Egypt. J. Bot., **Vol. 63,** No. 1, pp. 129-139 (2023)

Egyptian Journal of Botany
http://ejbo.journals.ekb.eg/

A New Reliable and Sensitive PCR Assay as an Early Diagnosis of Sex-Determination in Jojoba Plants Based on the Human SRY Gene

Mohei EL-Din Solliman[(1, 2)#], Mohammed Ba Abdullah[(3)], Hany S. Elbarbary[(4, 5)], Heba Allah A. Mohasseb[(1, 2)#]

[(1)]Plant Biotechnology Department, College of Agricultural and Food Sciences, King Faisal University, P.O. Box 400, Al-Ahsa 31982, Kingdom of Saudi Arabia; [(2)]Plant Biotechnology Department, National Research Centre, Dokki, Cairo; P.O. Box. 12622Egypt; [(3)]Biological Sciences Dept., College of Sciences, King Faisal University, P.O. Box 400, Al-Ahsa 31982, Kingdom of Saudi Arabia; [(4)]College of Medicine, King Faisal University, P.O. Box 400, Al-Ahsa 31982, Kingdom of Saudi Arabia; [(5)]Internal Medicine Departments, Faculty of Medicine, Menoufiya University, Menoufiya, Egypt.

JOJOBA (*Simmondsia chinensis* L.) is a dioecious perennial evergreen shrub that is native to the south-western deserts of North America, and has now been introduced to the Middle East. Currently, the global production of jojoba is low; this is mainly due to the high male-to-female ratio in plantations, since the plants are mainly established from seed. Hence, a proper male:female population ratio is almost impossible to maintain in the field, leading to reduced production. We report a breakthrough in the methodology for determining the sex of jojoba plants. Our research revealed that the jojoba genome contains a sequence of the SRY gene, which encodes a protein that is similar to that found in the human sex-determining region (SRY) gene. This region was amplified and matched with sequences that are found in papaya and humans. The jojoba-SRY (SRY gene) was amplified, and we closely matched its sequences with those in the papaya and human. The complete sequence of the DNA was deposited into the GenBank (MK991776, 360pb) database. Our newly developed method is rapid and straightforward, representing a breakthrough for sex determination strategies of jojoba plants at early developmental stages. The uniqueness of this approach is that it targets specific sequences in the SRY, a region that has been extensively studied in human genetics but is also apparently recognizable in plants. This useful molecular diagnostic tool is vital to agronomical breeding programs for sex determination in long-lived jojoba crops.

Keywords: Jojoba, Female, Male, Sex determination, SRY gene sequence.

Introduction

Jojoba (*Simmondsia chinensis* (Link) Schneider) is a desert-dwelling, dioecious perennial shrub that belongs to the order Caryophyllales, family Simmondsiceae, with 52 chromosomes that endure drought and salinity (Mills et al., 2004; Fernanda, 2007).

As a result of mixed pollination, the sex of the plant is impossible to determine before it flowers. The shrub produces about 700–800g of seeds per year. However, jojoba is known for its high content of secondary active ingredients, which are very beneficial for several vital industries; moreover, jojoba can be cultivated in most Arab nations, especially desert countries (Shehata et al., 2018). Dioecism, which is associated with sexual dimorphism, has always been a significant challenge in cultivating plant species, especially woody trees. In dioecious

#Corresponding authors emails: Prof. Mohei EL-Din Solliman email: msolliman@kfu.edu.sa; Dr. Heba Allah A. Mohasseb email: hmohasseb@kfu.edu.sa
Received 28/06/2022; Accepted 08/10/2022
DOI: 10.21608/ejbo.2022.147831.2027
Edited by: Prof. Dr. Reda M. Gaafar, Faculty of Science, Tanta University, 31527 Tanta, Egypt.

plants, the sex of the plant is usually difficult to identify, especially before flowering and during the early stages of development. The sex chromosomes in humans and other long-lived dioecious species are responsible for the determination of separate sexes. It is one of the biological systems that determines the outcome of sexual characteristics in an organism. In unisexual animals, chromosomes do not differ in morphology or number between males and females. Allosomes, or sex chromosomes, contain sex-determination genes that differ in morphology and number between males and females. Unisexual diploid individuals have two sex chromosomes, while the rest are autosomes (Solliman et al*.,* 2019).

Sex-linked markers in dioecious plants

In non-crop-producing plants, sex determination is still essential for environmental reasons. It is essential for population studies, which examine the ratio of male and female individuals and investigate factors that influence sex distribution. Population research determines which plants should be assigned the status of protected/endangered species. Therefore, having methods to determine sex in plants is important for many different fields, including agriculture, horticulture, ecology, and environmental protection (Solliman et al., 2019). Breeding jojoba plants is challenging because of their long phases of juvenility and dioecy. The mammalian sex-determining region (SRY) gene was identified more than three years ago, and has been extensively studied since. The SRY is a potential candidate for the mammalian testis-determining factor. The SRY appears to be part of a family containing several autosomal representatives (Mohasseb et al., 2009; Agrawal et al., 2007, 2008, 2011). Jojoba is a dioecious desert plant, family Simmondsiceae, with 2n= 52 chromosomes that tolerate drought and salinity (Mills et al., 2004; Fernanda, 2007). In order to obtain the most fruitful crops, many fruit-bearing female trees and a minimal number of male trees must be cultivated. For the most fruitful harvest, a small number of fruit-bearing female trees must be planted. However, it can be challenging to determine the sex of saplings before they are three years old. The earliest that female trees can be identified is when they are three years old and jojoba fruit begins to form (Drobnic, 2003).

Some molecules that are involved in sex determination have been identified in these individuals, including the SRY, which is crucial to the development of the testes. The human SRY is found on the Y chromosome. The female is called the homogametic sex because only one type of gamete (X-bearing) is produced (Cherif et al., 2016). A copy of the SRY is present on one chromosome in most XX men who lack a Y chromosome, which explains their maleness. However, because of the loss of the remaining X chromosome, the SRY may disrupt gene expression, altering development of the testes. DNA in the intracellular SRY binds and dramatically alters gene expression, which may affect testis development. Men without the Y chromosome retain a copy of the SRY on an X chromosome; thus, they are male (Solliman et al., 2019, 2021). A polymerase chain reaction (PCR)-based method was developed to rapidly detect the SRY in plants. DNA was isolated from human blood and bone, and from multiple samples of potential reassortant plants for PCR amplification, using A-satellite sequences within higher-order repeats on the human X and Y chromosomes (Iglesias et al., 1997; Solliman et al., 2002; Mahmoud et al., 2008; Solliman et al., 2019). However, these methods generally have not been applied for determining T-DNA insertion sites, since they are inefficient and/or complicated (Mohasseb et al., 2020). Conventional PCR allows sequences within known boundaries to be amplified. Several methods, including targeted gene-walking PCR (Parker et al., 1991), unpredictably primed PCR (Dominguez & Lopez-Larrea, 1994), and inverse PCR (Ochman et al., 1988; Triglia et al., 1988), have been developed to amplify DNA sequences that flank regions with known sequences. It is difficult to identify the gender of a reproductively inactive individual jojoba plant. Using the molecular marker SRY is an attractive approach for sex determination that our research group has successfully tested in mammals and other plants such as the date palm and jojoba. The goal of the present study was to test PCR amplification of the SRY as a method for determining sex in different sources of jojoba shrubs.

Materials and Methods

Isolation of plant DNA

All DNA isolation and purifications were performed according to the protocols that were described by our groups elsewhere (Solliman et al., 2019).

PCR (Polymerase chain reaction)

PCR reaction conditions and the SRY gene-walking strategy were conducted according

to several previously published protocols by our group on papaya (Solliman et al., 2019) and elsewhere (Parker et al., 1991; Dominguez & Lopez-Larrea, 1994; Solliman et al., 2019; Mohasseb et al., 2020). Mastermix was prepared using Thermo Scientific DreamTaq DNA polymerase, according to the manufacturer's instructions.

Early sex verification in dioecious plants

Initially, we tested DNA from females and males; different samples were randomly selected from dioecious plants grown at King Faisal University Research and Training Station. DNA from these samples was isolated, as previously reported (Mills et al., 2004; Mahmoud et al., 2008). Human DNA samples of males and females were obtained from the College of Medicine, KFU, Saudi Arabia. The publicly available program Primer 3 (https://primer3.ut.ee/) was employed for all of the oligo primers used in this study, targeting the male-specific SRY marker (Table 1).

As previously described, the newly designed SRY primers flanked the 300 to 550 bp region (Dominguez & Lopez-Larrea, 1994; Solliman et al., 2019).

Isolation of SRY gene

Restriction digestion: Jojoba adult shrub genomic DNA was restriction-digested overnight at 37°C, according to the manufacturer's instructions. The restriction enzymes used included *Bam*HI and *Bgl*II *Sou3*A. The following oligonucleotides were used: ADOP-32 and ADOP-27 primer sequences that are shown in Table 1; oligonucleotides ADOP-32 and ADOP-27 which were synthesized by Macrogen Inc. (Seoul, Republic of Korea).

Primary PCR amplification

An Applied Biosystems Veriti® thermal cycler was used for PCR amplification. Adaptor primer T7 sequences are listed in Table 1, and the SRY-2 used for amplification is shown in Table 1. The sequences of the SRY-1R and SRY_2R primers are shown in Table 1.

Secondary amplification

We used the remaining PCR sample from the primary amplification as a template, after purifying it via phenol extraction. The DNA purifications and PCR were amplified according to (Mahmoud et al., 2008; Solliman et al., 2019). The sequences of the SRY primers are shown in Table 1.

DNA sequencing

Sanger sequencing of the obtained PCR amplicons was conducted according to the instructions of Macrogen Inc. (Seoul, Republic of Korea).

Bioinformatics

Most of the sequence (DNA) analyses were performed using the CLCVector program and the GenBank database.

Results and Discussion

Jojoba is a dioecious desert shrub that produces high-quality oil equivalent to that of the sperm whale. This study aimed to characterize the jojoba SRY gene in order to overcome the sex determination problem.

For the very first time, we identified SRY-related sequences in jojoba plants. The present study included molecular techniques to determine with 100% certainty whether a jojoba plant was male or female, on the basis of our primers.

TABLE 1. Primer pairs employed in PCR genome-walking for the male-specific SRY marker

Oligo name	Sequences (5′—- 3′)
SRY_F1	5′-TCTTGCGTGGGGCACTTACAGCAACTC-3′
SRY_R1	5′-GCTGGAGGCAAGCGCCATAATCTGAG-3′
SRY_F2	5′-GTGATCCTGAAGCTGGTTTCTTGAG-3′
SRY_R2	5′-TCGCCTTCCGACTAGGTGAAGAG-3′
SRY_F3	5′-TCTTCGGAGCCCCCACTAACACCATCAC-3′
SRY_R3	5′-GGGTTATACAATATATCTTTTCCGTTAT-3′
ADOP-27	5′-CACTATACCCGCCGGCGGGCCCGCT-3′
ADOP-32	5′-AATACGACTCACTATAGGGCGGCCGCCCGGGC-3′
T7	5′-AATACGACTCACTATAGGGC-3′

Development of a technique to determine the sex of Jojoba plants

We investigated the occurrence of SRY-related sequences in jojoba cultivars for the first time. A polymerase chain reaction was used to isolate the jojoba-SRY gene, using homologs of the conserved motif of the SRY gene from humans.

PCRs using the SRY primers in different regions in humans and papaya were investigated for a possible primer that produces smaller fragments of the SRY. We decided to use the same reverse primer, SRY-R3, as in the previous papaya sequence published (Gangopadhyay et al., 2007). At the same time, for the forwarding primer we chose the ADOPTER sequences, mentioned in the Materials and Methods section, to be upstream from our reverse primers and as mentioned by Solliman et al. (2002), and Mahmoud et al. (2008). In our research, we developed a set of primers that targeted a section of the SRY gene to produce a 553bp male-specific PCR fragment that is unambiguous for gender identification results.

Isolation and sequencing of a partial fragment of the Jojoba SRY-gene

Jojoba genomic DNA was isolated from females and males of different cultivars in Alhassa Oasis, Saudi Arabia. The ADOP-32 primer contained sequences of the T7 primer. The 3'-end of the ADOP-27 primer was blocked to prevent extension during PCR amplification (Solliman et al., 2002; Mahmoud et al., 2008). The ligated DNA served as a template, and the SRY sequences were PCR-amplified with the T7 and SRY-1R primers. The amplified DNA became the template for the second PCR reaction with the T7 and SRY-2R primers (Fig. 1). A band of approximately 0.36 kbp was observed after digesting DNA with Sau3AI, which was sequenced (Figs. 2, 3, and 4) as described in the Material and Methods section with the steps to isolate the SRY gene). Cloning of the SRY gene was further confirmed with specific vector T7 and SP6 primers and subsequent sequencing (date not showing).

After additional PCR reactions with primers for different markers specific to the Y chromosome (e.g., the SRY and universal primer), the sex of the plant was identified as male. The SRY marker has previously been shown to yield a 200 to 553 bp PCR product that overlaps in size with some human and papaya sequences. Thus, additional PCR reactions had to be performed to determine the gender of the plant. The isolation of unknown DNA sequences that flank known regions is critical for gene analysis. Several protocols have been developed to isolate unknown DNA sequences (promoters) that are adjacent to known DNA sequences (cDNAs) by PCR (Hui et al., 1998).

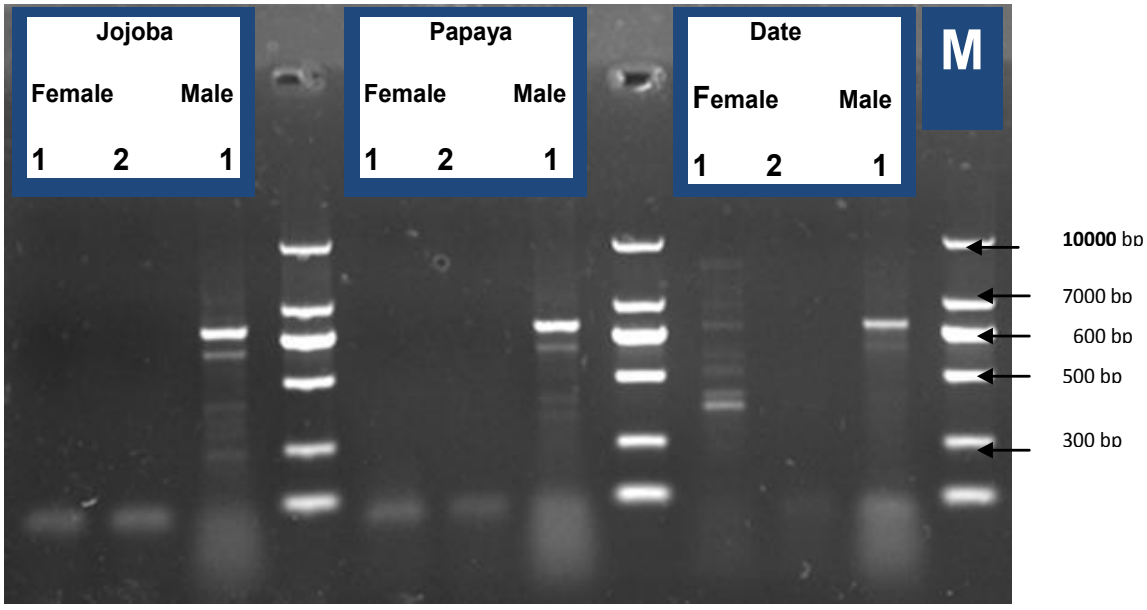

Fig. 1. PCR amplification for screening of the SRY gene with jojoba (job female 1,2 and job male 1), and comparisons with different varieties of dioecious plants: papaya (PyF 1,2, and PyM 1) and date palm (DF 1, 2, and DM 1), using specific primers SryF1+ SryR1 lane M DNA 100-bp marker

Isolated SRY DNA fragments were deposited into GenBank

A similar approach was adopted to isolate approximately 350 and 360bp lengths of DNA fragments of the jojoba-SRY gene by walking the genomic DNA using primers SRYR1, SRYR2, and SRYF1. The complete sequence of the DNA is presented in Figs. 2, 3, and 4. Furthermore, the sequence information was deposited in a public database, GenBank (Jojoba BankIt MK991776, 360 pb), as shown in Fig. 5.

Isolation and characterization of Jojoba SRY

A genomic fragment of approximately 360bp was amplified from different varieties of jojoba using the specific primers that were identified from human and papaya SRY sequence information. The SRY gene sequences were deposited with an accession number (Jojoba BankIt MK991776, 360pb) into the GenBank database. Our results showed isolation and characterization of different varieties of jojoba (Figs. 5 and 6).

Dioecy offers opportunities to explore the male and female programs separately, which can provide insight into the evolutionary, developmental, and molecular processes that lead to separate mechanisms for sex expression. We correctly identified the sex of 100 out of 100 individuals. These results show that our method is reliable for the sex determination of highly degenerated samples. Sequencing the coding exon region of the SRY gene in the five cases showed 99–100% alignment with the sequences of normal males. Genotyping was conducted for two closely related individuals with 50 normal male controls. Jojoba sex chromosomes are still indistinguishable, and our lab is currently investigating this. Therefore, the sex type of jojoba seedlings cannot be determined by cytological methods (Parasnis et al., 2000). Moreover, the sex type of jojoba seedlings cannot be determined either by embryo shape or morphology at the juvenile developmental stage. The propagation of jojoba is mainly carried out through seeds (Singh et al., 2008).

Sequencing analysis

The SRY fragment was 360bp in length. BLAST results showed that the predicted ORF had 98% similarity to the human published sequence, indicating a high degree of similarity with papaya, date palm, and human SRY. A 360bp fragment was obtained via PCR amplification.

Computer-aided sequence to identify Jojoba -SRY

A partial SRY gene sequence is shown in Figs. 4 and 5. Computer-aided sequence analysis of Jojoba-SRY revealed the ORF of the SRY. Figure 6 shows the homology searches with forwarding primers for the SRY. The alignment was very low; however, a very high degree of similarity was found upon comparison with the Phoenix dactylifera mitochondrion, complete genome sequence ID: (Accession number: gb|JN375330.1). BLAST was performed to show the homology with other multiple sequence alignments, as illustrated in Fig. 5.

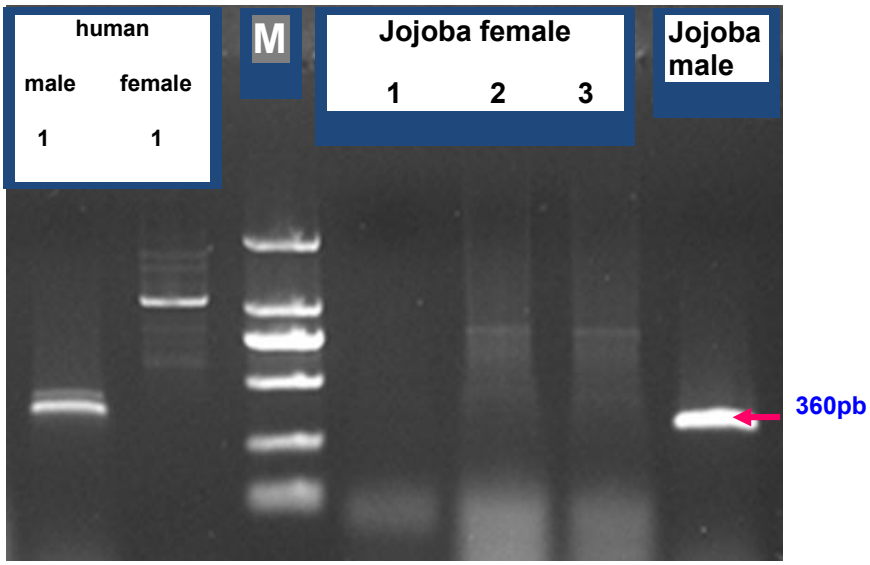

Fig. 2. PCR amplification for screening of the SRY gene in the human male (HM and HF), and compared with different female varieties of jojoba plants (JF 1, 2, and 3), and jojoba (JM 1), using specific primers universal SryF2+ SryR2 lane M DNA 100bp marker

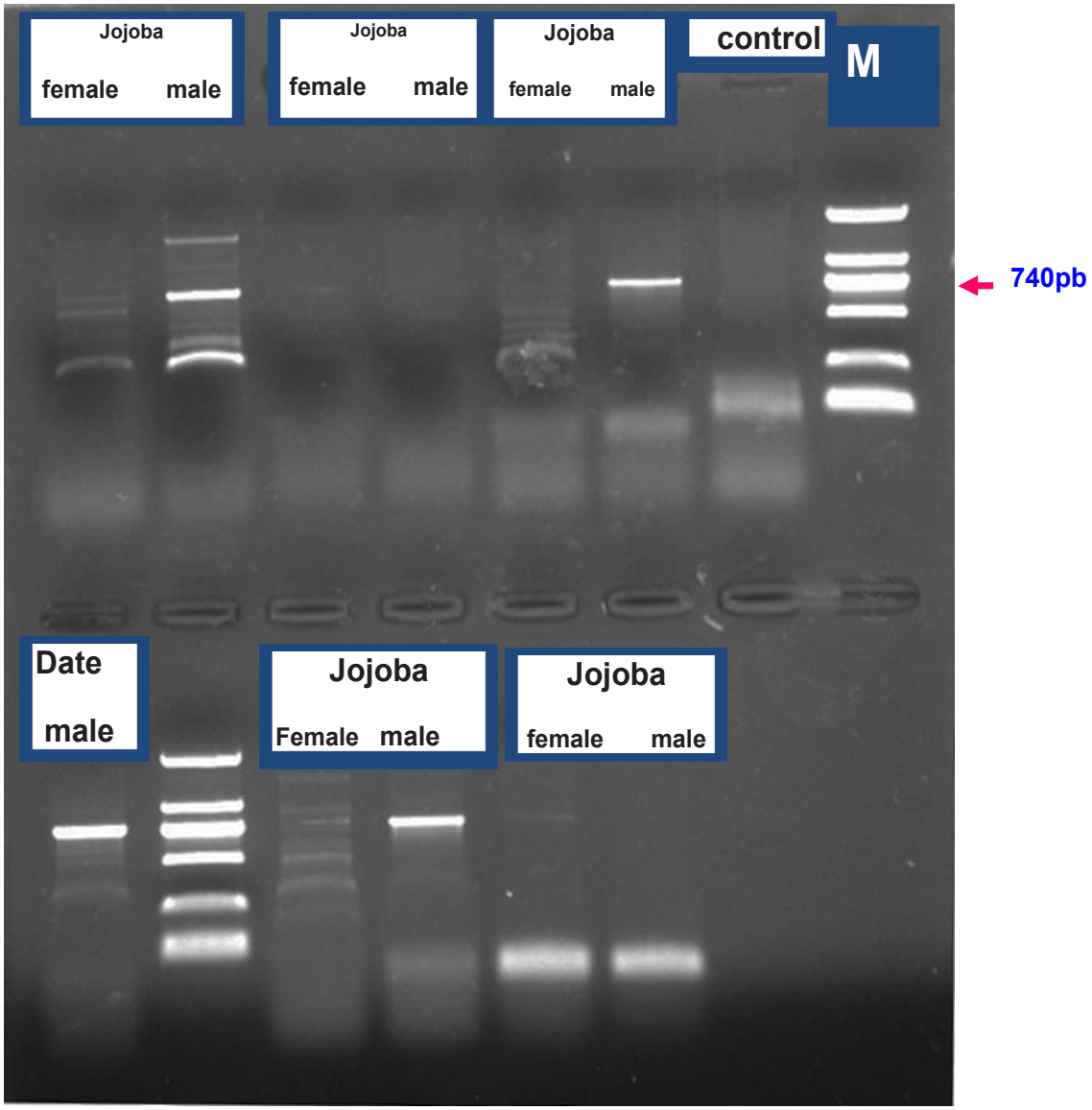

Fig. 3. PCR amplification for screening of the presence of the *SRY* gene with different varieties of jojoba using specific SryF3+SryR2 primers lane M DNA 1 kbp Marker

```
  1 atgcaatcat atgcttctgc tatgttaagc gtattcaaca gcgatgatta cagtccagct
 61 gtgcaagaga atattcccgc tctccggaga agctcttcct tcctttgcac tgaaagctgt
121 aactctaagt atcagtgtga aacgggagaa aacagtaaag gcaacgtcca ggatagagtg
181 aagcgaccca tgaacgcatt catcgtgtgg tctcgcgatc agaggcgcaa gatggctcta
241 gagaatccca gaatgcgaaa ctcagagatc agcaagcagc tgggatacca gtggaaaatg
301 cttactgaag ccgaaaaatg gccattcttc caggaggcac agaaattaca ggccatgcac
```

Fig. 4. The sequence was deposited in GenBank with the accession number (Jojoba BankIt MK991776, 360pb)

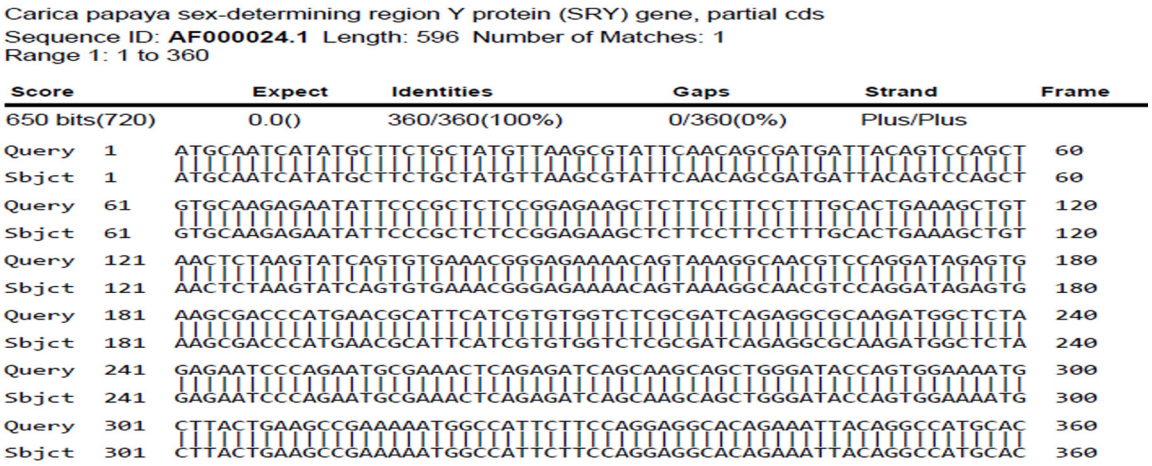

Fig. 5. Comparison of the DNA sequences of jojoba-SRY with SRY genes from papaya (accession number: gb|AF000024.1|CPAF000024) and homo sapiens (accession number: gb|JQ811918.1)

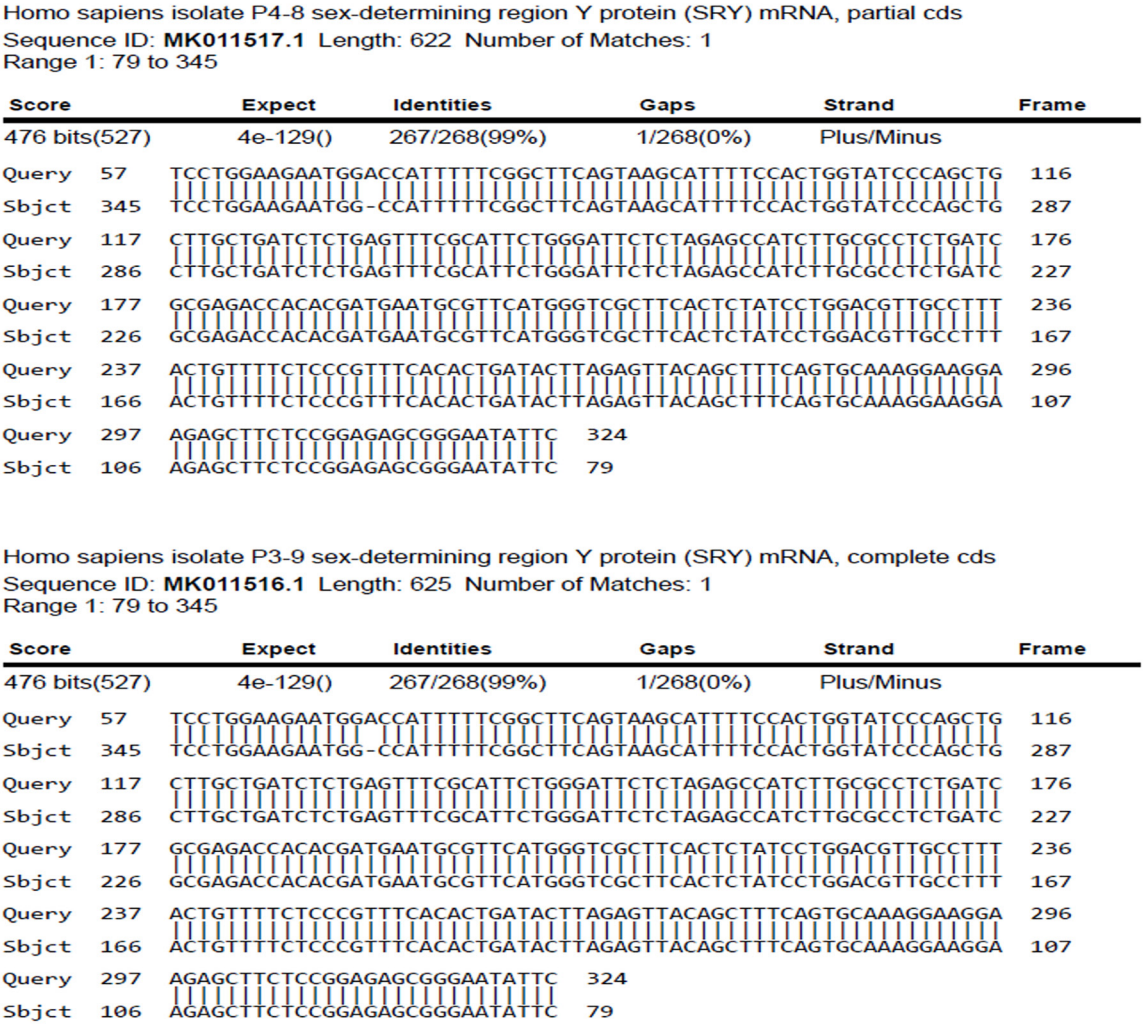

Fig. 6. Comparison of the DNA sequences of jojoba-SRY with SRY genes from humans (Homo sapiens clone 15 SRY (SRY) gene, complete CDs, accession number: gb|JQ811918.1)

The PCR primer pair consists of a forward PCR primer with 20 consecutive nucleotides of the sequence of flanking nucleotides. The sequence of the SRY-F1 primer is SRY_F 5′-AAACTTGATAGTTGTGTCACTCAT-3′. The sequence of the SRY-R1 primer is 5′-GGGCT-GTAAGTTATCGTAAAAGGAGC-3′. The sequence of the SRY_R2 is 5' CTAGCTGGTCAC-GTTGACCTTTTGTCC-3.

Sequences were compiled with the usage of Sequencher (model 4.1; Gene Codes, Ann Arbor, MI), and aligned with the usage of Clustal X (Thompson et al., 1997). The program Distance tree of results (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to decide the premiere for phylogenetic analyses. Each of the three genomic regions (SRY, 5′ flank, and 3′ flank) were analyzed, one by one, in addition to being input to a blended statistics set for phylogenetic reconstruction. The phylogenetic tree, based on the SRY coding region (360bp) and amino acid residues from translated SRY sequences, was highly similar to the nucleotide phylogeny (Fig. 7). Both the distance-based and maximum-likelihood (ML) analyses supported the same higher-order node that was observed in the nucleotide phylogeny, uniting lineage SRY in jojoba with SRY in humans and plants. We applied the present research for invention by providing PCR primer pairs to determine the sex of jojoba, wherein the PCR primer pairs are specific to the male. In this technique, partial sequences of the jojoba sex determination region of the Y chromosome (SRY) gene were amplified using nested PCR. Thus, our novel method represents a breakthrough in the sex determination of plants, and will help to identify the sex of saplings at early developmental stages. Our newly developed strategies for unambiguously selecting the female jojoba at a young age are simple and very important to breeders, limiting the plantation costs associated with inadvertently cultivating non-productive male plants. Early sex determination opens new perspectives for multiplying jojoba genotypes by seed, reintroducing biodiversity into jojoba groves, and for implementing genetic improvement programs all over the world. The SRY primers also relate to a method for determining the sex of a jojoba plant by detecting and using specific primers in the jojoba genome. In certain embodiments, the method is characterized through the amplification of a portion of genomic DNA of the jojoba tested, and using a pair of primers specific to the SRY gene to obtain the sex of the plant. The human SRY is like the SRY in mice and jojoba plants. Thus, a model for SRY function has been developed in plants. This finding has been particularly important for discovering interactions of the SRY with other genes in male sex determination. We have made great progress in our understanding of sexual dimorphism since the creation of the jojoba historically; the difference between the two sexes was confirmed to be due to the presence of the SRY gene in males. We have now accumulated additional molecular events that contribute to sex determination. In this method, all the template molecules are likely to be amplified linearly, leading to a lot of noise. In this study, we followed a protocol developed by Sullivan et al. (1993), and Reddy et al. (2002), that involved the use of restriction digestion of genomic DNA, followed by partial fill that prevents self-ligation between fragments. As mentioned above, the present study relates to molecular techniques that are specific to the sex of jojoba plants, and to the use of these techniques for distinguishing between male and female plants. The techniques make the early selection of female plants possible, thereby limiting plantation costs associated with the cultivation of non-productive male plants. Moreover, our techniques are «universal» in that they can be used regardless of the origin, variety, or cultivar of the jojoba.

Conclusions

The ability to determine the sex of jojoba plants in the early stages of development enables correct planning of a plantation. The amplified regions of the jojoba-SRY genes closely matched sequences of the SRY in the papaya and human. We designed two sets of primers that could amplify fragments of approximately 330bp and 470bp in dioecious male plants and human male samples, respectively. We used the PCR technique, with primers complementary to the SRY gene, to amplify the SRY fragment from genomic DNA extracted from male and female jojoba plants. The sequence analysis indicated that a conserved sequence related to the mammalian SRY gene. The existence of an SRY in male jojoba plants may enable sex determination and identification in all plant systems. The correct sex was detected in all tested jojoba plants. The sequences of the amplified regions closely matched published human and papaya SRY sequences. Fragments of about 360bp were isolated from jojoba via genomic walking.

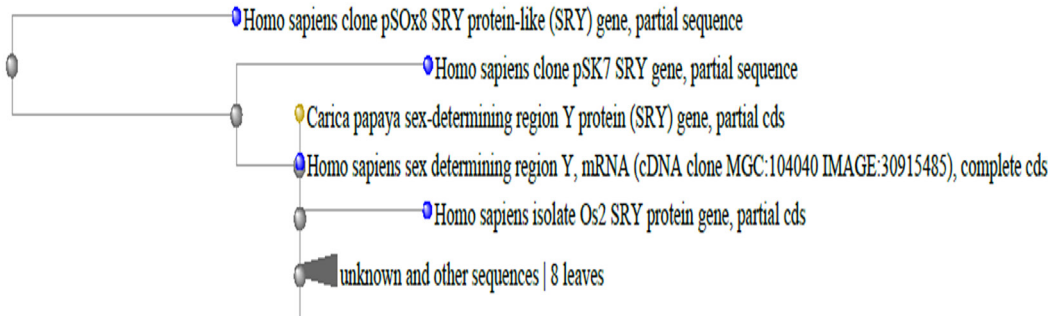

Fig. 7. Phylogenetic relationships of jojoba, *Carica papaya*, and Homo sapiens sex-determining region Y [Phylogenetic analysis of sex-determining region Y protein (SRY) gene and SRY gene proteins were performed separately, using all the reported members from Homo sapiens sex-determining region Y and *Carica papaya*. The tree was constructed using full-length amino acid sequences that were based on a neighbor-joining method with 100 bootstrap values]

Novelty statement: Dioecism, which is associated with sexual dimorphism, has always been a problem with cultivating plant species, especially woody trees. The present study was conducted with the purpose of developing, for the first time, reproducible and applicable techniques for the early diagnosis of sex in jojoba male plants, using simplex PCR and based on the SRY gene related to the human sex-determining region. In addition, the protocol was optimized to detect male jojoba plants, especially before flowering and during early stages of development.

Data availability statement: The data supporting the findings of this study are available in The National Center for Biotechnology Information: (https://www.ncbi.nlm.nih.gov/nuccore/MK991776.1?report=GenBank)

Acknowledgments: The authors extend their appreciation to the International Cooperation and Knowledge Exchange Administration at KFU project No. ICI-2019a.

Conflicts of interest: The authors declare no conflict of interest.

Authors' contributions: Mohei EL-Din Solliman and Heba A. A. Mohasseb conceived and designed the research. Mohei EL-Din and Heba Allah A. Mohasseb conducted experiments. Mohei EL-Din Solliman, Heba A. A. Mohasseb, Mohammed Ba Abdullah, and Hany Elbarbary analyzed the data. Mohei EL-Din Solliman, Heba Allah A. Mohasseb, and Mohammed Ba Abdullah wrote the manuscript.

Ethics approval: Not applicable.

References

Agrawal, V., Sharma, K., Gupta, S., Kumar, R., Prasad, M. (2007) Identification of sex in *Simmondsia chinensis* (jojoba) using RAPD markers. *Plant Biotechnology Reports*, **1**, 207- 210.

Agarwal, M., Shrivastava, N., Padh, H. (2008) Advances in molecular marker techniques and their applications in plant sciences. *Plant Cell Reports*, **27**, 617-631.

Agarwal, M., Shrivastava, N., Padh, H. (2011) Development of sex-linked AFLP markers in *Simmondsia chinensis*. *Plant Breeding*, **130**, 114-116.

Cherif, E., Zehdi-Azouzi, S., Crabos, A., Castillo, K., Chabrillange, N., Pintaud, J.C. (2016) Evolution of sex chromosomes before speciation in the dioecious *Phoenix* species. *Journal of Evolutionary Biology*, **29**, 1513–1522.

Dominguez, O., Lopez-Larrea, C. (1994) Gene walking by unpredictably primed PCR. *Nucleic Acids Research,* **22**, 3247-3248.

Drobnic´, K. (2003) Unusual failure of gender determination using amelogenin test, Poster Presented at Third European–American School in Forensic Genetics and Mayo Clinic Course in Advanced Molecular and Cellular Medicine, Sept 1–5, p. 36, Zagreb, Croatia.

Fernanda, G.M. (2007) Quality and chemical composition of residual cakes obtained by pressing jojoba seeds produced in La Rioja, Argentina. *Argentina. Journal of the Argentine Chemical Society*, **95**(1-2), 39-47.

Gangopadhyay, G., Roy, S.K., Ghose, K., Poddar, R.,

Bandyopadhyay, T., Basu, D., Mukherjee, K.K. (2007) Sex detection of *Carica papaya* and *Cycas circinalis* in the pre-flowering stage by ISSR and RAPD. *Current Indian Science India*, **92**(4), 524-526.

Hui, E.K., Wang, P.C., Lo, S.J. (1998) Strategies for cloning unknown cellular flanking DNA sequences from foreign integrants. *Cellular and Molecular Life Sciences*, **54**, 1403–1411.

Iglesias, V.A., Moscone, E.A., Papp, I., Neuhuber, F., Michalowski, S., Phelan, T., Spiker, S., Matzke, M., Matzke, A.J. (1997) Molecular and cytogenetic analyses of stably and unstably expressed transgene loci in tobacco. *Plant Cell*, **9**, 1251-1264.

Mahmoud, Eman A., Mohei El-Din, S.M., Aboul-Sound, M.A.M., Aboul-Enein, A.M., Sobhy, G.A., El-Shemy, H.A. (2008) Cloning of a novel antifungal promoter from *Phaseolus vulgaris* and the determination of its activity in stably transformed *Nicotiana tabacum* plants. *Current Issues in Molecular Biology*, **S1**, 53–61.

Mills, D., Zhou, Y., Benzioni, A. (2004) Improvement of jojoba shoot multiplication in vitro by ventilation *In Vitro Cellular & Developmental Biology - Plant*, **40**, 396–402.

Mohasseb, H.A., Moursy, H.A., El-Bahr, M.K. (2009) Sex determination of jojoba using RAPD markers and sry gene primer combined with RAPD primers. *Research Journal of Cell and Molecular Biology*, **3**, 102-112.

Mohasseb, Heba Allah A., Solliman, M., Al-Mssallem, I.S., Abdullah, M.B., Shehata, W.F., Hany, A.E. (2020) Salt tolerant phenomena, sequencing, and characterization of glyoxalase I (Jojo-Gly I) gene from Jojoba in comparison with other glyoxalase I genes. *Plants*, **9**(10), 1285.

Murray, M.G., Thompson, W.F. (1980) Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research*, **8**, 4321-4325.

Ochman, H., Gerber, A.S., Hartl, D.L. (1988) Genetic applications of an inverse polymerase chain reaction. *Genetics,* **120**, 621-623.

Parasnis, A.S., Gupta, V.S., Tamhankar, S.A., Ranjekar, P.K. (2000) A highly reliable sex diagnostic PCR assay for mass screening of papaya seedlings. *Molecular Breeding*, **6**, 337- 344.

Parker, J.D., Rabinovitch, P.S., Burmer, G.C. (1991) Targeted gene walking polymerase chain reaction. *Nucleic Acids Research,* **19**, 3055-3060.

Reddy, M.K., Nair, S., Sopory, S.K. (2002) A new approach for efficient directional genome walking using Polymerase Chain Reaction. *Analytical Biochemistry*, **306**, 154–158.

Shehata, W.F., Solliman, M.M., Mohasseb, H.A., Al-Khateeb, A.A., Aldaej, M.I., Alturki, S.M. (2018) Protocol of in vitro Jojoba (*Simmondsia chinensis* (Link) Schneider) callus induction. *Pakistan Journal of Biological Sciences*, **21**(8), 376-382.

Singh, A., Reddy, M.P., Patolia, J.S. (2008) An improved protocol for micropropagation of elite genotype of *Simmondsia chinensis* (Link) Schneider. *Biologia Plantarum*, **52**, 538-542.

Solliman, M. (2002) Isolation and characterization of plant promoters for high level and tissue-specific expression of foreign genes in transgenic plants. *Ph.D. Thesis. International Center for Genetic Engineering and Biotechnology*, 1998-2002, India.

Solliman, M., Mohasseb, Heba Allah A., Al-Khateeb, A.A., Al-Khateeb, S.A., Chowdhury, K., El-Shemy, H.A., Aldaej, M.I. (2019) Identification and sequencing of date-SRY gene: a novel tool for sex determination of date palm (*Phoenix dactylifera* L.). *Saudi Journal of Biological Sciences,* **26**(3), 514-523.

Solliman, M., Mohasseb, Heba Allah A., Al-Khateeb, A.A., Al-Khateeb, S.A., Wael, F.S. (2020) Induce new tomato varieties' resistance to fungal using Agrobacterium-mediated Transformation. *Fresenius Environmental Bulletin*, **29**(12), 11535-11544.

Sullivan, K.M., Mannucci, A., Kimpton, C.P., Gill, P.A. (1993) Rapid and quantitative DNA sex test: fluorescence-based PCR analysis X–Y homologous gene amelogenin. *BioTechniques*, **15**, 636– 641.

Thompson, J.D., Gibson, F., Plewniak, F., Jeanmougin, D., Higgins, G. (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, **25**, 4876–4882.

Triglia, T., Peterson, M.G., Kemp, D.J. (1988) A procedure for in vitro amplification of DNA segments that lie outside the boundaries of known sequences. *Nucleic Acids Research,* **16**, 8186.

اختبار PCR جديد موثوق وحساس كتشخيص مبكر لتحديد الجنس في نباتات الجوجوبا بناءً على جين SRY البشري

محيي الدين سليمان محمد سليمان[1، 2]، محمد محفوظ باعبد الله[3]، هاني سعيد البربري[4، 5]، هبة الله عادل أحمد محسب [1، 2]

[1]قسم التقنية الحيوية الزراعية - كلية العلوم الزراعية والغذائية، جامعة الملك فيصل، ص. ب 400، الأحساء 31982-المملكة العربية السعودية، [2]قسم التكنولوجيا الحيوية النباتية- المركز القومي للبحوث- الدقي- القاهرة- ص.ب. 12622- مصر، [3]قسم العلوم البيولوجية - كلية العلوم- جامعة الملك فيصل، ص. ب 400- الأحساء 31982- المملكة العربية السعودية، [4]كلية الطب- جامعة الملك فيصل، ص. ب 400- الأحساء 31982- المملكة العربية السعودية، [5]أقسام الطب الباطني- كلية الطب- جامعة المنوفية- المنوفية- مصر.

الجوجوبا (*Simmondsia chinensis* L.) هي شجيرة دائمة الخضرة ثنائية المسكن موطنها الصحاري الجنوبية الغربية لأمريكا الشمالية، وقد تم تقديمها الآن إلى الشرق الأوسط حاليا، يرجع انخفاض الإنتاج العالمي من الجوجوبا إلى ارتفاع نسبة الذكور إلى الإناث في المزارع، حيث يتم إنشاء النباتات أساسًا من البذور. ومن ثم، يكاد يكون من المستحيل الحفاظ على نسبة مناسبة من الذكور إلى الإناث في الميدان، مما يؤدي إلى انخفاض الإنتاج. وقد توصلنا الي طريقة للاختراق في منهجية تحديد جنس نباتات الجوجوبا. حيث كشف بحثنا أن جينوم الجوجوبا يحتوي على سلسلة من جين SRY، الذي يشفر بروتينًا مشابهًا لتلك الموجود في جين المنطقة المحددة للجنس البشري (SRY). تم تضخيم هذه المنطقة ومطابقتها مع سلاسل موجودة في البابايا والبشر. تم تضخيم جين jojoba-SRY (SRY)، وقمنا بمطابقة تسلسله عن كثب مع تلك الموجود في البابايا والبشر. ثم تم إيداع التسلسل الكامل للحمض النووي في قاعدة بيانات GenBank (MK991776،360pb). ودل ذلك على أن طريقتنا المطورة حديثًا سريعة ومباشرة، وتمثل اختراقًا لاستراتيجيات تحديد جنس نباتات الجوجوبا في مراحل النمو المبكرة. وهذا يعتبر نهجا متفردا وهو أنه يستهدف تسلسلات محددة في SRY، وهي منطقة تمت دراستها على نطاق واسع في علم الوراثة البشرية، ولكن من الواضح أيضًا أنها يمكن التعرف عليها في النباتات. وتعتبر أداة التشخيص الجزيئي الحيوية ذات المغزى لبرامج التربية الزراعية لتحديد جنس محاصيل الجوجوبا طويلة العمر.