DETERMINATION OFJOJOBA (*Simmondsia chinensis*) PLANT GENDER BASED ON A MALE-SPECIFIC DNA FRAGMENTS USING PCR ASSAY

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Sex determination in plants refers to the process by which plants develop either male or female reproductive organs. Unlike animals, where sex determination is primarily governed by genetic factors, plants exhibit a wide range of mechanisms for sex determination, including genetic, hormonal, and environmental influences (Tanurdzic and Banks, 2004). In some plant species, sex is determined by the presence of specific genes on sex chromosomes. For example, in the model plant *Arabidopsis thaliana*, the presence of Y chromosome determines maleness, while its absence results in femaleness. Similarly, in the dioecious plant *Silene latifolia*, males have XY chromosomes, while females have XX chromosomes (Irish and Nelson, 1989). In certain plant species, sex determination is influenced by environmental factors such as temperature, light, or nutrient availability (Grant *et al*., 1994). In some plant species, hormones are essential in determining sex. For instance, in zea mays, the hormone cytokinin stimulates the production of male flowers while cytokinin deficiency results in the growth of female flowers (Golenberg and West, 2013).The genetics, molecular biology, and biochemistry of genes controlling sexual determination in plants are still being analyzed to advance our understanding of sex determination in plants (Dellaporta and Calderon-Urrea, 1993). Jojoba, *Simmondsia chinensis*, is a dioecious, greatly diverse perennial shrub

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that is indigenous to the Sonoran Desert of North America. It is well-known for its oil, which is used in food and medicine, as well as for pharmaceutical use (Gad *et al*., 2021). As mentioned earlier, jojoba is a dioecious species (with separate male and female individuals) where the female plant produces seeds. Jojoba plant can face several problems, for example, the undesired male ratio, besides late flowering and production time of seeds. Also, when this jojoba plant is propagated through seeds, the male-to-female ratio will be about 50% or even more of these seedlings. Moreover, the gender can be determined morphologically solely after 3 to 4 years when the plants start to bear flowers. Though, if an efficient commercial yield is required, the male population should be no more than 10% of these seedlings (Al-Obaidi *et al*., 2017). Accordingly, all of these issues influence the cultivation of this plant, delay the ongoing breeding research, and affect oil production, which consequently affects the cost of the oil in the market. The success of growing jojoba, besides the whole jojoba industry and applications, depends on selecting genotypes of high yield and multiplying them via vegetative methods (Kumar *et al*., 2012). Consequently, it is critical to develop Molecular techniques for jojoba plant gender identification at any stage of growth. The creation of molecular markers for early sex identification in dioecious plants, such as jojoba, has been a priority in breeding projects. Several benefits over morphological indicators are provided by the use of DNA markers for sex determination in various crop species (Razumova *et al*., 2023). Many researchers investigated the use of molecular markers such as random amplified polymorphic DNA (RAPD), inter simple sequence repeat (ISSR) marker, and simple sequence repeats (SSR) for sex determination in jojoba. They revealed that such markers are useful approaches to distinguish between male and female jojoba plants (Agrawal *et al*., 2007).The accurate and efficient determination of jojoba gender is crucial for various aspects of plant research, breeding programs, and agricultural practices. The aim of this study is to investigate the use of male specific gene sequences to distinguish between male and female jojoba plants through PCR amplification using specific primers.

MATERIALS AND METHODS

Plant materials

Jojoba plants were kindly provided by Prof. Nahla El Awady, Horticulture Research Institute (HRI), Agricultural Research Center, Giza, Egypt. Twelve male and twelve female tree plants were used for the screening of sex-associated DNA markers. 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 –80°C prior to use.

DNA Isolation

Total genomic DNA was isolated from 1 g of leaf tissues of the selected

plants using the DNeasy Plant Mini kit (QIAGEN, Germany). The DNA was subsequently quantified by NanoDrop™ 2000 Spectrophotometer (Thermo Fisher Scientific, USA) and then tested for quality by agarose gel electrophoresis.

Retrieving DNA sequences and primers design

DNA fragments for male specific sequences were retrieved from Genbank with accession numbers (HQ166029.1, KP009976.1, and KP009975.1) then subjected to Primer3Plus online program to select primer pairs for the PCR analysis (Table 1).

PCR analysis

The PCR was carried out in a reaction volume of 25 µl using GeneAmp® PCR System 9600 Thermal Cycler (Fisher Scientific, USA). The reaction mixture contained one unit of Taq polymerase (GoTaq® DNA Polymerase, Promega), 30 ng of genomic DNA, 0.4 µM of each primer, 2.5 µl of 10X PCR reaction buffer and 0.2 mM of dNTPs. The PCR reactions were carried out at 94ºC for 5 min, followed by 35 cycles at 94ºC for 45 s, 56ºC for 45 s, 72°C for 1 min, and final extension at 72°C for 7 min. The amplified products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5ug/ml) in 1X TBE buffer then visualized on UV light and photographed using a Gel Documentation System (BIO-RAD 2000). Standard molecular weight marker, 50 bp DNA ladder, (NIPPON Genetics EUROPE) was also used in the agarose gel electrophoresis step.

RESULTS AND DISCUSSION

Young leaf tissues from 24 samples of both male and female plants were used to extract their genomic DNA according to manufacturer's instructions. The extracted DNA were tested for their purity and then used in the PCR analysis. Fig. 1 displays an example of analyzed DNA samples using a NanoDrop™ 2000 Spectrophotometer. Sample F1 has a concentration of 174.7 ng/µl and a ratio of 260/280 equal to 1.93, whereas sample M1 has a concentration of 87.9 ng/µl and a ratio of 260/280 equal to 1.86. All the tested samples had a ratio of 262/280 less than 2 indicating their purity.

In order to obtain PCR primers for amplifying distinctive products that distinguish between male and female jojoba plants, we retrieved three male-specific DNA sequences from Genbank with accession numbers (HQ166029.1, KP009976.1, and QP0009975.1). The obtained sequences were subjected to Primer3Plus online software (https://www.primer3plus.com/index.html) to generate PCR primers. For each sequence, we selected a pair of primers that amplify fragments less than 500 bp. As shown in Fig.2A, one distinct amplified fragment (281 bp) was produced by the PCR amplification

carried out using HQF and KP76R primers, and it was only found in the male samples and not the female ones.

Also the amplification using KP76F and KP76R primers produced a unique fragment (~326 bp) found only in male plant samples (Fig.2B). However, this set of primers amplified a fragment of about 275 bp that was present in the female samples, but not consistently across all the female samples used in this investigation. Additionally, using KP75F and KP75R primers resulted in two clear fragments; one was present in both male and female samples (~100 bp), and the other fragment (~150 bp) was present only in male samples (Fig. 2C). Due to the harsh environmental conditions, agricultural production in desert and semi-arid regions is limited, resulting in the underutilization of the majority of the land. However, recent advances in biotechnology and modern agricultural practices have opened up new opportunities for a range of human activities in these dry lands. Among these opportunities, jojoba is considered a promising oil seed crop for the economic development of arid and semi-arid regions (Al-Obaidi *et al*., 2017). To optimize productivity when growing jojoba plants, it is important to use the correct planting ratio of 10% male and 90% female (Tyagi and Prakash, 2004). Therefore, early identification of male and female plants is crucial for a successful plantation operation. Molecular markers, including the RAPD approach, have been used in many plants, such as *Mercurialis annua* (Khadka *et al*., 2002), *Carica papaya* (Deputy *et al*., 2002), and Date palm (Awan *et al*., 2017). Additionally, sex-linked loci in the plant genome have been detected using simple sequence repeats (SSR) and sequence-characterized amplified regions (SCAR), which have been utilized to isolate the related genes. In this study, we used a standard PCR technique to identify the gender type of the jojoba plant at any stage. To accomplish this, we designed PCR primers based on male specific sequences. It's interesting to note that the first set of PCR primers (HQF and HQR), used in this experiment, amplified a separate PCR product with ~281 bp fragment, clearly differentiating male and female jojoba plants. This fragment was only discovered in male samples. Also, primers KP76F and KP76R amplified ~ 326 bp product found only in male samples. Furthermore, the third set of primers (KP75F and KP75R) produced two clear fragments; one was present in both male and female samples (~ 100 bp), while, the other fragment (~150 bp) found only in male samples. Indeed, the three sets of primers used in this study clearly and simply differentiate between male and female jojoba plants. Similar findings were obtained by Ince *et al*., (2010) using Touch-down polymerase chain reactions to amplify jojoba DNA samples for gender identification. Moreover, Bhardwaj *et al*., (2010) analyzed a collection of male and female plants of 10 jojoba genotypes using RAPD and ISSR markers to compare their efficiency in detecting genetic polymorphism. ISSRs

showed superior efficiency over RAPDs due to the nonspecific results produced while employing the RAPD technique. RAPD and ISSR analyses yielded a scorable amplified products; however, about 60.7 and 69.3% were polymorphic. Contrary to our results, Bafeel and Bahieldin (2020) tested a variety of molecular markers (RAPD and ISSR) however, they were unable to identify the sex of jojoba plants in Saudi Arabia using these techniques. It's worth mentioning that Al-Dossary *et al*., (2021) reported the sequencing of male and female jojoba plants to define in more detail the differences between male and female plants on the genome level for the first time. This report will enrich the information about gender type in the jojoba plant and, as a result, develop more precise, conclusive methods for achieving this objective. Finally, the results of this study indicate the possibility of using simple PCR analysis to determine gender type in jojoba plants at any growth stage.

SUMMARY

Agricultural production in desert and semi-arid regions is limited due to harsh conditions, however, advances in biotechnology offer new opportunities. One promising crop is jojoba, which requires a specific male and female planting ratio for optimal growth. Therefore, early identification of the plants' gender is crucial for successful cultivation. This study developed PCR primers generated from male-specific DNA sequences that distinguish between male and female jojoba plants. One set of primers, HQF and HQR produced a distinct fragment (~281 bp) in male plant samples only. Also, KP76F and KP76R primers produced a unique fragment (~326 bp) found only in male plant samples. Furthermore, when using KP75F and KP75R primers, two fragments were produced, one was male specific (~150 bp) and the other fragment present in both male and female samples (~100).

Using these primers allowed rapid and reliable determination of jojoba gender, enabling early selection of female plants and reducing costs associated with non-productive male plants. Ultimately, simple PCR analysis can potentially determine gender type in jojoba plants at any growth stage.

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Table (1): Oligonucleotide primers used in this study.

Selected DNA sequences (accession number)	Primer name	Primer Sequence (5'-3')	(T_m)	Ref.
HQ166029.1	HQF HQR	AACGTGTAAGCTCGACCCAG GCTGTGGTCGATGTGGATCT	59.8ºC	Direct Submission
KP009976.1	KP76F KP76R	TTGCTGCGGACAACTTCTCA GAAGGCTTTTGCAGCGACTC	60ºC	(Heikrujam *et al.*, 2014a)
KP009975.1	KP75F KP75R	CAGATTGGGCTAGAGGCTGG CGAATCGCCTAACCCACCTT	60ºC	(Heikrujam *et al.*, 2014b)

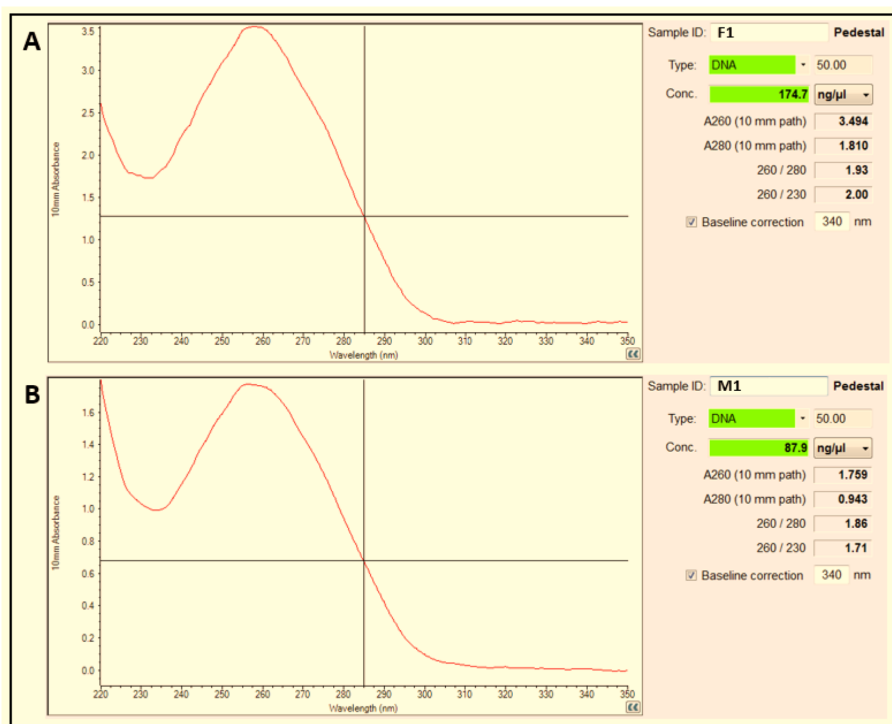

Fig. (1): Purity and concentration of selected DNA samples using NanoDrop™ 2000 Spectrophotometer. A: data obtained for F1 sample. B: data obtained for M1 sample.

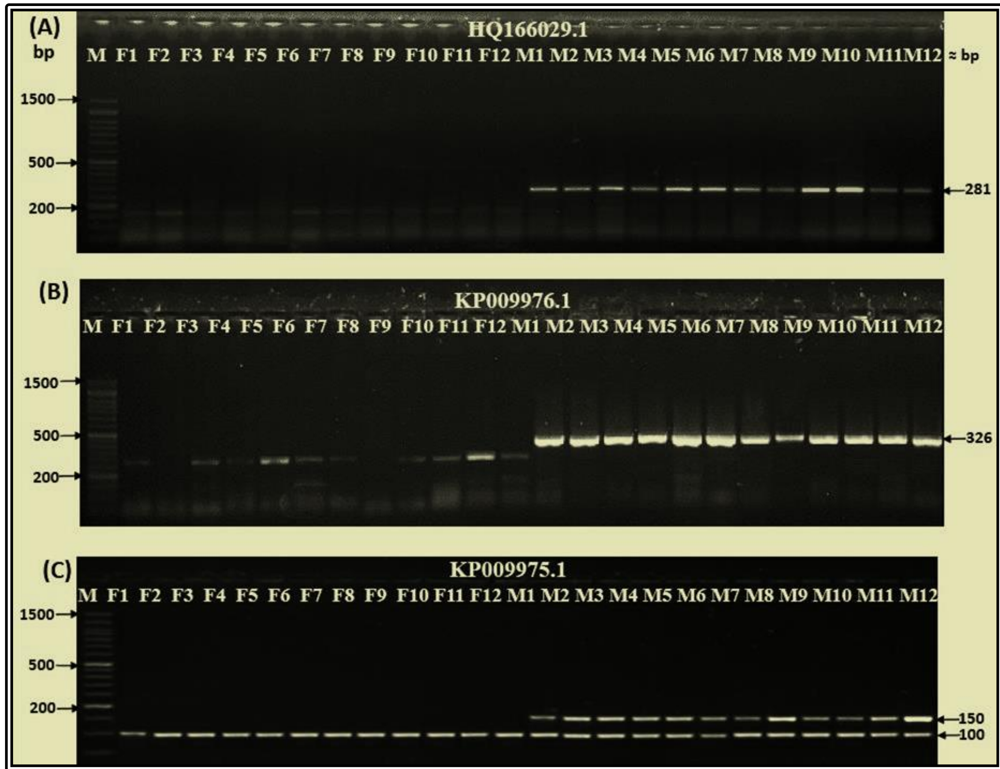

Fig. (2): PCR banding patterns of male and female jojoba plants. M: 50 bp ladder Marker. F1-F12: Female jojoba samples. M1-M12: Male jojoba samples. A: PCR amplification using HQF and HQR primers. B: PCR amplification using KP76F and KP76R primers. C: PCR amplification using KP75F and KP75R primers.