa* L. Mandolino *et al*. [52], Eucommia ulmoides Oliv. Xu *et al*. [53], Encephalartos natalensis, Prakash and Van Staden [54], Carica papaya Chaves-Bedoya and Nunenz [55]. A total of two RAPD primers were used to amplify the bulk DNA of male and female individuals, the number of total amplification products were 16 and the molecular weight size ranged from 280 bp to 1.340 bp. On the other hand, there were eight monomorphic bands and eight polymorphic bands with 50% polymorphic and primer OP-C5 was high polymorphic% (66.66%). While, primer OP-A1 produced low polymorphic (28.57%).

The two primers were found to have sex specificity in bulk analysis. Random decamer primer OPA-1 produced a unique band with 785 base pairs fragment in female and this band was absent in individual male (Fig. 4 and Table 13) and the other primer was found to produce a unique band with 485 base pairs fragment in male and this band was absent in individual female. In addition to this, many other bands were generated in both male and female samples. To confirm this observation, this primer was re-tested with the individuals of male and female samples of our jojoba cultivar.

SCoT-PCR Analysis: Eight SCoT primers were used to amplify the DNA of male and female individuals Fig. (5) and Table (14), the number of total amplification products were 45 with molecular weight size ranged from 130 bp to 1.500 bp. On the other hand, there were 18 monomorpic bands and 27 polymorphic bands with 60 % polymorphic and primer SCoT 7 was high polymorphic (100%). While, primer ScoT5 produced no polymorphic%.

Molecular Genetic Identification: Data presented in Fig. (5) and Table (14) illustrated that, SCoT 3, SCoT 7 and SCoT11 primers were found to have a sex specificity for male in analysis produced a unique band with molecular weight 840, 1080 and 730 base pairs respectively, in male individual DNA and this band was absent in individual female DNA. While, Primers (SCoT 2 and SCoT 5) were found to have sex specificity for female individual DNA analysis which produced a unique band with molecular weight 390 and 240 base pairs respectively and this band was absent in individual male DNA.

Our results confirmed with, a RAPD band from UBC of 354–560 bp was shown to be linked to a sex determination locus in Salix viminalis, Alstrom-Rapaport *et al*. [12]; two RAPD bands, 757 bp amplified with OPC-12 and 908 bp with OPA-10, were associated with male Piper longum, Banerjee *et al*. [56]; using 158 RAPD primers, a male-specific 2075-bp band was identified in Atriplex garretti, Ruas *et al*. [57] and a single 567-bp RAPD female sex-specific marker was identified following screening of 100 decamer primers in Trichosanthes dioica Roxb, Singh *et al*. [58].

CONCLUSIONS

Generally, it may be concluded that, the results of comparing jojoba male and female characters indicated that the male plants were superior than females. Moreover, the evaluated Male shrubs acquired highest values in vegetative growth characteristics, rooting percentage, fresh weight of roots, leaf protein, proline and chlorophyll b contents, These may favour the survival of males over females which explains the increasing of male/female ratio. These differences could be used in future for better understanding of the biochemical and physiological operating mechanisms in both sexes. Using genetic analysis is an important method to compare and differentiate between male and female plants, especially as it is difficult to distinguish between them at a young age.

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