Molecular Identification of Nine Okra Landraces by SDS-PAGE and RAPD Markers technique

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Abstract: Genetic identification of okra genotypes by fingerprinting techniques was performed on seed storage protein (SDS-PAGE) and leaf DNA (RAPD-PCR). Okra genotypes were collected from several geographical zone areas in Egypt and challenged to molecular marker analysis. Seed storage protein electrophoretic of SDS-PAGE indicated that the genotypes Aswan and Red, Tanta and Ityei El-Barud, Al-Sheikh Makram and Damanhur, Al-Hemaa and Al-Sabahia were closely related to each other. The nine okra genotypes were divided into main clusters A and B the first cluster A is further classified into two sub-clusters, a and b. Nine okra genotypes were assessed using 6 primers which amplified 61 bands with an average of 10.16 bands/ primer. Out of the total amplified bands, only 42 (68.58%) were polymorphic bands, 7 (11.47%) were monomorphic bands, 12 (19.67) positive unique bands and 1 (1.63%) negative unique bands. The dendrograms by UPGMA distinguished two main clusters, A and B. The first cluster A comprises two sub-clusters a and b. However, the main cluster B confined two main sub-clusters c and d. a considerable polymorphism appeared to exist, which showed genetic variability in okra genotypes that will be useful in the future for breeding program to obtained new genotypes with good traits.
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1. Introduction

Okra (*Abelmoschus esculentus* L.) belongs to *Malvaceae* family, while the scientific name of okra was changed from previously genus *Hibiscus* to recently genus *Abelmoschus*. Okra is mostly widely distributed and grown in Asia (India), Africa (Egypt, Ethopia, Ghana and Nigeria) and Southern America (Costa Rica), (Younis et al., 2015). Sodium dodecyle sulfate (SDS-PAGE) is widely used, not only for the determination of protein molecular weights but also for the various unknown proteins in addition to the identification and the quantification of newly biosynthesized proteins. Characterization of plant leaf or seed storage proteins by electrophoresis has proved to be a more rapid and a reliable alternative method for both initial identification and later verification of genotypes (Mohamed, 2000). Through readings of the several literatures on identification of proteins by SDS-PAGE, it was detected that the most research work were done on seed storage protein and little of them for leaf protein and the electrophoresis analysis permitted the identification of the unidentified samples (Dinlli and Bonetti, 1992). SDS-PAGE of soluble seed protein revealed some differences among the species in terms of the number of position and intensity of bands, while varietal discrimination based on SDS-PAGE could not be done. Similarity index calculated based on band homology established more divergence of the cultivated species with *A. moschatus* compared with *A. ficulneus* (Pal et al., 2002).

The molecular analysis of the genome at the DNA level can provide a greater advantage because DNA sequences are the same in all of the living cells of a plant, regardless of physiological or developmental stage of the tissue. The diversity based on phenotypic and morphological characters usually varies with environments and evaluation of traits requires growing the plants to full maturity prior to identification. Molecular markers have proven to be powerful tools in the evaluation of genetic variation and finding genetic relationships within and among species (Chakravarthi and Naravaneni, 2006). Among the several DNA based techniques, Random Amplified Polymorphic DNA (RAPD) is simple, less technology intensive, cheap and does not require pre-sequencing for designing primers. RAPD markers have been extensively used as a tool to estimate genetic diversity to determine intraspecific variations in different plant species (Mady et al., 2013, Mady et al., 2014 and Helaly et al., 2014). Diversity based on morphological characters usually varies with environments and evaluation of traits requires growing the plants to full maturity prior to identification of diverse genotypes. Now, the rapid development of biotechnology allows easy analysis of large number of loci distributed throughout the genome of the plants. Molecular markers have proven

to be powerful tools in the assessment of genetic variation and in elucidation of genetic relationships within and among species (Conrad and Osawaru, 2005, Chakravarthi and Naravaneni, 2006 and Mady et al., 2014).

Identification of genetic diversity was very important for develop the genetic variability of relatives crop varieties to improvement breeding programs (Parkash et al., 2011). Okra breeding program well done by several researchers to obtain heterosis and combining ability provides important information for improving economic characteristics (Obiadalla-Ali et al., 2013). The collection, maintenance, and assessment of the genetic diversity of landraces can help establish breeding programs to improve the studied landraces or to produce new hybrids that meet farm and market demands in terms of the disease resistance and fruit yield and quality (Mady et al., 2013). The objectives of this study were to conduct morphological characterization of 9 okra landraces collected from several geographical zone areas. The estimation of the genetic variation and genetic relationships among these landraces will be analysis using SDS-PAGE and RAPD marker techniques, and to develop genetic profiles for these landraces.

2. Material and Methods

A - Plant materials:

Seeds of nine okra (*Abelmoschus esculentus* L., Moench) genotypes were collected from different zone area which grown domestically in Egypt. Two of these genotypes called Al-Sabahia and Red were obtained from the Vegetable Research Department, Horticulture Research Institute, Agriculture ministry, Giza. While, the other 7 genotypes were collected from different locations in Eygpt and called with the same cultivation region such as Aswan, Al-Hemaa, Al-Sheakh Makram and Manshaa which obtained from Upper Egypt, were Tanta, Ityei El-Barud and Damanhur obtained from lower Egypt. The genotypes were analysed morphologically and chemically by Shalan et al., (2011). This study was carried out to follow the genetic diversity and identify genetically the relationship among all nine okra genotypes via using SDS-PAGE and RAPD techniques. The SDS-Page and RAPD experiments were done in Horticulture Research Institute and Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center, Ministry of Agriculture, Giza, Egypt.

B – Protein assay based on (SDS-PAGE)

Identification of nine Egyptian okra genotypes by Sodium Dodecyl Sulfate Poly Acrylamide Gel Electrophoresis (SDS-PAGE) was used to study the genetic background of the studied genotypes by their protein fingerprints. The seed storage proteins were analysed according to the method outlined by (Agueguia *et al*., 1994). Gel Analysis: Gels were photographed using a 35 mm colour film (200 ASA) and scanned with Bio-Rad video densitometer Model 620 USA, at a wave length of 577. Software data analysis for Bio-Rad Model 620 densitometer and computer was used as illustrated by the manufacturer.

C - DNA assay based on (RAPD-PCR)

The identification of DNA sequences in the investigated nine Egyptian okra genotypes were carried out according to the (RAPD-PCR) method which reported by Bagheri et al., (1995). Genomic DNA was extracted from okra seedlings leaves 7 days old using EZ-10 SPIN column genomic DNA isolation kit (BS425). 0.5 gm plant tissues were homogenized with 150 µl PCL solution (Plant cell lysis solution), then the mixture was incubated at 65°C for 20 min in 1.5 ml microfuge tube. Then the same volume of PCL was added, mixed and centrifuged at10.000 rpm for 5 min. The supernatant containing genomic DNA was transferred to a new tube and 300 µl of PB buffer was added and incubated 3 min. at room temperature, then centrifuged at 4,000 rpm for 2 min. Then the supernatant was removed and the pellet was washed two times with wash buffer. Finally, the pellet was resuspended in elution buffer and the columns were incubated at RT for 3 min then centrifuged at 10,000 rpm for one minute to elute DNA from the column.

Random amplified polymorphic DNAs (RAPDs) were produced by PCR using genomic DNA as templet and arbitrary primers **(**Welsh and Mcclelland 1990; Devos and Gale, 1992). Taq DNA polymerase was used to amplify DNA segments between closely spaced sequences (<2 kb) and complementary to the short random oligomers (10 mers). RAPD polymorphism results from changes in the primer-binding site in the DNA sequence. PCR products can be separated by gel electrophoresis. Six primers were used for RAPD-PCR analysis [OpA -10 (5′ CAGG GGT CTT G 3′), OpA-05 (5′ GTG ATC GCA G 3′), Op.C-02 **(**5′ GTG AGG CGT C 3′), OP.C-03 **(**5′ GGC GGT CTT T 3′), Op.E-05 **(**5′ TGA GCG GAC A 3′ and OP GO9 **(**5′ CTG ACG TCA C 3′)]. These primers were bought from (Operon Technologies, Alameda, USA). DNA amplification was carried out according to Williams *et al*., (1990). The amplification reaction was carried out in a 25 µl reaction volume containing 1×Taq polymerase buffer, 200 µ moles of each nucleotide in dNTPs, 1.5 mM $MgCl_2$, 2.5 unit Taq polymerase (Bio Allaiance), 25 p moles of decamer primer and 20 mg genomic DNA in a programmable thermal cycler (Techne).

Amplification reactions were cycled 45 times after an initial denaturation step for 3 min at 94 ˚C.

The Thermocycling profile was consisted of 1 min at 94 ºC (denaturation), 1 min at 36 ºC (annealing) and 2 min at 72 ºC (extension) with a final extension step for 5 min. Amplification products were mixed with loading buffer (2 µl 40% glycerol and 0.025% bromophenol blue) and fractionated on 2% agarose-1×Tris-acetate-EDTA-ethidium bromide gel electrophoresis in 1×TAE buffer at 120 V. RAPD bands were visualized and photographed on a UV transilluminator by a Polaroid camera.

Data were scored for computer analysis based on presence/absence of protein band or amplified products for each primer. Similarity matrix comparisons on pairs of genotypes based on shared polymorphic band over total number of bands were used to generate similarity coefficients according to Jaccard (1908). The similarity coefficients were then used to construct a dendrogram tree by UPGMA (unweighted pair-group method with arithmetical averages) using NTSYS-pc (Rohlf, 1993). Ploymorphism % counted as number of polymorphic amplicon fragments / total number of amplicon fragments * 100.

3. Results

This study focused to identify nine Egyptian genotypes by using seed storage protein (Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis SDS-PAGE) and Random Amplified Polymorphic DNA PCR (RAPD-PCR).

A - Seed storage protein based on (SDS-PAGE):

The protein content in the tested dry seeds of the various okra genotypes was extracted and analyzed by using SDS-PAGE technique. The obtained data show that the protein gel exhibits a maximum of 124 protein bands distributed over a wide range of molecular weights ranged from 206 to 33 kilo Dalton (KD) (Figure 1A). The data matrix of seed protein profile were coded and applied to computer software to determine the similarity between the studied okra genotypes. The output data (cluster analysis) of the nine Egyptian okra genotypes are summarized in Tables (1 and 2) and illustrated the dendrogram tree in Fig. (1B). From the above mentioned two tables the following notes are addressed: 1) The value of similarity among the different pairs of the studied genotypes fluctuates between 0.417 and 1.000. 2) The lowest similarity value (0.417) is scored between the two genotypes Al-Hemaa and Manshaa. 3) The highest similarity value (1.000) is detected between the two genotypes Aswan and Red.

Regarding the dendrogram tree, the analyzed data are discriminated into two clusters namely A and B. The first cluster A is further classified into two sub-clusters, namely a and b. The sub-cluster a joins both Al-Hemaa and Al-Sabahia genotypes, whereas the sub-cluster b is classified into two sub.sub-clusters namely c and d. the sub.sub- cluster c is classified into two sub.sub.sub-cluster namely e and f. the sub.sub.sub- cluster e joins both Tanta and Ityei El-Barud while the sub.sub.sub-cluster f joins both Aswan and Red genotypes. On the other hand, the sub.sub- cluster d comprised the genotypes Al-Sheikh Makram and Damanhur. However, the second cluster B comprised only Manshaa genotype.

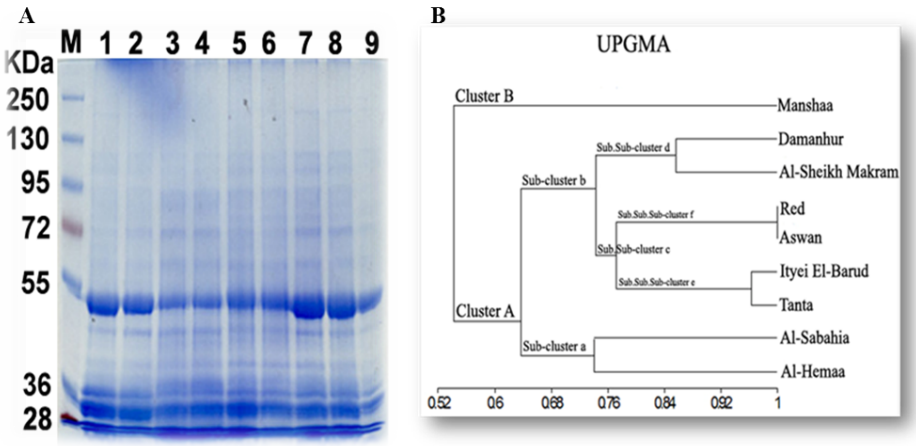

Figure (1): SDS-Page for okra protein analysis: A) Electrophoretic protein gel of the nine okra genotypes [Marker (M), Al-Hemaa (1), Al-Sabahia (2), Tanta (3), Ityei Elbarud (4), Alsheikh Makram (5). Damanhur (6), Aswan (7), Red (8) and Manshaa (9)]. B) Tree diagram from protein matrix of the various okra genotypes pair – groupaverage by unweighted.

Table (1): Similarity from protein matrix of the various Okra genotypes by Unweighted Pair-Group Mathematic Average (UPGMA).

Similarity matrix / Genotypes	G. 1	G. 2	G. 3	G. 4	G. 5	G. 6	G. 7	G. 8	G. 9
1- AL-Hemaa	1.00								
2- Al-Sabahia	0.74	1.00							
3- Tanta	0.64	0.59	1.00						
4- Ityei El-Barud	0.59	0.61	0.96	1.00					
5-Al-Sheikh Makram	0.71	0.52	0.79	0.81	1.00				
6- Damanhur	0.64	0.52	0.64	0.67	0.86	1.00			
7- Aswan	0.76	0.64	0.76	0.79	0.83	0.69	1.00		
8- Red	0.76	0.64	0.76	0.79	0.83	0.69	1.00	1.00	
9- Manshaa	0.42	0.43	0.58	0.61	0.58	0.58	0.56	0.56	1.00

Table (2): Relationships among the various okra genotypes by similarity matrix based on SDS-PAGE (UPGMA).

Nodes no.	**Group 1**	**Group 2**	**Similarity**	**Relationships**
1	G. 7 (Aswan)	G. 8 (Red)	1.000	2
2	G. 3 (Tanta)	G. 4 (Ityei El-Barud)	0.963	2
3	G. 5 (Al-Sheikh Makram)	G. 6 (Damanhur)	0.857	2
4	Node 2	Node 1	0.772	4
5	Node 4	Node 3	0.743	6
6	G. 1 (AL-Hemaa)	G. 2 (Al-Sabahia)	0.741	2
7	Node 6	Node 5	0.637	8
8	Node 7	G. 9 (Manshaa)	0.541	9

B - Leaf DNA based on (RAPD-PCR):

DNA was extracted from the leaves of the investigated okra genotypes and used as template from the PCR reactions. Amplification with 6- mer primers were used to detect RAPD-PCR reactions and generated reproducible and practically scorable RAPD. The exerted data in Fig. (2) and Table (3) discriminated further into conspicuous bands and absent ones where the conspicuous bandes were selected for further analysis. The number of amplified fragments of the studied genotypes is considerably varied according to the used primer. However, 4 amplicon fragments occurred in primer OpC-02, while 17 fragments in two primers OpC-03 and OpG-09. The effective 6 primers amplified 558 scorable bands (9 genotypes X 62 bands = 558) in all the investigated genotypes (Table 3). They are distinguished into 254 present bands and 304 absent bands. The obtained results show that the number of polymorphic fragments per primer ranged between 14 in Al-Hemaa (G.1) to 23 in Ityei El-Barud (G.4). On the other hand, it is obvious from the data summarized in Table (3) that the total number of amplified fragments is 62 throughout all the studied genotypes comprised 42 polymorphic bands, 12 positive unique bands, 1 negative unique band and 7 monomorphic bands.

The exerted results in Fig. (2) and Table (3) illustrate in detail the level of polymorphism which revealed by each RAPD primer. The highest percentage of polymorphism is recorded in primer OpG-09 (82.35%), meanwhile the lowest one was noticed in primers OpA-05 (41.67%). The results illustrate clearly the level of polymorphism as revealed by RAPD primers. However, the highest percentage of polymorphism occurred in primer No.6 OpG-09 (82.35%) meanwhile the lowest polymorphism was noticed in primer No. 1 OpA-05 (41.67%). The Unweighted Pair Group Method with Arithematical Averages data (UPGMA) expressed in the given diagram Table (4) and (5) show that there are two main groups 1 and 2. Group 1 included Al-Sabahia, Al-Sheahk Makram, Damanhur and AL-Hemaa genotypes while group 2 comprised Ityei El-Barud, Aswan, Red, Tanta and Manshaa genotypes. At the same time the highest node from the nine genotypes value (83.6 %) is recorded between Ityei El-Barud and Al-Sabahia genotypes while the Lowest one (58.2 %) is scored between Manshaa, Al-Hemaa and all genotypes.

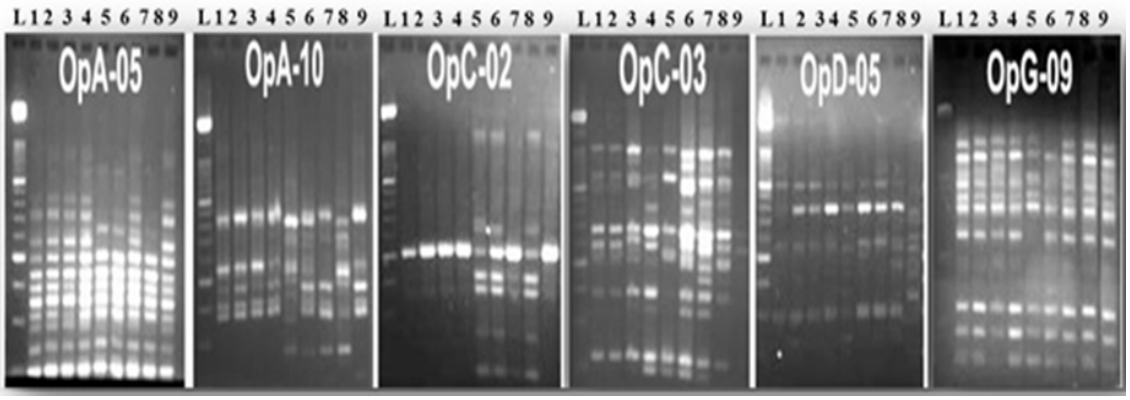

Figure (2): PCR product of genomic DNA of the nine okra genotypes [L= leader, Al-Hemaa (1), Al-Sabahia (2), Tanta (3), Ityei Elbarud (4), Alsheikh Makram (5). Damanhur (6), Aswan (7), Red (8) and Manshaa (9)] with the various primers (OpA-05, OpA-10, OpC-02, OpC-03, OpD-05 and PpG-09).

Table (3): The different fragments of the various Okra genotypes.

Primer name	Total fragments	Implication fragments				Polymorphism %
		Polymorphic bands	Monomorphic bands	Positive Unique bands	Negative Unique bands	
OPA-05	12	5	1	5	1	41.67 %
OpA-10	5	3	1	1	0	60.00 %
OpC-02	4	3	1	0	0	75.00 %
OpC-03	17	12	1	4	0	70.59%
OpD-05	7	5	2	0	0	71.43 %
OpG-09	17	14	1	2	0	82.35 %
Total	62	42	7	12	1	62*9 = 558 bands

Table (4): Similarity from DNA (RAPD) matrix of the various okra genotypes by Unweighted Pair-Group Mathematic Average (UPGMA).

Genotypes	**G. 1**	**G. 2**	**G. 3**	**G. 4**	**G. 5**	**G. 6**	**G. 7**	**G. 8**	**G. 9**
1- AL-Hemaa	1.00								
2- Al-Sabahia	0.65	1.00							
3- Tanta	0.55	0.75	1.00						
4- Ityei El-Barud	0.62	0.84	0.69	1.00					
5- Al-Sheikh Makram	0.55	0.57	0.54	0.60	1.00				
6- Damanhur	0.56	0.61	0.58	0.61	0.73	1.00			
7- Aswan	0.68	0.68	0.71	0.73	0.75	0.70	1.00		
8- Red	0.44	0.51	0.51	0.51	0.68	0.72	0.69	1.00	
9- Manshaa	0.49	0.58	0.58	0.58	0.48	0.59	0.63	0.66	1.00

The dendrograms of the nine okra genotypes shown by UPGMA in Fig. (3) distinguished two main clusters nominated as A and B. The first cluster A comprises two sub-clusters named a and b. The first sub-cluster a included only one genotype Al-Hemaa whereas the second sub-cluster b comprises two main sub.sub-cluster e and f. The sub.sub-cluster e joins both Al-Sabahia and Ityei El-Barud genotypes while the sub.sub-cluster f comprehends only Tanta genotype. On the other hand, the cluster B comprised two main sub-clusters mentioned c and d. The sub-cluster c included two sub.sub-cluster g and h. The sub.sub.-cluster g joins both Al-Sheikh Makram and Aswan genotypes while the sub.sub-cluster h included Damanhur and Red genotypes. Nevertheless, the sub-cluster d has only one genotype Manshaa.

Table (5): Relationships among the various okra genotypes by similarity matrix based on DNA (RAPD-PCR) primers (UPGMA).

Nodes no.	Group 1	Group 2	Similarity	Relationships
1	G. 2 (Al-Sabahia)	G. 4 (Ityei El-Barud)	0.836	2
2	G. 5 (Al-Sheikh Makram)	G. 7 (Aswan)	0.754	2
3	G. 6 (Damanhur)	G. 8 (Red)	0.724	2
4	Node 1	G. 3 (Tanta)	0.716	3
5	Node 2	Node 3	0.698	4
6	G. 1 (AL-Hemaa)	Node 4	0.609	4
7	Node 5	G. 9 (Manshaa)	0.592	5
8	Node 6	Node 7	0.582	9

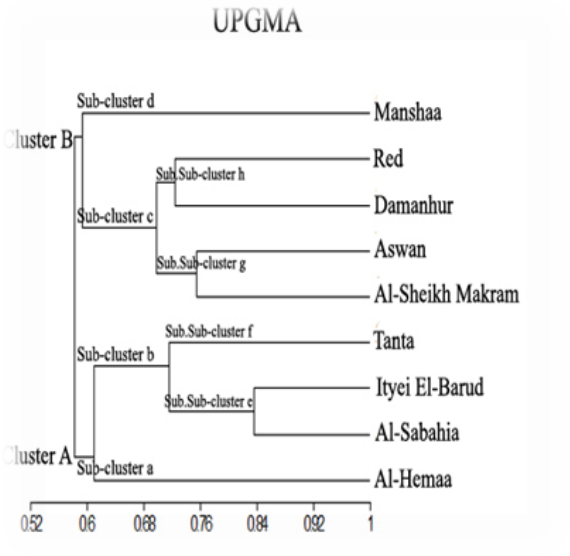

Figure (3): Tree diagram from DNA matrix of the various okra genotypes by unweighted pair group mathematic average.

4. Discussions

A - Seed storage protein based on SDS-PAGE

It is well known that protein is a quite distinguished character; to separate such individual bodies, solubility, hydration, molecular sieving and charge differences has been used. A combination of all them separate better than only one. Gel electrophoresis especially in polyacrylamide gel (PAGE), takes benefit of the parameters molecular size, shape and charge that mostly used today (Stegemann, 1984 and Cook, 1984).

Having a look to the obtained results of the various okra genotypes, it is obvious that all okra clearly differed in their relationship in SDS-page polymorphic bands, positive unique bands and negative unique bands. The positive and negative unique bands in the SDS-PAGE analysis enabled the studied okra genotypes to be distinguished from each other. The origin of these protein bands may be attributed to mutations at the priming site. Lack of studies with SDS-PAGE markers on okra, however, prevents a detailed genetic analysis of most traits having economic importance. These results indicated that the variability among collected okra genotypes due to different geographical zones (Mady et al., 2013, Mady et al., 2014 and Helaly et al., 2014).

Thus, the most important band is the positive unique one due to its presence in certain genotype and absence in another. This band, however, has a specific protein which in turn led to a specific mark. Nevertheless, it is well known that protein is a primary gene product which proves that there is probability of the consequence of specific gene arrangement, structure and activity which induced the appearance of the band. Whatever means, the synthesis of the specific protein may be induced by genetic diversity (Osanyinpeju and Odeigah, 1998) beside some different factors such as environmental and low-nutrient stresses (Nunez *et al*., 2001).

Evidences from our experiment showed that the tested genotypes were grown under the same environmental conditions and so the existed differences are related to the genetic diversity. In addition, the positive and negative unique bands ranked in the same degree of importance as the positive one. This may be due to the reason that it was a mark of the absence of specific protein which can helps in taxonomy and breeding purposes. The polymorphic bands are also very useful due to its appearance in some genotypes and contrarily absence in others. By far, these bands are used also for the identification of the various genotypes (Bakry, 2005).

In fact, the positive unique bands are the most important ones due to the presence of the certain unique sequences of protein in only one genotype which are considered as “labelling” characteristic to ensure distinction in every genotype. So, all the positive unique bands can be used in the identification of the close relationship, the protection of plant propriety rights and plant breeders (PBR) (Wright et al., 1984).

Generally speaking, the application of fingerprinting technique (seed storage protein band

SDS-PAGE) have been extensively applied for studies done on evolution, domestication and genetic diversity in wild and cultivated plant population in several crop plants (Odeigah et al., 1999 and Abdu 2006). It is also available to recognize the off-type seed. Thus, for these previous reasons the International Union for the Protection Of new Varieties (UPOV) recommended the seed storage protein method (SDS-PAGE) as one of the biochemical tests of cultivars identification (UPOV, 1991).

B - Leaf DNA based on (RAPD-PCR)

Nine okra genotypes were assessed using 6 primers which amplified 61 bands with an average of 10.16 bands/ primer. Out of the total amplified bands, only 42 (68.58%) were polymorphic bands, 7 (11.47%) were monomorphic bands, 12 (19.67) positive unique bands and 1 (1.63%) negative unique bands. This polymorphism was an indication of prevalence of moderate diversity among these 9 okra genotypes (Punitha and Raveendran, 2004). In okra 103 RAPD fragments were generated by 31 decamer primers in 43 genotypes of okra (Martinello et al., 2003). The cluster analysis showed a wide range of similarity ranging from 44.14 to 82.88% while 86 to 100% genetic similarity was observed using Sequence-related amplified polymorphism (SRAP) markers in okra among 23 genotypes (Gulsen et al., 2007). Martinello et al., 2003 reported that 103 bands in okra were generated from 31 primers in 43 okra genotypes. On the other hand, Vicente et al., (2008) they found that 110 (65.9%) of the total 167 bands produced by twenty ISSR (inter-simple sequence repeat) primers showed polymorphism with an average of 8.5 polymorphic bands per primer, however, in eggplant nine RAPD primers showed 95.3% (Sadder et al., 2007). Due to the importance of this DNA marker technique, the scientists used the RAPD technique as the best method in the field of plant identification (Garcia *et al*, 1998 and Lopez and Staub, 2001).

So, for the importance of this method it was choosen for the identification of the tested okra genotypes in our investigation. These results exhibited polymorphic bands, positive unique bands and negative unique bands among collected okra landraces due to the genetic variability dependent on grown in different geographical zone areas under different environmental condition it could be exhibit some of genetic changes when grown together under the same condition (Haq et al., 2012 and Haq et al., 2013). The dendrograms of the nine okra genotypes showed by UPGMA that the clusters were mostly formed based on the similarity of the genotypes collected from geographical origin areas. The genotypes from the different geographical origin mostly grouped together within the cluster dependents on the genotypes variability (Ahmadikhah et al., 2008, Lasalita-Zapico et al., 2010, Rahman et al., 2011and Tuhina-Khatun et al 2015).

DNA fingerprinting profiles of okra landraces collected from several geographical zone areas were quite similar, suggesting a close genetic relationship. The similarity in amplification profiles of most okra genotypes suggested that there is a degree of DNA conservation among the okra landraces. This genetic distance information could be useful in breeding programs in order to introduce ergonomically important genes (Hung et al., 2012). However, more extensive molecular data is needed in order to make a more general conclusion about the relationship among okra genotypes. The accessions with genuine labels are also necessary. Despite the strong homology that is exhibited by many of the okra genotypes, our work demonstrates that it is now possible to differentiate between closely-related cultivars. Furthermore, most of the cultivars that we studied can be individually characterized with cultivar-specific RAPD markers. Since the pedigree of our material is known, we were able to confirm that the okra genotypes relationships that were concluded from our RAPD data are extremely reliable (Martinello et al., 2003, Sadder et al., 2007, Gulsen et al., 2007, Vicente et al., 2008 and Haq et al., 2013).

Finally, the obtained information of the genetic relationships among the examined okra genotypes has several direct important applications for crop improvement. In plant breeding programs, estimates of the genetic relationships might have great importance in the selection of parents for hybridization, which reduce the number of genotypes needed to ensure sampling of broad range of genetic variability (Hung et al., 2012). However, the estimates of genetic distance (similarity) might also be useful for identifying heterosis groups for the crops in which this information is important. Molecular marker based technology has been proved a reliable strategy for detection of sex-associated markers in okra landraces. The RAPD marker technique is the cheapest, user friendly and reliable tool used for efficient fingerprinting of many plants (Helaly et al., 2014).

Conclusion

Both seed storage protein and DNA fingerprinting techniques that has been applied in this study proved to be very useful. The application of SDS-PAGE technique facilitates the accurate separation in a short time between any seed of different genotypes (cultivars, varieties, species, texa and accessions). From another point of view, the use of the rapid technique RAPD-PCR proved to be very efficient and sufficient to analyze and characterize all

the tested genotypes. Thus, the technique of RAPD-PCR can be recommended for the determination of genetic diversity among the various genotypes or species. Finally, the benefits from using the fingerprinting techniques in this trial is the protection of the Egyptian breeder's rights and the preservation of the Egyptian genotypes to be register as Egyptian varieties in the Egyptian Gene bank.

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