Morphological and Biochemical Markers for Genetic Diversity and Salt Tolerant In Some Barley Cultivars and Lines

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ABSTRACT

More than 280.000 barley accessions (*Hordeum vulgare* L.) were recorded in gene banks around the world. Most of the genetic diversity that local or traditional varieties of cultivated crops possess is being lost. The experiment was conducted at the Faculty of Agriculture (Saba Basha), Alexandria University, Egypt, during 2016 to characterize different barley cultivars and lines under different salt concentrations. Fourteen barely cultivars and eight lines were used in the current research. All the tested samples were sown in petri dish (15 cm dimeter) under different salt concentrations (0, 100, 200 and 300 Mm NaCl) using complete randomized design in three replicates. Morphological parameters were selected and measured after two weeks from each replicate. The morphological parameters were germination percentage (%), seedling height (cm) and root length (cm). Grains of each cultivar were squashed and total protein were sequentially separated. One dimensional SDS–polyacrylamide gel electrophoresis was performed to separate the total protein. The genetic relationships among cultivars and lines were measured based on morphological and biochemical markers aiming to use in the future breeding program based on genetic variations. The results revealed significant variations among the tested cultivars and lines under different salt concentrations. Line number 5 and 6 showed the highest mean values of morphological traits under high salt concentrations, while barely cultivars showed different response to salinity levels. The tolerant cultivars showed unique bands in total protein analysis that mean when barely plants subject to abiotic stress such as salinity, plants try to increase the total enzyme contact and some amino acid like proline as defense mechanism for protect the cell wall from damage, this fact was achieved during current results.

Key words: Barley, genetics, morphology, Salt stress, SDS

INTRODUCTION

Barley considers the crop grown on a large scale in regions like new reclaimed lands and in saline soils. Total cultivated area of barley differs from year to another year may be due to the rainfall amount in Egypt. Barely cultivated area in the Nile Valley gradually reduced, but enhanced in the reclaimed lands under different irrigation systems. Barley is one of the main cereals crop of the Mediterranean agriculture area (Harlan and Zohary, 1966). Barley crop is a salt-tolerant and economic crop in salinity affected arid and semi-arid regions of the world (Walia *et al*., 2006). Barley is diploid (2n=2x=14), good experimental plant model, mainly owing to its, self-fertility, large chromosomes, moderate genome size (5.3x109 bp), high degree of natural and easily inducible variance, hybridization, wide adaptability, and relatively limited space requirements (Zohary and Hopf, 1993).

Previous research detected that proteins account in barely is about 10% of the dry weight of mature barley grains, classified to storage and non-storage proteins. The major storage proteins in barley (prolamins) are called hordeins, (35-50%) of grain nitrogen (Kirkman *et al.*, 1982 and Kreis and Shewry, 1992) and divided to four groups of polypeptides called B, C, D and ã-hordeins. The electrophoretic separation of barley grain storage proteins can be used as an indicator for cultivar identification and wild barley biodiversity and phylogeny studies (El Rabey *et al*., 2002, El Rabey, 2004, El Rabey and Zayed, 2005 and El Rabey, 2008). The physiological and molecular markers could be used in screening different lines and cultivars for tolerance for salt stress during breeding programs of barley (Abdel-Hamid, 2014). Genetic relationship among 38 barley genotypes with the aid of RAPD, sequence tagged site (STS), simple sequence repeat (SSR) marker and revealed that RAPD marker could be utilized for estimating the relationships among cultivars and identification variety (Klara *et al*., 2007). Genotypic information is need in the form of markers for any quantitative trait loci involved (marker assisted selection) or of knowledge of the genes (Giora and Uri, 2012). In the present study, different morphological parameters and grain storage proteins electrophoresis (SDS–PAGE) have been used to characterize different barley cultivars and lines under different salt concentrations.

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MATERIALS AND METHODS

The experiment was conducted at the Faculty of Saba Basha, Alexandria University, Egypt, during 2016.

a- Morphological studies

Fourteen barely cultivars and eight lines (Table 1) were used in the current study. All the tested samples were sown in petri dish under different salt concentrations e.g. 0, 100, 200 and 300 Mm salt. After couples of weeks three major morphological parameters were selected and measured for all the tested samples using three replicates from each. The morphological parameters were germination percentage (%), seedling height (cm) and root length (cm). data were collected and analyses by statistical program Co-stat and LSD.0.05 was detected between the different values.

Table 1. Description and pedigree of barely cultivars and lines used in current study

Samples	Description and pedigree
Giza 2000	Six rows, Egyptian barley variety, late, productive in the favorable conditions, tolerant to salinity and to fungi diseases. It is issued from the following cross: Giza 117/Bahteem52//Giza118/FAO 86 Giza 121. ARC- Egypt
Giza 124	Six rows, Egyptian barley variety moderately productive in the favorable conditions and tolerant to fungi diseases. It is issued from the following cross: Giza 117/Bahteem 52// Giza 118/FAO 86. ARC- Egypt
Giza 123	Six rows, Egyptian barley variety, precocious, moderately productive in the favorable conditions and tolerant to salinity and fungi diseases. It is issued from the cross of: Giza 117 /FAO86. ARC- Egypt
Giza 129	Six rows, Egyptian naked barley accession, precocious, moderately productive in the favorable conditions and tolerant to drought and fungi diseases. ARC- Egypt
Giza 126	Six rows, Egyptian barley accession, late, productive in the favorable conditions and tolerant to drought and fungi diseases. It is issued from the following cross: Baladi, Bahteem/SD 729 Por 12769-BC. ARC- Egypt
Giza 127	Six rows, Egyptian barley accession, precocious, high productive in the favorable conditions and tolerant to fungi diseases. ARC- Egypt
Giza 128	Two rows, Egyptian barley variety, precocious, high productive in the favorable conditions. ARC- Egypt
Giza 125	Six rows, Egyptian barley accession, late, productive in the favorable conditions and tolerant to drought and fungi diseases. ARC- Egypt
Giza 136	Six rows, Egyptian naked barley accession, precocious, moderately productive in the favorable conditions. ARC- Egypt
Giza 135	Six rows, Egyptian naked barley accession, late, moderately productive in the favorable conditions. ARC- Egypt
Giza 134	Six rows, Egyptian barley variety, late, moderately productive in the favorable conditions and tolerant to salinity and fungi diseases. ARC- Egypt
Giza 133	Six rows, Egyptian barley variety and tolerant to salinity and fungi diseases. ARC- Egypt
Giza 132	Six rows, Egyptian barley variety and tolerant to fungi diseases.ARC- Egypt
Giza 130	Six rows, Egyptian naked barley accession, precocious, moderately productive in the favorable conditions and tolerant to drought and fungi diseases. It has been selected from the crosses ‘‘Comp.cross’’ 229//Bco.Mr./DZ02391/3/ Deir Alla 106 using the bulk method. ARC- Egypt
L1	AVT/ATIKI//M-ATT-73-337-1/3/ATHS /Lignee686/4/Kabaa ICARDA/CIMMYT Program
L2	JLB70-20/Sen ""S"//RIHANE-03 ICARDA/CIMMYT Program
L3	ACSAD 1182/4/Attiki//M-Att-73-337-1/3/Aths/ Lignee686 ICARDA/CIMMYT Program
L4	AlANDA/5//ATHS/4/Pro/Toll//Cer*2/Toll//3/5106/6/Baca""S"/3/AC253// C108887/C105761- ICARDA/CIMMYT Program
L5	CABUYA/ESMERALDA ICARDA/CIMMYT Program
L6	LIGNEE527/NK1272//JLB70-063/3/BAD ICARDA/CIMMYT Program
L7	ICARDA/CIMMYT Program
L8	ICARDA/CIMMYT Program

b- Biochemical studies (SDS–polyacrylamide Gel Electrophoresis)

Grains of each cultivar and lines were squashed and both water soluble and water-non-soluble protein and separated in 1.5 ml Eppendorf tubes. One dimensional SDS–Polyacrylamide gel electrophoresis was performed to separate both water soluble and water-non-soluble grain storage protein per the method of Laemmli (1970) on 15% polyacrylamide gels concentration against a broad range protein marker (212.000 – 2.340 kDa, New England).

c- Statistical Analysis

Data were statistically analyzed as complete randomized design (CRD) design experiments, using the CRD model as obtained by CoStat 1998-2005.Means were compared per Fisher test at (p=0.05) least significant difference (LSD) to estimate the significant differences among treatments (Steel and Trrie, 1982).

Protein gels were scored as 0/1 for absence/presence of the bands and the resulting of protein bands were analyzed using the NTSYS-pc2.0 software (Rohlf, 1998). Phylogenetic dendrograms were constructed using the UPGM A method (Unweighted Pair-Group Method with arithmetical algorithms Averages; (Sneath and Sokal, 1973)**.**

RESULTS AND DISCUSSION

A- Morphological characteristics

The current results will include two main subjects; salinity levels and cultivars/lines with three different structural parameters. Responses to NaCl in an early germination stage of the barley cultivars/lines were obtained by measuring parameters after four week germination in different concentrations of NaCl. Touching to the germination percentage, data in Table 2 indicated that all the lines showed 100% germination % under the control condition. Also, within the barley cultivars the germination % ranged from 73% (Giza 135), 75% (Giza 136) to 100%. No significant variations were obtained in cultivars and lines under tap water except cultivars (Giza 2000, Giza 129, Giza 125, Giza 136 and Giza 135) that demonstrated the lowest germination % value and rated from 73.8 to 85%. The general of a germination % under tap water was 95.72% with no significant values detected. With increasing in salinity levels from 0 to 100 Mm salt, the outcomes showed no significant change and the reduction was very low (Table 2). 16 of 22 tested samples almost were 100% germinated under 100 mm salt, while the other seven barely cultivars and lines ranged from 62.5 (Giza 135) to 87% (Giza 130). High significant variations were observed when salt levels increased up to 200 and 300 Mm salt as shown in Table 2, almost 25% was decreased in germination % in six cultivars and lines, i.e. (Giza 2000, Giza 129, Giza 126, Giza 133 and line 6) and 50% in (Giza 136, Giza 135 and Giza132), while the other samples showed normally germination % under the high salt concentrations.

Eight cultivars and lines showed almost 100% germination %, i.e. Giza 124, Giza 123, Giza 128, line 2, line 3, line 4, line 5 and line 7. In general, barely lines showed high salt tolerant comparing with cultivars (Table, 2). No significant variations were observed in the general mean for 100 and 200 Mm salt and were 91.64 and 91.20, in respect, while was 82.15 under the high salt level (300 Mm). When comparing barely cultivars and lines the data indicated that the lines are more salt tolerant based on the germination %. The data in Table 2 showed that cultivates Giza 136 and Giza 135 showed the lowest values (58.95 and 60.4%), followed by cultivars Giza 2000, Giza 132 and line 7 by 79.1, 77.65 and 83.75%. Some barely lines showed 100% germination % such as lines 2, 3, 5, 7 and the other ranged ~ from 85 to 95%.

Generally, data of Table (2) indicated that there was a reverse relationship between the salinity levels. However increasing salinity levels from 0 to 100 mM salt caused low reduction in germination %, whereas 200 and 300 mM salt had high decreasing in germination % as compared with control treatment in some barely cultivars and lines. Data for seedling height (cm) in Table (2) showed that all barely cultivars and lines have general mean 23.24 cm under tap water, while with the high salt concentrations reached to 9.60 cm with reduction in seedling height 58%. The average of seedling height in all cultivars and lines ranged from the lowest mean 18.73 cm (Giza 130) to 27.17 cm in line 6 with increase of salt levels from 0 to high level thus seedling highest was decreased almost 55% for all cultivars and lines. On the other hand, some cultivars showed low reduction for this character such as Giza 123 (38%), Giza 125 (50%), Giza 130 (35%), lines, 4 (53%), 6 (48%) and 8 (50%).

High significant variations were observed between the tested cultivars and lines and the highest value was recorded to the barely lines in range from ~16 to 19 cm comparing with control was from 22 to 27 cm, that indicated the reduction in seedling height was 30% under the high salt level. While, the reduction in barley cultivars was more that 45% in this character comparing with control.

Table 2. Morphological variation of different barely cultivars and lines as influenced by salinity concentrations during seedling growth stage

A- Cultivar and line	Germination percentage (%)					Seedling height (cm)					Root length (cm)				
	B- Salinity concentration (mm)														
	0	100	200	300	Average (A)	0	100	200	300	Average (A)	0	100	200	300	Average (A)
Giza 2000	85.0	83.5	73.4	74.5	79.1i	24.63	19.07	14.37	6.27	16.09^{fghi}	21.53	6.83	5.60	4.73	9.67^{a}
Giza 124	100.0	98.8	98.4	98.4	98.9^{ab}	21.93	16.37	9.53	9.80	14.41^{ij}	16.80	6.83	5.63	5.57	8.71^{abc}
Giza 123	100.0	98.4	98.2	98.4	98.7^{ab}	19.50	17.67	12.17	12.07	15.35^{ghi}	10.97	8.80	4.83	5.50	7.53^{cd}
Giza 129	81.6	100.0	93.0	70.8	86.4^{fg}	23.13	15.87	11.20	8.53	14.68^{hij}	14.37	5.87	6.43	4.37	7.76^{bcd}
Giza 126	98.4	98.6	95.4	75.0	91.8^{cde}	20.63	22.17	15.27	8.47	16.64^{efgh}	10.90	7.73	6.53	5.77	7.73^{bcd}
Giza 127	100.0	98.0	93.4	92.4	95.9^{abc}	21.60	18.93	11.07	7.67	14.82^{hi}	12.27	8.50	5.53	5.67	7.99^{bcd}
Giza 128	100.0	100.0	100.0	100	100^{ab}	21.83	10.30	11.57	7.07	12.69^{j}	15.43	4.27	6.17	4.83	7.68^{bcd}
Giza 125	98.5	92.5	91.8	80.8	90.9^{def}	21.70	22.47	15.17	11.00	17.59^{bcdefg}	14.83	11.27	5.50	6.20	9.45^{a}
Giza 136	75.8	75.6	46.0	44.2	60.4^{j}	24.33	19.73	13.90	8.53	16.62^{efgh}	13.80	8.57	6.23	7.00	8.90^{abc}
Giza 135	73.8	62.5	51.6	47.8	58.9^{j}	23.27	22.67	14.00	8.90	17.21^{cdefg}	11.87	7.33	3.73	5.33	7.07^{de}
Giza 134	97.0	98.4	89.9	83.6	92.3^{bcde}	23.60	20.17	16.70	8.50	17.24^{bcdefg}	11.73	10.17	8.60	3.67	8.54^{abc}
Giza 133	100.0	99.0	95.0	79.5	93.4^{cde}	22.07	17.67	13.97	10.70	16.10^{fghi}	14.37	8.17	6.23	4.83	8.40^{abcd}
Giza 132	95.8	87.0	77.6	58.15	79.6^{i}	22.37	19.80	15.57	6.77	16.13^{fghi}	14.37	8.70	7.57	3.73	8.59^{abc}
Giza 130	100.0	92.5	89.85	88.15	92.6^{cde}	18.73	15.57	15.63	12.00	15.48^{ghi}	11.60	4.60	6.73	7.33	7.57^{cd}
Line 1	100.0	100.0	100.0	60.0	90^{ef}	24.83	24.73	14.17	10.67	18.60^{abcdef}	12.50	8.73	5.83	3.77	7.71^{bcd}
Line 2	100.0	100.0	100.0	100.0	100^{ab}	26.33	19.53	12.00	5.67	15.88^{fghi}	8.83	6.03	4.50	3.90	5.82^{e}
Line 3	100.0	100.0	100.0	100.0	100^{ab}	25.80	22.33	18.10	10.97	19.30^{ab}	11.83	10.07	8.00	6.37	9.0^{7ab}
Line 4	100.0	100.0	100.0	100.0	100^{abc}	23.00	19.80	14.40	11.00	17.05^{defg}	13.70	5.43	6.40	4.77	7.58^{cd}
Line 5	100.0	100	100.0	100.0	100^{a}	22.80	23.50	18.73	10.30	18.83^{abcd}	9.53	8.50	7.73	5.17	7.73^{bcd}
Line 6	100.0	85.0	75.0	75.0	83.7^{gh}	27.17	22.50	14.87	11.03	18.89^{abcd}	13.67	8.50	7.83	5.60	8.90^{abc}
Line 7	100.0	100.0	100.0	100.0	100^{a}	26.10	24.40	13.97	12.50	19.24^{abc}	11.30	9.03	6.17	4.50	7.75^{bcd}
Line 8	100.0	100.0	100.0	80.0	95^{bcd}	26.00	26.00	14.33	12.83	19.79^{a}	13.73	6.70	5.83	4.50	7.69^{bcd}
Average (B)	95.72^{a}	91.64	91.20^{b}	82.15^{c}		23.24^{a}	20.06^{b}	14.12^{c}	9.60^{d}		13.18^{a}	7.76^{b}	6.25^{c}	5.14^{d}	
LSD at 0.05 (Salinity)	0.4152					0.880					0.5931				

*Mean values in the same column marked with the same letters are not significantly different at 0.05 level of probability.

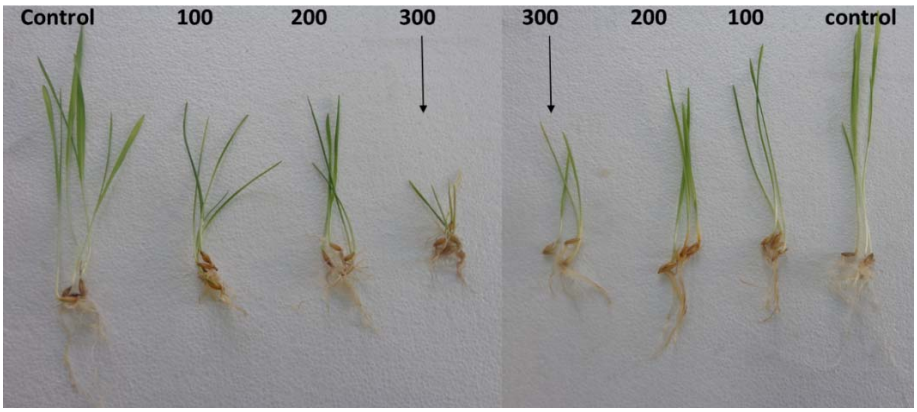

Figure 1. Example: response of barely cultivars and lines to different salinity levels

Finally, the overall of the cultivars and different salt levels for all the tested samples was 23.24 cm in control, 20.06 cm under 100 Mm salt, 14.12 under 200 Mm salt and the lowest value was 9.60 cm under the high salt level 300 Mm. In our study root length (cm) in all cultivars and lines showed considerable decrease with increasing salinity (Table 2). Increasing salt levels in germination % medium caused a marked inhibitory effect on root length of all barely cultivars and lines. The general mean of root length under control and salt level range from the highest value 13.18 cm in control to the lowest value 5.14 under 300 Mm salt. The root length reduction was ~50% under salt concentration comparing with tap water. The highest root length (cm) was recorded to Giza 130 by 7.33 cm, Giza 136 by 7 cm and the lowest value was 3.77 cm for line 1. High significant variations were observed between he controls and other salt levels with LSD=0.5932. The different in barely cultivars and lines response to high salt level as tolerant or susceptibility is observed in Figure (1).

These results indicate that early growth stage in barely is more responsive to salt stress particulenty in seedling length which is in accordance with Kingsbuvy and Gpstein (1984) pointing out that tolerance ability to salt stress can enhance with the age of wheat plant. Our results are compatible with data of Leonova *et al*. (2005), Iqbal *et al*. (2006) who found lesser reduction in plant length caused by salinity as a step toward adjustment. Also, inhibition of germination due to salinity has been reported by Megdiche *et al*. (2007), Abd El-Monem and Sharaf (2008), Afkari (2010), Khalid *et al.* (2010) and Mustafa *et al*. (2010).

B- Biochemical studies

Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The SDS polyacrylamide gel electrophoresis of the grain storage proteins in 22 barley cultivars and lines is shown in Figure 2. The distribution protein bands throughout the 22 barley cultivars, lines and their molecular weights are illustrated in Tables 3. Twenty protein bands were scored in the grains storage protein fraction, three out of them (170, 125 and 80 kDa) were common band and the other were polymorphic. The results based on protein pattern showed high significant variations between the barely cultivars and lines. This genetic diversity could be useful in breeding program for barely in Egypt. The cluster analysis of the twenty-two barely cultivars and lines divided to two main groups, A and B (Figure, 3) with genetic similarity 40%. The second group B includes two subgroups, B1 for Giza 126 and Giza127 (80%) similarity and B2 includes four barely cultivars i.e. Giza 126, 127 (100%); Giza 128 and 125 (100%). The similarity between these groups was 64%. On the other hand, for the first group A which includes two sub clustered A1 and A2 ((50%) showed that all the barely lines observed on separated cluster (A2) with 75% genetic similarity with different sub clustered ranged from 82 to 94%.

Results for A1 cluster divided to other sub-sub clustered A11 and A12 with 61% similarity and have the gullwing cultivars Giza 134 and 133 ((85%), Giza 124 (68%), Giza 132 and 130 (85%), Giza 123 and 135 (86%) and finally Giza 136 in separate cluster with 83%. The previous data showed that barely lines were observed in one cluster as showed in the morphological character under different salt concentrations (Table 2). Also, the high tolerance cultivars to salt stress were recorded in separate cluster (B) and the same results were observed in the morphological characteristics. The present results agree with Haddad et al. (2009) who obtained twenty-four protein bands from the SDS-PAGE of the water-soluble grain storage protein fraction. Their results showed that three out of them were common while the other 21 were polymorphic.

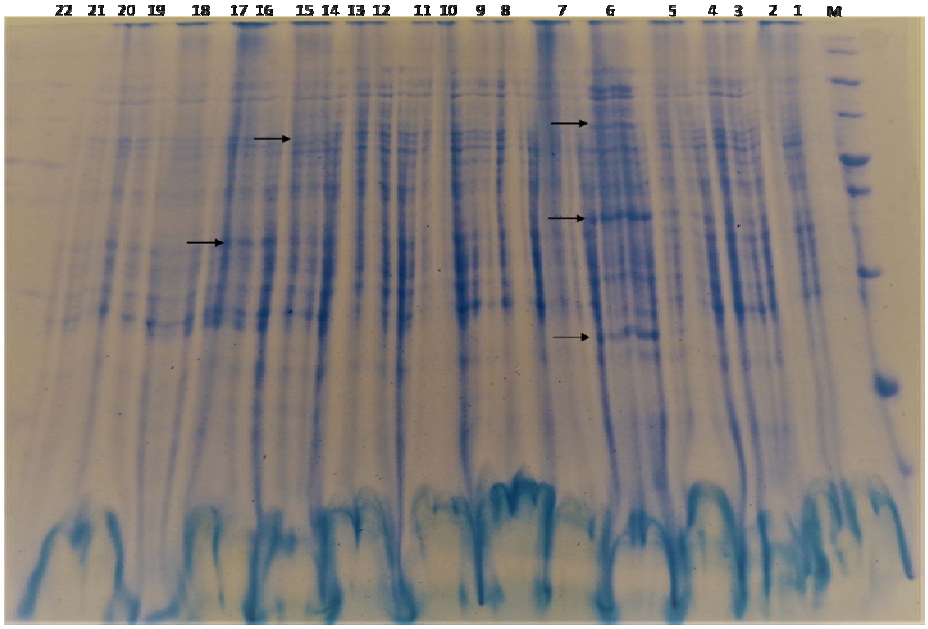

Figure 2. SDS–polyacrylamide gel electrophoresis pattern of barely cultivars and lines

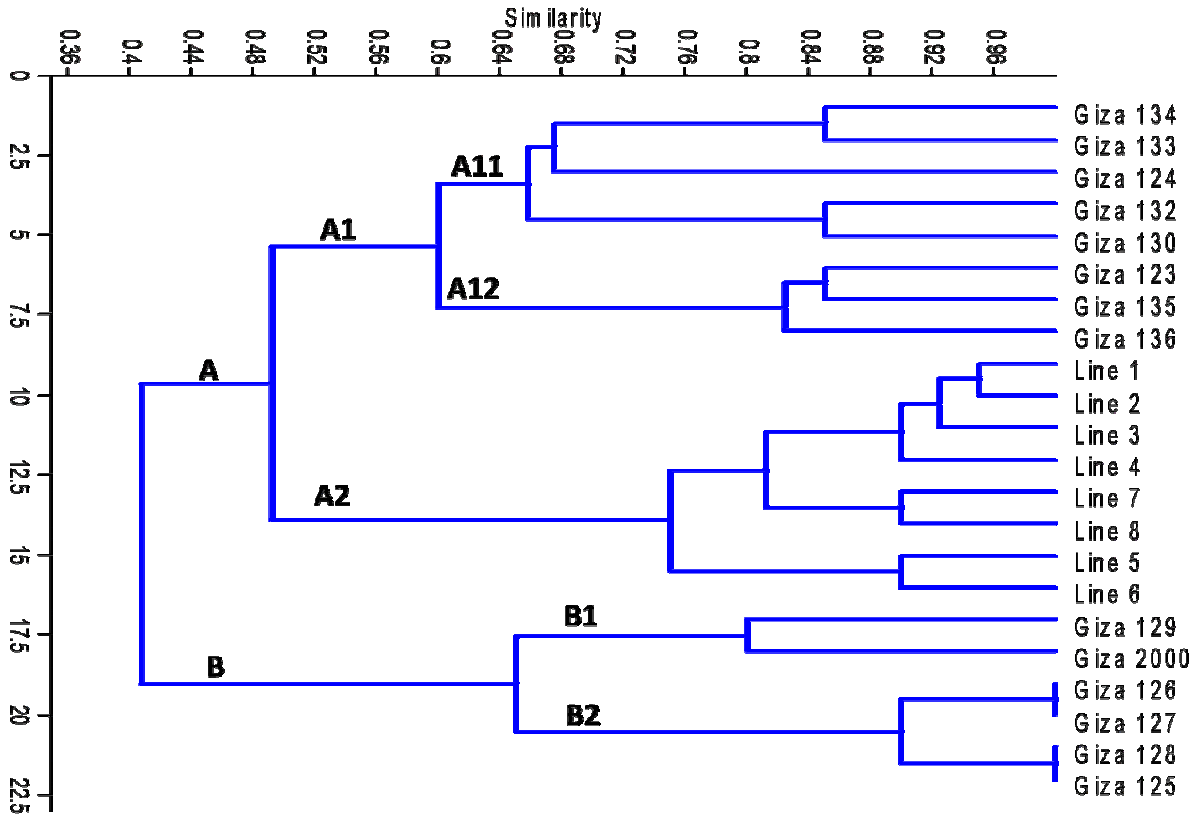

Figure 3. cluster analysis and genetic diversity of barely cultivars and lines based on protein pattern

The protein profile of the buffer soluble grain storage protein fraction showed 28 protein bands, four out of them were common and the other 24 were polymorphic. The genetic relationships of the seven Egyptian barley cultivars were investigated based on similarity of karyotypes and grain storage protein profiles using the NTSYS-pc2.

Grain storage protein electrophoresis are also a valuable evidence in cultivar identification and wild species phylogeny studies such as El Rabey et al. (2002), El Rabey (2004), El Rabey and Zayed (2005) and El Rabey (2008). Generally, twenty-four protein bands were obtained from the SD S-PAGE of the water-soluble grain storage protein fraction, three out of them were common while the other 21 were polymorphic. The 24 kDa and the 80 kD a band are characteristic to Line 3, and the 40.7 kDa band is characteristic to Giza 123. On the other hand, the protein profile of the buffer soluble grain storage protein fraction showed 28 protein bands, four out of them were common and the other 24 were polymorphic. The 27 kDa protein marker is characteristic to Line 35 cultivar.

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الملخص العربي

المعلمات المورفولوجية والبيوكيمائية للتنوع الوراثى وتحمل الملوحة فى بعض سلالات وانواع الشعير

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حاليا يوجد ما يقارب ٢٨٠٠ سلالة مختلفة من الشعير على مستوى العالم موجودة فى بنوك الجينات ومعظم هذة السلالات ناتجة من التهجين بين السلالات المنزرعة. أجريت هذة الدراسة بكلية الزراعة سابا باشا خلال العام ٢٠١٦ بغرض تقيم مدى التنوع الوراثى بين ٢٢ سلالة وصنف شعير منزرع فى مصر تحت ظروف الإجهاد الملحى معمليا. حيث زرعت جميع العينات فى المعمل فى أطباق بترى وبعد حدوث الانبات تم رى النباتات بتركيزات مختلفة من الملح هى ١٠٠- ٢٠٠- ٣٠٠ ملليموز ملح وكانت التجربة تصميم عشوائى تام. بعد اسبوعين من التعرض للملح تم قياس عدد من الصفات المورفولوجية وهى نسبة الانبات وطول البادرة وطول الجذر. هذا بالاضافة الى انة تم تحليل البروتين الكلى للبذور تحت درجات الملح المختلفة باستخدام بوكى اكراميد جيل لبيان اثر الملح على البادرات النامية. تم تقدير العلاقة الوراثية بين الاصناف والسلالات المستخدمة إعتمادا على الصفات المورفولوجية والبيوكيمائية حيث أوضحت النتائج ان هناك اختلاف معنوى ملحوظ لبعض الاصناف تحت درجات الملح المختلفة واظهر الصنف رقم ٥ اعلى مقاومة للاجهاد الملحى مقارنة بباقى السلالات والاصناف تحت الدراسة.