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Research Article
Isolation and Characterization of GPAT3 Gene from Jojoba Plant and its Inferior Early Diagnosis of Sex

[1,2]Mohei El-Din Solliman

[1]Department of Plant Biotechnology, College of Agricultural and Food Sciences, King Faisal University, P.O. Box 420, Al Ahsa 31982, Kingdom of Saudi Arabia
[2]Department of Plant Biotechnology, National Research Centre, Dokki, Cairo Governorate 12622, Egypt

Abstract

Background and Objective: In jojoba plants, the sex is usually difficult to identify, especially before flowering and during the very early stages of development. This stage is expected to facilitate breeding programs and adopt an invention and approach to isolate the GPAT gene identified between males and females: The study aimed at early diagnosis of sex in jojoba by sequence characterized by GPAT gene of sex-determining by simplex PCR. To prove the existence of the GPAT gene in male jojoba plants which may be the sex determination and identification in all plant systems. **Materials and Methods:** Initially, different primers were selected for the sex determination of jojoba samples using PCR-based amplification. The primers that can produce distinct DNA bands in males, not in females were selected for further experiments. The amplification of a male-specific GPAT marker situated in the sex determination region was amplified using specific primers. The newly designed GPAT primers flank region. **Results:** For the first time, separation and identified of the GPAT gene sequence of jojoba was done. The novel method represents a breakthrough in the sex determination of jojoba to identify sex at early developmental stages. This work provides a potentially useful diagnostic for determining sex in jojoba species. In this report, a breakthrough in the methodology for determining the sex of jojoba has been made. The amplified regions of the GPAT gene closely matched with sequences of GPAT in papaya and humans. **Conclusion:** The authors make an interesting finding by targeting the sequences in the GPAT gene and the final conclusion that PCR as a simple, rapid and reliable technique can complement and confirm sex by using specific primers pair according to our invention.

Key words: Jojoba, sex-determination, simplex PCR, GPAT marker, GPAT3 gene sequence

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Corresponding Author: Mohei El-Din Solliman, Department of Plant Biotechnology, College of Agricultural and Food Sciences, King Faisal University, P.O. Box 420, Al Ahsa 31982, Kingdom of Saudi Arabia

Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The jojoba is a perennial shrub that is desert dioecious. It is a member of the Simmondsiaceae family and order Caryophyllales. It has 52 chromosomes that allow it to withstand salinity and drought[1,2]. Because of mixed pollination, it is difficult to determine the sex of this plant before it flowers[3]. The shrub produces approximately 700-800 g of seed. Nonetheless, jojoba is regarded as a good plant because of its high content of secondary active constituents, which are highly advantageous for several crucial industries. In addition, it can be grown in most Arab countries, particularly deserts[4].

For environmental reasons, sex determination is still crucial in plants that do not produce crops. Determining a person's sex is essential for population studies, which examine the ratios of men and women and the variables that affect sex distribution and plants should be designated as endangered or protected depends on population research. Therefore, techniques for determining a plant's sex are extremely important for a variety of fields, such as ecology, horticulture, agriculture and environmental protection[2,5-7]. The jojoba (*Simmondsia chinensis* (Link) Schneider) plants have lengthy stages of juvenility and dioecy, making breeding difficult. Since its discovery more than three years ago, the mammalian sex-determining region (JoGPAT3) gene has been the subject of in-depth research. One possible candidate for the mammalian testis-determining factor is JoGPAT3. According to several autosomal representatives[7-10], JoGPAT3 appears to be a member of a family. The dioecious Jojoba plant, belonging to the Simmondsiaceae family, can withstand salinity and drought thanks to its 2n = 52 chromosomes[1,2]. The exact synchronization of developmental programs between sporophytic and gametophytic anther tissues is essential to the intricate process of pollen development. It is well known that lipid metabolism is highly active in both the developing microspores and the surrounding tapetal cells[11,12]. Due to its remarkable maturation process, which involves both structural and metabolic specialization, within a relatively short life span, the tapetum has been considered an attractive model system for studying various aspects of cellular activity. By comparing tapetal differentiation to the stages of microspore development, it is also easily monitored. The secretory type of tapetal cells in *Arabidopsis* anthers releases a variety of nutrients, lipids and proteins into the anther locule[13]. Both active secretion and eventually, the disintegration of the tapetal cells contribute to the release of nutrients from the tapetum. The successful production of pollen depends critically on the precise and timely progression of tapetal differentiation concerning microspore developmental stages[14]. Numerous cytoplasmic male sterility (CMS) mutants with anomalies in tapetal development demonstrate how particularly vulnerable tapetal development is to mitochondrial dysfunction[15]. Still, little is known about the molecular mechanisms underlying the majority of tapeta developmental defects. Even though lipid metabolism plays a major role in tapetal development, no evidence has been found to connect a de novo glycerolipid biosynthesis defect to any tapetal abnormality. Sequences that fall inside predetermined boundaries can be amplified using conventional PCR. To amplify DNA sequences that flank regions with known sequences, several techniques have been developed, including unpredictably primed PCR[16], inverse PCR[17] and targeted gene-walking PCR[5,18]. It is challenging to determine the gender of a reproductively dormant jojoba plant individual. Our research group has successfully tested the attractive molecular marker JoGPAT3 for sex determination in mammals as well as other plants such as the date palm and jojoba. The primary goal of the current study was to test the JoGPAT3 PCR amplification method for sex determination in various jojoba shrub sources. Here, reports the discovery of JoGPAT1, a novel JoGPAT gene from jojoba. Finally, it offers proof that JoGPAT1 is exclusively found in male jojoba plants, not in female plants. Numerous studies have demonstrated that GPAT1 deficiency affects tapetal differentiation, resulting in most microspores aborting before reaching maturity.

MATERIALS AND METHODS

Study area: This experiment was carried out at the Laboratories of the Department of Agricultural Biotechnology, Faculty of Agricultural and Food Sciences, King Faisal University, Al-Hassa, Saudi Arabia. Plant material was taken from grown male and female jojoba adult shrubs; the study was carried out from April, 2022 to October, 2023. Then the sterilization and primary cultivation of the excised explants were carried out as indicated in previous research of Solliman *et al.*[5].

Isolation of plant DNA: Genomic DNA was isolated on a mini-prep scale, as Murray and Thompson[19] mentioned with some modifications. Solliman *et al.*[5] performed all DNA manipulations and purifications according to the protocols.

Polymerase chain reaction: The Taq-polymerase, dNTPs (Deoxynucleotide Triphosphate) and convergent primers achieved amplification of the DNA fragment. Solliman *et al.*[5] performed the reaction conditions for PCR according to the protocols.

Table 1: Primer pairs were tested in PCR to analyze the amplification of a male-specific GPAT3 marker situated in the sex determination as follows

Primer	Sequences	Design
GPAT3F1	5'-GGCTGGTCTTAGGGTCGATG-3'	Forward-F1
GPAT3R1	5'-ATCATGGCCAGCAAGCATCT-3'	Reverse-R1
GPAT3F2	5'-AGAAAACCTGATATGCTCTCTG-3'	Forward-F2
GPAT3R2	5'-TGTGATGCACTTGGTAACTACT-3'	Reverse-R2

Table 2: Final primer pairs tested in the PCR for the amplification of a male-specific GPAT3 region

Primer	Sequences	Design
GPAT3F1	5'-GGCTGGTCTTAGGGTCGATG-3'	F1
GPAT3R1	5'-ATCATGGCCAGCAAGCATCT-3'	F

Method to early distinguish the gender of jojoba plants: At first, by testing the jojoba DNA from females and males, different 75 samples from each were collected. Two were randomly female and male cultivars of jojoba growing in King Faisal University, which grew in the Research and Training Station. The DNA from these samples was isolated according to the protocols by Solliman *et al.*[6].

Method to early distinguish the gender of jojoba plants: Initially, examined DNA from 300 separate samples of male and female jojoba plants. Two cultivars of jojoba, one male and one female, were cultivated randomly in the Research and Training Station of King Faisal University. These samples' DNA was extracted using procedures that followed Solliman *et al.*[2] and Solliman *et al.*[5] guidelines. The gpt3 sex determination region contains the male-specific GPAT3 marker, which was amplified using a newly created forward (F) and reverse (R) primer as shown in Table 1.

A male-specific GPAT3 marker situated in the Y chromosome sex determination region was amplified using a newly designed forward (F) and reverse (R) primer. Two primer sets were created that used exclusively in male dioecious plant samples, allowed us to amplify fragments of about 480 and 596 bps, respectively (Table 2).

The newly designed JoGPAT3 primers flank a 400 to 596 bp region as described by Lo *et al.*[20].

Isolation of JoGPAT3 gene

Map-based cloning: Genomic DNA was extracted from maize leaves using the CTAB method with some modifications[2,5,6].

Primary PCR amplification: Adaptor primer GPAT3 sequences are 5'-AATACGACTCACTATAGGGC-3' and JoGPAT3-2 (gene-specific primer, JoGPAT3) was used for amplification. The sequence of the JoGPAT3R1 primer is given: 5'-GGGCTGT AAGTTATCGTAAAAGGAGC-3' and GPAT3R3; 5'-CCTAGCTGGTC ACGTTGACCTTTTGTCC-3'.

Secondaries amplify: The remaining PCR sample of the primary amplification was used as a template after purification by phenol extraction. The DNA after precipitation was resuspended in 20 µL TE. Various dilutions of the excised band were PCR amplified using T7 and GPAT3-2R primers. The sequence of the GPAT3-2 primer is given: 5'-GGAGCATCTAGG TAGGTCTTTGTAGCC-3'.

DNA sequencing: Sequencing of genes was done at Macrogen Korea company.

Analysis of nucleotide sequences by homology and structural comparison of GPAT3-jojoba to other GPAT3 genes: Most of the sequence (DNA) analyses were performed using CLC Vector program and Genbank database. Homology research was done using FASTA. Multiple sequence alignment was done using CLUSTALW.

Homology and structural comparison of JoGPAT3 gene from jojoba: Most of the sequence (protein and DNA) analysis were performed using CLC vector program and GenBank database. Homology searches were done using FASTA and multiple sequence alignment was done using CLUSTALW 2.1 (default version).

RESULTS

These studies assumed that the following while looking for a plant GPAT based on the genomic data from rice, *Arabidopsis* and date palms: Two characteristics of a plant membrane-bound fatty acid acyltransferase are as follows: (1) It will be larger than sn-2 acyltransferases, which are typically around 300 amino acids long and (2) It will share certain conserved domains with other fatty acid acyltransferases.

This work pertains to molecular technique and uses our primer to distinguish between male and female jojoba plants and humans using the GPAT3 gene 100% of the time as shown in Fig. 1. The study also deals with the molecular method of jojoba plant sex determination as well as a particular primer and design for implementing the method in other plants that have distinct sexes. The presence of GPAT3-related sequences in jojoba plants for the first time.

ATGCCTATGTCCTCTCCTGTGGCAACTGCTTGTCATGGAGGGGAAGAGAGGCGCAGCATTGCCTGCGAAT
TTGAGGGTGCCCTCCTCATCTCAAAGAGACTCTTTGCCTACTTCATGCTTGTTGCTCTTGAAGCTGGTGG
CCCCATCAGGGCTCTTACACTGCTCCTAGTCTTCCCTCTCCTATGGTTGCTCGAGCTCGTCGGCCTTGAG
AGGATGGCTCTTCAACTGATGATCTTTGTATCCACGGCTGGTCTTAGGGTCGATGATCTGAAGGCTGTGG
CAAAGGCCACCCTGCCCAGATTTTATTTGGAAGACCTTAGGCAGAGAGCATATCAGGTTTTCTCGAGCTA
TGAGGGGAAGAAATTTGTGGTTACTTGTATCCCCAGGATCATGGTTGAGCCCTTCCTGAAGGAGTTCTTG
GATGTGGATCATGTGATTGGCGCCGAACTGAAGATGTTCAAAGATTGCTCTGTAGGCTTGGTGGCATCAC
CAGGAGTCATGTTAGGTGCCCGTCGGCTCCAAGCAGATAAGAGATTGGGCAGTGGGCTAAGAGATAAGAC
ATTCATGTTGCTGTGCAGGGAACACCACCCGATTCCGCCAGAAGAGACTTCCTCACCCTTGCGAAGAAAG
GATTATCCTAAGCCATTGATCTTCCATGATGGTCGTCTCATGGCTCATCCCACGCCATTGGCTTTCCTAG
CTGTTATCCTCTGGCTGCCCCTTGGCGCTCTCTTGGCCATTTACCGGATCCTTGTGGGTATCCTACTACC
GTACAAGATAGGCCTTGTGGGATCTGCAGTAACGGGCTTTCGCATTAGGGCCCAGTTTCCAAAAGCCCAA
CATTCTCTCAATACACCTGCAGCCGTGGGTGCGCCAAACAGGACGGGCACACTATATGTCTGCAATCATC
GGACACTTCTCGACCCAGTGATCGTATCAACAGCGATCCAACGAAAGGTGACTGCCGTTACATATAGTTT
AAGCAGATTCTCAGAGGTGATTTCTCCAATCCCAACCATCCGATTGACCAGGGACCGCTTCAAGGATGGT
GCTGCGATGCGATCACTGCTCAATCATGGTGATCTGGTAGTCTGCCCAGAGGGAACCACCTGCCGGGAGC
CATACCTCCTCCGATTCAGCCCCTTGTTTGCGGAGATCGCCGACAACATCGTTCCAGTCGCTGTTACAGC
TGAGGGTAGCATGTTCTATGGAACCACAGTCAGGGGGCACAAATGGTTGGATTCACTCTTCTTCCTAATG
AATCCTAGGCCTTGCTATCAGTTGCACTTCCTTGAAAGTATGGCAAGGGATCAAAGATCAAGCTTTGAAA
TTGCAAATGGCATCCAACGGTTGATTGGAGAAGCAGTTGGGTTCGAGTGCACCAATTTCACGCGGAAGGA
CAAATATCAGATGCTTGCTGGCCATGATGGTGCGGACACCAGGAGATGA

Fig. 1: Deduced amino acid sequence alignment of the members of the AtGPAT family in jojoba

A gene family in Arabidopsis, AtGPAT was found by a series of Basic Local Alignment Search Tool (BLAST) searches based on a query from a partial sequence spanning a region conserved between the yeast GPATs and other fatty acid acyltransferases. The seven members of this family, AtGPAT1 through AtGPAT7, have the four acyltransferase domains that have been previously identified based on amino acid sequence alignment (Fig. 1).

The residues His and Asp in block I, Gly in block III and Pro in block IV are completely conserved and have all been shown to be catalytically important locations.

Moreover, residues essential for G-3-P binding, such as Arg in block II and Gln and Ser in block III, are likewise shared by all Arabidopsis family members. Several blocks of conserved residues at the C terminus extend past areas known to be important for the function of the acyltransferase enzyme. The end of the N terminus exhibits significant sequence divergence, contributing to the variation in these members' sizes.

Development of a new technique for sex-determine of jojoba plants: The discovery of GPAT3-related sequences in jojoba cultivars was presented here for the first time. The Jojo-GPAT3 gene was isolated using the polymerase chain reaction from human homologs of the conserved motif of the GPAT3 gene.

Early in the jojoba cultivation process, farmers worldwide face a significant challenge when attempting to determine the sex of saplings. For example, a farmer may, for financial reasons, plant many productive jojoba female trees in their orchards while minimizing the number of jojoba male trees. This is because only female plants which may take up to three years to identify bear jojoba fruit until the trees begin to bloom.

Before the development of these new techniques, numerous attempts to determine the sex of jojoba plants at an early stage of growth have been made, with frustratingly unsuccessful results. A significant endeavor to comprehend the genetic underpinnings of dioecious plant sex determination and to devise strategies for early sex detection using molecular marker tools. To determine the sequences between the forward and reversed GPAT3 primers to identify a potential primer that can be used to create a smaller PCR in various human and papaya regions. The reverse primer choice is the same as that of the previously published papaya sequence. Our lab isolated several unidentified regions of known genes. It is common practice to determine the sex for plants, prenatal diagnoses and DNA analyses using PCR for sex determination. However, it is confirmed that this test is entirely trustworthy. An innovative set of primers that specifically targets a region of the JoGPAT3 gene yields a 553 bp male-specific PCR fragment that can be amplified through standard PCR protocol, providing rapid and clear results for gender identification.

Isolation and sequencing of a partial fragment of the jojoba GPAT3-gene: Male and female jojoba plants of various cultivars were used to isolate their genomic DNA in KFU. To

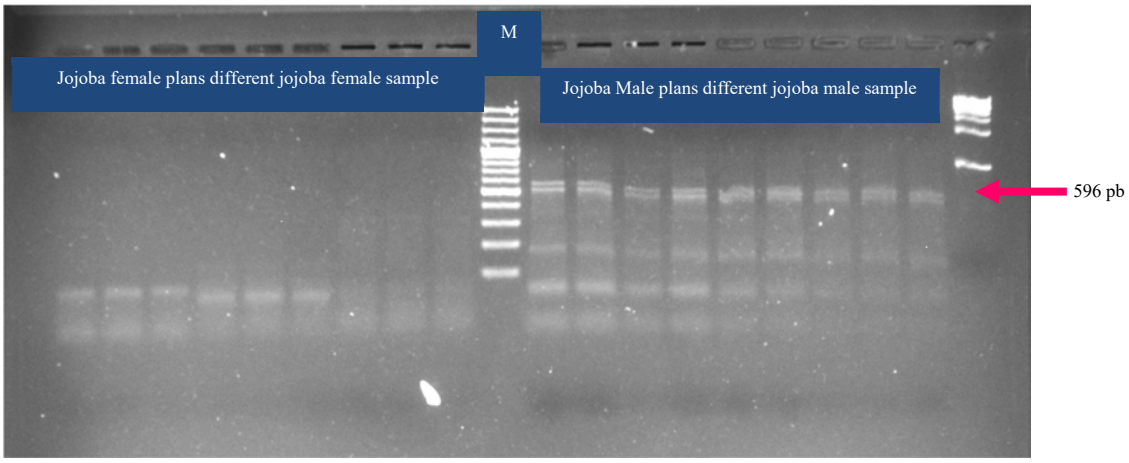

Fig. 2: Photograph of a gel showing DNA isolated from females and males of different cultivars Jojoba growing in Al-Ahsa Oasis KSA

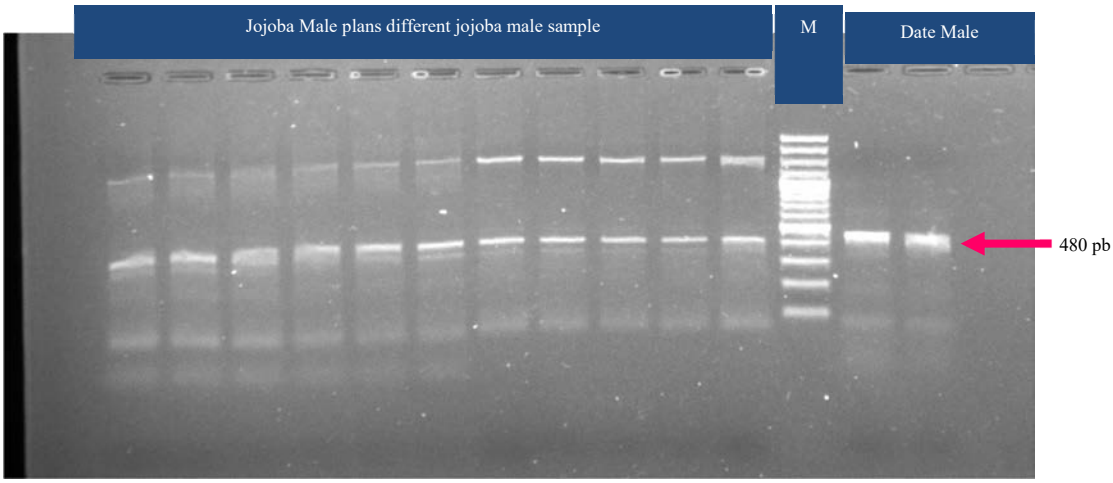

Fig. 3: PCR amplification for screening of the presence of GPAT3 gene with jojoba (jojoba male by F1 primer with R3 primer)

avoid extension during PCR amplification, the 3' end of the primer is blocked. The gpat sequences were amplified by PCR using gpat F1 and gpat R3 primers, using the DNA as a template. Using the GPAT3 F2 and GPAT3 R3 primers (Fig. 2), the amplified DNA served as the template for the second PCR reaction.

Further PCR reactions using primers for various markers specific to the JoGPAT3 have determined the sex of the plant to be male. It has previously been demonstrated that the GPAT3 marker produces a 500-596 bp PCR product that overlaps in size with certain date sequences (Fig. 3). Therefore, to ascertain the gender of the plant, these PCR reactions can be carried out.

A new approach was adopted to isolate the JoGPAT3 gene, which male and female jojoba plants can be identified. The GPAT3 gene separated in June 1990 was regarded as the best candidate for GPAT3. The GPAT3 gene is a single copy gene coding a peptide containing 400 conserved amino acids. Amplification of the male sample resulted in only one 480 to 596 bp band, which was putatively identified as the 480 bp GPAT3 amplification product (Fig. 2 and 3).

For gene analysis, it is essential to isolate unknown DNA sequences that border known regions. To separate unknown DNA sequences (promoters) from known DNA sequences (cDNAs) by PCR, several protocols have been developed.

Isolated JoGPAT3 DNA fragments were deposited into GenBank: A similar approach was adopted to isolate about 350 and 360 bp lengths of DNA fragments of the Jojoba-GPAT3 gene by walking the genomic DNA using primers GPAT3R1, GPAT3R2 and GPAT3F1 primer. The complete

Jojoba glycerol-3-phosphate acyltransferase 3 (gpat3) DNA,complete cds

ATGGCCACTGCTTCTCATGGAGAGGAAGAGAAGCGCAGTATTGCCTGCGAATTTGAGGGTGCCCTCCTCATCTCAAGGAGGC
TCTTTGCCTATTTCATGCTTGTTGCTCTTGAAGCTGGTGGCCCCATTAGGGCTCTCACACTGCTCCTAATCTTCCCTCTCCT
ATGGTTGCTCGAGCTCATCGGCCTTGAGAGGATGGCTCTTCAACTGATGATCTTTGTATCCACTGCGGGTCTTAGGGTCGAT
GATCTAAAGGTTGTGGCAAAGGCCATCCTGCCCAGATTTTATTTGGAAGACCTGAGGCAGAGAGCATATCAGGTTTTCTCGA
GCTATGAGGGGAAGAAATATGTGGTTACTTGTATCCCCAGGATCATGGTTGAGCCCTTCCTGAAGGAGTTCTTGGATGTGGA
TTATGTGATTGGCACCGAACTGAAGATGTTCAAAGATTGCTCTGTGGGTCTGGTGGCATCACCAGGAGTCATGTTAGGTGCC
CGTCGGCTCGAAGCAGTTCTATCCGTCGTGGAGGATGGTGAGGTGATCAGTGTTGGATTGGGCAGTGCACCAAGAGATAAGA
CATTCATGTTGCTGTATAGGGAACTCTACCCGATTCCGCCAGAAGAGAATTCCTCACCCCTGCGAAGAAAGGATTATCCTAA
GCCATTGATCTTCCATGATGGTCGTCTCGTGGCTCATCCCACGCCATTGGCTTTCCTAGCCGTGATCCTCTGGCTGCCCCTT
GGTGTTCTCTTGGCCATTTCCCGGATCCTTGTGGGTATAATACTACCCTACAAGATGGCCCTAGTGGGCTCTGCAGTAACGG
GCTTGCGCATTAGGGCCCACTTTCCAACAGCCCAACATGCTGTCAATACACCTGCAGCCGTGGGTGCACCAAACAGAAGGGG
CACAGTGTATGTCTGCAATCACCGGACACTTCTCGACCCTGTGATCGTATCAACAGCTATCCAACGAAAGGTGACTGCCGTT
ACATACAGTTTAAGCAGATTCTCAGAGGTGATTTCTCCAATTCCGACCATC

Fig. 4: Sequence of jojoba 1464 pb deposited in GenBank

sequence of the DNA is presented in Fig. 2-4. In addition, the sequence information was deposited in a public database GenBank (jojoba BankIt MK991776, 360 pb), as in Fig. 4.

Isolation and characterization of jojoba JoGPAT3: Approximately 1440 kb of genomic fragment amplified from various Jojoba types utilizing specified primers found in the date palm GPAT3 sequence data. The GenBank database will receive the genomic sequences. The study findings (Fig. 5a-b) demonstrated the isolation and characterization of various Jojoba types.

Dioecy provides chances to investigate the male and female programs independently, providing insight into the molecular genetics and evolutionary processes that result in distinct mechanisms for the sex expression system in plants. Like plant hormones, the mechanisms governing sex expression in plants can be either hereditary or epigenetic. Blooming plants often have an X-to-autosome ratio system and an active Y sex-determination mechanism. The field of sex determination has benefited from advances in both conventional and molecular methods. It was surprising to learn that just a small number of creatures have clearly defined sex chromosomes.

Positively, it would be beneficial for validation of sex before their sex expression at more significant perspectives. Therefore, the present review emphasizes the mode of sex determination among dioecious plants vis-a-vis summarizes the works related to gender-specific markers generated using male and female plants from agriculturally important dioecious crops. This new method was applied to a large number of jojoba individuals (male and female samples) whose sex had been identified morphologically. These findings demonstrate the validity of our approach to determining the sex of samples with significant degeneration. The five instances' coding exon region of the JoGPAT3 gene sequenced revealed 99-100% alignment with the sequences of males in the normal range. genotyping 50 healthy male controls and two closely related people. The sex chromosomes of jojoba are identical. Thus, cytological techniques cannot identify the sex type of jojoba seedlings. Furthermore, at the juvenile developmental stage, neither the shape nor the morphology of the embryo can reveal the sex type of jojoba seedlings. Jojoba is mostly propagated by seeds.

Sequencing analysis: The GPAT3 fragment was 560 bp in length. The GenScan and CLC analysis predicted that the DNA fragment had a 3' end that was homologous to the 3' ends of human and papaya GPAT3. The length of the full-length open reading frame (ORF) was 560 bp. The projected ORF was highly similar to human GPAT3, as evidenced by the BLAST results, which revealed 84% similarity to the published sequence amplified with human primers. A 560 bp fragment was amplified using PCR. A modified genomic "walking" approach that combines vectorette and suppression PCR "walking" and amplification produced the full-length sequence of GPAT3.

The GPAT3 was 560 bp long, including the open reading frame and presumed transcription start site. With a high degree of similarity to papaya GPAT3, the open reading frame encoded for 182 amino acids and had 86% identity to the published sequence amplified with papaya primers (protein_id = "AAB58342.1"). Extensive similarity was observed between the deduced DNA sequence and the human and papaya GPAT3 sequences (Fig. 6-7). Figure 7 showed the

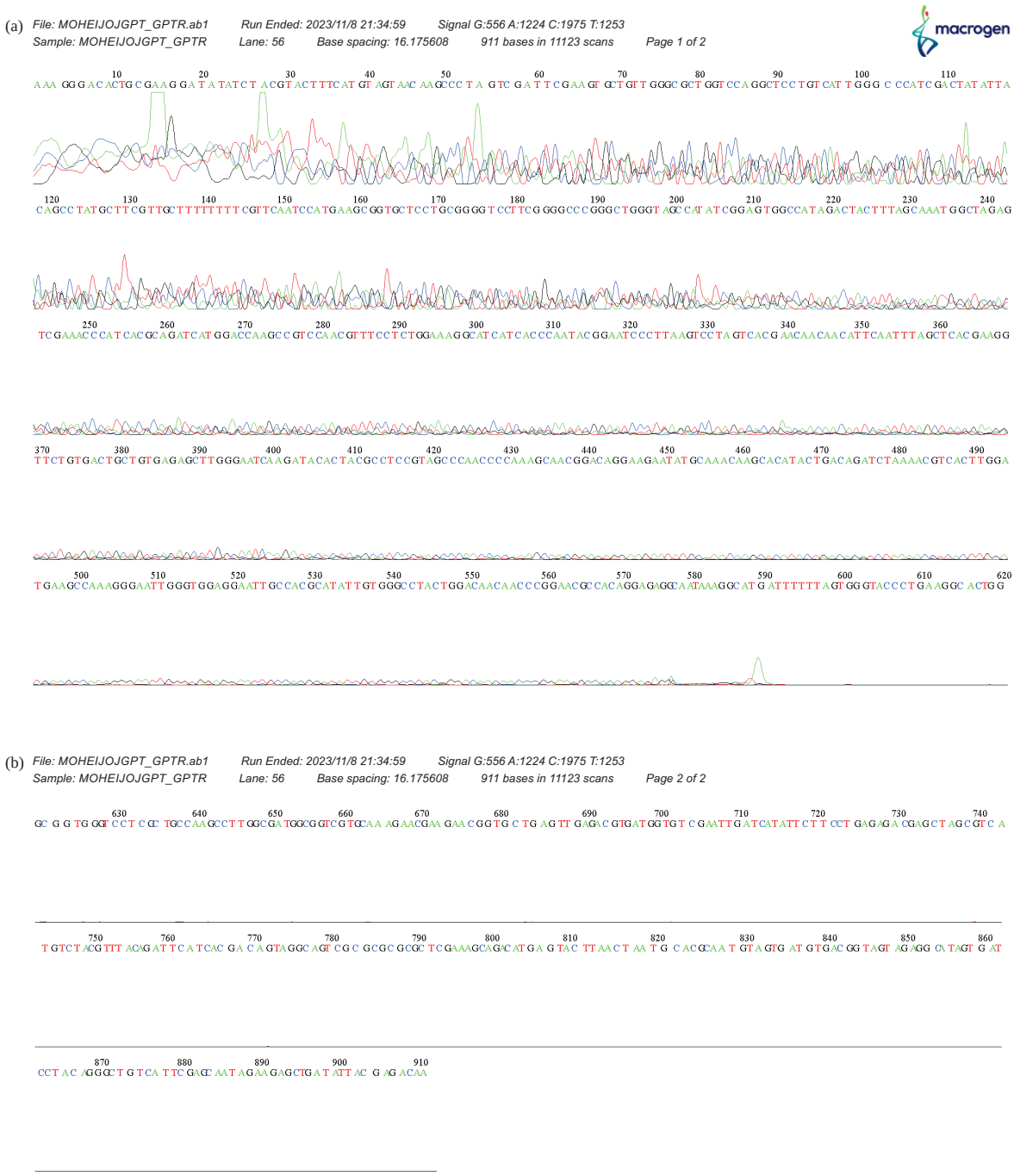

Fig. 5(a-b): (a) Reverse sequences of GPAT3 gene and (b) Forward sequences of GPAT3 gene

(a) A sex-determination gene from jojoba (GPAT3-jojoba). The nucleotide sequence of the GPAT3 partial gene sequence after being isolated from jojoba and sequencing using reverse primer and (b) A sex-determination gene from jojoba (GPAT3-jojoba)

comparison of the DNA sequences of jojoba glycerol-3-phosphate 2-O-acyltransferase JoGPAT3 (GPAT3) with the GPAT3 gene from *Ananas comosus* (glycerol-3-phosphate 2-O-acyltransferase 6-like, LOC109724820), mRNA). Sequence ID: XM_020253746.1 Length: 1637 Number of Matches: 1.

Sequence ID: MH680998.1 Length: 303122 Number of Matches: 2. The nucleotide sequence of the cloned GPAT3 Sequence Alignment Editor.

A PCR primer pair, as per our research and invention, is made up of a forward PCR primer that has 20 consecutive nucleotides from the flanking sequence. Here is the GPAT3-F1

Phoenix dactylifera clone dpS2X sex-determination region sequence
Sequence ID: MH680998.1, Length: 303122, Number of matches: 2
Range 1: 97783 to 98114 GenBankGraphics next match

```
Query  1130   GGGAGCCATACCTTCTCCGATTCAGCCCCTTGTTCGCCGAGATCGCCGACGACATCATTC  1189
              || |||||||||| |||  |||||||||||||||| |||||||||| ||| ||||| |||
Sbjct  98114  GGCAGCCATACCTCCTCTAATTCAGCCCCTTGTTCACCGAGATCGCGGACAACATCGTTC  98055

Query  1190   CGGTCGCTGTTGCAGTTGAGGGCAGCATGTTTTATGGAACCACAGTCAGGGGGCACAAAT  1249
              |||| |||||| |||| ||||| | |||||| ||||||||||||||  |||| ||||||
Sbjct  98054  CGGTGGCTGTTACAGT-GAGGGTACCATGTTCTATGGAACCACAGTC--GGGGAACAAAT  97998

Query  1250   GGTTGGATTCATTCTTCTTCCTCATGAATCCTAGGCCTTGCTATCAGTTGCACTTCCTTG  1309
              |||||||| |  ||||| |||| ||||||| |||||||||| |||||| ||||||||||
Sbjct  97997  GGTTGGATGCGCTCTTCCTCCTAATGAATCGTAGGCCTTGCTTTCAGTTACACTTCCTTG  97938

Query  1310   AAAGTATTACAAGGGATCAAAGATCAAGCTATGAAATTGCAAATGGCATCCAACGGTTGA  1369
               |||||| |||||||||||||||| |  |||||||||||||||||||| ||||||||||
Sbjct  97937  GAAGTATAACAAGGGATCAAAGATCCAATTATGAAATTGCAAATGGCATACAACGGTTGA  97878

Query  1370   TTGGACGAGCAATTGGGTTCGAGTGCACCAAGTTCACACGAAAGGACAAATATCAGATGC  1429
              |||||| || |||||||||||||||||||||| ||||| ||||||||||||||||||||||
Sbjct  97877  TTGGACAAGTAATTGGGTTCGAGTGCACCAATTTCACGCGAAAGGACAAATATCAGATGC  97818

Query  1430   TTGCCGGCCATGATGGTGCGGACACCAGGAGATGA  1464
              |||||||||||||||||| ||||||||||||||||
Sbjct  97817  TTGCCGGCCATGATGGTGCTGACACCAGGAGATGA  97783
```

Fig. 6: Comparison of the DNA sequences of JoGPAT3 gene from jojoba with *Phoenix dactylifera* clone dpS2X sex-determination region sequence

primer sequence: GPAT3_F 5'-AAACTTGATAGTTGTGTC ACTCAT-3'. The sequence of the GPAT3-R1 primer is given: 5'-GGGCTGTAAGTTATCGTAAAAGGAGC-3' and JoGPAT3_R2; 5'-CTAGCTGGTCACGTTGACCTTTTGTCC-3'.

The higher-order node that unites lineage GPAT3 in jojoba to GPAT3 in humans and plants in the nucleotide phylogeny was supported by both distance-based and maximum-likelihood (ML) analysis. This technique involved amplification of partial sequences of the Y chromosome (GPAT3) gene's major sex determination region using nested PCR.

All of the known members of the homo sapiens sex-determining region Y and *Carica papaya* were used in the separate phylogenetic analysis of the GPAT3 gene and the sex-determining region Y protein. Entire amino acid sequences were used to build the tree using 100 bootstrap values and the neighbor-joining technique.

Sequences were aligned using Clustal X and compiled using Sequencher, as described in (version 4.1; Gene Codes, Ann Arbor, MI). As seen in Fig. 8, the distance tree of results tool was utilized to choose the best for phylogenetic studies. Each of the two jojoba genomic regions (GPAT3, 5 flank and 3 flank) was analyzed separately and in a combined data set for phylogenetic reconstruction. Phylogenetic tree of the GPAT3 coding region as evolution in humans and plants. Data in Fig. 8 showed the maximum-likelihood phylogenetic tree derived by a heuristic search using the tree-bisection-reconnection branch-swapping method and distance tree of results.

DISCUSSION

The GPAT3 amplification only occurs in male jojoba samples, not in females. So, if the result of PCR amplification produced one band, then males and if no band then it is female. They further validated these primers to 315 jojoba samples of King Faisal University station region of the Kingdom of Saudi Arabia. Out of which, from 400 samples, by successfully identify the gender of jojoba which indicates that the technique is efficient in differentiating the sex of jojoba. Glycerol-3-phosphate acyltransferase (GPAT) catalyzes the initial step in the manufacture of almost all plant membrane phospholipids and storage triacylglycerols. These are the findings from several *in vivo*, *in vitro* and *in silico* studies conducted on Arabidopsis with the aim of designating AtGPAT9 for this crucial role. This gene is mostly found in single copies in most plant traits that are compatible with basic housekeeping functions. It has been substantially conserved throughout evolution[21-23].

The recently created methods for clearly choosing the female jojoba plant at a young age are highly significant and useful to breeders as they reduce the overall plantation expenses related to the other method of cultivating the non-productive male jojoba plants. The early sex determination of jojoba would also create new opportunities for global genetic improvement program implementation, jojoba genotype seeding and the restoration of biodiversity in jojoba groves. When expressed, the corresponding polypeptides have GPAT activity and transmembrane

Ananas comosus glycerol-3-phosphate 2-O-acyltransferase 6-like (LOC109724820), mRNA
Sequence ID: XM_020253746.1Length: 1637Number of Matches: 1

```
Query  26    CTGCTTGTCATGGAGGGGAAGAGAGGCGCAGCATTGCCTGCGAATTTGAGGGTGCCCTCC  85
             |||||| ||||| || | |||||| || |  |||||||   ||| | ||||| || || |
Sbjct  20    CTGCTTTTCATGTAGAGAAAGAGAAGCACTCCATTGCCATTGAACTGGAGGGAGCTCTTC  79
Query  86    TCATCTCAAAGAGACT-CTTTGCCTACTTCATGCTTGTTGCTCTTGAAGCTGGTGGCCCC  144
             || |||| | || ||| |||| ||||||| ||||  | || || ||||| |||||||||
Sbjct  80    TCCTCTCCA-GAAACTTCTTTCCCTACTTTCTGCTCATCGCCCTCGAAGCCGGTGGCCCC  138
Query  145   ATCAGGGCTCTTACACTGCTCCTAGTCTTCCCTCTCCTATGGTTGCTCGAGCTCGTCGGC  204
              ||||||||||   || |||||   ||  ||| |   |  |||  || | ||| |   |
Sbjct  139   CTCAGGGCTCTTCTTCTCCTCCTGTCCTATCCTTTATCACTGTTATTCAAACTCATTCTC  198
Query  205   CTTGAGAGGATGGCTCTTCAACTGATGATCTTTGTATCCACGGCTGGTCTTAGGGTCGAT  264
               ||| |   | ||||||||  ||||||||||  |||| || || || |||| |||
Sbjct  199   GATGAAAACGTAGCTCTTCATATGATGATCTTCATATCGACTGCCGGACTTATGGTTTCA  258
Query  265   GATCTGAAGGCTGTGGCAAAGGCCACCCTGCCCAGATTTTATTTGGAAGACCTTAGGCAG  324
             ||| ||||||| || || |||||||| || ||  | || | | || |||| || |||| |
Sbjct  259   GATGTGAAGGCGGTCGCGAAGGCCACACTTCCACGGTTCTTTCTGCAAGAGCTCAGGCGG  318
Query  325   AGAGCATATCAGGTTTTCTCGAGCTATGAGGGGAAGAAATTTGTGGTTACTTGTATCCCC  384
             ||  | ||  | || ||| |||  | ||  || |||||  | ||| || ||||||  | ||
Sbjct  319   AGCACGTACGAAGTCTTCGCGAAGTGTGGAGGTAAGAAGTATGTCGTGACTTGTTTGCCG  378
Query  385   AGGATCATGGTTGAGCCCTTCCTGAAGGAGTTCTTGGATGTGGATCATGTGATTGGCGCC  444
             ||| | ||||| || ||  || ||| ||||| ||||||||| ||||| |||||  || |
Sbjct  379   AGGGTTATGGTGGAACCAATCTTGAGGGAGTACTTGGATGTCGATCACGTGATCAGCACG  438
Query  445   GAACTGAAGATGTTCAAAGATTGCTCTGTAGGCTTGGTGGCATCACCAGGAGTCATGTTA  504
             ||  ||| | ||||    |   | | | | ||| |||||||  | |||||  |||||  |
Sbjct  439   GAGTTGAGGGTGTTTGGGGGCCGATGTCTCGGCCTGGTGGCTCCGCCAGGTATCATGGCA  498
Query  505   GGTGCCCG-----TCGGCTCC-------AAGCA--------------GATAAGAGATTGG  538
             ||   | |     ||  | ||       ||||              ||| |  ||||
Sbjct  499   GGCAGCAGAAGATTCACCGCCCTCATGGAAGCTCACGGGACTGATCGGATGATCGATTTC  558
Query  539   GCA----GTG-GGC--TAAGAGATAAGACATTCATGTTGCTGTGCAGGGAACACCACCCG  591
             | |    ||| |||  | |  | | || ||||| ||   ||||||  ||| || ||||
Sbjct  559   GGAATCGGTGCGGCATTTACTGCTCAGCCATTCTTGAGCCTGTGCCTGGAGCAGCACCTT  618
Query  592   ATTCCGCCAGAAGAGACTTCCTCACCCTTGCGAAGAAAGGATTATCCTAAGCCATTGATC  651
             ||||| ||||| |||| |  ||| || ||||   | ||  | ||||||||||| ||||||
Sbjct  619   ATTCCACCAGAGGAGAATAGCTCGCCATTGCCGCGGAACAACTATCCTAAGCCGTTGATC  678
Query  652   TTCCATGATGGTCGTCTCATGGCTCATCCCACGCCATTGGCTTTC-CTAGCTGTTATCCT  710
             ||||| || ||||||||| |||| |  ||   ||| | || |||  ||| |  || | ||
Sbjct  679   TTCCACGACGGTCGTCTCGTGGCACGACCAGGGCCGTCGGATTTTGCTATCA-TTCTTCT  737
Query  711   CTGGCTGCCCCTTGGCGCTCTCTTGGCCATTTACCGGATCCTTGTGGGTATCCTACTACC  770
             |||| | || || || |  ||||| ||||||| ||||||| | |||||  || | || ||
Sbjct  738   CTGGTTCCCTCTAGGTGTCCTCTTAGCCATTTTCCGGATCTTAGTGGGCCTCATCCTCCC  797
Query  771   GTACAAGATAGGCCTTGTGGGATCTGCAGTAACGGGCTTTCGCATTAGGGCCCAGTTTCC  830
              | |||  | |||||  | ||  |||| |  ||||||||||||||  ||||||| || ||
Sbjct  798   CTCCAATTTGGGCCTATTTGGTGCTGCGGCCACGGGCTTTCGCATACGGGCCCAATTCCC  857
Query  831   AAAAGCC--CAACATTCTCTCAATACACCTGCAGCCGTGGGTGCGCCAAACAGGACGGGC  888
              | ||||  | ||||| || |    |||||  ||     | ||| ||  | |  |  |||
Sbjct  858   TACAGCCTGCCACATT-TC-CGGGCCACCTCAAGTTACCGCTGCACCGGAAAAAAGTGGC  915
Query  889   ACACTATATGTCTGCAATCATCGGACACTTCTCGACCCAGTGATCGTATCAACAGCGATC  948
             ||||| ||||| |||||||||| ||| |||||||||| |||||| |||| || |   ||
Sbjct  916   ACACTGTATGTGTGCAATCATCAGACGCTTCTCGACCCTGTGATCATATCGACGGTTCTC  975
Query  949   CAACGAAAGGTGACTGCCGTTACATATAGTTTAAGCAGATTCTCAGAGGTGATTTCTCCA  1008
             || || ||| |  | || ||||| || || ||||||||||||||||  || | ||||||
Sbjct  976   CAGCGGAAGATCTCAGCTGTTACTTACAGCTTAAGCAGATTCTCAGAATTGGTCTCTCCA  1035
Query  1009  ATCCCAACCATCCGATTGACCAGGGACCGCTTCAAGGATGGTGCTGCGATGCGATCACTG  1068
             ||||| ||| ||||||| ||||||||||||  ||||| |||||   ||||||  |||||
Sbjct  1036  ATCCCGACCGTCCGATTAACCAGGGACCGCTGTAAGGACGGTGCGATGATGCGGCCACTG  1095
Query  1069  CTCAATCATGGTGATCTGGTAGTCTGCCCAGAGGGAACCACCTGCCGGGAGCCATACCTC  1128
             ||  |||| |||||||| || || ||||| ||||| ||||| ||||| || ||||||||
Sbjct  1096  CTTGATCAAGGTGATCTAGTGGTGTGCCCTGAGGGGACCACGTGCCGCGAACCATACCTG  1155
Query  1129  CTCCGATTCAGCCCCTTGTTTGCGGAGATCGCCGACAACATCGTTCCAGTCGCTGTTACA  1188
             ||||| || ||||| || ||||| |||||  ||||      | |  || || || |||  |
Sbjct  1156  CTCCGGTTTAGCCCGTTATTTGCAGAGATAACCGATGTGGTTGAACCGGTTGCCGTTCGA  1215
Query  1189  GCTGAGGGTAGCATGTTCTATGGAACCACAGTCAGGGGGCACAAATGGTTGGATTCACTC  1248
             ||  | || | ||||||||||  |||| ||  |||| ||||| |||||||| ||| ||
Sbjct  1216  GCCTACGGGACCATGTTCTATGGGTCCACGGTTCGGGGTCACAAGTGGTTGGACTCATTC  1275
Query  1249  TTCTTCCTAATGAATCCTAGGCCTTGCTATCAGTTGCACTTCCTTGA--AAGTAT--GGC  1304
             ||||| || |||||||| || |||   || ||||||| ||||||| ||    ||||  |||
Sbjct  1276  TTCTTTCTGATGAATCCGAGCCCTGCTTACCAGTTGGACTTCCTCGAGCCTGTATTCGGC  1335
Query  1305  AAGGGATCAAAGATCAAGCTTTGAAATTGCAAATGGCATCCAACGGTTGATTGGAGAAGC  1364
                   ||| | ||| |   |  ||  ||||||||  |||||||||||||||| || || ||
Sbjct  1336  ----TGTCAGAAATCGAAATACGATGTTGCAAATCACATCCAACGGTTGATCGGGGAGGC  1391
Query  1365  AGTTGGGTTCGAGTGCACCAATTTCACGCGGAAGGACAAATATCAGATGCTTGCTGGCCA  1424
               |||||||| |||||||||||||||||| |||||||| |||| |||||| || |||||
Sbjct  1392  GATTGGGTTCAAGTGCACCAATTTCACGCGAAAGGACAAGTATCGGATGCTCGCGGGCCA  1451
Query  1425  TGATGGTGCGGACACCAGGAGATGA  1449
             |||||||| ||| || || || |||
Sbjct  1452  TGATGGTGTGGATACAAGAAGCTGA  1476
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Fig. 7: Comparison of the DNA sequences of jojoba GPAT3 with GPAT3 gene from *Ananas comosus*

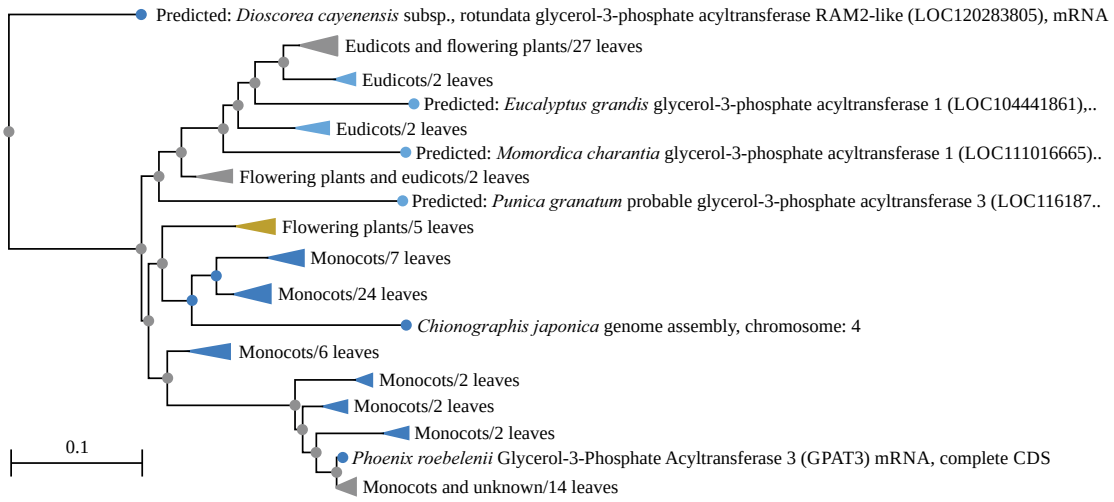

Fig. 8: Jojoba, *Carica papaya* and homo sapiens sex-determining region Y phylogenetic connections

domains. The current study focuses on the functional significance of one isoform, JoGPAT3. The Zhifu29 mentioned that disruption of the GPAT3 gene results in a massive arrest of pollen development. However, the phenotype can be recovered by introducing the gene into the mutant plant. This suggests that AtGPAT1 plays a crucial role in pollen development and male sex determination.

Consequently, a plant model of the GPAT3 function has been created. This has proven crucial in identifying how GPAT3 interacts with other genes to determine male sex. Since jojoba was first discovered historically, we have made tremendous strides toward understanding sexual dimorphism; the GPAT3 gene in males is the cause of the difference between the sexes. As of late, we have learned more about the molecular processes involved in determining sex[24-26].

As previously stated, the current effort is concerned with molecular methods unique to the sex of Jojoba plants and with applying these methods to differentiate between male and female plants. The early selection of female plants made possible by the approaches has the advantage of lowering the overall plantation expenditures related to the cultivation of the non-productive male jojoba plants. Furthermore, our methods are "universal" in the sense that they work with jojobas of any origin, variation or cultivar. Cultivating plant species has always been problematic due to dioecism, which is particularly linked to dimorphism in woody trees. Dioecious plants can be difficult to determine their sex, particularly in the early stages of development and before they flower. The sex of jojoba plants is only discernible during blooming, which occurs at about two years of age. As a result, it is nearly impossible to maintain a healthy population in terms of the male-to-female ratio in the field, which lowers production. Sequences of JoGPAT3 in date palms closely matched the amplified regions of the jojoba Glycerol-3-Phosphate Acyltransferase 3 (GPAT3) gene. In most plant systems, the presence of GPAT3 in male jojoba plants may result in sex identification and determination. The amplified regions' sequences closely matched the GPAT3 sequences of date palms and some plants that have been published. All the male samples and none of the female samples had a 1450 bp DNA fragment amplified. Consequently, this technique distinguished between the sexes of individual plants. In plant cells' extra plastidic compartments, glycerol-3-phosphate acyltransferase (GPAT; EC 2.3.1.15) mediates the first stage of glycerolipid biosynthesis. In this work, we report the molecular characterization of the JoGPAT gene family, a novel family of GPAT genes from Jojoba. The anther and pollen, two important structural elements of plant male reproductive organs, are made of lipid molecules. While our knowledge of the role of acyl lipids in plant reproduction has advanced, much remains unknown about the metabolic pathways of other lipid molecules, especially glycerolipids. Glycerol-3-phosphate acyltransferase was first characterized biochemically in extracts from animal and plant tissues in a series of reports beginning more than 60 years ago. In order to generate the sn-2 monoacylglycerol precursors that are needed for the extracellular polymerization of terrestrial plants' cutin and suberin barriers. Thus, it is likely that the GPAT1-GPAT8 family is not involved in the production of TAG and membrane lipids, which are essential for all living things[25-28]. Considering this, the current study was carried out to create repeatable and useful methods for the early detection of sex in male jojoba plants using simplex PCR, which relies on the GPAT3 gene linked to the human

sex-determining area that was initially supplied to the researcher. Furthermore, the procedure was designed specifically for male jojoba plants to identify the sex, particularly before flowering and in the early phases of growth. Dioecism, a condition linked to sexual dimorphism, has long been problematic for plant species that are cultivated, particularly woody trees[29,30].

CONCLUSION

The outcomes of the research also pertain to the above-discussed utilization of a sex-specific gene sequence in determining a jojoba's sex. The GPAT3 primers are also associated with a technique for identifying a jojoba's sex by looking for primers inside the genome. In some versions of the process, the GPAT3 gene-specific forward and reverse primers are used to amplify a specific region of the jojoba-tested genomic DNA to determine the sex of the plant. Thus, GPAT3 in humans is comparable to that in mice and jojoba plants. The authors make the interesting finding that such a tool can be developed by targeting the sequences in the GPAT gene, a region from jojoba plants. Finally, these results show that the PCR was a simple, rapid and reliable technique can complement and confirm sex determination in jojoba plants by using our specific primers pair according to our invention.

SIGNIFICANCE STATEMENT

The present work relates to the sex determination of the jojoba plants, particularly to gene sex determination of the jojoba plants based on the first identification of the GPAT gene in the jojoba plants. The jojoba plant is dioecious, having separate male and female plants. The JoGPAT1 is exclusively found in male jojoba plants, not in female plants. A method of determining the sex of the dioecious plants at an earlier stage would avoid the need to invest time and expense in growing and maintaining unwanted male dioecious plants. Thus, a sex-determination method for jojoba plants to solve the aforementioned problem is desired. The kits are provided for the sex determination of a jojoba plant. These and other features of the present invention will become readily apparent upon further review of the following specifications and drawings.

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