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Genetic Variability on some Genotypes of Chickpea (*Cicer arietinum* L.) Using RAPD-PCR Technique

[1]*Sahar F.M. El-Hefnawy and* [2]*Ahmed G. Helal*

[1]Faculty of Home Economic Al-Azhar University Nawag, Tanta, Egypt
[2]Field Crops Research Institute, Bahteem, Egypt

Abstract: The present investigation was carried to evaluate twenty four genotypes of chickpea to seven important agronomic traits. Twenty four genotypes were cultivated in 2016/2017 winter season, at Bahteem Research Station, in a randomized complete block design with four replications. Selection was practiced on the bases plant height, number of branches, number of fertilized nodes, number of pods per plant, number of seeds per plant, seed weight per plant, 100-seed weight. The results showed highly significant variations within genotypes, revealed a wide genetic variability for the studied traits and the possibility of genetic improvement, using genes in chickpea. Genetic diversity has been studied using RAPD-PCR technique and eight primers were used on five selected chickpea genotypes. The number of bands for each primer varied from a minimum of 1 band for the primer OPK08 to a maximum of 8 bands for the primer OPE12, with a polymorphic percentage of 6.7% to 31.6%. Genetic differences were observed among five of genotypes of chickpea 1, 6, 8, 10 and 12 respectively were selected for RAPD-PCR analysis. The results also showed that the degree of genetic similarity ranged between 0.78 and 0.97 where the highest was 0.97 between the genotypes 8, 10 and the lowest was 0.78 between genotypes 1, 12.

Key words: Chickpea (*Cicer arietinum*) · RAPD-PCR · Polymorphism · Genetic Similarity

INTRODUCTION

Chickpea (*Cicer arietinum*, L.) is one of the important plants in the world with the chromosome number of 2n = 16, in addition to growing for seed in Egypt. It is widely considered as a good source of protein, starch, cellulose, Chickpea is a rich source of vitamins, minerals fiber, nutrients and may provide a range of health benefits. It helps increase satiety, boost digestion, keep blood sugar levels stable, increase protection against metabolic syndrome and heart disease and more. Plus, it has low calories and filling. Starch in chickpeas is digested slowly and supports more stabilized blood sugar levels [1]. Furthermore, chickpea reflects in improving soil fertility, especially in dry areas, hence it contributes one of the most efficient fixers of the atmospheric nitrogen [2]. Genetic variability of crops plays an important role in sustainable development and food security. It is also important to selection the genotypes used in plant breeding programs when taken to improve the crop variety [3]. The use of molecular technologies can reduce the complexities of introducing a number of desirable traits into a single genotype. Likewise, molecular indicators can be used effectively in analyzes of genetic diversity and estimating genetic similarities [4]. Shengwu *et al*. [5] used RAPD markers for evaluation of genetic diversity of *B. napus* germplasm for China and Europe and reported the occurrence of a considerable genetic variation between Chines and European accessions. One of the important technologies Inter Simple Sequence Repeats (ISSR) that depends on the Polymerization Chain Reaction (PCR) and is considered one of the ideal technologies and it gives stable results even if it is implemented in repeaters or in multiple and fast places. It also requires a small amount of DNA, this technique was used to study the genetic diversity of rice [6] and potatoes [7]. Bhadana [8] worked to studying of genetic affinity between 14 species from sex *Cicer* 8 perennial species and 6 annual species including the cultivated species *C. arietinum*. When using a technique RAPD in the study, 12 primers were given in all 234 bands an average of 19.5 bands per primer. Genetic kinship tree

Corresponding Author: Sahar F.M. El-Hefnawy, Faculty of Home Economic, Al-Azhar University Nawag, Tanta, Egypt. E-mail: saharzena@hotmail.com.

showed that the species (Jaccard similarity index) was drawn using the Jacquard coefficient of similarity it was separated from each other according to the nature of growth and the geographical area, as it turned out that the species *C. reticulatum* It was nearest genetically to the species *C . echinospermum* of the rest of the other studied species, which indicates the possibility of transmission of genes between species. The aim of this research was to study genetic diversity in some genotypes of cultivated chickpeas by using RAPD-PCR and determine the degree of genetic similarity between them.

MATERIALS AND METHODS

Twenty four genotypes of *Cicer arietinum* L. were obtained from Agriculture Research Centre, Field Crops Research Institute, Genetic Resources Department, Bahteem, Egypt. The plan was sown in the season 2016 / 2017 at Bahteem Research Station in a randomized complete block design with four replications. The land plans twelve lines in two Kasbahs, each line consisted of one ridge of 3m length and 60 cm width. Hills were spaced at 20 cm with two seeds in hill and 32 plants per square meter, this means 140 thousand plants per acre. The experiment was cultivated in field plots with four replications. Each row contained 3m, the meter contained fifteen seeds. The average line included approximately 50 seeds and two hundred seeds for one variety. Ten plants were randomly assigned to each replication and the data were recorded to plant height (cm), number of branches/plant, number of fertile nodes, number of pods/plant, number of seeds/plant, seeds weight/plant, 100-seed weight (g). The data were subjected statistically analyzed, the analysis of variance (one way ANOVA) was done according to Snedecor and Cochran [9]. After that, five genotypes were selected to RAPD-PCR analysis, three of them had the highest estimate for the seven studied characteristics (6, 8 and 10) and two were the lowest for the studied characteristics (1and 12).

Seed Sterilization and Cultivation: Seeds were sterilized the by soaking them in 70% ethanol for 1 min, then transferred to three bowls each containing sterile distilled water, left in each bowl for 5 min and transferred. These seeds were placed in a container containing 5% chlorox for 5 min, then transferred once another to soak in distilled water three times each 5 min and then sown the seeds in special pots for 2- 3 weeks ago. Fresh leaves were taken in order to extract the DNA for genetic study.

Table 1: Origin of Chick pea genotypes were used in the present study and seed's size RAPD-PCR analysis

No.	Genotypes	Origin	Remarks
1	L.1	Land races El-menia	Small seed
2	L.3	Land races Assiut	Small seed
3	Giza 2	Egyptian variety	Small seed
4	Giza 531	Egyptian variety	Small seed
5	Giza 3	Egyptian variety	Small seed
6	Giza 195	Egyptian variety	Large seed
7	Giza 88	Egyptian variety	Small seed
8	Giza 4	Egyptian variety	Large seed
9	616	Syria ICARDA Aleppo	Large seed
10	F- 99-1- C	Syria ICARDA Aleppo	Large seed
11	Fo7- 196- C	Syria ICARDA Aleppo	Large seed
12	F79- 221-C	Syria ICARDA Aleppo	Large seed
13	Fo6- 74- C	Syria ICARDA Aleppo	Small seed
14	1131	Syria ICARDA Aleppo	Small seed
15	Fo8- 40- C	Syria ICARDA Aleppo	Large seed
16	Fo1- 31- C	Syria ICARDA Aleppo	Large seed
17	Fo5- 162- C	Syria ICARDA Aleppo	Small seed
18	Fo5- 19- C	Syria ICARDA Aleppo	Large seed
19	Fo7- 182- C	Syria ICARDA Aleppo	Large seed
20	Xo7- H- 11	Syria ICARDA Aleppo	Large seed
21	Fo3- 81- C	Syria ICARDA Aleppo	Large seed
22	Fo3- 119- C	Syria ICARDA Aleppo	Large seed
23	500 A- E	Land races Mallaney	Small seed
24	600 B- E	Land races Mallaney	Small seed

DNA Isolation: Total DNA was extracted from young leaves using the acetyl tri methyl ammonium bromide (CTAB) method [10]. Approximately 100- 150 mg of leaves were ground using 600 µL of preheated (65°C) extraction buffer (2% CTAB, 20 mM EDTA, 100 mM Tris-HCl, 1.4 M NaCl, 2% polyvinylpyrrolidone (PVP), 0.2% mercaptoethanol), transferred to a centrifuge tube (2 mL) and incubated for 30 min in a 65°C water bath, the samples were inverted every 5 min. An amount of 600 µL of chloroform-isoamyl alcohol (24:1) was added and mixed by inverting the tubes carefully for 10 times and the cells were centrifuged at 12000 rpm for 10 min at room temperature. The supernatant was collected and carefully mixed with two third volume of ice cold Isopropanol and the DNA samples were collected by centrifuging for 10 min. RNaseA (10 µg/ml) was added to the 50 µl of TE buffer (10 mM Tris and 0.1 mM EDTA) prior to dissolving the DNA to remove any RNA in the preparation and the mixture was incubated at 37°C for 30 min. A volume of 100 µl and 750 µl of 3 M sodium acetate and ice-cold absolute ethanol were added respectively.

Table 2: List of RAPD primers screened

Primer No.	Primer name	Sequence
1	OPE12	TTATCGCCCC
2	OPK08	GTGCAACGTG
3	OPK10	CACAGGCGGA
4	OPK19	GTGTGCCCCA
5	OPD08	CAGCACTGAC
6	OPO07	CCTCCAGTGT
7	OPO08	TTGGCACGGG
8	OPD07	CACCGTATCC

The DNA was collected by high-speed centrifugation for 10 min and then carefully washed with ice-cold absolute and 70% ethanol and centrifuged at 12000 rpm for 10 min. Finally, the samples were dried at room temperature and dissolved in 50-100 µl of TE buffer. The quality and concentration of DNA were determined by EMPLEN photometer P330.

PCR Condition: For DNA amplification, eight decamer RAPD (Table 1) primers (Operon, Germany) were used PCR was performed as follows: 94°C for 5 min; followed by 35 cycles of 94°C for 1 min, specific annealing temperature (Ta) according the primer sequence for 30 sec and 72°C for 3 min and the final extension step at 72°C for 10 min. Amplification was carried out in MJ Mini Bio RAD, thermal cycler in 25 µl reaction volume containing the following reagents: 1.0 µl of dNTPs (10 mM), 1.0 µl of $MgCl_2$ (25 mM), 5 µl of 10x buffer, 1.0 ul of primer (10 pmol), 1.0 µl of DNA (25 ng/µl), 0.3 µl of taq polymerase (5u/µl) and 15.7 dd.H_2O. The RAPD products were electrophoresed in 1.5 % agarose gel containing Red safe dye in TAE buffer (40 mM Tris-acetate, 20 mM glacial acetic acid, 1 mM EDTA, pH 7) at 75 V. The amplified bands were detected on UV-trans-illuminator, photographed by Gel documentation system UVITEC, UK). and according to analysis by Phoretix program 1D gel analysis software version 4.01. The genealogy tree was drawn between the five selected genotypes of chickpea (1, 6, 8, 10 and 12) Relying on the Jacquard coefficient of similarity (Jaccard similarity index) according to Jaccard [11]. It was separated from each other according to the nature of growth and the geographical area.

RESULTS AND DISCUSSION

Analysis of variance (ANOVA) for all studied characters across 2016/ 2017, out of the seven characters studied assuming least effect of changes on the genetic performance of the entries.

Data showed highly significant differences for seven studied character among genotypes, while in replications exhibited highly significant differences for all traits except number of branches/plant and number of fertile nodes. These results are in agreement with Mahdi and Shahram [12].

The success of breeding programs depends on the diversity within the clans and the selection of the desired inherited characteristics with the aim of genetic improvement.

The mean performance of twenty four genotypes are presented in table 4, it appears that genotype four was the highest plants (128.75 cm) followed by genotype six (100 cm) and the shortest length of genotype fourteen (56.25).

As for the number of branches, it was found that the sixth genotype was higher in the number of branches (10.25) followed by the genotype twenty three recording 8.5 branches and the lowest genotype in the number of branches was eleventh (2.75). The genotypes; two, seventeen and twenty-two were equal recoding 3.25 branches. On the other hand, the numbers of branches of the genotypes 13, 14, 15 and 16 were equal in, 4.25). Regarding the number of fertilized nodes, the fourteenth genotype had the highest (12.25) followed by the 23 genotype (11.25). Both ninth and twenty-fourth genotypes were equal (10.25) as well as the fifteenth and sixteenth genotypes (9.25) and the lowest number of fertilized nodes was in genotype in the twelve (3.75) The number of pods per plant was higher in the eighth genotype (129.25) and it was lower in the first genotype (27.75).

The eighth genotype recorded the highest number of seeds per plant (123.5) and the first genotype exhibited the lowest (25.75). The fourth and ninth genotypes recorded the same number of seeds per plant (46.75). The tenth genotype had the highest seed weight (38.55). The seventh genotype revealed the least seed weight (7.63). Weight of 100 seeds for each plant, the sixth genotypes recorded the highest weight of 100 seeds per plant (47.4 g) and the seventh genotypes had the lowest weight of 100 seeds per plant (20.25 g).

RAPD-PCR Analysis: Molecular and morphological analysis are among the most used tools for the estimation of genetic distances within a group of genotypes. Molecular markers provide an excellent tool for obtaining genetic information and their use in the assessment of genetic diversity Molecular markers are a useful complement to morphological and physiological

Table 3: Analysis of variance for seven studied traits in chickpea genotypes* and ** are significant at the 0.05 and 0.01 probability levels, respectively

S.O.V	d.f.	Plant Height(cm)	Number of branches/plant	No. of fertile nodes	No. of pods/plant	No. of seeds/plant	Seeds weight/plant	100-seed weight
Replications	3	40.28 **	0.455	0.316	14.51**	55.51**	11.97**	36.952**
Genotypes	23	1120.47**	12.587**	19.934**	2283.4**	2246.10**	315.83**	230.666**
Error	69	48.43	1.252	2.135	53.74	53.79	19.65	3.207

Table 4: The means performance of twenty four genotypes for seven studied traits in *Cicer arietinum* L.

	Traits						
Genotypes	Plant height(cm)	Number of branches/plant	No. of fertile nodes	No. of pods/plant	No. of seeds/plant	Seeds weight/plant	100-seed weight
1	95	4	4.75	27.75	25.75	7.73	30.4
2	80	3.25	4.5	60	60.25	16.88	28.45
3	120	4.5	7.25	53.5	39.75	11.63	29.53
4	128.75	7	7.75	51	46.75	21.73	46.83
5	73.75	6.75	8.5	76.25	81	17.48	21.5
6	100	10.25	9	51	59	27.85	47.4
7	82.5	5.25	6	34.5	33.5	7.63	20.25
8	90	4.75	8.25	129.25	123.5	37.95	30.75
9	90	6.25	10.25	48.5	46.75	15.9	34.15
10	93.75	5.25	8	90.5	89	38.55	43.5
11	68.75	2.75	4.25	38	40	13.98	35.25
12	71.25	2.5	3.75	31	29.5	9.2	31.5
13	63.75	4.25	10.5	98.75	97.5	20.83	21.48
14	56.25	4.25	12.25	37.25	36.5	8.12	22.28
15	72.5	4.25	9.25	56.25	54.25	12.03	22.3
16	76.25	4.25	9.25	37.5	35.25	8.45	24.53
17	72.5	3.25	6.75	46.5	48	9.85	20.9
18	67.5	3.5	7.25	40.75	39	8.33	21.8
19	76.25	4.5	9	48.75	45.5	12.35	27.35
20	92.5	4.75	6.5	36.5	34.5	9.33	29.98
21	87.5	4.75	8.5	44.75	44	12.68	29.2
22	85	3.25	7.75	35.75	48.25	14.45	30.48
23	93.75	8.5	11.25	52	53.25	25.2	31.28
24	92.5	3.75	10.25	40.25	37.25	10	27.45
LSD 0.05	9.817	1.578	2.061	10.341	10.346	6.254	2.526
C.V.%	8.2	23	18.4	13.9	14.1	28.1	6

characterization of cultivars because they are plentiful, independent of tissue or environmental effects and allow cultivar identification early in plant development [13].

In this study, genomic DNAs of five chickpea genotypes (1, 6, 8, 10 and 12) were extracted and compared by RAPD-PCR, using eight primers. The primers were (OPE12, OPK08, OPK10, OPK19, OPD08, OPO07, OPO08 and OPD07). For computer analysis to detect the differences between five genotypes, where intensive bands were considered when present as (1), while weak or absent bands were considered when absent as (0).

Fig. 1, 2, 3, 4, 5, 6, 7 and 8 Randomly amplified polymorphic DNA banding patterns of the five chickpea genotypes. M: DNA marker (Sizer 10.000), 1, 6, 8, 10, 12 Band sizes of the marker are expressed in bp.

Table 5 and Fig.1 showed the reaction of primer (OPE12) with the five genotypes, the primer yielded fifteen different bands with size ranged from 250 to 10, 000 bp. The results observed from the RAPD-PCR analysis is that all bands were present in all genotypes at size of 250, 450, 550, 600, 700, 1100, 1400, 1500 bp.

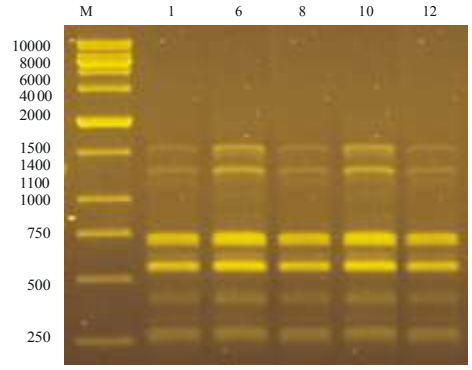

Fig. 1: RAPD-PCR polymorphism DNA with primer OPE12

As shown from Table 6 and Fig. 2 the reaction of primer (OPK08) with the five genotypes, the bands sizes ranged from 250 to 10, 000 bp. Three new bands were found at size of 3000, 750, 650. The band 3000 was appeared in genotypes (1, 8 and10) while the band 750 appeared in (1 and 6), also the band 650 appeared in (1, 6, 8 and 10) while the band 550 was presented only in genotype (1).

Table 5: Primer OPE12, molecular weights (bp) and presence or absence bands for five genotypes chickpea (*Cicer arietinum* L.)

		Genotypes				
Primer	MW	1	6	8	10	12
	10,000	0	0	0	0	0
	8,000	0	0	0	0	0
	6,000	0	0	0	0	0
	5,000	0	0	0	0	0
	4,000	0	0	0	0	0
OPE12	3,000	0	0	0	0	0
	2,000	0	0	0	0	0
	1,500	1	1	1	1	1
	1,400	1	1	1	1	1
	1,100	1	1	1	1	1
	1,000	0	0	0	0	0
	750	0	0	0	0	0
	700	1	1	1	1	1
	600	1	1	1	1	1
	550	1	1	1	1	1
	500	0	0	0	0	0
	450	1	1	1	1	1
	250	1	1	1	1	1
Total	8	8	8	8	8	

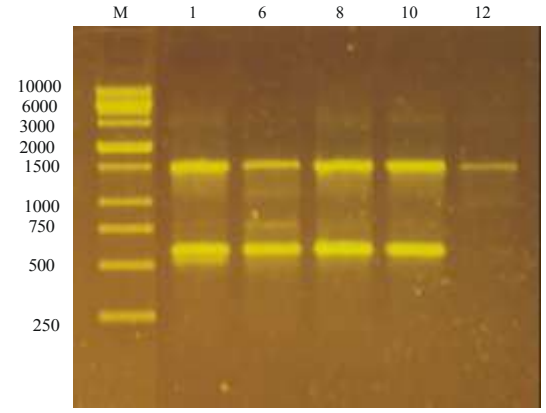

Fig. 2: RAPD-PCR polymorphism DNA with primer OPK08

Table 6: Primer OPK08, molecular weights bp and presence or absence bands for five genotypes chickpea (*Cicer arietinum* L.)

		Genotypes				
Primer	MW	1	6	8	10	12
	10, 000	0	0	0	0	0
	8, 000	0	0	0	0	0
	6, 000	0	0	0	0	0
OPK08	5, 000	0	0	0	0	0
	4, 000	0	0	0	0	0
	3, 000	1	0	1	1	0
	2, 000	0	0	0	0	0
	1, 500	1	1	1	1	1
	1, 000	0	0	0	0	0
	750	1	1	0	0	0
	650	1	1	1	1	0
	550	1	0	0	0	0
	500	0	0	0	0	0
	250	0	0	0	0	0
Total		5	3	3	3	1

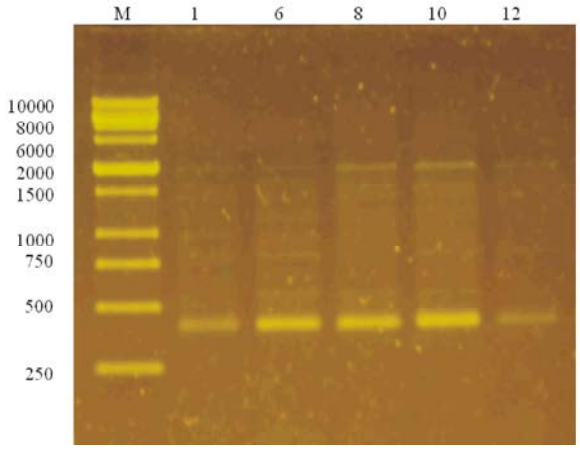

Fig. 3: RAPD-PCR polymorphism DNA with primer OPO07

Table 7: Primer OPO07, molecular weights (bp) and presence or absence bands for five genotypes chickpea (*Cicer arietinum* L.)

		Genotypes				
Primer	MW	1	6	8	10	12
	10, 000	0	0	0	0	0
	8, 000	0	0	0	0	0
	6, 000	0	0	0	0	0
OPO07	5, 000	0	0	0	0	0
	4, 000	0	0	0	0	0
	3, 000	0	0	0	0	0
	2, 000	1	1	1	1	1
	1, 500	0	0	0	0	0
	1, 000	0	0	0	0	0
	750	0	0	0	0	0
	500	0	0	0	0	0
	470	1	1	1	1	1
	250	0	0	0	0	0
Total		2	2	2	2	2

As shown from Table 7 and Fig. 3 the reaction of primer (OPO07) with the five genotypes, the bands sizes ranged from 250 to 10, 000 bp. One new band was appeared in all genotypes with size 470 and the band was presented in all genotypes with size 2000.

Table 8 and Fig. 4 showed the reaction of primer OPO07 with the five genotypes. The results showed that the fragments obtained from the RAPD-PCR sizes ranged from 250 to 10, 000 bp. One new band was appeared in all genotypes with size 680 and the band was presented in all genotypes with size 700, 1000 and 1500 bp. While the bands were absent in other genotypes.

Table 9 and Fig. 5 observed fragments obtained from the RAPD-PCR analysis for five genotypes, the bands were sizes 250 to 10.000 bp. One band was appeared in all genotypes with size at 750, 1400 and 2000 bp.

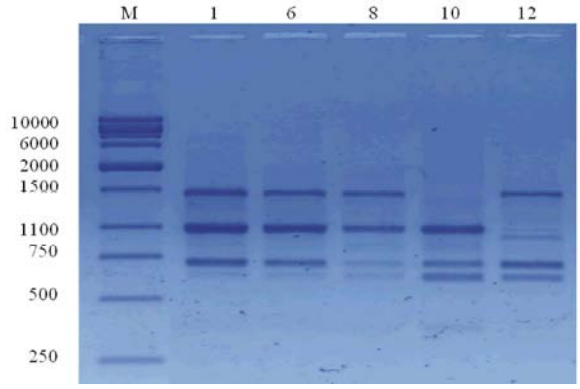

Fig. 4: RAPD-PCR polymorphism DNA with primer OPK10

Table 8: Primer OPK10, molecular weights (bp) and presence or absence bands for five genotypes chickpea (*Cicer arietinum* L.)

		Genotypes				
Primer	MW	1	6	8	10	12
	10, 000	0	0	0	0	0
	8, 000	0	0	0	0	0
	6, 000	0	0	0	0	0
OPK10	5, 000	0	0	0	0	0
	4, 000	0	0	0	0	0
	3, 000	0	0	0	0	0
	2, 000	0	0	0	0	0
	1, 500	1	1	1	1	1
	1, 000	1	1	1	1	1
	750	0	0	0	0	0
	700	1	1	1	1	1
	680	1	1	1	1	1
	500	0	0	0	0	0
	250	0	0	0	0	0
Total		4	4	4	4	4

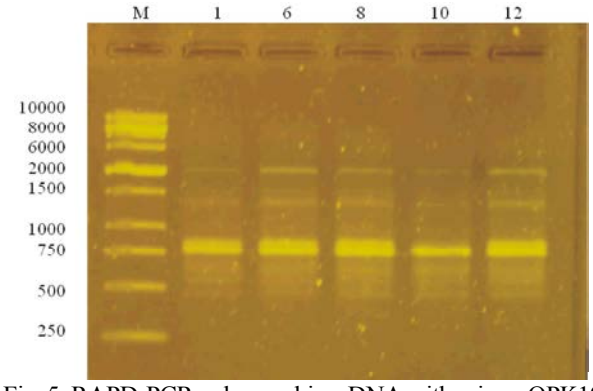

Fig. 5: RAPD-PCR polymorphism DNA with primer OPK19

As shown from Table 10 and Fig. 6 the reaction of primer with all genotypes that the bands ranged from 250 to10.000 bp. One band was appeared in genotype (1) at size 300 bp., while this band was absent in other genotypes. Two new bands were appeared with size 350 and 1100 bp in all genotypes.

Table 9: Primer OPK19, molecular weights (bp) and presence or absence bands for five genotypes chickpea (*Cicer arietinum* L.)

		Genotypes				
Primer	MW	1	6	8	10	12
	10, 000	0	0	0	0	0
	8, 000	0	0	0	0	0
	6, 000	0	0	0	0	0
OPK19	5, 000	0	0	0	0	0
	4, 000	0	0	0	0	0
	3, 000	0	0	0	0	0
	2, 000	1	1	1	1	1
	1, 500	0	0	0	0	0
	1, 400	1	1	1	1	1
	1, 000	0	0	0	0	0
	750	1	1	1	1	1
	500	0	0	0	0	0
	250	0	0	0	0	0
Total		3	3	3	3	3

Table 10: Primer OPD08, molecular weights (bp) and presence or absence bands for five genotypes chickpea (*Cicer arietinum* L.)

		Genotypes				
Primer	MW	1	6	8	10	12
	10, 000	0	0	0	0	0
	8, 000	0	0	0	0	0
	6, 000	0	0	0	0	0
	5, 000	0	0	0	0	0
	4, 000	0	0	0	0	0
OPD08	3, 000	0	0	0	0	0
	2, 000	0	0	0	0	0
	1, 500	0	0	0	0	0
	1, 100	1	1	1	1	1
	1, 000	0	0	0	0	0
	750	0	0	0	0	0
	500	0	0	0	0	0
	350	1	1	1	1	1
	300	1	0	0	0	0
	250	0	0	0	0	0
Total		3	2	2	2	2

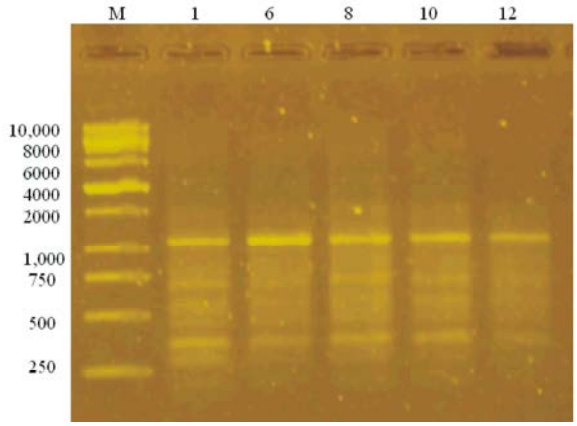

Fig. 6: RAPD-PCR polymorphism DNA with primer OPD08

Table 11 and Fig. 7. The results observed that the fragments obtained from the RAPD-PCR sizes ranged from 250 to 10.000 bp. Two new bands were presented with all genotypes at size 600 and 1900 bp. While two bands were appeared in all genotypes with size 700 and 1000 bp.

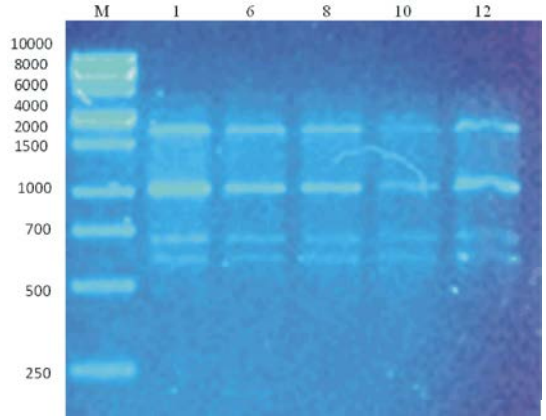

Fig. 7: RAPD-PCR polymorphism DNA with primer OPO08

Table 11: Primer OPO08, molecular weights (bp) and presence or absence bands for five genotypes chickpea (*Cicer arietinum* L.)

		Genotypes				
Primer	MW	1	6	8	10	12
	10, 000	0	0	0	0	0
	8, 000	0	0	0	0	0
	6, 000	0	0	0	0	0
OPO08	5, 000	0	0	0	0	0
	4, 000	0	0	0	0	0
	3, 000	0	0	0	0	0
	2, 000	0	0	0	0	0
	1, 900	1	1	1	1	1
	1, 500	0	0	0	0	0
	1, 000	1	1	1	1	1
	750	0	0	0	0	0
	700	1	1	1	1	1
	600	1	1	1	1	1
	500	0	0	0	0	0
	250	0	0	0	0	0
Total		4	4	4	4	4

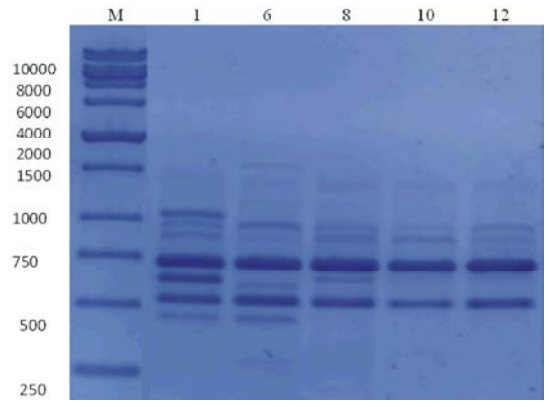

Fig. 8: RAPD-PCR polymorphism DNA with primer OPD07

Table 12: Primer OPD07, molecular weights (bp) and presence or absence bands for genotypes chickpea (*Cicer arietinum* L.)

		Genotypes				
Primer	MW	1	6	8	10	12
	10, 000	0	0	0	0	0
	8, 000	0	0	0	0	0
	6, 000	0	0	0	0	0
OPD07	5, 000	0	0	0	0	0
	4, 000	0	0	0	0	0
	3, 000	0	0	0	0	0
	2, 000	0	0	0	0	0
	1, 500	0	1	0	0	0
	1, 000	1	0	0	0	0
	950	1	1	1	1	1
	850	1	1	1	1	1
	750	0	0	0	0	0
	700	1	1	1	1	1
	650	0	0	1	0	0
	600	1	0	0	0	0
	530	0	1	0	0	0
	500	1	1	1	1	1
	400	1	1	0	0	0
	250	0	0	0	0	0
Total		7	7	5	4	4

From Table 12 and Fig. 8. The results deduced that one new band appeared in genotypes 1and 6 at size 400 bp. While this band was absent in other genotypes. One new band appeared in genotype 6 with size 530 bp and absent in other genotypes. Also one band appeared in genotype 1 with size 600 bp and absent in other genotypes. The band with size 650 bp was absent in all genotypes except for genotype 8. The band was appeared in all genotypes with size 850 and 950 bp. Also one band was appeared in genotype 1 with size 1000 bp, while this band was absent in other genotypes and one band was appeared in genotype 6 with size 1500 bp, while this band was absent in other genotypes.

The results obtained from Table 13 the cultivars including the present bands with different primers are confirmed by fingerprints and the percentage of polymorphism. The percentage of polymorphism was 28.6% in presence of primer OPK08, while the percentage of polymorphism was 6.7% with the presence of primer OPD08. The primer OPD07 showed higher polymorphism as its percentage 31.6%. The RAPD- fingerprints found different in five genotypes of chickpea. RAPD is an important tool used to identify polymorphism among the genotypes. These results agreement with Safari *et al.* [14] and Mahjoob *et al.* [15].

Table 13: Primers were tested for five chickpea genotypes by RAPD-PCR analysis that produced polymorphic bands and percentage of Polymorphism

Primers	Total	Monomorphic bands	Polymorhic bp.	N.	Polymorphism%
OPE12	18	8	-------	----	-------
OPK08	14	1	550 (1), 3000 (1, 8.10)	4	28.6%
OPK10	14	4	650(1, 6, 8, 10), 750 (1, 6)	-----	-------
OPK19	13	3	-------	-----	-------
OPD08	15	2	-------	1	6.7%
OPO07	13	2	300 (1)	----	-------
OPO08	15	4	-------	-----	-------
OPD07	19	4	-------	6	31.6%
			400 (1, 6), 530 (6), 600 (1), 650 (8), 1000 (1), 1500 (6)		

Table 14: Similarity coefficient among five chickpea genotypes calculated according to Jaccard coefficient based on using eight primers

	1	6	8	10	12
1	1				
6	0.81579	1			
8	0.81081	0.82857	1		
10	0.83333	0.85294	0.96774	1	
12	0.77778	0.84848	0.90323	0.93333	1

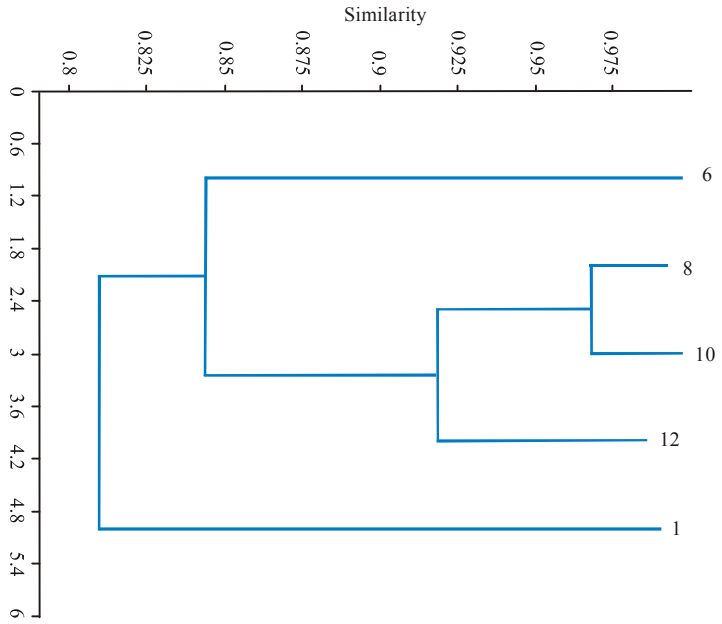

Fig. 9: Dendrogram based on Jaccard similarity coefficient five chickpea genotypes Generated using eight primers

Knowledge of the genetic diversity of a species is important for the choice of crossing parents in an accession and hybrid breeding. Heakel [16] showed that some genotypes of Egyptian canola by molecular markers related to high seed yield/plant and consequently oil percentage.

Depending on the results obtained by RAPD-PCR analysis and generally, it worth to mention that employing molecular markers, can help that breeder to introduce new breeding programs and can give a clue about the type and diversity of chickpea plants, which is dealing with. These results agreement with Abdelmigid [17].

Determine the Degree of Genetic Affinity Between the Five Genotypes of Chickpea: The genetic relationship between the five genotypes was studied by applying the matrix of compatibility percentages (PAV) Percent Agreement Values by Nei [18]. As the high values of this matrix indicates a genetic affinity, they arise between both genotypes and increase their genetic affinity increases. Cluster analysis using RAPD-PCR for five genotypes of chickpea with Jaccard's similarity coefficient ranging from 0.0 to 1.0 (Fig. 9 and Table 14). From Table 14 observed that the largest value of PAV it 0.96774 between genotypes (8 and 10), this indicates that they are highly

degree of genetic similarity. Followed by the two genotypes (10 and 12) equal 0. 93333, then he followed by two genotypes (8 and 12) equal 0.90323 respectively. While it was less valuable 0.77778 between two genotypes (1 and 12) this indicates that there is a genetic variation between them. The results agreed with Rao *et al.* [19] and Tarabain *et al*. [20].

Cluster Analysis: Clustering analysis allows the studied genotypes to be divided into groups that reflect the degree of genetic affinity between them and samples may collect within one group based on their original habitat or based on their origin and proportions. Cluster analysis of the results obtained with the aim of establishing the genetic affinity tree was conducted to determine the degree of genetic similarity (dendrogram). It was found that there are two main branches, the first branch included the model (1), while the second was divided into two sub branches, the first was (6) and second also divided two group one of them (12) and the other included two genotypes (8 and 10). Genotype (1) was the most genetically distant from the rest of the models studied genetic.

The cluster analysis of RAPD-PCR can be used to genetically differentiate between chickpea genotypes. However, more number of primers is suggested to be used this finding agreement with Safari *et al.* [14]. In general, diversity measurements were higher in the genotypes at which such a high level of genetic similarity may be used for selection of the materials in the breeding programs, where cultivars with high genetic distance can be used for this purpose.

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