Sex Determination of Jojoba Using RAPD Markers and Sry Gene Primer Combined with RAPD Primers.

1Heba Allah A. Mohasseb, 1Hamdy A. Moursy, 1Mohamed K. El-Bahr, 2Zakia M. Adam, and 1Mohei El-Din Solliman

1Plant Biotechnology Dept., and Vaccine Group, Central of Excellence for Advanced Sciences, National Research Center, Dokki, Giza, 12622, Egypt.
2Plant Dept., Faculty of Girls for Arts, Sciences and Education Ain Shams University, Cairo, Egypt.

Abstract: The random amplified polymorphic DNA (RAPD) technique was used to screen markers of sex determination in this species. A 520 and 650 bp RAPD marker, marker linked to sex determination in jojoba (male), was found in all the jojoba male but not in the female plants; its exclusiveness to male plants. Also, A 420 bp marker linked to sex determination in jojoba (female), was found in all the jojoba female. Our data of similarity index reveal that there is low relationship between male and female with most of RAPD primers. RAPD profiles of PCR products using primers sry combined with moh19 indicated that unique DNA fragment with 4 bands (1200, 700, 400 and 300 bp) is present in female clones and this band is completely absent in the male clone. However, other polymorphic bands were detected among either male or female clones with other primers tested. The primers sry combined with moh19 gave the highest numbers of bands (7) and percentage of polymorphism (33). We found that male and female are very similar in both in vitro and in vivo and using the sry combined with moh19 have similar patterns of bands. On other hand, the same primers (sry combined with moh19) possess more different bands that can differentiate between male and female almost (4) bands with female in both in vitro and in vivo. The random amplified polymorphic DNA (RAPD) molecular marker technique was used to determine the sex of male and female of jojoba. In the present study, DNA samples were extracted individually from male and female plants. After a total of 60 decamer primers had been tested, an approximate 19 primers can distinguish among male and female of jojoba. It is feasible to identify sex at the early stages of plant life, which is beneficial for improving breeding programs of this dioecious species.

Key words: CTAB, DNA extraction, RAPD, jojoba sex determination, Sry gene.

INTRODUCTION

Jojoba (Simmondsia chinensis L.) is a dioecious perennial evergreen shrub native to the south-western desert of North America and is now introduced to Egypt. This study was carried out to determine whether the RAPD method could be applied to the identification of the sex in jojoba plants. RAPD-PCR was employed to study polymorphic for DNA extracts from the male and female of jojoba plants. The results showed that RAPD could be used to identify the sexes of male and female in jojoba plants.

A novel strategy for DNA fingerprinting that us an arbitrarily short primer, generally of ten bases, for the low stringency amplification of multiple fragments from genomic DNA, was initially developed by both[1,2] demonstrated the utility of single short oligonucleotide primers of arbitrary sequence for the amplification of DNA segments distributed randomly through the genome. Also they showed that the pattern of amplified bands could be used for fingerprinting and[3] showed that the differences in the pattern of bands amplified from genetically distinct individuals behaved as mendelian genetic markers. Well saturated maps of the Arabidopsis[3] genomes have been constructed using RAPD technology. A single set of arbitrary-sequence (10-mers) may be used for mapping or fingerprinting any species[4,5,6,7,8].

In particular, the level of variation for RAPDs appears to be higher than comparable classes of molecular markers[9,10] RAPDs can be exploited as markers that segregate in a Mendelian manner[11,12].

The DNA fingerprints (DAFs) as revealed by RAPD have been found to be useful in a number of approaches in botanical research. For example, in the delimitation of infraspecific relationships in plants[13,14], genetic differentiation of populations[15], identification of genotypes[16], identification and classification of...
S. latifolia functions in suppression of time since the origin of dioecy. The Y chromosome degeneration may reflect one evolutionary repetitive sequences. The different degree of Y chromosome has not accumulated chromosome-specific repetitive sequences, whereas the Y chromosome of Silene latifolia has not accumulated chromosome-specific repetitive sequences. However, these floral organ identity genes show no evidence of sex chromosome linkage. The Y chromosome of Rumex acetosa contains Y chromosome-specific repetitive sequences, whereas the Y chromosome of Silene latifolia has not accumulated chromosome-specific repetitive sequences. The different degree of Y chromosome degeneration may reflect on evolutionary time since the origin of dioecy. The Y chromosome of S. latifolia functions in suppression of female development and initiation and completion of anther development. Analyses of mutants suggested that female suppressor and stamen promoter genes are localized on the Y chromosome. Recently, some sex chromosome-linked genes were isolated from flower buds of S. latifolia.

The objectives of this study were to investigate the ability of RAPD markers to discriminate among male and female cultivars of jojoba, and to determine the ability of RAPD markers to optimize identification of male and female of jojoba, and use for the first time the human sry sex determination gene primers to determine the sex gene in jojoba plant.

MATERIALS AND METHODS

Cultivars: Leaves of jojoba plants were collected from the Egyptian Natural Oil Company farm (Dr Nabil Sadek El Mogy), Ismailia, in July 2005.

DNA Extraction Protocol: To obtain fine powder, the powdered leaf sample was filtered using a 60-mesh sieve. A protocol using CTAB method with slight modification was followed. The standardized protocol for DNA extraction from jojoba leaf followed is given below:

1. To 500mg of ground leaf tissue, 20ml of extraction buffer (containing 3 per cent CTAB, 100mM Tris, 20mM EDTA, 1.4M NaCl, 2 per cent PVP and 1 per cent b-Mercaptoethanol) which was preheated to 65°C was added.
2. The tube containing the buffer and powdered leaf tissue was incubated for one hour at 65°C with intermittent shaking.
3. It was brought to room temperature and 6ml of CHCl₃: Isoamyl alcohol (24:1) was added to the tube, which was mixed well for 25-30 times by inverting the tube gently and spun at 6000 rpm for 10-15 minutes.
4. The supernatant was transfered to a fresh tube. This step was repeated to get a clear supernatant.
5. ½ volume of 5M NaCl was added and mixed well and to which 1volume of ice cod Propanol was added and left at 4°C overnight for precipitation of DNA.
6. The eppendorf tube was spun at 9000 rpm for 25 minutes. The supernatant was poured off and the DNA pellet was washed with 70 per cent ethanol and dried in a Vacuum drier.
7. The DNA was suspended in 300ml of TE (Tris-EDTA) buffer and transferred to an eppendorf tube.
8. 3ml of Rnase enzyme (1mg/ml) was added and incubated at 37°C for overnight.

...
9. 300ml of phenol was added, mixed well and spun at 5000 rpm for 5-10 minutes and the supernatant was transferred to another eppendorf tube. (The temperature of the centrifuge was maintained above 20°C).

10. 300ml of Phenol: Chloroform (1:1) was added to it, mixed well and spun at 5000rpm for 5 minutes and the supernatant was transferred to another eppendorf tube.

11. 300ml Chloroform was added, mixed well and spun at 5000rpm for 5 minutes and the supernatant was transferred to another eppendorf tube.

12. 1 volume of Prapanol was added to precipitate DNA. The tube was left at -20°C for overnight for fastening the precipitation.

13. The eppendorf tube was spun at 10,000 rpm for 20 minutes.

14. The DNA pellet was washed with 70 percent ethanol and dried. The pellet was resuspended in 300ml TE (Tris-EDTA) buffer.

15. 1/10th volume of 3M Sodium Acetate was added, mixed and spun at 5000rpm for five minutes. Finally the supernatant was transferred to another eppendorf tube.

Finally the quality and quantity of DNA was verified by electrophoresis on a 1.2 per cent Agarose gel.

**DNA Amplification Protocol:** The PCR programme included an initial denaturation step at 94°C for 2 mins followed by 45 cycles with 94°C for 1 min for DNA denaturation, annealing as mentioned with each primer, extension at 72°C for 30 seconds and final extension at 72°C for 10 minutes were carried out. The amplified DNA fragments were separated on 2% agarose gel and stained with ethidium bromide. Four 10-mer primers randomly selected were used in RAPD analysis. A 100 bp DNA ladder (Promga) was used as a Marker with molecular size of 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp. The amplified pattern was visualized on a UV transilluminator and photographed. The genetic similarity was calculated using the average linkage between groups according to [31]

**Electrophoresis:** The amplification products were analyzed by electrophoresis according to [32] in 2% agarose in TAE buffer (for each litre of 50X TAE Stock solution: 242 g Tris Base, 57.1 mL Glacial Acetic Acid and 100 mL 0.5 M EDTA), stained with 0.2 mg/ml ethidium bromide. Nucleic acids bands were photographed and detected under short wave UV light.

**Analysis of RAPD Results:** The RAPD bands were scored as presence (1) or absence (0). The index of similarity between each two varieties was calculated using the formula: \( B_a = \frac{2 N_a}{N_a + N_b} \) where \( N_a \) is the number of common fragments observed in individuals a and b, and \( N_a \) and \( N_b \) are the total number of fragments scored in a and b respectively (Lynch, 1990). The genetic similarity was calculated for each primer separately and average for all primers was carried out with each comparison. Dendrogram was constructed using the average linkage between groups [33].

**PCR-based Sex Determination in Jojoba by Sry and Random Primers:** In previous work we cloned the HMG-box region of the sheep, cattle, and goat SRY gene [34]. Using 5'-RACE from sheep testiccular RNA, we isolated a 400-bp fragment that hybridizes specifically with SRY. This fragment was used to screen a male sheep genomic library, and an insert of 12 kb containing the sheep. Sry gene was obtained and partially sequenced. From this sequence, oligonucleotide primers flanking the open reading frame (ORF) were defined to amplify genomic DNA from males and females.

The PCR conditions were: 30 cycles of 94°C for 1 min, 52°C for 1 min, 72°C for 1 min with Taq polymerase and the primer were used as following: Sry forward (5' TGCCA GGA GTATTGAGGG 3') and Sry reverse primers (5' CAGAGCA GTATTG 3').

**DNA Amplification:** DNA amplification was performed in a 12.5-μL reaction mixture containing 1× PCR buffer (50 mM Tris-HCl, pH 8.5, 1.5 mM MgCl2, 50 mM KCl, and 0.1% Triton X-100), 200 mM of each dNTP, 0.4 mM of each primer separately, 1 U Tsg DNA polymerase (Biobasic, Scarborough, Ont.), and 1–10 ng template DNA.

With an initial denaturation step of 94°C for 5 min, followed by 40 amplification cycles of 94°C for 1 min, 37°C for 2 min, and 72°C for 2 min, and a final extension at 72°C for 5 min. Negative controls were included for each experiment to test for contamination. DNA amplification products were separated on a 1.5% agarose gel, and visualized under 300 nm UV light after staining with ethidium bromide. Each PCR reaction was repeated at least once.

**RESULTS AND DISCUSSION**

Due to advances in molecular biology techniques, large numbers of highly informative DNA markers have been developed for the identification of genetic polymorphism. In the last decade, the random amplified polymorphic DNA (RAPD) technique based on the polymerase chain reaction (PCR) has been one of the most commonly used molecular techniques to develop
DNA markers. RAPD markers are amplification products of anonymous DNA sequences using single, short and arbitrary oligonucleotide primers, and thus do not require prior knowledge of a DNA sequence. Low expense, efficiency in developing a large number of DNA markers in a short time and requirement for less sophisticated equipment has made the RAPD technique valuable although reproducibility of the RAPD profile is still the centre of debate.

In a dioecious species, females and males may be subject to divergent directional selection due to differences in each sex's requirements for successful reproduction. For example, that sex with the higher reproductive cost may have undergone selection for a morphology that is more efficient for resource gain than that of the opposite sex.

*Simmondsia chinensis* (Simmondsiaceae), jojoba, is a dioecious evergreen shrub of the Sonoran Desert that is sexually dimorphic in some populations and isomorphic in other populations (fig. 1). The method involves mortar grinding of tissue, a modified CTAB extraction employing high salt concentrations and polyvinyl pyrrolidone, a RNase A treatment and successive isoamyl alcohol-chloroform extractions. The yield was approximately 15 mg DNA per 100 mg of initial fresh plant material. The genomic DNA obtained by this method was suitable to be used in simple sequence repeat and random amplified-polymorphic DNA reactions. This extraction method would allow the molecular analysis of shoots from different clones within jojoba male and female.

**Molecular Approach to the Study of Sex Determination in Jojoba:** An efficient protocol was developed for large scale of *In vitro* multiplication and regeneration of jojoba using seed as explants in jojoba and molecular approaches for sex determination. Random amplified polymorphic DNA (RAPD) technique was used to compare genetic material from male, female and unknown lines of jojoba.

Jojoba plants are strictly a dioeciously perennial tree native to USA. The female plants are economically more useful than the male plants. The random amplified polymorphic DNA (RAPD) technique was used to screen markers of sex determination in this species. A 520 and 650 bp RAPD marker, marker linked to sex determination in jojoba (male), was found in all the jojoba male but not in the female plants; its exclusiveness to male plants was confirmed by repeating the reaction more than one time (fig. 2 B and C) in the same figure A 420 bp marker linked to sex determination in jojoba (female), was found in all the jojoba female (fig. 2C).

Data of similarity index (Table 2) reveal that there is low relationship between male and female clones. Also, female clones are relatively closely related to each other and they have large number of homologous bands. Concerning the *in vitro* differentiated cultures, there was high similarity between each other. Due to the weak relationship of vitroplants to either male or female clones, we can not confirm clear link to sex or estimate the sex type of these unknown clones.

Sex-pooled DNA samples were prepared by mixing the DNA of ten male and ten female individuals, respectively. Among all the 19 primers screened so far, three bands, amplified by primers moh2 and moh3 appeared to be sex correlated in jojoba male and three bands amplified by primers moh1 and moh4, appeared to be sex correlated in jojoba female. The results showed that in each of the putative sex related bands, the correlation with sex is only partial, and some individuals have the marker-phenotype of the opposite sex. It is possible that the marker is linked to a sex-determining locus but not very tightly. We also observed that many of the RAPD bands screened in this experiment can be identified as species-specific (fig. 2, 3, 4 and 5).

A 1520 bp RAPD marker, marker linked to sex determination in jojoba (male), was found in all the jojoba male but not in the female plants; its exclusiveness to male plants was confirmed by repeating the reaction more than one time (fig. 3 C) in the same figure A 1100 bp marker linked to sex determination in jojoba (female), was found in all the jojoba female (fig. 3C).

All the primers (Table 1) examined produced different RAPD fragment patterns (Figure 2 to 5). The number of fragments generated per primer varied between 4 to 12. Genetic similarity estimated between different male and each female jojoba plants is presented in Table (2). The highest values of genetic similarity were observed between both male land 2 with female cultivars, and it ranged from 83.3 to 95.3%. This result reflects the similarity between male and female cultivars, but this data is not sufficient to identify unknown male and female from tissue culture tissue. Identification of male variety exactly needs more advanced molecular studies. We also observed that the small alterations in PCR parameters or quality of target DNA can alter RAPD patterns.

This result reflects the similarity with thus there may be reason to view with caution systematic conclusions based on RAPD analysis alone. On the other hand, the possibility of carrying out compatibility analysis with unlimited numbers of primers, each detecting variation at several regions in the genome, provides an advantage over other techniques. Even if some primers amplify identical regions of the genome or if the technique itself is noisy, it should be possible to build up quickly a consensus from patterns of inter-population variation. 

---

Fig. 1: The flowers and fruits of male and female jojoba. (A) Female flowers; (B) male flowers; (C) female fruit; (D) female Seed.

Fig. 2: RAPD patterns of male and female jojoba DNA samples generated by primers moh 1, moh 2 and moh 3. M is DNA marker, lanes 3, 6 and 9.

Fig. 3: RAPD patterns of male and female jojoba DNA samples generated by primers moh 4, moh 5, moh 6 and moh 7. M is DNA marker, lanes 3, 6 and 9.
**Table 1:** Primers used and their annealing temperatures.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence 5’ - 3’</th>
<th>Annealing Tm °C / Sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moh1</td>
<td>5’TGGCGACCTG3’</td>
<td>36°C</td>
</tr>
<tr>
<td>Moh2</td>
<td>5’GAGGGGCTC3’</td>
<td></td>
</tr>
<tr>
<td>Moh3</td>
<td>5’CCCTACCGAC3’</td>
<td></td>
</tr>
<tr>
<td>Moh4</td>
<td>5’TGCCTTCCG3’</td>
<td></td>
</tr>
<tr>
<td>Moh5</td>
<td>5’CACCTTCC3’</td>
<td></td>
</tr>
<tr>
<td>Moh6</td>
<td>5’GAGGAGAG3’</td>
<td></td>
</tr>
<tr>
<td>Moh7</td>
<td>5’GTGGCCGTCTC3’</td>
<td></td>
</tr>
<tr>
<td>Moh8</td>
<td>5’GTAGGGCGTC3’</td>
<td></td>
</tr>
<tr>
<td>Moh9</td>
<td>5’GGACCACAC3’</td>
<td></td>
</tr>
<tr>
<td>Moh10</td>
<td>5’TTGGCAAGG3’</td>
<td></td>
</tr>
<tr>
<td>Moh11</td>
<td>5’CTCAGTCGCA3’</td>
<td></td>
</tr>
<tr>
<td>Moh12</td>
<td>5’GGTGCGGGAAG3’</td>
<td></td>
</tr>
<tr>
<td>Moh13</td>
<td>5’GTTGACTGTG3’</td>
<td></td>
</tr>
<tr>
<td>Moh14</td>
<td>5’GCTAAATCCTAC3’</td>
<td></td>
</tr>
<tr>
<td>Moh15</td>
<td>5’GTTTCGCCCTC3’</td>
<td></td>
</tr>
<tr>
<td>Moh16</td>
<td>5’GTAGACCCGT3’</td>
<td></td>
</tr>
<tr>
<td>Moh17</td>
<td>5’AAGAGCCCGT3’</td>
<td></td>
</tr>
<tr>
<td>Moh18</td>
<td>5’GTAGGGCGATG3’</td>
<td></td>
</tr>
<tr>
<td>Moh19</td>
<td>5’AAGGGCCCGG3’</td>
<td></td>
</tr>
</tbody>
</table>

PCR-based Sex Determination in Jojoba by Sry and Random Primers: Fragment sizes (including the primers) produced using these two primer pairs are approximately 382 and 339 for the moh 4, moh 5 primers combined with SRY fragments, respectively (Figure 6-C). Every sample should produce at least one band and the absence of any amplification implies a failed PCR reaction. Testing the technique using samples of known males and females from jojoba species indicates that this sexing method is effective across a broad taxonomic range.

Two pairs of nested primers were derived from the 5 flanking sequence of the bovine SRY gene to serve as male-specific sexing primers, which were used for PCR-based sex determination of plants in combination with random primers as combined set of primer (Figure 6-A, B and C).

A sex-determination technique based on PCR amplification of genomic DNA extracted from jojoba tissue has been standardized in male and female samples from the Egyptian species. A Y-chromosome-specific region (SRY or Sex-determining Y-chromosome gene) of 210–224 bp size in the genome has been amplified (in males and female) using specific PCR primers (Figure 6-A and B).

A fragment of the Sry and random primers (one specific primer from sry gene with a random primers as PCR combined set primer) which gave region in the different size range 442–445 bp in both male and female and is also amplified (in both sexes) using another pair of primers simultaneously as positive controls for confirmation of sex. Molecular sexing was standardized in jojoba using random primers only or sry gene primer combined with the random primers.
Table 2: The sequence of the selected random primers, total number of amplification products per primer, number of polymorphic bands and percentage of polymorphism.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5' → 3')</th>
<th>Total number of bands</th>
<th>No. of polymorphic bands</th>
<th>Percentage of polymorphism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moh 1</td>
<td>5'TGGCGACCTG3'</td>
<td>4</td>
<td>3</td>
<td>75</td>
</tr>
<tr>
<td>Moh 2</td>
<td>5'GAGGCGTCGC3'</td>
<td>5</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Moh 3</td>
<td>5'CCTACCGAC3'</td>
<td>5</td>
<td>4</td>
<td>80</td>
</tr>
<tr>
<td>Moh 4</td>
<td>5'TGTTCCGCC3'</td>
<td>4</td>
<td>3</td>
<td>75</td>
</tr>
<tr>
<td>Moh 5</td>
<td>5'CACCTTTC3'</td>
<td>4</td>
<td>3</td>
<td>75</td>
</tr>
<tr>
<td>Moh 6</td>
<td>5'GAGGAGAG3'</td>
<td>6</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>Moh 7</td>
<td>5'GGTCCGTCC3'</td>
<td>4</td>
<td>5</td>
<td>80</td>
</tr>
<tr>
<td>Moh 8</td>
<td>5'GTGAGGCGTC3'</td>
<td>5</td>
<td>4</td>
<td>80</td>
</tr>
<tr>
<td>Moh 9</td>
<td>5'GACCCCAACC3'</td>
<td>7</td>
<td>6</td>
<td>85</td>
</tr>
<tr>
<td>Moh 10</td>
<td>5'TGGCACGGA3'</td>
<td>8</td>
<td>7</td>
<td>87</td>
</tr>
<tr>
<td>Moh 11</td>
<td>5'CCTAGTCGCA3'</td>
<td>4</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Moh 12</td>
<td>5'GGTGCGGGA3'</td>
<td>6</td>
<td>5</td>
<td>83</td>
</tr>
<tr>
<td>Moh 13</td>
<td>5'GGTGACTGTG3'</td>
<td>6</td>
<td>5</td>
<td>83</td>
</tr>
<tr>
<td>Moh 14</td>
<td>5'GCTAAATCTAC3'</td>
<td>7</td>
<td>6</td>
<td>85</td>
</tr>
<tr>
<td>Moh 15</td>
<td>5'GTTTGTCTCC3'</td>
<td>5</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Moh 16</td>
<td>5'GTAGACCGGT3'</td>
<td>4</td>
<td>3</td>
<td>75</td>
</tr>
<tr>
<td>Moh 17</td>
<td>5'AAGAGCGC3'</td>
<td>3</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Moh 18</td>
<td>5'GTAGGCACGT3'</td>
<td>5</td>
<td>6</td>
<td>83</td>
</tr>
<tr>
<td>Moh 19</td>
<td>5'AAAGGCCG3'</td>
<td>6</td>
<td>5</td>
<td>83</td>
</tr>
</tbody>
</table>

Fig. 6: RAPD patterns in 6 samples of jojoba obtained with the six primers. M is DNA marker, lanes 1 and 2 are the two samples of males and female by SRY primer + moh1 primer, lanes 3 and 4 are the sample of females and female by SRY primer + moh2 primer, lanes 5 and 6 the sample of tissue culture by SRY primer + moh3 primer, lanes 7 and 8 the sample of tissue culture by SRY primer + moh4 primer, lanes 9 and 10 the sample of tissue culture by SRY primer + moh5 primer and lanes 11 and 12 the sample of tissue culture by SRY primer + moh6 primer.

PCR appears to favour the amplification of the shorter fragment, i.e. the SRY fragment. Hence, false female positive tests never resulted from male DNA. This further supports the reliability of this system with two sets of Primers (Figure 6- A and C).

Testing the technique using samples of known sexes (determined by taking from the flowering plant at Notoil Company in Egypt) from jojoba male and female plants indicated that this sexing method was effective across a broad taxonomic range. The method can provide the secondary confirmation necessary for positive sex identification in jojoba plant propagated and regenerated plant in vitro, or a primary method where accurate field observation of sex is not possible in the first 6 month for seed germinated plant.

SRY gene was detected in male and female plant by specific primers were synthesized to generate a 260 bp and to use as sex determination in jojoba male and female with random primer could be useful for screening jojoba male and female plants for gender even before they reach reproductive maturity, resulting in considerable saving of time and economic resources. The genomic structures of SIX1 and SIY1 are very similar\cite{35,36}, consistent with the fact that recombination
between SIXI and SIY1 ended very recently[37]. Comparison of the genetic maps of the X chromosomes in these three dioecious species showed that the gene organization is highly conserved except for an inversion of the q arm between S. dioica and the other two species[38]. This high conservation is also known for the X-linked genes of eutherian mammals[39]. The genetic map of the X chromosome of S. latifolia was also compared with that of a homologous autosome of the related gynodioecious species S. vulgaris[39].

Comparison Between ex Vivo and in Vitro Jojoba Male and Female Plants: DNA isolated from adult female and male ex vivo, and two randomly selected clones of zygotic lines differentiated in vitro were subjected to RAPD analysis. Random primers (moh15, moh16 and moh17) were screened in RAPD analysis for their ability to produce sufficient amplification products (fig. 8 and 9).

The results of DNA fingerprints generated by PCR amplification using the three random primers are presented in Fig (8 and 9) and Table (2). The number of fragments generated per primer varied between 2 to 7. The total number of bands was 12 and the average percentage of polymorphism was 83.

The primers sry combined with moh19 gave the highest numbers of bands (7) and percentage of polymorphism (33). As shown in Fig (9) we found that male and female are very similar in both in vitro and in vivo and using the sry combined with moh19 are closely related to each other and have similar patterns of bands. On other hand, the same primes (sry combined with moh19) possess more different bands that can differentiate between male and female almost (4) bands with female in both in vitro and in vivo Fig (9).

RAPD profiles of PCR products using primers sry combined with moh19 indicated that unique DNA fragment with 4 bands (1200, 700, 400 and 300 bp) is present in female clones and this band is completely absent in the adult male clone. However, other polymorphic bands were detected among either male or female clones with other primers tested.

RAPD analysis showed a relatively closely relation between both female and male cultures, since they have large number of homologous bands with some primer. Although, there is low relationship between male and female, results of similarity could not confirm link to sex or estimate the sex type of unknown clones. RAPD analysis using common random primers proved that the field grown male and female plants were highly polymorphic. The potential of molecular markers in sex identification of in vivo grown and in vitro differentiated cultures of jojoba was investigated. In vitro lines were proliferated from both male and female of jojoba.

This method of DNA polymorphism analysis was developed independently by two different laboratories[1,2]. This procedure detects nucleotide sequence polymorphisms in a DNA amplification based assay using only a single primer of arbitrary nucleotide sequence using Polymerase Chain Reaction (PCR). In this reaction, a single species of primer binds to the genomic DNA at two different sites on opposite strands of the DNA template. If these primary sites are within an amplifiable distance thermocyclic amplification. The presence of each amplification product identifies complete or partial nucleotide sequence homology, between the genomic DNA and the oligonucleotide primer at each end of the amplified product. On an average, each primer will direct the amplification of several discrete loci in the genome, making the assay an efficient way to screen for nucleotide sequence polymorphism between individuals.

They concluded that, it is likely that these markers are linked to sex determining loci. Molecular markers have been used in breeding to diagnose and select a genotype based on a linked DNA marker, long before the phenotype is apparent. Marker-assisted selection would be very important in Jojoba breeding programs, as well as in cultivar improvement, because of their long juvenility period. We aim to find Random Amplified Polymorphic DNA (RAPD) markers linked to sex in Jojoba male and Jojoba female, the major wild species in Egypt used as rootstocks for Jojoba plant knowing the sex of a seedling may provide early selection in the nursery and help to establish and manage germplasm collections. Leaf samples were collected from ten male and ten female trees from each species[40,41].

ACKNOWLEDGMENTS

This work was supported by NRC. The authors are grateful to Dr. Nabil Elmogy for providing the semi-hardwood and seeds of jojoba plants. The authors are grateful also to Dr. Ahmed Magdi for his effort during the lab work.
Fig. 7: (A) The *In vitro* culture male and female of jojoba plant the sample used for RADP-PCR. (B) Female branch and male branch of *ex vivo* jojoba plant the sample used for RADP-PCR.

Fig. 8: RAPD profile of the *In vitro* (with moh15 primer; 1 male and 2 female) and *ex vivo* (with moh15 primer; 3 male and 4 female) culture male and female of jojoba amplified with moh15 RAPD primer. M: 100 bp ladder marker.

Fig. 9: RAPD profile of the *In vitro* (1 male and 2 female; by Sry forward primer with moh19 primer) and *ex vivo* (3 male and 4 female; by Sry forward primer with moh19 primer). M: 100 bp ladder marker.
REFERENCES


