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Classification of Some Cultivars Species of The Genus *Bougainvillea* Comm. ex Juss. Cultivated In Northern Iraq Using RAPD Markers

R. H. M. Al-Badrany [(1)*], A. M. Al-Mathidy [(2)]

[(1)*] The Iraqi Ministry of Education /Nineveh Education Directorate
[(2)] Department of Biology/ College of Education for Pure Sciences/ Mosul University/Iraq

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Correspondence:
Raad Hamad Mahmood Al-Badrany
Raadhamadm2@gmail.com

Abstract

The research dealt with a molecular taxonomic study of 6 cultivars of species of the genus *Bougainvillea* Comm. ex Juss., which included the cultivars 'Mary palmer', 'Rasberry ice', for the species *B. spectabilis* Willd, 'Royal purple', 'Formosa' for the species *B. glabra* Choisy, 'Thimma' for the species *B. peruviana* Hum. And Bon., and 'California gold' for the species *B. x buttiana* using eight different random primers for PCR, which was used to multiply and amplify the DNA fragments using random amplified polymorphic DNA (RAPD) markers. Through sample migration on an agarose gel, DNA was extracted from the leaves of cultivars and genetic diversity between amplified pieces for each cultivar was found. The findings indicated that differences in the locations on the genomes of the studied cultivars and the production of different bands revealed on the agarose gel, which reflects the magnitude of genetic variation depending on the primer used. As the primers produced (309) total bands resulting from (95) binding sites, including (12) monomorphic binding sites and (83) polymorphic binding sites distinct for the studied cultivars. The results of the cluster analysis (dendrogram) for the cultivars species of the genus *Bougainvillea* which depend on the values of the similarity showed the presence of clear variations among the studied cultivars.

The study concluded that the RAPD-PCR technique used in molecular classification based on molecular markers is a powerful tool for identifying and classifying cultivars of species of the genus *Bougainvillea* Comm. ex Juss. and detecting genetic relationships among them.

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1. Introduction

The genus *Bougainvillea* Comm. ex Juss. belongs to the (Nyctaginaceae) family, and includes (14) species, three of which are horticulturally important, namely *B. spectabilis* willd, *B. glabra* choisy and *B. peruviana* hum. And Bon., *Bougainvillea* is native to South America and is a common tropical and subtropical ornamental plant [1,2].

Bougainvillea is a group of popular ornamental plants grown mainly for its colorful bracts [3]. They are widely used in gardens [4], and in wide applications in green landscapes [5].

The differences in the *bougainvillea* cultivars are due to leaves, bracts, the color of the flower, the positions of the stamens and the flowering behavior of the cultivars, these morphological characteristics made the diagnosis and discrimination of *bougainvillea* cultivars difficult [6,7].

Molecular markers are important in plant systematics, they provide information about evolutionary relationships between and within species [8,9].

Using the RAPD markers in taxonomy of plant taxa, as the technique efficiently revealed the polymorphism among cultivars, which showed unique bands for cultivars, which helped in the molecular classification of the studied species [10, 11, 12], analysis of genetic plant species, as they are molecular markers of great value when they provide information about many unique sites randomly distributed across the genome, which helped in distinguishing cultivars species and analyzing the genetic relationships among them [13, 14],

genetic diversity evaluation among cultivars of species [15, 16], phylogenetic relationships between genotypes, varieties and cultivars [17].

Numerous studies in molecular systematic of Angiospermae have shown the importance of RAPD indicators in identification and taxonomy among taxa as Bamboo genera [18], Rose [19], *Punica granatum* L [20], *Citrullus* [21], *Cousinia* Cass [22], Palm [23], their molecular studies provided basic knowledge for understanding plant taxonomy through genetic variation that helped in identifying, distinguishing and classifying taxonomic ranks.

Found clear molecular variation among cultivars of the genus *Bougainvillea* Comm. ex Juss depend on RAPD indices [24, 25]

Recently, interest in planting *bougainvillea* bushes has increased because of their aesthetic value in gardens and landscapes, and because of this importance and the scarcity of molecular taxonomic studies that pertain to the cultivars of species of the genus *Bougainvillea*. Therefore, the current study aimes at molecular classification for some cultivars species of the genus *Bougainvillea* Comm. ex Juss. cultivated in Northern Iraq using RAPD markers.

2. Materials and methods

2.1 Plant materials

The study was based on fresh specimens of six cultivars of the genus *Bougainvillea*. species which were collected from Nineveh and Dohuk horticultural stations, and Planted in its nurseries and fields. During the growing season of (2023), the specimens were brought to the research center of the Department of Biology /College of Education for Pure Sciences/University of Mosul for study. Information was recorded for each specimen, which included the name of the species, cultivar, and collection site.it was photographed in full and as shown in Table (1) Figure (1).

As (4-5) young leaves were taken from the shoot apex and then placed in sterile and marked bags and transferred to the laboratory directly for DNA extraction from them.

Table 1: Names and locations collecting of the studied cultivars specimen of the *Bouginvillea* species

No.	Species	Cultivars	Locations collecting specimens
1.	***B. glabra***	ʿMary palmer’	Dohuk Horticultural station /Aqrah forest nursery (Dohuk)
2.		ʿRasberry ice’	Dohuk. Horticultural station/ Zahko Forest nursery
3.	***B. spectabilis***	ʿRoyal purple’	Nineveh Horticultural station
4.		ʿFormose’	Nineveh Horticultural station, Bagere fields (Dohuk)
5.	***B. peruviana***	ʿThimma’	Dohuk Horticultural station
6.	***B. x buttiana***	ʿCalifornia gold’	Nineveh Horticultural station

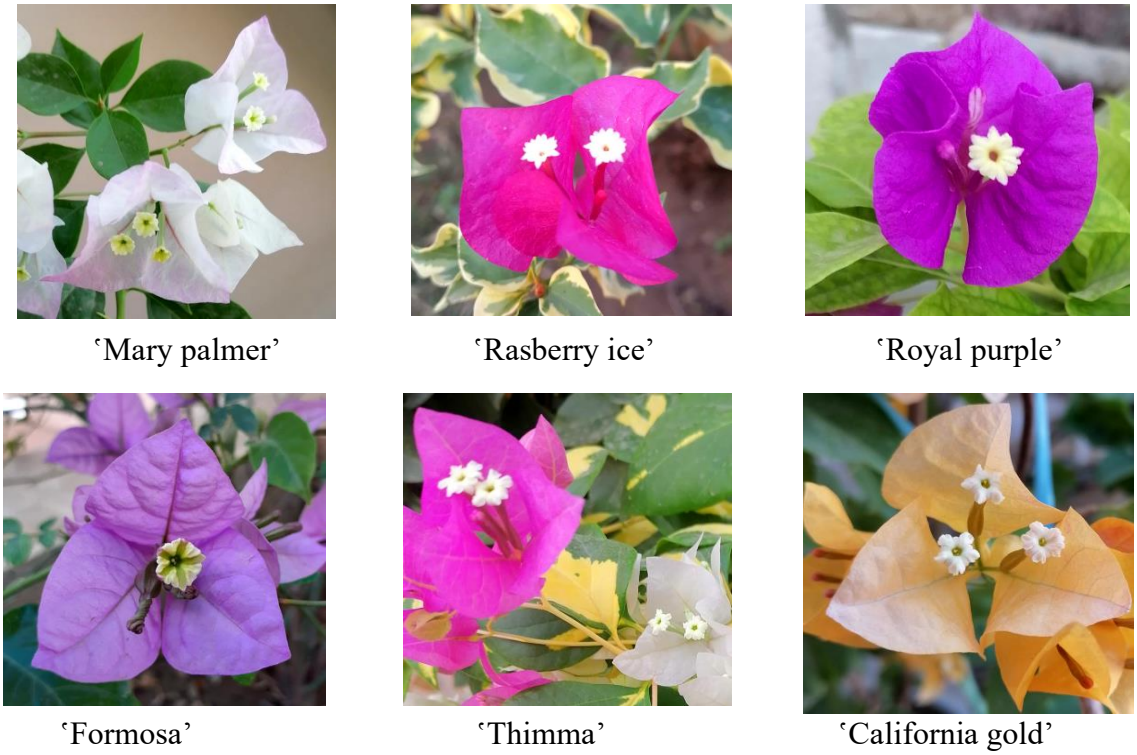

Figure 1. Names of cultivars *Bougainvillea* Comm. ex Juss

2.2 Genomic DNA Extraction:

DNA was extracted from young leaves of the cultivars of the genus under study using a special kit (plant genomic DNA extraction min kit, Farorgen, South Korea), where the extraction was carried out according to the instructions of the manufacturer company. The concentration and purity of the extracted DNA were measured using the device (Nanophotometer, Germany) and electrophoresis in agarose gel (1%).

2.3 Polymerase chain reaction

Eight random primers for RAPD markers were tested in the polymerase chain reaction for *Bougainvillea* Comm. ex Juss. cultivars using a final volume of 20 µl for the reaction. The mixture consisted of 2 µl nucleic acid in (50 ng/mL) concentration, 4 µl of the primer and 10 µl of the main mixture and filled the volume with ultrapure water. As for the reaction program (Table 2,3):

Table 2; Name of primers and sequence of nitrogenous bases of primers used study.

No.	Primer	Sequences 5'-3'
1.	OPA-4	AAT CGG GCTG
2.	OPA-10	GTG ATC GCAG
3.	OPA-12	TCG GCG ATAG
4.	OPA-19	CAA ACG TCGG
5.	OPC-10	TGTCTGGGTG
6.	OPH-14	ACC AGG TTGG
7.	OPH-17	CAC TCT CCTC
8.	OPW-11	CTG ATG CGTG

Table 3. Components of the amplification mix used in the study

No.	Stage	Temp. ℃	Time (Min.)	Cycles
1	Initial denaturation	95	10	1X
2	Denaturation	95	1	40X
3	Annealing	32	1	
4	Elongation	72	1.30	
5	Final Extension	72	10	1X
6	Terminal incubation	4	∞	

The amplifications were migrated in agarose gel (1%) for 1 hour, and then the bands were observed against the DNA Ladder by UV source. (Gel documentation system, Bio-Rad, USA)

2.4 Statistical Analysis:

The genetic similarity of the cultivars of the species of the genus under study was estimated using Jaccard's coefficient by converting the results of the RAPD markers that appeared in the agarose gel into characterization data was converted to similarity data, when the band is present (1) and (0) for its absence. The molecular sizes of the products of replication were estimated in comparison with the DNA Ladder, and to analyze this data, the similarity values were estimated in accordance with [26].

Using the following equation, the genetic distance was determined:

GD =1-2nxy/nx+ ny

nxy: the number of shared bands between models x and y that represent any two cultivars.

nx: the number of total bands in the model x

ny: the number of total bands in the model y

The Unweighted Pair Group Method with Arithmetic Average was used to create the dendrogram by considering genetic similarities [27], within the statistical program Numerical Taxonomy and Multivariate Analysis System (NTSYS-PC) [28]. Primer proficiency (%) was estimated using the equation [29], and the discriminatory ability (%) [30].

3. Results and discussion

The results showed the purity of DNA extracted from the young leaves of the cultivars species for the genus *Bougainvillea* Comm. ex. Juss. studied using the nanodrop device with finite drop and electrophoresis as having high purity ranged between (1.71-1.8) and electrophoresis on agarose gel shown in (Figure 2).

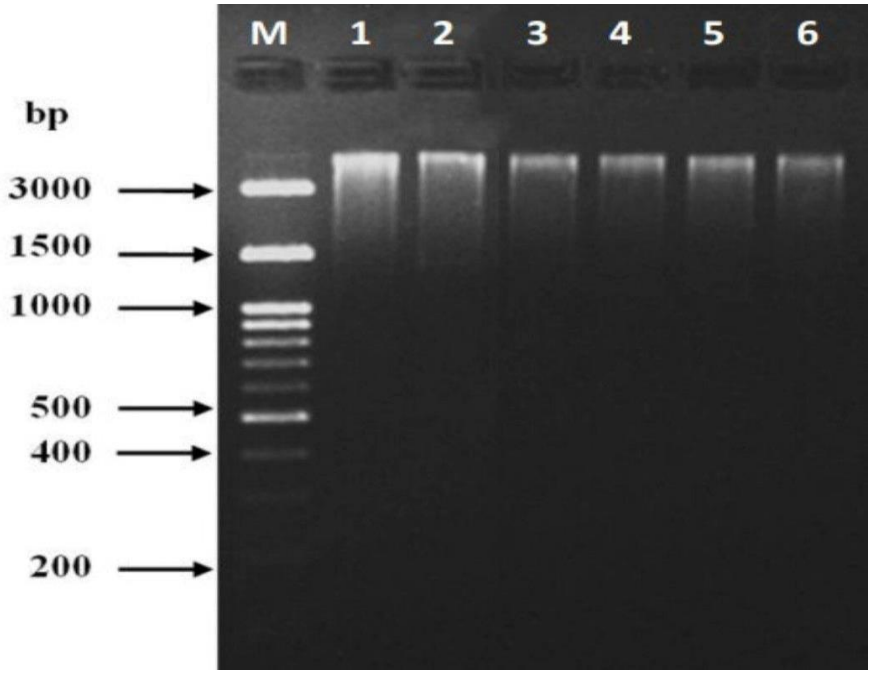

Figure 2. The genomic of studied cultivars of the species genus *Bougainvillea*

Lane M: DNA Ladder ;1- 'Mary palmer' ,2- 'Rasberry ice', 3 'Royal purple',4-'Formose',5- 'Thimma',6- 'California gold'

The results of the RAPD-PCR technique using (8) random primers (Table 4, Figure 3) showed that there were clear differences in locations on the genomes of the studied cultivars and the production of different bands

according to the primer used, as the total number of bands produced by the primers was (309), which resulted from (95) binding sites, including (12) monomorphic binding sites and (83) polymorphic binding sites distinct for the studied cultivars. The primer OPC10 scored the highest percentage of polymorphism reached (100%) and the lowest (80%) in the primer OPA-4and OPH-17. The primer OPC-10 was characterized by the highest discriminating ability (17.30 %) and OPA-10 had the highest primer proficiency (15.21%) while the primer OPA-4 recorded the lowest discriminating ability (8.02%) and primer proficiency (10.03%).
These results could identify a high level of polymorphism *Bougainvillea* cultivars [24, 25, 31].

Table 4. Results of the primers used in RAPD-PCR reactions.

No.	primer	bands size	No. of amplified local	No. of polymorphic local	No. of polymorphism local	Total bands of primer	Polymorphic bands	Polymorphism bands	Polymorphism %	Discriminating ability%	primer proficiency %
1	OPA-4	260-900	10	2	8	31	12	19	80.00	8.02	10.03
2	OPA-10	216-1490	17	2	15	47	12	35	88.23	14.77	15.21
3	OPA-12	200-1400	11	1	10	35	6	29	90.91	12.24	11.33
4	OPA-19	344-1109	8	1	7	32	6	26	87.50	10.97	10.36
5	OPC-10	257-1400	14	0	14	41	0	41	100	17.30	13.27
6	OPH-14	300-1500	12	2	10	45	12	33	83.33	13.92	14.56
7	OPH-17	300-1230	10	2	8	38	12	26	80.00	10.97	12.30
8	OPW-11	224-1420	13	2	11	40	12	28	84.61	11.81	12.94
Total			95	12	83	309	72	237		%100	%100

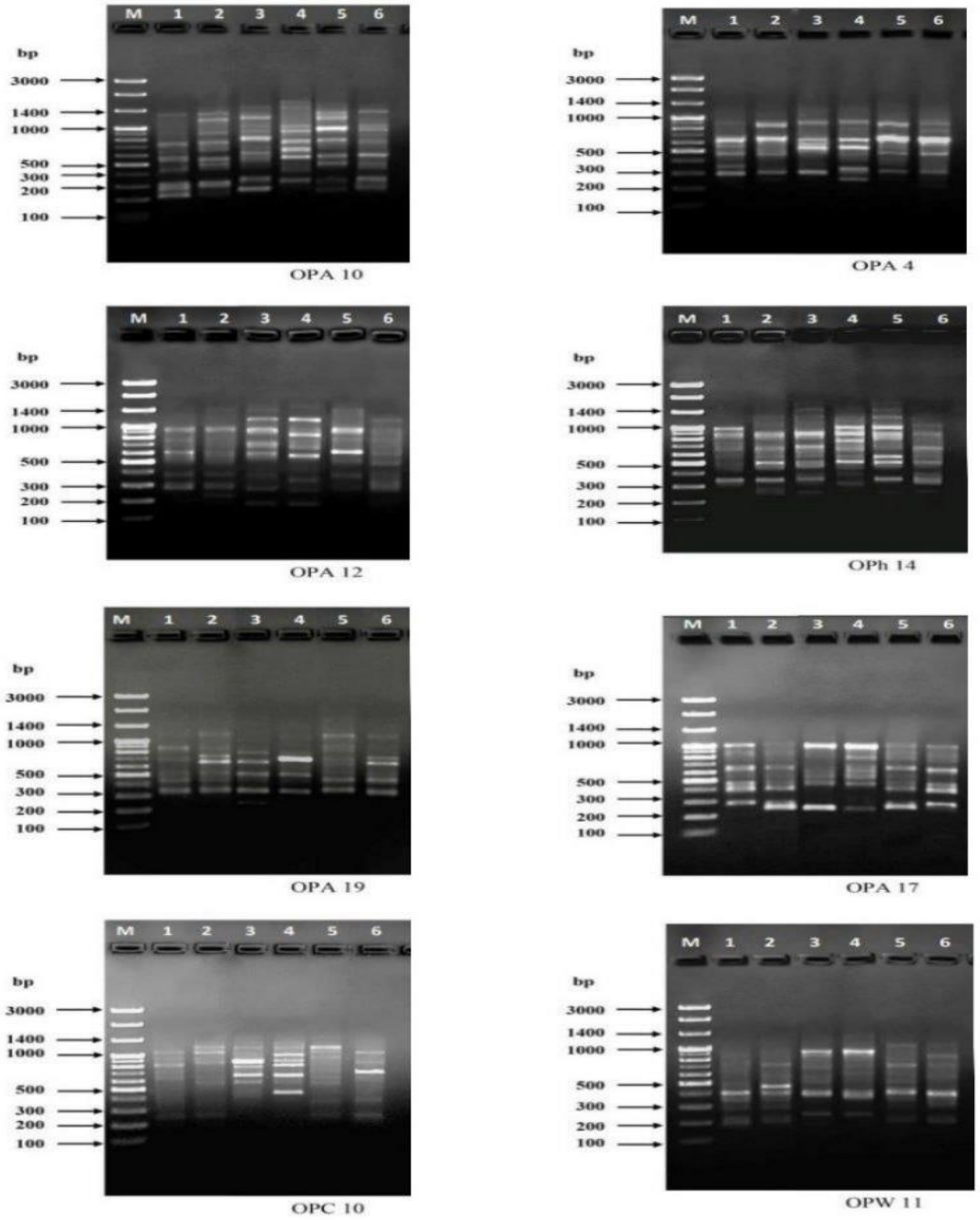

Figure 3. The amplification of products for the cultivars of the species of the genus *Bougainvillea* Comm. ex Juss. According to RAPD markers.

Lane M: DNA Ladder ;1- 'Mary palmer' ,2- 'Rasberry ice', 3 'Royal purple', 4-'Formose', 5- 'Thimma',6- 'California gold'

The results of the genetic analysis indicated a clear variation between the tested cultivars based on the values of similarity and genetic dimension, as it is noted from Tables 5and 6, that the highest value of the genetic similarity appeared (0.631) between *B. spectabilis* 'Rasberry ice' and *B. peruviana*'Thimma' indicating the least dimension value of (0.227) between the two studied cultivars, The lowest genetic similarity (0.282) between *B. spectabilis* 'Mary palmer' and *B. glabra* 'Formosa'as the highest genetic dimension value of (0.560). These results are consistent with [25, 32].

The results of the cluster analysis (dendrogram) based on genetic similarity between the cultivars of the genus *Bougainvillea*, showed the presence of clear variations between the cultivars of the species, as it was possible to separate them into two groups, the first main group included the two cultivars 'Royal purple' and 'Formosa' for the species *B. glabra* and second main group included the cultivar *B. peruviana* 'Thimma' which formed a first subgroup separate from the second subgroup which included 'Mary palmer' ,'Rasberry ice' of species *B. spectabilis* cultivars and B.*x buttiana* 'California gold' It is clear from cluster analysis that the cultivars belonging to the same species showed greater similarity than the cultivars belonging to other species, which helped in separating and isolating the studied cultivars as shown in (Figure 4), and these results are consistent with [24], when they used the RAPD markers in the study of genetic relationships among the cultivars of species of the genus *Bougainvillea*. Also, this result is consistent with [12, 16, 21, 22, 33] in that cluster analysis based on molecular markers helps in separating and isolating the taxa and finding genetic relationships among them.

Table 5. Genetic similarity matrix of the cultivars of the species of the genus *Bougainvillea* Comm ex. Juss. according to RAPD markers.

Proximity Matrix

Jaccard Measure

Cultivars	'Mary palmer'	'Rasberry ice'	'Royal purple'	'Formose'	'Thimma'	'California gold'
'Mary palmer'	1					
'Rasberry ice'	0.617	1				
'Royal purple'	0.324	0.411	1			
'Formose'	0.282	0.329	0.493	1		
'Thimma'	0.464	0.631	0.372	0.363	1	
'California gold'	0.565	0.619	0.373	0.364	0.559	1

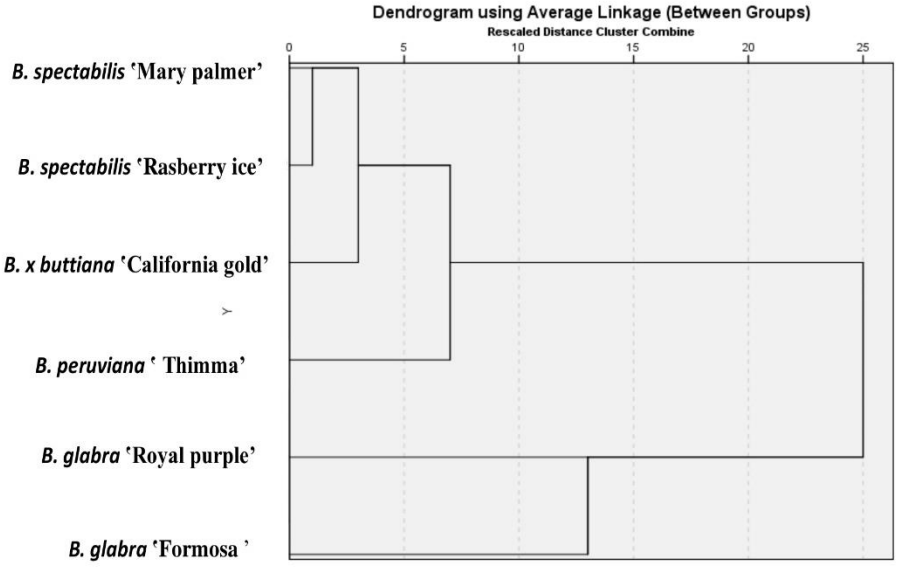

Figure 4. Dendogram between the cultivars of the species of *Bougainvillea* genus.

Table 6. Genetic dimension matrix of the cultivars of the species of the genus *Bougainvillea* Comm ex. Juss. according to RAPD markers.

Cultivars	ʻMary palmerʼ	ʻRasberry iceʼ	ʻRoyal purpleʼ	ʻFormoseʼ	ʻThimmaʼ	ʻCalifornia goldʼ
ʻMary palmerʼ	**0**					
ʻRasberry iceʼ	**0.237**	**0**				
ʻRoyal purpleʼ	**0.510**	**0.417**	**0**			
ʻFormoseʼ	**0.560**	**0.523**	**0.339**	**0**		
ʻThimmaʼ	**0.366**	**0.227**	**0.458**	**0.468**	**0**	
ʻCalifornia goldʼ	**0.278**	**0.235**	**0.456**	**0.486**	**0.283**	**0**

4. Conclusions

The RAPD-PCR technique used in molecular classification based on molecular markers is a powerful tool for identifying and classifying cultivars of species of the genus *Bougainvillea* Comm. ex Juss. and detecting genetic relationships among them. As a matter of fact, we get ahigh level percentage of polymorphism, which is, 80 -100 %, refer a high genetic diversity in the tested cultivars. The genetic diversity analysis is going to provide beneficial information for getting proper management and for its future utilization in basic and applied research.

Declaration of competing interest

All authors agree that no conflict of interest

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تصنيف بعض اصناف انواع الجنس *Bougainvillea* Comm. ex Juss. المزروعة في شمالي العراق باستخدام علامات RAPD

رعد حمد محمود البدراني[1]*، عامر محسن المعاضيدي [2]

[1] مديرية تربية محافظة نينوى، وزارة التربية العراقية

[2] قسم علوم الحياة، كلية التربية للعلوم الصرفة، جامعة الموصل

المستخلص

يتناول البحث دراسة تصنيفية جزيئية ل(6) اصناف من انواع الجنس *Bougainvillea* Comm. ex Juss والتي تضمنت الاصناف 'Mary palmer' 'Rasberry ice', *B. spectabilis* Willd للنوع 'Royal purple', 'Formosa' والصنفان *B. glabra* Choisy للنوع 'Thimma' والصنف *B. peruviana* للنوع 'California gold' والصنف Hum. & Bon. *B. x buttiana* للنوع باستخدام (8) بادئات عشوائية مختلفة لPCR والتي تم استخدمها لمضاعفة وتضخيم قطع الحمض النووي باستخدام واسمات DNA متعدد الاشكال العشوائية (RAPD) من خلال ترحيل العينات على هلام الاكاروز، تم استخلاص الDNA من اوراق الاصناف والتنوع الجيني بين القطع المتضخمة لكل صنف. اشارت النتائج الى وجود تباينات واضحة في المواقع على جينوم الاصناف المدروسة وانتاج حزم مختلفة تم الكشف عنها على هلام الاكاروز والتي تعكس مقدار التباين الوراثي بالاعتماد على البادئ المستخدم .اذ انتجت البادئات الثمانية (309) حزمة كلية ناتجة من (95)موقع ارتباط منها (12) موقع ارتباط احادي الشكل و(83) موقع ارتباط متعدد الاشكال مميزة للاصناف المدروسة . نتائج التحليل العنقودي (dendrogram) لأصناف انواع جنس الجهنمية والتي تعتمد على قيم التشابة اظهرت وجود اختلافات واضحة بين اصناف المدروسة. خلصت الدراسة الى ان استخدام تقنية RAPD-PCR في التصنيف الجزيئي استنادا الى المؤشرات الجزيئية اداة قوية لتشخيص وتصنيف اصناف انواع جنس الجهنمية وايجاد العلاقات الوراثية بينها.