

DEVELOPMENT OF AFLP, ISSR AND RAPD MARKERS FOR HIGH YIELD-RELATED TRAITS IN JOJOBA

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Jojoba (*Simmondsia chinensis*) is a long-lived dioecious evergreen shrub of arid zones with a life span of around 100 years; which has been vegetated as a cash crop. It is a native of Sonoran deserts of the South-Western United States of America, North-western Mexico and Baja California (Gentry, 1958; Benzioni, 1992). As a dioecious plant species, jojoba is obligatory outcrossing and consequently displays high genetic variability (Parker, 1990). The outbreeding of jojoba has given rise to highly heterogeneous seeds that supply us with a vast range of hybrid vigor and fertility.

Egypt produced 25 tons of jojoba oil resulted from 500 feddens. One square meter cultivated with jojoba giving cash return of 3 to 4€ in comparison with cotton which gained 0.75€. Consumption of mineral oil in Egypt recorded 400,000 tons/year, therefore, jojoba introduce a strategy solving these increments. Meanwhile, one hectare cultivated with jojoba yield an average of 1.64 tons of fruits in the Arabian countries, 4.56 tons in the European countries and 5.19 tons in the

USA (FAOstat, 2013). The least price of jojoba oil (10,000\$ /tones) that could be obtained from 3 or 4 feddens. Therefore, the successful improvement of jojoba industry depends on selection of high yielding genotypes, their multiplication through vegetative means and boosting yields by increasing fruit-bearing plants per unit area.

Morphological markers are based on visually accessible traits such as flower color, seed shape, growth habits, and pigmentation, and it does not require expensive technology but large tracts of land area are often required for these field experiments, making it possibly more expensive than molecular assessment. Agromorphological traits can help to enhance selection efficiency in crop improvement. Therefore, the evaluation and characterization on the basis of morphological and agronomic traits are considered the starting point of any breeding program (Fundora, 1998).

DNA markers offer several advantages in plant identification and genet-

ic studies over morphological markers. In comparison with morphological markers, DNA markers are variable and offer a higher level of allelic divergence, neutral to the environmental stresses, developmental stages and, not affected by other factors and genes (Karaca *et al.*, 2004). Molecular markers have protruded as a ravishing technology for estimating genetic fidelity (Joshi and Dhawan, 2007), molecular phylogenetics (Droogenbroeck *et al.*, 2004), genetic variations (Parsons *et al.*, 1997; Kremer *et al.*, 2005; Vijayan *et al.*, 2004; Atia *et al.*, 2016; Mokhtar and Atia, 2018), and disease resistance (Blaszczyk *et al.*, 2005; Perez de Castro *et al.*, 2007).

Although, molecular markers technology was employed successfully for characterization and identification of jojoba germplasm (Agarwal *et al.*, 2011; Sharma *et al.*, 2008); but, no one of those published reports assessed the genetic diversity or molecular characterization of jojoba strains in Egypt in term of yield improvement.

Therefore, the present study addresses an important area of jojoba genomics through combining different molecular marker techniques which are very powerful in the detection of polymorphism i.e. AFLP, ISSR and RAPD, associated with agronomical and morphological traits in order to develop specific markers that can be used to identify superior jojoba strains in early stages of growth.

MATERIALS AND METHODS

Plant Material

Fifty female strains of jojoba were selected from two different orchard locations (El-Kasasin and Cairo-Alexandria Desert road) in Egypt.

Evaluation of morphological and yield components traits

The selected strains were evaluated for their morphological, seeds characteristics and yield for two subsequent seasons (2015/2016) based on the following parameters:

- Morphological characteristics: average no. of branches, average leaf area (cm²), leaf fresh weight (g), leaf dry weight (g), average shoot length (cm), average leaf no./shoot, average no. internodes.
- Yield and seeds characteristics: Average seed length (cm), average seed diameter (mm), average seed weight (g), average seed volume, number of ridges per seed and seed yield/plant (kg).

Genomic DNA Extraction

Six jojoba strains representing the most extremes (highest and lowest) strains for each trait (Yield and average seed weight traits) were selected for molecular analysis. Fresh leaves samples from selected jojoba strains were collected to be used in high-quality genomic DNA extraction. The DNA extraction was

done using a DNAeasy Plant Mini Kit (QIAGEN, Santa Clarita, CA) according to the manufacturer's protocol.

AFLP analysis

The AFLP analysis was performed using the AFLP® Analysis System II (Invitrogen, USA) as described by Vos *et al.* (1995). Initially, 26 AFLP primer combinations (PCs) were tested against the DNA of the selected jojoba strains on agarose gel in order to check their amplification successfulness. Among these, only eight PCs given amplified products with a clear pattern. Therefore, these eight PCs were subsequently run on AFLP sequencing gels.

ISSR analysis

A total of 16 ISSR primers were tested against the DNA of the selected jojoba strains. The ISSR-PCR reaction primers were performed as described by Sharma *et al.* (2008).

RAPD analysis

RAPD-PCR was carried out as described by Adawy and Atia (2014). A set of 30 random 10-mer primers were applied against the selected jojoba strains in order to screening the polymorphism between and among them.

Data analysis

The generated/ amplified bands were scored visually. The bands were scored as present (1) or absent (0) to cre-

ate the binary data set. Polymorphism percentage was calculated by dividing number of amplified polymorphic bands by the total number of amplified bands by the same primer or primer combination. To estimate the genetic similarity, Dice coefficient was used (Jackson *et al.*, 2005). A dendrogram was generated by cluster analysis using the un-weighted pair group method of the arithmetic averages (UPGMA) for all different marker systems.

RESULTS AND DISCUSSION

During the last decades, the researchers interested in jojoba mostly selected superior female plants on the basis of many favorable traits such as natural resistance to pests, high harvest possibility, unique oil properties, and a strong plant structure. But, to the best of our knowledge no systematic or successful efforts have been undertaken yet to improve seed yield, oil and fatty acid content in jojoba.

Evaluation of morphological and yield components traits

Based on the evaluation of fifty female strains for their morphological, seeds characteristics and yield traits (seasons 2015/2016), six jojoba strains representing the most contrasting strains for each trait (Yield and average seed weight traits) were selected for molecular analysis (Table 1). Table (1) summarize the average values for the two seasons of morphological, seed characteristics and

yield components traits of the twelve selected jojoba strains.

The evaluation data for yield trait demonstrated that strain no. 8 exhibited the highest yield (7.700 kg) followed by strain no. 7 with (7.150 kg). While, strain no. 9 produced the lowest yield with (0.400 kg).

For the seed weight, strain no. 8, also, exhibited the highest average seed weight with (1.85 g/seed). While, strain no. 10 recorded the lowest average weight of seeds with (0.84 g/seed). In this respect, since the viability of seeds with weight less than one gram is often weak, moreover, their oil content was very low. Therefore, the selection of strains producing seeds with average weight greater than or equal one gram, will be preferable.

For better understanding the relationships between morphological, seed characteristics traits and yield, correlation coefficient between these traits and yield were estimated (Fig. 1). The correlation coefficient results revealed that the highest positive correlation was observed between average leaf area trait and yield (0.65) followed by leaf dry weight trait and yield (0.57). While, the lowest positive correlation was observed between average seed length trait and yield (0.14). On the other hand, the highest negative correlation was found between average seed diameter trait and yield (-0.38) followed by average shoot length trait and

yield (-0.31). While, the lowest negative correlation was observed between average seed weight trait and yield (-0.07) (Fig. 1).

Due to the wide variations in seed yield, selection of female plants is most important for increasing yields in future jojoba populations. Earlier efforts were made by the Central Salt and Marine Chemicals Research Institute (CSMCRI) to selecting jojoba female plants and exhibited continuous better performance over more than two decades. Besides high yield, the selected plants also had higher seed weight. They studied the connection between seed yield and other constituent traits, and correlation coefficients were estimated for all potential combinations.

In this context, earlier in the 1980s, many researchers (Abramovich *et al.*, 1976; Ramonet and Morales 1985; Yermanos, 1983) reported that there was no morphological trait recorded a considerable relationship with total output in jojoba. Whereas, Forti *et al.* (1985) reported an inverse correlation between plant volume and fruit yield in jojoba. Few years later, Ramonet-Razcon (1988) reported that there was a high relationship between the number of seeds per plant and seed production. At the same time, Purcell and Purcell (1988) observed large variations in jojoba plantations raised in Australia and made attempts to improve the species for higher yield. They made unsuccessful attempts to increase fruit set in jojoba through artificial pollination. A significant positive correlation between

seed yield and morphological traits were also documented (Chikara and Kumari, 1991).

Furthermore in Argentina, Tobares *et al.* (2004) conduct experiments to characterize superior jojoba genotypes and identify the agronomical practices that affect significantly growth, oil content and seed output. They studied both agronomical and chemical characteristics as descriptors to distinguish and select the jojoba clones using seed weight, yield, and wax content, which could allow for improvement by breeding and selection.

AFLP, ISSR and RAPD analysis

To ensure further development of jojoba as a commercial crop, it is crucial to identify the factors that contribute to the extreme variability between genotypes. Multidisciplinary approaches based on molecular genetics, biochemistry and agronomy are expected to provide accurate information to identify the genotypes with stabilized yields under various production systems. In particular, the generated DNA profiles or fingerprints of promising clones will facilitate the strategic planning to maximize the rate of improvement with the aim of choosing superior jojoba genotypes.

Screening of the selected twelve jojoba female strains using eight AFLP, 16 ISSR and 30 RAPD primers/primer combinations generated a total of 994 amplicons (of yield trait extremes individuals) and 976 amplicons (of seed

weight trait extremes individuals) (Table 2).

For AFLP analysis, twelve strains representing both yield and seed weight traits extremes (six strains for each trait) were amplified using eight AFLP primer combinations. In regard of yield, the eight AFLP PCs produced a total of 531 scorable bands, from which 149 bands (28.06%) were found to be polymorphic with an average 19 bands/PC (Table 2). The number of amplified DNA fragments per primer combination ranged from 42 bands (primer combination 6/4) to 89 bands (primer combination 2/2) and the percentage of polymorphism ranged from 14.2% (primer combination 6/4) to 42.7% (primer combination 2/2). While, regarding the seed weight trait, the eight AFLP PCs generated a total of 524 scorable bands, out of which 142 (27.09%) were found to be polymorphic with an average of 18 bands/PC (Table 2). The number of amplified DNA fragments per primer combination ranged from 42 bands (primer combination 6/4) to 91 bands (primer combination 2/2) and the percentage of polymorphism ranged from 14.2% (primer combination 6/4) to 43.9% (primer combination 2/2).

For ISSR analysis, in term of yield trait, the 16 ISSR primers produced a total of 138 scorable bands, out of which 49 bands (35.51%) were found to be polymorphic with an average 3 bands/primer (Table 2). The number of amplified DNA fragments per primer ranged from 3 bands (primer ISSR-5) to 12 bands (primers R1

and R8) and the percentage of polymorphism ranged from 0.0% (primers R2, R3, ISSR-2 and ISSR-5) to 63.6% (primer R7). On the other hand, in term of evaluating the extremes strains of the seed weight trait, they yielded a total of 135 scorable bands, out of which 42 (31.1%) were found to be polymorphic with an average of 3 bands/primer (Table 2). The number of amplified DNA fragments per primer ranged from 3 bands (primer ISSR-5) to 12 bands (primer R1) and the percentage of polymorphism ranged from 0.0% (primers R2, R6, R9, ISSR-2 and ISSR-5) to 66.6% (primer ISSR-9).

For RAPD analysis, the RAPD analysis of the yield extremes generated a total of 325 scorable bands, out of which 116 (35.6%) was found to be polymorphic with an average of 4 bands/primer (Table 2). The number of amplified DNA fragments per primer ranged from 5 bands (primer C3) to 16 bands (primers B17 and B18) and the percentage of polymorphism ranged from 0.0% (primers A3, C3 and C6) to 87.5% (primer B14). Meanwhile, the RAPD analysis of the seed weight trait extremes produced a total of 317 scorable bands, out of which 108 (34.0%) was found to be polymorphic with an average of 4 bands/primer. The number of amplified DNA fragments per primer ranged from 5 bands (primer C3) to 16 bands (primer B17 and B19) and the percentage of polymorphism ranged from 0.0% (primers A3, C3 and C6) to 75.0% (primer B14) (Table 2).

Molecular phylogeny of jojoba strains

A dendrogram based on UPGMA analysis of AFLP, ISSR, RAPD and combined data was constructed for both yield and seed weight extreme groups (Fig. 2).

For yield, the AFLP dendrogram comprised two main clusters; the first cluster grouped the yield superior strains (2, 7 and 8) in addition to strains 4 and 9. While, the second cluster comprised only the strain no. 13. The ISSR dendrogram comprised two main clusters; the first cluster grouped all yield superior strains (2, 7 and 8) in addition to strains 4. While, the second cluster comprised strain no. 9 and 13. The RAPD dendrogram comprised two main clusters, the first cluster grouped all yield superior strains (2, 7 and 8) in one subcluster, while, strains no. 4 and 9 were comprised in the second subcluster. The second cluster comprised only strain no. 13. Finally, the combined data dendrogram comprised two clusters, the first cluster grouped all yield superior strains (2, 7 and 8) in addition to strains 4 in one subcluster, while the second subcluster included strain no. 9. Whereas, the second cluster comprised only strain no. 13.

For seed weight, AFLP dendrogram comprised two main clusters, the first cluster grouped all superior strains for seed weight (5, 12 and 15) while the second cluster comprised the opposite extreme strains (1, 10 and 11). The ISSR dendrogram comprised two main clusters; the first cluster grouped the seed weight superior strains (5 and 12) in

addition to strains 1 and 11. While, the second cluster comprised strain no. 10 and 15. The RAPD dendrogram comprised two clusters, the first cluster includes two subclusters. The first subcluster grouped seed weight superior strains 5 and 12 in addition to strain no. 11. While, second subcluster included strains no. 1 and 10. Meanwhile, the second cluster included only strain no. 15. Finally, the combined data dendrogram comprised two main clusters, the first cluster grouped all superior strains for seed weight (5, 12 and 15) while the second cluster comprised the opposite extreme strains (1, 10 and 11).

In this context, selection based on molecular marker method relies on reproducibility and simplicity of the technique. The best-selected marker system should have minimum of both cost and labor needed as well as elevated precision and reliability. In this context, Bhardwaj *et al.* (2010) compare the efficiency and utility of ISSR and RAPD for detecting genetic polymorphism between 10 jojoba genotypes. They found that both ISSR and RAPD techniques were relatively informative in assessing the genetic diversity as well as genetic relationships among and within male and female plants of jojoba. They also reported that ISSR marker system generated a higher level of polymorphism in comparison to RAPDs system in jojoba. More recently, Arya *et al.* (2016) conducted a study aimed to correlate the molecular data of RAPD analysis with the phytochemical characters of 18 jojoba accessions at the intraspecific lev-

el. They reported that the evaluation of diversity with RAPD system combined with the analysis of fatty acids can reflect the most promising genotypes and consequently help in improvement of breeding programs and various commercial applications.

Superior jojoba strains identification by unique markers

The unique positive markers (UPM) were considered when band was present at least in two of the superior strains of yield or seed weight and absent in all opposite extreme strains. Meanwhile, unique negative markers (UNM) were considered when band was present at least in two of the opposite extreme strains and absent in all superior strains of yield or seed weight.

For yield, one AFLP PCs, three ISSR primers and six RAPD primers successfully identified 1, 4 and 7 unique positive markers, respectively. While, 2 AFLP PCs, 4 ISSR primers and 8 RAPD primers identified 4, 4 and 8 unique negative markers, respectively.

For seed weight, 4 AFLP PCs, 2 ISSR primers and 5 RAPD primers successfully identified 13, 3 and 5 unique positive markers, respectively. While, 6 AFLP PCs, 2 ISSR primers and 3 RAPD primers identified 14, 2 and 5 unique negative markers, respectively (Table 3).

Finally, fifty jojoba genotypes were evaluated for 13 morpho-agronomical traits. Two set of extreme

plants in term of yield and seed weight traits were molecularly characterized using 8 AFLP, 16 ISSR and 30 RAPD primers/primer combinations. These employed markers collectively revealed 28 unique (positive and negative) markers for yield trait and 42 unique (positive and negative) markers for seed weight trait. These developed unique markers represent promising milestones which can be used to identify superior jojoba genotypes in early stages of growth.

SUMMARY

Jojoba is an economic oil crop as its seeds store liquid wax (40-60% of dry weight). Due to wide variations in yield, selection of superior female plants is a big challenge to increase the yield in future populations. In this study, fifty female jojoba strains were evaluated for 13 traits representing morphology, seed characteristics and yield. Six strains representing the extremes for yield and seed weight traits were selected for molecular analysis. The selected strains were characterized using 8 AFLP, 16 ISSR and 30 RAPD primers/primer combinations. For yield, the AFLP, ISSR and RAPD produced 531, 138 and 325 total scorable bands with percentage polymorphism of 28.0, 35.5 and 35.6, respectively. While for seed weight, they generated 524, 135 and 317 total scorable bands with percentage polymorphism of 27.0, 31.1 and 34.0, respectively. The phylogenetic analysis for yield, successfully grouped the superior strains in one cluster while for seed weight, the superior strains were

grouped in another cluster except for ISSR dendrogram. These results represent the first study combining different molecular markers, agronomical and morphological evaluation of 13 traits aimed at developing unique positive and negative markers which can be used to identifying superior jojoba strains in early stages of development.

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Table (1): Morphological, seeds characteristics and yield component traits of the twelve selected jojoba strains.

| Strain No. | Y | ASW | ASV | ASD | ASL | NR | ANB | ALA | LFW | LDW | ASL | ALN | ANI |
|------------|------|------|------|-------|-------|------|------|------|-------|------|-------|-------|-------|
| 8 | 7700 | 1.85 | 1.80 | 11.83 | 17.27 | 4.00 | 5.25 | 6.55 | 10.90 | 2.93 | 14.87 | 34.00 | 17.00 |
| 7 | 7150 | 1.10 | 1.29 | 12.66 | 19.78 | 4.00 | 4.15 | 6.53 | 16.91 | 3.96 | 16.83 | 23.00 | 14.76 |
| 2 | 5800 | 0.93 | 1.00 | 11.40 | 16.24 | 3.00 | 2.43 | 5.67 | 11.59 | 2.22 | 15.72 | 29.00 | 12.76 |
| 11 | 5650 | 0.93 | 1.10 | 10.60 | 18.50 | 3.00 | 4.32 | 9.22 | 8.43 | 2.18 | 26.67 | 34.00 | 10.56 |
| 1 | 5550 | 0.93 | 1.00 | 9.37 | 17.72 | 3.00 | 3.40 | 5.34 | 14.03 | 2.93 | 23.11 | 21.00 | 13.06 |
| 10 | 5350 | 0.84 | 1.00 | 11.10 | 15.63 | 3.00 | 3.20 | 6.32 | 6.54 | 1.55 | 12.65 | 27.00 | 15.90 |
| 12 | 2900 | 1.26 | 1.40 | 12.32 | 17.01 | 3.00 | 2.10 | 5.07 | 15.60 | 2.78 | 15.78 | 15.00 | 9.32 |
| 14 | 2900 | 1.09 | 1.30 | 11.75 | 18.55 | 4.00 | 4.32 | 5.27 | 8.23 | 1.83 | 21.78 | 29.00 | 15.65 |
| 15 | 2250 | 1.78 | 1.80 | 15.25 | 20.33 | 3.00 | 1.75 | 4.96 | 11.20 | 2.34 | 18.29 | 16.00 | 8.00 |
| 3 | 2100 | 1.22 | 1.40 | 14.59 | 15.81 | 3.00 | 3.50 | 5.28 | 16.80 | 3.54 | 23.50 | 35.00 | 17.50 |
| 6 | 1950 | 1.24 | 1.30 | 11.67 | 16.91 | 3.00 | 4.25 | 3.91 | 6.82 | 1.31 | 22.85 | 23.00 | 6.70 |
| 13 | 1950 | 1.09 | 1.25 | 12.28 | 16.34 | 3.00 | 3.76 | 4.54 | 6.54 | 1.12 | 19.34 | 32.00 | 17.09 |
| 5 | 1550 | 1.73 | 1.70 | 13.86 | 18.16 | 3.00 | 3.00 | 3.56 | 6.43 | 1.34 | 16.98 | 17.00 | 8.50 |
| 4 | 1500 | 0.95 | 1.05 | 12.70 | 17.38 | 4.00 | 4.00 | 5.87 | 6.43 | 1.31 | 29.30 | 35.00 | 17.50 |
| 9 | 400 | 1.05 | 1.30 | 10.80 | 16.76 | 3.00 | 3.75 | 4.38 | 7.44 | 1.54 | 18.34 | 14.00 | 6.25 |

Y; yield, SW; average seed weight, ASV; average seed volume ASD; average seed diameter, ASL; average seed length, NR; number of ridges. ANB; average no. of branches, ALA; average leaf area (cm²), LFW; leaf fresh weight (gm.), LDW; leaf dry weight (gm.), ASL; average shoot length (cm.), ALN; average leaf no./shoot, ANI; average no. internodes

Table (2): Marker type, Primer/Primer combinations, total no. of bands, polymorphic bands and percentage of polymorphism of yield and seed weight traits.

| Marker Type | Primer/PC | Yield | | | Seed Weight | | |
|-------------|-----------|-------|-------------|--------------------|-------------|-------------|--------------------|
| | | Total | Polymorphic | % of Poly-morphism | Total | Polymorphic | % of Poly-morphism |
| AFLP | 1 / 4 | 56 | 18 | 32.1 | 56 | 18 | 32.1 |
| | 2 / 2 | 89 | 38 | 42.7 | 91 | 40 | 43.9 |
| | 3 / 8 | 74 | 19 | 25.7 | 72 | 17 | 23.6 |
| | 4 / 1 | 84 | 20 | 23.8 | 81 | 17 | 21.0 |
| | 4 / 2 | 60 | 19 | 31.6 | 60 | 19 | 31.6 |
| | 4 / 6 | 70 | 16 | 22.8 | 67 | 13 | 19.4 |
| | 6 / 4 | 42 | 6 | 14.2 | 42 | 6 | 14.2 |
| | 6 / 6 | 56 | 13 | 23.2 | 55 | 12 | 21.8 |
| Total | | 531 | 149 | 28.06 | 524 | 142 | 27.09 |
| ISSR | R1 | 12 | 6 | 50.0 | 12 | 4 | 33.0 |
| | R2 | 5 | 0 | 0.0 | 5 | 0 | 0.0 |
| | R3 | 7 | 0 | 0.0 | 8 | 1 | 12.5 |
| | R5 | 9 | 5 | 55.6 | 9 | 5 | 55.6 |
| | R6 | 5 | 2 | 40.0 | 5 | 0 | 0.0 |
| | R7 | 11 | 7 | 63.6 | 10 | 6 | 60.0 |
| | R8 | 12 | 2 | 16.7 | 11 | 2 | 18.2 |
| | R9 | 10 | 1 | 10.0 | 9 | 0 | 0.0 |
| | ISSR-1 | 10 | 2 | 20.0 | 10 | 3 | 30.0 |
| | ISSR-2 | 6 | 0 | 0.0 | 6 | 0 | 0.0 |
| | ISSR-3 | 9 | 4 | 44.4 | 8 | 2 | 25.0 |
| | ISSR-4 | 10 | 6 | 60.0 | 9 | 5 | 55.5 |
| | ISSR-5 | 3 | 0 | 0.0 | 3 | 0 | 0.0 |
| | ISSR-6 | 9 | 5 | 55.6 | 10 | 6 | 60.0 |
| | ISSR-8 | 8 | 2 | 25.0 | 9 | 3 | 33.3 |
| ISSR-9 | 7 | 5 | 71.4 | 6 | 4 | 66.6 | |
| | H24 | 5 | 2 | 40.0 | 5 | 1 | 20.0 |
| Total | | 138 | 49 | 35.5 | 135 | 42 | 31.1 |
| RAPD | A1 | 15 | 1 | 6.7 | 15 | 4 | 26.7 |
| | A2 | 11 | 4 | 36.4 | 11 | 3 | 27.3 |
| | A3 | 9 | 0 | 0.0 | 9 | 0 | 0.0 |
| | A4 | 11 | 4 | 36.4 | 10 | 5 | 50.0 |
| | A5 | 13 | 3 | 23.1 | 12 | 3 | 25.0 |
| | A7 | 11 | 2 | 18.2 | 10 | 1 | 10.0 |
| | A8 | 13 | 6 | 46.2 | 12 | 5 | 41.7 |
| | A11 | 6 | 2 | 33.3 | 9 | 6 | 66.7 |
| | A12 | 8 | 2 | 25.0 | 8 | 2 | 25.0 |
| | A15 | 14 | 10 | 71.4 | 13 | 7 | 53.8 |
| | A17 | 12 | 4 | 33.3 | 11 | 3 | 27.3 |
| | A19 | 10 | 7 | 70.0 | 10 | 5 | 50.0 |
| | B3 | 14 | 5 | 35.7 | 14 | 4 | 28.6 |
| | B4 | 8 | 4 | 50.0 | 8 | 3 | 37.5 |
| | B6 | 12 | 7 | 58.3 | 11 | 6 | 54.5 |
| B7 | 14 | 5 | 35.7 | 14 | 5 | 35.7 | |

Table (2): Cont'

| | | | | | | | |
|-------|-----|-----|------|------|-----|------|------|
| RAPD | B9 | 7 | 2 | 28.6 | 9 | 4 | 44.4 |
| | B10 | 12 | 5 | 41.7 | 11 | 2 | 18.2 |
| | B11 | 10 | 3 | 30.0 | 9 | 4 | 44.4 |
| | B12 | 9 | 3 | 33.3 | 8 | 2 | 25.0 |
| | B14 | 8 | 7 | 87.5 | 8 | 6 | 75.0 |
| | B17 | 16 | 3 | 18.8 | 16 | 3 | 18.8 |
| | B18 | 16 | 9 | 56.3 | 14 | 7 | 50.0 |
| | B19 | 15 | 5 | 33.3 | 16 | 5 | 31.3 |
| | B20 | 8 | 2 | 25.0 | 8 | 2 | 25.0 |
| | C2 | 9 | 1 | 11.1 | 9 | 2 | 22.2 |
| | C3 | 5 | 0 | 0.0 | 5 | 0 | 0.0 |
| | C6 | 6 | 0 | 0.0 | 6 | 0 | 0.0 |
| | C8 | 10 | 2 | 20.0 | 10 | 2 | 20.0 |
| A6 | 13 | 8 | 61.5 | 11 | 7 | 63.6 | |
| Total | | 325 | 116 | 35.6 | 317 | 108 | 34.0 |

Table (3): Marker type, unique positive markers (UPM), unique negative markers (UNM) and their corresponding sizes for yield and seed weight traits of the selected jojoba strains.

| Marker Type | Yield | | Seed Weight | |
|-------------|---|--|--|--|
| | UPM | UNM | UPM | UNM |
| AFLP | PC6/4 ⁷³⁰ | PC1/4 ³²⁰ , PC1/4 ⁶²⁰ , PC1/4 ²²⁵ , PC2/2 ²³⁶ | PC6/6 ²²⁵ , PC4/2 ²³⁰ , PC4/2 ³³⁰ , PC4/2 ³³⁵ , PC4/1 ²⁴⁰ , PC4/1 ⁶⁷⁰ , PC2/2 ¹⁵⁵ , PC2/2 ¹⁶⁰ , PC2/2 ¹⁶⁵ , PC2/2 ¹⁷⁵ , PC2/2 ²²⁵ , PC2/2 ³⁵⁰ , PC2/2 ⁴¹⁰ | PC1/4 ⁹⁰ , PC1/4 ¹⁷⁰ , PC6/6 ⁸⁰ , PC6/6 ⁵⁵⁰ , PC4/6 ¹⁴⁰ , PC4/6 ⁴¹⁵ , PC4/6 ⁶⁰⁰ , PC4/6 ⁶¹⁰ , PC4/2 ⁶⁸⁵ , PC4/2 ⁷²⁰ , PC4/1 ³⁶⁰ , PC4/1 ⁶⁹⁵ , PC3/8 ⁶⁵⁰ , PC3/8 ⁷⁸⁰ |
| ISSR | R7 ⁷⁵⁰ , R9 ¹⁰⁵⁰ , ISSR3 ⁵⁴⁰ , ISSR3 ⁵⁶⁰ | R5 ⁸⁰⁰ , ISSR4 ³²⁰ , ISSR6 ⁵⁹⁰ , ISSR9 ²⁸⁰ | R5 ³⁵⁰ , R5 ⁸⁰⁰ , ISSR4 ⁵⁰⁰ | ISSR8 ⁵⁰⁰ , ISSR9 ³⁰⁰ |
| RAPD | A15 ⁴⁰⁰ , B4 ⁴⁵⁰ , B7 ³⁶⁰ , B9 ⁵⁰⁰ , B18 ⁴⁰⁰ , B18 ⁴⁵⁰ , B19 ³⁴⁰ | A6 ¹¹⁰⁰ , A7 ²⁰⁰ , A19 ⁹⁵⁰ , B6 ⁴⁰⁰ , B14 ⁵²⁰ , B17 ²²⁰ , B19 ¹⁸⁰ , C8 ²⁰⁰ | B3 ⁷²⁰ , B4 ⁴⁵⁰ , B7 ²³⁰ , B18 ¹⁴⁰ , B19 ³⁴⁰ | B9 ⁷⁰⁰ , B18 ⁵⁴⁰ , B18 ⁹⁰⁰ , B18 ¹⁰⁰⁰ , C8 ²⁰⁰ |

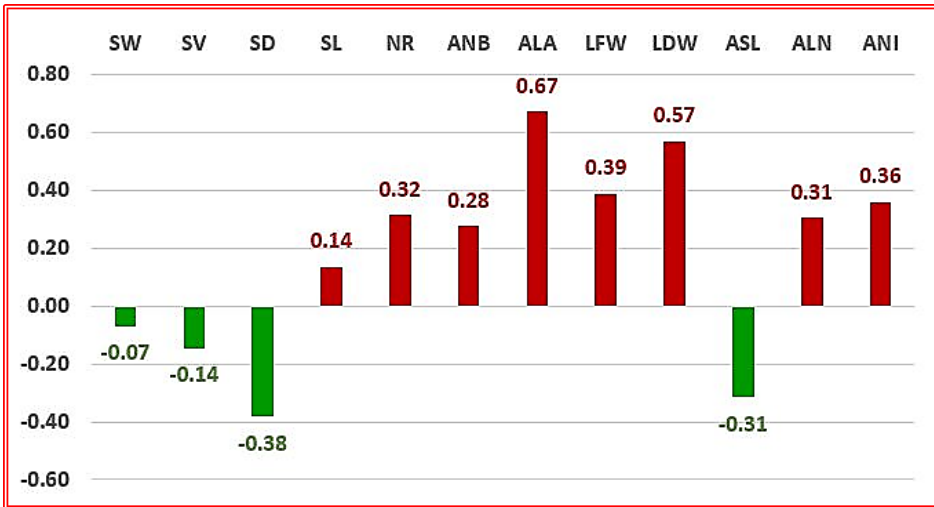


Fig. (1): Correlation coefficient of the morphological and seed characteristics traits in relative to yield of the twelve selected jojoba strains.

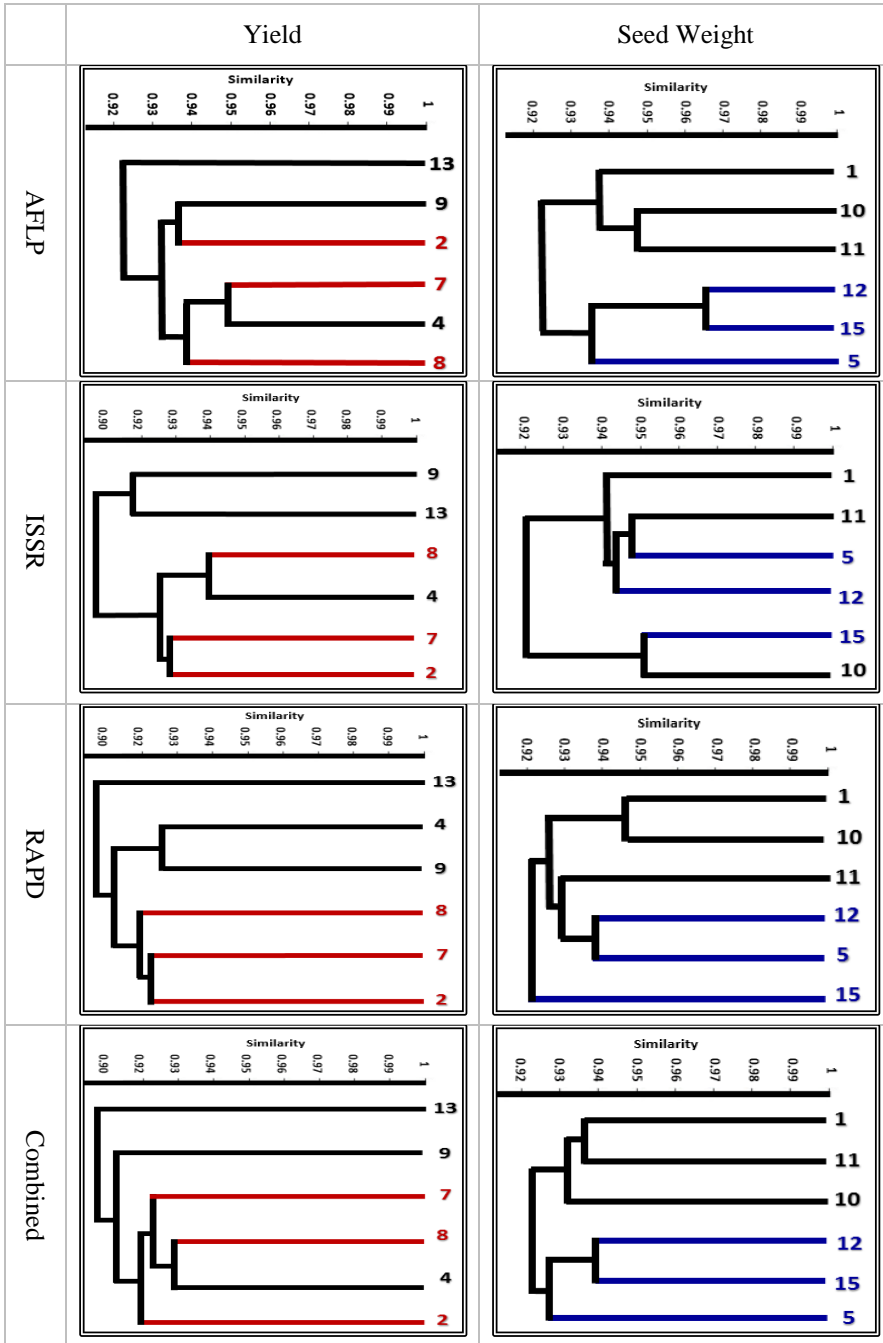


Fig. (2): Dendrograms depicting the genetic similarity among and between the selected six jojoba strains representing yield extreme strains (2, 7 and 8 Vs. 4, 9 and 13) and seed weight extreme strains (5, 12 and 15 Vs. 1, 10 and 11) based on AFLP, ISSR, RAPD and Combined data. (Superior yield strains: red font; superior seed weight strains: blue font).