https://doi.org/10.33805/2576-8484.199
Volume 5 Issue 1 | PDF 199 | Pages 6

Edelweiss Applied Science and Technology

Research Article ISSN: 2576-8484

Molecular Classification of *Vicia faba L* Genotypes by Using RAPD-PCR Markers

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Citation: Al-Talab N, Al-mathidy A and Esho K. Molecular classification of *Vicia faba L.* genotypes by using rapd-pcr markers (2021) Edelweiss Appli Sci Tech 5: 77-82.

Received: Oct 02, 2021

Accepted: Nov 15, 2021

Published: Nov 22, 2021

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Abstract

The research included the molecular classification study of seven genotypes of the bean *Vicia faba L.* (FBSPN2, TLD1266, TLD1814, TLB1266, Luzdeotono, favad and Histal. Using the RAPD technique for DNA, as 13 random primers were used, the products of inflation were transferred within the agarose gel, and the results of the study showed the possibility of separating the genotypes from each other and determining the degree of genetic variation between them, as the primers used produced (1002) packages of them (417 normal bundles and (585) mixed bundles. The genetic differences of the studied genotypes were determined to be distinguished by the number of bundles, as they reached (28) bundles, including (13) unique bundles and (15) absent bundles. The ILB1266 genotype showed the highest number of unique bundles, which It reached 4 bundles, while the cultivar Favad showed the absence of unique bundles in it, either bundles are absent. The genotypes (ILD1266, IILB1266, Luzdeotono) were distinguished for having the highest number, which amounted to (3) bundles. As for the FBSPN2 genotype, it did not have any absent bundle, and the primers varied. Of the resulting bundle sizes, their sizes ranged between bp (1925-130), and the highest value for the genetic dimension ranged between (0.110 - 0.269), as the lowest genetic dimension was between the two structures (FBSPN2 and ILD1266), which amounted to 0.110, and the highest value for the genetic dimension was (0.2 69) between the genotypes (ILD1266, HISTAL) (ILD1266, Luzdeotono) The Dendrogram shows the separation of the studied genotypes into two main groups, and each of them into two subgroups.

Keywords: Molecular, *Vicia faba L*, Genotypes, RABD, PCR.

Abbreviations: FAO-Food and Agriculture Organization, RAPD-Random Amplification of Polymorphic DNA, PCR-Perform Random Amplification.

Introduction

The faba bean (*Vicia faba L*) belongs to the legume family. It is one of the large families. Its genera is estimated at 727 and 19325 plant species. It is considered one of the most important crops because its seeds contain a high percentage of protein, ranging between 23-37%. In addition to containing quantities of soil, starch and some vitamins, plant residues are used after harvest as animal fodder or their dry seeds are used as a source of carbohydrate Ca, P, Fe, thyamin, neyasin, ascorbic acid and protein [1], in addition to being an important source for improving soil properties by fixing atmospheric nitrogen with root ganglia bacteria that coexist. Including FAO (2003) [2]. The bean plant also contains a number of amino acids, carbohydrates and vitamins. It is also used in the treatment of many pathological conditions such as kidney failure, kidney stones and eye diseases [3]. The genus *Vicia faba L.* is characterized by a lower number of chromosomes than other types of the genus (2N = 12), as the genome size is 13000 Mbp Genome. [4]. Studying the genetic dimension or genetic variation is an important step for the success of breeding programs in developing cultivars Distinguishing and knowing the degree of genetic kinship and the development of new strains Al-Ghamdi (2009) [5] has also published many studies and research that used molecular indicators in genetic selection or genetic purity of plant varieties and strains.

Randomly Amplified Polymorphic (RAPD) indicators were used for this purpose and used this technique for its ease and accuracy And the possibility of detecting the largest area of the plant genome and its low cost compared to other indicators [6,7] where RAPD-PCR indicators contribute to early detection and selection of the desired strains and genotypes for field crops by identifying varieties that prove their superiority even before planting them in a faster and more efficient way compared to traditional methods Al-Sakmani et al. (2018) [8], and the bean plant is distinguished by a wide range of genetic variances as well About the lack of molecular studies of the barley in the Iraqi country, especially the genetic structures and the varieties whose cultivation began to spread in the city of Mosul. That is why we decided to conduct this molecular study of genetic indicators in the bean to determine the variance and genetic imprint of the studied genotypes and to find the genetic dimension to it.

Materials and working methods

Sample collection

The plant samples represented by the young leaves of the genotypes of the bean were collected after a month and a half, i.e. 45 days from the date of planting, in the vegetable research fields of the

Department of Horticulture and Landscaping, College of Agriculture and Forestry, University of Mosul, during the agricultural season of fall 2020/2021, the seeds for all genotypes were sowing at 15th November 2021 [3]. 4-5 young leaves were taken from the developing apex and placed in sterile bags, then they were transferred to the laboratory directly to conduct the process of DNA isolation from them according to the method [9,10] as shown in **Table 1.**

No.	code	Entry	Pedigree	origin
1	P1	FBSPN2	WBR 2-7 x WRB 1-4 x local	Icarda
2	P2	S 2009, 175	ILB 1266-L28/05 x sel.99latt10418	Icarda
3	P3	ILD1814	Syrian LocaLarge	Icarda
4	P4	Aguadulce	ILB1266	Spain,Icarda
5	P5	Favada Orio	France	From local market
6	P6	Histal	Espanian	From local market
7	P7	Luzde otono	Espanian	From local market

Table 1: The genotypes used in the study and their symbols used.

Genome DNA extraction

DNA was extracted from young leaves using the Kit method, according to Shehab (2020) [11], after which the DNA was purified and examined, i.e. the so-called quality control tests, which included three stages: The first stage was to determine the concentration of the extracted DNA, and the second stage included determining its purity. The DNA extracted by reading the absorbance using a Bionanodrop spectrometer at the wavelength (260-280) nm, respectively, where the third stage included the migration of DNA within the agarose gel and 13 ten starters were used as shown in **Table (2)**.

No.	Primer	Sequence5-3	Resourcer
1	OPA-01	CAGGCCCTTC	Bukhari *et al.*, (2015)
2	OPA-03	ACTCAGCCAC	
3	OPA-04	AATCGGGCTG	
4	OPA-05	AGGGGTCTTG	Szilagyi *et al.*, (2011)
5	OPA-07	GAAACGGGTG	
6	OPA-09	GGGTAACGCC	
7	OPA-10	GTGATCGCAG	
8	OPA-11	CAATCGCCGT	
9	OPA-17	CACCGCTTGC	
10	OPB-10	CTGCTGGGAC	
11	OPC-08	TGGACCGGTC	
12	OPG-14	GGTGAGACC	
13	OPE-06	AAGACCCCTC	

Table 2 shows the random prefixes used in the study.

Performing RAPD-PCR reactions

The indicators of random amplification prepared by the Korean Macrogen company and shown in **Table (3)** were used, and the volumes and concentrations of the amplification mixture were determined according to the instructions of the Korean Macrogen company.

No.	Components	Concentration	Size (ml.)
1	Master mix	2x	10
2	Primer	20 Pmol/ml	2
3	$Mgcl_2$	25 mm	1
4	Water		5
5	DNA template		2
	Total		20

Table 3: Components of a random amplification mix.

The reaction components were mixed using a micro centrifuge for 5 seconds, then the samples were placed in the thermo cycle to Perform Random Amplification (PCR) by applying the program that is specified in the device for all subsequent reactions and for all the studied genotypes [12]. After the amplification process, samples that will be electrophoresed were prepared and prepared by mixing (4) microliters of DNA Ladder from 2 microliters of loading dye, which are placed in specific holes on one side of the agarose, and then 0.5 microliters of the polymerase chain products are withdrawn PCR is carried out in karose pits without dye because it has a special color. Then the electrophoresis is carried out at 80 volts for 60 minutes. After the migration is completed, the gel is transferred and immersed in a water basin containing distilled water, then exposed to a UV source on a UV-Transilluminotor, and then photographed Gel using a high-resolution digital camera.

Statistical analysis

Estimation of the relative efficiency and discriminatory ability of RAPD primers: the efficiency of each primer was calculated by the equation of Grudman et al. (1995) [13].

Efficiency = Number of packets per initiator / Total number of multiply packets per initiator x 100

While the discriminating ability was calculated based on the following equation:

Discriminative power = (Number of differentiated packets per prefix / Total number of variant packets of all prefixes) x 100

Genetic estimation

The genetic dimension of the studied genotypes was calculated and estimated by converting the results of the RAPD indicators that appeared into tables by placing (1) for the presence of the bundle and (0) for the absence and absence of the bundle, in order to find the genetic relationship between the genotypes. Statistical analyzes were also conducted by computer using the program mentioned by Rohlf (1993) [14].

Results and Discussion

This study was used to find the genetic fingerprint and genetic dimension for seven genotypes of bean using RAPD-PCR indicators (13) for primers on genome sites and to produce different packages, revealed on agarose gel, where all the primers used showed different packages between general and differentiated and that the use of a large number One of the primers in the RADP indicators is an indicator for increasing the high accuracy in deviating the genetic dimension, and this agrees with Tahi (2015) [15] in his study using 18 primers, of which 16 were primers that produced differentiated bundles and two initiators that produced general bundles for ten genotypes of bean, and this is in agreement with most researchers who used indicators The RAPD in the study of genetic variance [16-18).

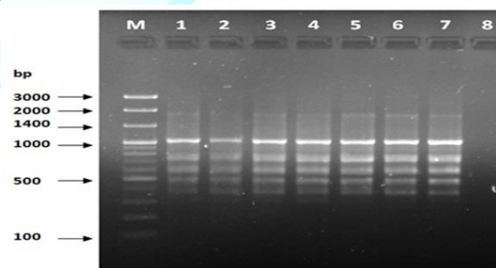


Figure 1: PCR for primer OPA-01.

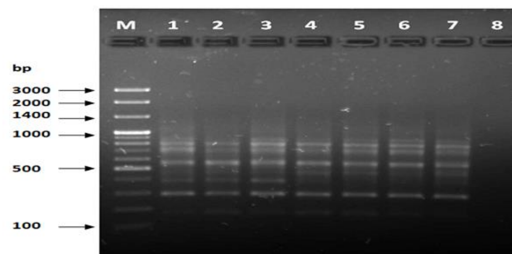


Figure 2: PCR for primer OPA-03.

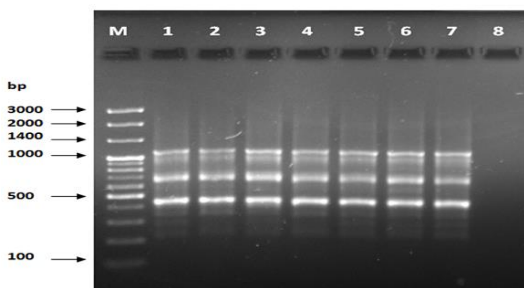


Figure 3: PCR for primer OPA-04.

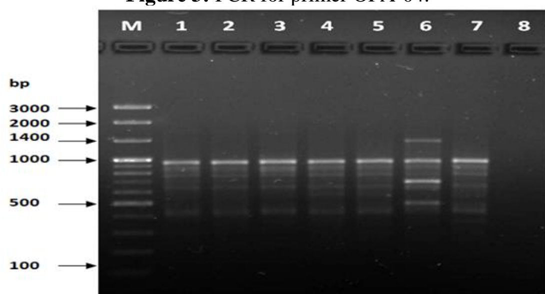


Figure 4: PCR for primer OPA-05.

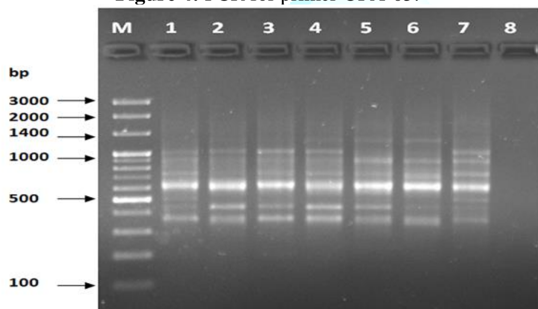


Figure 5: PCR of primer OPA-07.

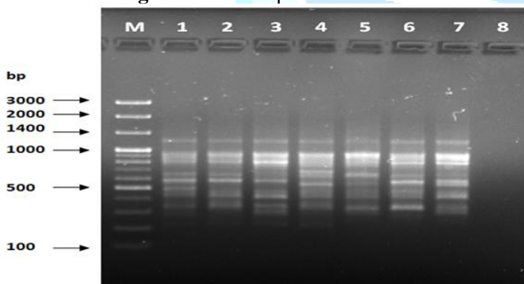


Figure 6: PCR of primer OPA-09.

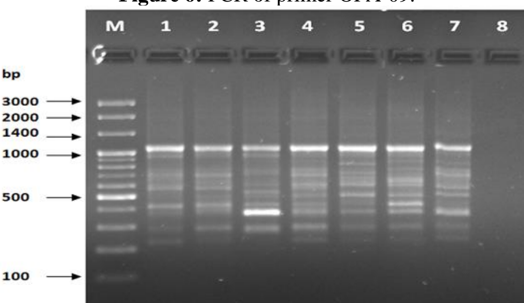


Figure 7: PCR of primer OPC-10.

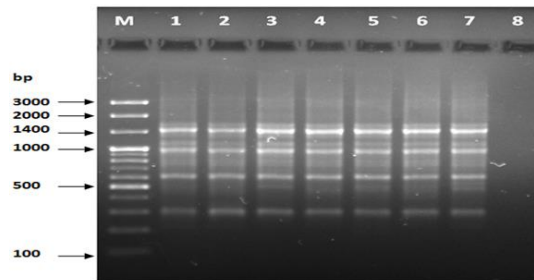


Figure 8: PCR of primer OPC-11.

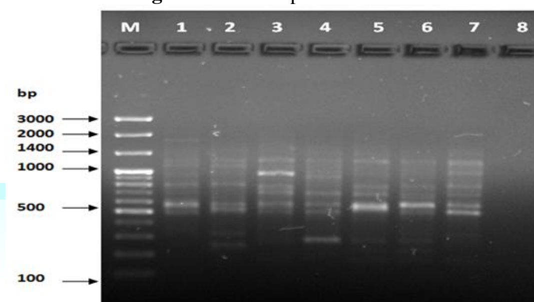


Figure 9: PCR for primer OPB-17.

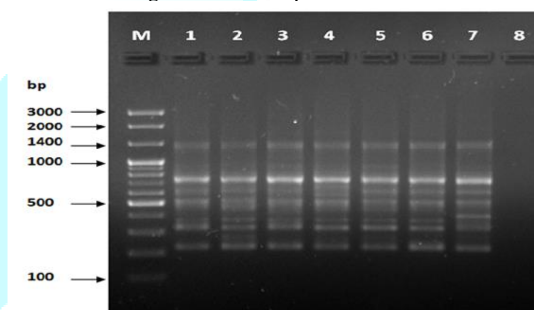


Figure 10: PCR for primer OPB-10.

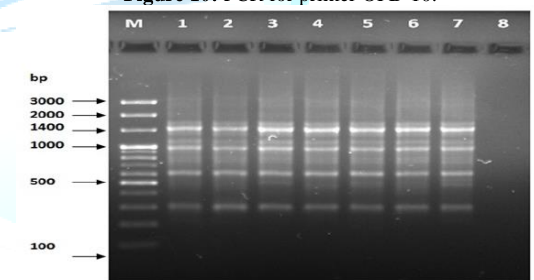


Figure 11: PCR for primer OPG-08.

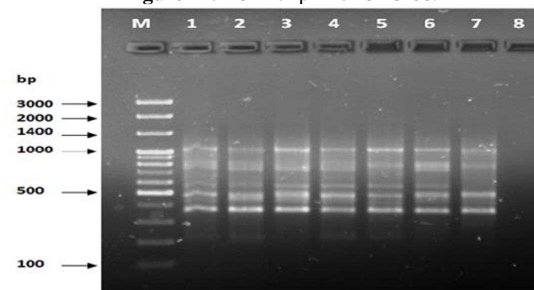


Figure 12: PCR for primer OPG-14.

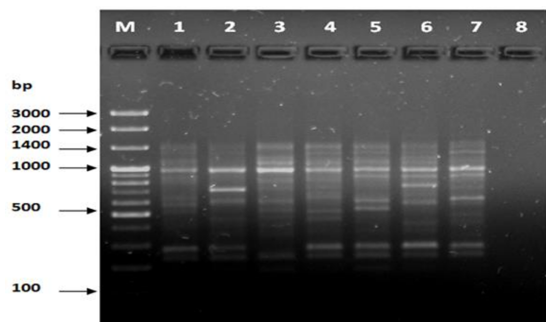


Figure 13: PCR for primer OPE-06.

Prefix results

The results of the used primers shown in **Table (4)** showed different types of packages, so the overall total of the sites that were exposed to the primers on the samples was (170) sites at a rate of (13.1) packages for each initiator, (48) of which are general sites and at a rate of (3.6) for each initiator and (122) Mutbaniya sites with a rate of (9.4) packets for each initiator. The initiator OPE-6 was distinguished by the highest number of sites that produced it, which amounted to (16) sites, while the initiators OPA-1, OPA-7 and OPG-14 recorded the least production of sites and amounted to (7) Location as shown in pictures (1, 3, 6, 12). The greater the number of divergent bundles, the greater the efficiency of the primers in determining the genetic dimension and the possibility of obtaining the genetic fingerprint of the genotypes, and this is consistent with the study of Bashher (2012) [19] which studied the imprint on 26 varieties of bean using 11 primers on the RAPD indicators, as the average of the bundles was (8.5) For each initiator, the average of the differentiated bundles is (5.5) packets. Variation occurs as a result of the occurrence of self-mutations or induced mutations that affected the distance between sites.

Genetics that occur naturally during the evolutionary process of living organisms and variation may have occurred as a result of deletion or addition at the site that binds Tingey, (1993) [20]. In addition, the total number of total bands that were produced from those sites shown in Table 4 is 1002 bands, of which 417 are general bands and 585 are polymorphic bands, as the initiator OPE-6 produced the highest number of packages produced amounted to 102 bands and the lowest number of packages for the initiator OPA -5 It reached 62 bundles, and the general variation rate of the produced primers was 56%.

One of the foundations on which the RAPD indicators depend is the number of bundles that a genome appears, which in turn represents the number of sites that the initiator finds and is associated with. The initiator Accordingly, the estimated statistics in this field indicated short random primers (9-15) bases that can be assigned (2-10) sites on the DNA of the genome of higher organisms such as plants, Borovkova et al.,(1997) [21]. Some genotypes were also distinguished by distinct bands (Unique bands) and Absalt bands, which are shown in **Table 5**. The total of distinct bands resulting from the initiators in the study was 28 distinct bands, 13 of which were unique bands and 15 absent bands, where the P4 genotype obtained the highest percentage. Of the unique bundles that reached 4 bundles, while the genotype P5 did not have unique bundles, and the absent bundles were characterized by the genotypes P2, P4 and P7 with the highest number of those bundles, which reached 3 bundles, in contrast, the genotype P1 did not have an absent bundle. Those bundles were distinguished Distinguishing and diagnostic characteristic of these genotypes because only those bundles appear in one structure, and this indicates a mutation in a particular site that led to the recognition of the initiator and the emergence of the unique bundle. As for the absent bundles, as well as the occurrence of a mutation in the locus of only recognizing the initiator in one genotype. Some previous researchers (Hormaza *et al*., 1998 [22], Al-Assi, 2002 [16] and Al-Qaisi, 2013) [17].

The produced primers also varied in the resulting bundle sizes shown in Table (5), they ranged between bp (1925-130), the lowest molecular size bp (130) in the OPB-6 initiator, while the highest molecular size was bp (1925) for the opa-17 primer, and the efficiency of the primers also varied. Proficiency in showing the variance between the studied genotypes was less efficient for the primers (OPG-14, OPA-4, OPA-1), which amounted to 5.73, while the highest efficiency was for the initiator OPE-6, which amounted to 3.11, which is shown in the pictures (2, 3, 4). Ability discriminatory for starters The OPG-6 initiator distinguished the highest discriminatory ability which was 17.57, while it was (0) zero in the OPA-4 initiator because the initiator did not show different sites.

From the results of the above RAPD, it was found that these primers have varying efficiency, but most of these primers had an appropriate efficiency, and as a result, the efficiency of the initiator may increase with the increase in the number of sites and the resulting bundles. Study through the resulting divergent beams Khierallah *et al*., (2014) [23].

Estimation of the genetic dimension based on RAPD indicators

The genetic distance was estimated from the results of the interactions of RAPD indicators among the studied cultivars of the bean using the NTSYS-PC genetic program. Version 210, which depends on the presence of bundles common to each genotype under study, and depends in its analyzes on the equation mentioned by (Nei *et al*, 1979) [24]. **Table 6** shows the values of genetic dimensions for the studied genotypes using 13 random RAPD primers. Between two structures, this indicates that the genetic distance between them should be equal to zero, and Table 7 shows the values of genetic similarity, as it represents the measure of the degree of genetic similarity or similarity between any two structures [23]. It was found through the values of the genetic dimension referred to in Table 6 that the values of the genetic dimension ranged between (0.110-0.269), where the lowest genetic dimension was between the structures P1 and P2, where it reached 0.110 and this is the highest similarity between the two structures, while the highest genetic dimension reached 0.269 between the structures genotypes (P6, P2) (P7, P2) This is the least genetic similarity between the genotypes among those values.

Cluster analysis

The studied genotypes were arranged based on the values of the genetic dimension, the genetic kinship tree, or the dendogram, and it depends on the genetic range in which the main groups are related, and therefore the presence of a section of the genotypes in a particular group indicates the extent of the similar genetic quantity to those genotypes in that group [25,26] Chart 1 shows a group analysis group for the synthesis of the genotypes of Bean *Vicia faba L* using cluster analysis, as it showed the genetic relationship between the studied structures using RAPD indicators, and it was found that they are divided into two main groups A and B, where group A was divided into two groups Major subgroup A1, A2, A1 included genotypes P7, P6, and major subgroup A2 included genotypes P5, P2 The second major group B was divided into two main subgroups B1, B2, where group B1 included genotypes P4, P3 and subgroup B2 included genotype P1 only.

Through these results, it was found that there is a degree of genetic similarity and clarity between the genotypes under study. The reason is due to two things. The first may be the differences between some structures in the phenotype, which is the result of the influence of the environment, while the second matter may show a genetically high

similarity between the structures that does not correspond to the phenotype. The reason for this is due to the non-coding regions of Abboud 2014, which is in agreement with many researchers, such as the study of (Tahir, 2015) [15], where he studied the genetic variation of ten varieties of broad beans cultured in Sulaymaniyah using 18 random primers in RAPD indicators, as well as the study of Basheer et al. 2012, [19] in which RAPD indicators were used to find the genetic variance of 26 cultivars of bean cultivars from different locations in Palestine using 26 random primers. It follows from this that the higher the number of primers used, the greater the efficiency of the indicator in determining genetic fingerprint and determining the genetic dimension, as the Increase in the number of Primers increase the number of linking regions according to the sequence of the primer used.

No.	Primer name	The number of location has produced	Number of originaLocation	Number of variation location	Total number of fragment for prime	Number of original fragments	Number of variation location	Number of unique fragment	Number of absent fragment	Variation percentage
1	OPA 1	7	6	1	73	36	37	1	0	14
2	OPA 3	8	3	5	82	30	42	0	1	63
3	OPA 4	7	7	0	84	42	42	2	1	0
4	OPA 5	8	4	4	62	29	33	0	0	50
5	OPE 6	16	3	13	102	38	64	1	4	81
6	OPA 7	7	2	5	64	27	37	0	2	71
7	OPC 8	8	5	3	77	36	41	0	1	38
8	OPA 9	11	4	7	81	31	50	2	0	64
9	OPA 10	10	2	8	79	26	53	0	0	80
10	OPB 10	9	5	4	75	35	40	1	1	44
11	OPA 11	12	2	10	85	29	56	2	1	83
12	OPG 14	7	3	4	77	35	42	0	2	57
13	OPA 17	12	2	10	71	23	48	4	2	83
	Total	122	48	74	1002	417	585	13	15	

Table 4: Results of the primers used in RAPD reactions.

			Unique and Absent Premium Packs														Primer efficiency (%)	Discriminatory ability of the primer (%)
No	Primers	molecular size (Mbp)	P1		P2		P3		P4		P5		P6		P7			
			U	A	U	A	U	A	U	A	U	A	U	A	U	A		
1	OPA1	320- 1700	0	0	0	0	0	0	1	0	0	0	0	0	0	0	5.73	1.35
2	OPA3	180- 830	0	0	0	0	0	0	0	0	0	0	0	0	0	1	6.55	6.76
3	OPA4	240-1075	0	0	0	0	0	0	0	0	0	0	2	1	0	0	5.73	0
4	OPA5	435-1500	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.55	5.41
5	OPE6	190-1500	0	0	0	1	0	1	0	0	0	0	1	1	0	1	13.11	17.57
6	OPA7	360-1250	0	0	0	2	0	0	0	0	0	0	0	0	0	0	5.73	6.76
7	OPC8	300-1500	0	0	0	0	0	0	0	0	0	0	0	0	1	0	6.55	4.05
8	OPA9	300-1230	0	0	0	0	1	0	1	0	0	0	0	0	0	0	9.01	9.46
9	OPA10	230-1080	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8.2	10.8
10	OPB10	130-1440	0	0	1	0	0	0	0	1	0	0	0	0	0	0	7.38	5.41
11	OPA11	224-1420	0	0	0	0	0	0	1	1	0	0	0	0	1	0	9.84	13.51
12	OPG14	220-1000	0	0	0	0	0	1	0	0	0	0	0	0	0	1	5.73	5.41
13	OPA17	244-1925	1	0	2	0	0	0	1	1	0	1	0	0	0	0	9.84	13.51
total			1	0	3	3	1	1	4	3	0	1	3	2	2	3	100%	100%

Table 5: Distinguishing packets, primer efficiency, and discriminating ability of the primer used.

	P1	P2	P3	P4	P5	P6	P7
P1	0						
P2	0.11	0					
P3	0.164	0.193	0				
P4	0.242	0.131	0.209	0			
P5	0.17	0.239	0.191	0.133	0		
P6	0.194	0.269	0.186	0.218	0.2	0	
P7	0.223	0.269	0.186	0.231	0.172	0.229	0

Table 6: Values of the genetic dimension of the genotypes of the bean plant.

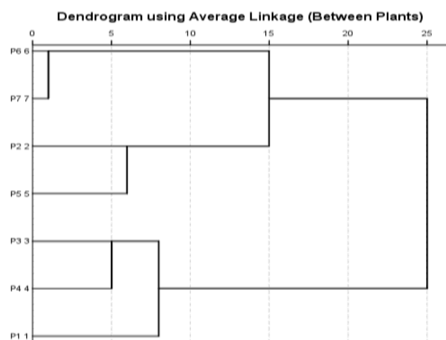
	P1	P2	P3	P4	P5	P6	P7
P1	1						
P2	0.895	1					
P3	0.848	0.824	1				
P4	0.785	0.877	0.811	1			
P5	0.843	0.787	0.826	0.875	1		
P6	0.823	0.764	0.83	0.804	0.818	1	
P7	0.8	0.764	0.83	0.793	0.842	0.795	1
S.A.	0.832	0.818	0.828	0.824	0.813	0.805	0.804

Table 7: Genetic similarity values for the genotypes of Beans.

	P1	P2	P3	P4	P5	P6	P7
P1	0						
P2	0.105	0					
P3	0.152	0.176	0				
P4	0.215	0.133	0.189	0			
P5	0.157	0.213	0.174	0.125	0		
P6	0.177	0.236	0.17	0.196	0.182	0	
P7	0.2	0.236	0.17	0.207	0.158	0.205	0

Table 8: values of genetic variation for the genotypes of the bean.

What determines the genetic proximity or distance between genotypes is the number of shared bundles, the greater the number of those the bundles say the genetic dimension. Those shared bundles indicate a similarity in the genetic material in that region of the genome for the studied genotypes, which may represent the similarity in phenotypic traits or in other traits related to productivity, reproduction, resistance to diseases, or similarity in genetic structure to the requirements of an environment suitable for growth or other characteristics other traits.



Scheme 1: Genetic relationship of bean genotypes based on the genetic dimension of RAPD indicators.

Conclusion

This study indicates that the primers used produced (1002) packages of them (417 normal bundles and (585) mixed bundles, The ILB1266 genotype showed the highest number of unique bundles, which It reached 4 bundles, The Dendrogram shows the separation of the studied genotypes into two main groups, and each of them into two subgroups.

Acknowledgments

The authors would like to express their utmost thanks to the Univ. of Mosul/College of Agriculture and Forestry for the facilities they have provided that have been helpful in improving this research paper's quality, and for DNA laboratory for analysis of DNA.

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