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Genetic Diversity of Three Medicinal Plants of Apiaceous Family

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ABSTRACT

Lots of debates has been risen up about the genetic rapport among species of Apiaceae family. Till now there is no quite suitable contemporary categorization at hand. This study aimed at sequencing the 18S ribosomal RNA gene (ITS gene) in three plants in this family, namely; *Apium graveolens, Petroselinum crispum* and *Coriandrum sativum*. This is to see if there is any genetic variation among them. Briefly, the plants were collected and their DNA was extracted, exposed to PCR amplification and Sangar sequencing to determine the genetic sequence of the 18S rRNA gene of each specie and compare it with a reference gene from the gene bank. The results showed that the gene was 99% identical with that of the standard of the gene bank for each of the Coriandrum *sativum* and *Apium graveolens*. While the percentage was100 % for *Petroselinum crispum*. This suggests that the mentioned gene was identical in the three species and excludes the incidence of any significant variation within this gene.

Keywords: - Ribosomal, Apiaceous, Coriandrum, Apium, Corriandrum, , compatible and gene bank. Gene variation.

INTRODUCTION

The Apiaceae (Umbelliferae) family is comprised of over 3,000 species of famous herbaceous plants with lots of medicinal effects. They are distributed widely over the sub-tropical, sub-temperate and temperate regions of the world. Plants of this family are famous nutritional, flavoring or medical agents as they are endowed with lots of phytochemicals with potent antimicrobial and anti-inflammatory properties. The most famous vegetable crops that belong to this family are carrot *(Daucus carota),* parsley (*Petroselinum crispum*), celery (*Apium graveolens*), Peruvian carrot (*Arracacia xanthorrhiza*) and coriander *(Coriandrum sativum)*[1]&[2].

It is expected to notice the presence of the genetic diversity among species of the same family due to the expected incidence of the genetic drift, gene flow or mutational changes. This results in the evolution of offspring generations with new characters [3]. The first involves changes in the allele frequency after having a random mating of the individuals [4]. On the other hand, the gene flow involves the transfer of the genetic materials by pollens in the winds or the migrating birds [5]. Meanwhile, random mutations may create new genes with unique characters in the subsequent generations resulting in deterioration or improvement in the aptitude of the organism to adapt to the surrounding environment. They are mostly induced by changes in the conditions of the surrounding environment, such as climate changes or exposure to toxins or pesticides. Such mutational changes are characterized by the occurrence of SNPs (Single Nucleotide Polymorphism); which involves substitution of one genetic codon by another one resulting in prominence of the interindividual differences [6] and [7].

In this study, the genetic variation in the 18 S rRNA gene in three species of Apiacea family, namely; *Corriander sativum, Apium gravedus* and *Petroselinuim crispum* was analyzed. They were procured from the same zone; farms around Baghdad city. It is noteworthy that this gene is one of the important ribosomal RNA molecules; that inter in the structure of the ribosomes. Its gene has a unique importance and was used extensively in the molecular analyses related to phylogenetic studies.

MATERIAL AND METHODS

Materials

Chamicals

DNA extraction kit was procured form (ZR Plant/Seed DNA MiniPrep). Meanwhile ethidium bromide, agarose , TAE loading buffer were procured form USA- pioneer. The Taq PCR was procured form (PreMix (Intron, Korea)) The forward and the reverse primers of the ITS gener were procured form IDT (Integrated DNA Technologies company, Canada).

Agarose gel electrophoresis

Plant material

Leaves of three plants; belong to the family of Apiaceous, were procured from several Iraqi farms around Baghdad city. They were namely; *Corriander sativum, Apium gravedus* and *Petroselinuim crispum*. They were identified by specialists in the herbarium center for botanical plants classification in Baghdad University. The leaves were washed out, were mixed with liquid nitrogen and then were pulverized using pestle and mortar.

Method

DNA extraction and purification

The DNA was extracted from the powdered leaves using the DNA extraction kit (ZR Plant/Seed DNA MiniPrep), and its purity was checked by agarose gel electrophoresis as previously prescribed (Voltage:70 and ethidium bromide was used as a loading dye, TAE was used as a loading buffer [9] to. Then the extract was exposed to the cyclical Polymerase Chain Reaction (PCR) for to amplify the 18S rRNA gene; a target gene for the different phylogentic studies to find the genetic diversity in the mentioned plants.

PCR amplification

Preparation of the primers

The two primers for the TSE **gene** were (***ITS1*** F: 5′-TCCGTAGGTGAACCTGCGG -3′) and (***ITS4*** R:5′ TCCTCCGCTTATTGATATGC-3′) with a genome size of 500-650 bp. They represent the forward and the reverse primers respectively and were supplied by IDT (Integrated DNA Technologies) company, Canada) [10] They were lyophilized and dissolved in a double deionized water (ddH_2O) to prepare a stock solution at a final concentration of 100 pmol/µl and were kept at -20 for the furture processes. Then working solution were prepared at a final volume 100 µl and a concentration of 10 pmol/µl using the double ionized water.

Gene amplification

The PCR mixture was prepared through mixing 1.5µl DNA, 5 µl of the Taq PCR PreMix (Intron, Korea), 1µl of each primer (10 pmol) and distilled water were added such that the final volume reached to 25µl using master reaction and a thermal Cycler (Gene Amp, PCR system 9700; Applied Biosystem). The details of the PCR amplification are illustrated in Table 1.

Table 1. The optimum condition of detection

No.	Phase	Tm (^{o}C)	Time	No. of cycle
1-	Initial Denaturation	95°C	3 min.	1 cycle
2-	Denaturation -2	95°C	45sec	
3-	Annealing	52°C	1 min	
4-	Extension-1	72°C	1 min	35 cycle
5-	Extension -2	72°C	7 min.	1 cycle

PCR products visualization

The PCR products were separated by agarose gel electrophoresis as mentioned above, stained with ethidium bromide and and under U.V (302nm) (Intron Korea), and they products were compared with a reference ladder

The KAPA Universal Ladder Kit (cat # KK6302) is designed for determining the approximate size and quantity of the double-stranded DNA on agarose gel. The kit contains eighteen DNA fragments (in base pairs): 100, 150, 200, 300, 400, **500**, 600, 800,**1000**, 1200, **1600**, 2000, 3000, **4000**, 5000, 6000, 8000, and 10000. The KAPA Universal Ladder contains four reference bands (500, 1000, 1600, and 4000) for orientation.

Sequencing and Sequence Alignment:

The item was sent to the national instrumentation place for natural administration (nicem) (http://nicem.snu.ac.kr/principle/?en_skin=index.html) for sequencing 3730XL sequencer, Applied Biosystem), In the mean time, the homology search was led utilizing Essential Nearby Arrangement Search Device (Impact) program which is accessible at the National Focus of Biotechnology Data (NCBI) (http://www.ncbi.nlm.nih.gov and BioEdit program. At last, the outcomes were contrasted and information got from the distributed Quality Bank; which is accessible at the NCBI online(www.ncbi.nlm.nih.gov).

RESULTS

Figure1 showed results for identification Coriandrum sativum, Apium graveolens ,and Petroselinum crispum respectively by using 18S ribosomal RNA gene (ITS)gene [ITS1 primer (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC 3)] Primers set supplied by IDT (Integrated DNA Technologies company, Canada)[10].

Figure 1. Agarose gel electrophoresis for 18S ribosomal RNA gene (650bp). Lane: M (M: 100bp ladder), Lane: 1(Coriandrum sativum), Lane: 2 (Apium graveolens),and Lane: 3 (Petroselinum crispum).

A- Coriandrum sativum

Results of the alignment of product amplification of the 18S ribosomal RNA gene for *Coriandrum sativum* showed one transversion A>C in Location (660 nucleotide) , two transitions A>G and G>A were observed in the locations; 652 and 665 nucleotide. Furthermore, the results suggest that the homology of the mentioned gene was 99% with the standard in the gene bank. The changes were observed in the segment between nucleotides (641 to 668) (figure 2) under sequence ID: KJ726639.1, and have number score (1120) bits.

When the value is near zero, this implies these

arrangements are indistinguishable. Besides, the bit Score is a factual proportion of the ethical closeness where the higher worth shows the serious extent of comparability (a class of <50 focuses). At last, the desire esteem (E) is a gauge of the occasions expected to get a similar comparability circumstantial. The lower the estimation of E the higher the level of similitude between groupings which give more prominent certainty.

Coriandrum sativum cultivar GS4 Multicut 18S ribosomal RNA quality, incomplete arrangement; interior interpreted spacer 1, 5.8S ribosomal RNA quality, and inside translated spacer 2, complete succession; and 28S ribosomal RNA quality, halfway grouping
Sequence ID: KJ726639.1.

Score	Expect	Identities	Gaps	Strand
1120 bits(1241)	0.0	625/628(99%)	0/628(0%)	Plus/Plus

```
Query 601  CTACCCGCTGAATTTAAGCCTATCGTAA  628
           ||||||||||| ||||||| |||| |||
Sbjct 641  CTACCCGCTGAGTTTAAGCATATCATAA  668
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Figure 2. Alignment analysis of the 18S ribosomal RNA gene of *Coriandrum sativum* as compared with the Gene Bank of the NCBI. Query represents a sequence from the sample; Subject (Sbjct) represents the database; obtained from the National Center Biotechnology Information(NCBI).

B- *Apium graveolens*

The sequencing and the BLAST analysis of the 18S ribosomal RNA gene also showed 99 % homology with the standard gene (figure3). There has been one Transition G>A in location (664) as seen along with utilization of the BLAST score . the ratio get 1087,and begin of nucleotide(65 to 669) under sequence ID: MH645762.1.

Score	Expect	Identities	Gaps	Strand
1087 bits(1205)	0.0	604/605(99%)	0/605(0%)	Plus/Plus

```
Query 541  GCGCTACACAATTTGTTCGCCTTAACTGTGACCCCAGGTCAGGCGGGACTACCCGCTGAA  600
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| 
Sbjct 605  GCGCTACACAATTTGTTCGCCTTAACTGTGACCCCAGGTCAGGCGGGACTACCCGCTGAG  664
```

Figure 3. Alignment analysis of the 18S ribosomal RNA gene of *Apium graveolens* as compared with the Gene Bank of NCBI. Query represents a sequence from the sample while Subject (Sbjt) 05).

C Petroselinum crispum

Results of the alignment of product amplification of the 18S ribosomal RNA gene for *Petroselinum crispum* did not show any point of transversion and the ITS gene was 100% homologous to that of the Gene bank.

Petroselinum crispum var. tuberosum DNA no. 31 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Sequence ID: GQ148799.1.

Score	Expect	Identities	Gaps	Strand
1005 bits(1114)	0.0	557/557(100%)	0/557(0%)	Plus/Plus

```
Query 361  AACCACTCATTCCTTGATTGGATGTGCTGGTATTTGGGCGGAAATTGGCCTCCCGTGCCT  420
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 393  AACCACTCATTCCTTGATTGGATGTGCTGGTATTTGGGCGGAAATTGGCCTCCCGTGCCT  452

Query 421  TGCTGCGCGGCTGGTGCAAAAGTGAGTCTCCGACGACGGACGTCGTGACATCGGTGGTTG  480
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 453  TGCTGCGCGGCTGGTGCAAAAGTGAGTCTCCGACGACGGACGTCGTGACATCGGTGGTTG  512

Query 481  TAAAAGACCCTCTTTTCTTGTCGCACGAATCCATGTCATTTTAGTGAGCTCGAGGACCCT  540
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 513  TAAAAGACCCTCTTTTCTTGTCGCACGAATCCATGTCATTTTAGTGAGCTCGAGGACCCT  572

Query 541  TAGGCGCAACAGACTTT  557
           |||||||||||||||||
Sbjct 573  TAGGCGCAACAGACTTT  589
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Figure 4. The alignment analysis of thew 18S ribosomal RNA gene of *Petroselinum crispum* as compared with the Gene Bank of NCBI. Query represents sequences; obtained from the sample; while Subject(Sbjt) represents a database of National Center Biotechnology Information (NCBI).

DISCUSSION

This study is the first study in Iraq that investigated the homology of the genetic sequence with the standard gene bank for three botanical species that belongs to the family of (Apeacea), namely; *Coriandrum sativum, Apium graveolens* ,and *Petroselinum crispum.* Such study highlights the importance of molecular markers for the evaluation of genetic diversity.

The genetic variability in *Corriandrum satuvum* was observed previously in lots of studies, such as; the study that was conducted by [11] which investigated the ubiquity of the single nucleotide polymorphism (SNP) points in the ribosomal ITS gene in 11 different strains of *Coriandrum sativum* in Indian. They were belonging to different agro-climatic zones assembled into same phylogenetic cluster. The study revealed that there had been no consistency in grouping of the plant's varieties indicating that coriander genetic diversity has wide genetic distribution across different agro-climatic zones. On the other hand, [12] observed the genetic variability using the Inter Simple Sequence Repeats (ISSR) technique. The study detected 1% variation in the TSE gene as compared with the data of the Gene Bank. The variation was represented by A>C, A>G and G>A; which were observed in the locations (660, 652 and 665 respectibvely) .

There are very few studies related to the genes sequencing of each of *Apium Graveolens* and *Petroselinum crispum*. It is crucial to pass through the genetic characters of these plants as they are rich with lots of medicinal phytemicals, such as flavonoids, carotenoids, coumarins and volatareile oils[13,14]. Most of the research work about them had concentrated on the physiological and the biochemical characters of the polants rather than on their molecular biology. This urges for performing the genome sequencing[15].

It is well known that studying the Inter Simple Sequence Repeats (ISSR) markers is crucial for the comprehension of the genetic linkage map construction, detection of the genetic diversity and in the molecular breeding. This can be due to their high degree of polymorphism and codominant inheritance [16]. Despite their value, few Inter Simple Sequence Repeats (ISSR) markers have been reported in celery, . In this study, the gentic diversity in the ITS gene; obtained from the mentioned plants was determined using Sanger Sequencing technique.

The study found 1% variation in the ITS gene for for Apium Graveolens (G>A in location (664 nucleotide). Meanwhile, there hasn't been any recorded change in *Petroselinum crispum*. This observation coincides with results of [17] study wherein the Real Time - PCR technique was used to determine the diversity in TUB-B,TUB-A ,and UBC genes.

CONCLUSION

The study suggests that there hasn't been any significant difference in the gene expression between the species of plant because they are of the same genetic linkage map.

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