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GENETIC DIVERSITY IN SOME WHEAT LANDRACES FOR SOME QUALITATIVE TRAITS AND PROTEIN FINGER PRINTING

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ABSTRACT

This experiment were conducted in Genetic Resources Research, Dept. FCR .ARC. Bahteem Research Station in 2015/2016 and 2016/2017 seasons; to using some genetic diversity parameters to evaluate wheat germplasm using varies methods. The results showed that the morphological character of wheat have a special role in determining the importance of each character for increasing yield.

Landraces and obsoleted a different species can be considered as availed portion of the gene pool. They proved to have a broader genetic base concerning, flag leaf anthocyanin coloration of auricles in the accessions No.1, 6, 7, 8,9and 10, this character is a unique marker distinguish durum germplasm, also showed that the dye of anthocyanin was associated with the seeding in durum until eleven days .This information would be have a value to the plant breeder for select the superior type for further testing. Proteins sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was used for each species and scored the presence or absence of each band was noted and entered in a binary data matrix. The overall results showed a low degree of heterogeneity; however different species revealed a differential protein banding pattern.

***Conclusively,** these results indicates that SDS –PAGE analyses of different species protein is useful to evaluate the genetic variability and different species identification, which can help in wheat breeding program.*

Key words. Genetic diversity, Landraces, Qualitative, Protein Finger Printing.

INTRODUCTION

Wheat is one of the most important and widely cultivated crops in the world, today most wheat grown is hexaploid, used for bred making and

other baked products (Debasis and Khurana, 2001). Genetic diversity is created by the nature and genetic recombination added by plant breeders for the varietal improvement,(Singh *et al.,* 2006). The knowledge of genetic diversity in a crop species is fundamental to its improvement. An assessment of the nature and magnitude of diversity among the genotypes will help to choose the better parents for hybridization. Bread wheat plays a major role among the few crop species, being widely grown as food sources and was likely a central point to the beginning of agriculture.

Morphological characters of wheat have a special role in determining the importance of each trait in breeding programs which at least led to improving the yield and introducing commercial varieties (Mollasadeghi *et al.,* 2011). Wheat yield is affected by many factors; genetic, environment and their interaction. The value of yield varied in dependence of yield attributes, such as stem height, leaf area, spike length, number of spikelets per spike and number of kernels per spike were also found associated with the vegetative growth period (Knezevic *et al.,* 2012).

The proteins of seed storage for wheat consider important sources of food and energy, being also involved in the determin the quality of bread making (Cooke and Law,1998). Acrylamide gel electrophoresis sodium dodecyl sulphate has become one of the most widely used techniques to separate and characterize the proteins in storage wheat (Bietz and wall, 1972) .They reported that two types of glutenin subunits were present in the wheat grains with both of low molecular weight (LMW)(10-70 KDa) and high molecular weight (HMW) glutenin subunits (80-130 KDa). The polyacrylamide gel electrophoresis has been used to show the different size of variation which exists between LMW and HML gluten in subunits and it has been suggested that deletions and insertion wit in the repetitive region are responsible for these variations with different length (Benmoussa *et al.*, 2000).

Therefore, the objective of this study was estimate the genetic diversity among wheat germplasm using various methods, the difference determine through in , morphological and protein finger printing were useful in the genetic purity of different wheat germplasm through identification the desirable genotypes for further utilization in plant breeding.

MATERIALS AND METHODS

The materials in the present study comprised of thirty wheat accessions as shown in Table (1). The thirty germplasm accessions were

Table (1): List of wheat species and landraces used in the study.

Serial No	Locations	Species	Chromosome No.	Origen
1	Sinai-Berar Allahfan (383)	Triticum aestivum	2n=42	Egypt
2	Sinai-Berar Allahfan (392)	Triticum aestivum	2n=42	Egypt
3	Sinai-Al-gora Al-Arish(430)	Triticum aestivum	2n=42	Egypt
4	18 km Marsa Matroh Salom high way (421)	Triticum aestivum	2n=42	Egypt
5	20km Marsa Matroh Salom high way(387)	Triticum aestivum	2n=42	Egypt
6	Introduction(12) (ICRDA)	Triticum durm	2n=28	ICRDA
7	Introduction(13) (ICRDA)	Triticum durm	2n=28	ICRDA
8	Introduction(14)(ICRDA)	Triticum durm	2n=28	ICRDA
9	Introduction(20)CIMMYT	Triticum durm	2n=28	CIMMYT
10	Introduction(21) (CIMMYT)	Triticum durm	2n=28	CIMMYT
11	ASSIUT (2908)	Triticum aestivum	2n=42	Egypt
12	ASSIUT (2979)	Triticum aestivum	2n=42	Egypt
13	ASSIUT (2990)	Triticum aestivum	2n=42	Egypt
14	ASSIUT (3101)	Triticum aestivum	2n=42	Egypt
15	Genetic stock (1080)	Triticum aestivum	2n=42	Egypt
16	ASSIUT (2795)	Triticum aestivum	2n=42	Egypt
17	ASSIUT (2801)	Triticum aestivum	2n=42	Egypt
18	Genetic stock (3201)	Triticum aestivum	2n=42	Egypt
19	Genetic stock (3292)	Triticum aestivum	2n=42	Egypt
20	Genetic stock	Triticum	2n=42	Egypt

	(3237)	aestivum		
21	ASSIUT (2968)	Triticum aestivum	2n=42	Egypt
22	Genetic stock (1053)	Triticum aestivum	2n=42	Egypt
23	Genetic stock (1054)	Triticum aestivum	2n=42	Egypt
24	Genetic stock (1057)	Triticum aestivum	2n=42	Egypt
25	Genetic stock (1060)	Triticum aestivum	2n=42	Egypt
26	Genetic stock (3420)	Triticum aestivum	2n=42	Egypt
27	Giza (168) local variety	Triticum aestivum	2n=42	Egypt
28	Seds (12) local variety	Triticum aestivum	2n=42	Egypt
29	Beni sweif (1) local variety	Triticum durm	2n=28	Egypt
30	Beni sweif (6) local variety	Triticum durm	2n=28	Egypt

SDS-protein electrophoresis:

obtained from the Genetic Resources Research Dept., Field Crops Research Institute (FCRI), Agricultural Research Center (ARC). The experiment was conducted in two seasons, of 2016/2017and 2017/2018, at Bahteem Research Station in a randomized complete blocks design with three replications. Each experimental plot consisted of four rows for each entry, 4m long and 20 cm width. Spacing between the plants within row was kept at 5cm.

All other agronomic practices were applied according to recommendations for wheat program**.** Coleoptile anthocyanin coloration, plant growth habit, flag leaf anthocyanin coloration of auricles plant. Frequency of plant with recurred flag leaves, flag leaf glaucosity of sheath, ear glauosity , culm glaucosity of neck, awns or curs and ear density were measurements as recommended scales that reported by IBGR (1985). For miniscale preparations of all thirty genotypes in wheat samples, 0.3 mg of leaf tissue (10 Days old) were grinded with phosphate buffer (pH:7) and pelted down then only 20ul with (80 ug) protein concentration was added to equal volume of Laemmli Sample

Buffer, 5µl of 10%SDS and 5µl of β–mercaptoethanol then boiling the mixture for 5 min and centrifugation 12000RPMfor 10 minutes to obtain the supernatant which contains protein fractionations. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (1970). Samples prepared by adding Protein fractionation electrophoresis was performed on 12% acrylamide gel using the apparatus manufactured by BioRad. Gels were analyzed using Total Lab TL100.

Data analysis

The photographs of SDS-PAGE gel was used to study the protein profile of the all accessions. The bands were designated on the basis of their molecular weight. The presence of protein band was scored as (+)positive and its absence(-)negative as shown in (Table 3- 5) only bright, clearly distinguishable bands were used in genetic analysis.

RESULTS AND DISCUSSIONS

The results revealed a significant variation among most of morphological characters in the different species in wheat germplasm . The range of variation observed among the germplasm for nine morphological characters are presented in Tables (2A, 2B and 2C) .Variation among germplasm was observed for coleoptile anthocyanin coloration, plant growth habit, flag leaf anthocyanin coloration of auricles of the plant. Frequency of plants with recurved, flag leaves , flag leaf glaucosity of sheath, ear glauosity, culm glaucosity of neck, ear density and awns or curs.

Wheat morphological of have a special role in determining the importance of each trait in increasing yield. Improvement based on breeding concept and use the large number of germplasm and best cultivar as a parent. The success in breeding program is not each because their were complex relationships between grain yield and it is yield components .The wheat yield is affected by many factors; genetic, environment and the interaction between genetic and environmental conditions (Epigenetic system).

Table(2A). Morphological characters of different studied species in wheat during 2016/2017 and 2017/ 2018growing seasons.

Accession .No.	Coleoptile anthocyanin coloration	Plant growth habit	Flag leaf anthocyanin coloration	Plant. frequency of plant with recurved flag leaves
1	Absent (very weak)	Erect	Absent (Very weak)	medium
2	Absent (very weak	Erect	Absent (Very weak)	Medium
3	Absent (very weak)	Erect	Absent (Very weak)	medium
4	Absent (very weak)	Erect	Weak	Medium
5	Absent (very weak)	Erect	Weak	Medium
6	Present	Erect	Strong	Low
7	Present	Erect	Strong	Low
8	Present	Erect	Strong	Low
9	Present	Erect	Strong	Low
10	Present	Erect	Strong	Low
11	Absent (very weak)	Erect	Moderate	medium
12	Absent (very weak)	Erect	Weak	medium
13	Absent (very weak)	Erect	Moderate	medium
14	Absent (very weak)	Erect	Weak	medium
15	Absent (very weak)	Erect	Weak	medium
16	Absent (very weak)	Erect	Absent (Very weak)	medium
17	Absent (very weak)	Erect	Moderate	medium
18	Absent(very weak)	Erect	Moderate	medium
19	Absent (very weak)	Erect	Moderate	Low
20	Absent (very weak)	Erect	Moderate	low
21	Absent	Erect	(Absent)	medium

	(very weak)		Very weak	
22	Absent (very weak)	Erect	(Absent) Very weak	low
23	Absent (very weak)	Erect	Weak	medium
24	Absent (very weak)	Erect	Absent (Very weak)	medium
25	Absent (very weak)	Erect	Strong	medium
26	Absent (very weak)	Erect	Weak	medium
27	Absent (very weak)	Erect	Strong	medium
28	Absent (very weak)	Semi erect	Strong	low
29	Present	Semi erect	Weak	medium
30	Present	Semi erect	Strong	low

Table (2B): Morphological characters of different studied species in wheat during 2016/2017 and 2017/ 2018growing seasons.

Accession No.	**Flag leaf glaucosity of sheath**	**Ear glaucosity**	**glum glaucosity of neck**
1	Very weak	Very weak	Absent or very weak
2	Very weak	Very Weak	Absent or very weak
3	Very weak	Very Weak	Absent or very weak
4	Very weak	Very Weak	Absent or very weak
5	Very weak	Very Weak	Strong
6	Very strong	Strong	Strong
7	Very strong	Strong	Strong
8	Very strong	Strong	Strong
9	Very strong	Strong	Strong
10	Very strong	Very strong	Very strong
11	Very weak	Very Weak	Weak
12	Very weak	Very Weak	Medium
13	Very weak	Very Weak	Absent or very weak
14	Weak	Very Weak	Medium
15	Weak	Strong	Strong
16	Medium	Medium	Strong
17	Weak	Very weak	Weak

18	Weak	Medium	Weak
19	Weak	Weak	Weak
20	Medium	Medium	Weak
21	Very weak	Very Weak	Absent or very weak
22	Weak	Medium	Medium
23	Weak	Medium	Medium
24	Medium	Weak	Weak
25	Weak	Weak	Medium
26	Very weak	Very weak	Weak
27	Very weak	Very Weak	Absent or very weak
28	Very weak	Very Weak	Absent or very weak
29	Very weak	Very Weak	weak
30	Very weak	Medium	Strong

Table (2C). Morphological characters of different studied species in wheat during 2016/2017 and 2017/ 2018growing seasons.

Accession No.	**Awns or curs**	**Spikelet**
1	Presence	Lax
2	Presence	intermediate
3	Presence	Dense
4	Presence	intermediate
5	Presence	Intermediate
6	Presence	Very dense
7	Presence	Very dense
8	Presence	Very dense
9	Presence	Very dense
10	Presence	Very dense
11	Absent	Intermediate
12	Presence	Intermediate
13	Presence	Intermediate
14	Presence	Intermediate
15	Presence	Intermediate
16	Presence	Intermediate
17	Presence	Dense
18	Presence	Intermediate
19	Presence	Dense
20	Presence	Very dense
21	Presence	Intermediate

22	Presence	Lax
23	Presence	Very dense
24	Presence	Lax
25	Presence	Intermediate
26	Presence	Intermediate
27	Presence	Very dense
28	Presence	Very dense
29	Presence	Very dense
30	Presence	Very dense

The present study was designed to find out the definite-distinguished characters of wheat (landraces, collected from different areas in Egypt and five lines from (ICRDA and CIMMYT) in addition to check varieties (local variety) in bread wheat (Triticum aestivum and Triticum durm) under study. Breeders need information to the field in specter. The breeders require these data for evaluation the genetic sources of their program.

In addition, it serves as a true witness in the course of breeder's right implementation. Field inspectors should have recognizable characters of the specific landrace, in addition, introduce and two cheek variety (local variety) under inspection spp that such standard of purity might be worked out properly. This could be carried out directly in the field or by taking sample for laboratory analyses. Certain morphological characters and protein SDS-PAGE aspects that needed to studied the furnish necessary data, in this respect.

Characterization of wheat under environmental conditions in Egypt, the value of nine traits was obtained for thirty accessions in the two seasons. The landraces usually have a breeder genetic base and can therefore prove an important valuable characteristics for breeding landraces and obsolete cultivars can be considered as a valuable portion and gene pool. Usually have a broader genetic base , in addition introduction and two check varieties in *Triticum aestivum* and two cheek *triticum durum,* phenotypic variation was derived from genotype, environment and their interaction between genetic and environment. These results are agreement with (Chapman ,2008; Joosen *et al.,* 2013).

The phenotypic variance of the same accession was derived from genetic, environment and interaction between genetic and environment. The results supported that variation existing the studied accessions is very important for breeding programs. The accessions revealed a wide range of

variations for morphological characters which have a potential to determine the best accessions for different environments. The variation was detected in the morphological characters of wheat germplasm collected from different regions of Egypt.

Data in Tables (2A, 2B and 2C) indicated that the coleoptile anthocyanin coloration for all different germplasm of wheat in durum (it is very strong accessions No. 6.7,8,9 and 10. Regarding o the habit of plant growth, erect except accession No. (29) Which were semi erect. Narrow variation existed for habit of plant growth.

Concerning to the flag leaf anthocyanin coloration of auricles No.(6,7,8,9and10) had a strong anthocyanin coloration which can be used as a unique character for this accessions in this stage, while accessions No. (1, 2, 3, 16, 21,22and 24) were very weak, and was moderate accession No. 11, 13, 17, 18,19and 20 in this germplasm.

The variation is useful trait for different germplasm identification. The frequency of plant with recurved flag leaves was classified to two groups , low and moderate . For the glancosity of sheath flag leaf classification three groups, absent (very weak), weak and moderate, the variation can be used as a unique characters for this accessions in this stage. These characters are important for the breeders in selection of promising plant material. Morphological diversity was detected for different traits in relation to regions altitudes and agro - ecological zones. These results are confirmed those obtained by (Hussein *et al* ., 1981) . Concerning ear glaucosity classification into five groups *i,e.,* absent (very weak) ,weak ,medium ,strong and very strong .

Regarding to the glum glaucousitiy of neck, classified to six groups, *i.e*. Absent, very weak weak, medium strong and very strong.

Awns or scars classified to into two groups, presence and absent. These results agreement with ear density was classified to four groups, lax, intermediate, dense and very dense, Hence the variation morphological diversity in Egyptian wheat genotypes in different relation to regions and altitude zone of origin. So this study was amid to estimate morphological diversity exists among wheat accessions collected over 30 years ago.

However, the genetic variation in current Egyptian wheat germplasm has over the millennia been presented by the farmers who keep the cereal from previous harvest years. So this study was aimed to identify the different species in wheat, in determine the importance of each character under study which increase yield in wheat.

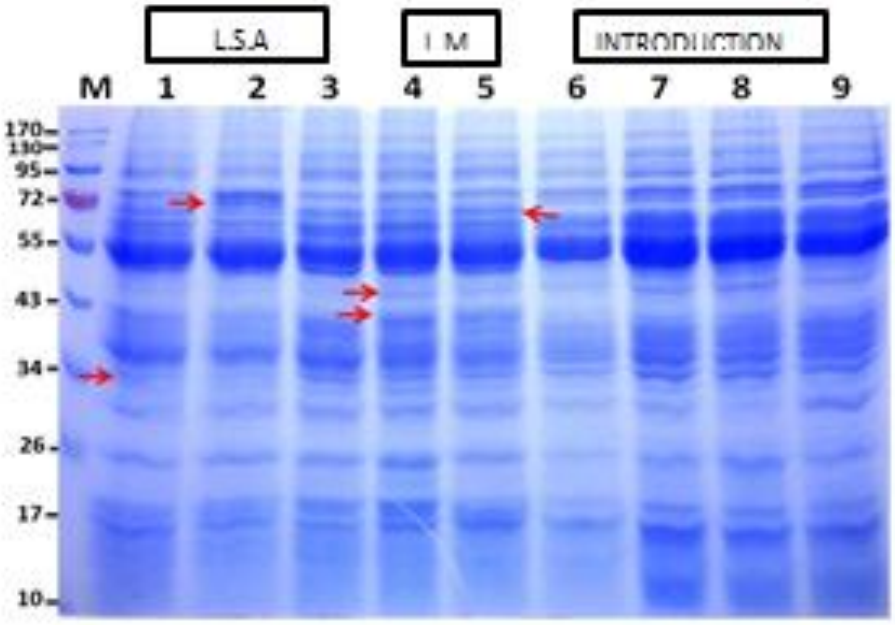


Figure (1): SDS-PAGE profile of seed storage protein from wheat genotypes from 1 – 9, Protein concentration 25ug/sample. Stained with coomassie blue on 12% acrylmide gel.

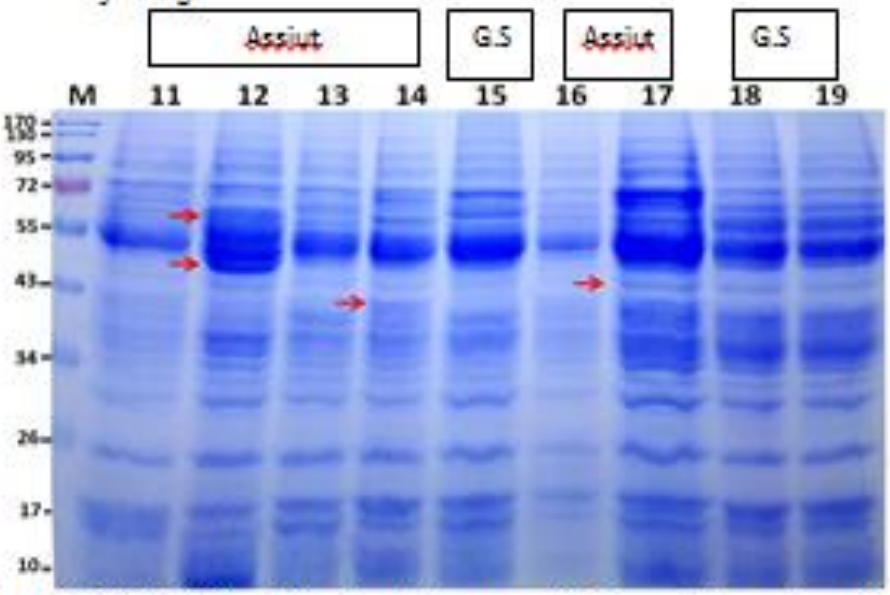


Figure (2): SDS-PAGE profile of seed storage protein from wheat genotypes from 11 - 19 , Protein concentration 25ug/sample. Stained with coomassie blue on 12% acrylmide gel.

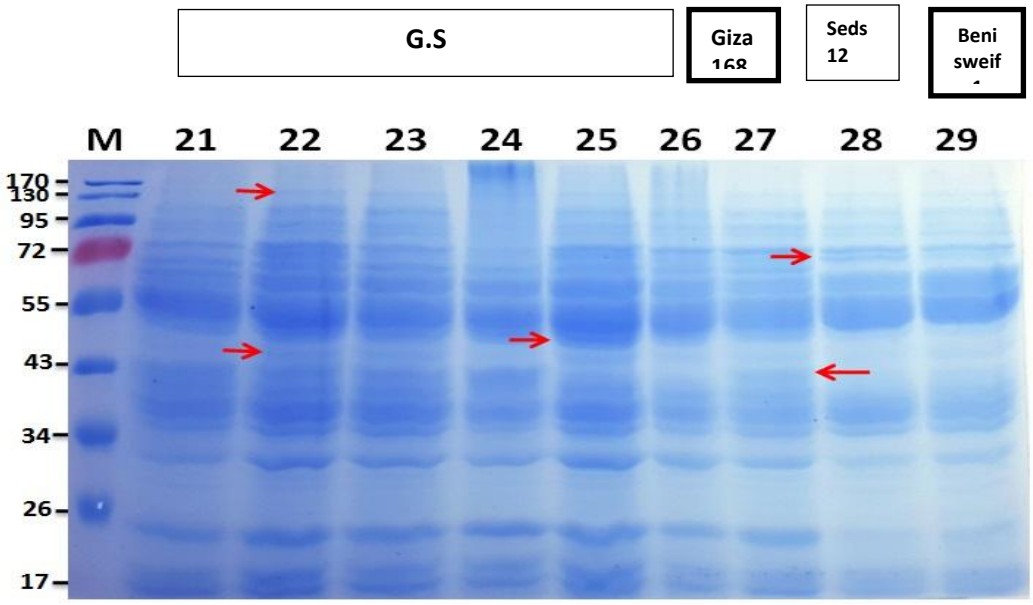
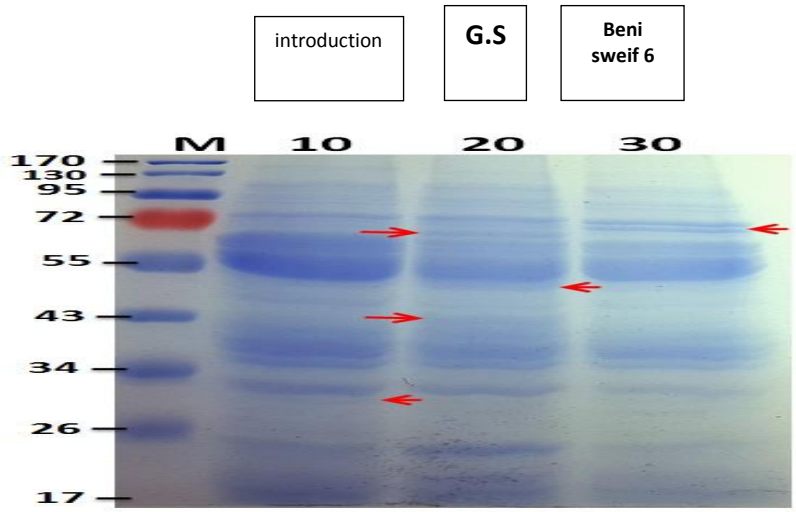


Figure (3**):** SDS-PAGE profile of seed storage protein from wheat genotypes from 21 - 29 Protein concentration 25ug/sample. Stained with coomassie blue on 12% acrylmide gel.



Figure(4**) .** Showed the SDS-PAGE profile of protein for seed storage from wheat genotypes which collected from different area of Egypt Protein concentration 25ug/sample, Stained with coomassie blue on 12% acrylmide gel.

The accession No.1 Sinai –Bear Aullahfan (112) gave a unique marker 34KD, while unique markers72 KD as polymorphism for accession No. 2 Sinai –Bera –Allahfan (1)were detected of them were polymorphic.

They also accession No. 4 18 km Mars matroh salom high way was found two unique markers 34, 50 KD, also accession No. 5 give unique markers 70 KD. Distinguish in two accession collection in Mars matroh salom high way, wherever the accessions No. 1,2, 3, 4, and 5 expected one origin. Also accession No.11,12,13 and 14 collected in assiut , the accession No.12 showed two unique markers 47 and 57 KD, also accession No. 14 give unique markers 39 KD.

Regarding the accession No. 17was gave unique marker45 KD. Meanwhile accession No. 22 genetic stock (GS) give the unique markers in 45 KD and 130 KD, also accession No. 25 give the unique marker 50 KD. Regarding to the Giza 168 local variety give unique marker 45KD, also Seds 12 local varieties give a unique marker 175KD.

The genetic stock gave three unique markers 30 KD, 34 KD and 65KD .Also Beni-Sewif 6 gave the unique marker 70KD. Meanwhile, local variety G168 gave the unique marker 30KD.

The results in Tables (3, 4 and 5) showed the highly genetic diversity among the wheat genotypes for protein bands polymorphism, where, two entries showed one polymorphism bands on the other side thirteen. The entries shoed two polymorphic bands , while six entries showed three polymorphic bands , finally nine entries showed four bands with different .Molecular weight ,72,55, 43,34 and 26 KD respectively.

Moreover ,the local variety showed highly variation where the Giza 168 local variety showed four bands with molecular weight 72, 43,34 and 26 KD respectively, the Seds (12) variety showed three with molecular weight 72,55 and 34 KD, respectively, while the Beni-Sweif 1 and 6 varieties showed the same two bands with molecular weight 55 and 34 KD respectively, that mean could be using the total soluble proteins in desitengwish among wheat genotypes.

Table (3): Molecular weights and polymorphic bands presence or absence in group1 genotypes of Wheat (*Triticum aestivum* L.)

Band MW	Genotypes									
	1	2	3	4	5	6	7	8	9	10
72	+	+++	+	+	++	+++	+++	+++	+++	+
55	-	-	-	-	-	-	+++	+++	+++	+++
43	-	-	-	++	-	-	-	-	++	-
34	-	-	-	+	-	-	+	+	+	-
26	+	+	+	+	+	-	+	+	-	+
total	2	2	2	4	2	1	4	4	4	3

Table (4): Molecular weights and polymorphic bands presence or absence in group2 genotypes of Wheat (*Triticum aestivum* L.)

Band MW	Genotypes									
	11	12	13	14	15	16	17	18	19	20
72	+	+	+	-	+	-	++	-	-	+
55	-	++	-	-	+	+	-	+	+	-
43	-	-	-	+	-	-	+	+	+	+
34	-	+	-	-	+	-	-	-	+	-
26	+	+	+	+	+	-	+	+	+	-
Total	2	4	2	2	4	1	3	3	4	2

Table (5): Molecular weights Wheat and polymorphic bands of the total protein in the third group of presence or absence of Wheat genotypes (*Triticum aestivum* L.)

Band MW	Genotypes									
	21	22	23	24	25	26	27	28	29	30
72	+	+	+	-	+	+	+	+	-	-
55	+	+	-	-	-	-	-	++	++	++
43	-	+	+	+	-	-	+	-	-	-
34	-	+	+	-	+	-	+	+	+	+
26	-	-	-	+	+	+	+	-	-	-
Total	2	4	3	2	3	2	4	3	2	2

Conclusively, these results indicates that SDS –PAGE analyses of different species protein is useful to evaluate the genetic variability and different species identification, which can help in wheat breeding program.

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التنوع الوراثى فى بعض سلالات ا لقمح على بعض الصفات الوصفية والبروتين والبصمة الوراثية للبروتين

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أجريت هذه الدراسة بقسم بحوث الأصول الوراثية معهد بحوث المحاصيل الحقلة ببهتيم خلال موسمى ٢٠١٥/٢٠١٦ و ٢٠١٦ / ٢٠١٧ لاستخدام بعض مقاييس التنوع الوراثى لتققيم بعض الأصول الوراثية فى القمح بإستخدام طرق متعددة .

ولقد أظهرت نتائج الصفات المورفولوجية فى القمح دورواهمية هذة الصفات فى زيادة المحصول .

كما أظهرت السلالات الاصول تنوع للبروتين والجينات المسئولة عن أفراز هدة البروتينات . وأظهرت صبغة الأثنوسيانين فى إذينات ورقة العلم فى الاصول الوراثية المستوردة رقم ١،٦،٧،٨،٩،١٠ وهذه الصفة معلمات فريدة فى القمح الرباعى ، كما أظهرت ان صبغة الأنثوسيانين مصاحبة للبادرات حتى عمر ١١ يوم . هذه العلامة يجب أن يأخذها المربى فى التمييزبين بعض المستورادات الوراثية . كما أن البصمة الوراثية للبروتين بإستخدام البولى أكريلاميد جيل SDS PAGE يعتمد على وجود (band) من عدمه فى تلك الاصول الوراثية . كما أظهرت النتائج إنخفاض نسبة الإختلافات الوراث.

التوصية: لذا توصى الدراسة باستخدام البصمة للاصول المختلفة وراثيا فى تقييم الاختلافات بين الاصول الوراثية وذلك للمساعدة فى برامج التربية لمحصول القمح.