USE OF RAPD IN DETERMINING THE GENETIC RELATIONSHIP BETWEEN SOME SPECIES OF FAMILY PLANTS CUCURBITACEAE GROWING IN IRAQ

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ABSTRACT

This research was done to conducted to determine TEN taxa of the Cucurbitaceae family were studied using the RAPD approach to examine taxonomic relationships and genetic diversity, including *Cucurbita pepo, Cucurbita maxima, Laganaria siceramia* L.C.V. local, *Laganaria siceramia* L.C.V. Syria, *Cucumis sativus, Cucumis melo, Cucumis melo flexuosus, Citrullus lanatus, Citrullus colocynthis, Luffa cylidrica*, DNA of fresh leaves was extracted using modified protocol of Kit due, invested DNA Marker based PCR technology using the RAPD Marker using Thirteen RAPD primers of (Operon) technology biologging to (Bio-RP1, Bio-RP2, Bio-RP3, Bio-RP4, Bio-RP5, Bio-RP6, Bio-RP7, Bio-RP8, Bio-RP9, Bio-RP10, UBC-13, OPC-12) sets. , where explained the relationship between taxa on the basis of the appearance and disappearance of the bands and showed that differences in the location on the genomes of the studied cultivars on the agarose gel .As the primers generated (92) total bands resulting from (225) binding sites ,including (92) polymorphic binding sites distinct for the cultivars under study . To determine the interspecific among the investigated taxa, cluster analysis of the diversity matrices was carried out.

Key words: molecular taxonomy, markers, genetic diversity, *Cucurbita, Cucumis, Citrullus, Luffa*.

استعمال تقنية التضخيم العشوائي متعدد الأشكال (RAPD) في تحديد العلاقة الجينية بين بعض أنواع نباتات عائلة القرعيات النامية في العراق

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المستخلص

تم إجراء هذا البحث لتحديد العلاقات التصنيفية والتنوع الوراثي لعشرة نباتات من نباتات العائلة القرعية Cucurbitaceae ، تمت الدراسة باستخدام مؤشرات التضخيم العشوائي Random Amplified DNA polymorphic (RAPD) حيث تم عزل الحامض النووي DNA وتضخيمه باستخدام تفاعل البلمرة المتسلسل (PCR). تشتمل الاصناف المدروسة على النباتات التالية: *Cucurbita pepo, Cucurbita maxima, Laganaria siceramia* L.C.V. local, *Laganaria siceramia* L.C.V. Syria, *Cucumis sativus, Cucumis melo, Cucumis melo flexuosus, Citrullus lanatus, Citrullus colocynthis, Luffa cylidrica*, ، وذلك باستخدام ثلاثة عشر بادئا عشوائيا وهي (Bio-RP1، Bio-RP2، Bio-RP3، Bio-RP2، Bio-RP3، Bio-RP5 -Bio-RP2 و Bio-RP6 و Bio-RP7 و Bio-RP8 و Bio-RP9 و Bio-RP10 و UBC-13 و OPC-12). ، حيث وضحت العلاقة بين الأصناف على أساس ظهور واختفاء الحزم على هلام الاكاروز التي تعكس الاختلافات في مواقع الارتباط على جينومات الأصناف المدروسة، ويتضح بان العدد الكلي للحزم (92) حزمة التي انتجت (225) موقعا، وكانت (92) موقعا متباينا مميزا للاصناف المزروعة قيد الدراسة. لتحديدالبعدالوراثي بين الاصناف، تم إجراء تحليل عنقودي لمصفوفات التنوع ، واظهرت النتائج التي تعتمد على قيم البعد الجيني وجود تباين وراثي بين الأصناف وقد تبين انها تنقسم إلى مجموعتين رئيسيتين تتكون المجموعة الأولى من *Cucumis sativus*، *Cucumis melo* and *Cucurbita pepo*، بينما تضمنت المجموعة الثانية على مجموعتين، والتي تم تقسيمها إلى مجموعتين فرعيتين، تحتوي على *Luffa cylidrica* and *Cucumis melo flexuosus* and *Citrullus lanatus*، اما المجموعة الفرعية الثانية التي تتضمن*Laganaria siceramia* L.C.V. local and Syria.

الكلمات المفتاحية: التصنيف الجزيئي، مؤشرات التضخيم، التنوع الجيني، *Cucurbita* ، *Cucumis*، *Citrullus* ، *Luffa*.

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INTRODUCTION

The Cucurbitaceae family is the second largest fruit, vegetable and the majority of which are herbaceous annual or perennial lianas, are the most significant food plants in the world, it one of the most economically important families of plants, containing about 115 genera and 960 species, there are contains a variety of vegetables or fruit crops, which are of great significance to the global or local economy. (15,25,30,39). Among them, luo-han-guo and bitter gourd have both employed as fruits and vegetables most of them have considerable economic value and the source of medicine (16,19). Plant taxonomy is a field of botany that focuses on identifying, characterizing, classifying, and naming plants mainly on the basis of their morphological traits. Comparative analysis of observable characteristics, including reproductive organs, stem anatomy, flower structure, and leaf shape, is used to classify plants into hierarchical groups that represent their evolutionary relationships.(19,30), and the use of molecular data has been era- splitting for taxonomy and may allow an accelerated pace of species discovery, that expansion of study transcription factors, enhancers, promoters, and translation of genetic code is still in a wide argument of different views in many living organisms for their poorly characterized at the molecular level (5, 11, 12). The foundational knowledge for comprehending taxonomy and domestication is provided by molecular data and evaluation of plants (4, 5, 7, 9). Understanding biology and genetics at molecular level has become very important for dissection and manipulation of genome architecture for addressing evolutionary and taxonomic questions (10, 21, 23, 26). Many molecular technologies of plants also rely on revealing variations at randomly picked anonymous sequences in genomes of plants. The aim is to give an accurate as possible view of the genome diversity, this is the case for amplified fragment length polymorphism (AFLP), which use primers with arbitrary sequence to amplify genomic regions, some multi-locus profiling techniques use a combination of AFLP associated with the revelation of either SSR loci (selective amplification of microsatellite polymorphic loci, SAMPL) (13, 14, 20, 22, 29). Among many DNA markers available, Random Amplified Polymorphic DNA (RAPD) is the simplest and cost – effective and can be performed in laboratory from most of its applications, in addition RAPDs can touch much of the genome and has the advantage that no prior knowledge of the genome under research is necessary. The RAPD technique is one of the important indicators in studying genetic variation between species and cultivars, determining their genetic identity, and drawing genetic maps, in the field of taxonomy of plants, RAPD is widely used in determining the genome of plants (2, 27, 31, 35). The use of RAPD markers to identify genetic variations is preferred over conventional morphology and biochemical markers sine these are completely devoid of any interference from environment effect and growth stages of experimental material thus marking them highly reliable (37) .The aim of this study is to identify the ten cultivars of Cucurbitaceae plants by distinguishing between them and clarifying their genetic relationships using RAPD analysis as a basic requirement for further crop improvement. It also compares the natural variation present in a collection of Iraqi landrace plants with other commercial cultivars and hybrids to determine how effective RAPD is at identifying Cucurbitaceae plants.

MATERIAIS AND METHODS

Plant material: The plants were cultivars in a field belonging to the University of Mosul, Collage of Agriculture and Forestry of Iraq in the middle of February 2021, of the plant samples. Healthy leaves of Cucurbitaceae plant which *Cucurbita pepo, Cucurbita maxima, Laganaria siceramia* L.c.v. local, *Laganaria siceramia* L.c.v. Syria, *Cucumis sativus, Cucumis melo, Cucumis melo flexuosus, Citrullus lanatus, Citrullus colocynthis, Luffa cylidrica.* Healthy leaves of the fresh leaf growths, free from path and insect infestations, were takes for each studied taxa plants.

Extraction of Genome DNA: complete genomic DNA was taken from leaf samples that were gathered independently from ten taxa of Cucurbitaceae (experiments were carried out in the laboratories of the Department of

Biology Collage of Science, University of Mosul). Plants tissue were turned into affine powder using liquid nitrogen, and bulked DNA extraction by using plant Genomic DNA Extraction Genomic DNA Min Kit (plant) by Gene aid, Korea in the Labe of DNA College of Science, Biology Department, Mosul University, Iraq. DNA quality was estimated by measured using the device Nano-drop Spectrophotometer (Bio-Nanodrop, England) in the wavelength (260) nm, which are (1.7-1.8) pure DNA and more than 2 are contaminated with another DNA (6).

Polymerase chain reaction

Thirteen random primers for RAPD markers were tested in the polymerase chine reaction for Cucurbitaceae plants cultivar. PCR amplification was performed using thirteen random deciders arbitrary (Bio-RP1, Bio-RP2, Bio-RP3, Bio-RP4, Bio-RP5, Bio-RP6, Bio-RP7, Bio-RP8, Bio-RP9, Bio-RP10, UBC-13, OPC-12) sets from the Bioneers Corporation (Korea) **(**23**)**, the primers were diluted according to the instructions of the supplied company by adding 340 ml D.D. Water to each primer from a final concentration of 100 pmol/ µl. DNA extraction was done by using the Min Kit (plant). Table 1 lists the primer sequences. The polymerase chain reaction was obtained from BiONNR (Accu Power PCR Premix) Korea, in a ultimate volume for the DNA amplifications was 20 µl tubes (containing: Top DNA polymerase 1U, dNTP(dATP, dCTP, dGTP, dTTP) 250 µM, Reaction Buffer with 1.5 Mm MgCl2 1x), than adding 5 µl DNA at concentration of 50 nanograms ,3 µl of the primer concentration 10 pmol/ µl (from each primer listed in Table 1) and 7 µl of the D.D.water, with mixing well using Vortex (2-3) seconds to complete the mixing of the reaction components, the reaction was carried out under sterile conditions to prevent contamination(36). The thermal cycler PCR System (Sen So Quest Gumbtt) Germany, was amplified according to the following programs and by type of prefix Table 2 **(**31, 25**).** After the completion of the PCR reaction was taken (5) µl of the reaction products carried out in the place assigned to the 1.5% agarose gel, DNA Ladder was carried and the samples were carried under 80 volt for 1 hours.

Table 1. Primer sequences for RAPD used for amplification

No.	Primers' names	Base sequence (5'-3 ')	Source
1	Bio-RP1	GGTTCGGGAA	(Probojati *et al*,2019)
2	Bio-RP2	GAAACGGGTG	
3	Bio-RP3	CACACTCCAG	
4	Bio-RP4	CAAACGTCGG	
5	Bio-RP5	GCATATCCGG	
6	Bio-RP6	CTGACCAGCC	
7	Bio-RP7	ACCAGGTTGG	
8	Bio-RP8	GGACCCCGCC	
9	Bio-RP9	GGCTGCAGAA	
10	Bio-RP10	CTTTCGTGCT	
11	UBC-13	CCTGGGTGGA	
12	OPC-12	TGTCATCCCC	
13	OPJ-5	CTCCATGGGG	

Table 2. Steps of the RAPD- PCR reaction

No.	Stage	Temp.°C	Time (Min.)	Cycles
I	Initial denaturation	95	10	1
II	Denaturation	95	1	40
III	Annealing	34	1	
VI	Elongation	72	1.30	
V	Final Extension	72	10	1
IV	Terminal incubation	4	∞	

Statical Analysis (Estimation of particle sizes) After the separate band by gel documentation done the photo cap in order to identify the bands, a software was utilized to determine the molecular size. Comparison of the size of the DNA ladder indicator with thermal PCR reactions. After translating the descriptive RAPD, that appeared in the agarose gel with a result into digital data by marking 1 when the band was present and 0 when it was absent, and organizing the data in a Table 3. That included Genotype Data, the results that were visible in the gel were evaluated. According to the following equation, all prefix results for the analyzed samples were combined, and the coefficient of genetic distance between samples was determined using the coefficient of 72:

Genetic Distance (G.D.) = 1- ($\frac{2*N_{xy}}{N_y+N_x}$)

As: G. Direpresents the Genetic Dimension. N_{xy}: it represents the number of bands common to the X and Y models, which represent two sample. N_x: it represents the number of total bands in the sample. (20). Scheme of cluster analysis using the pre-made Numerical Taxonomy System Program (NTSYS-PC) to determine a genetic tree's similarity or distance is described by (28).

RESUITS AND DISCUSSION

The results showed the purity of DNA extracted from the young leaves of the Cucurbitaceae plants studied using the Nanodrop device with finite drop and electrophoresis as having high purity ranged between (1.7 -1.8). The concentration of the extracted DNA was adjusted to 20 mg µl for the RAPD-PCR reaction in Figure 2. The results of the RAPD -PCR technique using (13) random primers (Table 3, Figure 2), the 225 amplified RAPD bands in total, which range from 100bp to 2000 kb size. The number of RAPD bands varied from 11(primers Bio-RP1, Bio-RP2, Bio-RP3) to 4(primers Bio-RP5, Bio-RP7), showed no bands in primer (UPC-13) (Table 3). That there were differences in locations on the genomes of the studied cultivars and the production of different bands produced by the primers was (92). N0 mono morphic were shown for the used 13 primers used in the current study, and (92) polymorphic binding sites distinct. The all primers scored the highest percentage of polymorphism reached (100%).

Table 3. Results of the primers used in RAPD -PCR reaction

No.	Primer	Bands size	No. of polymorphic local	Total bands of primer	Polymorphism %	Ability Discrimination %
1	Bio-RP1	200-1600	11	11	100%	12
2	Bio-RP2	200-2000	11	11	100%	12
3	Bio-RP3	100-1200	11	11	100%	12
4	Bio-RP4	100-1600	6	6	100%	6.52
5	Bio-RP5	300-1600	4	4	100%	4.35
6	Bio-RP6	100-1200	6	6	100%	6.52
7	Bio-RP7	300-600	4	4	100%	4.35
8	Bio-RP8	200-1200	8	8	100%	8.7
9	Bio-RP9	200-2000	8	8	100%	8.7
10	Bio-RP10	200-800	6	6	100%	6.52
11	UBC-13	100-1000	9	9	100%	9.78
12	OPC-12	200-1200	8	8	100%	8.7
13	OPJ-5	0	0	0	0	0
			92	92		

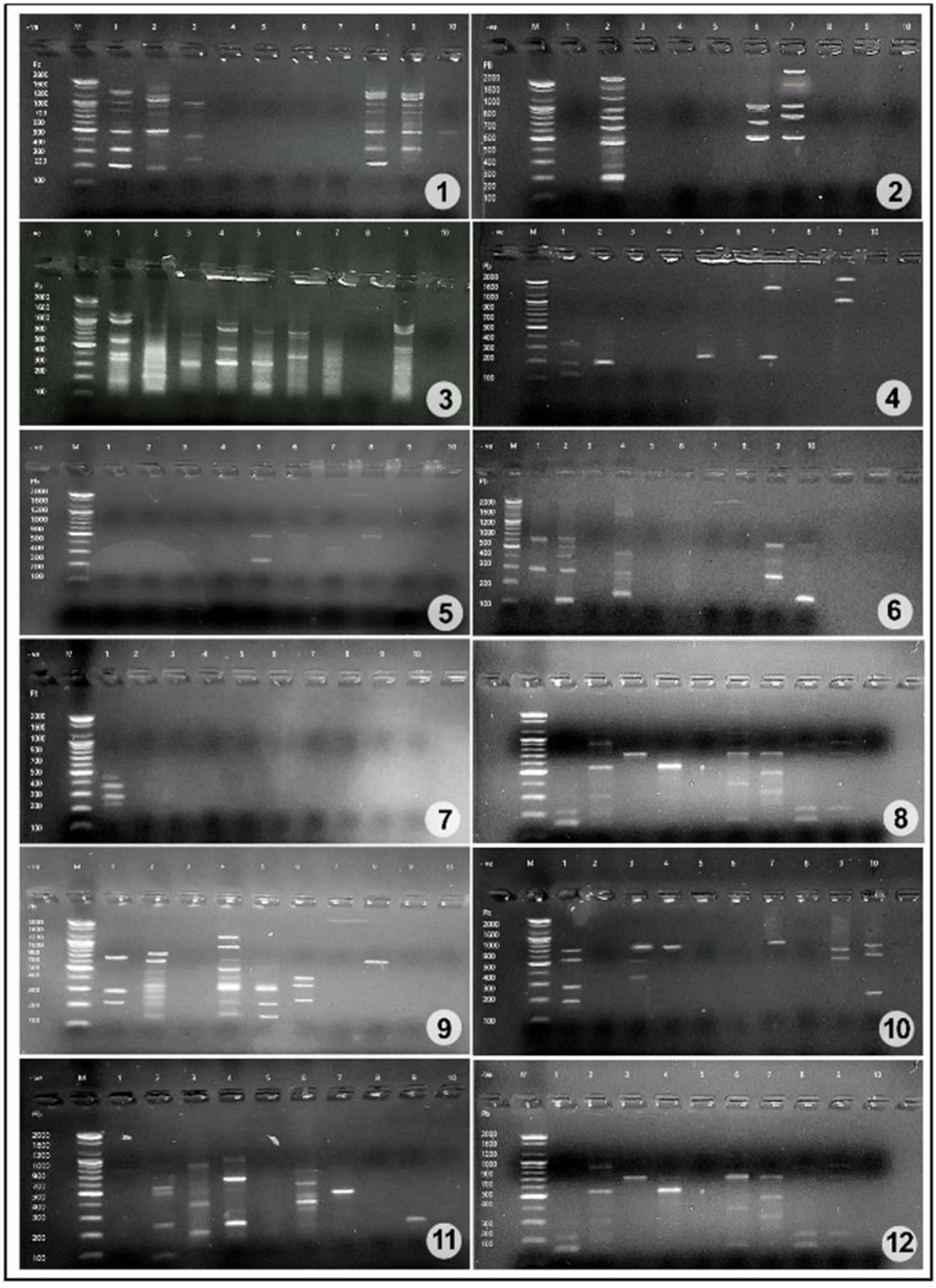

Figure 2. The amplification of products for the cultivars of the species of the cultivars of Cucurbitaceae according to RAPD markers

The Genetic distances between the analysis's subjects were determined using a straightforward matching procedure to produce a genetic distance matrix Table 4. Based on the values of the genetic dimension, the results of the genetic study showed a noticeable differences between the 10 cultivars; the genetic dimension coefficient values varied from 0.467- 1.That the highest value of the genetic dimension appeared between the cultivar *Cucurbita pepo*, with the cultivar *Luffa cylidrica with* a value of Laganaria *siceramia* L.c.v. local meaning 100% and the highest value of the genetic dimension

appeared between the cultivar *Citrullus lanatus* with the cultivar *Cucurbita pepo* and *Cucumis melo* with a value of 100%. The lowest value of (0.467) between the two cultivars *Laganaria siceramia* L.c.v. Syria and *Laganaria siceramia* L.c.v. local.

The results of the cluster analysis (dendrogram) of the cultivars of some Cucurbitaceae plants, which depend on the values of the genetic connections between the 10 taxa, Genotypes in Figure 4, show the presence of clear similarity value between the cultivars of the species in their genetic dimension, as there were basically divided into two main groups (I, II). The first (cluster I) consisted of (IA) included the cultivars *Cucumis sativus*, *Cucumis melo* and *Cucurbita pepo*, these two taxa isolated *Cucumis sativus* and *Cucumis melo*. The second cluster (II) contained two groups (IB, IC), which was divided into two sub-groups, IB containing *Luffa cylidrica Cucumis melo flexuosus* and *Citrullus lanatus*, these two taxa isolated *Luffa cylidrica Cucumis melo flexuosus* taxa dendrogram linkage joining. The second sub gropes IC, which separated into two clades containing *Laganaria siceramia* L.c.v. local, *Laganaria siceramia* L.c.v. Syria, *Citrullus colocynthis* and *Cucurbita maxima*, these two taxa isolated *Laganaria siceramia* L.c.v. local, *Laganaria siceramia* L.c.v. Syria the lowest value of the genetic dimension (0.467), demonstrated that the two taxa that were isolated from *Citrullus colocynthis*.

Table 4. Genetic dimension matrix of the cultivars of the Cucurbitaceae plants of the according to RAPD marker

Cutivars	1	2	3	4	5	6	7	8	9	10
1	0									
2	0.474	0								
3	0.843	0.864	0							
4	0.683	0.606	0.652	0						
5	0.818	0.769	1	0.744	0					
6	0.808	0.667	0.943	0.702	0.643	0				
7	0.849	0.77	0.889	0.75	0.862	0.622	0			
8	0.72	0.724	0.758	0.867	0.923	0.941	0.771	0		
9	0.467	0.588	0.814	0.709	0.778	0.773	0.867	0.667	0	
10	0.755	0.789	0.75	0.727	1	0.879	1	0.871	0.61	0

1-Laganaria siceramia L.c.v. local 2- Citrullus colocynthis 3- Luffa cylidrica 4 - Cucumis melo flexuosus 5- Cucurbita pepo 6- Cucumis sativus 7- Cucumis melo 8- Cucurbita maxima 9- Laganaria siceramia L.c.v. Syria 10- Citrullus lanatus.

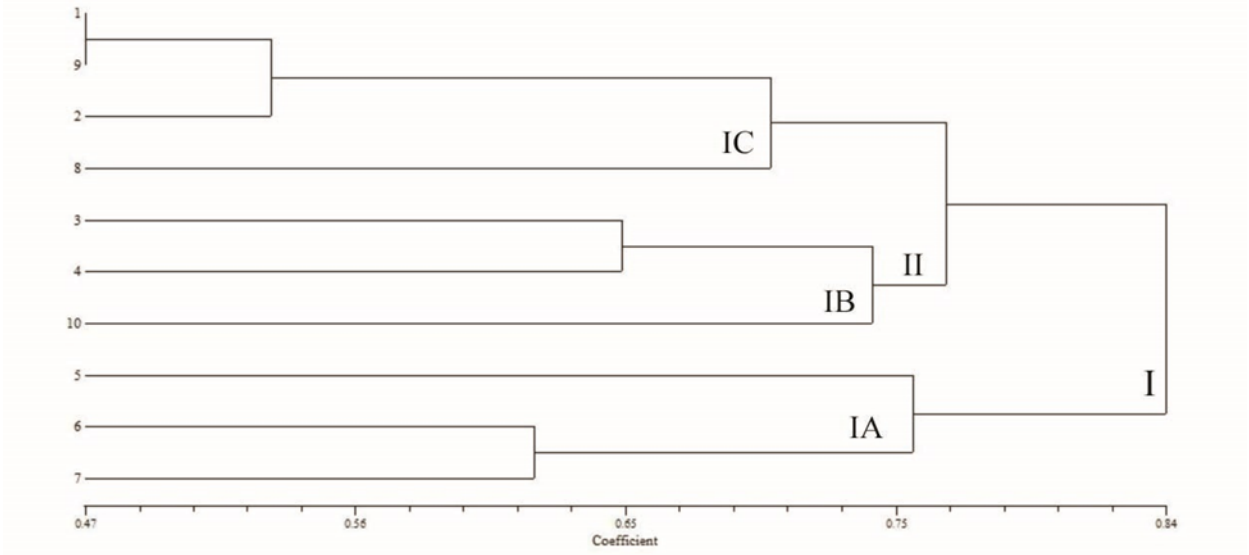

Figure 4: Dendrogram of the studied cultivars of the Cucurbitaceae plants.1-Laganaria siceramia L.c.v. local 2- Citrullus colocynthis 3- Luffa cylidrica 4 - Cucumis melo flexuosus 5- Cucurbita pepo 6- Cucumis sativus 7- Cucumis melo 8- Cucurbita maxima 9- Laganaria siceramia L.c.v. Syria 10- Citrullus lanatus.

In this study was isolated DNA (total genomic) by using KIT from the fresh leaves of Cucurbitaceae plant. RAPD numerous studies, both in the wild and in agriculture, have been conducted on population genetics plants (8,17,31). We could be anticipating a significant link or great genetic diversity among the species under study due to high repeatability and polymorphism levels. According to (3,18,28), the degree of variation in a particular genotype and the primer sequence determines the difference between reproducible bands based on each primer (18, 36). The results are consistent with, Phylogenetic analysis using RAPD (33, 34, 38, 40). Cucurbits' genetic diversity, as shown by RAPD analysis, may help breeders and genome researchers choose the right parents to cross to produce the right populations for breeding and genome mapping (24, 29). There could be a more direct relationship between the number of polymorphisms and the capacity to resolve genetic diversity among various genotypes. In this work, thirteen RAPD primers were utilized, and they are excellent for estimating genetic diversity between genotypes, than considered as a crucial instrument for tree species genetic enhancement (32). The knowledge will help breeders and managers create breeding and management plans that are better suited to react to selective pressure and secure the long-term survival of species. All research in This line of research will aid in future efforts to develop and breed plants in the Cucurbitaceae plant family. These results are consistent with (13), who reported that the fourteen genotypes of the Cucurbita species have levels of polymorphism that range from 69.8% to 97.0%, The results of (20) who indicated that *Cucurbita pepo* and Cucurbita maxima are highly polymorphic species, are consistent with the high level of RAPD markers polymorphism in Cucurbita species genotypes. According to the findings, RAPD variation may be able to discover enough variety in *Cucurbita pepo* or Cucurbita maxima for additional phylogenetic research and breeding studies. Additionally, these results supported the use of RAPD markers to identify genetic variation between closely related species at both the intraspecific and interspecific levels they also help identify taxa and duplicates and check for pollen or seed contamination during conservation efforts (1,35). The effective approach to understand evaluating germplasm material, or gene flow, in order to determine the species that might be further assessed, was offered by the trustworthy way of analysis utilized in the current study, and this rustle reveals a high degree of correlation among analyzed taxa. Our findings point to significant genetic diversity among cucurbit species and can be used to assess cucurbit diversity and choose a core collection to improve the effectiveness of managing germplasm for cucurbit breeding and conservation.

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CONCLUSION

We can conclude from the current study that there is significant genetic diversity among cucurbit species, which can be used to assess cucurbit diversity and select a core collection to improve the efficacy of managing germplasm for cucurbit breeding and conservation. The reliable method of analysis used in the current study provided an effective approach to understanding and evaluating germplasm material, or gene flow, to determine the species that might be further assessed, and this result reveals a high degree of correlation among analyzed taxa.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

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