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# Increase the Economic Value of the Jojoba (*Simmondsia Chinensis*) Yield Using Evaluation of Distinctive Clones Grown Under the Egyptian Environmental Conditions

**Esmail, A. E. G.[1], Amira, K. G. A.[2] and Adss, I. A. A.[3]**

[1]Pomology Department, National Research Center, Giza, Egypt; [2] Horticulture Department, floriculture Division, Agriculture Faculty, Damanhour University, Egypt; [3]Plant Pathology Department, Division of Genetics, Faculty of Agriculture, Damanhour University, Egypt.

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**Abstract** Jojoba (*Simmondsia chinensis*) is an industrial crop being grown in the arid and semiarid regions. Evaluation of ten jojoba clones that were selected from privet jojoba farm was studied to compare their growth parameters such as, tree volume, branch length (cm), branch diameter (mm), number of nodes forming branches, mean length for secondary branches per every branch, leaf area, chlorophyll (A and B), flowering date, flowering percentage, fruit set percentage and seed yield per plant (g). Moreover, seed samples were analyzed for oil content, protein content, minerals content and carbohydrates content. Finally comparing between the studied clones using the different of total protein band and Random Amplified Polymorphic DNA (RAPD) to determine genetic relationships among jojoba genotypes. All these parameters showed significant differences among the studied clones during both seasons except branch diameter (mm) and minerals percentage in the first season as the differences between clones were insignificant. It was observed that the maximum values corresponded to the economic parameters were recorded in clones EAI 1 and EAI 4 compared with the others. The study of the different of total protein bands showed the different of total protein bands in ten jojoba clones. Random amplified polymorphic DNA (RAPD) technique was used to investigate the patterns and distribution of genetic variability in studied clones. Cluster analysis was conducted to generate a dendrogram to elucidate the relationships among jojoba genotypes. The dendrogram data divided the jojoba genotypes into two main clusters. Genotypes EAI 1 and EAI 4 were found in the same sub-cluster using RAPD primers. These genotypes also had almost similar values for most traits such as the maximum values of seed yield per plant and seed oil content. It is concluded that jojoba plants in the natural habitat of Egypt belong to different genotypes.

**Keywords:** Jojoba, Distinctive clones

## Introduction

Jojoba (*Simmondsia chinensis*) is an industrial crop being grown in the arid and semiarid regions of southwestern US. The plant is a long-lived, dioecious perennial tree native to certain parts of the Sonoran Desert in

southern Arizona, southern California and northern Mexico (Nelson and Watson, 2001). Plantations of jojoba have been established in a number of desert and semi-desert areas, predominantly in Argentina, Australia, Israel, Mexico, Peru and the United States. The fruit is an acorn-shaped ovoid, three-angled capsule 1–2 centimeters (0.39–0.79 in) long, partly enclosed at the base by the sepals. The mature seed is a hard oval, dark brown in color and contains oil (liquid wax)(Phillips and Patricia, 2000).The female plants produce seed from flowers pollinated by the male plants. Jojoba leaves have an aerodynamic shape, creating a spiral effect, which brings wind-born pollen from the male flower to the female flower. In the Northern Hemisphere, pollination occurs during February and March. In the Southern Hemisphere, pollination occurs during August and September. The haploid number of jojoba is 13. Somatic cells of jojoba are tetraploid, the number of chromosomes is 2n = 4x = 52 (Hiroshi *et al.*, 1992). The product of primary interest is the seed oil, which is a unique liquid wax (commonly known as jojoba oil). Most of this oil consists of esters formed from acids and alcohols with chain lengths of 20 or 22 carbon atoms (Wisniak, 1987). Jojoba oil is used as a natural base for a wide range of cosmetic products because of its purity, lack of odor and stability. In addition, it also possesses heat resistant lubricating properties and is potentially useful in the chemical industry as a basic feedstock (Nelson and Watson, 2001) such as pharmaceuticals, lubricants, gear additives, extenders, anti-foaming agents, and in the wax and polish industries (US National Research Council, 1985 and Wisniak, 1987).

Selective breeding is developing plants that produce more seeds with higher oil content (Phillips and Patricia, 2000). Each plant is single-sex, either male or female, with hermaphrodites being extremely rare. As it occurs in other crops, the jojoba industry faces the challenge of finding ways to improve productivity and quality of the products. In addition, the seedlings cannot be sexed until the first flower buds appear 9 to 24 months after sowing (Dunstone and Begg, 1983). The pollen of the male trees is scattered for miles by the wind. Only female trees produce seeds (Gentry, 1958). Although jojoba plants start producing fruit in 3 years, full maturity takes 10 to 12 years, with the plant's life estimated to be 100 years (Verbanic, 1986). Only a small proportion (less than 1%) of the plant population originating from seeds of native plants has the potential of yielding economically acceptable yields (Purcell and Purcell, 1988). This outbreeding has resulted in highly heterogeneous seeds that provide a wide range of hybrid vigor and fertility. Ironically, the extreme genetic variation that was a major cause of failure in the seed-planted fields of the early jojoba pioneers will also be a key step for developing high yields in the future (Purcell *et al.*, 2000). Moreover, there is a lack of practical methods

for cultivar identification. Hence, the best method for jojoba improvement, in the short term, is the selection of plants with desirable characteristics and propagating them asexually. DNA-based genetic markers, such as restriction fragment length polymorphisms (RFLP) and random amplified polymorphic DNA (RAPD), have become more efficient, reliable and useful (Caetano-Anolles *et al.*, 1991 and Nybon, 1994). Amarger and Mercie (1996) have applied random amplified polymorphic DNA for the discrimination between two jojoba genotypes at the genomic level.

Random amplified polymorphic DNA (RAPD) technique was used to investigate the patterns and distribution of genetic variability in natural field-grown cuttings of jojoba plants (Gaber *et al*., 2007). The objective of this study was to evaluate jojoba plants for agronomic and yield characters to introduce jojoba as a commercial crop with the purpose of selecting superior jojoba genotypes suitable for EL-Behira governorate, Egypt.

## Materials and methods

During two successful seasons (2013-2014 and 2014-2015) the analyzing and comparing between ten jojoba clones selected from superior female plants were conducted. Individual plants were tagged in the privet farm and evaluated on the basis of high seed yield. Based on this selection criterion, ten most promising mother plants from individual plants were propagated by stem cuttings and planted in the privet farm in EL-Behira governorate, Egypt at the spring of 2005. On our experiment the evaluation of the best ten jojoba clones was carried out in two successive harvesting seasons 2013 and 2014. Plants of genotypes were planted in randomized complete block design (RCBD) with five replications per genotype. Distances between rows and within plants in rows were 3 and 2.5 m, respectively. Male plants were repeated one row every six female rows. Drip irrigation system was applied in the orchard, weed and pest control, and fertilization conducted following the standard agro-management practices. All the studied plants were subjected to the same condition including irrigation, farm practices and etc. The orchard soil analysis are given in (Table 1) and water irrigation analysis are given in (Table 2) according to procedures.

**Table 1.** Some physical and chemical analysis of the orchard soil

| parameters | pH | EC(dSm-1) | Ca++ | Mg++ | Na+ | K+ | HCO3- | Cl- | SO4= |
|---|---|---|---|---|---|---|---|---|---|
| values | 7.88 | 2.26 | 2.14 | 3.78 | 19.66 | 0.31 | 1.11 | 21.25 | 4.56 |

**Table 2**. Chemical characteristics of water weal used for the present study

| parameters | pH | EC(dSm-1) | Ca++ | Mg++ | Na+ | K+ | HCO3- | Cl- | SO4= |
|---|---|---|---|---|---|---|---|---|---|
| values | 7.45 | 3.44 | 5.42 | 3.64 | 21.88 | 3.24 | 1.98 | 29.24 | 3.25 |

***Parameters***

Parameters were recorded to achieve the objectives of this experiment for measuring plant growth and yield characters of jojoba genotypes. These parameters were measured as follows: For each genotype, five plants for every clone by randomly chosen (one plant per block from the inner rows) were tagged.

***Vegetative and reproductive measurements***

Data were collected from tagged plants of each clone for each block.

**1-Tree volume measurements** were taken for each plant in August of every season according to the following equation (Nelson et al., 1997): **Plant volume = [(length x width)/2] x height.**

**2-Branch characters:** Two branches were selected from the mid- level of the plants and the average of two branches characters were calculated for detailed analysis. On each branch the following data were collected:

**2.1: branch length (cm)**

**2.2: branch diameter (mm).**

**2.3: Number of node forming branches nodes**

**2.4: length of secondary branches per every branch**

**2.5: leaf area ($cm^2$):** This last character was calculated as mentioned by Koller (1972). One matured leaf was sampled from the second basal node on the main branch of each plant at each block in each clone. The leaves of each treatment were weighed. One square was taken from each leaf with a known area ($1cm^2$). The squares of each treatment were weighed. The total leaf area of each plant was calculating according to the following equation: **Leaf area ($cm^2$) = (Leaf weights x Square areas) / Square weights**

This procedure was repeated again using the leaves of the $4^{rd}$, $5^{th}$, $6^{th}$and $7^{th}$ basal nodes on the main branch. The average of leaf area was calculated.

**3- Chlorophyll A, B** were assayed in the commercial harvest stage. They were determined according to Wintermans and Mats (1965) as follows: half gram of fresh leaves was extracted by about 15 ml. of 85% acetone with 0.5g. calcium carbonate, the mixture was through a glass funnel and the residue was washed with a small volume of acetone and completed to 25 ml. The optical density of a constant volume of filtrate was measured at a wave length of 622 nm. for chlorophyll A, 644 nm. and for chlorophyll B using spectrophotometer. The following equation was used:-

Chl. A = 9.784 E.662 - 0.99E.644 = mg/gm.

Chl. B = 21.426 E.644 - 4.65 E. 662 = mg/gm.

**4-flowering**: three Branches per plant from each clone were tagged in December 2013 and 2014, and the number of floral buds was recorded. Every 15 days the number of open buds (with a visible stigma) was recorded, and the flowering percentage was calculated. The number of flowers that reaching mature fruits were also recorded.

**4.1-flowering date**: The numbers of days from first January until open the flowers was recorded. When 50% of the flowers were opened, the flowering date was calculated and recorded.

**4.2- Flowering percentage** was defined as the ratio of the number of flower buds to the number of nodes in the shoots of the current and previous year's growth (*Benzioni et al*., 1999).

**4.3- Fruit set percentage** was calculated as mentioned by Westwood (1978). The number of flowers that set fruits on the branch related to the basic number of flowers as given in the following equation:

**Percentage of fruit set = (No. of fruitlets x 100)/ Total No. of flowers**

**5- Seed yield:** Seed were harvested from the previous tagged plants by hand at full maturity. Harvested seeds were cleaned, dried and weighted (g). Seeds were hand-harvested every year in July and August from five plants per clone and used for determining seed yield plant$^{-1}$ (g) and analyzed for main components.

**6- Chemical analyses were performed following the AOAC (1995)**

**6.1: Oil content**: To determine oil content, seeds of each genotype were randomly selected, weighed, and dried at 50 ºC. The drying process was continued until the difference between the two successive weights was less than 1 mg. Five replications were used for this characteristic. The oil was extracted for 16 h with hexane with a Soxhlet apparatus.

**6.2: Crude protein:** Total organic nitrogen (N) was determined according to the method of Kjeldahl as indicated by (AOAC, 1995) for dry material. Crude protein content was obtained by multiplying the nitrogen (N) value by 6.25. Data represent the means of five replications.

**6.3: Mineral content:** To remove carbon, approximately 5 gm of each dry sample was ignited in a porcelain container and incinerated in the muffle furnace at about 550$^{o}$C. Mineral content was expressed as a percentage of dry matter.

**6.4: Total carbohydrates:** Total carbohydrates were estimated by the difference in the mean values, i.e., 100 - (sum of concentrations of protein, ash and lipid).

**7: RAPD markers**

Leaf samples were submitted to RAPD analysis by PCR amplification in a total volume of 25µl containing 2.5µl 10 x buffer, 2.5 µl 50mM MgCl2, 2.5 µl 4mM dNTPs,7 µl 50pmol primer,1 µl 10 ng of jojoba clones genomic DNA and 0.2 µl (5 units/ µl) Taq DNA polymerase (Promega Germany). The PCR program consisted on was applied : an initial denaturation cycle at 95 ºC for 5 min, 40 cycles at 95oC for 1 min, annealing at 30oC for 1 min and extension at 72oC for 1 min and finally an extra final extension step at 72oC for 10 min (Istock *et al*., 2001). Two µl of loading dye were added prior to loading of 10 µl sample per gel slot. Electrophoresis was performed at 100 volt with 0.5 x TBE as running buffer in 1.5% agarose. Gel was stained in 0.5 µg/cm3 (w/v) ethidium bromide solution and distained in deionized water. Finally the gel was visualized and photographed using gel documentation system.

Data obtained by RAPD-PCR DNA band patterns were scored for cluster analysis and dendrogram was constructed on the basis of the presence and absence of the amplified bands for each primer. A band present in jojoba clones was designated (1) and when bands, was used to generate similarity coefficients according to Jaccard (1980). The similarity coefficients were used to construct a dendrogram by UPGMA (Unweighted Pair-Group Method with Arithmetical Averages).

**Table 3.** List of primers name and their nucleotide sequences employed in the RAPD-PCR analysis.

| Primer number | Nucleotide sequence(5′ to 3′) |
|---|---|
| **OPA-02** | TG CGAGCTG |
| **OPA-04** | AATCGGGCTG |
| **OPC-O5** | GATGACCGCC |
| **OPC-09** | CTCACCGTCC |
| **OPO-12** | CAGTGCTGTG |

***Protein profile by sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE)***

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed for total proteins of the ten jojoba clones was carried out in 12% separating gel with a 5% stacking gel according to the method of Laemmli, 1970. The proteins bands were visualized by staining with 0.1% Coomassie brilliant blue R-250. Afterwards, the gels were distained in a methanol-acetic acid-water (3:1:6) mixture until protein bands became clearly visible.

***Statistical Analysis***

Fifty plants per clone were planted in completely randomized block design with five replications. Analysis of variance with SAS software (SAS Institute, 1988) was carried out on the test clones data. Clones' means were compared using the LSD test at 5% level of probability.

**Results and discussion**

**1-Tree volume:** Based on the analysis of variance for growth analysis characters, the results revealed the presence of highly significant differences among genotypes on tree volume at ten jojoba genotypes (Table 4). These indicate that the behavior of the genotypes differed from one to another under the same culture condition so the genotypes differed in their traits. Data in Table 4 showed that tree volume ranged from 2.73$m^3$ (EAI7 at first season) to 7.39 $m^3$ (EAI1 at the second season). No significant differences between the two clones EAI1 and EAI4 were detected for tree volume in both seasons, respectively.

**2-Branch traits:** Table 4 reveal significant differences among ten genotypes in branches vegetative traits (branch length, branch diameter, number of node forming branches and secondary branches length) except the differences between the values of branch diameter in the first season were insignificant.

**2.1: Branch length**: Genotype EAI 1 showing the highest significant branch length (60.60 cm) compared with all other studied clones in the first season. The same situation was observed with genotypes EAI 1 and EAI 4 in the second season as they recorded the maximum significant branch length too (64.38 and63.58 cm, respectively). On the contrary, Genotypes EAI 7 and EAI 9 showed the lowest significantly branch length for the first season (49.53 and

53.67 cm, respectively) and second season (50.85 and 54.57 cm, respectively). Insignificant differences between genotypes EAI 8 and EAI 9 were noticed. Increase in branch length is the result of cell division and elongation.

**2.2: For branch diameter (mm)**, the results in the first season showed insignificant differences between all studied genotypes. From another side, the differences between the studied genotypes in the second season were significant and EAI 1, EAI 3, EAI 4, EAI 6 and EAI 10 had the most thick branches (3.88, 3.52, 3.65, 3.29 and 3.87 mm, respectively) compared with the other studied genotypes.

**2.3**: **Number of node forming branches:** The results in the Table 4 showed that, genotypes EAI 1 and EAI 4 had the highest number of node forming branches (4). No significant differences were found between all studied genotypes except seventh one (EAI 7) which had the lowest value (2.2) in the first season for this trait and significantly differed with first and fourth clones. Moreover, genotype EAI 1, EAI 2, EAI 3 and EAI 4 in the second season gave the highest significant number of branched nodes (5.2, 4.0, 4.4 and 5.0, respectively).While the seventh clone gave the lowest number of branched node (2.8). These results indicated that each genotype has a different genetic character and their responses vary with climatic and soil conditions depending on genotype (Al-Soqeer, 2014).

**2.4: Secondary branches length:** The longest secondary branches were observed in the genotypes EAI 1, EAI 3 and EAI 4 (22.80, 21.0 and 21.4cm, respectively) in the first season and EAI 1, EAI 2, EAI 3 and EAI 4 (25.0, 23.0, 23.4 and 24.4 cm, respectively) in the second season. The shortest secondary branches were found in the genotype EAI 7 (18.2 and 19.6 cm) in both seasons, respectively (Table 4). These results indicated that each clone has a different genetic character and their responses vary under the same conditions depending on its genotype. Genetic differences among genotypes in plant height, number of branched nodes and plant diameters have previously been reported (Botti *et al.*, 1998; Benzioni *et al*., 1999; Tobares *et al*., 2004; Prat *et al*., 2008 and Al-Soqeer 2014).

**Table 4.** The mean of tree volume, branch length, branch diameter, branched nodes, secondary branches length and leaf area for ten jojoba genotypes.

| clones | Tree volume | | branch length(cm) | | branch diameter (mm) | | branched nodes | | secondary branches length(cm) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | First season | Second season | First season | Second season | First season | Second season | First season | Second season | First season | Second season |
| **EAI 1** | 5.86a | 7.39a | 60.60a | 64.38a | 3.50a | 3.88a | 4a | 5.2a | 22.80a | 25.0a |
| **EAI 2** | 3.94bc | 5.39c | 55.10bcd | 60.03c | 3.28a | 3.03bc | 3ab | 4.0abcd | 20.6bc | 23.0abcd |
| **EAI 3** | 4.33b | 6.39b | 55.94bc | 62.34b | 3.03a | 3.52abc | 3ab | 4.4abc | 21.0ab | 23.4abc |
| **EAI 4** | 5.78a | 7.35a | 57.00b | 63.58ab | 3.36a | 3.65ab | 4a | 5.0ab | 21.4ab | 24.4ab |
| **EAI 5** | 3.70c | 4.92cd | 55.02bcd | 58.68cd | 3.27a | 3.12bc | 3ab | 3.8bcd | 20.2bcd | 22.6bcde |
| **EAI 6** | 3.22d | 4.11de | 53.90cde | 57.00de | 3.34a | 3.29abc | 3ab | 3.8bcd | 19.6bcd | 21.8cdef |
| **EAI 7** | 2.73e | 3.55e | 49.53g | 53.67g | 2.85a | 2.89c | 2.2b | 2.8d | 18.2d | 19.6g |
| **EAI8** | 2.91de | 3.82e | 51.94ef | 55.71ef | 3.17a | 2.90c | 3ab | 3.0d | 18.8cd | 20.5efg |
| **EAI 9** | 2.85de | 3.79e | 50.85fg | 54.57fg | 2.98a | 2.93bc | 2.8ab | 3.0d | 18.4 d | 20.0 fg |
| **EAI 10** | 3.07de | 3.57e | 53.66de | 56.40e | 3.09a | 3.87a | 3ab | 3.4cd | 18.8cd | 21.2 defg |

Means in columns followed by the same letter are not statistically different at the 0.05 probability level.

**2.5: Leaf area**: It was found from Table 5 that the differences between plants leaf area were significant. In addition, the largest leaves were found in genotypes EAI4 (5.92 and 6.33for first and second seasons, respectively). The differences between the studied clones from first to seventh clone were insignificant in the first season and the same situation happened in the second season between the clones from first to sixth one. On the other side, the smallest leaf area was observed in genotype EAI10 (3.66 and 4.35 for both seasons, respectively). The differences among genotypes for vegetative traits (branches and leaf measurements) could be explained by the natural growth habits and branching of genotypes, concordant with the experience of Botti *et al*. (1998), Prat *et al*. (2008) and Al-Soqeer, 2014).

**3: Chlorophyll content**

**3.1: Chlorophyll(A) :** Regarding the both seasons, significant differences were observed among the studied clones in the chlorophyll a .the maximum values of chlorophyll a content were found in the first clone (0.876 and 0.898 mg/gm fresh weight) and second clone (0.848 and 0.876 mg/gm fresh weight) in the first and second seasons, respectively. Conversely, the minimum value for chlorophyll a content was observed in the ninth clone

(0.806 and 0.784 mg/gm fresh weight for first and second season, respectively) (Table 5).

**3.2: Chlorophyll (B):** It was observed that the first clone had the highest values of chlorophyll b (0.450 and 0.464mg/gm fresh weight during the first and second seasons, respectively) comparing with the other studied clones (Table 5). While the differences between the clones from first to ninth were insignificant in the first season. The same situation was observed between the first, second, third, fourth, sixth and seventh clone in the second one.

The average value for chlorophyll a content of ten clones (0.8321 and 0.8344mg/gm fresh weight for both season, respectively) and those of chlorophyll b (0.4140 and 0.4142 mg/gm fresh weight) were similar to the values reported by Ali et al., 2013 (0.800 and 0.400 mg/gm fresh weight for chlorophyll a and b, respectively.)

**Table 5.** The mean of leaf area, chlorophyll (A) and chlorophyll (B) for ten jojoba genotypes.

| Clones | leaf area($cm^2$) | | Chlorophyll(A) mg/gm | | Chlorophyll(B) mg/gm | |
|---|---|---|---|---|---|---|
| | First season | Second season | First season | Second season | First season | Second season |
| EAI 1 | 5.15ab | 5.75ab | 0.876a | 0.898a | 0.450a | **0.464a** |
| EAI 2 | 5.05ab | 5.19ab | 0.848ab | 0.876ab | 0.446a | **0.450ab** |
| EAI 3 | 5.06ab | 5.07ab | 0.8460ab | 0.856ab | 0.426ab | **0.438abc** |
| EAI 4 | 5.92a | 6.33a | 0.8360ab | 0.856ab | 0.432ab | **0.426abcd** |
| EAI 5 | 4.82ab | 4.96ab | 0.8312ab | 0.854ab | 0.408ab | **0.398bcd** |
| EAI 6 | 4.68ab | 4.82ab | 0.800b | 0.772b | 0.396ab | **0.408abcd** |
| EAI 7 | 4.42ab | 4.59b | 0.819ab | 0.824ab | 0.400ab | **0.416abcd** |
| EAI8 | 3.92b | 4.72b | 0.819b | 0.822ab | 0.416ab | **0.390bcd** |
| EAI 9 | 4.30b | 4.68b | 0.806b | 0.784b | 0.392ab | **0.384cd** |
| EAI 10 | 3.66b | 4.35b | 0.828b | 0.802ab | 0.374b | **0.368d** |

Means in columns followed by the same letter are not statistically different at the 0.05 probability level.

**4: Reproductive parameters**: Significant differences were presented among the clones in all reproductive parameters measured including flowering date, flowering percentage, fruit set percentage and plant seed yield.

**4.1: Flowering date**: Flowering date varied among the clones. Clone EAI 1 reached fifty percentage of flowering after 34.80 and 32.80 days (for

both season, respectively) from the beginning of January, following that clone EAI 4, EAI 3 then EAI 2 do that after 40.00, 44.20 and 47.40 days, respectively in the first season and the same situation occurred in the second season after 38.80, 42.00 and 44.00, respectively. While the clones EAI 9 had the longest period to reach fifty percent of flowering (67.40 and 64.40 day for first and second season, respectively) (Table 6). These results indicated that each genotype has a different genetic character and their responses vary with climatic and soil conditions depending on genotype (Al-Soqeer, 2014). Dormancy of jojoba flower buds is broken by exposure to temperatures between 5 and 20 $^{o}$C (Dunstone, 1980). Clones differ in the duration of low temperature required to break dormancy. Flower buds release from dormancy will complete morphogenesis and proceed to anthesis only if water is available and plants have accumulated a sufficient heat sum (Benzioni and Dunstone, 1985 and Ferriere *et al.*, 1989). Clones vary in their chilling requirement, which may affect time of anthesis. Environmental conditions known to affect the time of anthesis include radiation level and availability of nutrients in the soil (Benzioni and Nerd, 1989 and Dunstone 1988). Jojoba clones differ greatly in their chilling demands (Ferriere *et al.*, 1989 and Benzioni *et al.*, 1992). From that the clones such as EAI 1 its chilling requirement was presumably quite small so flowered earlier than the others. But on the contrary, some clones such as EAI 9 is needing very high chilling requirement so flowering late. Beside that, some clone such as EAI 5 had a moderate chilling requirement so that its flowering date was moderate between first and second mentioned groups and that was reflected on the other reproductive characters as shown in Table 6.

**4.2: Flowering percentage**: In the first season, the first and seventh clone recorded the highest and lowest percents of flowering (45.65 and 36.84 %, respectively) compared with all studied clones but the differences between the first, third and fourth clones were insignificant. Moreover, the first clones recorded the highest significant values for flowering percentage (49.24 %) in the second season. Add to that, clone EAI 7 had the minimum value for flowering percentage (42.38%) with insignificant differences between it and EAI 8, EAI 9 and EAI 10 clones (Table 6).

**4.3: Fruit set percentage:** Maximum fruit set percentages (95.30 and 96.17 %) were observed in the first clone for both seasons, respectively. The differences among first and fourth clones for fruit set percentage in the first season and among first, third and fourth clones in the second season were insignificant (Table 6). There were significant differences between abovementioned clones on one side and the other clones on the other side in each season alone. The smallest fruit set percentage values were noticed at seventh clone (89.74 and 84.11% for both seasons, respectively).

**4.4: Seed yield plant$^{-1}$**: The highest significant seed yield plant$^{-1}$(2600.40 and 2756.20 gm) was found in the first clone during the first and second season, respectively. From the other hand, the ninth clone had the lowest values regarding plant seed yield (1376.60 and 1424.40 gm/ plant for both seasons, respectively) compared with the other studied clones in both season, respectively. No significant differences were found among the clones sixth, seventh, eighth, ninth and tenth in the first season and between seventh, eighth, ninth and tenth clones in the second one (Table 6).

**Table 6.** The mean of flowering date, final fruit set, flowering percentage and seed yield /g/plant for ten jojoba genotypes.

| Clones | Flowering date | | Flowering percentage | | Final Fruit Set % | | Seed yield /g/plant | |
|---|---|---|---|---|---|---|---|---|
| | First season | second season | First season | second season | First season | second season | First season | second season |
| **EAI 1** | 34.80i | 32.80h | 45.65a | 49.24a | 95.30 a | 96.17a | 2600.40 a | 2756.20 a |
| **EAI 2** | 47.40f | 44.00e | 43.63b | 45.92bc | 92.35c | 94.42bc | 1589.20 d | 2244.80 c |
| **EAI 3** | 44.20g | 42.00f | 44.20ab | 46.60bc | 93.37b | 95.05ab | 1771.40 c | 2402.00bc |
| **EAI 4** | 40.00h | 38.80g | 45.12a | 47.50b | 94.55a | 95.82a | 2182.20 b | 2492.20 b |
| **EAI 5** | 50.60e | 46.60d | 43.33bc | 45.60cd | 92.05c | 94.12bc | 1580.40 d | 1984.40 d |
| **EAI 6** | 55.40d | 52.20c | 42.10cd | 44.10de | 91.63c | 93.98bc | 1401.60 e | 1747.00 e |
| **EAI 7** | 65.20b | 62.80a | 36.84f | 42.38f | 89.74 e | 84.11f | 1385.60 e | 1425.60 f |
| **EAI8** | 63.80b | 60.00b | 39.06e | 42.60ef | 90.02de | 91.91 d | 1387.60 e | 1474.60 f |
| **EAI 9** | 67.40a | 64.40a | 38.46e | 42.42f | 89.81de | 86.23e | 1376.60 e | 1424.40 f |
| **EAI 10** | 60.40c | 58.40b | 40.80d | 43.98ef | 90.68d | 93.43c | 1395.60 e | 1524.40 f |

Means in columns followed by the same letter are not statistically different at the 0.05 probability level.

The best vegetative growth traits which observed in the first and fourth clones contributed to the increase of the final fruit set, flowering percentage and the seed yield plant$^{-1}$. This results are in harmony with Benzioni *et al*. (1999) where they found that some clones exhibited excellent vegetative traits related to yield potential, such as, rapid growth and extensive branching .In previous studies, Mckelvie *et al.*, (1994) reported that yields of the new jojoba varieties, at an average density of 1250 plants per hectare, were yield at least 0.2 tons of seed per hectare in fourth year, gradually increased to 1.6 tons of seed per

hectare by twelfth year. After twelfth year, yields were expected to remain fairly constant. Ulger *et al.* (2002) found that seed yield of jojoba plants ranged from 0.02 to 0.5 kg per plant from the fourth year in Alata, Mersin, Turkey. Dunstone and Begg (1983) indicated that the first significant harvest was possible after four years from planting with yields of about 100-200 g per female plant. Ayerza (1996) and Benzioni *et al.* (1996) in Argentina and Israel, respectively, found that yields fluctuated between 705 and 148 g $Plant^{-1}$ in the third year of growth. Osman and Abo Hassan (2013) reported that average seed yield varied from 0.18 to 0.59 kg $plant^{-1}$ at Hail region in the fourth and fifth year, respectively. It was noteworthy that seed yield in our results exceeded the range of seed yield recorded in other studies.

**5: Mean seed components (Lipids, Proteins, Minerals and Carbohydrates):** The obtained data indicated that, the studied clones showed significant differences among them in lipids, proteins, minerals and carbohydrates, in both seasons except the differences between the percents of minerals in the first season were insignificant (Table 6).

**5.1: Seed oil content:** Jojoba is mainly considered a bi-purpose material and is used for oil extraction. The results in Table 7 revealed the presence of significant differences among the studied clones considering lipids and proteins. These variations may be attributed to genetic variability (Ayerza, 2001).The maximum mean value of lipids content was detected at the first clone (50.77 and 51.02%) during the first and second season, respectively compared with the other studied clones. Insignificant differences observed between the first, second, third and fourth clones in the first and second seasons. From the other hand, the minimum mean value of lipids was noticed in the tenth clone (48.31 and 47.72%) in both seasons, respectively (Table 7).The average value for lipids content varied from 48.31% (for genotype EAI 10 in the first season) to 51.02% ( for genotype EAI 1 in the second season). These values were in similar with the values reported by Perez-Gil *et al.* (1989) and Cappillino *et al.* (2003) (48.89% and 53.2%, respectively). As previously mentioned, the interest in jojoba seeds production is focused on the quantity and quality of their oil. Each clone showed a characteristic chemical composition depending on its particular genetic. Therefore, it was expected that different genome expressions were observed in the chemical parameters analyzed (Gayol *et al.*, 2004).

**Table 7.** The mean of carbohydrates, minerals, lipids and proteins for ten jojoba genotypes.

| Clones | Lipids% | | Proteins% | | Minerals% | | Carbohydrates% | |
|---|---|---|---|---|---|---|---|---|
| | First season | Second season | First season | Second season | First season | Second season | First season | Second season |
| EAI 1 | 50.77a | 51.02 a | 28.66bc | 29.48bc | 1.57a | 1.63de | 18.18de | **19.26cd** |
| EAI 2 | 49.95abc | 49.34abcd | 28.10bc | 29.20c | 1.51a | 1.65d | 21.80a | **20.36abcd** |
| EAI 3 | 50.03ab | 49.96 abc | 26.97c | 26.66d | 1.72a | 1.73b | 19.13cd | **20.98abc** |
| EAI 4 | 50.11ab | 50.22ab | 28.01bc | 27.36d | 1.63a | 1.66cd | 20.90ab | **20.11bcd** |
| EAI 5 | 49.3 bcd | 49.10bcd | 29.50b | 30.26b | 1.62a | 1.81a | 16.11f | **19.68bcd** |
| EAI 6 | 48.89 cd | 49.04bcd | 31.52a | 32.88a | 1.70a | 1.64d | 19.33cd | **17.80d** |
| EAI 7 | 48.85 cd | 48.34 cd | 28.29bc | 29.58bc | 1.53a | 1.75ab | 20.04bc | **22.94a** |
| EAI8 | 48.79 d | 48.08 d | 29.40b | 30.18b | 1.47a | 1.75ab | 19.46bcd | **20.77abc** |
| EAI 9 | 48.71 d | 48.02 d | 27.32c | 27.39d | 1.44a | 1.57e | 22.36a | **22.12ab** |
| EAI10 | 48.31 | 47.72 d | 32.47a | 33.14a | 1.46a | 1.72bc | 17.11ef | **18.00d** |

Means in columns followed by the same letter are not statistically different at the 0.05 probability level.

**5.2: Seed protein content:** There were significant differences among the analyzed clones in protein content. Regarding the first season the maximum significant values of protein content was observed in the tenth (32.47and 33.14a %) and sixth (31.52 and 32.88%) clones for both seasons, respectively with insignificant differences between them. On the other side, the minimum values were observed in the third clone (26.97 and 26.66%) compared with the other studied clones in both seasons, respectively. As mentioned above the average protein content ranged from 26.97 to32.47% in the first season and from 26.66 to 33.14 % in the second one (for the third and tenth clones, respectively) these values were higher than the values informed by Cappillino *et al.*, 2003 (15,2%) and by Wisniak, (1987) (14.9%).

**5.3: The minerals content** was varied from 1.44% (clone EAI 9) to 1.72% (EAI 3) in the first season. While in the second one, varied from 1.57% (clone EAI 9) to 1.81% (clone EAI 5) (Table 7). Moreover, the mineral content was similar to the results reported by Al-Soqeer *et al.* (2012) where they found that mineral content ranged from (1.61 to 1.93%) in their study.

**5.3: Seed carbohydrates content %:** During the first season, the ninth clone had the highest percentage of carbohydrate (22.36%) with insignificant differences between second, fourth and ninth clones. The same

situation was noticed at the seventh clone which recorded the maximum value of carbohydrate content (22.94%) in the second season and no significant differences were detected between second, third, seventh, eighth and ninth clones in the same season too. From the other hand, the lowest value of carbohydrate percent was found in the fifth and sixth clones (16.11 and 17.8% during first and second seasons, respectively) (Table 7). These differences in carbohydrate percent value due to the differences between clones are in agreement with Al-Soqeer *et al.* (2012).

**6: The phenotypic correlation coefficients** among different plant characters of ten jojoba genotypes are presented in Table 8. There was highly significant positive correlation of seed yield between and each of flowering date, final fruit set %, flowering percentage %, tree volume (m), branch length (cm), number of nodes forming branches and secondary branches length (cm). These results reveal that selection should be practiced for flowering date, final fruit Set %, flowering percentage %, tree volume (m), branch length (cm), branched nodes and secondary branch length (cm) genotypes to select high yielding jojoba genotypes.

The same results were observed between seed oil content and each ofseed yield**,** final fruit set %, flowering percentage %, tree volume (m), branch length (cm), number of nodes forming branches and secondary branch length (cm). These results obvious that selection should be practiced for seed yield**,** final fruit set %, flowering percentage %, tree volume (m), branch length (cm), number of nodes forming branches and secondary branches length (cm) (Table 8).

**Table 8.** Simple correlation coefficients of various characters among ten jojoba genotypes.

| | Parameters | Seed yield | | Oil content | |
|---|---|---|---|---|---|
| | | **First season** | **Second season** | **First season** | **Second season** |
| **1** | Tree volume (m) | 0.853 ** | 0.884 ** | 0.615 ** | **0.598 ** ** |
| **2** | Branch length(cm) | 0.803 ** | 0.894 ** | 0.490 ** | **0.572 ** ** |
| **3** | Branch diameter(cm) | -0.076 | -0.189 | -0.105 | **-0.177** |
| **4** | Number of nodes forming branches | 0.339 ** | 0.651 ** | 0.174 | **0.446 ** ** |
| **5** | Secondary branches length(cm) | 0.566 ** | 0.752 ** | 0.594 ** | **0.411 ** ** |
| **6** | Leaf area ($cm^2$) | 0.104 | 0.045 | 0.136 | **0.102** |
| **7** | Flowering date | -0.709 ** | -0.885 ** | -0.655 ** | **-0.546 ** ** |
| **8** | Flowering percentage % | 0.681 ** | 0.846 ** | 0.531 ** | **0.579 ** ** |
| **9** | Final Fruit Set % | 0.831 ** | 0.709 ** | 0.646 ** | **0.505 ** ** |
| **10** | Seed yield(g) | - | - | 0.613 ** | **0.543 ** ** |

**7: Fingerprinting of ten jojoba clones Using Random Amplified Polymorphic DNA (RAPD):** Using five primers in RAPD-PCR showed clear difference among the ten studied ten jojoba clones on the basis of amplified product band patterns observed with each primer. The amplification profiles with the primers and are shown in Figure 1. All of these primers succeeded to give polymorphic patterns among jojoba clones. Also, high similarity was observed between 1 and 4 jojoba clones (These two jojoba clones were moderately in protein content) and 2 and 3 jojoba clones (These two jojoba clones were low in protein content) followed by 6 and 10 jojoba clones (These two jojoba clones were high in protein content).

***Cluster analysis of RAPD results***

The RAPD band patterns were analyzed using UPGMA method to generate a dendrogram indicating the relationship between ten jojoba clones. The presence or absence of any particular DNA bands was the only factor considered in the computer analysis. The generated dendrogram showed linkage distance (Figure2) indicating that the ten jojoba clones were classified into two main clusters. Cluster A includes two sub-clusters. Sub- cluster 1 was divided into two groups. Group 1 includes clone7, and 9. Group 2 includes clone 8. Sub- cluster 2 includes clones 6 and 10.Cluster B includes two sub-clusters, sub- cluster 1 was divided into two groups, group 1 includes clones 1and 4. Group 2 includes clones 2 and 3, sub- cluster 2 includes clone 5.

***The differences in total protein band in the jojoba clones***

Figure 3 showed the differences in total protein bands in ten jojoba clones. New bands were appeared; also intensity was increased in some clones compared with each other. Jojoba clones1 and 2 increased the intensity band at 80 kDa. 7 and 8 clones showed new band at 90kDa, while5, 6 and 10 clones had new band at 65kDa.

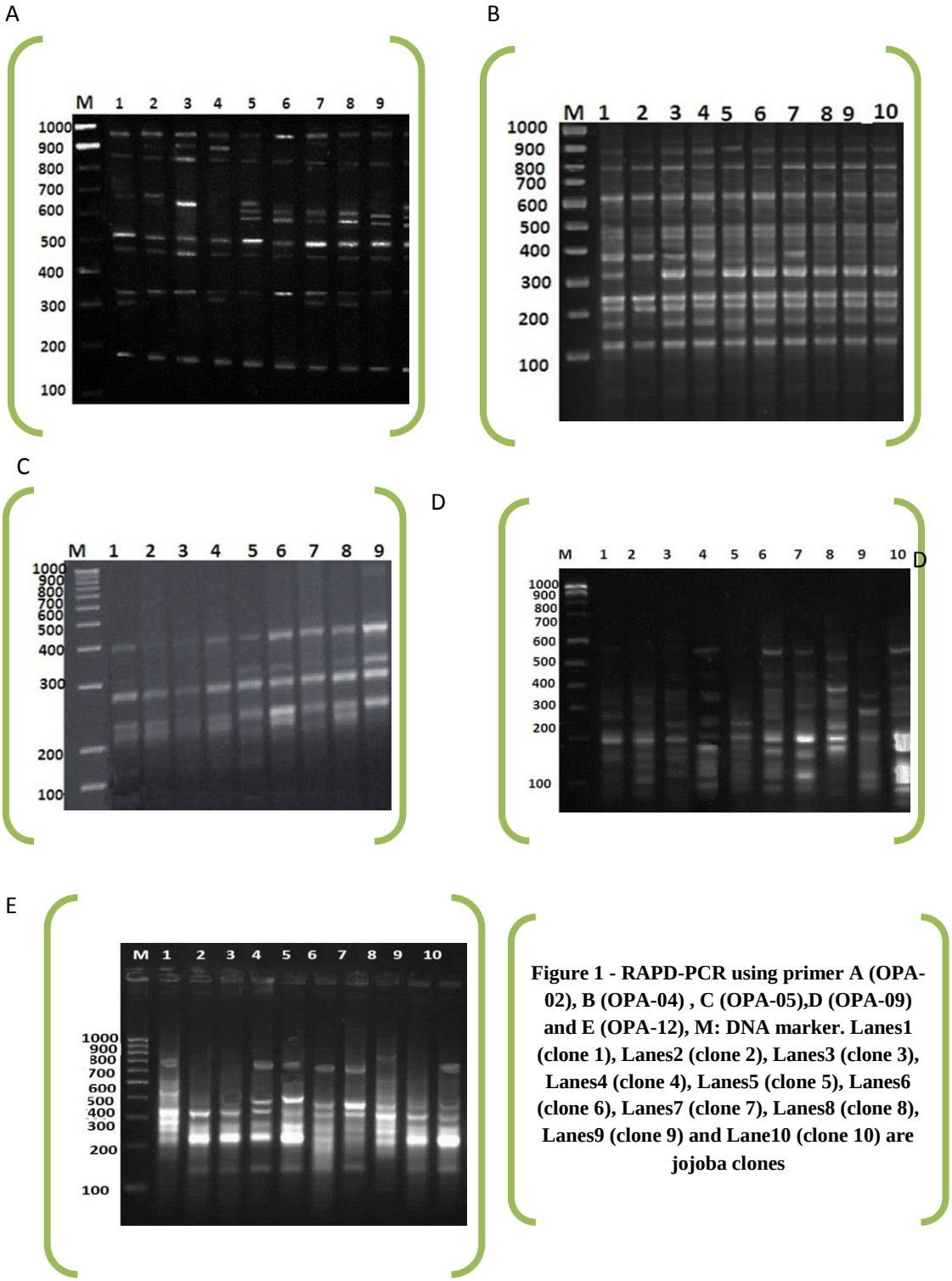


**Figure 1 - RAPD-PCR using primer A (OPA-02), B (OPA-04) , C (OPA-05),D (OPA-09) and E (OPA-12), M: DNA marker. Lanes1 (clone 1), Lanes2 (clone 2), Lanes3 (clone 3), Lanes4 (clone 4), Lanes5 (clone 5), Lanes6 (clone 6), Lanes7 (clone 7), Lanes8 (clone 8), Lanes9 (clone 9) and Lane10 (clone 10) are jojoba clones**

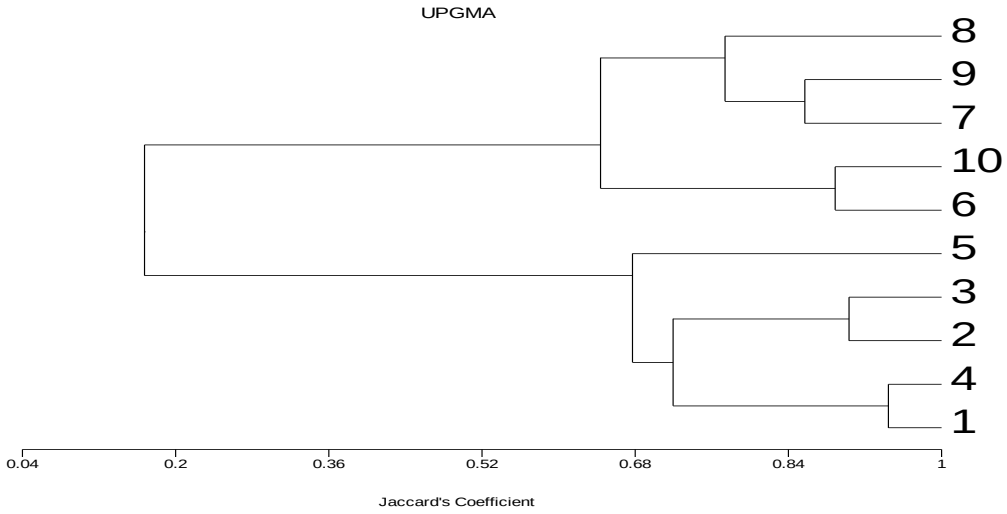


**Figure 2.** Dendrogram obtained by clustering (UPGMA method) based on the band pattern obtained by the RAPD-PCR analysis for ten jojoba clones according to Jaccard index

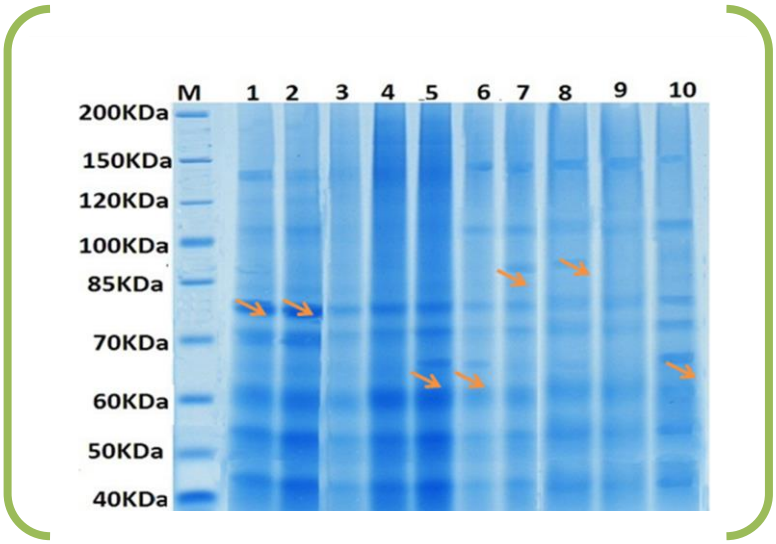


**Figure** 3. The SDS-PAGE of total protein profile of ten jojoba clones) protein molecular weight standard, Lanes 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 are jojoba clones

## Conclusion

The variations observed in the studied characters were principally due to clone differences. It is concluded that jojoba shows good establishment under El-Behera region of Egypt. Genotypes EAI 1 and EAI 4 recorded the highest value for plant vegetative and flowering growth, plant seed yield, fruit set percentage and seed oil content. For proteins content, genotype EAI 10 and EAI 6 had the highest values. For seed carbohydrates content, clone EAI 9 and EAI 7 recorded the highest value in the first and second seasons, respectively. First and second genotype recorded the highest chlorophyll content. Moreover, the genotypes EAI 1 and EAI 4 flowered early. The results obtained in this work indicated that there is a large genetic variability among jojoba clones established at El-Behara Region, which could permit improvement by selection and breeding. That was seen in the results of cluster analysis of RAPD results which indicated that there is genetic rapprochement between first and fourth clones because they were found in the same sub-cluster using RAPD primers.The results of this study revealed that clones EAI 1 and EAI 4 recommended for commercial production in El-Behara Region.

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