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Genetic Diversity Of Egyptian Cowpea (*Vigna unguiculata* (L.) Walp.) Landraces and their Genetic Relationships Based on Seed Storage Protein and Isozymes

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THE CURRENT research was performed to study the genetic diversity of 19 cowpea landraces (*Vigna unguiculata* (L.) Walp.) from 7 localities in Egypt using seed storage protein and three isozymes (α-esterase, β-esterase, and peroxidase) gel electrophoresis. The results of the seed protein pattern of the studied landraces revealed a total number of 29 bands with molecular weights ranged from 7 to 265kD (one unique, 26 polymorphic, and 2 monomorphic bands) were detected. The unique band with a molecular weight of 205kD was detected in only one landrace. The number of bands in different 19 landraces ranged from 9-14 bands per landrace. The highest number of bands (14 bands) was detected in one landrace only (Sohag number 2) and the lowest number of bands (9 bands) was detected in 3 landraces (numbers 4,7 and 8 from Sohag, Luxor, and Dakahlia, respectively).

Banding patterns of three isozymes (α- esterase, β-esterase and peroxidase) in Egyptian cowpea landraces showed that seven loci were present in all studied landraces of cowpea, one locus of α-esterase, five loci of β-esterase and one locus peroxidase. The number of loci varied in different landraces and ranged from 2-6 loci for α-esterase isozyme, 5-7 loci of β -esterase isozyme and 1-5 loci for peroxidase. The α-esterase and peroxidase banding patterns showed higher polymorphism in cowpea landraces compared with β-esterase.

By using the combined protein and isozyme data, high variability among landraces was obtained and analyzed by the *NTSYS*-pc software program.

Keywords: Genetic diversity, Seed storage proteins and isozymes, *Vigna unguiculate.*

Introduction

Cowpea *(Vigna unguiculata* (L.) Walp.) belongs to the family Fabaceae. It is an annual, self-pollinated, leguminous crop**.** It is cultivated around the world (Asia, South America and tropical Africa) not only as a grain but also as a vegetable and fodder crop. The cowpea seed contains 20.42-34.60% of proteins (Bdalla et al., 2001). Also, the cowpea seed protein contents showed great variability among cultivars or varieties and ranged from 15.06 to 38.5% (Hall et al., 2003; Patil, 2012; Afiukwa et al., 2013; Itatat et al., 2013; Oke et al., 2015). Variations were also observed in the protein content, proteins fractions and other seed characteristics among other cowpea varieties by Freitas et al. (2004), Bekhit (2007), Ajeigbé et al. (2008), Vasconcelos et al. (2010) and Alghamdi et al. (2019).

Genetic diversity is key to the development of any crop plant. The use of sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of seed proteins is a reliable, also, relatively inexpensive way to establish genetic markers for the identification and genetic analyses of several important agricultural commodities (Oppong-Konadu et al., 2005). SDS-PAGE is considered a simple and accurate test for the identification and

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characterization of different species (Lioi et al., 1999). Seed proteins are extremely stable and are not affected by environmental conditions (El-Ghamery et al., 2003). Besides, it can reveal a considerable degree of polymorphism and a simple genetic control subject to minimum environmental influence (Tripathy et al., 2010). Despite the significant difference in seed protein content among cowpea varieties, some studies have recorded three to seven genes for genetic control of protein accumulation by Fernandes et al. (2012).

In *Vigna* spp seed storage protein markers were successfully used to resolve taxonomic relationships and classify cultivated varieties (Rao et al., 1992). However, the total seed protein contents and banding pattern in cowpea estimated by using of gel electrophoresis significantly differ among cowpea varieties or genotypes as recorded by many investigators (Tripathy et al., 2010; Win et al., 2011; Sharma, 2012; Choudhary, 2013; Alege et al., 2014; Satpute & Pardhe, 2014; Koolwal, 2015; Mahfouz, 2015; Animasaun, 2017; Hayette & Aissa, 2017; Koolwal & Jangid, 2017; Sharma & Krishna, 2017; Alghamdi et al., 2019).

Isozymes are classified as multiple molecular forms of a single enzyme have slightly different compositions of amino acids due to differences in DNA nucleotide sequence which code for protein (Micales et al., 1986). Isozyme analysis is well suited for the study of genetic diversity because 1) isozymes usually display high levels of variation between species, 2) they are represented by multiple loci distributed over the genome, and 3) the analysis are relatively easy to apply to large numbers of individuals (Morden et al., 1989). These markers are stable because they are not more easily and accurately affected by environmental influences, since the does not wait until the plants reproduce (Kumari, 2017).

Isozymes were used to assess genetic affinities among *Vigna* species (Pasquet & Vanderborght, 2000; Ghafoor et al., 2001; Sidhu, 2003). Isozymes have been useful in the study of *V. unguiculata* and the wild form (Panella & Gepts, 1993). The variation of isozymes in different wild and cultivated accessions of *Vigna unguiculata* was scored at 17 putative loci to determine genetic relationships within this species (Sonnante et al., 1996). Moreover, in Pasquet (1999) studies, the genetic was evaluated using an allozyme analysis in 199 accessions of wild and cultivated cowpea. Esterase (EST) and peroxidase (POX) were studied in twenty accessions of cultivated cowpea from different countries. Reis & Frederico (2001) and Gaafar (2006) reported esterase isozymes in different species of *Vigna* are stable characteristic which are not affect by environmental factors. Bekhit (2007) studied the banding patterns of isozymes (esterase and peroxidase) to identify two cowpea cultivars and the results revealed the banding patterns of esterase were in slightly higher polymorphism. Kouam et al. (2012) examined the genetic structures in 35 wild cowpea using ten enzyme systems. The results revealed 21 isozyme loci, of which 9 showed polymorphism.

This study aims to analyze the genetic relationships and level of genetic diversity between 19 different landraces of cowpea by using seed storage proteins and isozymes properties in order to select some of these landraces may be used for crossing programme for improvement in cowpea yield potential.

Materials and Methods

Seeds of 19 landraces of *Vigna unguiculata* from 7 localities in Egypt were obtained from National Gene Bank (NGB), Agricultural Research Center (ARC), Giza, Egypt (11 landraces) and from Agricultural Research Station Kafr El-Sheikh (Sakha) (8 landraces). In Table. 1, the list of landraces with their selection source is provided.

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	Qaha 21	Kaha
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	Asiut	-----		

SDS-PAGE was carried out under Laemmeli (1970) procedure with minor modification defined by Tripathy et al. (2010), Sharma (2012), Choudhary (2013). The electrophoresis was carried out on BioRAD vertical gel electrophoresis equipment (Model: Protein II Xi Cell) along with its cooling unit with a power supply maintained at 30 mA for four and half hours. gels were stained after electrophoresis by using Coomassie brilliant blue R 250 followed by distaining step and finally washing in tap water. The dissociated polypeptides molecular weights were determined by using standard marker.

Extraction of Isozymes from seeds homogenizing 0.5g of seeds samples using a mortar and pestle in 1mL extraction buffer (10 percent glycerol). The extract was then transferred into clean Eppendorf tubes and for 5min. centrifuged at 10.000rpm. The supernatant was transferred to new clean Eppendorf tubes and held at –20°C until it was used for electrophoretic analysis. 20µL extract of each sample was combined with 5µL of bromophenol blue and the mixture was then added to each well. After electrophoresis, the gel was stained according to enzyme system with the appropriate substrate. Peroxidase (POX) investigation was made according to Soltis et al. (1983) and α- and β- esterase investigation was made according to Scandalios (1964).

Similarity matrices as computed by the program were used to construct the UPGMA (Sneath & Sokal, 1973). Dendrogram was constructed to elucidate the diversity among the accessions studied. The thoroughly distained gels were put in clear transparent polythene bags for further use in photography. Gels were scored for the presence (1) and absence (0) of every protein subunit band. These binary data were used to analyze using NTYSYS –pc, (Version 2.1 software) (Rohlf, 2002). The SIMQUAL sub- programme was used to calculate the Jaccard's coefficient (Jaccard, 1908).

Results

The results of the electrophoresis of total storage proteins of 19 landraces of cowpea are shown in Fig. 1. The electrophoresis pattern of the studied landraces revealed a total number of 29 bands (one unique in the landrace no.4, 26 polymorphic bands in the rest of landraces) were detected with molecular weights ranged from 7 to 265kD and 2 common bands (monomorphic bands) with MW of 12 and 18kD were detected in these studied samples. The unique band with molecular weight of 205kD was detected in the one landrace. The 26 detected bands were polymorphic with different molecular weight ranged from 7 to 265kD. The highest number of bands (14 bands) was detected in one landrace while the lowest number of bands (9 bands) was detected in 3 landraces.

The number of storage seed protein bands recorded in different landraces of cowpea collected from different localities of Egypt is given in Table 2. From the results, it was noticed that some bands are present in about more than half of the studied Landraces (10-19), the molecular weight ranged from 12-105kDs. For example, band no. 27, 28 with molecular weight of 18, 12kD was detected in all 19 studied landraces. Band no.13 with a high molecular weight of 105kD was detected in 11 landraces. Band no. 23, 24 with molecular weight of 40, 35kD was detected in 10 landraces, respectively. But band no. 20 with a high molecular weight of 50kD was detected in 2 landraces no.12, 18. Band no.4 with molecular weight of 254kD was detected in landrace no.1 and considered as unique band for this Landrace. Moreover, the results showed different number of bands ranged from 2, 3, 4 and 5 were observed in different landraces ranged from 2-8 landraces. In the other hand bands were present in few landraces (4, 20) with molecular weight of 205, 50kD were recorded.

The results of α-esterase enzyme of different 19 landraces of cowpea are shown in Table 3 and represented in Fig. 2. It was noticed that seven loci of α-esterase named (EST1, EST2, EST3, EST4, EST5, EST6 and EST7 were appeared in different landraces of cowpea. One locus of α-esterase named EST3 was present in all different landraces of cowpea as monomorphic locus but locus EST7 was recorded in landrace no.14 as unique locus. On the other hand, the number of loci of α-esterase isoenzyme was varied with different landraces and ranged from 2-6 loci (Table 3). The higher number (6 loci) was recorded in one landrace no. 14 while the lowest number 2 loci was recorded in six landraces.

Gel stained for β-Esterase isozymes of different 19 landraces of cowpea are shown in Table 3 and represented in Fig. 3 displayed 7 zones of activity. It was found that seven loci of this isozyme. Five of them were recorded in all landraces studied as monomorphic locus. EST6 and EST7 as polymorphic loci were present in 9 and 4 landraces, respectively. The number of loci of β-esterase isozyme were varied with different landraces and ranged from 5-7 loci (Table 3). The higher number (7 loci) was recorded in one landrace no. 1 where's the lowest loci number (5 loci) was registered in seven landraces.

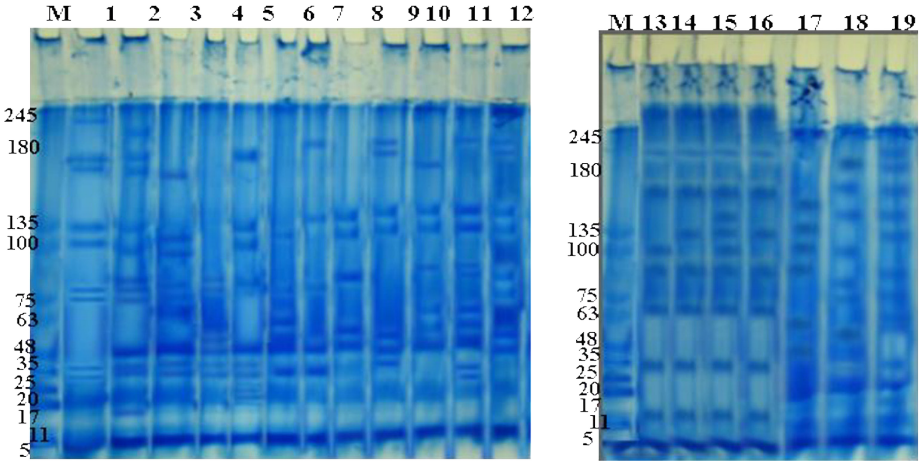

Fig.1. The SDS Polyacrylamide gel electrophoresis pattern of seeds protein from different localities (1-19) landraces of cowpea (*Vigna unguiculata*)

TABLE 2. Percentage of polymorphism and number of totals, polymorphic, monomorphic and unique bands from 19 different landraces of cowpea (*Vigna unguiculata*)

Landraces	**Sohag**				**Qena**	**Luxor**		**Dakahlia**		**Asyut**	**Kaha**	**Kafr El Sheikh**							
Samples No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Total No of amplified bands	11	13	11	9	10	10	9	9	11	10	14	13	10	10	11	10	10	11	13
Total within the Landrace	21				10	15		13		10	14	24							
No. of monomorphic bands	4				0	4		6		0	0	1							
No. of polymorphic bands	9				10	0		0		10	14	19							
No. of unique bands	8				0	11		7		0	0	4							
% of polymorphism	80.95				100	73.33		53.84		100	100	95.83							

In this study, peroxides enzyme showed distinct seven loci as given in Table 4 and represented in Fig. 4. It was found that only one locus (POX1) was present in all different landraces of cowpea. POX2 and POX3 loci were found in 11 and 5 landraces, respectively. POX4 locus as a unique locus was only found in landrace no. 7, while POX5 and POX7 loci were present in two landraces. POX6 locus was found in 3 different landraces. On the other hand, the number of loci of peroxidase isoenzyme were varied in the landraces and ranged from 1-5 loci (Table 4). The higher number (5 loci) was recorded in one landrace no. 7 while the lowest number (1 locus) was recorded in 5 landraces.

The neighbor joining (NJ) distance tree constructed of combined results (Fig. 5) showed that the 19 landraces of cowpea divided into two groups at a distance of 8 on similarity distance scale. Each group was further subdivided into two clusters at distance of 6.97.

The Jaccard's pairwise similarity coefficients (Fig. 6) based on protein profile and isozymes revealed the overall range of genetic similarities ranged from 0.54 to 0.90 in 19 landraces of cowpea which indicates there was a high variability among the landraces. The average genetic similarity among these 19 landraces was 0.72. Lowest value of similarity showing maximum divergence such as between landrace no.3 and 16. Highest value of similarity between landrace no. 1 and 2 and between landrace no 14 and 15.

TABLE 3. α- and β-Esterase isozymes loci pattern of 19 different landraces of cowpea (*Vigna unguiculata*)

Landraces of cowpea	α- and β- Esterase's (Est) loci															
	1		2		3		4		5		6		7		Total	
	α-	β-	α-	β-	α-	β-	α-	β-	α-	β-	α-	β-	α-	β-	α-	β-
1	+	+	+	+	+	+	+	+	-	+	-	-	-	-	**4**	**5**
2	+	+	+	+	+	+	+	+	-	+	-	-	-	-	**4**	**5**
3	+	+	+	+	+	+	+	+	-	+	+	-	-	-	**5**	**5**
4	+	+	-	+	+	+	-	+	-	+	-	-	-	-	**2**	**5**
5	+	+	-	+	+	+	+	+	-	+	-	-	-	+	**3**	**6**
6	+	+	-	+	+	+	-	+	-	+	-	-	-	-	**2**	**5**
7	+	+	-	+	+	+	+	+	-	+	-	-	-	+	**3**	**6**
8	+	+	-	+	+	+	-	+	-	+	-	-	-	+	**2**	**6**
9	+	+	-	+	+	+	-	+	-	+	-	-	-	-	**2**	**5**
10	+	+	-	+	+	+	-	+	-	+	-	+	-	-	**2**	**6**
11	+	+	-	+	+	+	+	+	-	+	-	+	-	-	**3**	**6**
12	+	+	-	+	+	+	-	+	-	+	-	-	-	-	**2**	**5**
13	+	+	+	+	+	+	+	+	-	+	+	+		-	**5**	**6**
14	+	+	+	+	+	+	+	+	-	+	+	+	+	+	**6**	**7**
15	+	+	+	+	+	+	+	+	-	+	-	+	-	-	**4**	**6**
16	-	+	-	+	+	+	+	+	+	+	-	+	-	-	**3**	**6**
17	-	+	-	+	+	+	+	+	+	+	-	+	-	-	**3**	**6**
18	-	+	-	+	+	+	+	+	+	+	-	+	-	-	**3**	**6**
19	-	+	-	+	+	+	+	+	+	+	-	+	-	-	**3**	**6**
No. of Landanraes	**15**	**19**	**6**	**19**	**19**	**19**	**13**	**+**	**4**	**19**	**3**	**9**	**1**	**4**	**61**	**107**

The presence (+) and the absence (-) of loci bands.

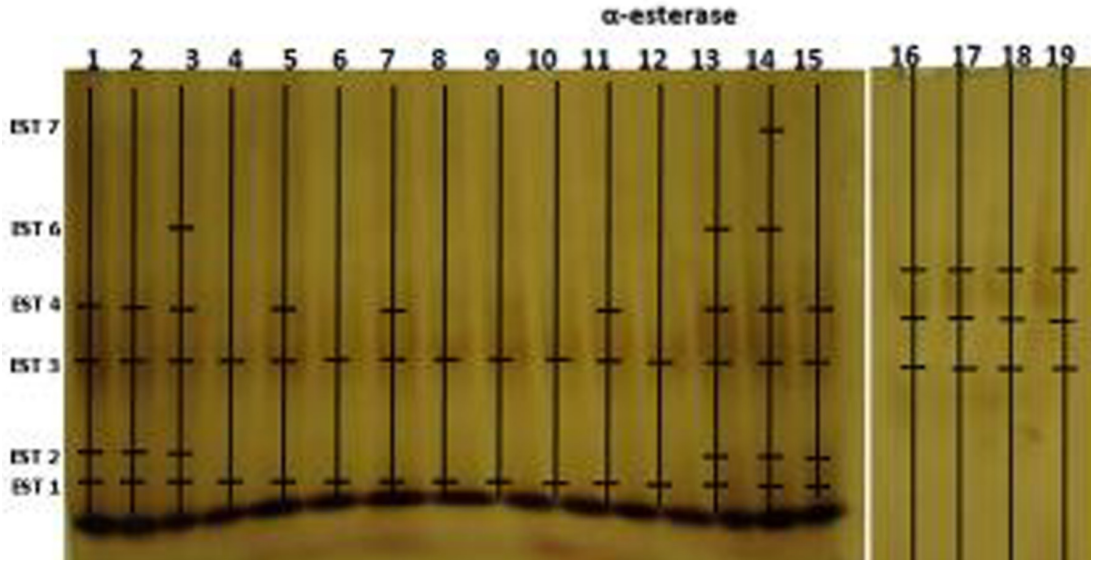

Fig. 2. The α-esterase loci pattern of different samples (1- 19) landraces of cowpea (*Vigna unguiculata*)

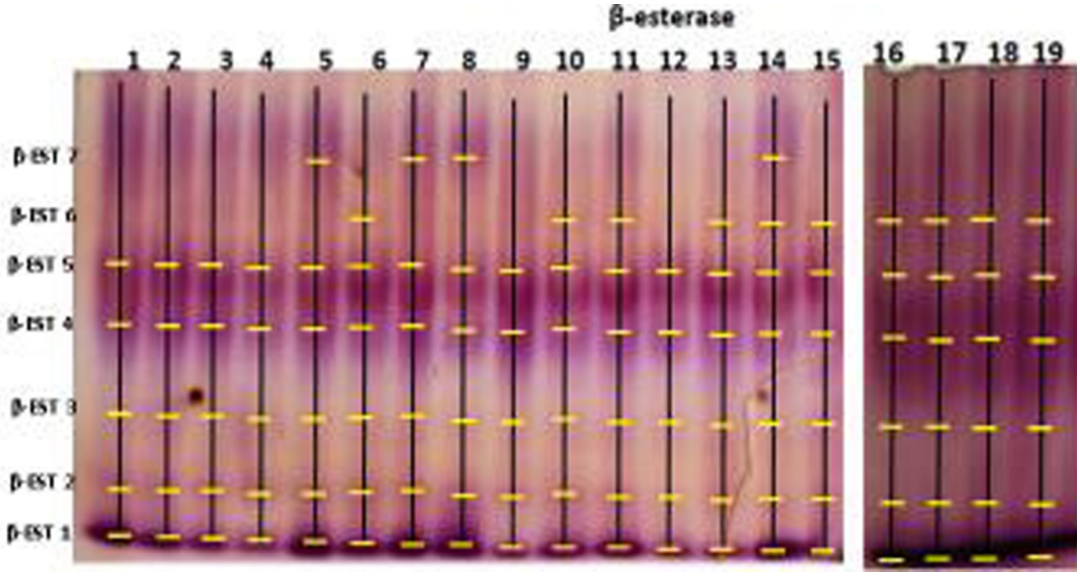

Fig. 3. The β -esterase loci pattern of different samples (1- 19) landraces of cowpea (*Vigna unguiculata*)

TABLE 4. Peroxidase isozymes loci pattern of 19 different landraces of cowpea (*Vigna unguiculata*)

Cowpea landraces	Peroxidase isozymes (Per) loci							No. of loci
	1	2	3	4	5	6	7	
1	+	-	-	-	-	-	-	1
2	+	+	-	-	-	-	-	2
3	+	-	-	-	-	-	-	1
4	+	+	-	-	-	-	-	2
5	+	-	-	-	-	-	-	1
6	+	-	-	-	-	-	-	1
7	+	+	-	+	+	-	+	5
8	+	+	-	-	-	-	-	2
9	+	-	-	-	-	-	-	1
10	+	+	+	-	-	-	-	3
11	+	+	+	-	-	-	-	3
12	+	+	-	-	-	-	-	2
13	+	+	+	-	-	-	-	3
14	+	+	+	-	-	-	-	3
15	+	+	+	-	-	-	-	3
16	+	+	-	-	+	-	+	4
17	+	-	-	-	-	+	-	2
18	+	-	-	-	-	+	-	2
19	+	-	-	-	-	+	-	2
No. of landraces	19	11	5	1	2	3	2	43
Locus type	M	P	P	U	P	P	P	-

The presence (+) and the absence (-) of loci bands.

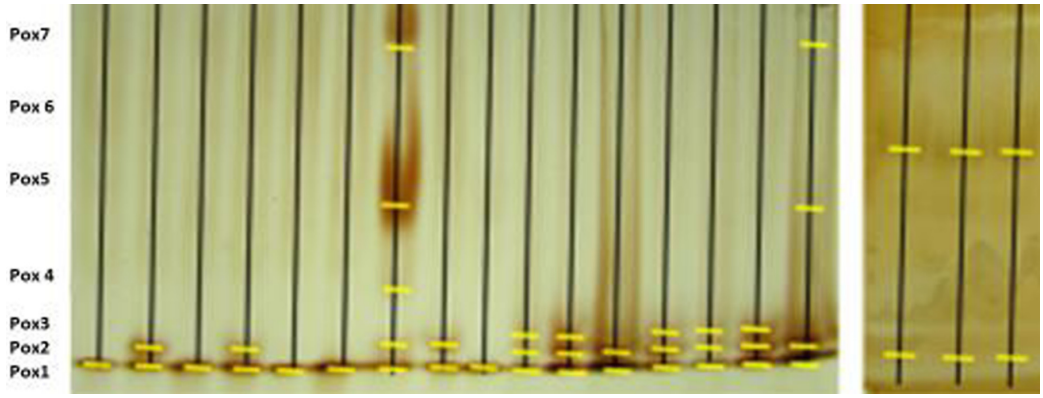

Fig. 4. The peroxidase loci pattern of different samples (1- 19) landraces of cowpea (*Vigna unguiculata*)

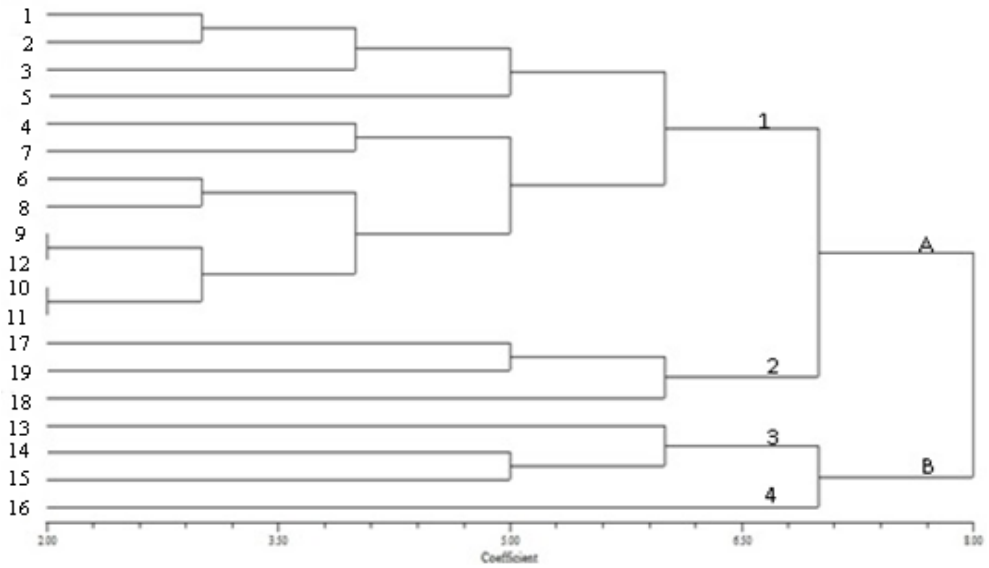

Fig. 5. The relationships of the cowpea landraces based on combined protein and isozymes data and expressed as NJ tree constructed using NTSYS-pc software

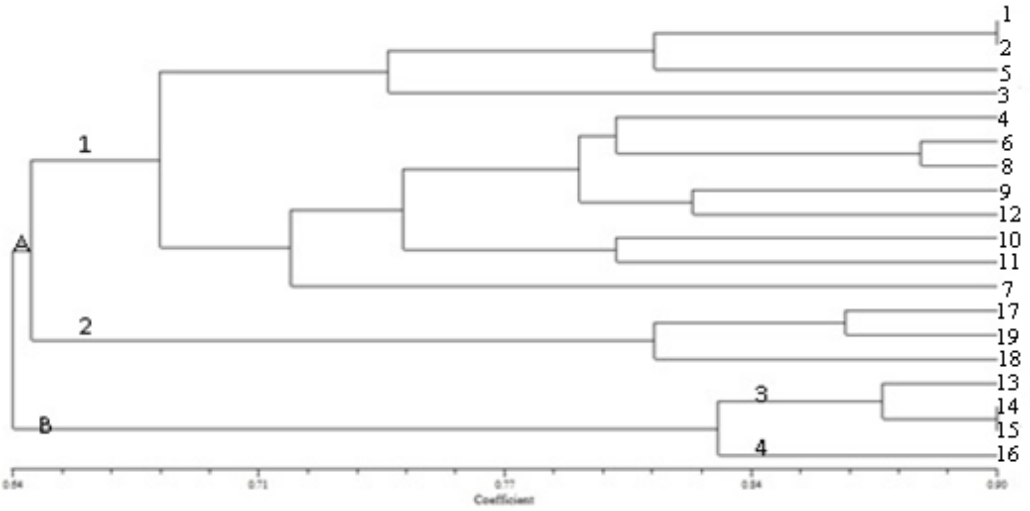

Fig. 6. Dendrogram of 19 different cowpea landraces generated by Jacquard's similarity coefficient and the unweighted pair group method with arithmetic average (UPGMA) clustering methods based on combined protein and isozymes analysis

Discussion

The seed storage proteins are non-enzymatic and have the sole purpose of providing proteins (source of sulpher and nitrogen) needed during germination and establishment of a new plant. Seed storage proteins polyacrylamid gel electrophoresis (SDS-PAGE) can be used economically to determine genetic variation and relationship in germplasm and also to distinguish mutants from their parent genotypes (Hameed et al., 2009). Seed storage proteins profiling based on SDS-PAGE can be used for various purposes, such as varietal identification, biosystematics analysis, determination of phylogenetic relationships between different species, generating of revealent information to complement assessment and passport data (Malviya et al., 2008). The electrophoresis pattern of the studied landraces revealed a total number of 29 bands (one unique band, 2 common bands and 26 polymorphic bands) were detected with molecular weights ranging from 265 to 7 kDs. The variation in protein content obtained in this study was similar to the previous study on accessions of cowpea (Odeigah & Osanyinpeju, 1996; Valizadeh, 2001; Shuaib & Zeb, 2007; Afiukwa et al., 2013; Itatat et al., 2013; Omonhinmin & Ogunbodede, 2013; Alege et al., 2014; Lukong et al., 2014; Satpute & Pardhe, 2014; Mahfouz, 2015; Hayette & Aissa, 2017; Koolwal & Jangid, 2017).

The maximum number of bands (14 bands) was detected in 2 landraces and 13 bands in 2 landraces and the lowest number of bands (9 bands) was detected in 3 landraces. In the study, a wide range of protein peptides (low to high molecular weights) demonestrated the potential for discriminating used cowpea landraces and can generate additional variability to complement established varieties. Thus, profiling of total seed storage proteins through SDS-PAGE for differentiating cowpea landraces is well established (Ghafoor et al., 2002; Satpute & Pardhe, 2014; Hayette & Aissa, 2017; Koolwal & Jangid, 2017). Genetic variation in a set of landraces has an important role in identification of landraces. Electrophoretic seed storage proteins analysis was directly related to the genetic background of the proteins that reveal genetic diversity (Javid et al., 2004; Afiukwa et al., 2013; Itatat et al., 2013).

In this study the landraces from different localities showed different percentage of polymorphism in electrophoretic banding pattern of seed storage proteins. This percentage ranged from 53.84 to 95.83 in five localities and 100% in two localities. Seed storage proteins polymorphism is associated with the genetic background of proteins (Iqbal et al., 2005) and can be used as a potential molecular marker for varietal identification and economic characterization of cowpea landraces (Oppong-Konadu et al., 2005). In this investigation, two common bands (monomorphic bands) with molecular weight of 12 and 18kDs were observed in all studied landraces but the unique band with molecular weight of 205kDs was detected in one sample (landrace no. 1). The landraces no. 12 and 18 can be differentiated from the rest landraces by presence only a polypeptide band with molecular weight of 50kDs. Such specific protein markers could be reliably used for varietal certification and maintenance of pure seeds in seed multiplication programme. Similarly, (Odeigah & Osanyinpeju, 1996) noted two main total seed protein electrophoretic pattern with respect to 39 and 20kD which were present in six out of the 20 accessions of *Vigna unguiculata* analyzed. Finally, protein electrophoresis is a powerful tool for population genetics with reference to genetic diversity and phylogenetic relationship and the SDS-PAGE technology is particularly considered as a reliable way because storage proteins are largely independent of environmental fluctuations.

Isozymes are polymorphic and have proven to reliable markers since they are stable characteristics than morphological characters and do not change with environmental condition. In addition, electrophoresis of plant enzymes is more rapid than field-testing and can be detected with a small amount of tissue extract (Wagner & McDonald, 1982). In the analysis of isozymes, many distinct bands with little back ground staining is excellent enzyme for use in taxonomic studies (Kuhus & Fretz, 1978). This is consistent since esterase produce many bands which could be arbitrary divided into active regions (fast moving bands and slow moving bands), and the sufficient variability between accessions of the species could be allowed the use of isozyme analysis as a system for cultivars identification (Eeswara & Peiris, 2001). Also, Selvi et al. (2003) concluded that variation in isozymes (peroxidase and α-esterase) can be exploited in order to understand the nature of genetic variation and relationships between *Vigna unguiculata* accessions.

In the light of the results of this study, three different isozymes (α-esterase, β-esterase and Peroxidase) were selected to obtain better insight into genetic relationships within and between 19 different landraces of cowpea. It was noticed that seven loci of α-Esterase enzyme; one was present in all as monomorphic, one unique locus and the rest five loci were polymorphic with a high polymorphism in contrast to the 7 loci of β-Esterase where five of these loci were monomorphic,

two polymorphic and no unique locus was detected. In this respect, Hameed et al. (2009) reported that the number of isozymes bands ranged from three to six bands and Gaafar (2006) reported that the cultivated form the Egyptian *Vigna unguiculata* had two loci of esterase enzyme (Est 2 and Est 9). Maithreyee (2011) reported that the cowpea cultivars experinced a strong polymorphism in esterase isozyme profile of 32 with respect to number, position and intensity of bands was noticed among the cowpea cultivars.

The results of both types of esterase enzymes in this study revealed the banding patterns of esterase were in slightly higher polymorphism in cowpea cultivars in accordance of (Bekhit, 2007) who studied the banding patterns of esterase in two cowpea (*Vigna unguiculata*) cultivars, Moreover, study of Reis & Frederico (2001) showed that accessions of cowpea were monomorphic for almost all loci tested for eight enzyme systems except for the esterase. Also, a total nine bands of esterases in ten genotypes were observed with 28.57% polymorphism (Anatala, 2012).

Peroxidase is one of the important enzymes present in all tissues and performs catalytic functions. Also in this study, seven loci of the isozymes of peroxides were detected in different landraces of cowpea; one locus (Per1) was monomorphic band, five loci were polymorphic and one unique locus was also detected. Of these five loci, two bands (Per 5 and 7) were only observed in two landraces. This result was in agreement with findings of Jaaska & Jaaska (1988), Prestamo & Manzano (1993). Similar results was also observed in case of peroxidase banding patterns in two cultivars of Egyptian cowpea (*Vigna unguiculata*) (Bekhit, 2007), a total six alleles were generated peroxidase isozymes in ten genotypes of cowpea with 17.67% polymorphism (Anatala, 2012), two peptides peroxidase isozymes had been identified in cowpea root (Albuquerque et al., 2015) and three peroxidase isoforms cowpea (*Vigna unguiculata*) (Wabale, 2018).

Genetic diversity as mentioned by Van Hintum (1995) is the extent to which material differs within a group of plants. The similarity index ranged from 0.57 to maximum 1.0 on the base of isozymes between the different landraces. The clustering analysis indicated that landrace only one landraces had maximum variability compared to other landraces. A similar result was observed by Anatala (2012) in that isozymes markers were proficient to distinguish ten cowpea genotyps. So, a low genetic variability at the isozyme level was seen in the cowpea landraces used in this study because a single domestication event is involved in the origin of this crop as reported by Wamalwa et al. (2016) and the low genetic divergence observed in this study is in consistent with the findings of Padulosi & Ng (1993) who attributed it to this crop's self-pollination nature. Given that the accessions were from different regions, it could also indicate high-gene flow within regions and limited time for significant genetic differentiation along geographical lines as indicated by Karuma et al. (2008).

Finally, the combined results of Jaccard's pair-wise similarity coefficients and UPGMA dendrograms constructed on the basis of combined data of seed storage proteins and isozymes revealed that the landraces no.1 and 2 are actually the same accession, despite being collected from the same region and showed highest variability with other landraces followed by the landraces no.14 and 15 hich could be exploited to improve the cowpea germplasm. Higher values of similarity index between any of two landraces indicate a close relationship between them. Lowest value of similarity index showing maximum divergence such as between landrace no.3 and 16 and any of other landraces. A similar result of similarity range was observed in different previous studies (Omonhinmin & Ogunbodede, 2013; Alege et al., 2014; Lukong et al., 2014; Satpute & Pardhe, 2014; Mahfouz, 2015). On the basis of Jaccard's coefficient the landrace pairs which found to exhibit maximum divergence was recoded. Therefore, this pairs can be said to be diverse pairs and hybridization between them may result into new recombinants of desirable protein types in according to Win et al. (2011), Sharma (2012) and Choudhary (2013). This study has showen that although the cowpea genetic base is small, there is some degree of variation between cowpea accessions that can be exploited to boost cowpea crop as a food safty measure similar to Wamalwa et al. (2016) results.

Conclusion

The overall results of this experiment revealed the genetic variations of cowpea landraces (*Vigna unguiculata* (L.) Walp.) that result in high variability among landraces when using combined protein and isozyme data. In addition, -esterase and peroxidase banding patterns in cowpea landraces showed higher polymorphism than -esterase.

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Authors contribution: Author A, conceived of the presented idea and wrote the manuscript with support from Author B. Author B and D developed

the theory and performed the computations. Authors C and D verified the analytical methods. All authors discussed the results and contributed to the final manuscript.

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التنوع الجيني لسلالات اللوبيا المصرية وعلاقاتها الوراثية اعتمادا على بروتينات البذور والنظائر الانزيمية

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تم إجراء البحث الحالي لدراسة التنوع الجيني لـ 19 سلالة من اللوبيا من 7 مواقع في مصر باستخدام التفريد الكهربي لبروتين البذورالمخزن وثلاثة مشابهات انزيمية (α-esterase و β-esterase و peroxidase). وقد أظهرت نتائج التفريد الكهربي لبروتين البذور للأنواع الأصلية المدروسة إجمالي عدد 29 حزمة بروتينية بأوزان جزيئية تراوحت من 7 إلى 265 كيلو دالتون (حزمة فريدة و 26 متعددة الأشكال و 2 أحادي الشكل). تم اكتشاف الحزمة الفريدة ذات الوزن الجزيئي البالغ 205 كيلو دالتون في سلالة واحدة فقط . وقد تراوح عدد الحزم في 19 سلالة من اللوبيا من 14-9 حزمة لكل سلالة. أيضا تم الكشف عن أكبر عدد من الحزم البروتينية (14 حزمة) في سلالة واحدة فقط (سوهاج رقم 2) وتم الكشف عن أقل عدد من الحزم البروتينية (9 حزمة) في 3 سلالات (الأعداد 4 و7 و 8 من سوهاج والأقصر والدقهلية، على التوالى).علي الجانب الاخرأظهرت أنماط الثلاثة مشابهات انزيمية في السلالات المدروسة من اللوبيا المصرية أن سبعة مواضع موجودة في جميع السلالات المدروسة من اللوبيا وموضع واحد من α-esterase وخمسة مواضع من β-esterase وأيضا موضع واحد من ال peroxidase. في المقابل تنوعت عدد المواقع في السلالات المختلفة وتراوحت بين 6-2 مواضع ل α-esterase ، و7-5 مواضع ل β-esterase و5-1 مواضع peroxidase. وقد أظهرت أنماط النطاقات α-esterase و peroxidase تنوع عالي في سلالات اللوبيا مقارنة مع ال β-esterase. وفي النهاية مع استخدام البيانات المجمعة للبروتين والإيزوزيم ، تم الحصول على تباين كبير بين السلالات الأصلية أثناء تحليلها بواسطة برنامج NTSYS-pc.