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Full Length Research Paper

Sex determination of jojoba (*Simmondsia chinensis* cv. Arizona) by random amplified polymorphic DNA (RAPD) molecular markers

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Jojoba (*Simmondsia chinensis* (Link) Schneider) is a dioecious shrub that produces fruits in female plants. Its seeds stores liquid wax which is used in cosmetic, pharmaceutical and plastic industries. This species is generally propagated by seed. The sex of seedlings is not distinguishable by cytological and seed cultivation methods. This investigation was carried out to study the sex-specific random amplified polymorphic DNA (RAPD) markers in thirteen 4-year-old jojoba plants from two provinces of Iran and DNAs of those populations were extracted by CTAB method. Out of the 20 tested primers, two primers, namely F1 and F10, produced 460 and 680 bp fragments, respectively and were importantly recognized to distinguish between female and male plants, accordingly. Also, the results of the ratio difference test showed that, more efficient sex determination of jojoba seedlings is done using both F1 and F10 primers due to gene cooperation between them. The preliminary results of this study for sex determination would help the recognition of potential fruit-bearing seedlings for having high yield per hectare in horticultural systems. Furthermore, the findings would help saving time and economic resources in jojoba breeding programs.

Key words: Jojoba, sex determination, dioecy, random amplified polymorphic DNA (RAPD) molecular marker.

INTRODUCTION

Sexual reproduction is a prominent feature of the life cycle in most animals and plants (Ming et al., 2007). The pistil and stamen are the parts of the flower sheltering ovules and producing pollen grain, respectively. In most typical dioecious plants, pistil and stamen develop as separate individuals, which are distinguished as “pistillate” and “staminate” plants (Xu et al., 2004). The genetic basis of sex determination in dioecious plants may be extremely diverse. Some dioecious species have heteromorphic sex chromosomes (for example, *silene latifolia*), whereas in other species, sex is determined by one or several autosomal nuclear loci, possibly influence by cytoplasmic genes (Alstrom-rapaport et al., 1998). Jojoba (*simmondsia chinensis*) a dioecious species, is an important crop shrub that produces commercially valuable seeds in female plants. It is now the only species belonging to the genus *Simmondsia* in family of *Simmondsiaceae*. Its seeds stores liquid wax which is used in cosmetic, pharmaceutical and plastic industries (Tyagi and Prakash, 2004). It has promising physical properties, such as high viscosity index, high flash and fire points, high dielectric constant and high stability and freezing point that can be used in various industries (Agrawal et al., 2007). Due to its potential to make canopy and eliminating windy erosion in desert regions in

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Abbreviations: RAPD, Random amplified polymorphic DNA; **CTAB,** cetyl trimethyl ammonium bromide; **SCAR,** sequence characterized amplified region.

Iran, its adaptation was initiated by Shahsavand Hasani et al. (2006).

Jojoba sex chromosomes are not distinguishable. Therefore, sex type of jojoba seedlings cannot be determined by cytological methods (Parasnis et al., 2000). Also, sex type of jojoba seedlings can not be determined either by embryo shape or morphology at the juvenile developmental stage. Propagation of jojoba is mainly through seeds (Singh et al., 2008). Therefore, three to four years are required for this shrub to reach the flowering stage of its life cycle and it is a slow growing and the ratio of male to female plants in the field is around 5:1 (Agrawal et al., 2007). In general, male plants are not useful commercially, therefore, the farmers eliminate a considerable number of male plants and this increases production costs.

The last decade has witnessed an increasing number of research efforts directed at identifying and characterizing molecular markers and genes involved in plant dioecy (Kafkas et al., 2001). Random amplified polymorphic DNA (RAPD) is a simple identifier of polymorphism and has been used for identification of dioecious cultivars in phylogenetic studies (Xu et al., 2004), selection of traits of interest (Yakubou et al., 2005) and classification of plants at the genus and species levels (Wong et al., 2004; Bouza et al., 2006). The objective of this study is to develop a sex-specific RAPD marker linked to sex determination in jojoba seedlings.

MATERIALS AND METHODS

Plant materials

Plant materials consisted of leaves from 11 different jojoba genotypes (cv. Arizona) collected from plants after the complete expression of the sexual phenotype in two locations. The first group is made up of 6 plants from 4 year old jojoba in Kerman University, Iran, including 3 male and 3 female plants. The second group of jojoba plants (cv. Arizona) was obtained from agriculture research centre in Fars province, Iran, consisting of 7 plants including two male and five female plants. The leaf samples of these plants were stored in an ice box or liquid nitrogen before transporting to the laboratory for further processing and then all specimens stored at -80°C before extracting DNA for screening sex linked DNA marker by RAPD analysis.

Genomic DNA isolation

Total genomic DNA was isolated from 1 g of leaf tissues of female and male jojoba plants using the standard CTAB method with minor modifications (Saghari-maroof et al., 1984). The plant tissues were ground to a fine powder in liquid nitrogen, transferred to eppendrof tubes and 700 ml of warm extraction buffer was added. The mixture was incubated at 65°C for 30 min. Chloroform: octanol (24:1) was added and the solution was mixed by inversion and centrifuged at 10000 rpm for 10 min at room temperature. The chloroform: octanol step was repeated. The aqueous phase was transferred to another tube. DNA precipitated with equal volume of isopropanol, mixed and incubated at -24°C for 20 min then centrifuged at 10000 for 10 min. The isopropanol was removed and the DNA pellet was rinsed with 75% ethanol. Then, chilled ethanol (-24°C) was added to DNA precipitation and centrifuged at 10000 rpm for 5 min. Finally, the precipitated DNA was air-dried and dissolved in TE buffer.

Spectrophotometric trials

DNA concentration was determined in a Biowave S2100 spectrophotometer. Also, the purity of DNA was checked by OD260/280 and through gel electrophoresis with 0.8% agarose gel. In all cases, the extracted DNA was diluted to a final concentration of 50 ng/µl and then was used for polymerase chain reaction amplification (PCR).

RAPD marker analysis

Twenty primers from Cinagene Company (Iran) were used for RAPD analysis. The PCR was performed in a reaction volume of 25 µl using the eppendrof thermal cycler. The reaction mixture contained one unit of *Smar Taq* polymerase (Cinagene Company), 50 ng of male or female genomic DNA, 0.4 µM of RAPD primer, 2.5 µl of 10 × PCR reaction buffer (500 mM KCl, 100 mM Tris HCl) and 1.5 mM $MgCl_2$, 0.2 mM of each dNTP. The RAPD-PCR reactions were carried out at 94°C for 5 min, followed by 40 cycles at 94°C for 45 s, different annealing temperature with various type of primer for 45 s, 72°C for 1 min, and final extension at 72°C for 7 min. Amplification products were electrophoresed in 1% agarose gel and visualized by ethidium bromide staining in 0.5 x TBE for 3 h at 80 V. Standard molecular weight markers were also used in the electrophoresis trail.

RESULTS

The results of primers polymorphism information content (PIC) of 13 jojoba plants showed that PIC ranged from 0.32 to 0.89 (Table 1). The highest and lowest amounts were observed in primer 396 and 391, respectively. Having the Higher amount of PIC value is related to the power of primer for detection of Genetic variability in the population, (Mohammadi-nejad et al 2008). Hence, primer 396 (5'-GAATGCGGAG-3') was identified as the best primer for genetic diversity in jojoba plants. The results of RAPD primers indicated 79 bands for sex-specific DNA markers. 21 monomorphic bands were identified in male and female plants. Furthermore, 58 polymorphic bands were determined out of them and 56 bands had no role in sex determination, since they were found in both male and female plants. Two other bands where detected by F1 (5'-AGGAGTCGGA-3') and F10 (5'- GGGCCACTCA-3') primers, these bands showed different sex patterns in male and female plants. 460bp fragment (F1 primer) was present in individual female but absent in individual male plants (Figure 1). This band was called “female sex-specific band”.

F10 primer detected a 680 bp fragment, only in male plants (Figure 2). Furthermore, statistical analysis of the ratio difference showed that, the difference of 460 bp fragment presence in female plants was significant, since this band was detected in 7 out of 8 female plants. Therefore, this band can be referred to as, sex- specific

Table 1. Number of alleles and polymorphism information content (PIC) value of rapid markers for 11 jojoba genotypes.

PIC	Number of alleles	Frequency of main alleles	Marker
0.83	9	0.22	F1
0.40	3	0.66	F2
0.87	9	0.16	F3
0.46	3	0.61	F5
0.72	7	0.38	F7
0.48	5	0.66	F8
0.79	9	0.27	F10
0.32	3	0.77	391
0.85	12	0.27	392
0.89	13	0.16	396
0.87	10	0.16	56
0.77	7	0.33	69
0.54	5	0.61	53
0.82	8	0.22	62
0.59	5	0.55	54

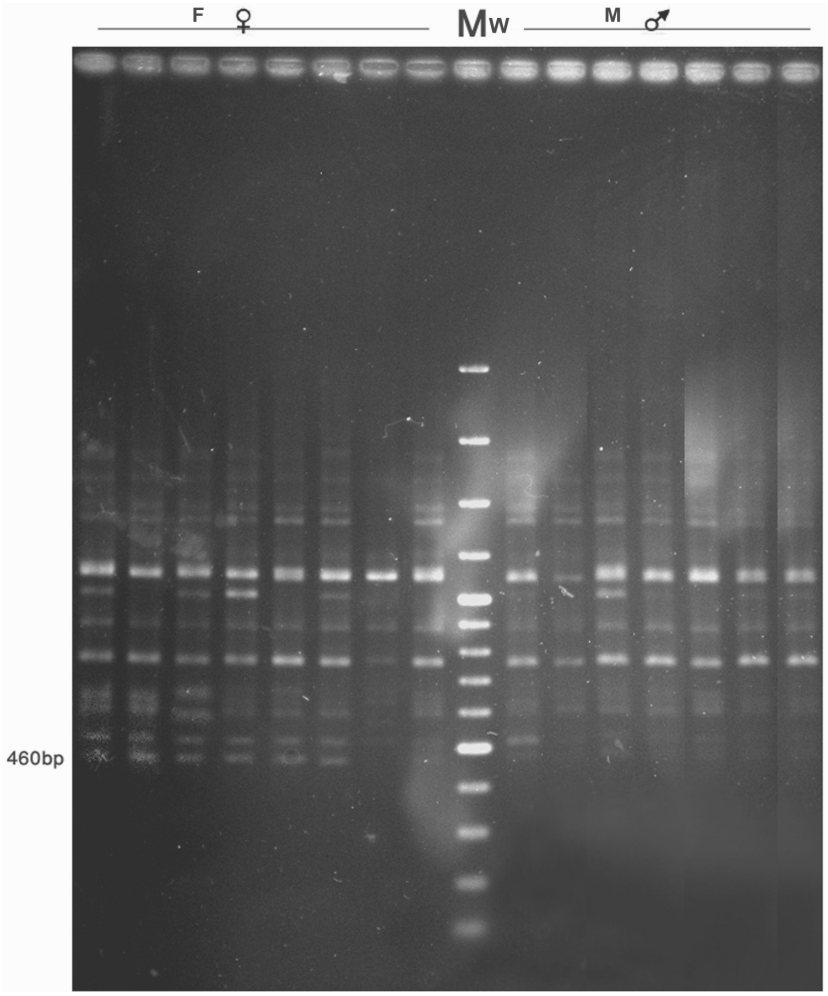

Figure 1. RAPD banding patterns of male and female jojoba plants (cv. Arizona) obtained by the arbitrary primer F1 which indicates a 460 bp sex-linked band in female plants. (F, female plants; M, male plants; Mw: 100 bp ladder marker).

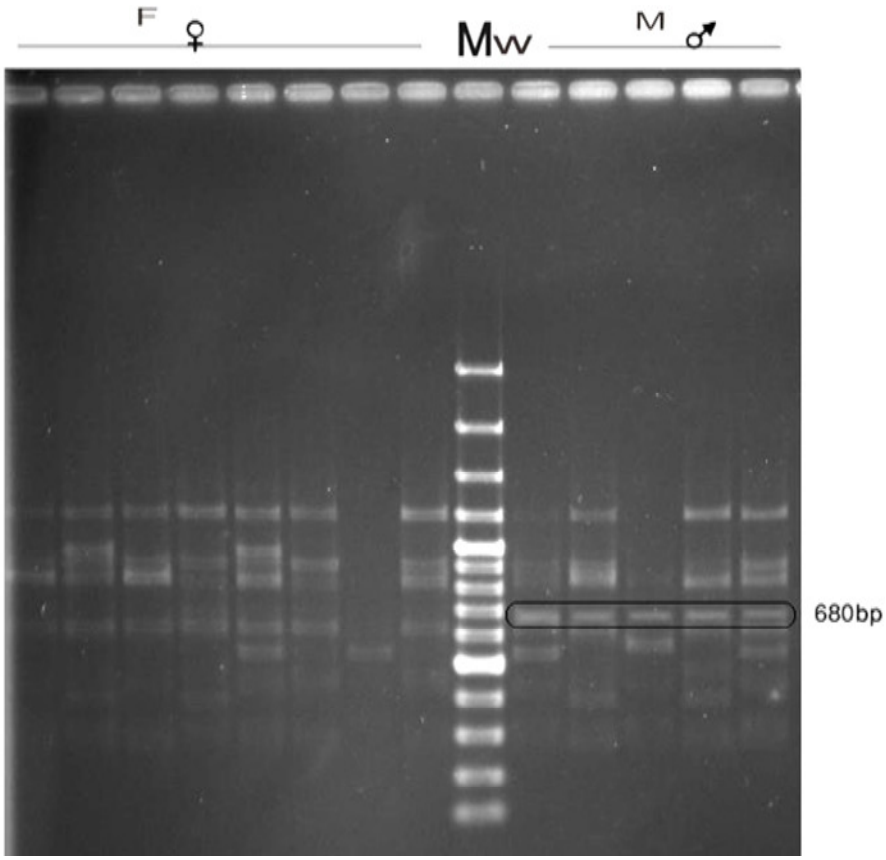

Figure 2. RAPD banding patterns of male and female jojoba plants (cv. Arizona) obtained by the arbitrary primer F10 which indicates a 680 bp sex-linked band in male plants. (F, female plants; M, male plants; Mw, 100 bp ladder marker).

band in female plants. The ratio difference test indicated that, the difference of 680 bp fragment presence in male plant was not significant and it is referred to as, male sex-specific band. Since F1 and F10 primers, were able to differentiate male and female plants from each other, with 460 bp fragment present in seven female plants and absent in five male plants and 680 bp fragment absent in seven female plants but present in five male plants; it is therefore recommended that, both F1 and F10 primers be used together in the sex determination of jojoba seedlings.

DISSCUSION

Due to the absence of genetic information on sex determination in dioecious plants, the use of molecular markers for discriminating between staminate and pistillate genotype is worthwhile (Xu et al., 2004). In recent decades, the use of molecular markers in sex determination have been increased, for example amplified fragment length polymorphism (AFLP) markers were developed in *Asparagus officinallis* and *Ficus fulva* (Spada et al., 1998; Parrish et al., 2004), DAF marker in papaya (Somsri et al., 1998) and RAPD markers *in Papaya*, *Cannabis sativa* and *Populus tomentosa* (Lemos et al., 2000; Deputy et al., 2002; Mandolino et al., 2002; Hou et al., 2009). The most common marker for sex determination is RAPD marker. Agrawal et al. (2007) introduced a 1400 bp RAPD fragment for sex determination in jojoba plants but in the present research this primer (OPG-5) was not able to detect male and female plants in few individuals of jojoba seedlings. Harvey et al. (1997) have used the primer F1 for sex identification in *Actinidia chinensis* species and was able to detect female plants from male ones. These results show the possibility of similar conserved sequences of *A. chinensis* and *S. chinensis* genomes and perhaps also exist in many other dioecious species.

In *Humulus lupulus* (Polley et al., 1997) and *Asparagus* (Jiang, 1997) utilized 1000 and 760 primers for sex identification, respectively. One primer was only used for designing sex-specific SCAR primers. Hormaza et al. (1997) used 400 RAPD primers for sex determination in pistacia and found one band in female plants. In *Salix viminalis* also 350 random decamer primers were tested and only one band was detected (Alstrom-Rapaport et al., 1998). Agrawal et al. (2007) worked with 72 markers

in jojoba and found only one sex-specific RAPD marker, whereas in this study we used 20 RAPD markers in jojoba and found one sex-specific marker for male and one for female. Furthermore, in pepper two female-specific bands was obtained using random decamer primers (Banerjee et al., 1999). Shirkot et al. (2002) also reported six female-specific markers and two male-specific markers in *Actinidia deliciosa*. Also, in *S. viminalis* two female-specific markers were found (Gunter et al., 2003).

The results of this study support that, sex determination in jojoba may be affected by one or several autosomal loci, or several loci possibly when an epistatic manner is involved in sex determination. The preliminary results of this study for sex determination would help in the recognition of potential fruit-bearing seedlings for having high yield per hectare in horticultural systems. Furthermore, the findings would help save time and economic resources in jojoba breeding programs.

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