

A new reliable and sensitive PCR assay as an early diagnosis for Sex-determination in Jojoba plants based on the human SRY gene

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Abstract

Background

Jojoba (*Simmondsia Chinensis* L.) is a dioecious perennial evergreen shrub native to the south-western desert of North America and has now been introduced to Middles East. Currently, there is low production of jojoba globally, mainly due to the high male to female ratio in the plantations since they are mainly established from seed. Hence, a proper population stand in terms of the male: female 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 the sequence of SRY gene, which encodes a protein similar to that found in the human Sex-Determining Region (SRY) gene. This region was amplified and matched with sequences found in papaya and human.

Results

The jojoba-SRY regions (SRY gene) was amplified, and we closely matched with sequences of SRY in the papaya and human. The complete sequence of the DNA was deposited in GenBank (BankIt MK991776,3 60pb). Our newly-developed method is rapid and straightforward, representing a breakthrough for sex determination strategies of jojoba plants to identify sex at early developmental stages.

Conclusion

The uniqueness of this approach is that it targets specific sequences in SRY, a region extensively studied in human genetics but also apparently recognizable in plants. This useful molecular diagnostic tool is vital in agronomical breeding programs for sex determination in long-lived jojoba crops.

Background

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

Due to mixed pollination, the sex of the plant is impossible to determine before it flowers. The shrub produces about 700–800 gm seeds per year. However, jojoba is known for being a good plant due to its high content of secondary active ingredients which are very beneficial for several vital industries; besides that, it can be cultivated in most Arab countries, especially desert ones [Shehata et al., 2018]. Dioecism, associated with sexual dimorphism, has always posed an immense challenge for cultivating plant species, especially in woody trees. In dioecious plants, the sex of the plant is usually tricky 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 determine the outcome of sexual characteristics in an organism. In

unisexual animals, chromosomes do not differ in morphology or number in males and females. Allosomes or sex chromosomes differ in morphology and number in males or females and contain sex-determination genes. Unisexual diploid individuals have two sex chromosomes, and the rest are autosomes [Solliman *et al.*, 2019].

Sex-linked markers in dioecious plants

In non-crop-producing plants, sex determination remains 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. Thus, methods for sex determination in plants are of high significance to many different fields, including agriculture, horticulture, ecology, and environmental protection [Solliman *et al.*, 2019]. Breeding Jojoba plants are challenging because of the 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. SRY is a potential candidate for the mammalian testis-determining factor. SRY appears to be part of a family with several autosomal representatives [Mohasseb *et al.*, 2009, Agrawal *et al.*, 2007, Agrawal *et al.*, 2008, Agrawal *et al.*, 2011]. Jojoba is a dioecious desert plant, family Simmondsiaceae, with $2n = 52$ chromosomes that tolerate drought and salinity [Mills *et al.*, 2004 and Fernanda, 2007]. 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 female trees can be identified when they are three years old when jojoba fruit begins to form [Drobnic, 2003].

Some molecules involved in sex determination have been identified in these individuals, including SRY, which is crucial to the development of testes. Human SRY is found on the Y chromosome. Female is called the homogametic sex because only one type of gamete(X-bearing) is produced [Cherif *et al.* 2013]. A copy of SRY is present on one chromosome in most XX men lacking a Y chromosome explaining maleness. However, because of the loss of the remaining X chromosome, SRY may disrupt gene expression, altering testis development. DNA in Intracellular SRY binds and dramatically alters gene expression, which may affect testis development. Men without a Y chromosome retain a copy of SRY on Y chromosome, So, they are male [Solliman *et al.*, 2019 and Solliman *et al.*, 2021]. A Polymerase chain reaction (PCR)-based method was developed to detect SRY in plants rapidly. DNA was isolated from blood, bone, and multiple other samples of potential reassortant plants for PCR amplification using A-satellite sequences within higher-order repeats on the human X and Y chromosomes [Solliman *et al.*, 2019, Iglesias *et al.*, 1997, Solliman *et al.* 2002, and Eman *et al.*, 2009]. However, these methods generally have not been applied for determining T-DNA insertion sites because 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 and Lopez-Larrea 1994], and Inverse PCR [Triglia *et al.*, 1988 and Ochman *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 of jojoba plants. The molecular marker SRY is an attractive approach for sex determination that our research group has successfully tested in mammals and other plants like the date palm and jojoba. The goal of the present study was to test the PCR amplification of SRY as a method for determining sex in different sources of jojoba shrubs.

Results

Jojoba is a dioecious desert shrub that produces high-quality oil equivalent to the sperm whale. This work aimed to characterize the jojoba SRY gene to overcome this problem.

For the very first time, we have identified SRY-related sequences in jojoba plants. The present study includes molecular techniques and determines with 100% certainty whether male or female in jojoba plants by using our primers.

1-Development of a technique to determine the sex of Jojoba plants

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

PCR with the SRY primers in different regions in humans and papaya were investigated for a possible primer that produces smaller fragments of 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 forwarding primer, we choose the ADOPTER sequences as we mention in the material and methods to be upstream from our reverse primers and as mentioned by [Solliman *et al.* 2002 and Eman *et al.*, 2009]. In our work, we developed a set of primers that target a section of the SRY gene to produce a 553-bp male-specific PCR fragment that is unambiguous and gender identification results.

2-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 and Eman *et al.*, 2009]. The ligated DNA served as a template, and the SRY sequences were PCR-amplified with T7 and SRY-1R primers. The amplified DNA was the template for the second PCR reaction with the T7 and SRY-2R primers (Fig. 1). A band of approximately 0.36 kb was observed after digesting DNA with Sau3AI, which was sequenced (Figs. 2, 3, and 4) as shown in the material and methods for 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).

The sex of the plant has been identified as male after additional PCR reactions with primers for different markers specific to the Y chromosome (e.g., SRY and universal primer). The SRY marker has been previously shown to yield a 200- to 553-bp PCR product that overlaps in size with some human and papaya sequences. Thus, additional PCR reactions must 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].

3-Isolated SRY DNA fragments were deposited into GenBank.

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

4-Isolation and characterization of Jojoba SRY

A genomic fragment of approximately 360 bp was amplified from different varieties of Jojoba using the specific primers identified from the human and papaya SRY sequence information. The SRY gene sequences were deposited by the 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, giving an insight into the evolutionary, developmental, and molecular processes leading to separate mechanisms for sex expression. We could correctly identify 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 of two closely related individuals with 50 normal male controls. Jojoba sex chromosomes till now are not distinguishable and our lab doing this part of research nowadays. Therefore, the sex type of jojoba seedlings cannot be determined by cytological methods [Parasnis et al., 2000]. Also, the sex type of jojoba seedlings cannot be determined either by embryo shape or morphology at the juvenile developmental stage. Propagation of jojoba is mainly through seeds [Singh et al., 2008].

5-Sequencing analysis

The SRY fragment was 360 bp 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 360 bp fragment was obtained by PCR amplification.

6-Computer-aided sequence to identify Jojoba -SRY

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

Discussion

The PCR primer pair consists of a forward PCR primer consisting of 20 consecutive nucleotides of the sequence of flanking nucleotides. The sequence of the SRY-F1 primer is SRY_F 5'-AAACTTGATAGTTGTGTCATCAT-3'. The sequence of the SRY-R1 primer is 5'-GGGCTGTAAGTTATCGTAAAAGGAGC-3'. SRY_R2; 5' CTAGCTGGTCACGTTGACCTTTTGTCC-3.

Sequences had been 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>) becomes used to decide the premiere for phylogenetic analyses. Each of the 3 genomic regions (SRY, 5' flank, and 3' flank) become analyzed one by one in addition to in a blended statistics set for phylogenetic reconstruction. The phylogenetic tree based on SRY coding region (360 bp) 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 observed in the nucleotide phylogeny uniting lineage SRY in jojoba to SRY in humans and plants. We apply the present work 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 by nested PCR. Thus, our novel method represents a breakthrough in the sex determination of plants and will help identify the sex of saplings at early developmental stages. Our newly developed strategies for unambiguously selecting the female jojoba at a young age are very sample and very important to breeders, limiting the plantation costs associated with the cultivation of the non-productive male plants. The early sex determination would open new perspectives for multiplying by seed the jojoba genotypes, reintroducing biodiversity into Jojoba groves, and implementing genetic improvement programs all over the world. The SRY primers also relate to a method for determining the sex of a Jojoba by detecting and using specific primers in the Jojoba genome. In certain embodiments, the method is characterized in that the amplification of a portion of genomic DNA of the Jojoba tested, using a pair of primers specific to the SRY gene, to obtain the sex of the jojoba tested. So, human SRY is like SRY in mice and jojoba plants. Thus, a model for SRY function has been developed in plants. This has been particularly important for discovering interactions of 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 have 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 work 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 have the advantage of allowing the early selection of female plants and therefore limiting the plantation costs associated with the cultivation of the non-productive male plants. Also, our techniques are that they are "universal" in that they can be used regardless of the origin, variety, or cultivar of the jojoba.

Conclusions

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

Materials And Methods

1.1 Isolation of plant DNA

All DNA isolation and purifications were performed according to the protocols described by our groups elsewhere [Solliman *et al.*, 2019].

1.2 PCR (Polymerase chain reaction)

PCR reaction conditions and SRY gene-walking strategy were conducted according to several previously published protocols by our group on papaya [Solliman *et al.*, 2019] and elsewhere [Solliman *et al.*, 2019, Mohasseb *et al.*, 2020, Parker *et al.*, 1991, Dominguez and Lopez-Larrea 1994]. Mastermix was prepared by using Thermo Scientific DreamTaq DNA Polymerase according to the manufacturer's instructions.

1.3 Early sex verification in dioecious plants

At first, we tested DNA from females and males; different samples were randomly selected from dioecious plants growing in King Faisal University Research and Training Station. DNA from these samples was isolated as previously reported [Mills *et al.*, 2004 and Eman *et al.*, 2009]. Human DNA samples for 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 the oligo primers used in this study targeting the male-specific SRY marker (Table 1).

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

Oligo name	Sequences (5'–à3')	Combination
SRY_F1	TCTTGCGTGGGGCACTTACAGCAACTC	A
SRY_R1	GCTGGAGGCAAGCGCCATAATCTGAG	
SRY_F2	GTGATCCTGAAGCTGGTTTCTTGAG	B
SRY_R2	TCGCCTTCCGACTAGGTGAAGAG	
SRY_F3	TCTTCGGAGCCCCCACTAACACCATCAC	C
SRY_R3	GGGTTATACAATATATCTTTTCCGTTAT	
ADOP-27	5'-CACTATACCCGCCGGCGGGCCCGCT-3	
ADOP-32	5'-AATACGACTCACTATAGGGCGGCCCGCCGGGC-3'	
T7	5'-AATACGACTCACTATAGGGC-3	

As previously described, the newly designed SRY primers flank from 300 to 550-bp region [Solliman *et al.*, 2019 and Dominguez and Lopez-Larrea 1994].

1.4 Isolation of SRY gene

Restriction digestion: Jojoba adult shrub genomic DNA was restriction-digested overnight at 37°C according to the manufacturer's instructions. Restriction enzymes used included *Bam*HI and *Bgl*II *Sou*3A. The following oligonucleotides were used: ADOP-32 and ADOP-27 primers sequences are given in table 1; oligonucleotides ADOP-32 and ADOP-27 were synthesized by Macrogen Inc. (Seoul, Republic of Korea).

1.4.1 Primary PCR amplification

Applied Biosystems Veriti® thermal cycler was used for PCR amplification. Adaptor primer T7 sequences are given in table 1, and SRY-2 was used for amplification is given in table 1. The sequence of the SRY-1R SRY_2R primers were given in table 1.

1.4.2 Secondary amplification

The remaining PCR sample of the primary amplification we used as a template after being purified by phenol extraction. The DNA purifications and PCR were amplified was done according to [Solliman *et al.*, 2019 and Eman *et al.*, 2009]. The sequence of the SRY primers is given in table 1.

1.5 DNA sequencing

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

1.6 Bioinformatics

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

Declarations

Novelty Statement: Dioecism, which is associated with sexual dimorphism, has always been a problem with cultivating plant species, especially woody trees. Accordingly, the present study was conducted to develop reproducible and applicable techniques for early diagnosis of sex in jojoba male plants by simplex PCR depending on the SRY gene related to the human sex-determining region as presented here for the first time. In addition, protocol optimized for jojoba male plants to determine the sex, especially before flowering and during the very 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>)

Ethical Approval

This study was approved by the university human research ethics committee and all procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

The authors declare that they have no competing interests.

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.

Informed Consent and Consent for publication: Informed consent was obtained from all individual participants included in the study.

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Figures

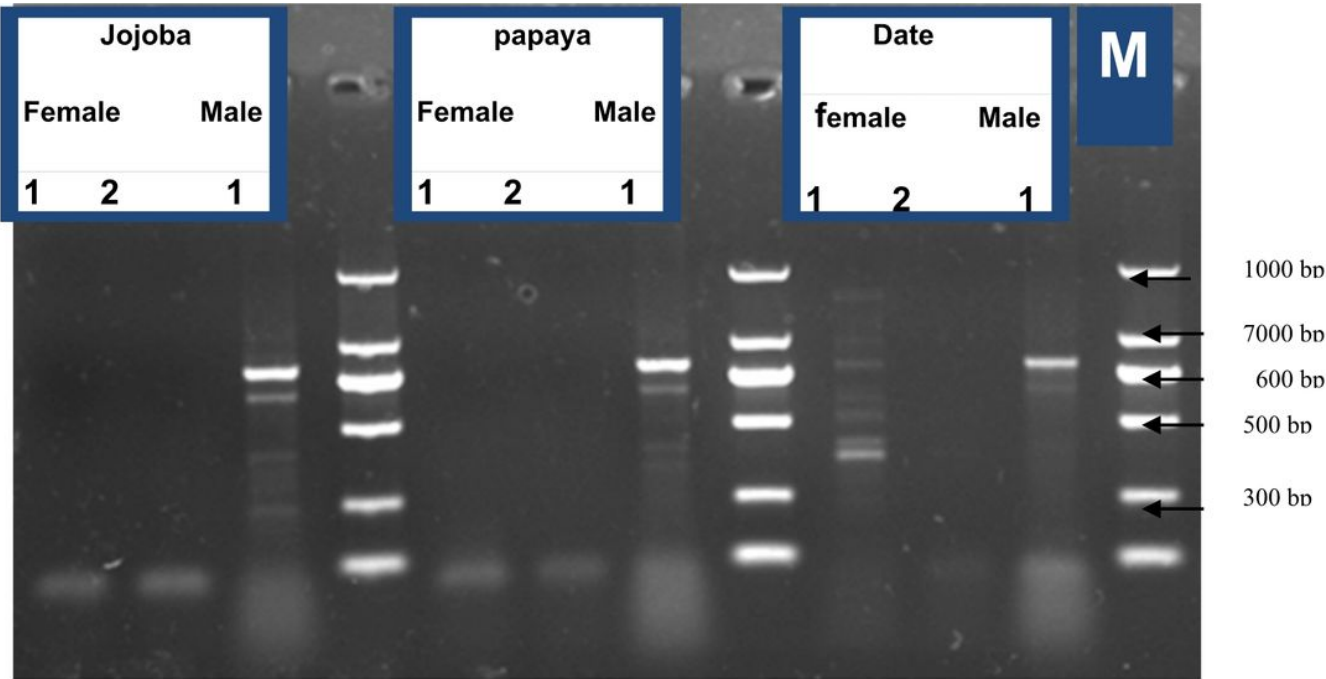


Figure 1

PCR amplification for screening of the presence of SRY gene with jojoba (job female 1,2 and job male 1), and compere with different variety of dioecious plant: 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.

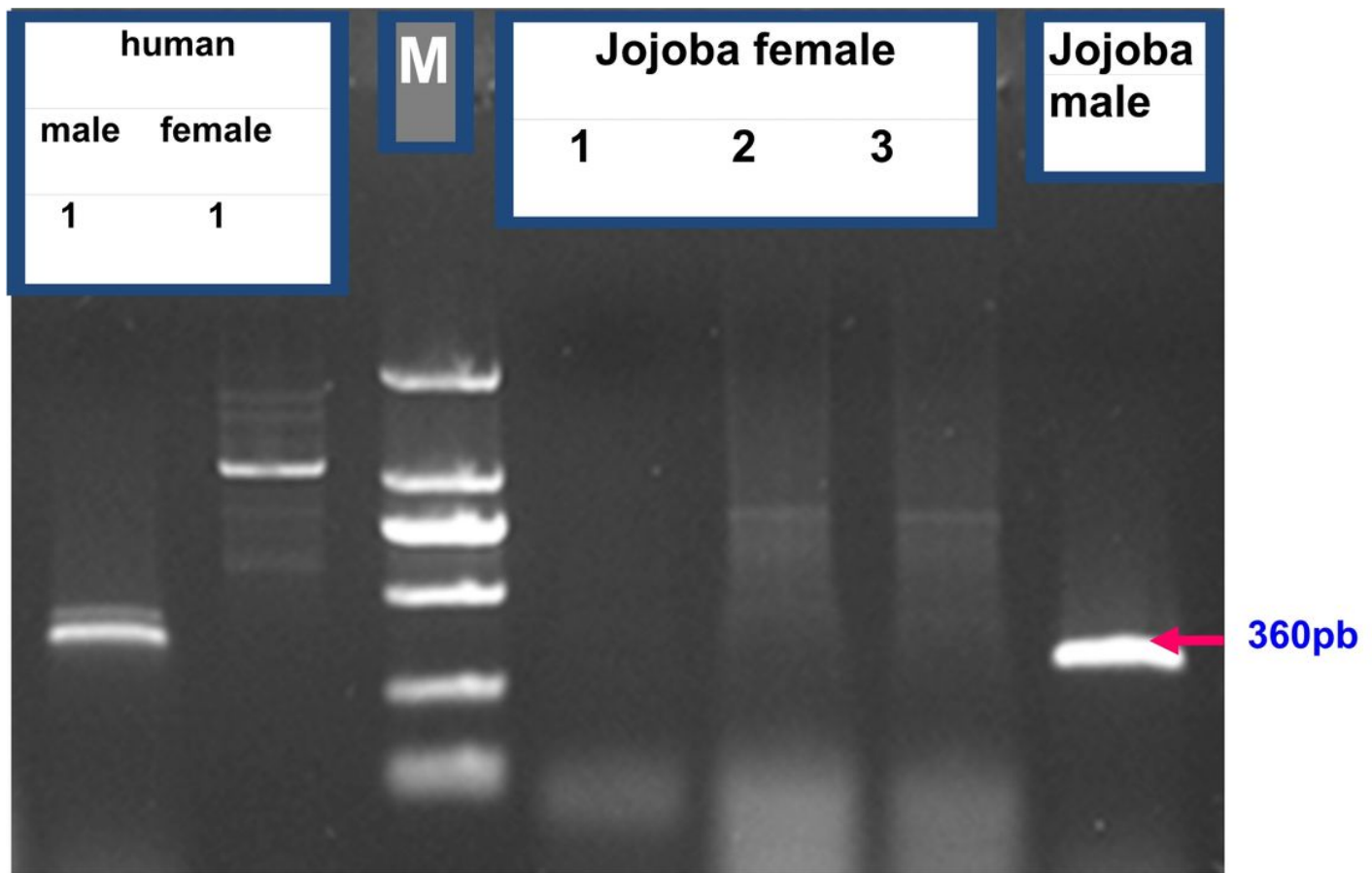


Figure 2

PCR amplification for screening of the presence of SRY gene with human male (HM and HF) and compare with different female variety of jojoba plant (JF 1, 2 and 3), and jojoba (JM 1), using specific primers universal SryF2+ SryR2 lane M DNA 100 bp Marker.

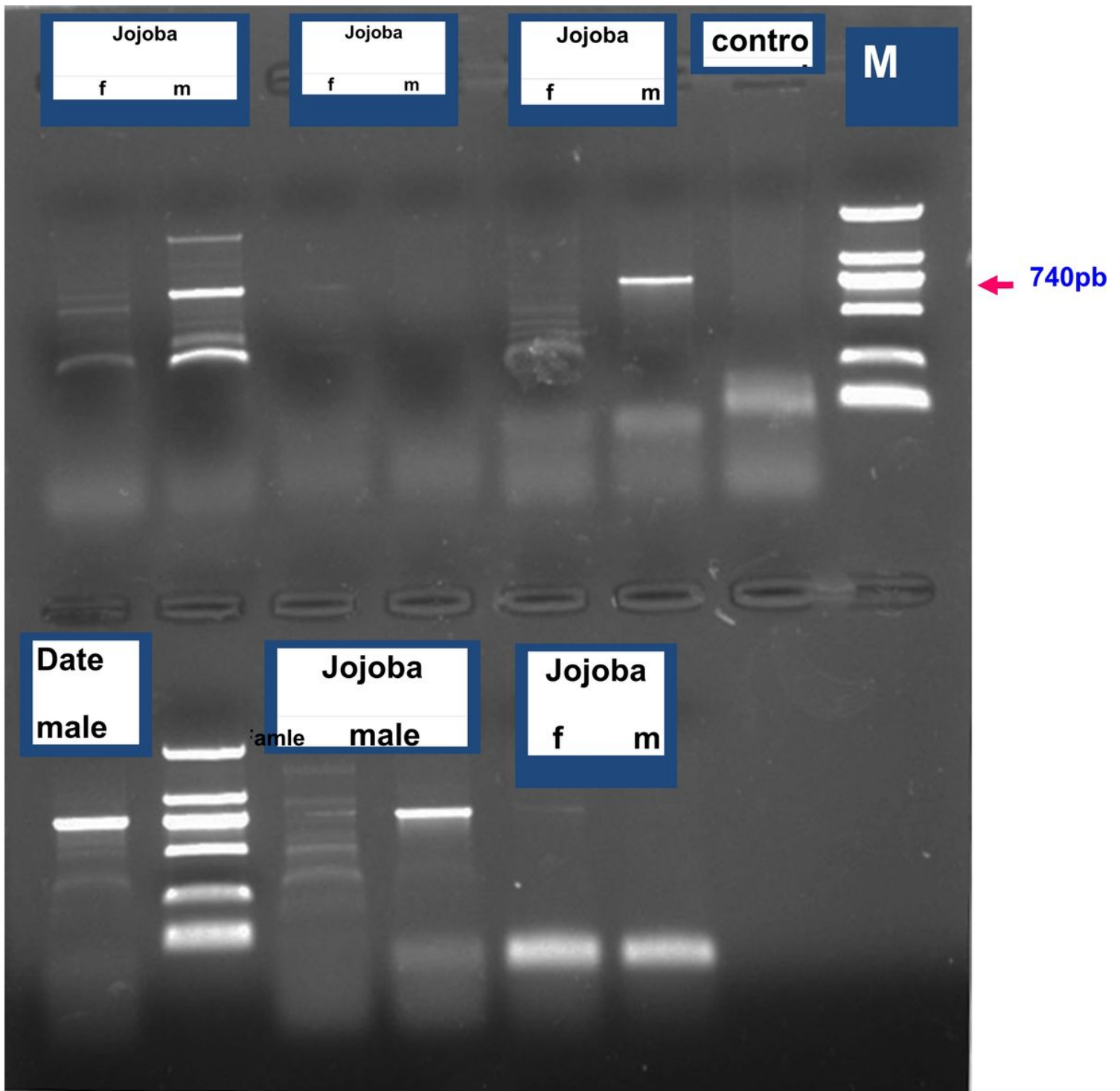


Figure 3

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

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

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Figure 4

The sequence was deposited in Genbank with the Accession number (Jojoba BankIt MK991776, 360pb).

Carica papaya sex-determining region Y protein (SRY) gene, partial cds

Sequence ID: **AF000024.1** Length: 596 Number of Matches: 1

Range 1: 1 to 360

Score	Expect	Identities	Gaps	Strand	Frame
650 bits(720)	0.0()	360/360(100%)	0/360(0%)	Plus/Plus	
Query 1	ATGCAATCATATGCTTCTGCTATGTTAAGCGTATTCAACAGCGATGATTACAGTCCAGCT	60			
Sbjct 1	ATGCAATCATATGCTTCTGCTATGTTAAGCGTATTCAACAGCGATGATTACAGTCCAGCT	60			
Query 61	GTGCAAGAGAATATTCCCGCTCTCCGGAGAAGCTCTTCCTTCCTTTGCACTGAAAGCTGT	120			
Sbjct 61	GTGCAAGAGAATATTCCCGCTCTCCGGAGAAGCTCTTCCTTCCTTTGCACTGAAAGCTGT	120			
Query 121	AACTCTAAGTATCAGTGTGAAACGGGAGAAAAACAGTAAAGGCAACGTCCAGGATAGAGTG	180			
Sbjct 121	AACTCTAAGTATCAGTGTGAAACGGGAGAAAAACAGTAAAGGCAACGTCCAGGATAGAGTG	180			
Query 181	AAGCGACCCATGAACGCATTATCGTGTGGTCTCGCGATCAGAGGCGCAAGATGGCTCTA	240			
Sbjct 181	AAGCGACCCATGAACGCATTATCGTGTGGTCTCGCGATCAGAGGCGCAAGATGGCTCTA	240			
Query 241	GAGAATCCCAGAATGCGAAACTCAGAGATCAGCAAGCAGCTGGGATACCAGTGAAAAATG	300			
Sbjct 241	GAGAATCCCAGAATGCGAAACTCAGAGATCAGCAAGCAGCTGGGATACCAGTGAAAAATG	300			
Query 301	CTTACTGAAGCCGAAAAATGGCCATTCTTCCAGGAGGCACAGAAATTACAGGCCATGCAC	360			
Sbjct 301	CTTACTGAAGCCGAAAAATGGCCATTCTTCCAGGAGGCACAGAAATTACAGGCCATGCAC	360			

Figure 5

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

Homo sapiens isolate P4-8 sex-determining region Y protein (SRY) mRNA, partial cds

Sequence ID: MK011517.1 Length: 622 Number of Matches: 1

Range 1: 79 to 345

Score	Expect	Identities	Gaps	Strand	Frame
476 bits(527)	4e-129()	267/268(99%)	1/268(0%)	Plus/Minus	
Query 57	TCCTGGAAGAATGGACCATTTTTCGGCTTCAGTAAGCATTTTCCACTGGTATCCCAGCTG	116			
Sbjct 345	TCCTGGAAGAATGG-CCATTTTTCGGCTTCAGTAAGCATTTTCCACTGGTATCCCAGCTG	287			
Query 117	CTTGCTGATCTCTGAGTTTCGCATTCTGGGATTCTCTAGAGCCATCTTGCGCCTCTGATC	176			
Sbjct 286	CTTGCTGATCTCTGAGTTTCGCATTCTGGGATTCTCTAGAGCCATCTTGCGCCTCTGATC	227			
Query 177	GCGAGACCACACGATGAATGCGTTTCATGGGTCGCTTCACTCTATCCTGGACGTTGCCTTT	236			
Sbjct 226	GCGAGACCACACGATGAATGCGTTTCATGGGTCGCTTCACTCTATCCTGGACGTTGCCTTT	167			
Query 237	ACTGTTTTCTCCCGTTTCACACTGATACTTAGAGTTACAGCTTTCAGTGCAAAGGAAGGA	296			
Sbjct 166	ACTGTTTTCTCCCGTTTCACACTGATACTTAGAGTTACAGCTTTCAGTGCAAAGGAAGGA	107			
Query 297	AGAGCTTCTCCGGAGAGCGGGAATATTC	324			
Sbjct 106	AGAGCTTCTCCGGAGAGCGGGAATATTC	79			

Homo sapiens isolate P3-9 sex-determining region Y protein (SRY) mRNA, complete cds

Sequence ID: MK011516.1 Length: 625 Number of Matches: 1

Range 1: 79 to 345

Score	Expect	Identities	Gaps	Strand	Frame
476 bits(527)	4e-129()	267/268(99%)	1/268(0%)	Plus/Minus	
Query 57	TCCTGGAAGAATGGACCATTTTTCGGCTTCAGTAAGCATTTTCCACTGGTATCCCAGCTG	116			
Sbjct 345	TCCTGGAAGAATGG-CCATTTTTCGGCTTCAGTAAGCATTTTCCACTGGTATCCCAGCTG	287			
Query 117	CTTGCTGATCTCTGAGTTTCGCATTCTGGGATTCTCTAGAGCCATCTTGCGCCTCTGATC	176			
Sbjct 286	CTTGCTGATCTCTGAGTTTCGCATTCTGGGATTCTCTAGAGCCATCTTGCGCCTCTGATC	227			
Query 177	GCGAGACCACACGATGAATGCGTTTCATGGGTCGCTTCACTCTATCCTGGACGTTGCCTTT	236			
Sbjct 226	GCGAGACCACACGATGAATGCGTTTCATGGGTCGCTTCACTCTATCCTGGACGTTGCCTTT	167			
Query 237	ACTGTTTTCTCCCGTTTCACACTGATACTTAGAGTTACAGCTTTCAGTGCAAAGGAAGGA	296			
Sbjct 166	ACTGTTTTCTCCCGTTTCACACTGATACTTAGAGTTACAGCTTTCAGTGCAAAGGAAGGA	107			
Query 297	AGAGCTTCTCCGGAGAGCGGGAATATTC	324			
Sbjct 106	AGAGCTTCTCCGGAGAGCGGGAATATTC	79			

Figure 6

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

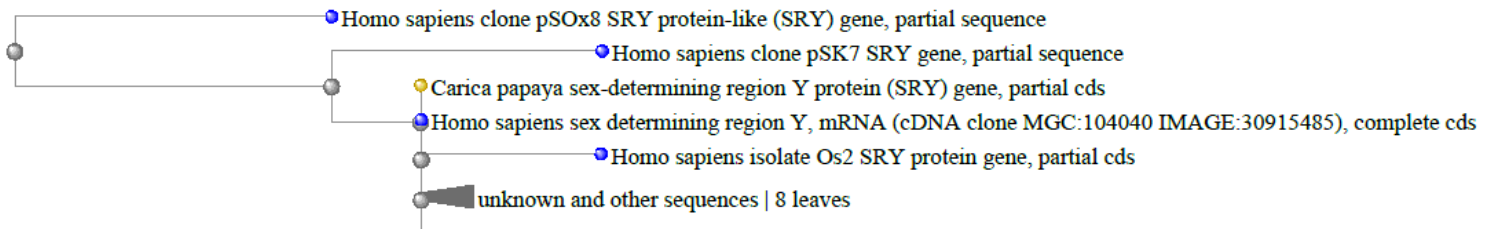


Figure 7

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 based on a neighbor-joining method with 100 bootstrap values.