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Research Article

DNA Fingerprinting of Sex in Jojoba (*Simmondsia chinensis*) Grown under the Semi-arid Conditions of Sudan

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Abstract
Jojoba cultivation and production face the challenge of establishing ways to identify the sex at early stage of plant growth. The present study was carried out to identify sex of jojoba at the seedling stage under Sudan condition. Two DNA markers, ISSR (UBC807) and RAPD (OPG-5), were used for sex identification of jojoba genotypes: two known male and females genotypes and four unknown genotypes. ISSR marker, UBC807 was successfully amplified a unique male-specific band at 1200 bp, while RAPD marker, OPG-5 could not amplify a unique band within jojoba sex. The result clearly indicates that ISSR-UBC807 marker can be used for sex identification of jojoba at seedlings stage, a finding that could make the commercial cultivation and production of jojoba possible in Sudan.

Key words: DNA markers, Goat nut, Male/Female identification, ISSR, RAPD

Introduction

Jojoba (*Simmondsia chinensis* (Link.) Schneider is dioecious wind- pollinated perennial shrub species. It is a native of the Sonoran Desert of Arizona and arid California of the United States, as well as Northern Mexico [1, 2]. The crop received great attention as industrial seed-oil crop in several subtropical countries in South America, Africa, India, the Middle East and Australia ([3, 4, 5]. Its valuable oil in the seed contains 45-50% of seed weight [6]. The oil in the seed, classified as liquid wax, is totally different from other vegetable oils. It is easy for squeezing and refining [7], is chemically resembles the sperm whale with important industrials uses as lubricant, medicinal and pharmaceuticals purposes [8, 9] as well as in cosmetics [10]. In addition, seed pulp is used for livestock feed and as soil enhancer [5, 11]. Accordingly, jojoba could be used as an alternative to sperm whale oil; therefore, contributes to restrict whale hunting [12].

For its cultivation, its deep tap root system, the waxy layer on the leaves and its resistant to salinity, render jojoba to be grown under marginal soil conditions [13]. Because the current energy crises, drought problems and restrictions on whaling and whale products boosted the economic importance of jojoba.

Jojoba was introduced to Sudan in 1978 by UNDP in a joint venture program with Sudanese government to i) study the domestication of the crop as a new plant species in different climatic and edaphic conditions in Sudan, ii) as a new cash crop that suites the less advantageous areas, iii) to enrich the endangered areas, and v) to study the possibilities of using jojoba as a soil stabilizer against desert creep [13]. Fortunately, successful cultivation has been observed in many part of Sudan, especially at Arkaweet area of the

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Red Sea State [13], where the plantation that established since the late seventies is still surviving. However, the main problem that faces jojoba cultivation is a male biased population due to the difficulties associated with the dioecious nature of its pollination as it is difficult to differentiate between male and female plants at the early stage of jojoba growth [14]. For good yield, jojoba seeds should be grown in a ratio of I male: 5 female. Moreover, it is also difficult to use Biochemical and cytological analysis for sex determination [15].

Recenlty, DNA marker has been used as a useful tool for sex determination of jojoba plant and numbers of DNA markers have been identified as sex-specific markers in jojoba plant [14, 16, 17, 18]. [16] reported on UBC-807, an ISSR marker is a male-specific marker in jojoba plant that could amplify the fragment of 1200 base pair. Moreover, [14] developed a male-specific RAPD (OPG-5) which produced a unique band at 1400 base pair. Although jojoba genotypes were introduced to Sudan since 1978, and there is will for economic seed yield increase; however, no research has yet been done to identify jojoba sex at the early stage of jojoba plant growth under the semi-arid conditions of the Sudan.

The objective of the present study was to revalidate ISSR and RAPD markers for sex determination of jojoba genotypes.

Material and Methods

Plant materials

Young leaves from eight jojoba genotypes (two males and two females from the fully differentiated plant) collected from Arkaweet, red sea state, Sudan as well as young leaves from four unknown jojoba seedlings grown in the green house of the faculty of Agriculture, University of Khartoum, Sudan were used.

Genomic DNA extraction

Genomic DNA was isolated from young leaves of the known male and female plants as well as from the unknown jojoba plants using modified CTAB method of [19]. Leaf samples were ground to fine powder using liquid nitrogen in sterilized pestle and mortar. Five grams of tissue powder were mixed with 10 ml of pre-warmed CTAB extraction buffer (65°C) plus PVP (Polyvinylpyrrolidone) in sterilized 15 ml Falcon tubes and shacked several times. The mixture was incubated in water bath at 65°C for one hour. Contents of the tubes were gently mixed at an interval of 10 minutes by inverting them several times. After incubation, the samples were cooled for 5 minutes at room temperature (25°C) and then 10 ml chloroform: isoamylalcohol (24:1) mixture was added. The mixture was mixed gently by inverting the tubes several times. The samples were centrifuged for 10 min at 8000 rpm in REMI C24 at room temperature. The upper aqueous layer was transferred to a new sterilized Eppendorf tubes. The extracted DNA was precipitated with equal volume of ice cold isopropanol followed by centrifugation at 10000 rpm for 5 min and then the upper aqueous layer was dropped out. DNA pellets were washed twice with Wash I solution (Ethyl alcohol 76.0 % and 0.2 M Sodium acetate (pH 5.2)) for 20 min and Wash II solution (10 mM Tris (pH 8.0) and 1 mM EDTA (pH 8.0)) for 2 min respectively and centrifuged for 5 min at 4000 rpm in each. DNA samples were dried at room temperature for one hour and subsequently dissolved in appropriate volume of sterilized double distilled water.

RNase A treatment

DNA samples were treated with RNase A solution (50 µg/ml) and incubated at room temperature for overnight to remove RNA contamination from DNA samples. DNA was again extracted by adding equal volume of chloroform: isoamylalcohol (24:1 v/v) mixture. Samples were mixed well and then centrifuged at 10000 rpm for 10 min. Supernatant was transferred to new Eppendorf tubes. 1/10 vol. of 3M sodium acetate (pH 6.8) and 2 vol. of chilled absolute alcohol was added, followed by centrifugation to pellet down the DNA. Pellet was then washed with 70% alcohol, air-dried and finally dissolved in 50 µl of sterilized double distilled water. DNA concentration and purity were measured using Nanodrop.

Preparation of bulked DNA sample

Bulked DNA samples were prepared by mixing 10 µl of genomic DNA from three male and female plants in separate tubes.

PCR amplification

Two primers $OPG5_{1400}$ and UBC-807 were used for sex determination of jojoba genotypes as shown in Table 1. PCR reactions were carried out in 20µl volume containing 2µl of DNA template, 0.4µl of each primer (10-20 picomole) and 4µl of 5x HOT Master Mix Ready (BioDyne Company). PCR reaction mixture was incubated at 95°C for 5 minutes as an initial step. Then the PCR conditions for $OPG5_{1400}$ and UBC-807 primers were accomplished following the methods of [14] and [16]. PCR amplification of $OPG5_{1400}$ primer was carried out with one cycle consisting of 60 second at 94°C for denaturation, 30 sec at 36°C for annealing and 60 sec at 72°C for extension, and 45 cycle of 5 sec at 94°C, 15 sec at 36°C and 60 sec at 72°C and a final cycle of 7min at 72°C. Thereafter, PCR amplification of UBC-807 primer was carried out with a preliminary cycle of 180 sec at 94°C, followed by 35 cycles of 20 sec at 94°C,

60 sec at 50°C and 90 sec at 72°C; Then, a final cycle of 7 min at 72°C. PCR products were separated by gel electrophoresis on 0.8 % agarose gels, stained with ethidium bromide and visualized with the UV trans-illuminator.

Table 1
Primers sequences used for jojoba sex determination

Marker	Sequences	Type
$OPG5_{1400}$	5- CTGAGACGGA-3 Male specific References [14]	RAPD
UBC-807	5-AGA GAG AGA GAG AGA GT-3 Male specific References [16]	ISSR

Results and Discussions

Jojoba has received great attention worldwide due to the multi-purpose uses of its valuable oil from the seed. Although Sudan is not within the belt where jojoba is grown naturally, the successful establishment and production of seeds from the crop that grown at different parts in Sudan, especially at Arkwakeet, indicates that the country could have a real potential investment possibilities for production of seeds and oil if the problem associated with identification of male and female plants at the early stage of development is solved. DNA markers have been extensively used in sex determination of dioecious plant such as *Phoenix dactylifera* [20], *Carica papaya* [21] and *Simmondsia chinensis* [14, 16, 17, 18].

In the present study, the RAPD primer $OPG5_{1400}$) was in variance to the result of [14] to amplify a unique male-specific fragment as shown in Figure1. The deviation of the observed results from the expected one may be caused by either differences in the used genotypes, differences in genotype x environment interaction and/or PCR conditions under which the amplification was carried out.

On the other hand, ISSR primer (UBC-807) showed a clear male- specific band in bulk male and all individuals male at 1200 bp as shown in Figure 2. This clear male-specific band was entirely absent in bulk female and individuals female genotypes as shown in Figure 2. Moreover, the young unknown jojoba genotypes/plants from the greenhouse displayed variation in their sex gender, tree of them were males and one was a female plant. Our results are in agreement with that of [16] and [18] in their study to differentiate between male and female plants in jojoba. Thus, confirming the presence of male bias phenomenon in jojoba when seeds are used for propagation. The present study indicates the usefulness of ISSR primer in sex determination in jojoba plant at the early stage of development. This finding could make the commercial cultivation and production of jojoba possible in Sudan.

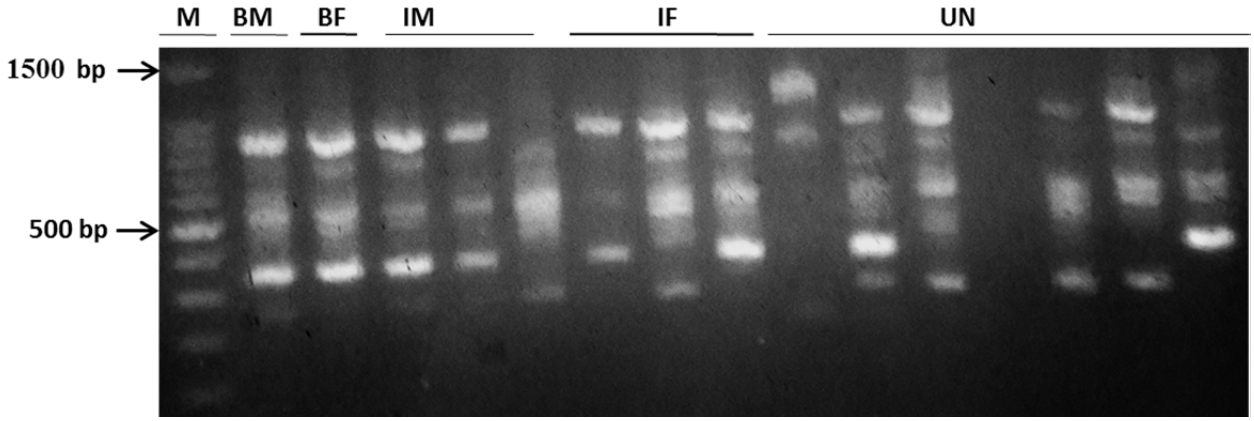

Figure 1. RAPD banding pattern of jojoba plant obtained by $OPG5_{1400}$ primer (5- CTGAGACGGA-3) Does not amplify a unique male-specific fragment (M, marker; BM, bulk of male; BF, bulk of female; IM, individuals of male; IF, individuals of female; UN, unknown sex plants)

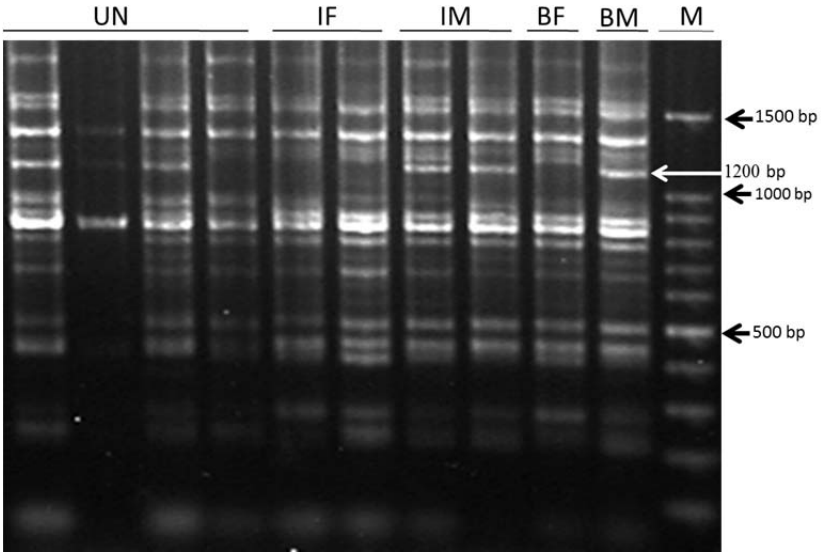

Figure 2. ISSR banding pattern of jojoba plant obtained by UBC-807primer (5-AGA GAG AGA GAG AGA GT-3) shows male specific at 1200bp (M, marker; BM, bulk of male; BF, bulk of female; IM, individuals of male; IF, individuals of female; UN, unknown sex plants)

Conclusions

From the results of the present study it is concluded that UBC807 primer is useful in identifying the sex of jojoba plant at the early stage of seedlings. Therefore the result could contribute to the encouragement of jojoba cultivation and production in Sudan. We recommended performing sequencing for a male specific band obtained by UBC807 marker and designing male –specific primers for sex identification of jojoba plant at early stage.

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