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DETERMINATION OF GENETIC VARIATION OF EGYPTIAN COWPEA (*VIGNA UNGUICULATA* (L.) WALP.) USING RAPD-PCR PATTERNS

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ABSTRACT

Cowpea (*Vigna unguiculata* (L.) Walp.) is the most important grain legume crop grown in tropical and subtropical regions. Seeds of cowpea have a high nutritional value containing high amount of protein (23–29%). Seeds of 19 landraces of cowpea (*Vigna unguiculata*) were obtained from gene bank of Cairo University (11 landraces) and from Agricultural Research Station Kafr El-Sheikh (Sakha) (8 varieties). The seeds planted till maturity. Random amplified polymorphic DNA (RAPD) was assayed to determine the genetic variation of 19 varieties of cowpea cultivated in different regions of Egypt. A total of five random primers were used in the study. Amplification of genomic DNA of Egyptian cowpea landraces with these RAPD primers yielded 54 fragments that could be scored, of which 45 were polymorphic. Number of amplified fragments with random primers ranged from 4 (OP 856) to 14 (OP 880). Percentage polymorphism ranged from 75% (OP 856) to a maximum of 100% (OP 889, OP 868, Op 808 and OP 880), with an average of 95%. A dendrogram produced by the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) based on Jaccard"s similarity coefficient revealed that the cowpea varieties divided into two groups which showed variability that may be exploited for crop improvement. The overall range of genetic similarities ranged from 0.48 to 0.93 in 19 landraces of cowpea which indicates there was high variability among the varieties. Based on genetic distance in RAPD analysis the landraces no. 9 and 12 appeared as most divergent and could be used in breeding programme of cowpea. It could be concluded that RAPD markers are important tools for genetic analysis for cowpea(*Vigna unguiculata* (L.) Walp.).

Keywords: Cowpea, *Vignaunguiculata*, Genetic diversity, RAPD

1. INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.), a true diploid ($2n = 2x = 22$) species, belongs to the family Leguminosae (Fabaceae), tribe Phaseoleae, genus *Vigna*, and section Catiang (Verdcourt, 1970). Cowpea (*Vigna unguiculata* (L.)Walp.), an annual crop, is one of the most important and widely cultivated legumes in the world, particularly in Africa, Latin America, and some parts of Asia and the United States (Xiong *et al*., 2016).

Cowpea (*Vignaunguiculata*(L.)Walp.)is an important indigenous African grain legume grown in places with severe weather conditions in the tropics and sub-tropics in Africa, Asia and South America (Singh *et al.,* 1997 and Ba *et al.,* 2004). Cowpea (*Vigna unguiculata* (L.)Walp.)is widely grown by subsistence farmers in West and East Africa where its grain and leaves are sources of highly valuable food, due to their high contents of proteins, minerals and vitamins. There are several diverse uses of cowpea due to which the varietal requirement in terms of plant type, seed type, maturity, pattern of use and growth are diverse from region to region. Therefore cowpea breeding programme becomes more complex and no single variety can be suitable for all the objectives (Barrett, 1987). Thus, there is need to develop varieties suitable for a specific region and or use.

Traditionally, diversity within and between varieties was determined by assessing difference in morphology. Morphological identification requires the plant to grow to full maturity and is difficult in case of natural populations, because morphological characters are influenced by environmental factors. Cowpea is primarily a self-pollinating crop and its genetic base is considered to be narrow (Fana*et al.,* 2004). However, application of

biochemical and genetic techniques are important potential tools for the study of wild and domesticated species.

DNA fingerprinting distinguishes different plant varieties according to their DNA variations at a set of genetic loci. One of the powerful and convenient molecular marker systems is Random Amplified Polymorphic DNA (RAPD) analysis. It is widely used for the genetic mapping, taxonomic and polygenic studies of many plants. Their environmental stability and nearly unlimited availability have made RAPD markers an ideal tool for plant investigation (Miller and Tamksleu, 1990). RAPD, among the popular techniques, is considered a useful tool to distinguish between different botanical species due to its low cost, assay is rapid and easy, needs a small amount of plant material with prior sequence information (Marieschi *et al*., 2009).

Genetic diversity plays an important role in the success of any breeding program (Ali *et al.,* 2007). Knowledge of genetic diversity in available varieties and genotypes is very useful for plant improvement all over the world, promoting the efficient use of genetic variations in breeding programs throughsupporting proper selection of cross combination among large sets of parental genotypes (Mafakheri *et al.*, 2017). In RAPD analysis, genomic DNA is amplified by PCR using short primers with an arbitrary sequence resulting in multiple amplicons of different lengths that are analyzed by gel electrophoresis. This result is characteristic fingerprints of genomic DNA. Therefore, it identifies the polymorphisms of the nucleotide sequence spread randomly over the entire genome (Sucher and Carles, 2008). RAPDs have advantages such as rapidity, requiring little genomic DNA as template and they are able to detect variation in coding and noncoding regions of the genome (Gajera *et al*. 2010). However, most RAPD loci are assumed to possess only two alleles and segregate as dominant markers, leading to an under estimation of the genetic diversity.

In cowpea a small number of investigations have been done via RAPDs (Akundabweni, 1995, Mene´ndez *et al,*. 1997, Mignouna *et al,*. 1998, Lakhanapaul *et al.*, 2000, Tosti and Negri, 2002, Fall *et al.,* 2003 and Ba *et al.*, 2004). Later on, the potential application of RAPD techniques in determining genetic diversity among cultivated cowpea varieties Random Amplified of Polymorphic DNA (RAPD) have been widely used for genetic diversity studies (Zannou *et al.,* 2008, Nkongolo*et al.*, 2009, Ghalmi*et al.,* 2010, Kumari *et al.,* 2012, Patil *et al.,* 2012, Prasanthi *et al.,* 2012, Huaqiang *et al*., 2013, Mahfouz, 2015, Patil *et al.,* 2015, Srujana and Bhavani, 2016, Udensi *et al.,* 2016, Nagalakshmi *et al.,* 2017, Khalafalla, 2018 and Damarany *et al.,* 2018). Thus, the objective of the present study was to evaluate the genetic variation between 19 landraces of *Vigna unguiculata* collected from different locations in Egypt using DNA molecular marker (RAPD-PCR) of total genomic DNA.

2. MATERIALS AND METHODS:

2.1. Plant Materials:

Seeds of 19 landraces of *Vigna unguiculata* were obtained from Gene Bank of Cairo University (11 landraces) and from Agricultural Research Station Kafr El-Sheikh (Sakha) (8 landraces). List of the studied landraces were given in Table1.The morphological character measurements of the collected landraces of cowpea were taken after grown in the Botanical Garden of the Botany Department, Faculty of Science, Tanta University, till flowering and maturity.

2.2. Methods:

2.2.1. RAPD Analysis:

2.2.1.1. DNA Extraction and Purification

Genomic DNA was extracted from the young leaves of 19 cultivated landraces of *Vigna unguiculata* at Biotechnology Lab, Botany Department, Faculty of Science, Kafr El-Sheikh University, then frozen in liquid nitrogen, ground with mortar and pestle, and total genomic DNA was isolated by genomic DNA and Total RNA Co-extraction KIT (Spin-

Table 1. List of the studied 19 landraces of cowpea (*Vigna unguiculata*) and their different locations.

No.	Code	Location	No.	Code	Location
1	1111776	Sohag	11	Kaha 21	Qaha
2	1111777	Sohag	12	Sakha 1	Kafr El -Sheikh
3	1111782	Sohag	13	Sakha 28	Kafr El -Sheikh
4	1111784	Sohag	14	Sakha 3	Kafr El -Sheikh
5	1111826	Qena	15	Sakha 53	Kafr El -Sheikh
6	1111766	Luxor	16	Sakha 56	Kafr El -Sheikh
7	1111825	Luxor	17	Sakha 2	Kafr El -Sheikh
8	1111785	Dakahlia	18	Sakha 4	Kafr El -Sheikh
9	1111786	Dakahlia	19	Sakha 5	Kafr El -Sheikh
10	1111832	Asyut	----------------------		

column) BioTake Corporation. Purity of DNA was determined according to (Sambrook *et al.*, 1989) by measuring its optical density in spectrophotometer at 260 nm/280 nm ratios and quality of DNA samples was checked by loading them on 0.8% agarose gel and observed it on UV illuminator.

2.2.1.2. PCR Amplification:

PCR amplification carried out according to Williams *et al*. (1990). After screening a total of five primers of Operon Technologies Inc. 14-19 bp oligonucleotides (CA, USA) were used for analysis (Table 2). These primers produced informative and polymorphic products obviously visible by the agarose gel electrophoresis. Agarose gel electrophoresis is the most effective way of separating DNA fragments of varying sizes ranging from 100 bp to 25 kb (Sambrook and Russell, 2001). RAPD amplifications were performed in a 25 ml volume containing 12.5 µl of master mix green, 7.5 µl water free nuclease, 3 µl primer mix and 2 µl of DNA sample. Amplifications were performed using a Gene Amp PCR System 9700 (Applied Biosystems, CA, USA). The DNA amplification was carried out using thermal cycler**.** The amplification was programmed after an initial strand separation cycle at 94ºC for 5 min followed by 40 cycles; each cycle included a denaturation step at 92ºC for 1 min, an annealing step at 42º for 1 min and 10 s and a polymerization step at 72ºC for 2 min with a final extension step at 72ºC for 5 min. The amplification products were separated on 1.5% agarose gels and stained with ethidium bromide. The RAPD fragments were photographed using a UV trans-illuminator and analyzed with a gel documentation system**.** DNA ladder (50 bp) mix was used as standard DNA with molecular weights of 1500, 1400, 1200, 1150, 1000, 800, 525, 500, 280, 400, 225, and 100 bp. The run was performed for about 30 min at 80 V in mini submarine gel BioRad.

Table 2. The random primers codes and sequences.

No.	Name	Sequence (5'–3')
1	OP 889	ACACACACACACAC
2	OP 868	GAAGAAGAAGAAGAAGAA
3	OP 856	ACA CAC ACA CAC ACA CCA
4	OP 808	AGAGAGAGAGAGAGAGC
5	OP 880	GGAGAGGAGAGGAGA

2.3. Data Scoring and Statistical Analysis of RAPD Data:

RAPD bands were scored using binary matrix "1" for presence and "0" for absence. Data was analyzed using NT-SYS-pc version 2.1 (Rohlf *et al,*. 2002). Pair-wise genetic similarities between varieties were estimated using the Jaccard‟s similarity coefficient (Jaccard *et al*., 1908). A dendrogram was constructed based on similarity coefficient values by adopting the sequential hierarchical agglomerative non-overlapping (SHAN) clustering technique of unweighted pair group method of arithmetic mean (UPGMA) which is a variant of the average linkage clustering algorithm (Sneath and Sokal, 1973).

3. RESULTS:

3.1. RAPD analysis:

The results revealed that the five primers produced a total of 54 bands for the 19 landraces of cowpea (Table 3 and Figures 1&2). Of these bands, 45 are polymorphic, one monomorphic and 7 unique. The mean polymorphism for all bands taken together was 95% (46/54). The highest number of bands (14) was produced by the primer OP 880 where the lowest number (4) was produced by the primer OP 856. Both OP 889 and OP 868 primers produced 13 bands each in contrast to primers OP 808 produced 10 bands. The numbers of polymorphic bands varied between primers; Primers OP 889, OP 868 and OP 880 produced 12 polymorphic bands, whereas the profile produced by primer OP 808 showed only 8 polymorphic bands. Primer OP 856, showed one polymorphic bands. The polymorphism percentage of 100% was recorded with the primers OP 889, OP 868, OP 808 and OP 880 and 75 % with primer OP 856.

The five used primers showed variation with regard to average band informative (Avlb) and resolving power (Rp). The Avlb and Rp values of these polymorphic primers have been depicted in (Table 3). The primer OP 856 showed the lowest Avlb (0.1312), while the highest Avlb (0.510) was exhibited by primerOP 889. Rp ranged from 0.526 to 6.632.

The RAPD fingerprinting produced by primer OP 808 generated a total of 10 bands ranging in size between 105 bp and 505 bp (Table 3 and Figure 1*)* including 8 polymorphic bands and two unique bands. The highest molecular size of the RAPD products produced by this primer (505 bp) was scored in five varieties of 9, 10, 11, 12 and 16. Two unique bands were observed with molecular weight of 400 and 360 bp in varieties no. 13 and 14, respectively.

A total of four bands were generated with primer OP 856.The number of bands is varied in size ranged between 150 bp and 350 bp (Table 3 and Figure 1) including one polymorphic band and two unique bands and one monomorphic band. The unique band with molecular weight of 190 bp was observed in varieties no. 7. The highest molecular size (350 bp) was scored in one variety of no.11 and was a common band for this variety. The polymorphic band was observed with molecular weight of 230 and present in varieties no. 7 and 12.

The size of the amplified bands ranged from 90 bp to 590 bp with primer OP 889 and this primer revealed a total number of 13 (Table 3 and Figure 2). These bands include 12 polymorphic bands and one unique band. The highest molecular size of the RAPD products produced by this primer (590 bp) was scored in four landraces of 9, 16, 18 and 19. One unique band was observed with molecular weight of 140 in variety no. 10.

Table 3. The number of polymorphic bands and percentage of polymorphism in the RAPD profile of 5 primers in the genome of different 19 landraces of *Vigna unguiculata*.

(AvIb)	Resolving power (Rp)	% Poly-morphism	Number of mono-morphic bands	Number of unique bands	Number of polymorphic bands	Total No. of bands	Sequence	Primer code	Range of MWt (KDs)	serial
0.510	6.632	100	0	1	12	13	$(AC)_7$	OP 889	590-90	1
0.478	6.211	100	0	1	12	13	$(GAA)_6$	OP 868	600-150	2
0.132	0.526	75	1	2	1	4	$(A C)_8$ CA	OP 856	350-150	3
0.326	3.263	100	0	2	8	10	$(AG)_8$ C	OP 808	505-105	4
0.451	6.316	100	0	2	12	14	$(GGA)_3(GA)_3$	OP 880	455-110	5
-	-	-	1	8	45	54	**Total**			
0.379	4.5896	-	-	-	-	-	**Mean**			

(AvIb) Average of informative bands for a primer

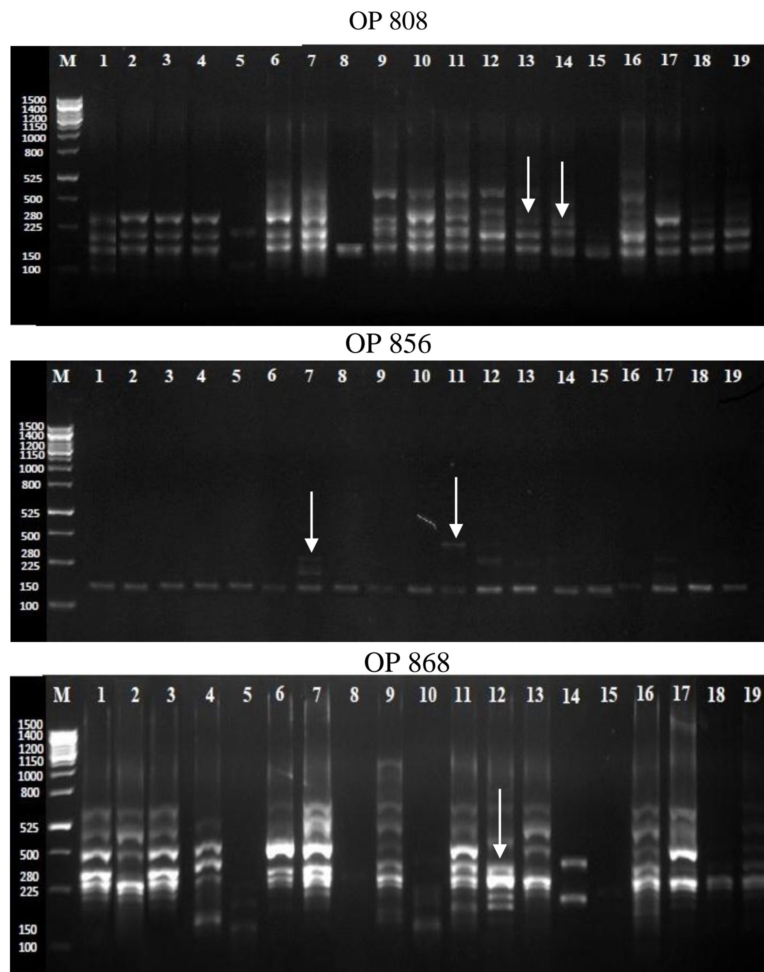

Fig. 1.DNA fragment profile produced in 19 varieties of cowpea using RAPD Primer (OP 808 and OP 856). (Unique bands are indicated by arrows).

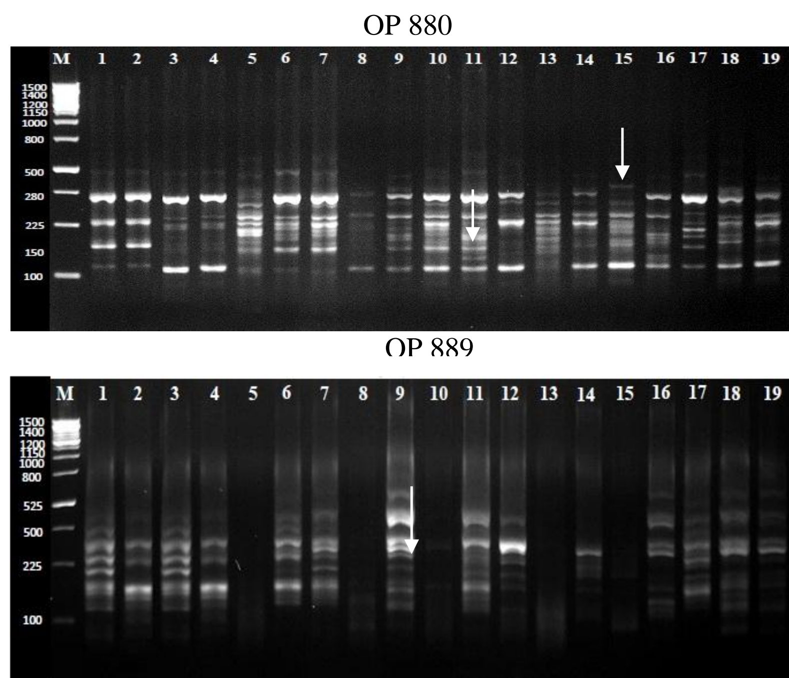

Fig. 2.DNA fragment profile produced in 19 varieties of cowpea using RAPD Primer (OP 868, OP 880 and OP 889). (Unique bands are indicated by arrows).

3.3. Cluster analysis:

The Jaccard's similarity indices based on RAPD profiles were subjected to UPGMA analysis sub programme of SHAN (Fig. 3). Data analysis resulted in a dendrograme with two major clusters sharing o.63 similarity coefficient. Cluster analysis revealed that landraces of cowpea under study fell into two groups, minor group A (6 landraces) and major group B (13 landraces). Group A divided into two sub groups 1 & 2; first subgroup (1) had only three varieties and was subdivided into 2 clusters. First cluster $1a_1$ containing one separate variety no. 13. Second cluster $1a_2$ had two varieties no. 8 and no. 15 with 0.89 similarity coefficient. Second sub group (2) containing 3 landraces and was further subdivided into two clusters; the first one $2a_1$ contains two landraces together no. 10 and 14 with similarity coefficient 0.83 and a separate landrace no.5 in the second cluster $2a_2$. The second group B includes 13 landraces *viz.*1, 2, 3, 4, 6, 7, 9, 11, 12, 16, 17, 18 and 19. This group B subdivided into 10 clusters; three of these clusters included 2 landraces together such as 18 & 19, 6 & 17 and 1 & 3. The rest landraces of this group were in separate clusters.

4. DISCUSSION:

Genetic variation is a prerequisite for genetic improvement of agricultural crops. But proper use of genetic diversity within varieties collections needs a good knowledge about their characteristics. Characterization of accessions, varieties or genotypes is traditionally based on morphological and agronomic traits, which is of high interest for plant breeders. Presence of genetic variation in crop populations is not simply detected by morphological characteristics existence in the collected cowpea varieties assessed inthe studied varieties. Use of molecular markers in plant diversity studies is increased for detection ofdifferences in crop populations at the DNA level. Cowpea (*V. Unguiculata* (L.)Walp.) is an important resource for local agriculture in Egypt and has been cultivated in many countries. In this study, the objective was to examine the relationship and genetic variation among 19 Egyptian cowpea varieties. Cowpea has most likely been domesticated only once (Pasquet, 1999). Relatively low levels of genetic diversity and variation both within and between cultivated cowpea may be a result of an initial, single bottleneck (Pasquet, 2000). Previous studies using RAPD typically indicate little variation within and between cowpea accessions (Doebley, 1989, Pasquet, 2000, Li *et al.*, 2001, Tosti and Negri, 2002 and Ghalmi *et al.,* 2010).

The two repeated types (GA and CA) of the primers revealed approximately equal levels of polymorphism.The two primers containing AC repeats taken together and the primer containing GA repeatsgenerated 13 and 8 of the polymorphic bands, respectively. The other two primers of the used five primers containing GGA and GAA repeats produced equal number of bands (12) with 100% of polymorphism. Our results confirming the results of a previous study (Ajibade *et al.*, 2000 and Ghalmi *et al.,* 2010) in that the repeated sequences CA and GA produced a more important number of polymorphisms and which means that they are appropriate for phylogenetic analysis in *Vigna*.

Markers of DNA have been used successfully to assess molecular polymorphism in cowpea (Lakhanapaul *et al.,* 2000).The RAPD analysis revealed substantial polymorphism in cowpea (Ghalmi *et al.,* 2010 and Patil*et al.,* 2015). This marker system was found to good in the detection of polymorphism andwas efficiency in distinguishing the cowpea genotypes (Singh *et al.,* 2014). The use of appropriate statistical method especially in case of RAPD analysis is very important to make genetic variation more definitive. According to Peleg *et al.,* (2008) and Nevo *et al.* (2012), genetic diversity study is very critical for the study of crop evolution and genetic improvement of crop plants. This interestingly helps in the identification and correct interpretation of the associations between functional variation as well as molecular genetic diversity. The result of the current work

Table 4. Jaccard's similarity coefficient for different cowpea varieties based on RAPD data analysis.

Variety code	Variety 1	Variety 2	Variety 3	Variety 4	Variety 5	Variety 6	Variety 7	Variety 8	Variety 9	Variety 10	Variety 11	Variety 12	Variety 13	Variety 14	Variety 15	Variety 16	Variety 17	Variety 18	Variety 19
Variety1	1.00																		
Variety2	0.91	1.00																	
Variety3	0.93**	0.87	1.00																
Variety4	0.76	0.81	0.83	1.00															
Variety5	0.54	0.59	0.57	0.63	1.00														
Variety6	0.78	0.76	0.85	0.80	0.57	1.00													
Variety7	0.72	0.74	0.80	0.78	0.56	0.72	1.00												
Variety8	0.65	0.74	0.65	0.70	0.70	0.65	0.59	1.00											
Variety9	0.69	0.67	0.69	0.70	0.48*	0.69	0.70	0.56	1.00										
Variety10	0.57	0.67	0.65	0.70	0.78	0.61	0.67	0.70	0.59	1.00									
Variety11	0.74	0.76	0.70	0.69	0.54	0.78	0.61	0.61	0.69	0.57	1.00								
Variety12	0.67	0.69	0.74	0.69	0.57	0.70	0.69	0.61	0.61	0.69	0.63	1.00							
Variety13	0.67	0.76	0.74	0.72	0.72	0.67	0.65	0.80	0.57	0.72	0.59	0.67	1.00						
Variety14	0.59	0.65	0.67	0.69	0.69	0.59	0.65	0.72	0.54	0.83	0.48	0.67	0.78	1.00					
Variety15	0.61	0.70	0.57	0.63	0.74	0.54	0.52	0.89	0.52	0.70	0.50	0.57	0.76	0.72	1.00				
Variety16	0.76	0.78	0.72	0.67	0.52	0.65	0.67	0.67	0.74	0.59	0.69	0.69	0.69	0.57	0.63	1.00			
Variety17	0.74	0.83	0.78	0.80	0.65	0.85	0.80	0.72	0.65	0.65	0.74	0.67	0.70	0.59	0.61	0.69	1.00		
Variety18	0.67	0.72	0.70	0.72	0.65	0.67	0.69	0.72	0.61	0.69	0.52	0.70	0.74	0.74	0.69	0.80	0.70	1.00	
Variety19	0.69	0.74	0.76	0.78	0.63	0.69	0.70	0.74	0.63	0.67	0.61	0.69	0.83	0.72	0.67	0.67	0.67	0.67	1.00

**=The highest similarityvalue *= The lowest similarity value

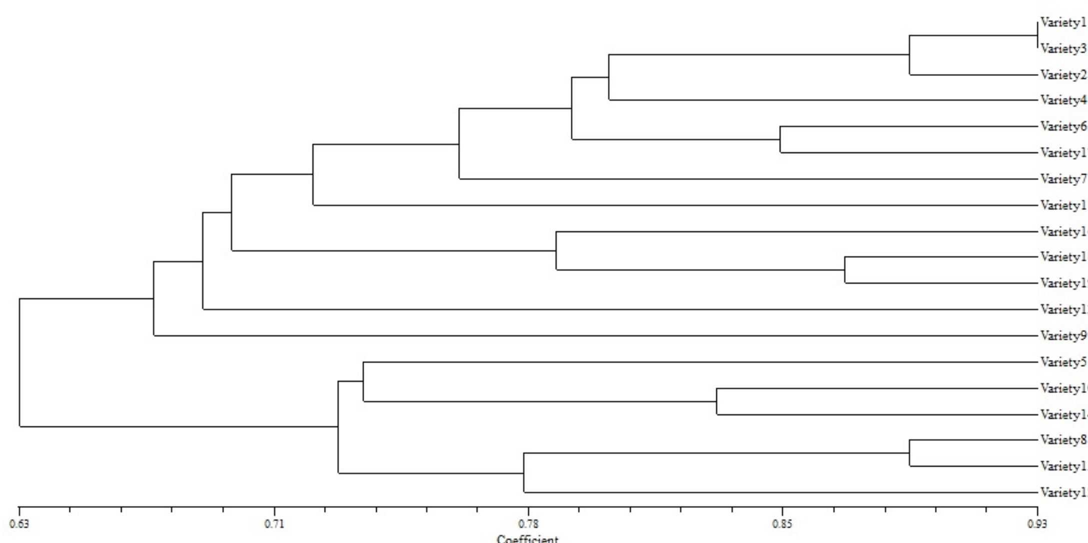

Fig. 3. Dendogram of 19 different cowpea varieties generated by Jacquard's similarity coefficient and the unweighted pair group method with arithmetic average (UPGMA) clustering methods based on RAPD analysis

on RAPD showed that of the 54 bands generated, 45 were polymorphic with percentage polymorphism that ranged from 75-100%, with average polymorphism of 95%. This high level of polymorphism results (95%) among 19 cowpea varieties similar to that obtained by many investigators which ranged between 55% to 95% such as 64.5% (Sharawy and El-Fiky, 2003), 55.0% (Zannou *et al.,* 2008), 58.44% (Ghalmi *et al.,* 2010),95% Karuppanapandian *et al*., 2006), 65% (Patil *et al.,* 2012),90.0% (Prasanthi *et al.,* 2012), 71.2% (Patil *et al.,* 2013), 76% (Patil *et al.,*2015), 55% (Khan *et al*., 2015), 78.83% (Kolade*et al.*, 2016), 86.1% (Udensi *et al*., 2016), 88% (Pradeepkumar *et al.,* 2017), 90% (Khalafalla, 2018) and 80.3% (Damarany *et al.,* 2018). The percentage polymorphism obtained from our study is quite high, which indicates the primers used were good, revealing genetic diversity in the cowpea accessions studied, but in contrast other studies with RAPD markers described lowers levels of polymorphism, e.g., 12% (Mene´ndez *et al.,* 1997) and 18.5% (Tosti and Negri, 2002). In this respect, Ba *et al*., (2004) concluded that this high genetic polymorphismwas based on the use of pre-screened, highly-informative primers. The individuals belonging to the same landrace gave the same genetic profiles which underlined their genetic homogeneity (Ghalmi *et al*., 2010).The observed primers that gave 100% polymorphism in our results had the repeated sequences as previously reported by Ghalmi *et al.* (2010), Ajibade *et al.* (2000) and Prasanthi *et al.,* (2012). It might probably be that the ability to resolve genetic variation in any crop species germplasmis more directly related to the number of polymorphism detected by the marker techniques as well as the percentage of polymorphic RAPDs. .

Moreover, in this study, only one or two unique DNA fragments with different molecular weight were detected in all varieties. Similarly, to the results obtained by Khalafalla (2018) which showed the presence of (1-5) unique fragments of different sizes for particular genotypes of cowpea, This unique band could be used as positive DNA markers for the variety identification and discrimination as concluded by Damarany *et al.* (2018). As well as the absence of a common band in some cultivars but not in the others is referred to be a negative marker (non-amplified fragment) and could be also used as negative DNA markers which is might be useful for future breeding programmes and derivation of plant lines (Saker, 2005 and Damarany *et al.*, 2018).In this respect, Hadrys *et al*.(1992) reported that the monomorphic bands are constant bands and cannot be used to study diversity while polymorphic bands revealed differences and

can be used to examine and establish systematic relationship among the genotypes

A number of studies have been undertaken to assess the genetic variant and phylogenetic relationship in plant genetic resources. Several workers have reported the usefulness of RAPD technique in studying the diversity of crop genotypes. Using RAPD markers in population genetic analysis of biodiversity, relationships among species at different levels, to identify cultivars and to reveal phylogenetic relationships among them was reported by many authors (Ba *et al.,* 2004, Abd El-Hady *et al.,* 2010, Motagi *et al.,* 2013 and Anatala *et al.,* 2014). The results of this study indicated that RAPD are sufficiently informative and powerful to assess genetic variability in *V. unguiculata* (L.) Walp. in similar to the results of Patil *et al.*(2015).

The highest value of similarity coefficient (0.93) was detected between varieties 1 and 3 indicates the less divergence and could not be used in hybridization programme.These results are in accordance to the studies of phylogenetic diversity and relationship in cowpea by using RAPD polymorphic DNA marker (Karuppanapandian *et al*., 2006 and Prasanthi *et al.,* 2016). The lowest value of similarity coefficient (0.48) was evident between varieties no. 5 and 9 and between no. 11 and 14 indicates more divergence and superior lines could be developed by using these parents in breedin gprogramme. Such information may be useful for selecting the diverse parents and monitoring the genetic diversity periodically in the breeders working collection of cowpea. The RAPD analysis revealed substantial polymorphism in cowpea (Patil *et al.,* 2015). The technique may be used to obtain reasonably precise information on genetic relationship among the cowpea genotypes. The UPGMA is based on the assumption that mutation rate among different genotypes is constant and this has been widely used for analysis of genetic variation in plants. This method has been employed in present study during analysis of RAPD polymorphism. Wild relatives, exotics and mutant lines appeared to be good sources for genetic variation Nagalakshmi *et al.*(2017).

The dendrogram based on Jaccard's similarity coefficient was constructed using UPGMA after analysis of banding patterns generated by all the varieties with 5 primers across the 19 cowpea varieties. The dendrogram and similarity coefficient values of genetic similarity varies between 0.48 and 0.93 give an idea about the nature of the individual sample in the whole sample set. The cluster analysis was carried out based on the RAPD profile and it was observed that, the varieties no. 9 and 12 occupied a unique position and was most diverse from rest of 17 varieties of cowpea. As well as the results based on the RAPD profile broadly grouped the 19 cowpea varieties into two main groups (A and B). The first group (A) was formed by six varieties and the rest of 13 varieties were included in to second group (B). These groups were further divided into subgroups and clusters revealing sufficient amount of diversity within the each cluster.These results are in agreement with Zannou *et al*., (2008) and Patil (2012) and in similar to the study of Motagi *et al.*, (2013) which revealed that the genetic similarity co-efficients among genotypes varied between 0.44 to 0.82. Based on UPGMA and SAHN clustering cowpea genotypes grouped into two main clusters. Cluster II comprised of two genotypes, while other nineteen genotypes were grouped under cluster I. Also, A. dendrogram of genetic similarity was constructed based on 23 polymorphic bands of RAPD obtained from 5 primers using UPGMA revealed a separation of 13 cowpea accessions with the similarity coefficient ranged between 0.54 to 1.00 (Sarutayophat *et al.,* 2007). Recently, Khan *et al.* (2015) reported that the dendrogrambased on RAPD analysis indicated segregation of six germplasms of cowpea into two main clusters; the main clusters are further divided into sub clusters. In general, it is concluded that the genetic variation obtained in this study might be useful in future cowpea improvement programmes and selection in cowpea may be

based on the phenotypic traits as well as on the molecular markers. Hence, studies on molecular markers are quite useful in analyzing the genetic variation in cowpea.

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يعتبر نبات اللوبيا (*Vigna unguiculata* (L.) Walp) أهم محصول بقولى يزرع في المناطق الاستوائية وشبه الاستوائية . تحتوي بذور اللوبيا على قيمة غذائية عالية من البروتين تقدر ب (٢٣-٢٩%). تم الحصول على ١١ سلالة من اللوبيا (*Vigna unguiculata*) من بنك الجينات بجامعة القاهرة و ٨ سلالات من محطة البحوث الزراعية بكفر الشيخ (سخا). تم زراعة البذور حتى النضج. فى هذه الدراسة تمت معايرة DNA متعدد الأشكال العشوائي (RAPD) لتحديد الاختلاف الجيني في ١٩ سلالةً من اللوبيا المنزرعة في مناطق مختلفة من مصر. تم استخدام خمس بادئات عشوائية (primer) في الدراسة. أعطى تكبير الحمض النووي الجينيومى لأصناف اللوبيا المصرية ٥٤ شاظيه والتى تم تسجيلها مع هذه البادئات، منها ٤٥ شاظيه كانت متعددة الأشكال. تراوح عدد الشظايا المكبرة مع كل بادئ من ٤ شظايا (OP 856) إلى ١٤ شاظية (OP 880) وتراوحت نسبة تعدد الأشكال بين ٧٥% (OP 856) إلى ١٠٠% كحد أقصى مع (OP 889, OP 860, Op 808, OP 880) بمتوسط ٩٥%. أظهرالتحليل العنقودى بإستخدام نظام ال (UPGMA) على أساس معامل تشابه جاكارد أن أصناف اللوبيا تنقسم إللى مجموعتين أظهرتا إختلافات يمكن استغلالها لتحسين المحاصيل. تراوح المدى الكلي لأوجه التشابة الوراثي من ٤٨,. إلى ٩٣,. في ال ١٩ صنفا من اللوبيا فى هذه الدراسة، مما يشير إلى وجود إختلافات عالية بين الأصناف. واستناداً إلى المسافة الجينية في تحليل RAPD ، ظهر الصنفان ٩ و١٢على أنهما أكثر تباعدًا ويمكن استخدامهما في برنامج تربية اللوبيا. مما سبق يمكن الاستنتاج أن دلائل ال RAPD من أهم ألادوات المستخدمة للتحليل الوراثي للوبيا.