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Full Length Research Paper

Molecular-based marker for sex differentiation of jojoba *in vivo* and *in vitro* Iraqi cultivars using RAPD- PCR technique

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The objective of this study was to distinguish between males and females of jojoba (*Simmondisia chinensis)* using Random Amplified Polymorphic DNA (RAPD) markers in order to detect any DNA variation. A callus was produced from both male and female jojoba leaves, using MS medium with BA 0.5 and 2 to 4, D 2.5 mg/l respectively. Genomic DNA of jojoba plant was extracted using CTAB method. The concentration of DNA ranging between 100 to 150 µg with DNA purity ranging from 1.01 to 1.19. About 0.7 g of male and female plant leaves, and calli were used. Eight different random primers were evaluated for their usefulness in detecting DNA variation between male and female of jojoba. This involved optimization of RAPD reaction condition including, DNA template, *Taq* polymerase, and primers. Five primers showed no amplification and represent a mean of distinguishing between male and female. One primer (C5) showed amplification and represent a mean of distinguishing between the two sexes from the fresh sample tissue; the callus results show no significant difference. The results demonstrated the feasibility of using RAPD-PCR in distinguishing between jojoba sexes.

Key words: RAPD- PCR, *Simmondsia chinensis*, sex differentiation.

INTRODUCTION

Simmondsia chinensis (Link) Schneider, a multi-use dicot shrub of dry areas, has emerged as a cash crop. It is being cultivated for its seeds which store liquid wax whose properties are similar to spermaceti (Sperm whale oil), a substitute for petro products and precious high-priced lubricants. Jojoba is a slow-growing desert shrub having a male biased (5:1; male:female ratio) population.

Since there is no method available to determine the sex at the seedling stage for our Iraqi cultivars, current investigations have been carried out to generate a sex-specific random amplified polymorphic DNA (RAPD) marker in jojoba which is based on the PCR amplification of random locations in the genome of plant.

Development of molecular-base strategies for the early sex identification of dioecious taxa has been a priority in breeding programs for their greater economic potentials, epically in such plants; gender influences economic values, breeding schemes and opportunities for commercial harvest. Moreover, studies on marker technology regarding dioecy in general would provide insight for a better understanding of the developmental as well as the evolutionary pathways of dimorphism (Kremer et al., 2005).

Native Americans extract the oil from jojoba seeds to treat sores and wounds centuries ago. Collection and

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processing of seed from naturally occurring stands in the early 1970's marked the beginning of Jojoba domestication. In addition, the ban on the importance of sperm whale products in 1971 led to the discovery that Jojoba oil is in many regards superior to sperm oil applications in the cosmetics and other industries (Hartwell, 1971).

Today 40000 acres of Jojoba are under cultivation in the southwestern U.S. Much of the interest in Jojoba worldwide is the result of the plant's ability to survive in the harsh desert environment. The utilization of marginal land that will not support more conventional agricultural crops could become a major assert to the global agricultural economy. The oldest commercial Jojoba plantings in the U .S. were established in the late 1970's and presently the production of jojoba oil of thousands of tons per year. The major world producers are the United States and Mexico, with considerable quantities of oil being exported to Japan and Europe (Braun et al., 1979).

Jojoba seed contains a light – gold colored liquid wax ester which is the primary storage lipid of the plant. This is unlike conventional oil seed crops, such as soybean, corn, olive, or peanut which produce oils as the primary storage lipid. Jojoba wax (called oil) makes up 50% of seed dry weight. The physical properties of Jojoba oil are: high viscosity, high flash and fire point, high dielectric constant, high stability and low volatility. Its composition is little affected by temperature up to 300°C. Jojoba oil contains straight – chained of 20 and 22°C fatty acids and alcohols and two unsaturated bonds, which make the oil susceptible to many different types of chemical manipulations. The extracted oil is relatively pure, non – toxic, biodegradable, and resistant to rancidity (Brown and Dwyer, 1988).

Most jojoba oil produced in the U.S. today is sold at a high price for use in cosmetics and hair care products. As many as 300 products containing jojoba have appeared in the U.S. As the supply of oil increases and price decreases, more uses will become economically feasible. For example, the viscosity index of jojoba oil is much higher than that of petroleum oil; therefore, it may be used as a high temperature and high pressure lubricant. The stability of jojoba oil makes it attractive to the electronic and computer industries and since jojoba oil contains no cholesterol or triglycerides and is not broken down by normal metabolic pathways, it may become an important low-calorie oil for human consumption. The oil can be used as an antifoam agent antibiotics production and as a treatment for skin disorders. Other proposed uses include candles, plasticizers, detergents, fire retardants, transformer oil, and for the leather industry (Brown and Dwyer, 1988).

Tissue culture techniques have been used to propagate this plant. MS medium is characterized by rich concentration of nitrate, potassium and ammonia such that other mediums are all converted to the original quantities of MS (Skoog and Miller, 1957). The most striking components for all media is the growth regulators, normally auxins and cytokinins were being used to produce callus tissues (Anderson, 1975; Yeoman and Macleod, 1977).

MATERIALS AND METHODS

Plant material

Jojoba cultivars, procured from the Baghdad University green house facility, were used for the screening of sex-associated DNA markers by RAPD analysis. Leaf material was picked from fully developed, field grown plants after the complete expression of the sexual phenotypes, and the individual samples were stored at -70 prior to use.

Callus induction and DNA isolation

MS media were prepared as in (Skoog and Miller, 1957). 100 mg/l of myo - inositol were added, pH were adjusted to 5.7 ±1 using 1N NaOH and HCL. Plant growth regulators 2, 4-D and BA in different concentrations were added. Cultures were kept in 28-32 °C. About 0.7 g from both freeze – dried callus and freeze dried leaf were used for DNA Isolation. According to the protocol mentioned in (Saghai-Maroof et al., 1984) with modification, the samples were grinded with 0.3 g of alumina in small mortar and pestle. The powder was placed on Eppendorf tube. 600 µl of 1 × CTAB extraction buffer was added, and then the mixture gently dispersed with plastic stirring rod, the mixture incubated for 20 min in 56°C, 600 µl of chloform – octanol (24 to 1) were added, emulsified by shaking. The samples were centrifuged for 2 min in micro centrifuge and the aqueous phase was then removed. The denatured protein interface was washed in the original tube with 100 µl of extraction buffer and, then re- centrifuged .The aqueous phase was subsequently removed. 70 µl of 10% CTAB were added to the pooled aqueous phase to mix. Debris in the aqueous phase was removed, and an equal volume of precipitation (600 µl) was added, mixed and left for 20 min at room temperature. The samples were centrifuged (2000 g, 15 min) to collect the precipitate. The supernatant was poured off and the pellet was drained. The nucleic acid/CTAB pellet was re dissolved in 400 µl of 1 N NaCL, and then heated to 56ºC. The solution was removed to a sterile Eppendorf tube, 800 µl of ethanol was added when the pallet was fully dissolved, and the samples were placed in – 20°C over night. The precipitated nucleic acid pallets were dried (air dry) for 54 min and then washed with 65% ethanol for 1 min. The nucleic acid redissolved in 50 µl of sterile distilled water. The samples were then stored in –20°C and the DNA measured by spectrophotometer.

RAPD marker analysis

The following reagents were mixed in sterile 1.5 ml Eppendorf tube, since four Jojoba samples were analyzed; it was easier to prepare a master mixture of reagents and the enzyme enough for all DNA samples. It was gently spun down in a 1.5 ml – Eppendorf tube. 2 µl of the master mixture was aliquot into other tubes. 2 µl of DNA template solution was added to each tube and mixed gently. The reaction mixture was overlaid with 20 µl of mineral oil. The tubes were placed in the thermal cycler to carry out amplification of genomic DNA of Jojoba. The amplification program was run as follow: 1 cycle of 94°C for 2 min, 40 cycles of 92°C for 1 min, 36°C for 1 min, 72°C for 1 min, 1 cycle of 72°C for 10 min, from the reaction mixture (Table 1), the amplified DNA was withdrawn into

Table 1. represents the component required for RAPD-PCR reaction.

Addition order	Component	Volume (µl)	Concentration
1	10 XPCR buffer	2.5	1X
2	dNTPs	2.5	0.2 mM
3	Primer	2	10 Pmol
4	Tag Polymerase	0.2	1U
5	S.D.W	15.8	-----
6	DNA	2	25-50 ng
Total		25	

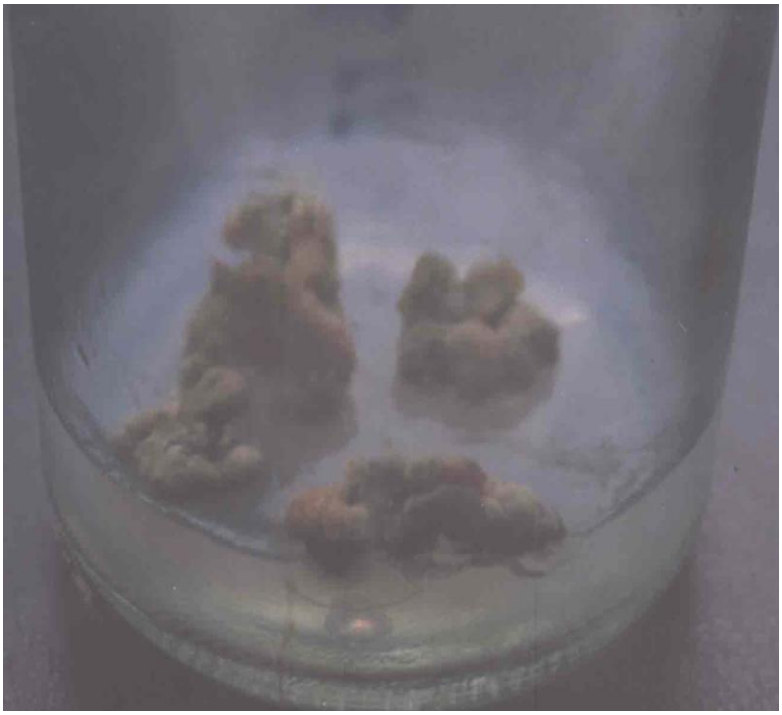

Figure 1. Callus cultures originated from leaf explants grown on maintenance medium containing 0.5 mg/l BA and 2.5 mg/l 2, 4-D. Cultures were continuously cultured on fresh medium at 21 days intervals (16 h light/daily).

another tube and analyzed by gel electrophoresis (1.2%) (Understander, 1990).

RESULTS AND DISCUSSION

In callus induction experiment three parts of *S. chinensis* (leaf, root and stem) were used as explants for each male and female using differing combination between the growth hormones 2, 4-D and BA. The leaf explants only gave response on both male and female parts. The effect of the combination between different concentrations of 2, 4-D and BA on the response (%) to callus induction on jojoba leaf male explants is shown Figure 1 and Table 2.

The interaction between the two growth regulators achieved 83.3% response that was in a combination of 0.5 mg/l BA and 2.5 mg/l 2, 4-D. Explants that were not treated with hormones did not show any induction of callus. The effect of different concentration of BA and 2, 4-D on the percentage of leaf female explants responded to callus induction as shown in Table 3.

Results on Tables 2 and 3 showed that the best callus induction response for both explants (male, female) occurred at a combination of 0.5 mg/l BA and 2.5 mg/l 2, 4-D. Also these tables showed that the responses of female explants were higher than those explants from male plant when using the same concentration. Callus induction requires a balanced ratio for auxins and

Table 2. Effect of different concentration 2, 4-D and BA. On the response (%) of callus induction *S. chinensis* male leaf explants (n = 12).

2,4-D (mg/l)	BA (mg/l)		Mean
	0.0	0.5	
0.0	0.0	8.33	8.33
1.0	16.33	25.0	15.66
1.5	8.33	25.0	16.65
2.0	16.33	41.6	28.96
2.5	33.3	83.3	58.30
Mean	11.59	36.64	

Table 3. Effect of different concentration of 2, 4-D and BA on the response (%) of callus induction *S. chinensis* female leaf explants (n =12).

2,4-D (mg/l)	BA (mg/l)		Mean
	0.0	0.5	
0.0	0.0	16.66	16.66
1.0	16.66	25.0	20.83
1.5	16.66	33.3	24.98
2.0	25.0	50.0	37.5
2.5	41.6	91.6	66.6
Mean	19.98	43.31	

cytokinins. However, in a number of plant species, callus induction favors higher auxins than cytokinins (Yeoman and Macleod, 1977).

DNA isolation from plants

Genomic DNA from *S. chinensis* plants were isolated according to the procedure described by Saghai-Maroof et al. (2007) (Figure 2), although, PCR technique do not require a large quantity and high purity DNA suitable quantity of DNA approximately (100 to 200 µg) from 0.7 g of both fresh plant tissue (male and female leaves) and callus induced in culture (also from male and female explants were isolated, the purity of isolated DNA in male fresh plant was 1.19, female fresh plant 1.15, male callus 1.15, and for female callus 1.11. The extraction buffer containing CTAB helped buffer cell lyses and the CTAB complex formed the Nucleic acid / CTAB complex which does not dissolve in solvents. Also, nucleic acid can be separated from other cell components by chloroform – octanol precipitation.

RAPD Results

Sex-specific markers in dioecious taxa which could be generated through DNA analysis using PCR technology have been proven to be a reliable strategy; as such, markers for sex prediction can be analyzed at any developmental stage of growth. The RAPD technique (Williams et al., 1990) is a simple identifier of polymorphism and has been used to screen markers of sex determination in several plants, that is, *Salix viminalis* L. (Alstrom-Rapaport et al., 1998), *Actinidia chinensis* (Gill et al., 1998), *Asparagus* (Jiang and Sink, 1997), *Myristica fragrans* Houtt. (Ganeshaiah et al., 2000), *Eucommia ulmoides* Oliv. (Xu et al., 2004), *Encephalartos natalensis* (Prakash and Van Staden, 2006), *Carica papaya* (Chaves- Bedoya and Nunenz, 2007). Eight primers of oligonucletides were screened for RAPD analysis using genomic DNA isolated from four samples Jojoba plant. The results were obtained from PCR reaction using these primers and were classified into two groups:

In the first group, no amplification was detected. In spite of repeating a number of experiments, similar results were obtained using these five primers. These primers include (A10, N7, N16, O2, and E13). The second group of RAPD results produced amplified products using four primers that were (A1, A18, D20 and C5).

Only one primer (C5) was found to have sex specificity in mass analysis for the sample of callus tissue. Random decamer of this primer gave difference between the female and male sample at the presence of two bands (about 1.5 and 0.85 kbp) in the female and absence of these bands for male. Also, this primer showed that the

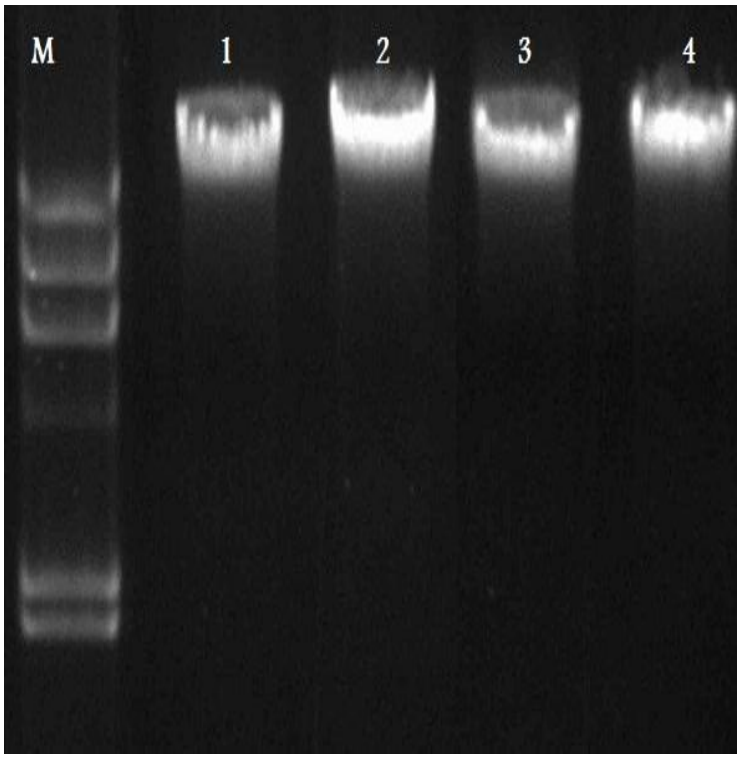

Figure 2. the DNA isolation from four S. Chinensis. Electrophoresis was performed on (1.2%) agarose gel and run with 5 volts/cm. The lanes (1, 2, 3, 4) represent the female, male fresh plant, female, male callus, respectively, Lane M: 100 bp DNA ladder (Promega).

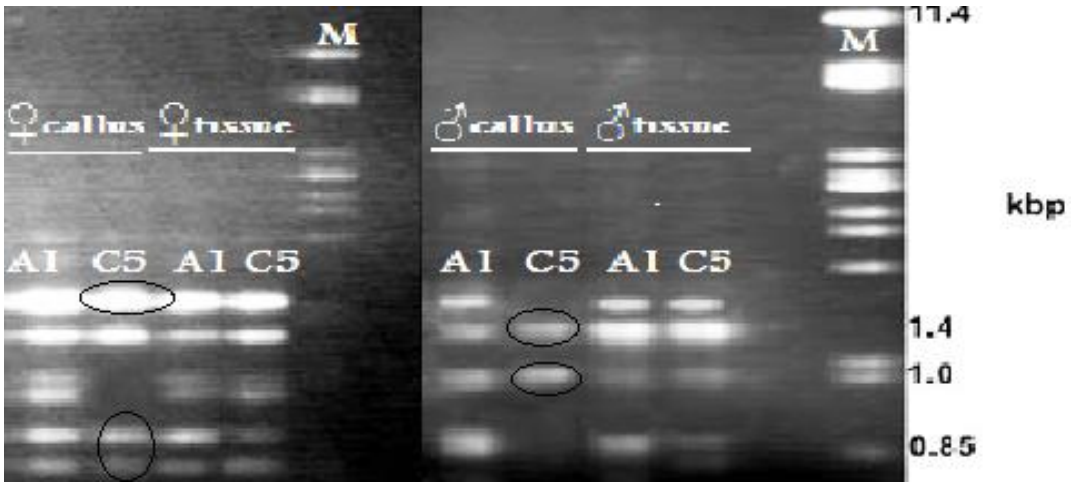

Figure 3. RAPD pattern of *S. chinensis* obtained with A 10 and C5 primers electrophoresis was performed on (1.2%) agarose gel and run with 5 volt/cm. The lines (A, B, C, D) represent female, male fresh plant, female, male callus and tissue respectively. Line M indicates the λ DNA *Pst* 1 from Fermentas.

band was around 1.0 kbp for the male sample and absent for this band for the female sample (Figure 3). In addition to this, many other bands were generated in both male and female samples. To confirm this observation, this primer was re-tested with the individuals of male (fresh tissue and callus) in four biological replicates each. Thus,

(C5) primer could be recognized as a putative sex-linked marker for four jojoba tissue culture cultivars.

The differentiation of sex by using specific markers in dioecious taxa through DNA and PCR tools showed consistent strategy, because like these markers, sex prediction can be analyzed different at developmental stages of growth. RAPD-DNA base marker (have been used previously in different plant species such as, *Asparagus* (Jiang and Sink, 1997), *Cannabis sativa* L. (Mandolino et al., 1999), *C. papaya* (Urasaki et al., 2002; Chaves- Bedoya and Nunenz, 2007), *M. fragrans* Houtt. (Ganeshaiah et al., 2000), *E. ulmoides* Oliv. (Xu et al., 2004), *E. natalensis* (Prakash and Van Staden, 2006).

Our findings considered the first report describing a method for sex identification of jojoba in Iraq, which might be helpful in identifying the sex in *S. chinensis* at the early stage of seedling by its commercial growers and for further plant breeding systems.

REFERENCES

Alstrom-Rapaport C, Lascoux M, Wang YC, Roberts G, Tuskan GA (1998). Identification of a RAPD marker linked to sex determination in the basket willow (Salix viminalis L.). J. Hered., 89: 44-49.

Anderson WC (1975). propagation of Rhododendron part 1 .Development of culture medium. Proceed. Int. Plant Propagat. Soc., 25: 129-134.

Braun W R, and Espericueta BM (1979) . Organic production Biomesay Jojoba (Simmondsia Chenensis Link) in the coastal desert of Sonora, pp. 557-72.

Brown J, Dwyer K (1988). The Jojoba potential: An Overview , proceedings from the 7th international conference on Jojoba Its Uses , Phoenix , AZ.

Chaves-Bedoya G, Nunenz V (2007). A SCAR marker for the sex types determination in Colombian genotypes of *Carica papaya*. Euphytica, 153: 215-220.

Ganeshaiah KN, Ravishankar KV, Anand L, Shibu MP, Shaanker U (2000). Identification of sex-specific DNA markers in the dioecious tree, nutmeg (*Myristica fragrans* Houtt.). PGR Newsletter, 121: 59-61.

Gill GP, Harvey CF, Gardner RC (1998) Development of sex-linked PCR markers for gender identification in *Actinidia*. Theor. Appl. Genet., 97: 439-445

Hartwell, J.L. (1971). Plants used against cancer, a survey. Lloydia J. Bacterio., 92(3): 723-726.

Jiang C, Sink KC (1997). RAPD and SCAR markers linked to the sex expression locus M in *Asparagus*. Euphytica, 94: 329-333.

Kremer A, Caron H, Cavers S, Colpaert N, Gheysen G, Gribel R, Lemes M, Lowe AJ, Margis R, Navarro C, Salgueiro F (2005). Monitoring genetic diversity in tropical trees with multilocus dominant markers. Heredity, 95: 274-280.

Mandolino G, Carboni A, Forapani S (1999). Identification of DNA markers linked to the male sex in dioecious hemp (Cannabis sativa L.). Theor. Appl. Genet., 98: 86-92

Prakash S, Van Staden J (2006). Sex identification in Encephalartos Enatalensis (Dyer and Verdoorn) using RAPD markers. Euphytica. 152: 197–200.

Skoog F, Miller, CO (1957). Chemical regulation of growth and organ formation in plant tissue cultured in vitro. Symp. Exp. Biol., 11: 118-130.

Undersander DL, Lueschen WE, Smith LH, Kaminski AR, Doll JD, Kelling KA, Oplinger ES (1990). Sorghum – for syrup Alternative Field Crops. Available at (http://www.hort.purdue.edu/newcrop/afcm/index.html).

Urasaki N, Tokumoto M, Tarora K, Ban Y, Kayano T, Tanaka H, Oku H, Chinen I, Terauchi R (2002) A male and hermaphrodite specific RAPD marker for papaya (Carica papaya L.). Theor. Appl. Genet., 104: 281-285

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984). Ribosomal DNA spacer-length polymorphism in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc. Natl. Acad. Sci. USA, 81: 8014-8018.

Williams JGK, Rubelik AR, Livak KJ, Rafalski A, Tingey SV (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18: 6531-6535.

Xu WJ, Wang BW, Cui KM (2004). RAPD and SCAR markers linked to sex determination in *Eucommia ulmoides* Oliv. Euphytica, 136: 233-238.

Yeoman MM, Macleod AJ (1977). Tissue culture techniques. In:Street H. E. (ed). Plant tissue and cell culture. Blackwell Scientific Publisher, Oxford.