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GENETICAL STUDIES ON WHEAT DROUGHT TOLERANCE USING MOLECULAR AND BIOCHEMICAL MARKERS

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ABSTRACT

Two field experiments were carried out at the Experimental farm of Gemmeiza Agricultural Research Station, Agricultural Research Center, Egypt, during three successive seasons; 2011/2012, 2012/ 2013 and 2013/2014. Four wheat genotypes (*Triticum aestivum* L.) were used in this study namely, Gemmeiza 9, Gemmeiza 11, Misr 1 and Gemmeiza line 22, which represents a wide range of drought tolerance variability and crossed to obtain F1 seeds of two crosses (Gemmeiza 9 × Misr 1) and (Gemmeiza 11 × Gemmeiza line 22). F1 plants were self-pollinated to produce F2 seeds and evaluated in two experiments. The first experiment (normal conditions) was irrigated four times after planting irrigation, the second experiment (drought conditions) was given one surface-irrigation, 30 days after planting irrigation. Presence of genes responsible for drought tolerance is basic requirement for improving any crop species including wheat. The objective of this study was conducted to assess genetic studies among two populations of bread wheat genotypes using SSR markers and SDS-PAGE aiming to developing wheat cultivars and achieving sustainability in wheat production in Egypt. The results showed that the crossing between Gemmeiza 11 and Gemmeiza line 22 which having drought tolerance in addition to good gluten strength can be used in breeding programs in future. the electrophoretic profiles of the studied genotypes of both wheat crosses revealed that the total number of protein banding patterns was twenty seven. These bands were widely distributed among wheat genotypes, having a wide range of molecular weights ranging from 16 to 127 KD. Meanwhile, band 27 in the first cross and band 10 in the second cross were unique bands characterizing the parents Gemmeiza 9 and Gemmeiza 11 and serving as markers for drought breeding.

Key words: wheat, *Triticum aestivum* L., SSR, SDS PAGE, drought tolerance

INTRODUCTION

Triticum aestivum L. is the common wheat belongs to the Poaceae family which is one of the most significant and diverse families of kingdom Plantea. Wheat is nature's unique gift to humankind as it produces excellent source of nutrition in terms of carbohydrates, minerals and proteins (Hitesh and Renu 2009). Globally wheat is being cultivated over an area of 218 million hectares with a production of 713 million tonnes (FAO, 2013). In Egypt, wheat is cultivated in about 1.42 million hectares (3.049 million faddans). The local production is about 8.8 million tonnes covering less than 53.3% of local consumption (FAO, 2013). This reflects the size of the problem and the efforts needed to increase wheat production (Gad, 2010). Recently, the breeding programs are played a great role in replacing landraces by highly-yield of genetically enhanced wheat varieties. (Al-Rawashdeh and Al-Rawashdeh, 2011). Morphological, physiological and cytogenetical plant traits are used at present as a selection criteria, which are not stable and greatly affected by environmental conditions. Selection based on biochemical markers, seed storage proteins and molecular markers are more stable than those

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abovementioned traits (Farshadfar *et al*., 2003). Many kinds of molecular markers based on various DNA analysis methods are being used in present day breeding programs, molecular marker techniques, which are presently available to identify the variability, diversity and similarity at molecular levels (Mukhtar *et al*., 2002 ; Malik *et al*., 2010). New molecular tools such as simple sequence repeats (SSR) have now provided the opportunity to monitor genetic integrity at the genotype level and laboratory tests are available to determine any unintentional genetic erosion or change in genetic identity. SSRs, used in common wheat, is often not developed from the genes themselves because the cloning of genes in wheat is complicated by its allohexaploid nature and large genome size. In contrast, drought tolerant responsible genes based SSR markers will be more polymorphic than other markers (Kassa *et al.*, 2006).

Sodium dodecyl sulphate poly acrylamide gel electrophoresis (SDS-PAGE) is used as a biochemical marker for the evaluation of genetic diversity because of its simplicity and effectiveness for describing the genetic structure crop germplasm. The analysis of storage protein variation in wheat has proved to be useful tool not only for diversity studies but also to optimize variation in germplasm collections (Masood *et al.,* 2000). Also large number of germplasm lines can be characterized by biochemical markers in short period of time. In addition, the data for storage protein reflects more truly the genetic variability because biochemical markers are a direct product of genes and the environment does not influence their expression (Masood *et al.,* 2004).

Therefore, this research aimed to assess genetic studies among two populations of bread wheat genotypes using SSR markers and SDS-PAGE aiming to developing wheat cultivars and achieving sustainability in wheat production in Egypt.

MATERIALS AND METHODS

The present study was carried out at the Experimental Farm of El-Gemmeiza Agricultural Research Station, Egypt during three successive seasons; 2011/2012, 2012/ 2013 and 2013/2014. Four wheat genotypes (*Triticum aestivum* L.) used in this study were Gemmeiza 9, Gemmeiza 11, Misr 1 and Gemmeiza line 22, which represents a wide range of drought tolerance variability. Genotype seeds were obtained from Egyptian Agricultural Research Center (ARC), Wheat Research Department. The origin, characterization and pedigree of the four genotypes are presented in Table 1. In 2011/ 2012 growing season, the four parental genotypes were planted and were crossed to obtain F1 seeds of two crosses (Gemmeiza 9 × Misr 1) and (Gemmeiza 11 × Gemmeiza line 22). In the second season (2012/2013), the hybrid seeds were sown. F1 plants were selfg-pollinated to produce F2 seeds. In the third season (2013/2014), the obtained seeds of both populations, *i.e.* P_1, P_2, F1 and F2 were planted and evaluated using a randomized complete block design. Each plot was consisted of 30 individual guarded plants for P_1, P_2 and F1 and 300 plants for F2 in two separate irrigation regime experiments. The first experiment (normal conditions) was irrigated four times after planting irrigation, *i.e.* five irrigations during the whole season. The second experiment (drought condition) was given one surface-irrigation, 30 days after the planting irrigation (two irrigations during the whole season).

Bulked Segregant Analyses (BSA)

Bulked-segregant analyses (BSA) was used in conjunction with SSR analysis (Michelmore *et al*., 1991) to find markers linked to drought tolerance genes. Tolerant and sensitive bulks were prepared from F2 individuals each of ten sensitive and ten tolerant F2 plants, based on phenotypic assessments for drought. SSR primers were then, applied on the parents, F1 and the two F2 bulked DNA samples.

PCR amplification

A set of three SSR markers, were used to create the molecular marker data and presented in Tables 2 and 3.

DNA extraction and molecular technique (SSR) were done according to the method outlined by Sehgal *et al.* (2012). While the procedure of SDS-PAGE was conducted according to Slađana *et al.* (2011) as well as Bradova and Chroma (2008).

Table 1. Pedigree, origin and characterization of studied wheat genotypes

No.	Genotype	Pedigree	Origin	Character
1	**Gemmeiza 9**	ALD"S"/HUAC"S"//CMH-74A630/5X CGM4583-5GM-1GM-0GM	Egypt	T
2	**Gemmeiza 11**	BOW"S"/KVZ//7C/SERI 82/3/GIZA#168/SAKHA#61 CGM7892-2GM-1GM-2GM-0GM	Egypt	S
3	**Misr 1**	OASIS/SKAUZ//4*BCN/3/2*PASTOR CMSSOOYO1881T-050M-030Y-030M-030WGY-33M-0Y-0S	Egypt	S
4	**Gemmeiza Line 22**	KAUKO/CMH82-493//YRR/3/SAKHA#93 CGM8322-1GM-2GM-1GM-1GM-0GM	Egypt	T

T: drought tolerance S: drought sensitivity

Table 2. SSR-primers associated with important traits selected for the current study

No.	Primer	Trait category	References
1	**TSM0120/1RS Rye**	Drought tolerance	Kofler *et al.* (2008)
2	**Glu-A3d/Glu-A3**	Gluten strength (LMW)	Zhang *et al.* (2004)
3	**UMN19/Glu-A1**	Gluten strength (HMW)	Liu and Anderson (2008)

Table 3. Primers name and sequences of the SSR loci reaction

No.	Primer	Sequence
1	**TSM0120**	F: ACGACGTTGTAAAACGACCCGCCGTCCTCCTCCT R: AGACGGCAGGCATGGAT
2	**Glu-A3d**	F: ACGACGTTGTAAAACGACACCAGTTATTCATCCATCTGCTC R: GTGGTTTCGTACAACGGCTCG
3	**UMN19**	F: ACGACGTTGTAAAACGACCGAGACAATATGAGCAGCAAG R: CTGCCATGGAGAAGTTGGA

Molecular analysis was carried out at Molecular Genetic Lab. National Gene Bank, Giza, Egypt.

Biochemical analysis was carried out at Biotechnology Lab. National Research Center Giza, Egypt.

RESULTS AND DISCUSSION

Simple Sequence Repeats (SSR) Finger Printing

Figs. 1-3 and Table 4 illustrate SSR-PCR banding patterns of wheat genotypes, P1, P2, F1, F2 tolerant bulk and F2 sensitive bulk of two crosses "Gemmeiza 9 × Misr 1" and "Gemmeiza 11 × Gemmeiza line 22" using three SSR primers.

The SSR analysis revealed that only two primers gave bands as shown in Table 4, primer TSM0120/1RSRye with molecular weight of 361 and primer UMN19/Glu-A1 with molecular weight of 377. The first primer was available in screening drought tolerance or drought susceptible in DNA bulks or in the parents. However the second primer Glu-A3d was also available for gluten strength. Meanwhile the SSR primer Glu-A3d/Glu-A3 was not able to give any bands in any genotype of both wheat crosses.

The data showed that the SSR primer TSM0120/1RSRye was identified at Gemmeiza 9, F1 and F2 tolerant bulk of the wheat cross "Gemmeiza 9 × Misr 1" and also was expressed in Gemmeiza line 22 and F2 tolerant bulk in the second cross. These results may suggest that the banding expression of this SSR primer seem to be a marker for drought tolerance in wheat.

While, the SSR primer UMN19/Glu-A1 was detected among either one or both parents, besides F2 sensitive bulk of both studied wheat crosses, suggesting that the banding expression of this primer may serve as marker for drought sensitive in wheat.

Generally, these results may gave attention or highlight on the good characteristics of Gemmeiza 11 and Gemmeiza line 22 which having drought tolerance in addition to good gluten strength which can used in breeding programs in future.

These results may demonstrate that SSR markers combined with bulked segregant analysis can be used as indicator for drought tolerance in wheat. Our results appeared to be in agreement with those reported by Roussel *et al.* (2005) and Malik *et al.* (2010).

Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE profiles of the studied wheat genotypes of both crosses are given in Figure 4 and diagrammatically shown in Figure 5 and illustrated in Table 5.

The electrophoretic profiles of the studied genotypes of both wheat crosses revealed that the total number of protein banding patterns was twenty seven. These bands were widely distributed among wheat genotypes, having a wide range of molecular weights ranging from 16 to 127 KD.

Fourteen bands out of twenty seven bands were detected in all genotypes of both wheat crosses, representing common bands and having numbers of 1, 2, 3, 6, 7, 9, 12, 13, 14, 15, 18, 19, 20 and 21.

Also, bands 17 and 24, bands 4, 8 and 22 were common in all genotypes of the first and second crosses, respectively.

Interestingly, band 27 in the first cross and band 10 in the second cross were unique bands characterizing the parents Gemmeiza 9 and Gemmeiza 11 and serving as markers for drought breeding. Moreover, none of genotypes in both crosses possessed all bands, but bands distributed among genotypes ranging from 18 bands to 22 bands, with average frequency of 0.666. The relative frequency distribution of SDS-PAGE bands, their molecular weights and their polymorphic state are given in Tables 5, 6 and 7. The data showed that total polymorphism among studied wheat genotypes for protein banding was about 48.14 %, reflecting the possibility of using it in wheat breeding programs.

In this connection, Dvořáček and Čurn (2003) evaluated protein fractions as biochemical markers for identification of spelt wheat cultivars. Also, Shuaib *et al.* (2007) studied seed storage protein using SDS-PAGE among wheat varieties for characterization these varieties. Likewise, Kaleem *et al.* (2008) used SDS-PAGE technique in studying genetic diversity in wheat. Our findings seem to be in parallism with such reports Demirevska *et al.* (2008) and Najaphy *et al.* (2014).

Table 4. The SSR amplified fragments obtained from the DNAs of four wheat parents and their subsequent F1 plants and their tolerant and sensitive F2 plants

Primer	Bands and molecular weight	Cross 1: Gemm.9 × Misr 1					Cross 2: Gemm.11 × Gemm. Line 22				
		P1	P2	F1	F2 Bulk		P1	P2	F1	F2 Bulk	
					T	S				T	S
TSM0120	Band at 361	1	0	1	1	0	0	1	0	1	0
Glu-A3d	Band at 0	0	0	0	0	0	0	0	0	0	0
UMN19	Band at 377	1	1	0	0	1	1	0	0	1	1

T: Drought tolerance S: Drought sensitivity 1: Present bands 0: Absent bands

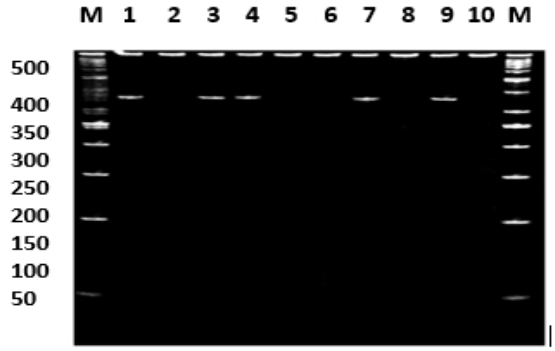

Fig. 1. SSR-PCR banding patterns of wheat genotypes under drought stress using primer : TSM0120

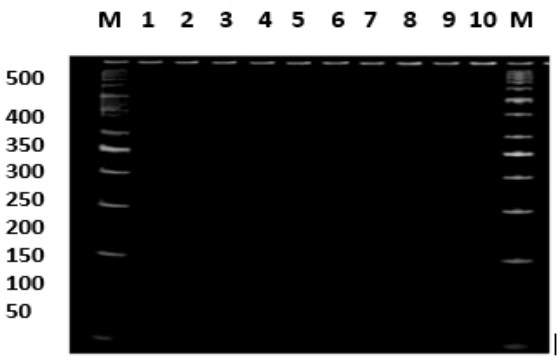

Fig. 2. SSR-PCR banding patterns of wheat genotypes under drought stress using primer Glu-A3d

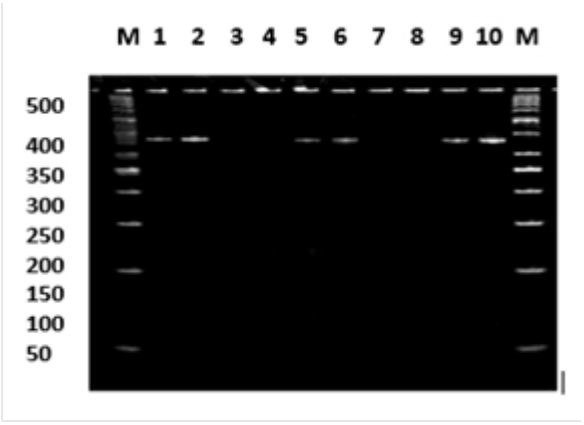

Fig. 3. SSR-PCR banding patterns of wheat genotypes under drought stress using primer UMN19

Lane M-kb: molecular –size ladder 1: Gemmeiza 9 2: Misr 1 3: F1 4: F2 Tolerant 5: F2 Sensitive 6: Gemmeiza 11 7: Gemmeiza Line 22 8: F1 9: F2 Tolerant 10: F2 Sensitive.

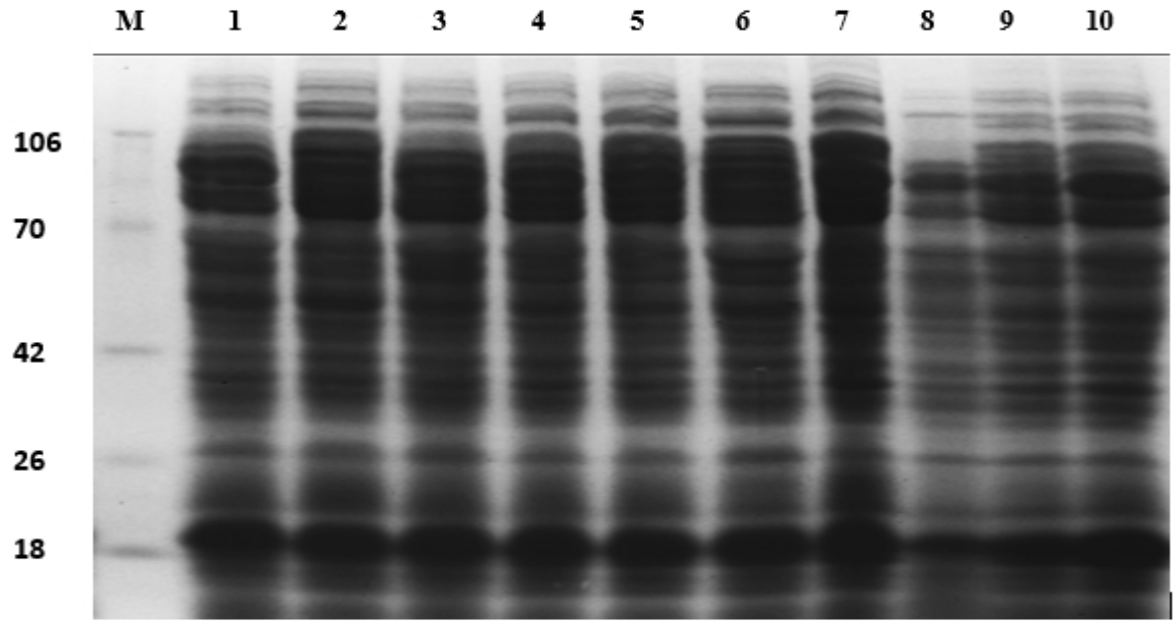

Fig. 4. SDS – PAGE profiles of wheat genotypes associated with drought tolerance in wheat

M: Molecular weight 1: Gemmeiza 9 2: Misr 1 3: F1 4: F2 Tolerant 5: F2 Sensitive 6: Gemmeiza 11 7: Gemmeiza Line 22 8: F1 9: F2 Tolerant 10: F2 Sensitive

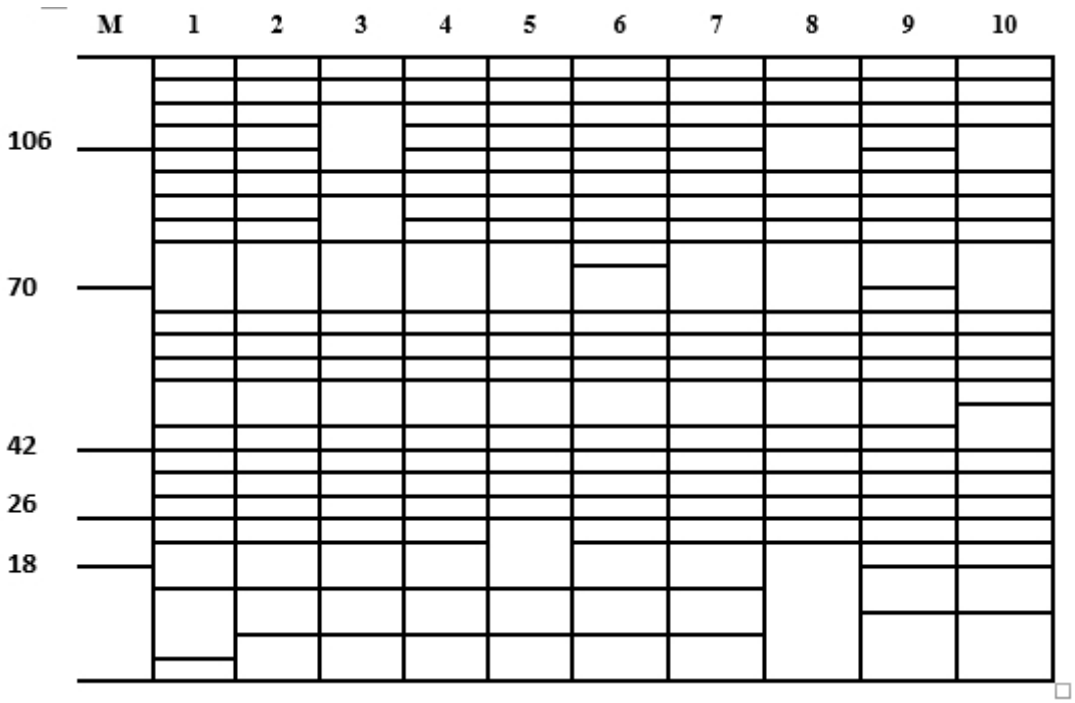

Fig. 5. SDS – PAGE diagram of wheat genotypes associated with drought tolerance in wheat

M: Molecular weight 1: Gemmeiza 9 2: Misr 1 3: F1 4: F2 Tolerant 5: F2 Sensitive 6: Gemmeiza 11 7: Gemmeiza Line 22 8: F1 9: F2 Tolerant 10: F2 Sensitive.

Table 5. SDS-PAGE protein bands of wheat genotypes of both crosses

Band No.	MW	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7	Lane 8	Lane 9	Lane 10
1	**127.858**	1	1	1	1	1	1	1	1	1	1
2	**125.165**	1	1	1	1	1	1	1	1	1	1
3	**116.799**	1	1	1	1	1	1	1	1	1	1
4	**113.733**	1	1	0	1	1	1	1	1	1	1
5	**106.131**	1	1	0	1	1	1	1	0	1	0
6	**95.925**	1	1	1	1	1	1	1	1	1	1
7	**91.44**	1	1	1	1	1	1	1	1	1	1
8	**84.65**	1	1	0	1	1	1	1	1	1	1
9	**80.691**	1	1	1	1	1	1	1	1	1	1
10	**77.948**	0	0	0	0	0	1	0	0	0	0
11	**77.534**	0	0	0	0	0	0	0	0	1	0
12	**66.624**	1	1	1	1	1	1	1	1	1	1
13	**61.841**	1	1	1	1	1	1	1	1	1	1
14	**51.332**	1	1	1	1	1	1	1	1	1	1
15	**47.647**	1	1	1	1	1	1	1	1	1	1
16	**45.058**	0	0	0	0	0	0	0	0	0	1
17	**44.819**	1	1	1	1	1	1	1	1	1	0
18	**42.383**	1	1	1	1	1	1	1	1	1	1
19	**38.003**	1	1	1	1	1	1	1	1	1	1
20	**34.716**	1	1	1	1	1	1	1	1	1	1
21	**32.396**	1	1	1	1	1	1	1	1	1	1
22	**22.381**	1	1	1	1	0	1	1	1	1	1
23	**17.152**	0	0	0	0	0	0	0	0	1	1
24	**17.016**	1	1	1	1	1	1	1	0	0	0
25	**16.438**	0	0	0	0	0	0	0	0	1	1
26	**16.35**	0	1	1	1	1	1	1	0	0	0
27	**16.22**	1	0	0	0	0	0	0	0	0	0
Total		21	21	18	21	20	22	21	18	22	20

Table 6. Relative frequencies and polymorphism distribution for SDS-PAGE bands in different wheat genotypes

Band No.	RF	MW	Frequency	Polymorphism
1	0.121	127.858	1.000	Common bands
2	0.129	125.165	1.000	Common bands
3	0.155	116.799	1.000	Common bands
4	0.165	113.733	0.900	Polymorphic
5	0.191	106.131	0.700	Polymorphic
6	0.229	95.925	1.000	Common bands
7	0.247	91.440	1.000	Common bands
8	0.276	84.650	0.900	Polymorphic
9	0.294	80.691	1.000	Common bands
10	0.307	77.948	0.100	Monomorphic
11	0.309	77.534	0.100	Monomorphic
12	0.366	66.624	1.000	Common bands
13	0.394	61.841	1.000	Common bands
14	0.464	51.332	1.000	Common bands
15	0.492	47.647	1.000	Common bands
16	0.513	45.058	0.100	Monomorphic
17	0.515	44.819	0.900	Polymorphic
18	0.536	42.383	1.000	Common bands
19	0.577	38.003	1.000	Common bands
20	0.611	34.716	1.000	Common bands
21	0.637	32.396	1.000	Common bands
22	0.776	22.381	0.900	Polymorphic
23	0.876	17.152	0.200	Polymorphic
24	0.879	17.016	0.700	Polymorphic
25	0.892	16.438	0.200	Polymorphic
26	0.894	16.350	0.600	Polymorphic
27	0.897	16.220	0.100	Monomorphic

Table 7. Protein banding polymorphism for wheat genotypes and their crosses.

Common bands	14
Monomorphic bands	4
Polymorphic bands	9
Total polymorphic bands	13
Total number of bands	27
Polymorphism (%)	48.148%
Mean of band frequency	0.666

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دراسات وراثية على التحمل للجفاف فى القمح باستخدام الواسمات الجزيئية والبيوكيميائية

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تم تنفيذ تجربتين حقليتين فى المزرعة البحثية لمحطة البحوث الزراعية بالجميزة، مركز البحوث الزراعية، مصر خلال ثلاث مواسم زراعية وهي ٢٠١٢/٢٠١١ ، ٢٠١٣/٢٠١٢ و ٢٠١٤/٢٠١٣، حيث تم استخدام اربعة تراكيب وراثية لقمح الخبز (*Triticum aestivum* L.) وهي جميزة ٩، جميزة ١١، مصر ١ و سلالة ٢٢ جميزة، وتم اختيارهم بناءًا على التنوع الكبير فى التحمل للجفاف وتم التهجين بينهم للحصول على الهجينين (جميزة٩ × مصر ١) و(جميزة ١١ × سلالة ٢٢ جميزة) وتم زراعتهم للحصول على حبوب الجيل الثانى وتقييمهم فى تجربتين، التجربة الأولى (الظروف العادية) اربعة ريات بعد رية الزراعة، والتجربة الثانية (ظروف الجفاف) رية واحدة بعد ٣٠ يوم من رية الزراعة، وجود جينات تحمل الجفاف هو شرط أساسي لتحسين القمح، حيث تهدف هذه الدراسة لتقييم التراكيب الوراثية لاثنين من عشائر قمح الخبز باستخدام الواسمات الجزيئية SSR والبيوكيميائية SDS-PAGE وذلك بهدف تحسين أصناف القمح لتحقيق أعلى إنتاجية للقمح في مصر، وأثبتت النتائج أن الهجين (جميزة ١١ × سلالة ٢٢ جميزة) يتحمل الجفاف بالإضافة إلى قوة الجلوتين حيث يمكن استخدامه في برامج التربية مستقبلًا، وأظهر التفريد الكهربي لكلا العشيرتين أن العدد الإجمالي لحزم البروتين كان سبعة وعشرين حزمة، وتوزعت هذه الحزم على نطاق واسع من التراكيب الوراثية، ووجود مجموعة كبيرة من الأوزان الجزيئية تتراوح بين ١٦-١٢٧ كيلودالتون، وكانت الحزمة رقم ٢٧ في الهجين الأول والحزمة رقم ١٠ في الهجين الثاني مميزة للآباء جميزة ٩ وجميزة ١١ ويمكن استخدامها كواسمات للتربية للجفاف.

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٢- أ.د. ممـــدوح كـــامـــل علـــي أميـــن أستاذ الوراثة المتفرغ - كلية الزراعة - جامعة الزقازيق.