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Identification The Genetic Dimension For Several Genotypes Of Cowpea (Vigna Unguiculata L.)

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Abstract

Eight genetic types of cowpeas were used which (Max , JA20 , JA10 , Ramshorn , GLS/14 , Italia , Black eyes , AL- hokool) to study genetic diversity and determine it،s genetic dimension, DNA is isolated from the leaves of the genetic types and the fit amount is obtained which conceutration and parity , (15) Random praimer form RAPD pointers were used , The total of pundles produced was (127) bunds , (60) were dissimilar bundles , The (OP-G9) Primer had the highest formal multiplicity which (85.71) , while the lowest of Formal multiplicity which (%0) in (OP – VO2) Primer . (OP – 1O2) Primer had the highest efficiency (9.45) , (OP – VO2) had the Lowest discriminative ability which was (%0) and the highest discriminative ability was (11.67) in (OP – 1O2) , (OP – G11) Primers , The study also showed that the values of genetic dimension was (0.05 – 0.21) , The lowest genetic dimension in (V5 – V7) types which (0.05) , and that was the highest genetic similarity between the two types , while The highest genetic dimension was (0.21) between (V4 – V1) types and that was the lowest genetic similarity.

Keywords: genetic dimension, cowpea, Vigna unguiculata L.

Introduction

The cowpea belong to Fabaceae Family, and it is one of the important economic crops for it high nutritional value Matlab etal (1989), The cowpea is plant in spring and autumn (Al-Rikabi and Jasim , 1981), The cowpea under cultivation in the world is estimated (12.5) million hectares (16800) Dunams of cowpea were planted in Iraq in (2015) with yield of (1272.2) K.g . Dunam^{-1} (Yearly Statistical Abstract , 2015) , In recent years there has been increased interest in the study of genetic diversity which is important in plant breeding programs for the purpose of selecting parents for hybrids and describing the genetic material molecular indices based on RAPD analysis have there for been studied , especially after qualitative advances in the use of molecular methods in recent years , for describing genetic types and the differences between varity and wild species cowpea as well as determining the degree affinity between varities , for that there many studies have been done In assessing genetic diversity in five RAPD, Ahmed etal (2010) indicated that (16 bundles) were shown at arte of four bundles per primer , with a percentage of Polymorphisms (37.5%) , In studying the genetic variation of (14) varities of cowpea and by used (13) primer by RAPD , mendes etal . (2015) founder that (257) sites with polymorphisms (96.11%) and the molecular size for primers was (200 – 2000 pb) . Patil etal . (2015) while studying the genetic variation of (20) varities of cowpeas by the RAPD technique , they observed (194) identification sites , (152) which were formal sites , and an average percentage of multiple forms was (78.83%) , The rate of genetic similarity

among the species studied was (0.53 – 0.79) . Nagalakshmi etal. (2017) reported on the genetic diversity of (13) varities of cowpea using (6) primers , The primers in this study showed (39) Identification sites , The polymorphism rate was (71.42 for OP – A12 Primre) and (100%) for (OP – A13 , OP – A6 , OP – A19) Primers , The results which (Shahatha , 2018) mentioned it when studied genetic diversity on the cowpeas crop using primers the primers produced (73) Identification sites , of which (56) were general and (17) were heterogenus (differentiation) , with (540) of total bundles (448) were general and (92) were dissimilar . The study showed alower genetic distance between (V1 , V8) varieties were the highest genetic distance between (V5 , V4) , the lowest efficiency of the primers was (2.96) while the highest was (8.88) , the highest discriminative potential was (38.04) and the lowest was (0) . The objective of the study is to determine the genetic dimension of the genetic types involved in the study according to the RAPD biomarkers , in order to provide an indication and explanation of the genetic behavior of these hybrids and an acceptable explanation of the hybrid power .

2-: Research Materiales and Methods

The study was conducted in (wahaj aldna) Laboratory , Plant samples were collected for to (4 – 5) young infection – free Leaves were taken from agrowing top for plant and placed in special labeled bags that were transferred directly to the laboratory to isolate DNA from the genetic types described in (Table 1).

(Table 1) The genotypes involved in the study

The sequence	Genotype
1	ALhokool
2	Black eyes
3	Italia
4	Gls /14
5	Ramshorn
6	JA10
7	JA20
8	Max

2-1 : The Method of Extraction DNA

1 (gm) was taken of the young leaves and washed with distilled water , then dried and then added liquid nitrogen on them , then the parts of leaves are crushed until they turn in to white powder , then the powder is trans ferred to sterile tubes and (8 ml) of the extraction solution (dissolution solution) is added at atemperature (65 m) and tube is turned until it is homogeneous , then it is trans ferred to the incubator in the water bath at the temperature of (65 m) for a period (90 – 60 minutes) , then the tube is left for (15 minutes) to gain room temperature and then added (5ml) from (chloroform) and shaking for (15-20 minutes) , and placed in the centrifuge at (4000 cycle / minute) then the upper fine water layer is drown by aspecial absorbent , the same amount of the same solution is added and the former phase is returned under the same conditions . Then the refrigerated isopropanol solution is sdded and the tubes are calmly flipped when awhite mass appears , which is fliments of DNA and transferred it to another tube containing (300 – 400) microliters of solution , and the filaments are kept at temperature (-20m).

2-2 : RAPD – PCR reactions

Agarose gel was prepared at a concentration (1%) to transfer the DNA and obtain a concentration of 1% (1g) of the agarose powder was dissolved to (100 ml) of (TBE 1X) using heat source , cooled to (55m) , then poured in to aspecial sink so that bubbles are not formed , Then is left until the gel solidifies and then it is placed on electric with voltage difference of (3) volts / cm^{-1} after the electrodes are placed , where the transfer should be towards the positive electrode has to take (1.5 – 2 hours) (15) primers was used randomly as shown in table (2) .

This process is based on the RAPD – PCR interaction program

1- First initial denaturation of (95m) for a period of (3) minutes .
2- Ordinary denaturation at atemperature of (95 c) for (1minutle).
3- cohesion (primer bonding) at atemperature of (36c) for one minute .
4- Elongation at atemperature of (72c) for one minute .
5- Final elongation at atemperature (72 m) for (10) minutes .

After the program is completed , the pipes are lifted out of the device and kept frozen , with (5) microliters drown in , and the mixture is loaded in to a (2%) agarose gel pit then added ethidium bromide for one hour with moved it after exposed to the uv source on the ur_ transilluminator and photographed the gel using a high – definition digital camera and stored the images on computer for statistical analysis.

2-3: Statistical analysis of the result of RAPD

The bands appearing in the gel were coding by (1) and (0) which presented appering of bands and not appering (Nei and Lei , 1979) .

2-4 : Estimate the efficiency discriminatory ability and formal pluralism of the primers used in the RAPD

1- Primer efficiency = number of bands produced from each primer / Total bands of primer X 100 .

2- percent of discriminating ability per primer = number of bands wich formal pluralism per primer / number of bands which formal pluralism for all primer X100 .

3- Percentage of formal pluralism bands = number of formal pluralism bands per primer / Total of bands for primer X100 .

4- formal pluralism is determined by horizontal line of sight of all sampels of on primer , the differented bands one from another was consider like one bands (with Formal pluralism) . (AL – Assie etal , 2012) , (Bian , Mahmudal , 2010) .

(Table 2) Primers used in the PCR - RAPD study

	Primer	Sequence
1	Op-MO6	CTGGGCAACT
2	OP-R14	CAGGATTCCC
3	OP-M20	AGGTCTTGGG
4	OP-I02	GGAGGAGAGG

5	OP-V02	AGTCACTCCC
6	OP-V19	GGGTGTGCAG
7	OPG-2	GGCACTGAGG
8	OP-M14	AGGGTCGTTC
9	OP-PO4	GTGTCTCAGG
10	OP-H01	GGTCGGAGAA
11	OPG-6	GTGCCTAACC
12	OPG-9	TGTAGCTGGG
13	OPG-11	TCCGCTGAGA
14	OPG-16	CAAGGTGGGT
15	OPA-8	GTGACGTAGG

3-1 : Result of the RAPD primers which used in study

The result shown in (table 3) pointed to that the primers in study produced (127) bands which presented total bands , (60) bands was differentiated from total , The primer (OP – 102) Was produced the highest number of bands (12) followed by primer (OP – a8) which produced (11) bands , The primers (OP- G6 , OP – G 9 , OP – PO4 , OP V02 , OP – M20) Produced the lowest number of bands which was (7) bands , while the primers (OPG – 11 , OP – 102) Produced the highest number of differentiated bands which was (7) bands and the lowest in the primer (OP – V02) which was (0) , The greater number of bands mean that more efficient of the primer in determining the genetic dimension and the fingerprinting of ,

(Table 3)) Shows the products of the prefixes from the total and varied packages with their

efficiency ratios and their differential ability for the studied samples .

Name of the initiator	The total number of packages	The number of varying package	For initiator plurality %	Of Initiator efficiency %	Of the initiator discriminating power %
op-m06	8	3	37.5	6.30	5
op-r14	9	6	66.67	7.09	10
op-m20	7	4	57.14	5.51	6.67
op-l02	12	7	58.33	9.45	11.67
op-v02	7	0	0	5.51	0
op-v19	8	2	25	6.30	3.33
op-g2	9	2	22.22	7.09	3.33
op-m14	7	3	42.86	5.51	5
op-p04	7	4	57.14	5.51	6.67
op-G9	7	6	85.71	5.51	10
op-G11	10	7	70	7.87	11.67
op-G16	9	4	44.44	7.09	6.67
op-a8	11	6	54.55	8.66	10
op-h01	9	4	44.44	7.09	6.67
op-g6	7	2	28.57	5.51	3.33
Total	127	60			

the study genotypes and that similer with the result of (Basheer etal , 2012) , The variation may be produced as a result of induced mutation or self – mutation that influence the distance between genetic sites which happened in development process in living organisms .The variation may have been caused by addition and deletion at sities to which is associated (Tingey and Tufo , 1993) The highest formal pluralism produced in (OP –G9) Primer which was (85.71%) followed by (OP – 102) was (58.33%) , The lowest formal pluralism was (0) in (OP – V02) Primer , while (OP – 102) recorded the highest efficiency (9.45%) , The primers (OP – G6 , OP – G9 , OP – PO4 , OP – M14 , OP – V02 , OP – M20) recorded the lowest efficiency (5.51%) from the result we can concluded that when the differential bands in creased the primer efficiency

in creased too . The discriminating ability of the primers varied , the lowest of it recorded in (OP – V02) primer (0) , which the highest was (11.67%) in (OP – G11 , OP – 102) and that agree with (Sahatha , 2018) .

3-2: Genetic dimension based on moleculer indicators

The phenol genetic dimension was estimated using genetic program NTSYS – PC version 2.1 , which is based on the amount of similarity between the genetic types (varieties) and it is analyses based on (Nei , 1979) , The similar genetic dimision value in (Table 4) , when a random primers (15) is used , this indicates that the genetic dimension between the two types (varieties) should be equal to zero , Relativity represents ameasure of the genetic similarity of any two individus khierallah etal (2014).

(Table 4) The values of the genetic dimension of the eight genotypes included in the study depending on the molecular indicators .

8	7	6	5	4	3	2	1	
							0	1
						0	0.14433	2
					0	0.12264	0.1215	3
				0	0.11628	0.097436	0.20812	4
			0	0.13744	0.087719	0.10577	0.12381	5
		0	0.058296	0.12381	0.10132	0.11111	0.12919	6
	0	0.057269	0.052632	0.10698	0.060345	0.11321	0.1215	7
0	0.09009	0.10599	0.10092	0.15122	0.11712	0.13861	0.18627	8

so they are equal when there is no genetic variation between genetic genotypes , and this is shown when individuals share common bands and there is no differential between them when more primers are used for different areas of correlation depending on the sequence of the primer . what determines the genetic dimension between genotypes is the number of samller bands , The greater numbers of samller bundles mean , The dimision between the genotypes is decrease con verdly bundles that share each other indicate that the genetic material in genomic region and that may represented similarities in phenotype , or it could be the similarity of the non – coding regions that have no genetic expression . while distance genetic genotypes that share the lowest number of bundles and that may be resulted to differentiation in nucleotides sequences in genom (al-zhairi ,2014) . The values of the genetic dimension ranged from (0.05 – 0.21) , with the lowest genetic dimension (0.05) which was between (V5 , V7) and that the highest similer between the two genotypes in study . The highest genetic dimension was (0.21) between (V1 , V4) and that considered the lowest genetic dimension between the two genotypes , The value of the genetic dimision is shown in (Table 4) .

The scheme (1) The genetic relationship between the eight genotypes based on molecular markers

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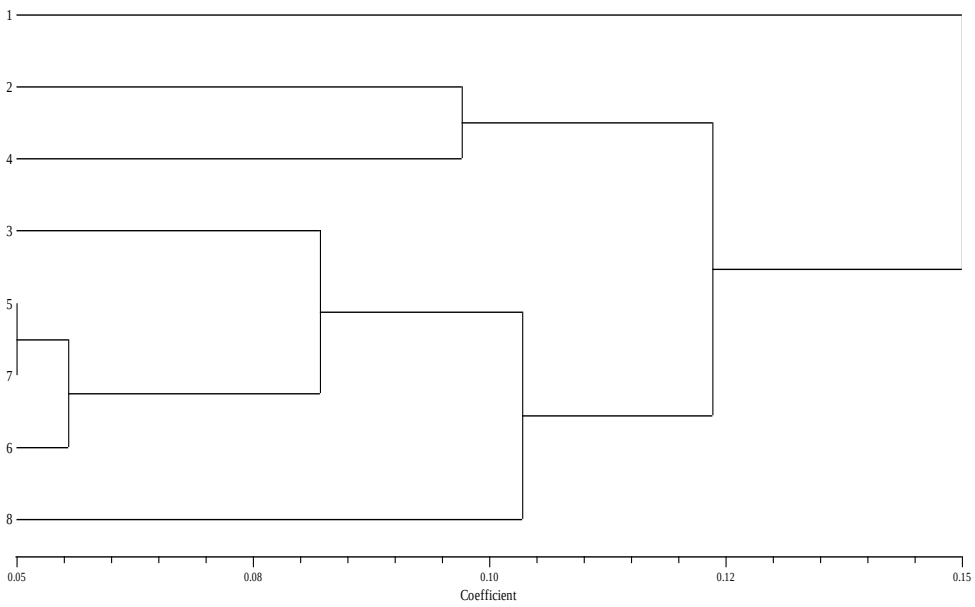
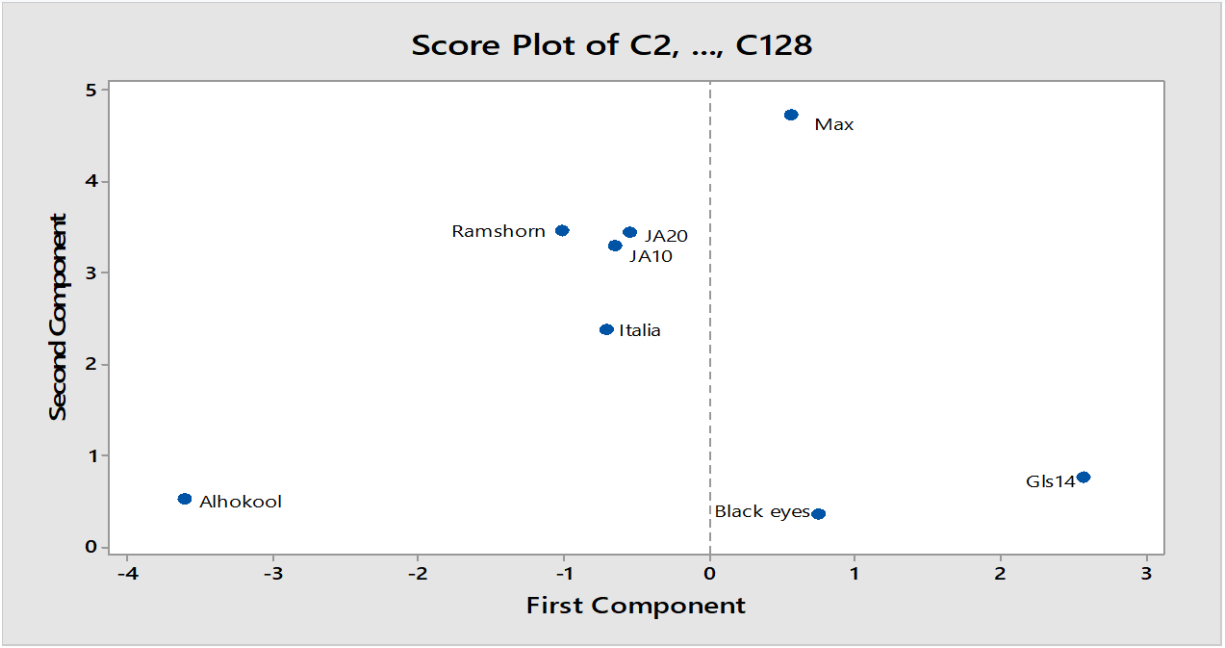


The scheme (2) PCA analysis using RAPD



3-3 : Cluster analysis

The arranged of genetic genotypes by bands on the value of genetic distance in genetic tree or cluster analysis scheme depends on the genetic dimension which the major groups are related , thus , the existence of a section of genetic genotypes is indicates the quantum of genetic similarities of this types (varieties) in that group (al-zaidi etal , 2016) . The cluster analysis in scheme (1) for eight genotypes of cowpea explain that two major groups were split in to two groups . The first group involved (AL- hokool) type while the secondary major group split to two subgroups , the first of them contained (Black eyes , GLS / 14) types while the secondary (sub group) classified to two ander subgroups , The first involved the (max) type while the sub second consist of (Itali , Ramshorn , JA20 , JA10) . The result show a degree of genetic similarity or different between the genetic genotypes involved in the study , due to two factors , firstly the difference between the eight genotype in phenotypes may be a result of environment impact , which is happened in many breeding programs and the secondary yea son may be belong to the high genetic

similarity between the genetic genotype and that different from the first yea son which depend on phenotypes and that due to the similarity of coding and non – coding genes (abboud etal , 2014) . and that agree with several researchers who used RAPD indicators like (Sahatha , 2018) . when he studied genetic diversity in a cowpea crop using (15) random primers on eight genotype and the primers have shown competence in finding the genetic dimension (patil , etal. 2015) . when they studied the genetic diversity by used (20) primers on (20) varieties of cowpea . and the componeut for all consistent which depend . the result of primers bundles which is also an indicator of the relationship between the genetic genotype in this study (scheme 2) and it explain the result of cluster analysis which split to four groups major group involved of (AL- hokool) type and the second major group consist of (Itali , JA10 , JA20 , Ramshorn) , while the (Max) type was in sub - group and the genotype (Black eyes , GLS / 14) were another sub – group

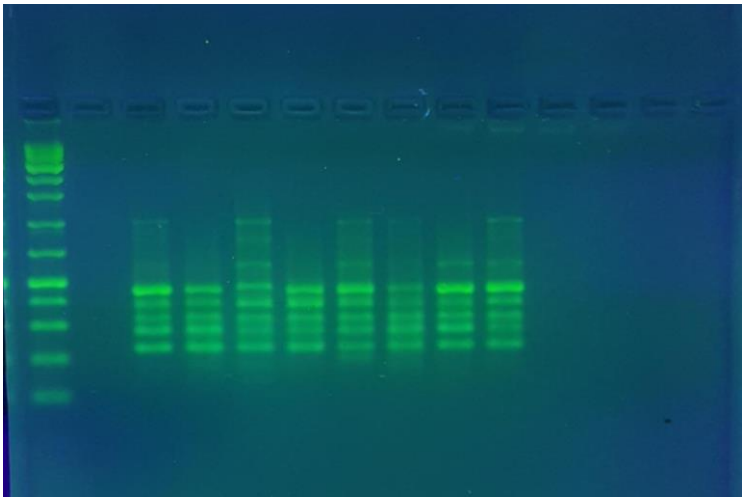


Figure (1) PCR product of primer OP-MO6The product was electrophoresis on 2% agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hours. N: DNA ladder

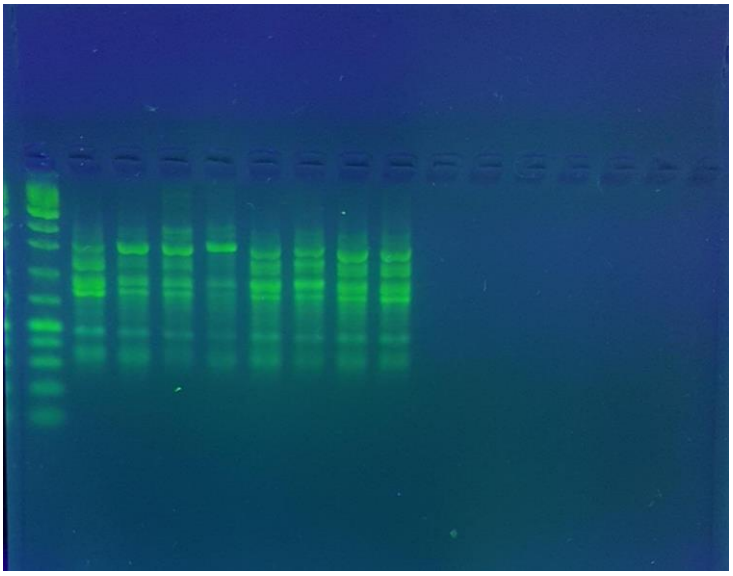


Figure (2) PCR product of primer OP-R14The product was electrophoresis on 2% agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hours. N: DNA ladder.

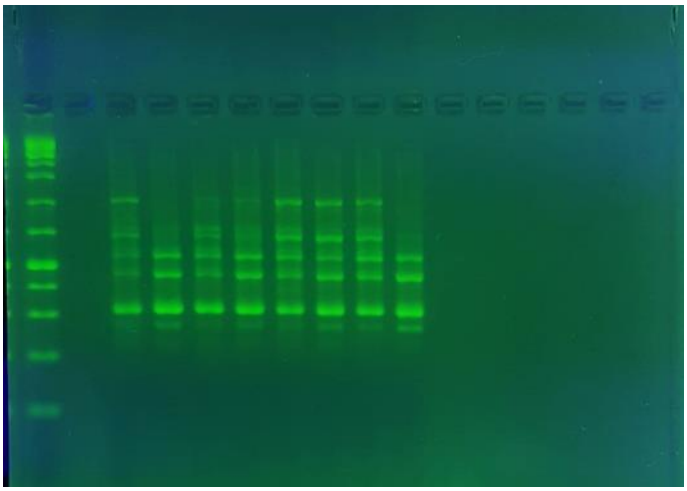


Figure (3) PCR product of primer OP-M20 The product was electrophoresis on 2% agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hours. N: DNA ladder .

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