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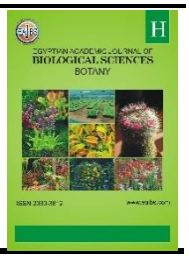

Molecular Characterization of a Number of Local and Cultivated Varieties of Eggplant Using RAPD Markers in Salah Al-Din – Iraq

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ABSTRACT

Genetic relationships among 12 varieties of eggplant (*Solanum melongenaL.*) were studied using fourteen Randomly Amplified Polymorphism D.N.A. (RAPD) markers. The local varieties of eggplant include Wisam, Mahaly, Nasr, White, and Alrafidien, while cultivars varieties include four Turkish (1,2,3,4) and three Italian (1,2,3). Randomly Amplified Polymorphism D.N.A. (RAPD), one of the markers that depend on PCR (Polymerase Chain Reaction), is used to find genetic distance and cumulative analysis among twelve samples of eggplant. D.N.A. was extracted from the plant leaves, and then the RAPD technique was d by using fourteen primers; the products then were electrophoresed on a1.5% Agarose gel; it was shown that the primer which gave more bands was OP D-10. It gave 67 bands; its efficiency was 7.5, while its differentiation capacity was 6.4. The variable rate for this primer was 67%. On the other hand, the less producible primer was OP D-18. It gave 23 bands; its efficiency was 5.6, while its differentiation capacity was 4.7, and the variable rate for this primer OP D-18 was 100%. It must be noted the appearance of unique bands. It was 10 bands among studied eggplant varieties, while absent bands that appeared in this study were 7 bands. The fifth sample had a higher number of unique bands, it was 4 bands;while the samples had reached a lower number of individual bands it`s (2-6-8-9-11-12)it had one amazing band only.The sample thathada higher number of absent bands was the sixth (Turkish 1) with 5 absent bands;while the sample thathad the lower number of absent bands was the fifth(Iraqi Al-Rafidain) whichhad one absent band only.The genetic tree which depended on RAPD primers areshow that the genetic distance values rangedbetween(0.0596-0.9351)where it was a lower genetic distance between (Iraqi Nasr &Iraqi White) it`s (0.596); While it was a higher genetic distance between (Iraqi Al-Rafidain& Turkish 2), it`s(0.9351).

INTRODUCTION

Solanaceae is a large plant family. Brinjal eggplant (*Solanum melongena***)** is the fifth more economical important vegetable in Solanaceae after tomato, pepper, potato, and tobacco. (Knapp *et al.*,2004)**.** Eggplant is common in many essential diets in many communities, especially in Africa, India, and Bangladesh, It`s considersa leaf vegetable(champan,2019). It`s a vital source of fibers and metals (Iron, Calcium, Potassium,

Magnesium, Zink, Phosphorous) and vitamins (C, B6, B12, E, D, A) (Magioli and Mansur,2005).

Identifying genetic origins is necessary for farmers to produce new varieties or to further improve existing ones depending on consumer requirements or challenges of growth conditions. Molecular markers have tremendous potential to explore genetic diversity by discovering multiple species; they are helpful tools for reproduction, genotyping, and genome identification(Brown *et al.*,2014).Various studies have been conducted to determine eggplant genetic diversity using the (RAPD Markers) Randomly Amplified Polymorphism D.N.A. (Ranil *et al.*,2017).RAPD markers depend on the Polymorphism Chain Reaction (PCR) technique which wasdiscovered by American scientists (Williams *et al.*,1990). The foundation for action is itsdouble target with short random primers (it`s a habit 10 nitrogenous base) high content from cytosine and guanine range 50-70, Primers are bind to their complementary sites on the D.N.A. It works dependent on the number of gene binding sites of the primer on D.N.A. and the distance between them (Weigand *et al.*,1993). RAPD markers are characterized by complete dominance, multiple sites detectable, easy, fast, inexpensive and need a small amount of D.N.A. (Al Sugmainy RZ,2020). RAPD have been used to examine the genetic diversity of many plant species (Thormann *et al.*,2004).

The study aims to find the genetic relationship and dimension among some local and cultured (*Solanum melongenaL.)* in Salah Al-Din _Iraq.

MATERIALS AND METHODS

Collection and Cultivation Samples:

Eggplant seeds obtained from agricultural offices officially licensed by the Iraqi Ministry of Agriculture, we got five local varieties and seven imported varieties of *Solanum melongena*. Local types included (Wisam, Mahaly, Nasr, White, in addition Al-Rafidain) While imported varieties included four Turkeys and three Italians (Table 1).

Eggplant seeds were planted onthe fourteenth of October in plastic agricultural plates which have holes by dimensions (3×3) cm, it`s filled with unique agricultural soil called Peitmos, the seeds put in a deep 1 cm and sprayed by water, after that I covered the dishes with plasticcover, after two weeks the seeds begin germination.

Table (1): The studied samples of eggplant.

NO.	Studied varieties
1	Iraqi Wisam
2	Iraqi Mahaly
3	Iraqi White
4	Iraqi Nasr
5	Iraqi Al Rafidain
6	Turkish1
7	Turkish2
8	Turkish3
99	Turkish4
10	Italian1
11	Italian2
112	Italian3

Genome D.N.A. Extraction:

The genome is isolated from the young eggplant leaves using the CTAB method (Huang *et al.*,2013). Take about 1 gm. of young leaves of eggplant. After the genomic extraction is complete the extracted genome is kept in deep freeze for later use in the Randomly Amplified Polymorphism Reaction (RAPD) process.

Measuring Genome Concentration and Purity:

The concentration of the genome was measured as purity estimate by Nanodrop device, where the absorption of ultraviolet light has been calculated by Spectrophotometer device on wavelength (260) nanometer, we took from D.N.A. extract (1) microl and put the genome in this device and switching it. And the purity was estimated in the following relation: Absorption on 260nm. Absorption on 280nm. (Maniatis *et al.*,2001). The samples gave a range of purity (1.8-2.3) and range of concentration (36-246) (Table 2).

Table 2: Concentration and purity of the D.N.A. of samples studied**.**

NO.	Varieties	DNA con. ng	OD. 260/280
1	Iraqi Wisam	41	2
2	Iraqi Mahaly	55	1.8
3	Iraqi White	145	2
4	Iraqi Nasr	110	2
5	Iraqi Al-Rafidain	95	2
6	Turkish 1	36	1.8
7	Turkish 2	77	2
8	Turkish 3	50	2.2
9	Turkey 4	246	2
10	Italian 1	39	2.3
11	Italian 2	50	2
12	Italian 3	131	2

RAPD_PCR Primers and Program:

RAPD reaction had done based on (Williams et al.,1993) on (12) varieties of eggplant, by using premix was provided from Bioneer association, Distilled water provided from U.S.A I.N.C. and (14) primers of RAPD Provided by Operon Technique Inc. (Table 3).

Table (3): The RAPD primers.

NO.	Primers	Sequence 5`→→→ 3`
1	OP A-02	CAGGCCCTTC
2	OP B-04	GGACTGGAGT
3	OP B-20	GGGAGCATCC
4	OP C-08	AACGGTGGCC
5	OP H-16	GTCCGATACA
6	OP C-10	CACACTCCAG
7	OP D-10	TGGACCGTGC
8	OP D-18	CCTTGACGCA
9	OP J-04	GAGAGCAACC
10	OP A- 04	AGCAACCTTGA
11	OP B-12	CGGAACAGCA
12	OP D-12	TCTCAGCTGG
13	OP D-03	GGACACCGGT
14	OP C-16	CCATTGCGGG

Prepared the main reaction mixture RAPD-PCR (master reaction) by mixing the components PCR premix with 2µ of RAPD primer with 3µ of genome isolated from eggplant leaves with 15µ of distilled water to the mixture. The tubes are then placed on the polymer device. After the reaction ends, lift the tubes off the machine and freeze until it`s electrophoresis on an Agarose gel to determine the molecular size of the D.N.A. (Al Sugmainy ,2020).

D.N.A. Molecular Size Estimation:

To determine the D.N.A. molecular size, It`s pulled by a pipette 5 microliter from tubes and put in gel pits to perform electrophoresis on Agarose gel, after the electrophoresis time is over, the gel is lifted and put in the UV-transilluminator and filming the gel with a digital camera to see the bands clear.

Statistical Analysis:

RAPD outcome got and quoted bytables depending on the presence or absence of genome band of samples studied, where the band presence symbol is NO. one while the band absence symbol is zero, after that count the genetic distance coefficient &similarity coefficient using Nei`s coefficient 72 (Nei and Li 1979), after that run the statistical analysis and drawing the genetic tree in between entries in UPGMA method by NTSYS-pc, in Computer.

RESULTS AND DISCUSSION

It`s found in this studying the concentration of the D.N.A. target has a significant impact on shown bands density on Agarose gel because of the increase in the number of sites recognized by primer, as a result, duplicates increase, at times the products get dispersed, andthey give rise to smear among the bands (Becerril *et al.*,2002). It`s used in RAPD markers primers consisting of 10 nitrogenous bases where it`s in one copy can recognize different locations on D.N.A., these locations produce other packets at electrophoresis on Agarose gel, it can also detect variations among samples (Waly *et al.*,2012).

RAPD markers areuse in genetic variation among twelve eggplant varieties (local &cultured). Table (4), shows 14 randomly primers which all have links on D.N.A. and produced different bands seen on Agarose gel with the presence of D.N.A. marker (ladder 100 bp), the primers produced monomorphic and polymorphic bands, the sum of the sites that primers learned on D.N.A. 92 sites10 main sites and 82 polymorphic sites, (OP D-10) has the highest number of productive sites up to 9 sites. In comparison, the two primers (OP D-12 &OP D-03) have the lowest number of functional areas to 5 sites, total bands from these sites was 601 including 120 leading bands and 481 polymorphic bands, the (OP D-10) primer has the highest number of bands up to 67, while the (OP D-18) primer has the lowest number of bands up to 23. Diversity ratio of productive primers were 89%.

This study has been characterized by Unique and Absent bands, the total ratio of unique bands was 10, while the total percentage of absent bands was 7. The fifth sample has more significant number of individual bands up to 4, while the (2^{nd},6^{th},8^{th},9^{th},11^{th},12^{th}) pieces had one unique band only. the 6^{th}sample had a more significant number of absent bands up to 6, while the 5th had one. These bands were a distinguishing feature of these varieties. Still, the appearance of bands in a single class indicates a mutation in a specific site which led to the primer was recognize these sites without the other one and the formation of the unique band. Absent bands are caused by a mutation in a primer learning site in one variety only these obscure the appearance of the band, and this conforms to the findings of (Sambrook *et al.*,1989).

Table 4: Results of RAPD Primers of the studied varieties.

N0.	Primer name	Location NO.	Monomorphic location NO.	Polymorphic location NO.	Bands summation	Monomorphic band NO.	Polymorphic band NO.	Unique bands number	Absence band number	Polymorphism percent
1	P 1 OP A-02	6	2	4	52	24	28	-	-	67%
2	P 2 OP B-04	6	-	6	49	-	49	-	1	100%
3	P 3 OP B-20	7	1	6	46	12	34	2	-	85%
4	P 4 OP C-08	6	-	6	46	-	46	-	-	100%
5	P5 OP H-16	6	-	6	38	-	38	1	2	100%
6	P 6 OP C-10	8	-	8	58	-	58	1	-	100%
7	P 7 OP D-10	9	3	6	67	36	31	-	2	67%
8	P 8 OP D-18	6	-	6	23	-	23	2	-	100%
9	P 9 OP J-04	8	-	8	47	-	47	-	1	100%
10	P10 OP A-04	8	-	8	32	-	32	2	-	100%
11	P11 OP B-12	6	-	6	28	-	28	2	-	100%
12	P12 OP D-12	5	2	3	51	24	27	-	-	60%
13	P13 OP D-03	5	1	4	28	12	16	-	1	80%
14	P14 OP C-16	6	1	5	36	12	24	-	-	83%
	Total	92	10	82	601	120	481	10	7	89%

Table (5) shows the RAPD primers varied in molecular sizes of the resulting bands, ranging from (100-2000) bp; it's the smallest molecular size was 100 bp of the (OP D-10) While the most significant molecular size was 2000bp of the (OP A-02, OP B-20, OP C-08, OP D-10, OP J-04). For proficiency of primers, less proficiency was 3.9 in (OP D-03), While the higher proficiency was twelve in (OP D-12). The (O.P. D- is distinguished (12) in (OP C-10).

Table 5: Show distinguish bands, efficiency, and recognition of the capacity of RAPD primer.

NO	Primer Number	Molecular size	Studied variety																								Efficiency	Recognizing capacity
			1		2		3		4		5		6		7		8		9		10		11		12			
			unique	Absence	Unique	Absence	Unique	Absence	Unique	Absence	unique	Absence	Unique	Absence	Unique	Absence	Unique	Absence	Unique	Absence	Unique	Absence	Unique	Absence	Unique	Absence		
1	P1 OP A-02	200-2000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6.8	5.8
2	P2 OP B-04	350-1500	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	12	10
3	P3 OP B-20	200-2000	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	8.3	7
4	P4 OP C-08	400-2000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	11.7	9.5
5	P5 OP H-16	300-1500	-	-	-	-	-	-	-	-	1	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	9.3	7.9
6	P6 OP C-10	300-1500	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14.2	12
7	P7 OP D-10	100-2000	-	1	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	7.5	6.4
8	P8 OP D-18	300-1500	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	5.6	4.7
9	P9 OP J-04	200-2000	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	11.5	9.7
10	P10 OP A-04	300-1500	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	7.8	6.6
11	P11 OP B-12	200-1500	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	6.8	5.8
12	P12 OP D-12	500-1500	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6.6	5.6
13	P13 OP D-03	300-1250	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.9	3.3
14	P14 OP C-16	300-1500	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5.8	4.9
Total			-	1	1	-	-	-	-	-	4	1	1	5	-	-	1	-	1	-	-	-	1	-	1	-		
			1		1		-		-		5		6		-		1		1				1		1			
			17																									

RAPD results appear for the 12 varieties (Fig.1) of eggplant are random, and a genetic distance is not possible by chance, enhancing the presence of known sites (Sambrook *at el.*,1989).

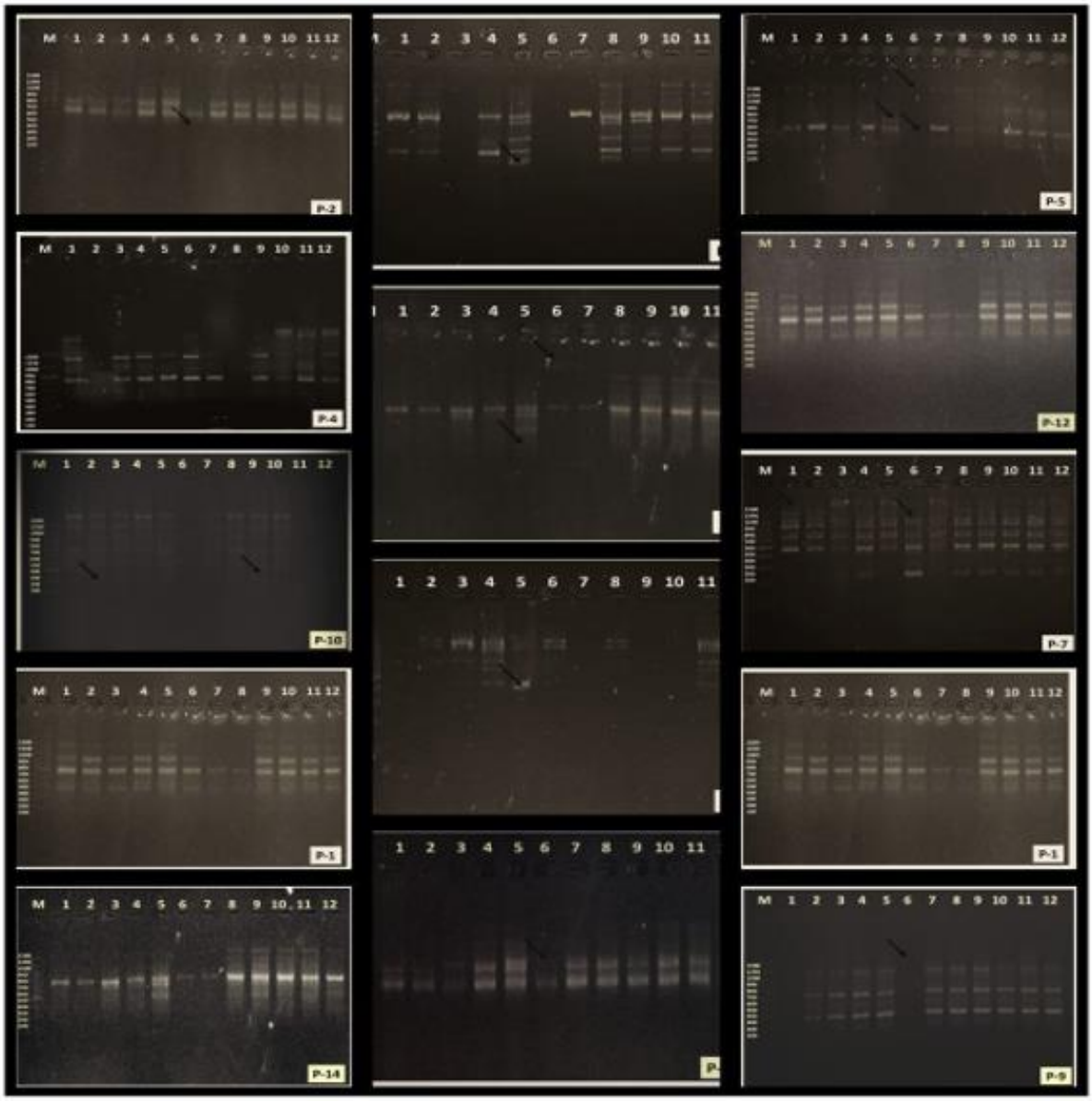

Fig.1: RAPD electrophoresis for 12 eggplant samples on 1.5% Agarose gel by using 14 primers

Estimation of the Genetic Dimension:

Dimension and genetic relationship are essential to plant breeders because whatever happens in improving plant crops the genetic dimension between genetic sites leads to increased yield and improved it. (Islam *et al.*,2014).

Estimated the genetic dimension of results of the RAPD markers for imported and local eggplant varieties by using a genetic program (NTSYS-PC. version 2.10) based on shared bands between varieties; it`s factoring is based on a law (Nei and Li 1979). The genetic dimension of the radiator scores ranged from (0.9351 – 0.0596) (Table 6). It was less genetic dimension (0.596) between (Iraqi White and Iraqi Nasr), which is the highest genetic similarity of the two varieties. In contrast, the highest genetic dimension (0.9351) was between (Iraqi Al-Rafidain – Turkish 2), which is the less genetic similarity, the genetic dimension of the other varieties ranging from (0.9351-0.0596).

If the genetic material of any two classes is identical, the genetic dimension must be zero (Khierallah *et al.*,2014)

Table 6: The genetic affinity and distances rates between 12 eggplant varieties.

	1	2	3	4	5	6	7	8	9	10	11	12
1	0.0000											
2	0.1286	0.0000										
3	0.1488	0.1035	0.0000									
4	0.2037	0.1514	**0.0596**	0.0000								
5	0.2814	0.2119	0.2119	0.2257	0.0000							
6	0.4525	0.4174	0.3529	0.3138	0.3810	0.0000						
7	0.4831	0.5999	0.6487	0.6918	**0.9351**	0.6442	0.0000					
8	0.2237	0.1949	0.1398	0.1496	0.1829	0.3466	0.7030	0.0000				
9	0.1953	0.2024	0.1430	0.1739	0.2173	0.3362	0.5368	0.1055	0.0000			
10	0.2776	0.2699	0.2452	0.2869	0.3331	0.3343	0.4966	0.2289	0.1809	0.0000		
11	0.3104	0.3251	0.2725	0.3169	0.3104	0.4657	0.6970	0.3015	0.2558	0.3499	0.0000	
12	0.3638	0.3164	0.2623	0.3363	0.4974	0.4296	0.5431	0.3707	0.3279	0.3466	0.1987	0.0000

Cluster Analysis:

Based on the genetic dimension derived from the genetic structures of the studied varieties painted, a Dendrogram it`s a diagram that shows the evolutionary relationship of organisms that have arisen from a common ancestor (Singh *et al.*,2006). Creation of the Dendrogram group for twelve eggplant varieties by using cluster analysis (Fig. 2), and it turns out that the studied varieties were divided into two main clusters, the first central cluster including (Turkish 2) only, While the second main cluster separated into two sub-clusters, the first sub-cluster includes (Italian 1), while the second sub-cluster divided to two sub-clusters, the first includes (Turkish 1) the and the second is separated into two sub-clusters, the first includes (Italian 2 and Italian 3). The second was divided into two sub-clusters the first includes (Iraqi Al-Rafidain, Turkish three, and Turkish 4) and the second includes (Iraqi Wisam, Iraqi Nasr, Iraqi Mahaly, and Iraqi White).

The results show a genetic difference between the genetic structures for studied varieties. This is consistent with the phenotype of quantitative traits of the genetic structures. The reason for this is genetic differences in phenotypic structures by environmental factors, and the second reason is the high genetic similarity, which isn`t consistent with phenotypes (Al Sugmainy,2020).

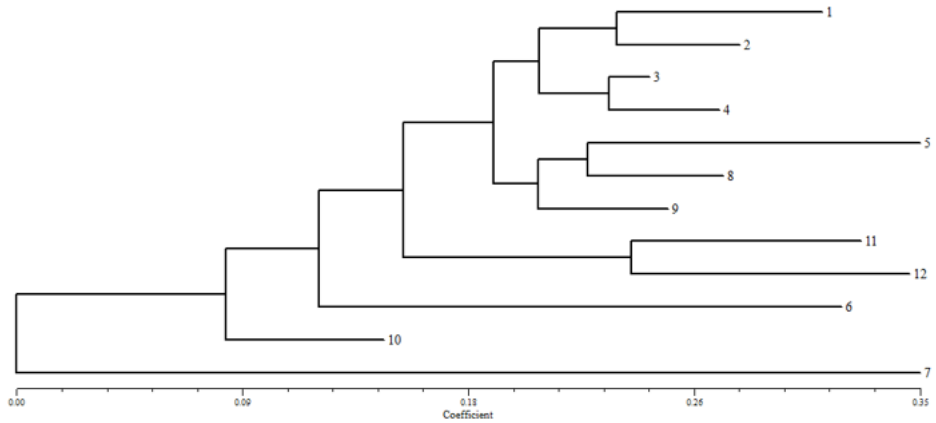

Fig. 2: The Dendrogram 12 genotypes of eggplant based on 14 RAPD primers.
1/Iraqi Wisam,**2**/Iraqi Mahaly**, 3**/Iraqi White,**4**/Iraqi Nasr,**5**/Iraqi Alrafidain, **6**/Turkish 1, **7**/Turkish 2, **8**/Turkish 3, **9**/Turkish 4, **10**/Italian 1,**11**/Italian2 **12**/Italian 3.

Conclusion:

Local and genetically imported *Solanum melongena L.*varieties had been studied using RAPD-PCR technology, where this technique revealed the genetic relationship and

genetic convergence between local and imported eggplant varieties, a number of RAPD primers had been shown to distinguish a number of samples studied dependent on unique, and absence bands, the(OP D-10) is the most important because it produced the most bands where it produced 67 bands, the analysis shows that the two nearest varieties(0.0596) were (Iraqi Nasr and Iraqi White). Still, the different varieties (0.9351) were (Iraqi Al Rafidain and Turkish 2).

REFERENCES

Al-Sugmainy R. Z, Abed, A. M, Aldoori MAM Alazawy Adnan F. (2020). Detection of phenotype and genetic effect of chloroform flax seeds extract Leishmania Tropica .897 -903.

Becerrill C, Ferrero M, Castano A. (2002). Detection by RAPD of genetic alteration in vitro: amplification and conservation conditions of D.N.A. extracts. *Toxicology* Mechanisms and *Methods*,12(2):155-67.

Brown, A., D. Anderson, K. Racicot, S. J. Pilkenton and E. Apostolidis (2017). "Evaluation of phenolic phytochemical enriched commercial plant extracts on the in vitro inhibition of α-glucosidase." *Frontiers in nutrition,* 4: 56.

Chapman, M. A. (2019). Introduction: The importance of eggplant. The Eggplant Genome, Springer: 1-10.for agricultural research in the dry area. Alppo. Syria. Spooner, D., R

Huang, Q.X.; Wang, X.C.; Kong, H.; Guo, Y.L. and Guo, A.P. (2013). An efficient D.N.A. isolation method for tropical plants. *African Journal of Biological Sciences*, 12(19): 2727-2732.

Islam Z. Siddiqua MK, Hasan M, Nahar MA, Amirulalam MD, Shamsuzzaman SM *et al.*, (2014). Assessment of genetic diversity of brinjal (Solanum melongena L.) germplasm by RAPD markers. *Research on Crops,* 15(2):416-422.

Khierallah S. M.; Alsammaraie, S. K. I., and Mohammed, H. I. (2014). Molecular characterization of some Iraqi date palm cultivars using RAPD and ISSR markers. *Journal of Asian Scientific Research,*4(9):490-503.

Knapp S, Bohs L, Nee M, Spooner DM (2004). Solanaceae – a model for linking genomics with biodiversity. *Comparative and Functional Genomics,* 5: 285-291.

Knapp, S., M. W. Chase and J. J. Clarkson (2004). "Nomenclatural changes and a new sectional classification in Nicotiana (Solanaceae)." *Taxon,* 53(1): 73-82.

Magioli, C. and E. Mansur (2005). "Eggplant (Solanum melongena L.): tissue culture, genetic transformation and use as an alternative model plant." *Acta Botanica Brasilica,* 19(1): 139-148.

Maniatis, T.; Fritsch, E.F. and Sambrook, J. (2001). In Vitro Application of D.N.A. by the Polymerase Chain Reaction, in Molecular Cloning: A Laboratory Manual. 2nd Cold Spring Harbor

Nei, M. and Li, W.H. (1979). Mathematical model for studying genetic variation in terms of restriction Endonucleases. *Proceeding of the National Academy of Science,* U.S.A. 74,5269-5273.Cited by Henry, R.J. (1997

Ranil, R., J. Prohens, X. Aubriot, H. Niran, M. Plazas, R. Fonseka, S. Vilanova, H. H. Fonseka, P. Gramazio and S. Knapp (2017). "Solanum insanum L. (subgenus Leptostemonum Bitter, Solanaceae), the neglected wild progenitor of eggplant (S. melongena L.): a review of taxonomy, characteristics and uses aimed at its enhancement for improved eggplant breeding." *Genetic resources and crop evolution,* 64(7): 1707-1722

Sambrook, J.; Fritsch, E. and Maniatis, T. (1989). Molecular cloning laboratory manual. 2nd ed. Cold Spring Harbor Laboratory New York.

Singh, P. K., S. K. Dasgupta and S. K. Tripathi (2005). Hybrid vegetable development, C.R.C. Press synoptic survey. Linnean Society symposium series.

Thormann, C.E., M.E. Ferreira, L.E.A. Camargo, J.G. Tivang and T.C. Osborn. (2004). Compare RFLP and RAPD markers to estimate genetic relationships between cruciferous species. Theoretical and Applied Genetics. 88.973-980.

Waly, C.; Bordeos, A.; Madamba, M.R. Baraoidan, M.; Ramos, M.; Wang, G.L.; Leach, J.E. and Leung, H. (2012). Rice lesion mimic mutants with enhanced resistance to diseases. *Molecular Genetics and Genomics*, 279:605-614.

Weigand, F., Baum, M., and Udupa, S. (1993). D.N.A. molecular marker techniques. Technical Manual No. 20. International center

Williams, J. G. K., Hanafey, M. K., Rafalsky, J. A., & Tingey, S. V. (1993). Genetic analysis using random amplified polymorphic DNA markers: Methods in Enzymology. *Recombinant DNA*, 68-77.

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 functional as genetic markers. *Nucleic Acids Research*, 18(22):6531-6535.