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Using Single Nucleotide Polymorphism (SNP) in Sex Determination of Date Palm

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Abstract: The date palm is one of the most important fruit types in the Arabian Peninsula and the Islamic nation because of its suitability to the environmental conditions and its psychological status among the Arab people. The palm tree is a dioecious plant so many varieties have emerged. It was important to find genetic diversity and DNA fingerprints in the early stages. Therefore in this paper used Three specific primers of Single Nucleotide Polymorphism (SNPs) used to sex discriminate on three Male- and three female- date palm by specific haplotypes were identified by screening SNPs, The results of sequence alignment showed that the sequences of DNA fragments resulting from the design of the sex-linked primer gene (TBL3) differ in some sites, as sites 432 and 433 have identical bases G and C, respectively, in male cultivars of date palm, while the same two sites carry the base A in the female cultivars. As for the sequence of DNA fragments resulting from the design of the gene primer (TBL5), the alignment process showed that locus 693 was unified in one sex and differed with the other sex, by diagnosing the T base in male cultivars and the G base in female cultivars, and the 904 loci were distinguished in The male cultivars have the base G, while the female cultivars have the base A. The study showed the power of SNP technique in determining sex in date palm at the genome level.

Keywords: Date palm**,** DNA**,** male cultivars , sequences**,** sex discriminate**,** SNP**.**

Introduction

Dates palm is one of the most important fruit trees in the Arabian Peninsula. They are adaptable to live in a warm and dry environment and it may be given very complete food. The palm tree is Unisexual, dioecious plant, male flowers on plant and female flowers on another plant. it must be pollinated by the human to give an economic produced.

The propagation process in date palms is still carried out by the offshoots. Seed propagation has many determinants. In addition to the fact that the resulting plants are not identical with the mother plant due to the genetic isolation resulting from cross-pollination, in addition, many of the resulting plants are male. Approximately 50% of the plants are male, this matter limited to use seeds in order to obtain new varieties of high quality or for the productive purpose, in addition, long time to confirm the resulting plant is male or female, If any farmer wishes to produce plants from seeds to obtain the fruits to benefit from them in the Transformative Industries, Those are the biggest determinants [1, 2]

So many research has used many techniques in trying to diagnose and identify sex in the palm, but most of the research has been not encouraging results [3, 4, 5,6].Therefore, the research aims at using SNP markers to gender identification in date palms as a modern and accurate technique.

Material and methods

Twenty male and 20 female date palm cultivars were selected for the purpose of examining the primers efficiency of SNP technique. Three males and three females were selected from among these trees for use to find sequencing and sex identification. The male cultivars are (Ghannamy akhadir ،Khadairi ،Smismy), and female cultivars are (Brtgala ، Sakri ، Abd-alhadi). Using liquid nitrogen and Promega kit DNA plant extract to extract DNA from young date palm leaves.

The SNP primers used is (1). TBL3 (F-TCTTGATAGAGGTTCTTGGGTG R-ACACTATTGTCCTTGCTTCCAGT) (2). TBL5 (F-TGTATTGTCAGGGCACACAGA R-CAATGTCCCACACTCTCACCT), then used the following program for PCR, Pre-denaturation 94C to 4 min, denaturation 94c to 45s, Annealing 62C to 45s, Extension 72C to 30s, elongation 72c to 7min this for 40 circles. The detection of the PCR product by agarose 1% by electrophoresis. The bands were extracted from gel and purification by Top PCR purification Kit (TGk 1007) then sent to Microsynth AG company to find sequencing by DNA Analyzer ABI 3730XL (Thermo Fisher Scientific). The result was analysis by NSBI Database, T-COFFEE Multiple Sequence Alignment, and Bioedit software**.**

Result and Desiccation

The first and second primer was selected to represent a larger segment for DNA and maybe take a more representative to sex. Both the Reverse and Forward sequences were compared to ensure that the sequences are complementary, and the existence of gaps and make sure readings BLAST was carried out for the fragment of SNP, which has a length of 455bp with sequence 1_20488_F3 in FASTA format based on National Center for Biotechnology Information NCBI showed that the sequence 1_20488_F3 corresponds to 16 locations in NCBI (figure 1). As shown in figure (2) the match percent around 97% to 100%, it was the lowest ratio of 97% in the DNA fragment take Accession No. KY677843.1 which return to fragment in length 950bp for definition sex in date palm by transduction beta-like protein 3 gene encoded using mRNA, These proteins are present in many real nuclei and cover a wide range of functions including regulatory units of transducers in signal transduction, pre-RNA processing, and cytoskeletal synthesis . Which is illustrated by [7] Depending on the sex-linked Tormozembryo Defective (TOZ19) gene in Aspen (Populus trichocarpa), a poplar or black cotton, a broad-leafed tree that is native to Western North America. It is used for timber and is best known as a typical organism in plant biology. The sequencing of its high-genome sequence has published a whole genomic in 2006 and was the first sequenced gene genome [8] . Cited by [7] and which Use BLAST for used TOJ19 against the date palm genome, a presumed gene for beta 3 protein (TBL3) was found in date palm trees that were very similar to the TOZ19 gene then applied on three male trees and four female trees. Based on the multiple nucleotide sequence obtained, haplotypes of male and female palms were identified by examining multiple nucleotide profiles (SNPs).

It also corresponds with the African oil palm Elaeis guineensis with the same percentage of 97% with the same gene transduction beta-like protein 3 genes with a size of 3048bp gene and its coding area of 100-2796, which carries Accession No. XM_010914433.2, which can be used as a model for a match as shown in Table (3). It is clear that the 97% match ratio was within the sequence between 302-430 for DNA sequence 1_20488_F3 versus 1240-1368 in the length of 3048 Accession No. XM_010914433.2. and between 1302-1430 of the length of 3063 for Accession No.KY677843.1. It is worth mentioning that this sequence is associated with 12 sequences mentioned in the NCBI with a match of 98% and 99%, as shown by the Figure(2), which include the characterization of the gene with the relationship in the varieties Samani, Zaghloul, Hayani, Orabi and three varieties of date palm males not specified.

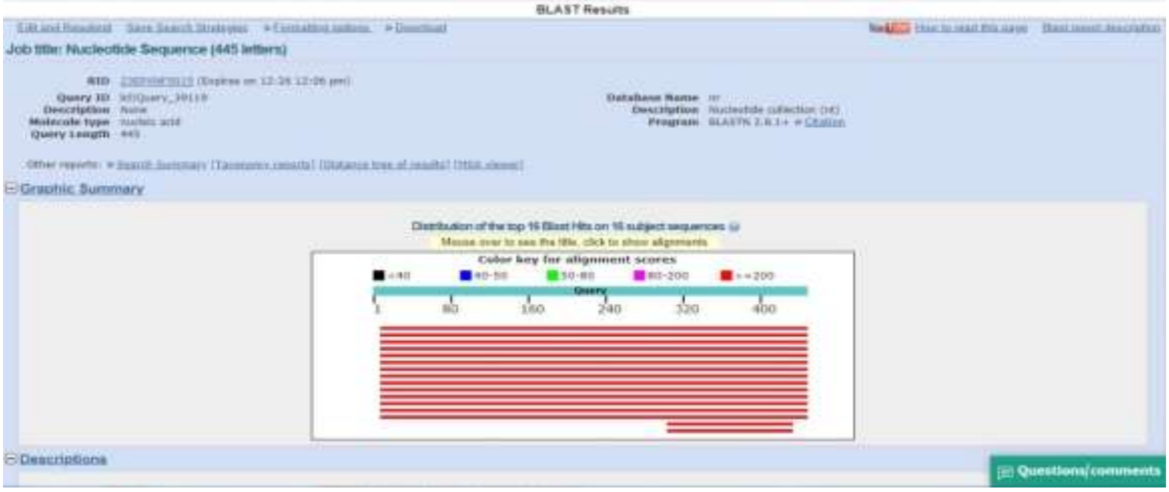
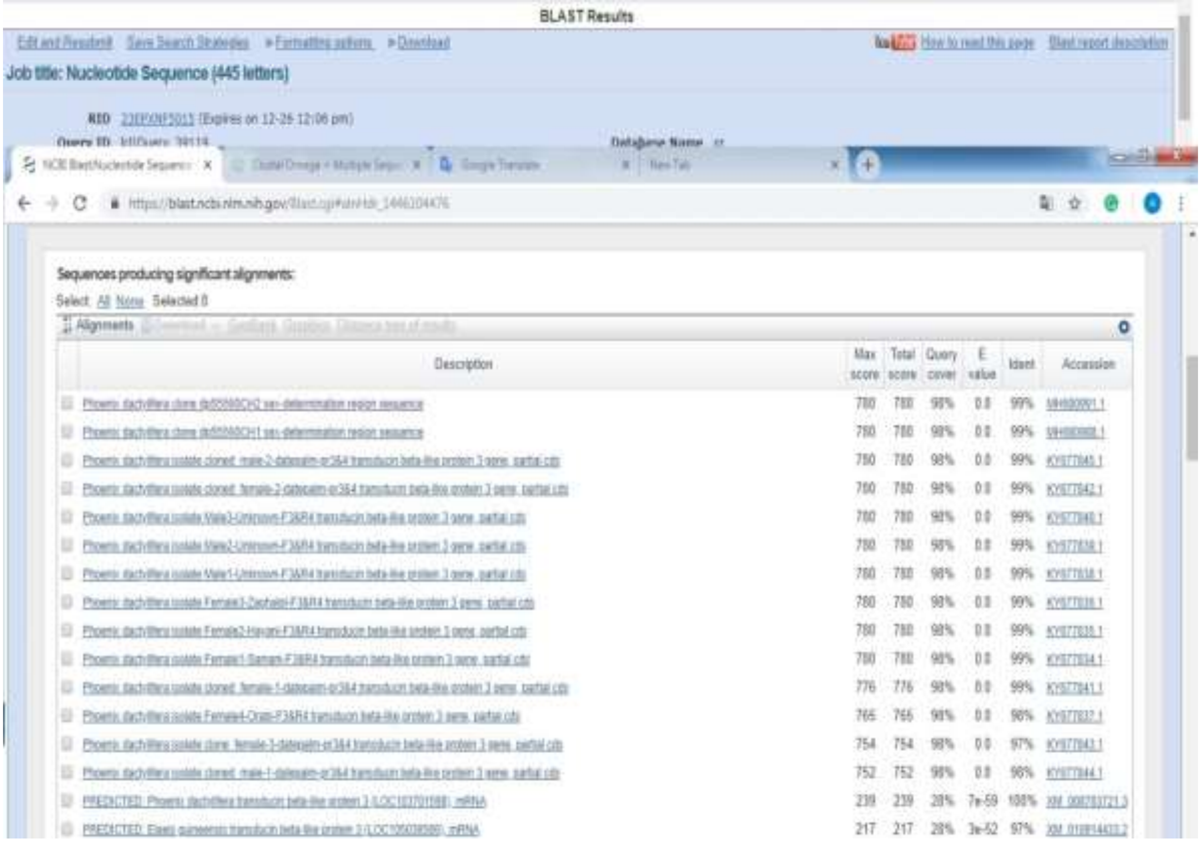


Figure (1) Distribution of top 16 Blast Hits on 16 subject sequins of Date palm to 1_20488_F3



BLAST Results

Job title: Nucleotide Sequence (445 letters)

RID 23EFXNF5013 (Expires on 12-26 12:06 pm)

Sequences producing significant alignments:

Select: All None Selected 0

	Description	Max score	Total score	Query cover	E value	Ident	Accession
☐	Phoenix dactylifera clone [illegible] sex-determination region sequence	780	780	98%	0.0	99%	MH680991.1
☐	Phoenix dactylifera clone [illegible] sex-determination region sequence	780	780	98%	0.0	99%	MH680988.1
☐	Phoenix dactylifera isolate cloned_male-2-datepalm-pr364 transducin beta-like protein 3 gene, partial cds	780	780	98%	0.0	99%	KY677845.1
☐	Phoenix dactylifera isolate cloned_female-2-datepalm-pr364 transducin beta-like protein 3 gene, partial cds	780	780	98%	0.0	99%	KY677842.1
☐	Phoenix dactylifera isolate Male3-Unknown-F3&R4 transducin beta-like protein 3 gene, partial cds	780	780	98%	0.0	99%	KY677840.1
☐	Phoenix dactylifera isolate Male2-Unknown-F3&R4 transducin beta-like protein 3 gene, partial cds	780	780	98%	0.0	99%	KY677836.1
☐	Phoenix dactylifera isolate Male1-Unknown-F3&R4 transducin beta-like protein 3 gene, partial cds	780	780	98%	0.0	99%	KY677838.1
☐	Phoenix dactylifera isolate Female3-[illegible]-F3&R4 transducin beta-like protein 3 gene, partial cds	780	780	98%	0.0	99%	KY677839.1
☐	Phoenix dactylifera isolate Female2-Hayani-F3&R4 transducin beta-like protein 3 gene, partial cds	780	780	98%	0.0	99%	KY677835.1
☐	Phoenix dactylifera isolate Female1-Samani-F3&R4 transducin beta-like protein 3 gene, partial cds	780	780	98%	0.0	99%	KY677834.1
☐	Phoenix dactylifera isolate cloned_female-1-datepalm-pr364 transducin beta-like protein 3 gene, partial cds	776	776	98%	0.0	99%	KY677841.1
☐	Phoenix dactylifera isolate Female4-[illegible]-F3&R4 transducin beta-like protein 3 gene, partial cds	765	765	98%	0.0	98%	KY677837.1
☐	Phoenix dactylifera isolate clone_female-3-datepalm-pr364 transducin beta-like protein 3 gene, partial cds	754	754	98%	0.0	97%	KY677843.1
☐	Phoenix dactylifera isolate cloned_male-1-datepalm-pr364 transducin beta-like protein 3 gene, partial cds	752	752	98%	0.0	98%	KY677844.1
☐	PREDICTED: Phoenix dactylifera transducin beta-like protein 3 (LOC103701598), mRNA	239	239	28%	7e-59	100%	XM_008783721.3
☐	PREDICTED: Elaeis guineensis transducin beta-like protein 3 (LOC105038580), mRNA	217	217	28%	3e-52	97%	XM_010914433.2

Figure(2) sequences production significant alignments to 1_20488_F3

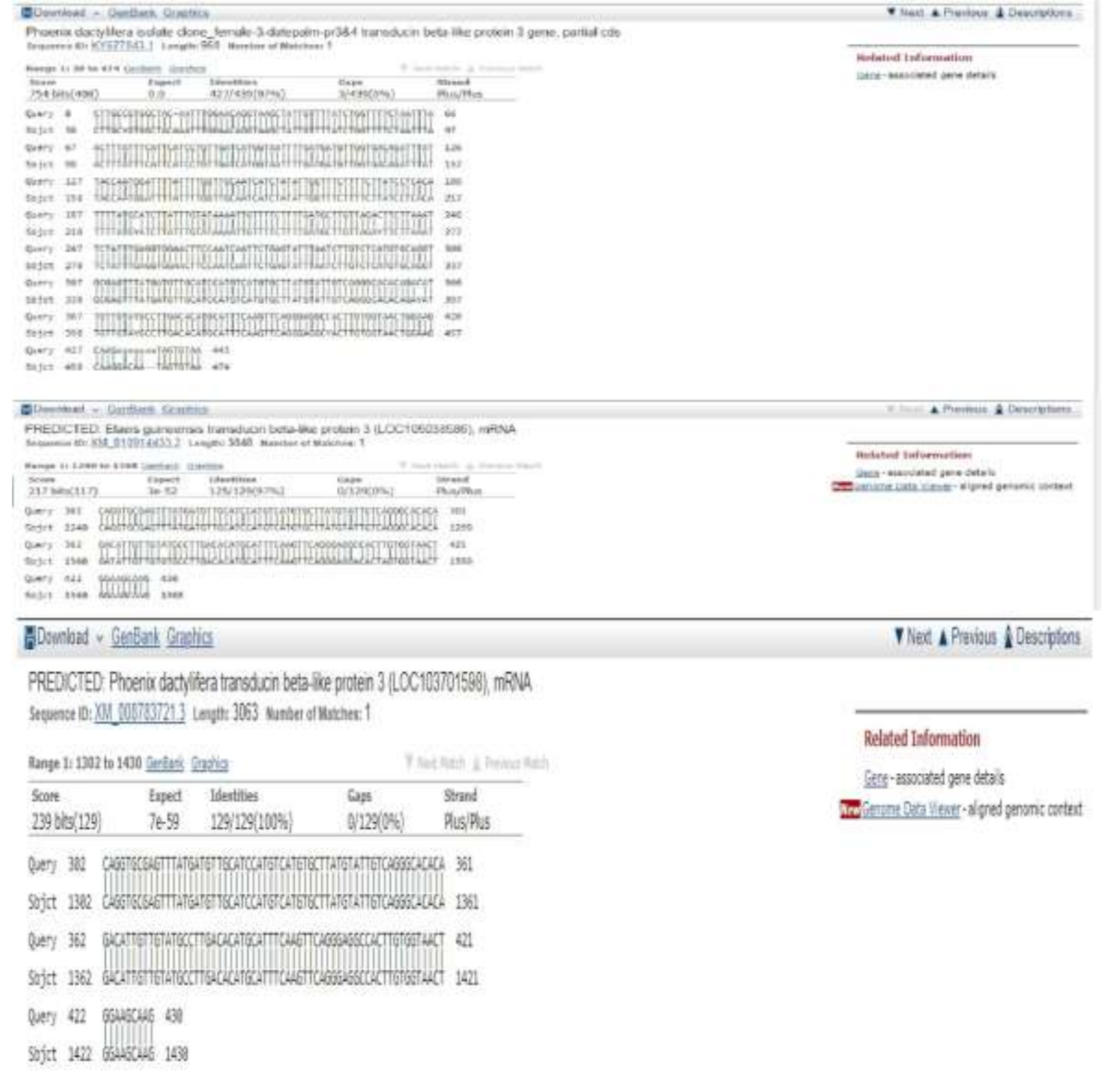
The highest match result for sequence 1_20488_F3 is 100% in fragment sequence Accession No. XM_008783721.3, which has an identification number for the gene 103701598, the match between 302-430 versus sequence 1302-1430, there is 129 matches between the two series. Which is the gene for transducin beta-like protein 3 and the gene-ID update: 103701598, updated on 18-Oct-2018 in the NCBI. The 99% match mentioned by [9], which holds Accession No. MH680991.1 and MH680988.1, it is noted that the matching sequence is 8-445, corresponding to sequence 1116955 - 1116535, corresponding to 434 bases of 439 base with 3 gaps Gaps and 1120081-1119645 with 434 match and 3 gaps for two sequences respectively Figure (3). This was described by [9] when examining the CYP703 and GPAT3 genes, which are single-molecule genes present in males and critical to the development of male flower in single-nucleus and absent in females.

PREDICTED: Phoenix dactylifera transducin beta-like protein 3 (LOC103701598), mRNA

Score	Expect	Identities	Gaps	Strand
239 bits(129)	7e-59	129/129(100%)	0/129(0%)	Plus/Plus

PREDICTED: Elaeis guineensis transducin beta-like protein 3 (LOC105038580), mRNA

Score	Expect	Identities	Gaps	Strand
217 bits(117)	3e-52	125/129(97%)	0/129(0%)	Plus/Plus



Phoenix dactylifera isolate clone_female-3-datepalm-pr3&4 transducin beta-like protein 3 gene, partial cds

```
Score          Expect   Identities      Gaps          Strand
754 bits(408)  0.0      427/439(97%)    3/439(0%)     Plus/Plus
```

PREDICTED: Elaeis guineensis transducin beta-like protein 3 (LOC105038586), mRNA

Sequence ID: XM_010914433.2 Length: 3540 Number of Matches: 1

```
Score          Expect   Identities      Gaps          Strand
217 bits(117)  3e-52    125/129(97%)    0/129(0%)     Plus/Plus
```

PREDICTED: Phoenix dactylifera transducin beta-like protein 3 (LOC103701598), mRNA

Sequence ID: XM_008783721.3 Length: 3063 Number of Matches: 1

Related Information

Gene - associated gene details

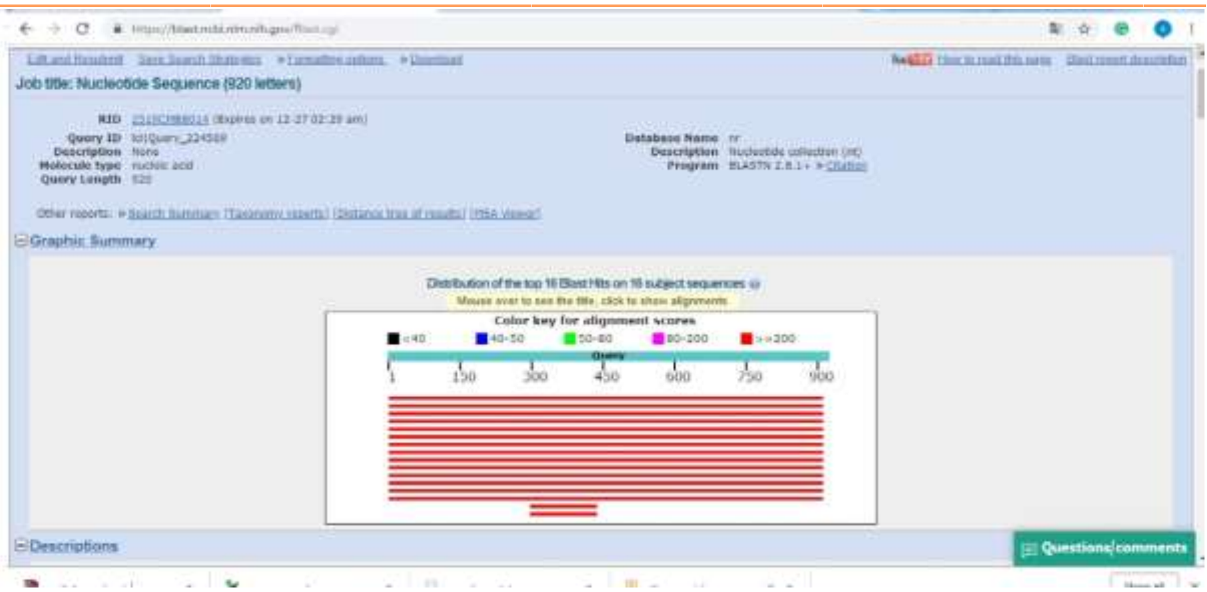
Genome Data Viewer - aligned genomic context

Range 1: 1302 to 1430 GenBank Graphics

```
Score          Expect   Identities      Gaps          Strand
239 bits(129)  7e-59    129/129(100%)   0/129(0%)     Plus/Plus

Query  302   CAGGTGCGAGTTTATGATGTTGCATCCATGTCATGTGCTTATGTATTGTCAGGGCACACA  361
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1302  CAGGTGCGAGTTTATGATGTTGCATCCATGTCATGTGCTTATGTATTGTCAGGGCACACA  1361

Query  362   GACATTGTTGTATGCCTTGACACATGCATTTCAAGTTCAGGGAGGCCACTTGTGGTAACT  421
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1362  GACATTGTTGTATGCCTTGACACATGCATTTCAAGTTCAGGGAGGCCACTTGTGGTAACT  1421

Query  422   GGAAGCAAG  430
             |||||||||
Sbjct  1422  GGAAGCAAG  1430
```

Figure (3) specific element of fragment to 1_20488_F3

When BLAST for other segment 7_20488_F3 has a length of 920bp in the FASTA sequence based on NCBI, the results show that the sequence 7_20488_F3 has a genetic match with 16 sequences (Figure 4) Where it ranges from 97% to 100% in sequences that own Accession No. XM_010914433.2 and XM_008783721.3 respectively. It is worth mentioning that 13 of these sequences have a common relationship resulting from a gene-like transduction beta-like protein 3 gene (sex gen) and 2 sequences of genes related to the sex determination sequence region, Is related to sex determination in African oil palm (figure, 5).



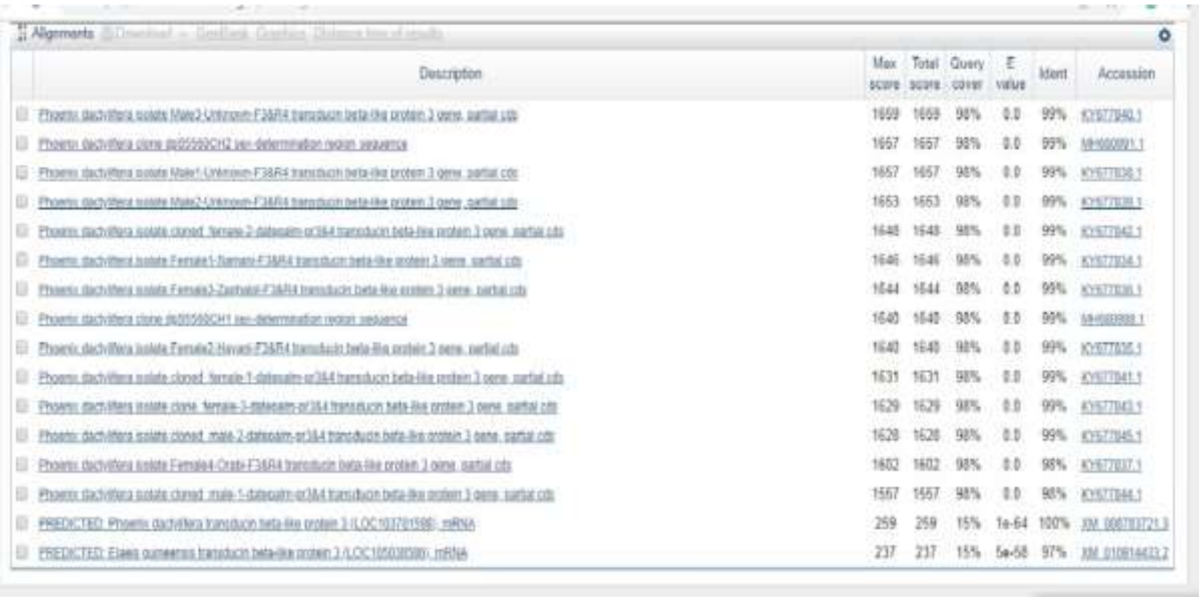
Job title: Nucleotide Sequence (920 letters)

RID [illegible] (Expires on 12-27 02:29 am)

Query ID lcl|Query_224589
Description None
Molecule type nucleic acid
Query Length 920

Database Name nr
Description Nucleotide collection (nt)
Program BLASTN 2.8.1+

Figure (4) Distribution of top 16 Blast Hits on 16 subject sequins of Date palm to 7_20488_F3



	Description	Max score	Total score	Query cover	E value	Ident	Accession
☐	Phoenix dactylifera isolate Male3-Unknown-F3&R4 transducin beta-like protein 3 gene, partial cds	1659	1659	98%	0.0	99%	KY677840.1
☐	Phoenix dactylifera clone dp05560CH2 sex-determination region sequence	1657	1657	98%	0.0	99%	MH600091.1
☐	Phoenix dactylifera isolate Male1-Unknown-F3&R4 transducin beta-like protein 3 gene, partial cds	1657	1657	98%	0.0	99%	KY677838.1
☐	Phoenix dactylifera isolate Male2-Unknown-F3&R4 transducin beta-like protein 3 gene, partial cds	1653	1653	98%	0.0	99%	KY677839.1
☐	Phoenix dactylifera isolate cloned_female-2-datepalm-pr3&4 transducin beta-like protein 3 gene, partial cds	1648	1648	98%	0.0	99%	KY677842.1
☐	Phoenix dactylifera isolate Female1-Barhi-F3&R4 transducin beta-like protein 3 gene, partial cds	1646	1646	98%	0.0	99%	KY677834.1
☐	Phoenix dactylifera isolate Female3-Zahdi-F3&R4 transducin beta-like protein 3 gene, partial cds	1644	1644	98%	0.0	99%	KY677836.1
☐	Phoenix dactylifera clone dp01560CH1 sex-determination region sequence	1640	1640	98%	0.0	99%	MH600090.1
☐	Phoenix dactylifera isolate Female2-Hayani-F3&R4 transducin beta-like protein 3 gene, partial cds	1640	1640	98%	0.0	99%	KY677835.1
☐	Phoenix dactylifera isolate cloned_female-1-datepalm-pr3&4 transducin beta-like protein 3 gene, partial cds	1631	1631	98%	0.0	99%	KY677841.1
☐	Phoenix dactylifera isolate clone_female-3-datepalm-pr3&4 transducin beta-like protein 3 gene, partial cds	1629	1629	98%	0.0	99%	KY677843.1
☐	Phoenix dactylifera isolate cloned_male-2-datepalm-pr3&4 transducin beta-like protein 3 gene, partial cds	1628	1628	98%	0.0	99%	KY677845.1
☐	Phoenix dactylifera isolate Female4-Orabi-F3&R4 transducin beta-like protein 3 gene, partial cds	1602	1602	98%	0.0	98%	KY677837.1
☐	Phoenix dactylifera isolate cloned_male-1-datepalm-pr3&4 transducin beta-like protein 3 gene, partial cds	1567	1567	98%	0.0	98%	KY677844.1
☐	PREDICTED: Phoenix dactylifera transducin beta-like protein 3 (LOC103701598), mRNA	259	259	15%	1e-64	100%	XM_008783721.3
☐	PREDICTED: Elaeis guineensis transducin beta-like protein 3 (LOC105038580), mRNA	237	237	15%	5e-58	97%	XM_010914433.2

Figure (5) specific element of fragment to 7_20488_F3

The use of nucleotide sequences in determining sex

The alignment of DNA sequences from both primers can determine sex by drawing on the SNP diagnostic precision in identifying one of the identical nucleotides similar in the palm cultivars under one sex and different with another sex. Noted from Sequencing aliment programs Figure (6), the sequences of DNA fragments from the primer (1) contain a high matching segment in both date palm sexes. It is also noted that sites 432 and 433 have a sexual correlation in the G or C bases in the cultivars Male while the same site carries A in the female cultivars.

C:\Users\msn\Desktop\احمد يوسف حلف السكوتر\aliment_1_6 from bioedit.fas

6 total sequences

Mode: Select / Slide

Selection: 0

Sequence Mask: None

Numbering Mask: None

Start ruler at: 1

Untitled

6 total sequences

shade threshold 100%

Mode: Select / Slide

Selection: 0

Position: 2: 2_20488_F3.fasta 340

Sequence Mask: None

Numbering Mask: None

Start ruler at: 1

Figure (6) sequence alignment by bio edit and t-Coffe software for 1_20488_F3 fragment

As for the DNA sequence from primer 2, it is noted from figure (7) that there a high marching in nucleotides sequence of this segment in the cultivars ender same sex, It can pointed site 693 was united in one sex and differed with the opposite sex, It noted T base in the male cultivars while G base in the female cultivars, and in 904 in the male cultivars carrying the G base while in the female cultivars is A base.

Concluded from this work that the use of the nucleotide sequence extracted from the SNP primers can be a clear evidence of sex diagnosis, and in some respects agrees with what [7] reported that the active sites were 562, 581 and 712, This segment is identical to the specific sex segments in the African oil palm and this is demonstrated by [10] in finding pieces of genomic DNA compatible with sex in the oil palm.

Figure (7) sequence alignment by bio edit and t-Coffe software for 7_20488_F3 fragment

Conclusion:

Determination of sex in date palm is a major challenge because it is no vegetative way can determine the sex in date palm before the fruiting stage, the way SNP method is effective and accurate has succeeded in identifying the sex based on one of the genes determining the sex in the palm plants

Acknowledgments

All the appreciation and thanks go to the Biotechnology Laboratory - Palm Research Center - University of Basra. and The Department horticulture & landscap for supporting this work.

Supplementary materials

Supplementary data can be found with this article online at
https://www.ncbi.nlm.nih.gov/nuccore/LC619214.1/
https://www.ncbi.nlm.nih.gov/nuccore/LC619215
https://www.ncbi.nlm.nih.gov/nuccore/LC619216
https://www.ncbi.nlm.nih.gov/nuccore/LC619217
https://www.ncbi.nlm.nih.gov/nuccore/LC619218

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