RAPD AND ISSR ANALYSIS OF THE GENETIC RELATIONSHIP AMONG SOME SPECIES IN RUTACEAE IN AND APICEAE IN IRAQ.

H. J. M. Altameme **I. A. Ibraheam**
Assist Prof. **Assist. Prof.**
Department of Biology/ College of Science for Women/ University of Babylon, Hilla, Iraq.
Wsci.huda.j@uobabylon.edu.iq **Wsci.israa.a@uobabylon.edu.iq**

ABSTRACT

Random amplified polymorphic DNA (RAPD) technique was used as a tool for assessing genetic diversity and species relationships among five species from Rutaceae family [Sour orange (*Citrus aurantium* L.); Sweet Orange (*Citrus sinensis* (L.) Osbeck); Mandarin (*Citrus reticulata* Blanco); Pummelo (C*itrus maxima* (Burm.) Merr.) and Grapefruit (*Citrus paradisi* Macf.)] and four species from Apiaceae [Carrots (*Daucus carota* L.; Celery (*Apium graveolens* (Mill.) Pers.); Parsley (*Petroselinum crispum) and* Dill (*Anethum graveolus* L.)]. These plants were collected from a different region at Hilla city in Iraq. A total of 50 polymorphic amplified products from 170 bands were obtained from eight primers (OPC2, OPC8, OPC14, OPB11 ,OPB18 ,BH10 ,BH11 and BH14) in *Citrus* species and the value of Jaccard's coefficient ranged from 0.246 to 0.690. In contrast of 81 polymorphic amplified products from 129 bands in four species in Apiaceae and the value of Jaccard's coefficient ranged from 0.080 to 0.269. Based on the bivariate (1-0) data and genetic similarity with the use of the UPGMA cluster method, the dendrogram separated the studied species. Therefore, it could be concluded that RAPD technique an efficient technique for studying the molecular characterization and used for resolving relationships among plant populations.

Keywords: genetic study, genetic similarity, polymorphism, citrus, *daucus.*

تحليل التضاعف العشوائي المتعدد الاشكال للعلاقات الوراثية بين بعض انواع العائلتين السذابية والخيمية في العراق.

هدى جاسم محمد التميمي اسراء عدنان ابراهيم
استاذ مساعد استاذ مساعد
قسم علوم الحياة/ كلية العلوم للبنات/ جامعة بابل/ الحلة / بابل/ العراق

المستخلص

اعتمدت تقنية تضاعف العشوائي المتعدد الاشكال لسلسلة الدنا كأداة لتقييم التنوع الجيني والعلاقات الوراثية بين خمسة انواع من العائلة السذابية [النارنج (*Citrus aurantium* L.) والبرتقال (*Citrus sinensis* (L.) Osbeck) واللالنكي (*Citrus reticulata* Blanco) والسندي (C*itrus maxima* (Burm.) Merr.) والكريب فروت (*Citrus paradisi* Macf.)] وأربعة أنواع من العائلة الخيمية [الجزر (*Daucus carota* L.) والكرفس (*Apium graveolens* (Mill.) Pers.) والبقدونس (*Petroselinum crispum)* و الشبنت (*Anethum graveolus* L.)]، اذ جمعت هذه النباتات من مناطق مختلفة من مدينة الحلة في العراق. وقد تم الحصول على 50 حزمة متضاعفة عشوائيا متعددة الاشكال من أصل 170 حزمة باستخدام ثمانية بادئات عشوائية (OPC2وOPC8 و OPC14 و OPB11 و OPB18و BH10و BH11 و BH14) في انواع جنس الحمضيات المدروسة وكانت قيمة معامل Jaccard لها تتراوح بين 0.246–0.690 مقارنة مع 81 حزمة متضاعفة عشوائية متعددة الاشكال من أصل 129 حزمة بين الانواع الاربعة في العائلة الخيمية وقيمة معامل Jaccard لها يتراوح بين 0.080–0.269. وحسب بيانات ذات المتغيرين (0–1) والتشابه الوراثي باستعمال طريقة العنقوديةUPGMA امكن رسم المخطط الشجيري للفصل بين الانواع المدروسة. لذا يمكننا القول بان تقنية تضاعف العشوائي المتعدد الاشكال لسلسلة الدنا RAPD يمكن أن تكون تقنية فعالة لدراسة التوصيف الجزيئي وتستخدم لتسوية العلاقات بين المجتمع النباتي

الكلمات المفتاحية: دراسة وراثية، التماثل الوراثي، تعدد الاشكال، الحمضيات، الجزر.

***Received:27/7/2018, Accepted:2/12/2018**

INTRODUCTION

Botanical flora of Iraq included several plant families that studied morphology and some of them chemically in some detail (16). However, there are some problems related to the classification of certain species and species within the tribes and subfamilies. Genetic divergence and convergence between two genotypes using biotechnologies, which provided modern methods of detection and differentiation between genotypes and show the extent of genetic divergence between them. Molecular taxonomy is one of the most important aspects of evolution in the last decade, with the application of DNA or RNA data to help solve most taxonomic problems by diagnosing or inferring the relationship between living organisms. Molecular taxonomists believe that molecular data are more likely than phenotypic data to know the true ethnic origin of organisms because they reflect changes at the gene level and didn't directly affected by environmental changes such as those with phenotypic traits (18). Several molecular technologies have been implemented in the plant classification, such as protein techniques, which include amino acid sequencing techniques and the electrical transfer of Enzyme electrophoresis, as well as DNA-related technologies, including Restriction Fragment Length Polymorphisms (RFLPs), Random Amplified Polymorphic DNA (RAPDs), Amplified Fragment Length Polymorphisms (AFLPs), and the sequencing of the DNA(DNA Sequences) . For this reason, molecular taxonomy has made tremendous achievements through all the data that have become available over the last 50 years in plant classification (6). In this study, RAPD based on polymerase chain reaction (PCR) was adopted because this technique is fast, easy and requires less time (32) to study the molecular variations and relations among five species of the *Citrus* species of the Rutaceae family and four species belonging to the Apiaceae. *Citrus*, which includes mandarin, orange, lemon and grapefruit, has a high economic and nutritional value. It belongs to Aurantioideae, one of the seven subfamilies of the Rutaceae family. There are nine species spread in Iraq from this genus (2). The species of *citrus* also have the status of Asexual reproduction and increase in the frequency of mutation and cross compatibility. This has led to significant appearance and environmental variations between species, which had an impact on the selection of species in many molecular studies (1,5,10, 15, 20,24, 25) and many other international studies compared to Iraq, which did not promote such studies except for a study (3) which deals with 14 species and hybrids spread in the eastern regions of Iraq using the RAPD technique. Apiaceae (Umbelliferae) family includes carrots, celery, coriander, dill and presley, which are among the global families with 300 genera, 3000 species (7), 60 genera and 143 species in Iraq, which are economic and medical importance. As a vegetable and exporter of resin paints as well as used as medical drugs and as a source of perfume and oils (5). The family was divided into 3 subfamilies, Hydrocotyloideae, Saniculoideae and Apioideae, in about 12 tribes. However, the relations between phylogeny and evolution among the major and most complex species of Apioideae remain unclear despite molecular studies such as (8, 9, 11, 12, 23, 29) . Due to the lack of molecular studies in Iraq for the different species under study. This study aims to study genetic diversity and determine the genetic relationship between species based on the degree of genetic similarity between them and determine the DNA of each species under study using RAPD technology.

MATERIALS AND METHODS

Plant sampling:

This study was carried out in the Biotechnology Laboratory of the College of Science for Women -University of Babylon during the period January-June 2016. Plant specimens were collected from different regions of Babil province. These were five species belonging to the *Citrus* in Rutaceae family, and four different species belonging to the Apiaceae family (Table 1). These species were identified according to Flora of Iraqi (30).

DNA extraction:

Fresh leaves were selected for each type and then cut into very small pieces. It was placed in a ceramic vase and samples were crushed with the addition of liquid nitrogen continuously until it became very fine powder.

(0.05 g) per sample and followed the DNA extraction and purification method attached to the extraction kit used (Promega, USA) Genomic ® Wizard.

Interaction of random multiplication of DNA fragments using PCR-RAPD technology

The amplification reaction method was adopted according to (32). Eight random primers were chosen: OPC2, OPC8, OPC14, OPB11, OPB18, BH10, BH11 and BH14, The polymerase chain reaction was performed in a volume of 25 microliter per sample consisting of 50 ng of DNA and 250 µm of each of the four nucleotides (dTTP / dATP / dCTP / dGTP) and 10 bicomol from each one of the Taq DNA polymerase polymerase, The thermal cycler PCR System (Verity, Applied Biosystem) was amplified according to the following programs and by type of prefix (Table 2). The DNA amplification products obtained from the use of the above prefixes for the species under study were carried out in the place assigned to the 1.3% agarose gel, DNA Ladder was carried and the samples were carried under 75 volt for 3-4 hours. With a special camera in the Gel documentation system.

Statistical analysis

Statistical analysis of the degree of genetic similarity between the samples studied by reading the DNA bands by binary characters where the appearance of a band was given the number (1) while the absence of the band was given the number (0) ,Then analyzed the results using the statistical program past software ver. 1.92. The phylogenic tree was plotted between the studied samples of RAPD markers according to the Jaccard coefficient of genotype similarity (UPGMA) in the unweighted pair-group method using an arithmetic average (14). The following indicators were measured:

Polymorphism% = (No. the polymorphic bands of random primer / the total number of bands of the same primer) × 100.

Efficiency of primer =(No. the polymorphic bands to each primer / total number of bands to the same primer) × 100

Discriminating power of primer = No. the polymorphic band to each primer /total number of the polymorphic band to all primer X 100 %. by (13).

RESULTS AND DISCUSSION

Previous studies have shown that molecular marker techniques can overcome many of the limitations of morphological and biochemical techniques and can detect DNA-level variations (31). Although there are many plant encyclopedias, many of which have been described morphologically and chemically characters, some species are still ambiguous, so random samples from two different plant families have been selected in an attempt to find genetic affinity and divergence between them by using eight random primers,

Table 1. Species under study with common names, families, subfamilies, tribes and subtribes

No.	Scientific name	Common name	Family	subfamily	tribe	Subtribe
1	*Citrus aurantium* L.	Sour orange	Rutaceae	*Aurantioideae*	*Citreae*	*Citrinae*
2	*Citrus sinensis* (L.) Osbeck	Sweet Orange				
3	*Citrus reticulata* Blanco	Mandarin orange				
4	*Citrus maxima* (Burm.) Merr.	Pummelo				
5	*Citrus paradisi* Macf	Grapefruit				
6	*Daucus carota* L.	Carrots	Apiaceae	*Apioideae*	*Scandiceae*	*Daucinae*
7	*Apium graveolens* (Mill.) Pers.	Celery			*Apieae*	-
8	*Petroselinum crispum*	Parsley				
9	*Anethum graveolus* L.	Dill				

Table 2. Special program for the randomization of PCR-RAPD in thermal circulation according to the type of primers

Name of Primer	Initial denaturation		Denaturation		Annealing		Extension		Final extension	
	Temp C°	Time (s)	Temp C°	Time (s)	Temp C°	Time (s)	Temp C°	Time (s)	Temp C°	Time (s)
OPC2	1				44				1	
OPC8	94	60	94	30	4	60	72	120	72	300
OPC14	1				40				1	
	94	240	94	60	36	60	72	120	72	120
OPB11	1				45				1	
OPB18	95	300	94	60	36	60	72	120	72	240
BH10	1				40				1	
BH11	94	240	94	60	40	60	72	120	72	300
BH14										

The random multiplication results showed the distribution of 170 polymorphic amplified products spread in five species of *Citrus* (sour orange, sweet orange, mandarin orange, pummelo and grapefruit). The highest numerical value was 36 bands at the OPC8, while the lowest number of bands at the OPB11 was only 6. The total number of polymorphism fragments for all selected primers was about 50 at the percent 42%. In Table 3, it is clear that the number of bands produced in each random primers is different. In primer OPC2, the total number of bands was 22, only 9 bands were Polymorphism (40.91%), and OPC8 has 36 bands, only 14 are polymorphic (38.89%) which is higher than the rest of the primers used. The primer OPC14 has 80% of the heterogeneous bands of 8 out of 10 bands. While there were 6 and 20 bands in the OPB11 and OPB 18 with a percentage of about 66.67% and 50% for the polymorphic bands respectively. Also the results showed that the primers BH10, BH11 and BH14 had a total number of bands of 27, 25 and 24 fragments and percentages of heterogeneous bands 14.82, 28 and 16.67%, respectively.

Table 3. Details of RAPD and ISSR amplifications between the studied *Citrus* species of the Rutaceae family.

Primer	Primer sequences 5' to 3'	No. of total amplified bands	No. of polymorphic bands	No. of Monomorphic bands	Polymorphism %	Primer efficiency (%)	Primer discriminatory power (%)
OPC2	GTGAGGCGTC	22	9	0	40.91	12.94	5.29
OPC8	TGGACCGGTG	36	14	1	38.89	21.18	8.24
OPC14	TGCGTGCTTG	10	8	0	80.00	5.89	4.71
OPB11	GTAGACCCGT	6	4	0	66.67	3.53	2.35
OPB18	CCACAGCAGT	20	10	0	50.00	11.77	5.88
BH10	GAGAGAGAGAGACC	27	4	3	14.82	15.88	2.35
BH11	GTGTGTGTGTGTCC	25	7	2	28.00	14.71	4.12
BH14	CTCCTCCTCGC	24	4	3	16.67	14.12	2.35
	Summation	170	50	9	-		
	Average	21.25	7.5	1.13	42		

As for the random polymerization products of the species belonging to the Apiaceae family (carrots, celery, parsley and dill), it was less than the previous, 129 bands distributed to the four species. Depending on the type of primers used, there were 81 fragments at 64.68% of polymorphism. The lowest number of bands was at the OPC14 of 12 fragments and the highest number of bands appeared on the OPC2 primer with about 21 bands. The percentage of polymorphism was limited between 38.1-83.82% in the OPC2 and OPC14 respectively as lower and upper limits, while the rest of the ratios of the other primers overlapped between these values. As for monomorphic bands, they did not appear in all types of primers except for the primer OPC2, in which only one band was observed in the four species. The efficiency of the primers used was fairly similar. The start of the OPC14 was less efficient at 9.30%, followed by the efficiency of the primers BH10 and OPB11 by 10.08 and 10.85% and 18OPB with the efficiency of 11.36% and continuing to reach the highest efficiency of the OPC2 primer of 16.28%. The results showed that the

discriminatory power of each primer was the same in OPC2, BH10 and BH14, approximately at 9.88%, and increased to 12.35% in OPC8, OPC14, 13.58 and 14.82% in OPB11 and 18 OPB respectively, reaching a high of 17.28% The primer BH11. (Table 4) (Figure 1).

Table 4. Details of RAPD and ISSR amplifications between four species of the Apiaceae family.

Primer	Primer sequences 5' to 3'	No. of total amplified bands	No. of polymorphic bands	No. of Monomorphic bands	Polymorphism %	Primer efficiency (%)	Primer discriminatory power (%)
OPC2	GTGAGGCGTC	21	8	1	38.1	16.28	9.88
OPC8	TGGACCGGTG	17	10	0	58.82	13.18	12.35
OPC14	TGCGTGCTTG	12	10	0	83.33	9.30	12.35
OPB11	GTAGACCCGT	14	11	0	78.57	10.85	13.58
OPB18	CCACAGCAGT	15	12	0	80	11.63	14.82
BH10	GAGAGAGAGAGACC	13	8	0	61.54	10.08	9.88
BH11	GTGTGTGTGTGTCC	20	14	0	70	15.20	17.28
BH14	CTCCTCCTCGC	17	8	0	47.06	13.18	9.88
	Summation	129	81	1	-		
	Average	16.13	10.13	0.13	64.68		

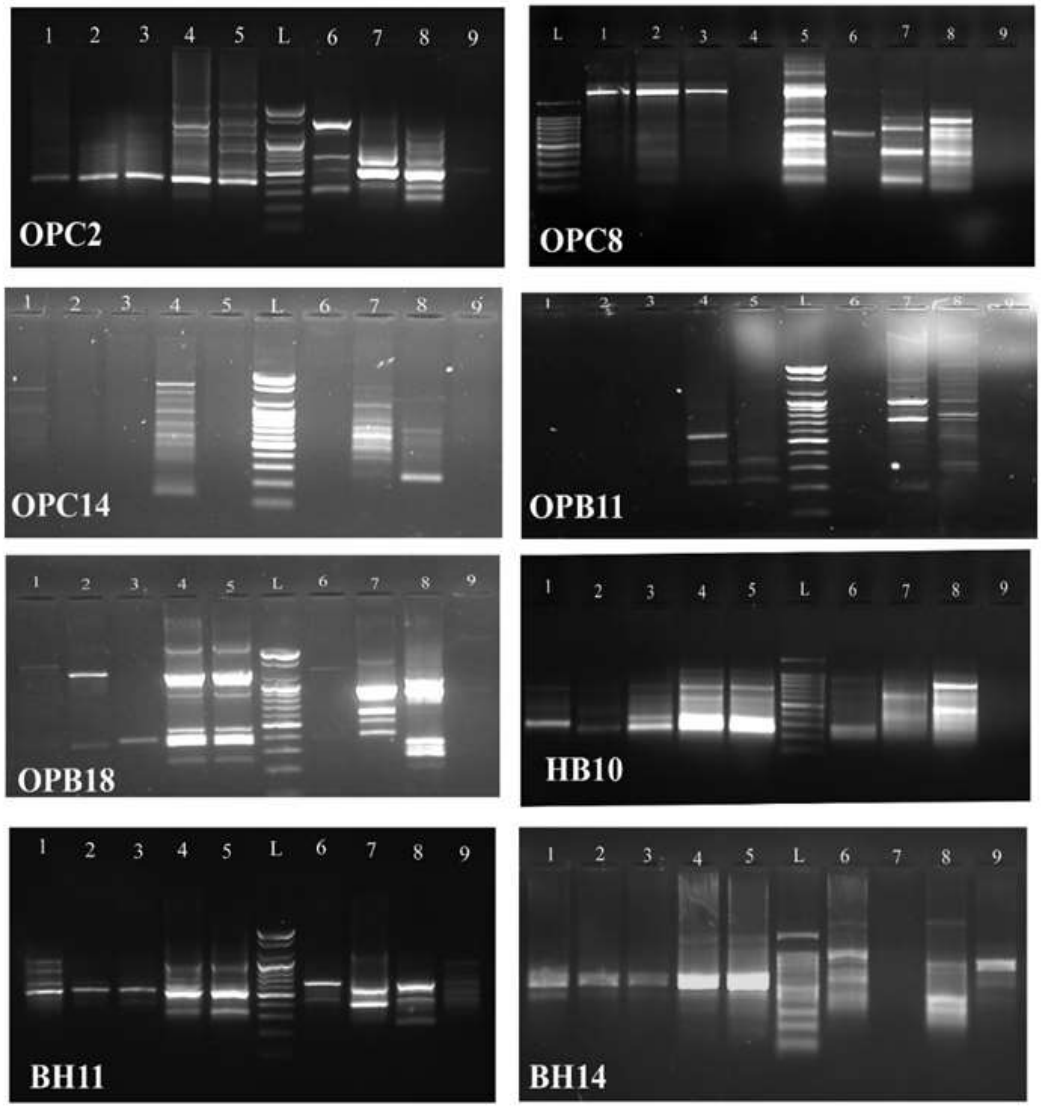

Figure 1. Banding patterns of RAPD and ISSR fragments of Rutaceae and Apiaceae individuals. Lane L molecular size marker one step 100 bp ladder (Promega). 1: *Citrus aurantium* 2*: Citrus sinensis, 3: Citrus reticulata, 4: Citrus maxima,* 5*: Citrus paradise, 6: Daucus carota,* 7*: Apium graveolens,* 8*: Petroselinum crispum,* 9*: Anethum graveolus*

It is clear that the primer OPC14 has the highest percentage of polymorphism in both the Rutaceae and Apiaceae families, as well as the absence of monomorphic bands between the two families, indicating that each family has a genetic imprint that differs from the other family. This study agrees with the results of others researches (21) which pointed that the high efficiency and discriminatory power of primers are important in obtaining fingerprints for each taxon. It is worth mentioning that the distance or proximity to the genetic structure is determined by the number of joint bands. The more the number of band leads to a less genetic dimension, and the smaller the number of bands, leads to the greater the distance between the genotypes. The common band indicates a similarity in the genetic material in that region of the studied genome, which may represent similarities in phenotypic or anatomical characteristics or similarities in the environment (4). Dendrogram diagram was derived from the results obtained from the PCR-RAPD, indicated by the convergence and divergence of genotypes between species. Table 5 and Figure 2 shows that the *Citrus* species had the highest similarity between *Citrus aurantium* and *Citrus reticulata* at 0.690, While the lowest value of the similarity is 0.246 between the two types *Citrus sinensis* and *Citrus maxima*, so Figure 2 shows that the dendritic cluster was in the first two groups due to the correlation of the two types of *Citrus aurantium* and *Citrus reticulata* and are associated with *Citrus sinensis*, the second group is composed of the two types *Citrus maxima* and *Citrus paradise*. This result is consistent with the study of other research (3) except for grapefruit with orange and pummelo. The reason may be due to the different primers used and therefore may have an effect on the location of one species within the group. On the other hand, Scora (28) mention there are three real and essential species of *Citrus* species in Subspecies: *Citrus*: *Citrus* (*Citrus medica*), mandarin (*Citrus reticulata*) and pummelo (*Citrus maxima*). The rest of the cultured species are only hybrids of species from the three main mentioned. While a recent molecular study using the ITS and AFLP method has confirmed that pummelo and mandarin are of maternal and paternal origin respectively from sweet orange and Sour orange and grapefruit is a hybrid of pummelo and sweet orange, which represented both mother and male parents, respectively (19). Also some other research (22) noted that the genetic distance between species or different species has increased during the evolutionary diversity, while the distance between species within the Intera-species have increased because of geographical isolation, The geographical location may also be attributed to the reason for the existence of variations between taxa and hybrids in one species. This is confirmed by a study of Hussein (17) after calculating the percentage of the genetic dimension among the varieties of roses, when indicating that this genetic difference is due to different geographical locations and to the processes of education and improvement carried out on its. It was also shows in Table 6 and Figure 3 that the highest value of the genotype was 0.269 for *Daucus carota* and *Anethum graveolus* and the lowest value was observed for *Apium gravelolens* and *Anethum graveolus*. This genetic similarity showed the association of the two species, *Daucus carota* and *Anethum graveolus*, as the first group, the two types of *Apium graveolens* and *Petroselinum crispum*, as a second group in the dendritic cluster of the Apiaceae family. This study agreed with the results of the previous genetic studies, although the method of evaluation of the genetic material was different, (27) using the AFLP method of the carrot plant (29) of *Anethum graveolus* in a similar manner to the previous study, On the other hand, some researchers (26) showed that the spatial change of parsley species had a significant effect on the number of random multiplication bands using RAPD method, in contrast to the study of celery (32) when it was indicated as a two chromosomal plant (2N) Polymorphism between its varieties and its characteristics. This is an important feature to know the genetic map of the plant for the purposes of gene cloning and Breeding. The results show that the markers can estimate the magnitude of variations at the molecular level through all studied genetic sites. This reflects the reality of these markers and their comprehensiveness for all areas of the

organism's genetic material. By using eight random primers in two different families and giving clear variations in the number of identical and heterogeneous bands gave evidence that RAPD-PCR is a successful method of detecting genetic variations between species as well as not requiring prior genetic information about the organism

Table 5. Similarity Matrix computed with the Jaccard coefficient

Species	*C.aurantium*	*C.sinensis*	*C.reticulata*	*C.maxima*	*C.paradisi*
C.aurantium	**1**	**0.594**	**0.690**	**0.295**	**0.328**
C.sinensis		**1**	**0.643**	**0.246**	**0.345**
C.reticulata			**1**	**0.255**	**0.309**
C.maxima				**1**	**0.540**
C.paradisi					**1**

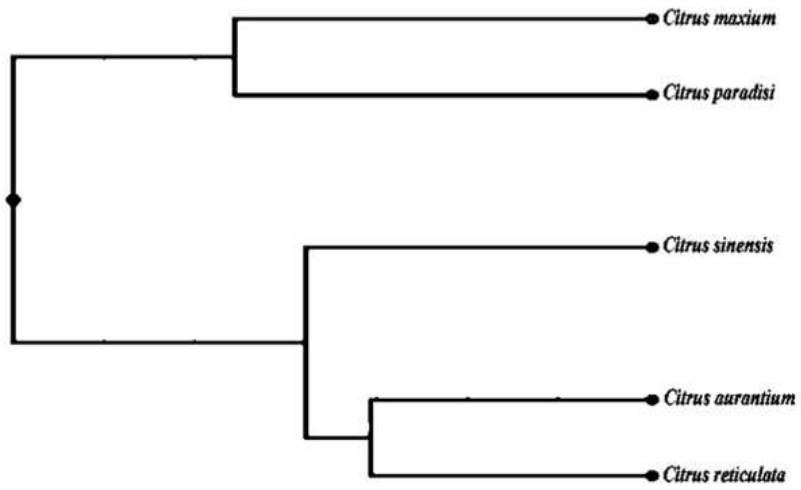

Figure 2. UPGMA dendrogram indicating the genetic relationships among *citrus* species based on RAPD and ISSR markers

Table 6. Similarity Matrix computed with the Jaccard coefficient

Species	*Daucus carota*	*Apium graveolens*	*Petroselinum crispum*	*Anethum graveolus*
Daucus carota	**1**	**0.163**	**0.213**	**0.269**
Apium graveolens		**1**	**0.267**	**0.080**
Petroselinum crispum			**1**	**0.109**
Anethum graveolus				**1**

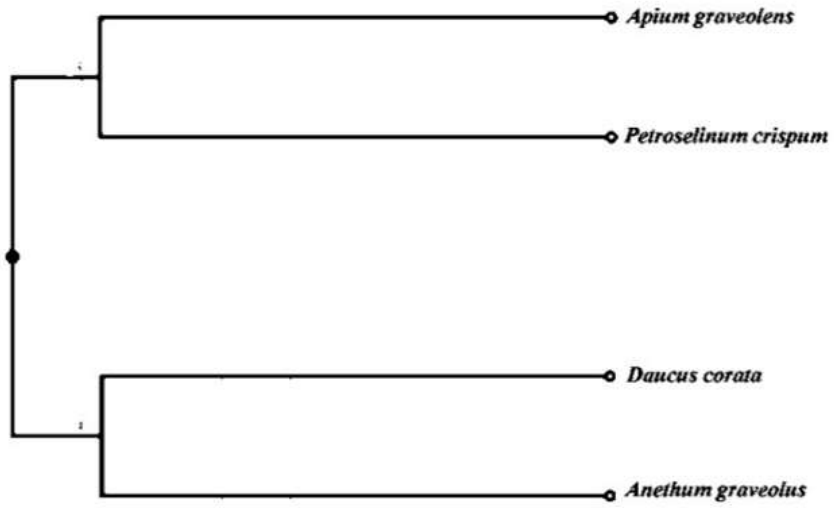

Figure 3. UPGMA dendrogram indicating the genetic relationships among species in Apiaceae based on RAPD and ISSR markers

REFERENCES

1. Akhter, S.; M.J. Ferdous; M.R. Hossain and G. Rabbani . 2009. Molecular characterization of Jamir (*Citrus jambhiri*) accessions of Bangladesh through PCR based RAPD markers. J. Agrofor. Environ. 3 (1): 21-24.
2. AL- Musawi, A. H. 1987 . Plant Taxonomy, Univ. of Baghdad, pp. 379.
3. Al-Anbari, A.K.; N. Kanawapee; T.A. Al-Kazragi; H. Al-Jewari; A. Al-Mashhadani; S. Barusrux; P. Pornponugrungrueng and P. Theerakulpisut 2014. Genetic diversity of *Citrus* (Rutaceae) in Iraq based on Random Amplified Polymorphic DNA (RAPD) markers. African Journal of agricultural research. 9(11):1012-1019
4. Al-qaisy, E. Kh. Kh; A.A. Al–assy, and M.Y. Al-fahady. 2014. Studying genetic distance for some inbreed line of *Zea mays* by using genetic marker (RAPD). Journal of Tikrit University For Agriculture Sciences, Special Issue of the Third Specialized Conference / Plant Production in 26-27 / 3/2014. pp: 303-315
5. Cabrita, L.; P. Elisiario; J. Leitao and A. Guerrerio. 2001. Assessment of the genetic relationships among *Citrus* species and varieties by isozyme and RAPD markers. Acta Hort., 546: 177-181
6. Crawford, D. J. 2000. Plant macromolecular Systematics in the past 50 years: one view. Taxon, 49: 479-501
7. Cronquist, A. 1981. An Integrated System of Classification of Flowering Plants. Columbia University Press, New York, USA
8. Downie, S. R., and D. S. Katz-Downie. 1996. Amolecular phylogeny of Apiaceae subfamily Apioideae: Evidence from nuclear ribosomal DNA internal transcribed spacer sequences. Am. J. Bot. 83: 234–251
9. Downie, S. R.; D. S. Katz-Downie and K. Cho. 1996. Phylogenetic analysis of Apiaceae subfamily Apioideae using nucleotide sequences from the chloroplast *rpo*C1 intron. Molecular phylogenetic and evolution 6(1):1-18
10. Federici, C.T.; D.Q. Fang; R.W. Scora and M.L. Roose. 1998. Phylogenetic relationships within the genus *Citrus* (Rutaceae) and related genera as revealed by RFLP and RAPD analysis. Theor. Appl. Genet., 94: 812-822
11. Fu, C.; Y.Qiu and H. Kong 2003. RAPD analysis for genetic diversity in *Changium smyrnioides* (Apiaceae), an endangered plant. Bot.Bull. Acad.Sin. 44:13-18
12. Gaudeul, M.; I. Till-Bottraud; F. Barjon and S. Manel. 2004. Genetic diversity and differentiation in *Eryngium alpinum* L. (Apiaceae): comparison of AFLP and microsatellite markers. Heredity, 92, 508–518
13. Grudman.H.; C. Schneider; D. Hartung; F. Daschner and T. Pith. 1995. Discriminatory power of three DNA typing techniques for *P.aeruginosa*. Clin.Microbiol.,3:528-532
14. Hammer, Ø.; D. A. T. Harper and P .D. Ryan 2001. PAST: palaeontological statistics software package for education and data analysis. Paleontologia Eletronica. 4(1):1-9.
15. Hamza, E. M. 2013. Genetic diversity of some *Citrus* varieties based on microsatellite and RAPD molecular markers in Egypt. World Journal of Agricultural Sciences 9 (4): 316-324
16. Harborne, J. B.; D. Boulter, and B.L. Turner. 1971. Chemotaxonomy of the Leguminosae. Academic Press, London
17. Hussein, J. K. 2011. The genetic distance of *Rosa* spp. Using RAPD. The Iraqi Journal of Agricultural Sciences 42 (2): 71-79
18. Judd, W.S.; C.S. Campbell; E.A. Kellogg and P.F. Stevens. 1999. Plant Systematics. A phylogenetic Approach. Sinauer Associates, USA
19. Li, X. and R. Xie. 2010. The origin of cultivated *Citrus* as inferred from internal transcribed spacer and chloroplast DNA sequences and amplified fragment length polymorphism fingerprints. J. Am. Sco. Hortic. Sci. 135(4):341-350
20. Luro, F.; F. Laigret and J.M. Bové. 1992. Application of Random Amplified Polymorphic DNA (RAPD) to *Citrus* Genetic and Taxonomy, 1: 225-228. In: International Citrus Congress, 7, Italy, 1992; Proceedings Italy: International Society of in Citriculture
21. Mahenthiralingam, E.; M. E. Campell; J. Foster; J.S. Lam and D.P. Speert. 1996. Random amplified polymorphic DNA typing of *Pseudomonas aeruginosa* isolates recovered from patients with cystic fibrosis. Journal of Microbiology 9: 1129-1135
22. Mei Z.; L. Yang; M.A. Khan; M. Yang; C. Wei; W. Yang; C. Wei; W. Yang; X. Peng; M.

Tania; H. Zhang; X. Li and J. Fu . 2014. Genotyping of *Ganoderma* species by improved random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) analysis. Biochem Syst Ecol.56:40-48

23. Mei, Z.; C. Zhang; M.A. Khan; Y. Zhub; M. Tania; P. Luo and J. Fu. 2015. Efficiency of improved RAPD and ISSR markers in assessing genetic diversity and relationships in *Angelica sinensis* (Oliv.) Diels varieties of China. Electronic Journal of Biotechnology 18: 96–102

24. Munankarmi, N.N.; R.L. Shrestha; N. Rana; J.K.C. Shrestha; S. Shrestha; R. Koirala and S. Shrestha. 2014. Genetic diversity assessment of acid lime (*Citrus aurantifolia* Swingle) landraces of eastern Nepal using RAPD markers. Int. J. Appl. Sci. Biotechnol., 2(3): 315-327

25. Penjor, T.; M. Yamamoto; M. Uehara; M. Ide; N. Matsumoto; R.Matsumoto and Y. Nagano. 2013. Phylogenetic relationships of *Citrus* and its relatives based on matK gene sequences. PLoS ONE 8(4): e62574. doi: 10.1371Journal.pone.0062574

26. Said-Al Ahl, H. A. H.; M. Abou-Ellail and A. O. Elsayed. 2016. Harvest date and genotype influences growth characters and essential oil production and composition of *Petroselinum crispum* plants. J. Chem. Pharm. Res., 8(5):992-1003

27. Santos, C.A.F. and P.W. Simon. 2002. Some AFLP amplicons are highly conserved DNA sequences mapping to some linkage groups in tow F2 populations of carrot. Genet. Mol. Biol. 25(2): 195-201

28. Scora, R.W. 1975. On the history and origin of *Citrus*. Bulletin of the Torrey Botanical Club. 102(6): 369-375

29. Solouki, M.; S. B. Hoseini; B. A. Siahsar and A. Tavassoli .2012. Genetic diversity in dill (*Anethum graveolens* L.) populations on the basis of morphological traits and molecular markers. African Journal of Biotechnology. 11(15): 3649-3655

30. Townsend, C.C. and E. Guest. 1980 "Flora of Iraq. Vol. four, part one, Cornaceae to Rubiaceae". Ministry of Agriculture and Agrarian Reform, Republic of Iraq, pp:627

31. Verma, S.; S . Singh; S. Sharma,; S.K. Tewari; R.K. Roy; A.K. Goel and T.S. Rana. 2015. Assessment of genetic diversity in indigenous turmeric (*Curcuma longa*) germplasm from India using molecular markers. Physiol Mol Biol Plants. 21(2):233-42

32. Williams, J.G.K.; A.R. Kubelik; K.J. Livak; J.A. Rafalski; and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18: 6531-6536

33. Yang X. and C. F. Quiros. 1995. Characterizing the celery genome with DNA-based genetic markers. J.Am.Sco. Hortic.Sci. 120(5):747-751.