### Comparative assessment of genetic diversity of peanut (Arachis hypogaea L.) genotypes as revealed by RAPD, AFLP and SDS- protein markers

(Received: 01. 11. 2015; Accepted: 09. 11.2015)

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#### ABSTRACT

The present work was conducted to evaluate RAPD, AFLP and SDS protein marker systems for their ability to detect genetic diversity among ten peanut (Arachis hypogaea L.) genotypes and to compare the efficiency of these marker types in the classification of accessions according to the gene pool of peanut genotypes. The polymorphic fragments were obtained on the basis of 10 differentiating primers using the RAPD method, 2 differentiating primer combinations using the AFLP, and SDS protein. The ten RAPD primers produced 38 polymorphic bands, while AFLP primer combinations produced 30 polymorphic bands, and SDS protein pattern produced 4 polymorphic bands. RAPD data analysis showed that the genetic similarity among ten peanut accessions ranged from 75.3 to 98.7%, while the AFLPs generated data showed that the highest genetic similarity value was 97.8% and the lowest value was 74.7%, and the SDS protein showed that the highest genetic similarity value was 100% and the lowest value was 71.4%. The dendrogram generated with hierarchical UPGMA (Un-weighted Pair Group Method with Arithmetic Averages) cluster analysis of the Jaccard's similarity coefficient matrices revealed two major clusters, which were identified.

Key words: Groundnut accessions, Molecular markers, Polymorphism, Genetic similarity.

#### INTRODUCTION

eanut, or groundnut (Arachis hypogaea L.) is an important oilseed crop which is widely consumed by humans. Argentina, China and the United States are the largest exporters of peanuts while the European Union and Asia tend to be the largest peanut importers (Revoredo and Fletcher, 2002). Peanut oil, peanut butter and peanut seeds are highly nutritious for human consumption. The seeds contain approximately 45-51% oil and most of their oil content consists of oleic and linoleic acids (Lopez et al., 2000). Arachis hypogaea L. is grown throughout the world as a source of oil and hypogaea protein. The Arachis is an allotetraploid (AABB, 2n = 4x= 40 chromosomes), resulting from duplication of the cross between AA and BB wild type species (Leal-Bertioli et al., 2009).

The classification of groundnut only on the basis of their morphological characteristics is not sufficient, therefore the assessment of variation at gene level during germplasm collections and pedigree construction is necessary. The use of molecular markers will be helpful for the collection of advanced and novel genotypes of groundnut. DNA- based markers provide accurate knowledge at gene level, which was not possible with phenotypic markers (Altinkut *et al.*, 2003).

Molecular markers have been used for tagging of important traits of groundnut in interspecific introgression populations. Many markers have been identified which were associated with resistance to late leaf spot (Mace et al., 2006). Despite significant physiological, agronomic and morphological variation, the peanut exhibits variations at low level by RAPD (Mondal and Badigannavar ., 2010), SSR (Raina et al., 2001) and AFLP (Herselman, 2003). The deficiency of molecular variation in cultivated peanut is somewhat surprising since there is a wide range of variation in morphological characters such as seed size, seed coat color, maturation time and disease resistance (Hopkins et al., 1999). The lack of polymorphic markers has hindered cultivated peanut, in comparison with other crops, being enhanced by molecular techniques such as marker-assisted selection (MAS), resistance gene cloning, genetic mapping and evolutionary studies (He et al., 2003). The objective of thise study was to investigate and compare molecular profiles among the cultivated peanut genotypes using RAPD,AFLP and SDS-protein molecular marker techniques.

#### MATERIALS AND METHODS

Ten elite peanut (Arachis hypogaea L.) genotypes, as illustrated in Table (1), provided by the Oil Crops Research Dept., field crops Research Institute, ARC, Giza, Egypt, were utilized to assess the phylogenetic relationships and genetic diversity among these accessions using data generated from RAPD, AFLP and SDS-protein molecular marker techniques. This study was conducted the two successful seasons during of 2013/2014 and 2014/2015 in the greenhouses and laboratories of Department of Genetics Engineering, and Genetic Faculty of Agriculture, Benha University, Egypt.

#### **1.Genomic DNA extraction**

Genomic DNA was extracted from youngest leaves using DNeasy® Plant mini Kit for DNA isolation from plant tissue with modifications by adding 5 mg/ml PVP to the extraction buffer.

Table (1): Cultivar number, name and origin of ten elite peanut (Arachis hypogaea L.) genotypes.

Cultivar no.	Cultivar name	Cultivar origin
1	Giza 6	Egypt
2	R92	USA
3	G23	USA
4	Ismailia	Egypt
5	NC12	USA
6	M 420	India
7	M511	China
8	M432	India
9	Ecr 538	USA
10	Ecr 563	USA

### 2. Random amplified polymorphic DNA (RAPD)

A total of ten random 10-mer primers as indicated in Table (2) were used in the detection of polymorphism among cultivated peanut genotypes. Reactions were carried out in a total volume of 25  $\mu$ l containing 30 ng of genomic DNA as a template, 30 pmoles of random primer, 2 mM of dNTP's mix (dATP, dCTP, dTTP and dGTP, ABgene, Surrey, UK), 10 X PCR buffer, 25 mM MgCl<sub>2</sub>, and 2 units Taq DNA polymerase (Promega, USA). Amplification were carried out in a thermo cycler (UNO II Biometra) programed for 94°C for 4 min, (one cycle); followed by 94°C for 45 sec, 36°C for 1 min, and 72°C for 1 min (35 cycle); 72°C for 10 min (one cycle) ,then 4°C (infinitive).

The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5  $\mu$ g/ml), and visualized with ultraviolet light and photographed. DNA fragment sizes were determined by comparison with the 1Kb DNA ladder marker(Promega USA).

Primer	Sequence (5' - 3')	Primer	Sequence (5' - 3')
OPA-09	GGGTAACGCC	OPB-08	GTCCACACGG
<b>OPA-19</b>	CAAACGTCGG	OPF-19	CCTCTAGACC
<b>OPA-20</b>	GTTGCGATCC	<b>OPF-20</b>	GGTCTAGAGG
OPB-05	TGCGCCCTTC	<b>OPZ-01</b>	TCTGTGCCAC
<b>OPB-07</b>	GGTGACGCAG	<b>OPZ-02</b>	CCTACGGGGA

Table (2): Sequence of ten decamer arbitrary (10-mer) primers assayed in RAPD- PCR marker.

## **3.** Amplified fragment length polymorphism (AFLP)

AFLP analysis was performed using the Invitrogen AFLP<sup>®</sup> Analysis system I, and AFLP Starter primer kit (Cat # 10544-013 and respectively, 10483-014. Invitrogen Corporation, Carlsbad, California, USA) as indicated in Table (3). Amplicon template preparation, pre-amplification and selective amplification were conducted according to manufacturer's protocol of the BRL AFLP analysis system I kit (Gibco, USA) for small genome using a total of 250 ng of genomic digestion. DNA in EcoR1(E)/Mse1(M)Amplification products were electrophoresed in 6% (w/v) denaturatig polyacrylamide sequencing gels for 2h at 2000W, and DNA

bands were visualized using silver staining according to the methods of Vos *et al.*, (1995).

#### 4. SDS-protein electrophoresis

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (1970), as modified by Studier (1973).

#### 5. Data analysis

Amplified products for RAPD, AFLP and SDS-protein markers were visually examined for each primer or each primer combination. Also, Gel Documentation System (Gel-Doc 2000, with Diversity Database Fingerprinting Software, version 2.1, Bio-Rad Laboratories, Hercules, California, USA) was used for gel analysis, scoring, data handling, cluster analysis and construction of dendrograms. Pair-wise comparisons of peanut genotypes were used to determine similarity coefficients according to Dice's similarity coefficient (DSC's). The similarity coefficient was then used to construct dendrogram using the Un-weighted Pair Group Method with Arithmetic Averages (UPGMA) employing.

Table (3): Sequence of different AFLP primers used.

GAGTCCTGAG
GGACTCAT
GACTGCGTACC
TACGCAGTCTAC
TCCTGAGTAA
TCCTGAGTAA <u>CAA</u>
TCCTGAGTAA <u>CAG</u>
GTACCAATTC
GTACCAATTC <u>AAG</u>

#### **RESULTS AND DISCUSSION**

## 1. Identification of random amplified polymorphic DNA (RAPD)markers

A set of twenty 10-mer oligonucleotides primers was pre-screened for the ability to detect polymorphism in ten Peanut (*Arachis hypogaea* L.) genotypes. Any fragment thought to be artifact or difficult to be scored was not included in the data set. Out of 20 prescreened primers, 10 primers successfully amplified bands and were highly informative. The ten primers that generated reproducible and scorable polymorphic markers were selected for further analysis.

They produced multiple band profiles with a number of amplified DNA fragments ranging from 8 to 14, with an average of 11 bands per primer , while the number of polymorphic fragments ranged from 3 to 6

with an average of 4.5 polymorphic bands per primer (Table 4). A maximum number of 14 fragments were amplified with primers OPB-07 and the minimum number of 8 fragments were amplified with primers OPA-09, OPA-20, OPB-05, OPF-20 and OPZ-01 as shown in Table (4). The total number of reproducible fragments amplified by the ten primers reached 102 bands, of which 38 were polymorphic fragments. This represented a level of polymorphism of 37.25 %, which indicates a low level of polymorphism among the genotypes studied. The size of the amplified fragments also varied with the different primers and ranged from 100 to 1500 bp as shown in Fig (1). The obtained results are in good accordance with those of Lan et al.( 2007); Yu et al.( 2007); Kumar et al.( 2007) and Zitoun et al.(2008). All these authors demonstrated that used primers produced reliable and reproducible banding patterns and the number, size of amplified DNA fragments and the percentage of generated polymorphic bands differed with the different primers tested. Al-Saghir and Abdel-Salam (2015) evaluated the genetic diversity of the twenty peanut accessions using random polymorphic amplified DNA (RAPD) molecular marker to evaluate RAPD markers to be used in peanut as genetic markers and improved such techniques as suitable strategies peanut for germplasm characterization. Twenty-seven RAPD primers produced 210 amplification products of which 80 (36.4%) were polymorphic.

Table (4): Levels of polymorphism, total number of bands, monomorphic bands, polymorphic bands and percentage of polymorphism as revealed by RAPD markers among the ten cultivated peanut genotypes.

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No.	Primers	Total number of bands	Mono morphic bands	Poly morphic bands	% of polymorphism
1	OPA-09	8	2	6	75.00
2	OPA-19	10	7	3	30.00
3	OPA-20	8	4	4	50.00
4	OPB-05	12	8	4	33.33
5	OPB-07	14	10	4	28,57
6	OPB-08	10	6	4	40.00
7	OPF-19	11	7	4	36.36
8	OPF-20	8	5	3	37.50
9	OPZ-01	8	5	3	37.50
10	OPZ-02	13	10	3	23.08
-	Fotal	102	64	38	37.25

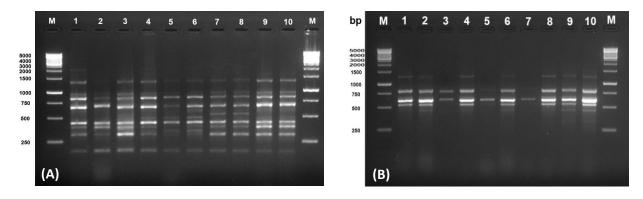


Fig. (1): RAPD profiles of ten cultivated peanut genotypes (1 - 10) as detected with primers (A)OPA-20, (B) OPF-19. DNA molecular weight standards (M) 1 kp DNA ladder.

The genetic similarity among ten peanut (Arachis hypogaea L.) genotypes was estimated in terms of using Dice's similarity coefficients (DSC's) to compute the similarity matrix based on the scored RAPD data matrix. This similarity matrix was used to generate a dendrogram using the UPGMA method. RAPD data analysis show that the genetic similarity among ten peanut genotypes ranged from 75.3 to 98.7%, with an average value of 87%. In addition to , RAPD analysis, the highest similarity level (98.7%) was detected between Ecr 538 and Ecr 563, which are closely related accessions. While, the lowest genetic similarity (75.3%) was detected between Ecr 563 and Giza 6 genotypes.

A dendrogram separated ten peanut (*Arachis hypogaea* L.) genotypes into two major clusters, the first cluster contained Giza 6 accessions collected from Egypt while the second cluster contained the remaining cultivated forms of peanut, that could be divided into two sub clusters. Peanut cultivars

(Ecr 538 and Ecr 563) are grouped together in the first sub cluster, while peanut cultivars (R92, G23, Ismailia1, NC12, M420, M511 and M432) grouped together in the second subcluster as shown in Fig (2).

These results are in congruence with those obtained by Al-Saghir and Abdel-Salam (2015)who showed that a **UPGMA** dendrogram clustered the 20 peanut accessions. Two major clusters resulted ; One cluster included by itself (S1 to S8) and the second cluster included the rest of the accessions (S9 to S20). The third cluster had two groups of Arachis duranensis. The genetic distance between first and the second cluster was 6.6. Moreover, a significant genetic variation was detected in peanut accessions different which could reflect genetic backgrounds. Cluster analysis showed that samples S1 to S8 and S9 to S20 were genetically close 96% (they showed similarity).

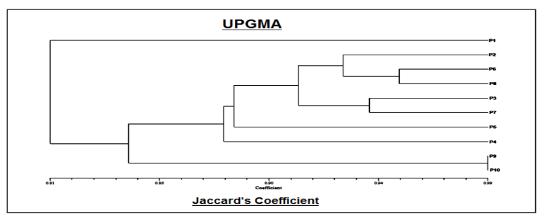


Fig. (2): Dendrogram for the ten peanut genotype accessions constructed from the RAPDs generated data using UPGMA method and similarity matrices computed according to DSC's

Sitthiwong et al. (2005) stated that RAPD analysis provides a specific technique and reliable method in classifying pepper (Capsicum annuum L.) accessions. Aros et al. (2006) concluded that RAPD markers allowed between discrimination all commercial varieties and wild species of alstroemeria. Cai et al. (2007) stated that the generated phylogenetic tree by adopting the UPGMA method to describe the relationships within and among species or cultivars of cherry was generally in agreement with taxonomical classification. Hadian et al. (2008)summarized that a UPGMA dendrogram separated the 28 accessions of Satureja hortensis into three main groups in 0.40 distance unit. There was little relationship between genetic divergence and geographical origins, so that the population from similar geographical places belonged to separate clusters. Zitoun et al. (2008) reported that a constructed dendrogram showed that most of Tunisian olive accessions are closely related to olive genotypes originating from the Eastern Mediterranean and some are clustering with genotypes originated from the Western Mediterranean.

#### **2. Identification of amplified fragment** length polymorphism (AFLP) markers

Two EcoR1 and Mse1 selective primer combinations were used for fragment amplification. All AFLP polymorphisms were scored as dominant markers. Sequences of selective primers used in assaying peanut genotypes are given in Table (5). AFLP primer combinations used generated 91 of selectively amplified DNA fragments and the identification of 30 DNA fragments (32.9%) polymorphic markers as shown in Table (5). On average 45.5 distinguishable bands were observed after selective amplification with each primer combination, and an average of 32.9 % of these AFLP bands were found to be polymorphic among peanut genotypes, while, the percentage of polymorphism ranged from 26.2 to 38.8%.

The highest percentage of polymorphism (38.8%) was obtained with primer combination (E-AAG X M-CAA), while, the lowest percentage (26.2%) was obtained with the primer combination (E-ACA X M-CAG). However, the maximum number of bands per primer combination was found to be 49 which was generated with primer combination (E-AAG X M-CAA), whereas, the minimum numbers of bands per primer combination

were 42 obtained with primer combination (E-ACA X M-CAG), (Table 5 and Figure 3).

This confirms the high multiplex ratio produced by AFLP markers. The size of

selectively amplified DNA fragments ranged in size from 50 to 900 bp as shown in Fig (3).

 Table (5): Levels of polymorphism, total number of bands, monomorphic bands, polymorphic bands and percentage of polymorphism as revealed by AFLP markers among the ten peanut genotypes.

Primer Combinations	Total number of bands	Monomorphic bands	Polymorphic bands	% of polymorphism
$E_{AAG}X\ M_{CAA}$	49	30	19	38.8
$E_{ACA}XM_{CAG}$	42	31	11	26.2
Total	91	61	30	32.97

Based on AFLP analysis, the highest similarity percentage (97.8%) detected by the AFLP assay was between M 420 and M 432; both share the same genetic background and geographical region since they were collected from India. While the lowest percentage of similarity (74.7%) was detected between Giza 6 (Egypt) and Ecr 563(USA) peanut cultivars. A constructed dendrogram separated ten types of peanut into two major clusters, according to the geographic region and to classical botanical classification of cultivated forms of peanut. The first cluster contained Giza 6 and Ismailia 1, while, the second cluster contained the other cultivated forms of peanut ; the second cluster is divided into two sub clusters. The first sub cluster contains Ecr538 and Ecr 563; the second sub cluster is divided into two sub sub clusters; first sub-sub- cluster contains R92, while G23, NC12, M 420, M511and M432 grouped together in the second sub- sub cluster as shown in Fig (4).

These results are in good accordance with the results obtained by Adewale *et al.* (2012) who characterized 80 AYB accessions(African yam bean) using five AFLP primer combinations (E-AAC/M-CAG, E-ACT/M-CAG, E-AGC/M-CAG, EACG/M-CAT and E-ACG/M-CTG) which generated a low percentage polymorphism of 26% due to the many monomorphic fragments obtained (168). This revealed higher similarities across some of the genomic loci of the tested population of AYB.

Maras et al.(2008) evaluated AFLP (Amplified Fragment Length Polymorphism) and SSR (Simple Sequence Repeat) marker systems for their ability to detect genetic diversity within a set of 29 common bean accessions. The ten AFLP primer combinations produced 112 polymorphic bands, while 14 SSR primer pairs generated 100 polymorphic bands. Ojuederie et al. (2014)evaluated 40 accessions of Sphenoshylis stenocarpa hochst ex using AFLP markers to generate a total of 1730 amplified fragments across the African yam bean accessions; of which 1640 fragments polymorphic were (95.2%). Genetic fingerprinting using AFLP markers has been reported in peanut (Herselman, 2003) and various crops; Dolichos Bean (Venkatesha et al., 2010); Velvet bean (Capo-chichi et al., 2003); and in assessment of genetic diversity in sesame (Sesamum indicum L.) (Ali et al., 2007). It has also been used successfully for analyzing genetic diversity in some other plant species such as Yam Bean (Sphenostylis stenocarpa) (Aremu and Ibirinde, 2012).

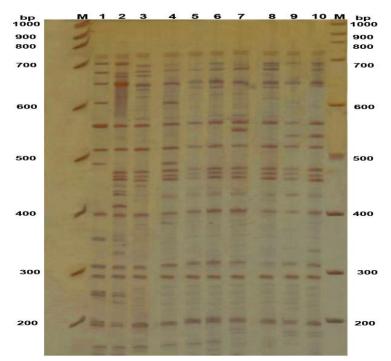


Fig. (3): AFLP profiles of ten peanut genotypes (1 - 10) as revealed by AFLP primer combination (E-AAG X M-CAA). (M) DNA molecular weight standards 100 bp DNA ladder.

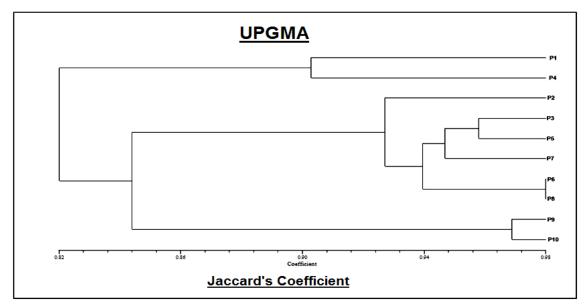


Fig. (4): Dendrogram for the ten peanut genotype accessions constructed from the AFLPs generated data using UPGMA method and similarity matrices computed according to DSC's.

# 3. Molecular fingerprints based on SDS-protein.

The electrophoretic banding patterns of proteins extracted from the youngest leaf of the ten peanut genotypes are shown in Fig (5). The results of SDS-PAGE revealed a total number of 14 bands with molecular weights (MW) ranging from about 20 to 200 kDa, which were not necessarily present in all genotypes. Data revealed 10 common bands (monomorphic), while the remaining 4 bands were polymorphic with 28.6 % polymorphism. The densitometric analysis of the SDS-protein banding patterns of the studied genotypes were found to be useful in varietals identification of the studied peanut genotypes.

The similarity relationships ranged between 71.4 and 100%. The highest similarity index (100%) was recorded between most of them, while the least similarity index (71.4%) was observed between genotypes Giza 6 and Ecr 563. Dendrogram for the ten genotypes is presented in Fig (6). The genotypes were separated into two main clusters. The first cluster contained Ecr563 while, the second cluster contained all the rest forms of peanut genotypes, that could be divided into two sub clusters. First sub cluster contained Giza 6 and Ismailia 1 genotype while second sub cluster contained remaining genotypes.

Electrophoresis of proteins is a powerful tool for the detection of genetic diversity, especially SDS-PAGE protein seeds as a reliable technology used in the screw. Detectors molecular markers, such as protein marker can be as useful and reliable tool in the identification of genetic variation and kinship relations varieties and plant species used (Criley *et al*, 2008) and (Tamkoc and Arslan 2010). Soybean protein (Razavizadeh and Ehsanpour, 2012), oats (Dvoracek *et al.*, 2003), pistachio species (Ehsanpour *et al*, 2010) and brassica cultivars (Rahman *et al.*, 2004) have been used. Protein markers can act effectively to study the genetic variation of germplasm for its utilization in crop breeding programs (Javaid *et al.*, 2004). This study showed that diversity exists for protein profiles and seed storage proteins have potential for aiding species classification and for serving as markers for hybridization studies.

The obtained results are in good accordance with those obtained by Masoomeh et al. (2015) who used Seed storage protein electrophoresis for identification of 11 groundnut (Arachis hypogaea L.) cultivars. To survey genetic relation and diversity among peanut genotypes, protein bands were analyzed with the Total lab software and results showed that the 17 visible major bands were shown on the vertical gel. Increase of expression levels in a specific protein (band No.11 approximately 26 kDa weight) was expressed in all cultivars. Cluster analysis with UPGMA method on the basis of protein bands and cut the dendrogram at 50% similarity, divided the cultivars into 4 groups. According to the results, genotypes 7, 8, 9, 10 and 11 in the first group, genotype 3, 4 and 5 in the second groups and genotypes 1 and 2, were in the third and fourth groups, separately.

Berber and Yasar (2011) characterized 28 bean cultivars grown in Turkey by using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) technique. The numerical analysis of SDS-PAGE of seed protein profiles showed that each cluster had slight discriminative protein banding. For example, the cluster I include 5 of reference bean cultivars (lines 15, 17-20), sharing many protein bands. All members of the subcluster IIa were from Black Sea region, whereas the subcluster IIc consisted in Gina standard cultivar together with all genotypes from the Eastern Anatolia part of Turkey, having the highest intra-cluster similarities (between 95.54 and 100%). In general, the results of this study showed that the proteins of peanut cultivars were suitable for evaluation of

genetic diversity and dividing the cultivars in separate groups.

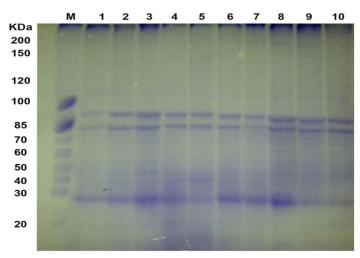


Fig. (5): SDS-protein banding patterns among ten peanut genotypes.

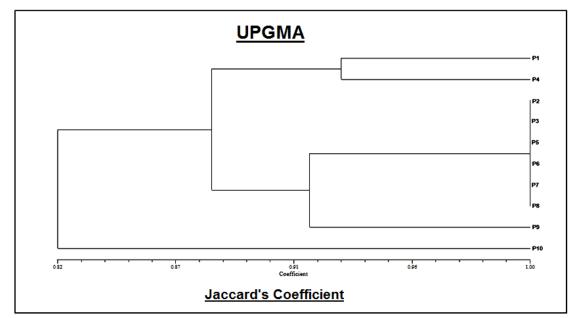


Fig. (6): Dendrogram for the ten peanut genotype accessions constructed from the SDS protein generated data using UPGMA method and similarity matrices computed according to DSC's.

The data summarized the total number of reproducible fragments amplified by the ten RAPD primers reached 102 bands, of which were polymorphic fragments. This 38 represented a level of polymorphism of 37%. The number of amplified DNA fragments ranged from 8 to 14, with an average of 11 bands per primer while the number of polymorphic fragments ranged from 3 to 6 with an average of 4.5 polymorphic bands per primer, which indicates a low level of polymorphism among studied genotypes. The results of SDS-PAGE revealed a total number of 14 bands with molecular weights (MW) ranging from about 20 to 200 kDa, which were not necessarily present in 10 genotypes. Data revealed 10 common bands (monomorphic), while the remaining 4 bands were polymorphic with 28.6 % polymorphism. Two AFLP primer combinations generated a total of 91 amplified fragments DNA of which 30 were polymorphic. This represented a level of polymorphism of 32.5 % among peanut genotypes. The highest percentage of polymorphism (38.8%) was obtained with primer combination (E-AAG X M-CAA), while, the lowest percentage (26.2%) was obtained with the primer combination (E-ACA X M-CAG). However, the maximum number of bands per primer combination was found to be 49 which was generated with primer combination (E-AAG X M-CAA), whereas, the minimum numbers of bands per primer combination were 42 obtained with primer combination (E-ACA X M-CAG) which indicates a very high level of polymorphism among studied genotypes.

In conclusion, the differences between RAPD, AFLP and SDS protein reflect the extent of genome coverage and different evolutionary properties. The genomic distribution of RAPD and SDS protein is random, whereas it is reasonable to expect that AFLPs should have complete genome (Nowosielski et al ., 2002 and Lanteri et al., 2004). Although RAPD and SDS protein markers have been extensively used for peanut fingerprinting and genetic analysis, AFLP analysis provides а higher level of polymorphism than RAPD and SDS protein. AFLP markers are advantageous because they reveal a large number of reproducible markers, thereby increasing the probability of identifying polymorphic markers, even among closely related genotypes such as in peanut (Ojuederie genotypes al., 2014). et Comparisons of the efficiency of RAPD, SDS protein and AFLPs in assessing the genetic diversity of peanut have generally shown that AFLP is the preferred technique. Geleta et al. (2005) in a comparison of AFLP and RAPD to evaluate genetic diversity in sorghum, found that AFLP was the best suited molecular assay for accurate fingerprinting.

The obtained results of the molecular markers study concluded that DNA markers, i.e., RAPD, AFLP and SDS-protein represent an efficient tools for estimating the genetic relationships within and among crop species and their wild relatives, *i.e.*, peanut genotype and generate fingerprints for each accession, in addition to identifying specific markers which might be closely related with useful traits. This is essential for the maximization of benefits and utilization of plant genetic resource collections and could be of great benefit in genotype identification and protection, germplasm conservation and breeding programs.

#### REFERENCES

Adewale, B.D.; Dumet, D.J.; Vroh-Bi, I.; Kehinde, O.B.; Ojo, D.K.; Adegbite, A.E. and Franco, J.(2012). Morphological diversity analysis of African yam bean and prospects for utilization in germplasm conservation and breeding. Gen. Res. Crop Evol., 59(5):927-936.

- Ali, G.M.; Yasumoto, S. and Seki-Katsuta, M. (2007). Assessment of genetic diversity in sesame (*Sesamum indicum* L.) detected by Amplified Fragment Length Polymorphism markers. Elec. J. Biotech., 10(1):12-23.
- Al-Saghir, M. G. and Abdel-Salam, A. G.(2015). Genetic diversity of peanut (*Arachis hypogea* L.) cultivars as revealed by RAPD markers. Amer. J. Plant Sci., 6: 2303-2308.
- Altinkut, A.; Kazan, K. and Gozukirmizi, N. (2003). AFLP marker linked to water-stress-tolerant bulks in barley (*Hordeum vulgare* L.). Genet Mol Biol., 26: 77-82.
- Aremu, C.O. and Ibirinde, D.B. (2012). Biodiversity Studies on Accessions of African Yam Bean (*Sphenostylis stenocarpa*). Int. J. Agric. Res., 7:78-85.
- Aros, D.; Meneses, C. and Infante, R. (2006). Genetic diversity of wild species and cultivated varieties of *alstroemeria* estimated through morphological descriptors and RAPD markers. Sci. Hortic., 108: 86–90.
- Berber, I. and Yasar, F. (2011). Characterization of bean (*Phaseolus* vulgaris L.) cultivars grown in turkey by SDS-PAGE of seed proteins. Pak. J. Bot., 43(2): 1085-1090.
- Cai, Y.L.; Cao, D.W. and Zhao, G.F.(2007). Studies on genetic variation in cherry germplasm using RAPD analysis. Sci. Hortic., 111: 248–254.
- Capo-chichi, L.J.; Weaver, D.B. and Morton, C.M. (2003). The use of molecular markers in the study of genetic diversity in mucuna tropical and sub-tropical. Agroe., 1 (2-3):309-318.
- Criley, R.A.; Roh, M.S.; Kikuchi, M. and Manshardt, R.M. (2008). A

Comparison of *Gardenia augusta* cultivars using isozymes and RAPD markers. Acta Hort., 766: 461-468.

- **Dvoracek, V.; Curn, V. and Moudry, J.** (2003). Suitability of oat-seed storageprotein markers for identification of cultivars in grain and flour samples. Plant, Soil Environ., 49 (11): 486-491.
- Ehsanpour, A. A.; Shojaie, B. and Roatami, F. (2010). Characterization of seed storage protein patterns of four Iranian Pistachios using SDS-PAGE. Nat. Sci., 2(7): 737-740.
- Geleta,L.F.; Labuschagne,M.T. and Viljoen,C.D.(2005).Genetic variability in pepper (*Capsicum annuum* L) estimated by morphological data and amplified fragment length polymorphic marker. Biodivers.Conserv.,14:2361-2375.
- Hadian. J.; Tabatabaei, **S.M.F.**; Naghavi, M.R.; Jamzad, Z. and Masoumi, T.R. (2008).Genetic diversity of Iranian accessions of hortensis L. based Satureja on horticultural traits and RAPD markers. Sci. Hort., 115: 196-202.
- He, G.; Meng, R.; Newman, M.; Gao, G.; Pittman, R. N. and Prakash, C. S. (2003). Microsatellites as DNA markers in cultivated peanut (*Arachis hypogaea* L.). BMC Plant Biol., 3, 3.
- Herselman, L. (2003.) Genetic variation among southern African cultivated peanut (*Arachis hypogaea* L.) genotypes as revealed by AFLP analysis. Euphytica., 133: 319-327.
- Hopkins, M. S.; Casa, A. M.; Wang, T.;
  Mitchell, S. E.; Dean, R. E.; Kochert,
  G. D. and Kresovich, S.
  (1999).Discovery and characterization of polymorphic simple sequence repeats (SSRs) in peanut. Crop Sci., 39:1243–1247.

Arab J. Biotech., Vol. 18, No. (2) July (2015):

- Javaid, A.; Ghafoor, A. and Anwar, R. (2004). Seed storage protein electrophoresis in groundnut for evaluating genetic diversity. Pak.J. Bot., 36 (1): 25-29.
- Kumar, N.; Lakra, W.S.; Majumdar, K.C.;Goswami, M. and Ravinder, K. (2007).Genetic diversity in the Indian population of *Penaeus monodon* as revealed by mtDNA sequence analysis. Aquac. Res., 38:862-869.
- Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London)., 227: 680-685.
- Lan, G.B.; Liao, S.M. and Yan, B. (2007). Effect of water temperature on larval development and metamorphosis of *Sipunculus nudus*. J.Fish. China, 31(5):633-638.
- Lanteri, S.; Saba, E.; Cadinu, M.; Mallica, G.M.; Baghino, L. and Portis, E. (2004): Amplified fragment length polymorphism for genetic diversity assessment in globe artichoke. Theor. Appl. Genet., 108: 1534–1544.
- Leal-Bertioli, S.; Jose, A.C.; Alves-Freitas, D.M.; Moretzsohn, M.C.; Guimaraes, P.M.; Nielen, S.; Vidigal, B.S.; Pereira, R.W.; Pike, J.; Favero, A.P.; Parniske, M.; Varshney, R.K. and Bertioli, D.J. (2009). Identification of candidate genome regions controlling disease resistance in *Arachis*. BMC Plant Biol., 9: 1-12.
- Lopez, Y.; Nadaf, H. L.; Smith, O. D.; Connell, J. P.; Reddy, A. S. and Fritz, A. K. (2000).Isolation and characterization of the Delta(12)-fatty acid desaturase in peanut (Arachis search hypogaea L.) and for polymorphisms for the high oleate trait in Spanish market-type lines. Theor. Appl. Genet., 101: 1131–1138.

- Mace, E.S.,; Phong, D.T.; Upadhyaya, H.D.,; Chandra, S. and Crouch, J.H. (2006). SSR analysis of cultivated groundnut (*Arachis hypogaea* L.) germplasm resistant to rust and late leaf spot diseases. Euphytica, 152: 317-330.
- Maras, M.; Sustar-Vozlic, J. ; Javornik, B. and Meglic, V. (2008). The efficiency of AFLP and SSR markers in genetic diversity estimation and gene pool classification of common bean (*Phaseolus vulgaris* L.). Acta Agric. Slovenica, 91(1): 87-96.
- Masoomeh, J.; Mehran, G. and Fatemeh, J.(2015). Seed storage protein electrophoresis for identification of some Groundnut (*Arachis hypogaea* L.) cultivars. Inte'l Res. J. of Appl. Basic Sci., 9 (10): 1718-1721.
- Mondal, S. and Badigannavar, A.M. (2010). Molecular diversity and association of SSR markers to rust and late leaf spot resistance in cultivated groundnut (*Arachis hypogaea* L.). Plant Breed., 129: 68-71(4).
- Nowosielski, J.; Podyma, W. and Nowosielska, D. (2002). Molecular research on the genetic diversity of polish varieties and landraces of *Phaseolus coccineus* L. and *Phaseolus vulgaris* L. using the RAPD and AFLP method. Cel. Mol. Biol., 7: 753-762.
- Ojuedrie, O. B.; Balogun, M. **O**.; Fawole, I.; Igwe, D. O. and Olowolafe, M.O.(2014). Assessment of the genetic diversity of African yam bean accessions (Sphenostylis stenocarpa) using Amplified Fragment Length Polymorphism (AFLP) markers. Afr. J. biotech.,13 (18):1850-1858.
- Rahman, M.; Mukhlesur, D. and Yutaka, H. (2004). Genetic diversity in Brassica species using SDS- PAGE analysis. J. Biol. Sci., 4: 234-238.

- Raina, S.N.; Rani, V.; Kojima, T.; Ogihara, Y. and Singh, K.P. (2001). Devarumath RM: RAPD and ISSR fingerprints as useful genetic markers for analysis of genetic diversity, varietal identification, and phylogenetic relationships in peanut (*Arachis hypogaea* L) cultivars and wild species. Genome, 44: 763-772.
- Revoredo, C. L. and Fletcher, S. M. (2002). World peanut market: an overview of the past 30 years. Georgia Agric. Exper. Station Res. Bull., 437:1–24.
- Sitthiwong, K.; Matsui, T.; Sukprakarn, S.; Okuda, N. and Kosugi, Y. (2005). Classification of pepper (*Capsicum annuum* L.) accessions by RAPD analysis. Biotech., 4: 305–309.
- Studier, F.W. (1973). Analysis of bacteriophage T7 early RNAs and proteins of slab gels. J. Mol. Biol., 79: 237-248.
- Tamkoc, A. and Arslan, E.(2010). Comparison of agronomic characters, total seed storage proteins and their use for genotypes discrimination in the kentucky bluegrass (*Poa pratensis* L.). Biotech. Biotec Eq., 24(1): 1573-1576.

- Venkatesha. **S.C.;** Gowda. **P.H.:** Ganapathy, K.N.; Gowda, M. and Ramachandra, R. (2010). Genetic Fingerprinting in Dolichos Bean using AFLP Markers and Morphological Int'l. J. Biotech. Biochem., Traits. 6(3):395-404.
- Vos, P.; Hogers, R.; M. Bleeker, R.; Reijans, M.; Van de Lee, T.; Hornes, M.; Freijters, A.; Pot, J.; Peleman, J.; Kuiper, M. and Zabeau, M. (1995).
  AFLP: a new technique for DNA fingerprinting. Nuc. Acids Res., 23: 4407-4414.
- Yu, Z.N.; Wei, X.H.; Kong, X.Y. and Yu, S.S. (2007). Application of the first internal transcribed spacer (ITS-1) of ribosomal DNA as amolecular marker to population analysis in *farrer's Scallop* Chlamys farreri. Acta Oceanologica Sinica., 26(1):93-100.
- Zitoun, B.; de- Caraffa, V.B.; Giannettini, J.; Breton, C.; Trigui, A.; Maury, J.; Gambotti, C.; Marzouk, B. and Berti, L. (2008). Genetic diversity in Tunisian olive accessions and their relatedness with other Mediterranean olive genotypes. Sci. Hort., 115: 416–419.

### الملخص العربي

# التقييم النسبى للإختلافات الوراثية لبعض أصناف الفول السودانى بإستخدام الواسمات الجزيئية RAPD, AFLP and SDS- protein

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أجريت هذه الدراسة لتقدير مدى قدرة الواسمات الجزيئية RAPD, AFLP and SDS- protein على تقدير مستوي الإختلافات الوراثية بين مجموعة من أصناف الفول السودانى المنزرعة في مصر والمستوردة وكذلك إجراء مقارنة ما بين الإختبارات الثلاثة لتقدير مدى قدرة كلاً منهما في تقسيمهما طبقاً للأصل الجيني لمنشئهم وكانت الشظايا الناتجة من إستخدام ١٠ بادئ RAPD (٢٠٢) شطية من شطايا AD و ١٩ شطايا من شطايا من شطايا ATL من مادئات الناتجة من إستخدام ١٠ بادئ RAPD (٢٠٢) شطية من شطايا AD و ٩٩ شطايا من شطايا من شطايا ATL من المنظايا الناتجة من إستخدام ١٠ بادئ RAPD (٢٠٢) شطية من شطايا ATL و ٩٩ شطايا من شطايا من معددة المظاهر بادئات ATL وقد أوضحت نتائج تحليل البيانات الخاصة بـARP وجود ٢٨ شظايا من شطايا من شطايا من معددة المظاهر بينما كانت ٢٠ متابع متعددة المظاهر ظهرت من نتائج تحليل بيانات AFLP و ٤ شطايا من مطايا من معددة المظاهر بينما كانت ٢٠ معددة المظاهر طهرت من نتائج تحليل بيانات AFLP و ٤ شطايا من مطايا من معددة المظاهر بينما كانت ٢٠ متابع متعددة المظاهر طهرت من نتائج تحليل بيانات AFLP و ٤ شطايا من معددة المظاهر بينما كانت تعام ٢٠ بالغايا متعددة المظاهر طهرت من نتائج تحليل بيانات AFLP و ٤ شطايا متعددة المظاهر بين أصناف الفول السودانى تتراوح ما بين ٢٠ ٧٠ % لي ١٠ ٨٠ % بينما كانت ٢٠ % % مع AFLP مع بين أصناف الفول السودانى تتراوح ما بين ٢٠ ٧٠ % مع SDS - وقد تم تحليل بيانات AFLP % مع AFLP % من نتائج تحليل بيانات الفول السودانى تتراوح ما بين ٢٠ ٧٠ % مع SDS - وقد تم تحليل علاقات درجة القرابة والتراثى الوراثى بيناء على مصفوفات التشابه المأخوذة من كل نوع من الواسمات الجزيئية بإستخدام طريقة UPGMA الوراثى بناء على مصفوفات التشابه المأخوذة من كل نوع من الواسمات الجزيئية بإستخدام طريقة لاوراثي الوراثى بناء على مصفوفات التشابه المأخوذة من كل نوع من الواسمات الجزيئية بإستخدام طريقة للوراثى الوراثى بناء على مصفوفات التشابه المأخوذة من كل نوع من الواسمات الجزيئية بإستخدام طريقة للوراثي الوراثي بناء المريخي القرابة الوراثية .