Molecular Characterization of Selected Genera of the (Apiaceae) Family using SSR Molecular Markers

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Abstract

The conducted study which included the study of a few selected races from canopy plants from the chemical perspective which included *Apium graveolens L*, *Anethum graveolens L, Coriandrum sativum L, Petroselium crispum L, cyminum Cuminum L, Foeniculum vulgare L, Pimpinella anisum L, Daucus carrot L and Ammi majus L.* The DNA was detected and extracted and Conducted molecular tests on the DNA using (SSR Simple Sequence Repeats) using the polymerization device (PCR) and electrophoresis process on Agarose Gel.

Five selected and specific prefixes were used which approved its effectiveness in giving multiple shapes (Polymorphic) among the studied plants of 95%, as packs were obtained from a total number of 14 out of 53 different pack with an arithmetic average of 2.8 for each prefix. Cluster analysis and phylogenetic tree have shown that there is a genetic affinity between 0.84615-0.125 according to the two variables data (1-0) the genetic similarity using cluster analysis (UPGMA)has allowed to draw the portfolio diagram among the studied races, It can be said that the SSR Simple Sequence Repeats can be an effective technique for studying molecular characterization and can be used to separate the genetic relations in the plant world.

Key words: *canopy plants (Apiaceae), multiple shapes, genetic study.*

Introduction

Canopy plants (Apiaceae) is one of the largest plant groups which carries an economical importance which are used as spices like (cumin, fennel, coriander etc…) and vegetables like (celery, carrots, dill and parsley) and medical crop regardless of the taste and flavor that it adds to food, moreover its high oil content , antioxidants, minerals and vitamins that are important to our health, which makes it a potential treasure for neutral materials (1) and it contributes in peoples everyday life in an essential way like celery, parsley, cumin, anise, carrot and dill (2) and several more add to wild species.

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Uses of molecular markers in the evolution and past development of plant science, Initial evolutionary studies were completely dependent on geographical and morphological changes between organisms. Molecular markers are widely used nowadays (3) Molecular studies on phylogeny are largely based on chloroplast genome sequence data due to their simple and stable genetic nature making them ideal markers in evaluating plant strains (4) Assessment of genetic diversity. Recent developments in molecular markers and genome sequencing present a great opportunity to investigate genetic diversity in very large genetic material (5) Assessment of genetic diversity is very useful in studying plant evolution and comparative genomics, which helps to understand the structure of different populations (6) Genetic markers have been successfully applied in identifying genetic diversity and classifying genetic material (7).

Genetic mapping uses methods to locate a gene as well as to determine the distance between two genes.

Genetic mapping is the main area of research in which molecular markers are used today. The principle of genetic mapping is the chromosomal recombination during meiosis, which leads to the separation of genes. The markers located near the gene in question are known. On the same chromosome with linked markers [8].

Materials & Methods

Plant Material:

The vegetative leaves were taken from the plants at the age of 3-4 weeks from the growth phase in the afternoon period when the period of division of the cells is large, especially the healthy young leaves free from pathological, fungal and insect infections, then the leaves were taken to the stage of crushing with liquid nitrogen at a temperature (-169C) A ceramic mortar was used in which the leaves were placed, then liquid nitrogen was poured at a very low temperature in a certain amount, to crush the leaves with the hand in the ceramic mortar to break the walls of plant cells. Then the samples were preserved in freezing by marked boxes for molecular examination and DNA extraction tests(DNA)[9].

Deoxyribonucleic acid extraction (DNA):

DNA extraction was performed from plant samples belonging to the studied Apiaceae family using Protocol(plant)Genomic DNA Mini Kit, The procedures mentioned in the DNA extraction protocol were followed and According to the instructions attached to the company producing extraction, The DNA is isolated from leaves free of pathological infections, and its quality is determined and detected in the acarose gel at a concentration of 1% containing ethidium bromide 0.5 µg / ml, and the TBE x1 solution in the electrophoresis device under voltage (80-90 volts).

Simple Sequence Repeat(SSR Technique):

After isolating the DNA (DNA) from the papers of the genera of the studied paraglider family, the DNA is duplicated using (5) SSR primers Table (2) which shows the nucleotide sequence of the primers used in the study. The polymerase chain reaction (PCR) was performed so that the final size of the reaction is (20µl), the PCR reaction mixture was prepared using the (PerMIX) AccuPower® PCR kit equipped by the Korean company BIONEER, Table (1).

Table (1) reaction mix materials of (PCR)

No:	PCR master mix	(Volume)
1	DNA template	2 µl
2	Forward primer	1.5 µl
3	Revers primer	1.5 µl
4	PCR water(D .W)	15 µl
5	Total	20 µl

Table (2)Primers which used in the study[10].

No	Primer	Sequence	Product size bp
1	Apium F	5- GCT CTG GAA ACG TGA ACT GGA -3	261
	Apium R	5- TGG CCA ATG TCT TTC CGC AT -3	
2	Petrosi F	5- TTG TTT GGA CCC ACC ATT GC -3	289
	Petrosi R	5-CGA ATC ATC TCC CTG CAC CT -3	

***Cont ...* Table (2)Primers which used in the study[10].**

3	Spia F	5- GTT CGA GTA CCA GGC G -3	230
	Spia R	5- CTT CCC TTG ACC TTC CG -3	
4	Cau F	5- CCA CAG CCA CAA CCA TTT C -3	234
	Cau R	5- CCA TCA ACC TCT AAC GCC -3	
5	Cor F	5- GTC GCT TCT TGA CTT CAG -3	252
	Cor R	5- CCA ACC TCA TAA CAC CTC AC -3	

PCR Thermo cycler conditions:

The reaction is performed in a PCR machine according to the following program:

1 - Denaturation: an initial mutant of the DNA strings at a temperature of (95 ° C) for a period of (3) minutes and a number of cycles of (35), then a second mutant at a temperature of 95 ° C for 45 seconds to separate the DNA strings from each other.

2. Annealing: According to the coalescence temperature of each user (58-47) for 45 seconds.

3. Extension: the initial extension at a temperature of 72 degrees C for a period of 1 minute and the final expansion at a temperature of 72 degrees C for a period of (3-5) minutes.

Results and Discussion

Polymorphism resulting from PCR-SSR technique:

When studying the genetic variation of the Apiaceae family genera, the technique of PCR-SSR was mainly relied upon by adopting five prefixes for all nine samples of the research paper used, and depending on those five prefixes, 14 packets or doubling distributed over all samples as they appeared during the electrophoresis process on gel-bands. For the nine samples approved in the thesis, 5 polymorphic prefixes were given, and the polymorphic percentage was 95%, Table (3). The number of packets for each initiator ranged between two packages as the least number of packages in the Petrosi initiator and (4) packages as the highest number with the initiator Spia with an average of 2.8 packages per initiator This study was consistent with what was indicated by [11].

The compatibility efficiency of the primers (primers) was almost similar to some extent. First, both the Petrosi and Cor initiator were less efficient, as their ratio appeared to be 14.28% for both, followed by the Apium and Cau efficiency of 21.42%, then the Spia initiator at 28.57% showed the highest efficiency of All the prefixes in Table (3), and the discriminative ability of each primer was as follows: 15.38% for each of the Petrosi and Cor initiators, while the Apium, Spia and Cau prefixes gave a capacity of 23.07%, which is the highest among the five types of prefixes in Table (3), and from It is clear that the initiator Spia gave the highest percentage of efficiency than the rest of the other prefixes used in this thesis and this study came in line with other results and research [12], regarding the study of the Apiaceae family indicated that high efficiency and differential ability of the prefixes to obtain genetic markers for all species [13], indicating that the distance between them is equal or that the distance between them and the genetic proximity is determined from the embryonic structure by calculating the number of domains obtained and apparent in the electrophoresis process, that the number of shared beams is what determines The genetic proximity or distance between the genotypes, the greater the number of bundles, the less the genetic dimension, and vice versa, because the common bundles indicate a similarity in the genetic material that may show similarities in the phenotypic characteristics related to productivity, reproduction, resistance, diseases, etc. A number of bundles with each other is due to the presence of differences in the sequence of nucleotides in the genome of the genotypes.

Here, the importance of using different primers targeting several regions of the plant genome appears to show the difference that is found between the genotypes, which represents similarities in the phenotypic or anatomical characteristics and similarities in the environment [14], Photo capt software was used to calculate the molecular size to detect the bundles resulting from the PCR reactions and compare them to the size of the DNA Ladder [15]. The SSR results that appeared in the gel were analyzed after converting the results. The characterization was based on numerical data by setting (1) when the beam was present and (0) when it was absent on the acarose gel. The genetic dimension factor between the samples was calculated using a factor of 72 s'Nie [16] according to the following equation:

$$\textbf{Genetic Distance} = 1-\left(\frac{2*N_{xy}}{N_y+N_x}\right)$$

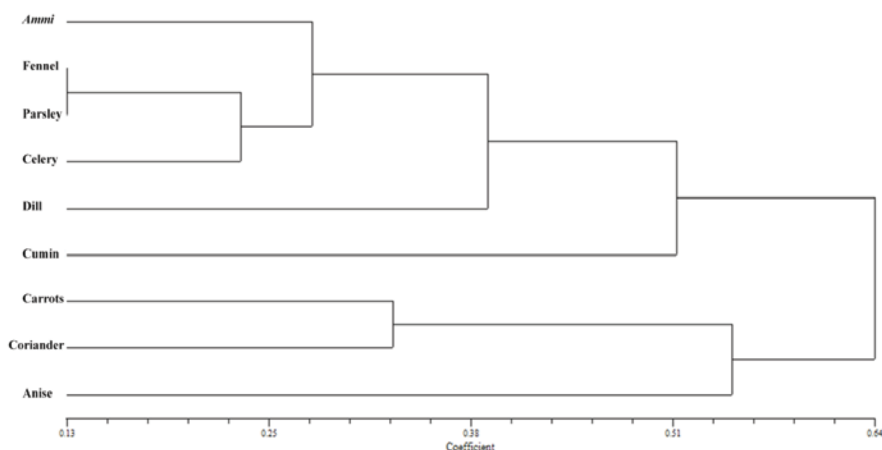
As: G. D. represents the genetic dimension, N_{xy} represents the number of packages shared between the x and y models that represent two samples, N_x represents the total number of packets in the sample x and N_y represents the total number of packets in the sample y.

Table (3) shows the details of the SSR amplification for five primers of the Apiaceae family

Initiator name	**The total number of packages**	**The number of varying packages**	**% For initiator plurality**	**% Of starter efficiency**	**% Of the initiator discriminating power**
Apium	3	3	100	21.42857	23.07692
Petrosi	2	2	100	14.28571	15.38462
Spia	4	3	75	28.57143	23.07692
Cau	3	3	100	21.42857	23.07692
Cor	2	2	100	14.28571	15.38462
The summation	14	13	95		
The Average	2.8	2.6			

UPGMA cluster analysis of Apiaceae family samples resulting from the use of SSR technique:

Cluster analysis allows dividing the studied samples into groups, and the division of studied plant samples into groups of the degree of genetic affinity between them reflects that the factor that collects samples in one group originates from the original habitat of plants or can be traced back to the origin from which they originated and their lineage. Which was obtained with SSR technology and through it, a genetic kinship tree (Dendrogram) can be created that works to determine the degree of genetic kinship and draw the cluster analysis scheme according to the method of UPGMA [17], using the ready-made program NTSYS-pc (Numerical Taxonomy System) to obtain a tree Kinship or genetic dimension (Figure 1).



(Figure 1) UPGMA cluster analysis of the studied samples using SSR technology (Celery, Dill, Coriander, Parsley, Cumin, Fennel, Anise, Carrots, *Ammi*).

Dendritic analysis of the genera of the Apiaceae family based on the results of the PCR-SSR technique

The genetic dimension between the plant samples of the studied Apiaceae genera was estimated by using the Photo capt statistical program, depending on the ratio of genetic affinity between the studied genera, as in Table (4). The results showed that the highest value of a genetic dimension of (0.846) was recorded between the two samples (*coriander* and *Ammi*), and the lowest value of a genetic dimension of (0.125) was recorded between the two samples (parsley and Fennel), that these samples were divided into two clusters, which included the first cluster of samples (*Ammi*, Fennel, parsley, celery, dill, cumin) where it was found. The highest percentage of genetic similarity and affinity between parsley and the Fennel was 87.5%, the percentage of genetic similarity between celery and the *Ammi* was 76.19%, while the percentage of similarity between celery and dill and cumin with dill was 71.42% and 60%, respectively, while The second cluster included the following genera (carrots, coriander, and anise). The degree of genetic affinity between coriander and carrots was the degree of similarity between them: 66.67% and anise with coriander. As for anise and carrots, the percentage of kinship between them was 50% and 40% respectively, which is evident from the above The degree of kinship (each of them converges a genetic distance) was between parsley and the Fennel, and the lowest degree of kinship (each of them diverged from a hereditary distance) between coriander and *Ammi,* as the ratio was (15.38%), as each falls in a different group from the other within the same family The reason is due to the occurrence of genetic mutations or environmental factors, although the method of evaluating the genetic material was different [18].

Table (4) the values of the molecular analysis of the genera of the Apiaceae family showing the genetic affinity

	Ammi	Carrot	anise	Fennel	Cumin	parsley	coriander	Dill	Celery
Ammi	0								
Carrot	0.71429	0							
Anise	0.6	0.6	0						
Fennel	0.36842	0.55556	0.6	0					
Cumin	0.52941	0.42857	0.75	0.5	0				
Parsley	0.2381	0.63636	0.66667	0.125	0.57143	0			
Coriander	0.84615	0.33333	0.5	0.75	0.66667	0.8	0		
Dill	0.52941	0.42857	0.5	0.33333	0.4	0.42857	0.66667	0	
Celery	0.2381	0.63636	0.5	0.25	0.57143	0.22222	0.8	0.28571	0

Conclusion

All the prefixes used gave multiplexed packages in the polymerase chain reaction. The prefixes used in this research proved their effectiveness in giving a morphological polymorphism among the genders of the studied Apiaceae family with a percentage of (95%), which supports the claim of the importance of relying on molecular indicators, especially SSR technology in determining kinship Genetic between plant species.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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