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GENETIC RELATIONSHIPS OF SOME BARLEY CULTIVARS BASED ON PHENOTYPIC, SEED QUALITY AND MOLECULAR ANALYSES

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ABSTRACT

The genetic diversity and relationships among ten barely cultivars were evaluated using morphological traits, seed quality, protein banding patterns and random amplified polymorphic DNA (RAPD) markers. Eight morphological traits (plant height, spike length, No. of spikes/plant, No. of grains / spike, 100 kernel weight, biological yield, grain yield /plant and harvest index %) and seed quality traits (germination %, seed vigor index, accelerated ageing, electrical conductivity, seedling characters and seedling vigor index) were analyzed in a two- winter seasons experiments: 2008-2009 and 2009-2010 with 10 barely cultivars. This study showed that barley cultivars differed significantly with respect to the studied attributes. Giza 123 gave the highest value for seven characters namely i.e., plant height, spike length, number of spikes/ plant, number of grains/ spike, 100- kernel weight, grain yield, and HI%. Giza 2000 gave the highest value for five characters i.e., plant height, spike length, number of grains/ spike 100-kernel weight, and HI%, while Giza 126) gave the highest value for four characters i.e., spike length, number of spikes/ plant ,number of grains/ spike, and HI%. Meanwhile, Giza 124 gave the highest value for three characters i.e., spike length, number of grains/ spike and HI%. Whereas Giza 127 gave the highest value for two characters i.e., biological yield and grain yield. Meanwhile Giza 132 gave the highest value in HI% only. Giza 118, Giza 124, Giza 126 and Giza 132 recorded the highest values for five characters, namely germination %, coleoptiles length, radical length, seed vigor index and seedling vigor index in both seasons. Giza 124 gave the highest value of accelerated ageing % in both seasons and gave the lowest value of electrical conductivity which indicated that Giza 124 is tolerant to heat and high vigor but Giza 129 gave the highest value of electrical conductivity which indicated that this genotype has the lowest vigor and lowest germination. Thus, these varieties may be useful for plant breeder. The patterns of seed protein studied by SDS-PAGE indicated that each genotype was characterized by proteins with specific molecular weight. Significant genetic variability among the cultivars was obtained at both the morphological and molecular level. RAPD primers that were used for the analysis enabled the estimation of DNA polymorphism of the cultivars. Similarity index estimated among the ten barely cultivars based on morphological and protein banding patterns indicated that Giza 117 and Giza 118 are the closest cultivars. Dendrograms based on morphological traits, protein banding patterns and RAPD and also the combined analysis across the protein and RAPD-PCR indicated that Giza 117 and Giza 118 are located in the same group.

Key Words: ***Barley, Fingerprint, Protein electrophoresis, RAPD, Morphological attributes.***

INTRODUCTION

Barley (*Hordeum vulgare* L.) is the world's fourth most important crop after wheat, maize and rice. It is the most widely grown cereal crop in regions which are not suitable for growing other crops (Bidinger *et al* 1977). In Egypt, barley is one of the most suitable winter cereal crops growing mainly under rain fed conditions with limited water supply, or in soils suffering from salinity (Abd-Alla *et al* 2007). The new varieties exhibit good performance under different environmental stresses, i.e. salinity, alkalinlty, water shortage and poor soil fertility.and showd high yielding ability under the environmental conditions prevailing within the target regions. But local varieties are susceptible to the main diseases and lodging, Mansor *et al* (1975) found that G.117 and G.118 were sensitive to diseases and moderate loading than G.119, El -Sayed *et al* (1992) found that G.119 was the lowest grain yield than the new barley cultivars under rain fed conditions. Meanwhile, Megahed and Gaafer (2003) reported that G.118 and G.123 were highly tolerant under soil salinity. The new varieties were resistant to the main barley diseases and out yielded the commercial. Several authors, such as Ahmed (1986), Assad (1989) and Abd El-Rahman (2003), reported that G.123 was tolerant under soil salinity and new reclaimed land area, while G.124 tolerant to heat stresses (Abo- El-Enin *et al* 1998). On the other hand, El-Sayed *et al* (1992) found that G.126 was superior under water deficient conditions, meanwhile El-Sayed *et al* (1995) studied the new two rowed cultivar G.127 that characterized by short growing season, high grain quality and malting industry. Moreover, El-Sayed *et al* (2002) suggested using G129 hull-less barley variety for human consumption because it has high content of soluble dietary fibers and B-glucan as compared to other cereals. The new varieties G.132 and G.2000 have good performance characters under the adverse conditions and reclaimed land (Ahmed *et al* 2007).

The germination test aimed at determining the maximum germination potential of a seed lot, which in turn can then be used to compare the quality of different lots and estimate the field planting value (ISTA 1993).

Seedling measurements include seedling growth test as determined by measuring the linear growth and seedling dry weight, which has been used successfully on barley and wheat (Perry 1977). Also seedling dry weight is closely related to seed vigor (Edlje and Burris 1970). The electrical conductivity test measures electrolyte leakage of the seeds (Hibbard and Miller 1982) or from a single seed (Hepburn *et al* 1984).

The introduction of polyacrylamide gel electrophoresis (PAGE) technique was developed first by Davis (1964) and Ornstein (1964). The use of SDS-PAGE and its theoretical background of molecular measurements

have been discussed by Andrews (1986). The SDS-PAGE protein was found to be a useful technique in cultivar identification (Abdel-Tawab *et al* 1993).

Currently, the technique of the RAPD markers (Randomly Amplified Polymorpliic DNA) based on the polymerase chain reaction (PCR) was proposed by Williams *et al* (1990) to amplify DNA sequences with a single short (9-10 bp) primer of arbitrary nucleotide sequence. It requires small amounts of DNA, easy to perform and reveals dominant molecular markers of ultimate potentialities in several fields including systematics and evolution (Witkus *et al* 1994), gene mapping (Komatsuda *et al* 1997) and genetic diversity (Baum *et al* 1997 and Mohamed 2004). RAPD markers have been used to determine genetic relationships in several plant species; examples include flax (Fu *et al* 2001 and Diedericlisen and Fu 2001), sorghum (Dahlberg *et al.* 2002), and date palm (Soliman *et al* 2003). The genetic diversity in barley and the relationship of barley cultivars have been addressed by using biochemical evidence derived by the electrophoretic separation of the seed protein hordein (Faccioli *et al* 1999) and different isozymes (Volis *et al* 2001). Evidence obtained from electrophoretic separation of the seed protein hordein was also combined with morphological criteria to investigate genetic diversity in 49 accessions of naked barley (Atanassov *et al* 2001). Morphological criteria were also combined with the DNA fingerprinting methodology AFLP (Amplified fragment length polymorphism) to illustrate the genetic relationships of 30 cultivars of spring two-rowed and six-rowed barley from Europe and America (Schut *et al* 1997) and to finger print 15 cultivars of Egyptian barley (El-Rabey *et al* 2002).

RAPD markers have been widely used to study genetic diversity in barley in combination with other molecular markers (Russell *et al* 1997 and Fernandez *et al* 2002). Examples of the use of RAPD markers to study genetic aspects of barley also include the study of Baurn *et al* (1997) on the genetic diversity among wild barley (*Hordeum spontaneum*) in the near east and the investigation by Hang *et al* (2000) on 16 cultivars of two-rowed and six-rowed barley in North America. Similar studies utilizing RAPD markers have been carried out on barley from Syria (Showman *el al* 2001), the spring barley in Europe (Kuczynska *et al* 2001) and the hull-less barley from the Tibet region (Yu *et al* 2002).

Concerning to RAPD analysis, Williams *et al* (1990) was embraced in different laboratories, especially those in the developing countries, due to its low cost compared to other DNA-based techniques, such as amplified fragment length polymorphism (AFLP) Vos *et al* (1995) and simple sequence repeats (SSR) Bruford and Wayne (1993). Besides, RAPD protocol is fairly simple, while protocols like AFLP and SSR are technically demanding (Karp *et al* 1997). The aim of the present study is to characterize

some barley cultivars and determine their relationships, based on morphological criteria, protein electrophoresis and RAPD-PCR markers.

MATERIALS AND METHODS

This study was carried out in the laboratories of the Department of Cell Res., Field Crops Res. Institute and the Department of Seed Technology Research. Field experiments were carried out at the experimental farm of Barley Res. Dept., Field Crops Res. Institute, at Bahteem Experimental Station, ARC, Egypt. Ten Egyptian barley cultivars were used as shown in Table 1.

Field experiments

Ten barley cultivars (Table 1) were grown up to the seed maturity stage in a randomized complete block design with three replications during the two successive winter seasons i.e. 2008/2009 and 2009/2010. The following traits were measured; plant height (cm), spike length (cm), No. of spikes/plant, No. of grains / spike, 100 kernel weight (g), biological yield (g), grain yield /plant (g) and harvest index (%). The data of the two seasons were statistically analyzed by MStatC computer program as separate seasons and combined analysis across seasons and the differences among means were compared using LSD at 5% according to Snedecor and Cochran (1981).

Table 1. Name, Pedigree and type of the ten cultivars used this study

Cultivar	Pedigree	Type	Characteristics
Giza 117	Baladi 16 / Palestine 10 (G.I.11191)	Six rowed	Local varieties (Commercial)
Giza 118	Beecher (Introduced from USA)	Six rowed	
Giza 119	Baladi 16/Gem (G.I. 7243)	Six rowed	
Giza 123	Giza 117/ FAO 86	Six rowed	Salt tolerant
Giza 124	Giza 117/Bahteem 52// Giza 118/FAO 86	Six rowed	Heat tolerant
Giza 126	Baladi Bahteem/S D729-Por12762 BC	Six rowed	Drought tolerant
Giza 127	W12291/Bags//Harmal-02	Two rowed	Malting industry
Giza 129	Deir Alla 106/Cel//AS 46/Aths*2	Six rowed	Hull-less barley
Giza 132	Rihane-05//AS 46/Aths*2Athe/Lignee 686	Six rowed	New variety
Giza 2000	Giza 117/Bahteem 52//Giza 118/ FAO 86 / 3/ Baladi 16/Gem.	Six rowed	Drought tolerant

Laboratory experiments

Germination test

The germination test is ordinarily performed pure on the seed of the crop kinds that constitute five percent (or more) of the sample after all inert matter and other crops and weed seeds were removed.

a- **Standard germination:** 50 pure seeds of each cultivar and three replications were placed in Petri dishes containing filter paper soaked with distilled water. The Petri dishes were placed in an incubator at 25± 1°C for 7 days. Normal seedlings were counted according to the international rules of ISTA (1993). Germination percentage was calculated using the following formula outlined by Krishnasamy and Seshu (1990).

$$\text{Germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Number of tested seed}} \times 100$$

b- **Seed vigor index** was calculated using the following formula (Copeland 1976)

$$\text{Seed vigor index} = \frac{\text{Number of seeds germinated (1}^{st}\text{ count)}}{\text{Number of days to first count}} + \frac{\text{Number of seeds germinated (last count)}}{\text{Number of days to last count}}$$

c- **Accelerated ageing germination %:** The accelerated ageing test is useful in determining the storage potential of seed lots. High temperature (approximately 42°C) and a relative humidity close to 100 % is used on a sample of any particular seed lot to obtain a pre-view of what the germination of that lot may be if it is put into storage for six months, one year or longer. The seeds were kept in an ageing chamber at 45°C and 100 % relative humidity for 3 days. After ageing, the seeds were sun dried. Seed survival percentage was determined by the standard germination test at 20 °C and the mean normal seedling percentage was calculated Association of Official Seed Analysis (AOSA 1983).

d- **Electrical conductivity test:** The conductivity test provides a measurement of electrolyte leakage from plant tissues. Seed lots having high electrolyte leakage are classified as low vigor, while those having low electrolyte leakage are considered as high vigor. The electrical conductivity of the leachate was determined according to procedures described by AOSA (1983). Four sub- samples of 50 seeds of each cultivar were weighed and placed into plastic cups with 250 ml of distilled water, and held at 20°C. After 24 h, the electrical conductivity of

the leachates was determined using EC meter. The mean values were expressed in μS $cm^{-1}g^{-1}$ seed weight.

$$\mu S\,cm^{-1}g^{-1} = \frac{\text{Conductivity for each flask } (\mu s)}{\text{Weight of seed sample (g)}}$$

e- **Seedling characters:** Normal seedlings obtained from standard germination test were used for seedling evaluation according to the rules of the Association of Official Seed Analyst (AOSA 1983). Seedling shoot and root length were measured after 7 days of germination test. Twenty-five seedlings from each Petri dish were randomly selected and shoot and root lengths of individual seedling were recorded. The shoot and root were also dried at 70 °C for 72 h, then the dry weight of seedling was determined. In addition seedling growth rate was calculated.

f. Seedling vigor index was calculated using data recorded on germination percentage and seedling growth according to International Seed Testing Association (ISTA 1985) by the following formula:

Seedling vigor index = seedling length (cm) X germination percentage

Electrophoresis of total proteins

Soluble proteins were extracted from seeds and SDS-PAGE was conducted according to the protocol described by Laemmli (1970). After the protein has been established, it was stained, photographed and compared with the patterns of known proteins (Protein ladder).

RAPD-PCR Analysis

a. Genomic DNA extraction

Young and fresh leaf samples were collected separately from 10 plants for each cultivar. All selected leaves were normal and free from any pathogenic symptoms. Leaf samples were saved in ice box and quickly transported to laboratory. Plant tissues were ground under liquid nitrogen to a fine powder, then bulked DNA extraction was performed using DNeasy plant Mini Kit (QIAGEN).

b. Polymerase chain reaction (PCR)

PCR amplification was performed using five random 10 mer arbitrary primers (Table 2) with the following sequences. All primers were used for PCR following the protocol of Williams *et al* (1990).

Table 2. Random primer names and their nucleotide sequences for RAPD-analysis

No	Name	Sequence
1	A12	5`-TCGGCGATAG-3`
2	A13	5`-CAGCACCCAC-3`
3	A14	5`-TCTGTGCTGG-3`
4	A15	5`-TTCCGAACCC-3`
5	A18	5`-AGGTGACCGT-3`

Amplification was conducted in 25 µl reaction volume containing the following reagents: 2.5 µl of dNTPs (2.5 mM), 2.5 µl $Mgcl_2$ (2.5 mM), and 2.5 µl of 10 x buffer, 3.0 µl of primer (10 pmol), 3.0 µl of template DNA (25 ng / µl), 1 µl of *Taq* polymerase (1U/ µl) and 10.5 µl of sterile dd H_2O. The DNA amplifications were performed in an automated thermal cycle (model Techno 512) programmed for one cycle at 94° C for 4 min followed by 45 cycles of 1 min at 94° C, 1 min at 36° C, and 2 min at 72° C. the reaction was finally stored at 72° C for 10 min. Amplified products were size-fractioned using ladder marker (100bp) Fermentas CO, by electrophoresis in 1.5 % agarose gels in TBE buffer at 120 V for 1 h. the bands were visualized by ethidium bromide under UV florescence and photographed.

Genetic relationships

Similarity indices were calculated and the consensus tree was developed based on the morphological traits, protein and RAPD banding patterns of the ten selected barley cultivars and the combined analysis across protein and RAPD analyses using SPSS statistical analysis program (version 10) to study the genetic relationships among them.

RESULTS AND DISCUSSION

Performance of the varieties

Ten local barley varieties were used in these investigations that possess different attributes to adapt the prevailing environmental conditions and have high yielding potential

The results of the performance of the ten barley varieties for different characters are presented in Table (3) showed that means of yield and yield components in both years were significant by different for all studied characters.

Table 3. Mean performance for yield and yield components of 10 barley cultivars during 2008/2009 and 2009 / 2010 and combined across seasons.

Genotypes	Plant height (cm)			Spike Length(cm)			No. of spikes/plant			No. of grain / spike		
	S1	S 2	Combined	S1	S 2	Combined	S1	S 2	Combined	S1	S 2	Combined
Giza 117	75.00	73.33	74.17	6.00	5.00	5.50	27.00	24.00	25.50	32.67	26.00	29.33
Giza 118	88.33	81.67	85.00	6.33	8.00	7.17	30.00	29.00	29.50	30.67	26.67	28.67
Giza 119	90.00	86.67	88.33	8.00	7.33	7.67	32.33	32.67	32.50	29.33	26.67	28.00
Giza 123	100.00	93.33	96.67	10.00	9.33	9.67	48.00	37.00	42.50	44.00	41.00	42.50
Giza 124	90.00	90.00	90.00	8.00	9.00	8.50	38.00	32.67	35.33	38.00	35.00	36.50
Giza 126	93.33	90.00	91.67	9.00	8.00	8.50	41.67	37.67	39.67	38.67	36.67	37.67
Giza 127	88.33	83.33	85.83	8.33	8.00	8.17	30.33	25.33	27.83	31.33	29.33	30.33
Giza 129	88.33	86.67	87.50	7.33	7.33	7.33	37.33	32.00	34.67	32.67	30.33	31.50
Giza 132	80.00	78.33	79.17	8.00	7.67	7.33	40.67	32.67	36.67	36.00	32.00	34.00
Giza 2000	100.00	90.00	95.00	9.00	8.33	8.67	40.67	32.00	36.33	41.33	32.00	36.67
Mean	89.33	85.33	87.33	8.100	7.700	7.900	36.60	31.50	34.05	35.47	31.57	33.52
L.S.D at 0.01												
Genotype (G)			3. 94			0.29			1. 24			1.10
Year (Y)			4. 60			0.56			2. 78			2. 44
G X Y			6.50			0.92			3. 93			3. 45
C.V.%			13.36			15.29			15.22			10.65

Table 3. Continued

Genotypes	100 Kernel weight (g)			Biological yield (g)			Grain yield /plant (g)			Harvest index (%)		
	S1	S 2	Combined	S1	S 2	Combined	S1	S 2	Combined	S1	S 2	Combined
Giza 117	3.90	3.50	3.90	140.00	120.00	130.0	32.00	25.00	28.50	23	21	22
Giza 118	3.67	3.23	3.45	150.00	126.67	138.33	31.67	24.00	27.83	21	18	20
Giza 119	3.70	3.27	3.83	133.33	123.33	128.33	33.33	30.00	31.67	25	24	25
Giza 123	4.60	3.80	4.20	113.33	110.00	111.67	36.00	32.67	34.33	32	30	31
Giza 124	4.20	3.33	3.79	126.67	123.33	125.00	36.67	31.33	33.00	27	25	26
Giza 126	4.2	3.67	3.93	123.33	143.33	118.33	35.00	28.67	31.83	28	25	27
Giza 127	3.37	3.00	3.18	143.33	130.00	136.67	36.67	32.67	34.67	26	25	26
Giza 129	3.87	3.57	3.72	133.33	113.33	123.33	36.67	29.00	32.83	28	25	27
Giza 132	4.13	3.53	3.83	126.67	116.67	121.67	36.33	30.67	33.50	29	26	28
Giza 2000	4.17	3.83	4.00	123.33	113.33	118.33	35.00	30.00	32.50	28	26	27
Mean	3.98	3.47	3.73	131.33	119.00	125.17	34.73	29.40	32.07	27	25	26
L.S.D at 0.01												
Genotype (G)			0.021			3.50			2.12			0.009
Year (Y)			0.047			7.82			0.95			0.004
G X Y			0.066			11.06			3.02			0.013
C.V.%			13.36			10.99			10.22			9.30

Table 4. Pairwise similarity matrix among the ten barley cultivars based on morphological traits

Cultivar	1	2	3	4	5	6	7	8	9
2	1.00								
3	0.90	0.91							
4	0.87	0.87	0.85						
5	0.79	0.78	0.82	0.85					
6	0.79	0.78	0.82	0.84	1.00				
7	0.79	0.79	0.81	0.84	1.00	1.00			
8	0.78	0.78	0.82	0.84	1.00	1.00	1.00		
9	0.77	0.78	0.73	0.74	0.84	0.84	0.84	0.83	
10	0.72	0.72	0.76	0.75	0.85	0.83	0.87	0.87	0.79

Where: 1= Giza 117, 2= Giza 118, 3= Giza 119, 4= Giza 123, 5= Giza 124, 6= Giza 126, 7= Giza 127, 8= Giza 129, 9= Giza 132, 10= Giza 2000

129, between Giza 126 and each of Giza 127 and Giza 129 and between Giza 127 and Giza 129).

Based on morphological traits cluster analysis according to UPGMA was used to construct a dendrogram (Fig. 1) showing GD interrelationships among the cultivars. From Fig. 1, we can see two major clusters. The first cluster consists of three cultivars: Giza 119 alone in one subcluster, while Giza 123 and 124 in the other subcluster.

The remaining seven cultivars are grouped into the second cluster, which is further divided into two sub-clusters, one of them including Giza 117 and Giza 126, whereas the remaining cultivars are grouped in the other subcluster, which is devided into two sub-sub clusters, one of them included Giza 129 alone, as shown in Fig(1).

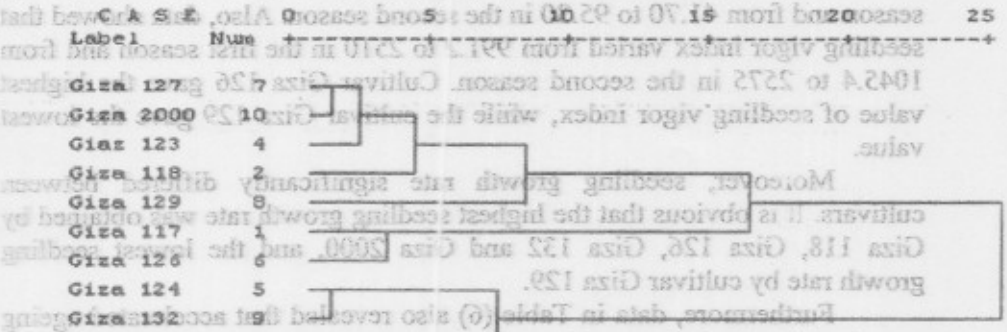

Fig. 1. Dendrogram illustrating genetic relationship among ten barley cultivars generated by UPGMA cluster analysis based on morphological traits of the two seasons (combined analysis).

Standard germination and seedling growth

The standard germination percentage presented in Table (5) indicated that it ranged from 48 to100% in the first season and 49 to100% in the second season. The highest value of standard germination percentage was obtained from cultivars Giza 118, Giza 124, Giza 126 and Giza 132, while the lowest value appeared in cultivars Giza 119 Giza 129. In general, the germination test values were less than 90% in eight cultivars, indicating that the quality of seed lots were not subjected, to deterioration and the performance was not impaired (Hampton and Coolbear 1990). Also, data in Table (5) showed that coleoptile length ranged from 11.40 to14.47 cm in the first season and from 11.33 to14.67 cm in the second season. Cultivar Giza 126 gave the highest value in coleoptiles' length, but cultivar Giza 127 gave the lowest value in both seasons.

Radical length ranged from 8.40 to11.00 cm in the first season and from 9.40 to 11.10 in the second season.

Seedling fresh weight ranged from 0.270 to 0.380 g in the first season and from 0.250 to 0.370 g in the second seasons. Cultivars Giza 118, Giza 132 and, Giza 2000 gives the highest values of seedling fresh weight, while cultivars Giza 119 and Giza 129 gives the lowest value for seedling fresh weight in both seasons.

Seedling dry weight was influenced by barley cultivars in both seasons. Cultivar Giza 123 gave the highest seedling dry weight. The lowest seedling dry weight was obtained by cultivars Giza 119 and Giza 129 in both seasons. Therefore, cultivars which contained high germination and high seedling fresh and dry weights are considered to have greater vigor than others (Abd-Alla *et al* 2007).

Seed vigor index (Table 6) ranged from 41.07 to 94.57 in the first season and from 41.70 to 95.30 in the second season. Also, data showed that seedling vigor index varied from 991.2 to 2510 in the first season and from 1045.4 to 2575 in the second season. Cultivar Giza 126 gave the highest value of seedling vigor index, while the cultivar Giza 129 gave the lowest value.

Moreover, seedling growth rate significantly differed between cultivars. It is obvious that the highest seedling growth rate was obtained by Giza 118, Giza 126, Giza 132 and Giza 2000, and the lowest seedling growth rate by cultivar Giza 129.

Furthermore, data in Table (6) also revealed that accelerated ageing was significantly influenced by cultivars in both seasons. Accelerated aging ranged from 4.67 to 45.67% in the first season and from 7.00 to 44.00% in the second seasons. The highest value of accelerated ageing was obtained from cultivar Giza 124 while cultivars Giza 119, Giza 127 and Giza 129 gave the lowest values.

Table 5. Germination percentage, cleoptile length, radical length, seedling fresh weight and seedling dry weight of 10 barley cultivars during 2008/2009 and 2009/2010 and combined across seasons .

Varieties	Germination %			Cleoptile length (cm)			Radical length (cm)			Seedling fresh weight (g)			Seedling dry weight (g)		
	S1	S 2	Combined	S1	S 2	Combined	S1	S 2	Combined	S1	S 2	Combined	S1	S 2	Combined
Giza 117	94.0	97.0	95.5	12.5	12.17	12.4	9.3	10.0	9. 7	0.360	0.322	0.340	0.037	0.034	0.036
Giza 118	100.0	99.0	99.5	13.2	14.37	13.8	10.1	9.9	10.0	0.340	0.330	0.330	0.035	0.038	0.037
Giza 119	61.0	59.0	60.0	11.8	11.80	11.8	10.3	9.7	10.0	0.360	0.320	0.340	0.023	0.024	0.023
Giza 123	93.0	91.0	92.0	13.3	13.60	13.5	8.7	9.4	9.0	0.3800	0.350	0.370	0.037	0.039	0.038
Giza 124	100.0	100.000	100.0	11.9	13.33	12.6	10.1	9.5	9.8	0.360	0.360	0.360	0.035	0.035	0 035
Giza 126	100.0	100.00	100.0	14.5	14.67	14.5	10.7	10.8	10.7	0.320	0.250	0.290	0.036	0.037	0.036
Giza 127	97.0	97.0	97.0	11.4	11.33	11.4	10.2	10.5	10.4	0.350	0.330	0.340	0.032	0.031	0.032
Giza 129	48.0	49.0	48.5	12.3	12.33	12.3	8.4	9.6	9.0	0.270	0.250	0.260	0.022	0.023	0.022
Giza 132	98.0	100.0	99.0	13.7	13.83	13.8	10.0	11.1	10.6	0.330	0.370	0.350	0.037	0.038	0.037
Giza	98.0	99.0	98.5	12.2	13.00	12.6	11.0	10.7	10.8	0.320	0.360	0.340	0.035	0.035	0.035
Mean	88.9	89.1	89.0	12.7	13.04	12.9	9.9	10.1	10.0	0.339	0.324	0.332	0.033	0.033	0.033
L. S.D at															
Genotype			2.42			0.49			0.33			0.19			0.002
Year (Y)			1.08			0.22			0.15			0.008			0.001
G X Y			3.43			0.69			0.47			0.027			0.003
C.V. %			1.74			2.44			2.14			3.73			4.24

Table 6. Seed vigor index, seedling characters and seed vigor of 10 barley cultivars during 2008-2009 and 2009-2010 and combined across seasons.

Varieties	Seed vigor index			Seedling vigor index			Seedling growth rate			Accelerated aging			Electrical conductivity		
	S1	S 2	Combined	S1	S 2	Combined	S1	S 2	Combined	S1	S 2	Combined	S1	S 2	Combined
Giza 117	70.2	72.2	71.2	2049.4	1984.6	2017.0	3.13	3.17	3.15	37. 7	34.3	36.0	62.2	61.9	62.1
Giza 118	94.6	92.3	93.4	2322.5	2399.2	2360.9	3.33	3.47	3.40	30.0	31.0	30.5	42.0	42.3	42.3
Giza 119	46.8	42.3	44.6	1339.5	1045.4	1192.5	3.20	3.07	3.13	6.0	13.7	9.8	73..7	72.3	73.0
Giza 123	81.9	82.7	82.2	2032.3	2086.7	2059.5	3.17	3.30	3.23	20.0	23.7	21.8	38.7	41.1	39.9
Giza 124	94.6	94.6	94.6	2197.7	2272.7	2235.2	3.17	3.27	3.22	45.7	44.0	44.8	33.3	35.0	34.2
Giza 126	94.6	94.6	94.6	2510.0	2575.0	2542.5	3.57	3.53	3.55	28.7	30.0	29.3	46.1	36.9	41.5
Giza 127	91.1	94.3	93.2	2090.7	2114.5	2192.6	3.10	3.10	3.10	12.0	15.0	13.5	44.1	32.3	38.2
Giza 129	41.1	41.7	41.4	991.2	1286.7	1138.9	2.97	3.10	3.03	4.0	7.6	5.8	75.2	78.0	76.6
Giza 132	93.3	95.3	94.3	2305.7	2460.0	2382.9	3.37	3.70	3.53	33.0	33.7	33.3	40.9	40.8	40.9
Giza	92.7	91.6	92.1	2258.9	2342.4	2300.7	3.30	3.37	3.33	25.7	27.0	26.3	38.9	37.1	38.0
Mean	80.1	80.2	80.2	2009.8	2056.7	2033.3	3.23	3.31	3.27	24.4	25.9	25.2	49.5	47.8	48.7
L.S.D at															
Genotype			2.79			100.56			0.077			1.13			1.68
Year (Y)			1.25			44.99			0.034			0.51			0.75
G X Y			3.90			142.22			0.109			1.60			2.37
C.V %			2.23			3.17			1.51			2-88			2.21

It was possible to identify the barley cultivars as following, seed of Giza 117, Giza 118 and Giza 119 contained proteins with molecular weights of 102.413 and 98.761 KDa while, the cultivar Giza 132 is characterized by proteins with molecular weights of 96.137 and 49.324 KDa, cultivar Giza 123 is characterized by proteins with molecular weights of 98.761 and 49.324 KDa (Table 7).

Table 7. Molecular weights (M.W) of soluble protein bands extracted from ten barley cultivars by SDS-PAGE.

No. of bands	M.W.	1	2	3	4	5	6	7	8	9	10
1	121.297	1	1	1	1	1	1	1	1	1	1
2	113.56	1	1	1	1	1	1	1	1	1	1
3	110.229	1	1	1	1	1	1	1	1	1	1
4	106.228	1	1	1	1	1	1	1	1	1	1
5	102.413	1	1	1	0	0	0	0	0	0	0
6	98.761	1	1	1	1	0	0	0	0	0	0
7	96.137	0	0	0	0	0	0	0	0	1	0
8	89.124	1	1	1	1	1	1	1	1	1	1
9	85.618	1	1	1	1	1	1	1	1	1	1
10	83.582	1	1	1	1	1	1	1	1	1	1
11	73.872	1	1	1	1	0	0	0	0	0	0
12	66.588	1	1	1	1	1	1	1	1	1	1
13	53.508	1	1	1	1	1	1	1	1	1	1
14	50.127	1	1	1	1	1	1	1	1	1	1
15	49.324	1	1	0	1	0	0	0	0	1	0
16	48.095	1	1	1	1	1	1	1	1	1	0
17	43.754	1	1	1	1	1	1	1	1	1	1
18	41.15	1	1	1	1	1	1	1	1	1	0
19	35.5	0	0	0	1	1	1	1	1	1	1
20	32.93	1	1	1	1	1	1	1	1	1	1
21	29.556	1	1	1	1	1	1	1	1	0	1
22	25.809	1	1	1	1	1	1	1	1	1	1
23	15.737	1	1	1	1	1	1	1	1	1	1
Total No. of bands		21	21	20	21	18	18	18	18	19	16

Where: 1= Giza 117, 2= Giza 118, 3= Giza 119, 4= Giza 123, 5= Giza 124
6= Giza 126, 7= Giza 127, 8= Giza 129, 9= Giza 132, 10= Giza 2000

The electrophoretic differences observed in this study should provide a supplemental means for cultivar identification (Fig. 2). These findings indicated clearly that protein electrophoretic analysis is an important tool for the identification of barley cultivars.

Genetic distances (GD) among the ten barely cultivars pairs based on protein banding pattern were expressed in the form of a matrix, as shown in Table (8) (pairwise similarity matrix). The values of GD among the cultivars ranged from 0.81 (between Giza 200 and each of Giza 117 and Giza 118) to 1.00 (between Giza 117 and Giza 118, between Giza 124 and each of Giza 126, Giza 127 and Giza 129, between Giza 126 and each of Giza 127 and Giza 129 and between Giza 127 and Giza 129).

Table 8. Similarity matrix among the ten barley cultivars based on protein banding pattern.

Cultivar	1	2	3	4	5	6	7	8	9
2	1.00								
3	0.98	0.98							
4	0.95	0.95	0.93						
5	0.87	0.87	0.90	0.92					
6	0.87	0.87	0.90	0.92	1.00				
7	0.87	0.87	0.90	0.92	1.00	1.00			
8	0.87	0.87	0.90	0.92	1.00	1.00	1.00		
9	0.85	0.85	0.82	0.90	0.92	0.92	0.92	0.92	
10	0.81	0.81	0.83	0.87	0.94	0.94	0.94	0.94	0.86

Where: 1= Giza 117, 2= Giza 118, 3= Giza 119, 4= Giza 123, 5= Giza 124
6= Giza 126, 7= Giza 127, 8= Giza 129, 9= Giza 132, 10= Giza 2000

Cluster analysis according to UPGMA based on protein banding patterns across of the two seasons for ten barely cultivars was used to construct a dendrogram (Figure 3) showing GD interrelationships among the cultivars. There were two major clusters. The first cluster is devided into two subclusters; the first included cultivar Giza 132, and the other culster is devided into two sub-sub cluster, one of them included Giza 2000, while the other sub-sub cluster included the remaining cultivars. The remaining four cultivars are devided into two sub-clusters, one of them included Giza 123 alone, while the other is devided again into two sub-sub clusters.

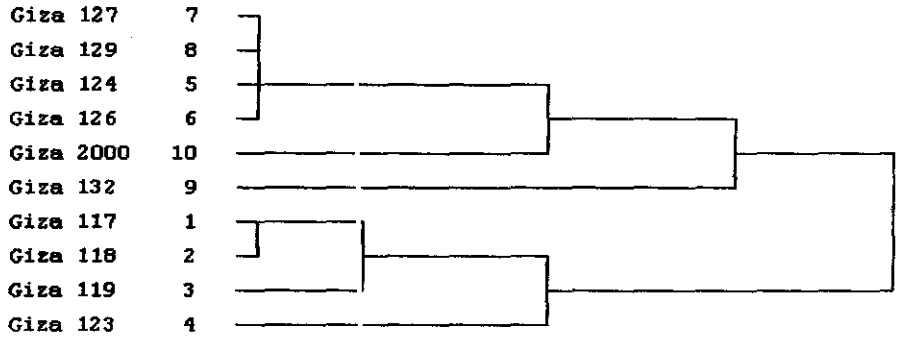

Fig. 3. Dendrogram of the genetic distances between ten barley cultivars based on protein banding patterns of the two seasons (combined analysis).

RAPD analysis

Five RAPD primers were used for DNA polymorphism assessment in ten barely cultivars. In the DNA polymorphism analysis, only clear and reproducible bands were used. Of the 48 amplified RAPD fragments, 33 were polymorphic(Table 9). The average number of polymorphic fragments per primer varied from 3 to 11, averaging 6.6, while the size of the fragments ranged between 0.5 and 12.0 kbp. The average percentage of polymorphism was calculated for each primer individually and ranged from 33.3 to 85.7% (Table 9).

Genetic distances (GD) among the ten barely cultivars pairs based on RAPD-PCR analysis were expressed in the form of a matrix, as shown in Table (10) (pairwise similarity matrix). The values of GD among the cultivars ranged from 0.67 (between Giza 118 and each of Giza 124 and Giza 132) to 0.96 (between Giza 123 and Giza 126) and to 0.94 (between Giza 117 and Giza 118).

Cluster analysis according to UPGMA based on RAPD-PCR analysis was used to construct a dendrogram for ten barely cultivars (Fig. 5) showing GD interrelationships among the cultivars. Looking at Figure (5), we can see that there are two major clusters. The first cluster included Giza 132 alone, while the remaining nine cultivars are grouped the second cluster, which is further divided into two sub-clusters, one of them including Giza 119 and Giza 124, whereas the remaining cultivars are grouped in the other subcluster, which is devided into two sub-sub clusters, one of them included Giza 117 and Giza 118, as shown in Fig. (5).

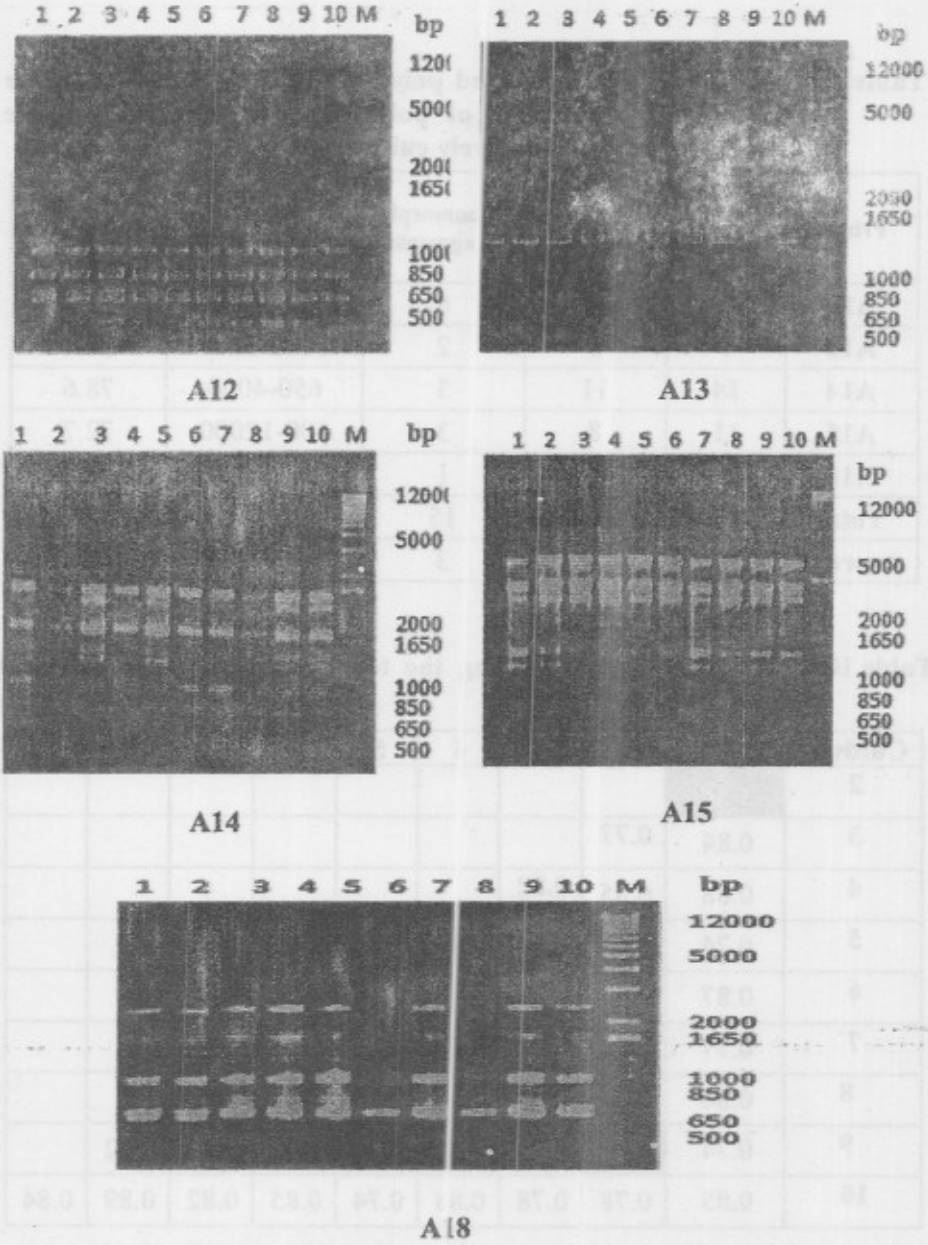

Fig. 4. RAPD amplification products generated by primer A12, A13, A14, A15 and A18. The order of samples: Lane M, 100 bp ladder; lane 1, Giza 117; lane 2, Giza 118; lane 3, Giza 119; lane 4, Giza 123; lane 5, Giza 124; lane 6, Giza 126; lane 7, Giza 127; lane 8, Giza 129; lane 9, Giza 132 and lane 10, Giza 2000

Table 9. Number of amplified and polymorphic fragments, their size range and percentage of polymorphism detected by five RAPD primers in 10 barely cultivars.

Primer	Amplified fragments	Polymorphic fragments	Monomorphic fragments	Fragment size range (bp)	Percentage of polymerrphism %
A12	9	3	6	500-1650	33.3
A13	7	5	2	1000-3200	71.4
A14	14	11	3	650-4000	78.6
A15	11	8	3	500-12000	72.7
A18	7	6	1	500-3000	85.7
Total	48	33	15		
Average	9.6	6.6	3		68.34

Table 10. Similarity matrix among the ten barley cultivars based on RAPD –PCR analysis.

Cultivar	1	2	3	4	5	6	7	8	9
2	0.94								
3	0.84	0.77							
4	0.88	0.85	0.82						
5	0.74	0.67	0.83	0.81					
6	0.87	0.84	0.81	0.86	0.87				
7	0.77	0.73	0.76	0.86	0.81	0.88			
8	0.80	0.77	0.74	0.83	0.76	0.83	0.83		
9	0.74	0.67	0.80	0.72	0.69	0.72	0.78	0.82	
10	0.85	0.78	0.78	0.88	0.74	0.85	0.82	0.89	0.84

Where: 1= Giza 117, 2= Giza 118, 3= Giza 119, 4= Giza 123, 5= Giza 124
6= Giza 126, 7= Giza 127, 8= Giza 129, 9= Giza 132, 10= Giza 2000

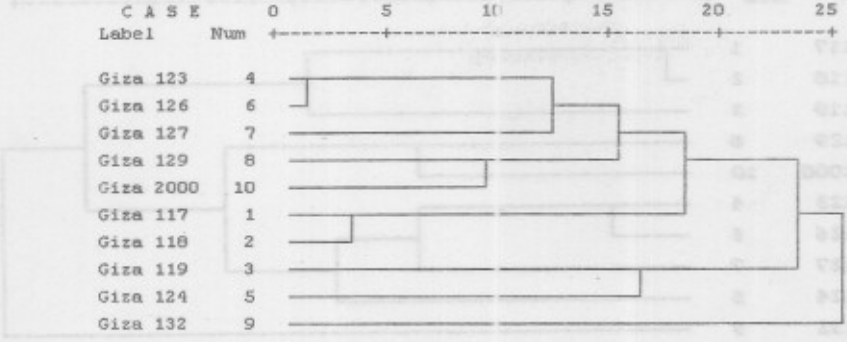

Fig. 5. Dendrogram of the genetic distances between ten barley cultivars based on RAPD-PCR.

The results of the similarity index and consensus tree of the combined data based on protein banding patterns and RAPD-PCR analysis for the ten barley cultivars (Table 11 and Fig. 6) showed that the lowest genetic similarity (0.74 and 0.75) was observed between Giza 118 and each of Giza 124 and Giza 2000, while the highest genetic similarity (0.96 and 0.95) was scored between Giza 117 and Giza 118 and between Giza 123 and Giza 126 T-9 and each of RT-11 and RT-6.

Based on the combined data across protein banding patterns and RAPD-PCR analysis, the consensus tree divided cultivars into two main clusters; the first included Giza 132. The other cluster was divided into two main sub-clusters; the first one included Giza 117, G 118 and Giza 119, while the second was divided into two main sub-sub-clusters, which consisted of the remaining barely cultivars. The genetic distance between Giza 117 and Giza 118 is very close

Table 11. Similarity matrix among the ten barley cultivars based on Protein and RAPD –PCR analysis (combined analysis).

Cultiv	1	2	3	4	5	6	7	8	9
2	0.96								
3	0.90	0.85							
4	0.91	0.89	0.86						
5	0.79	0.75	0.86	0.85					
6	0.87	0.85	0.84	0.91	0.91				
7	0.80	0.78	0.81	0.88	0.88	0.92			
8	0.83	0.80	0.79	0.87	0.80	0.88	0.89		
9	0.78	0.74	0.81	0.79	0.78	0.79	0.83	0.85	
10	0.84	0.79	0.80	0.88	0.81	0.78	0.86	0.90	0.85

Where: 1= Giza 117, 2= Giza 118, 3= Giza 119, 4= Giza 123, 5= Giza 124
6= Giza 126, 7= Giza 127, 8= Giza 129, 9= Giza 132, 10= Giza 2000

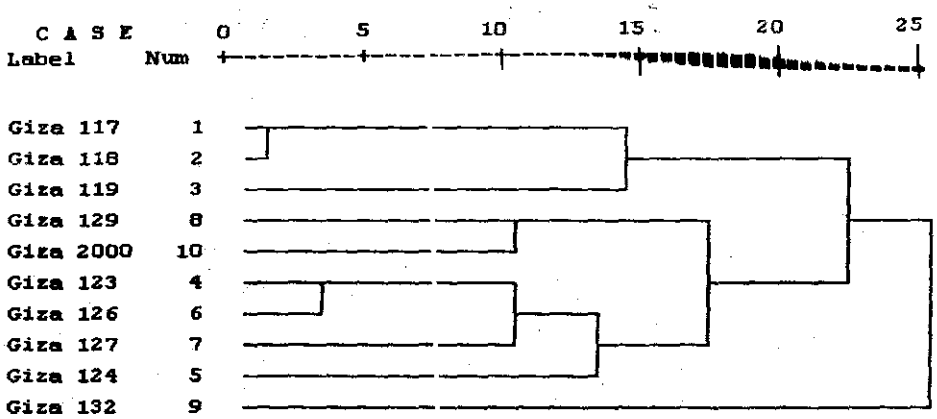

Fig. 6. Dendrogram of the genetic distances between ten barley cultivars based on protein and RAPD-PCR of the two seasons (combined analysis).

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العلاقات الوراثية لبعض أصناف الشعير على أساس الشكل المظهري وجودة البذور والتدليل الجزيني

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تم تقييم عشرة اصناف من الشعير ثلاثة اصناف تجارية قديمة و سبعة اصناف جديدة منها الصنف جيزة ١٢٧ ثنائى الصف وبقية الاصناف سداسية وهذه الاصناف الجديدة تتحمل الظروف البيئية غير الملائمة وذلك لتقييم درجة القرابة من حيث التركيب الوراثى بين الاصناف والاختلافات المورفولوجية ودراسة المحصول ومكوناته فى هذه الاصناف العشرة . وقد اقيمت تجربة حقلية فى موسمى ٢٠٠٨/٢٠٠٩، ٢٠٠٩/٢٠١٠ فى تصميم قطاعات كاملة العشوائية فى ثلاث مكررات وعند الحصاد تم اخذ القراءات على النباتات الفردية . وهى طول النبات (سم)، طول السنبلة (سم)، عدد السنابل لكل نبات فردى، عدد الحبوب لكل سنبلة، وزن مائة حبة (جم)، وزن المحصول البيولوجى (جم)،وزن محصول لحبوب (جم) و دليل الحصاد كنسبة مئوية. كذلك تم التقييم المعملى قبل الزراعه لتقدير النسبه المئويه للانبات القياسى وصفات البادرة ودرجة التوصيل الكهربى وكذلك اختبار الشيخوخة. ويمكن تلخيص النتائج فيما يلى: اظهرت الدراسة وجود معنوية عالية لتفوق الاصناف الجديدة على الاصناف التجارية القديمة فى كل الصفات تحت الدراسة، تفوق الصنف جيزة ١٢٣ فى سبع صفات وهى طول النبات وطول السنبلة وعدد السنابل وعدد حبوب السنبلة ووزن المائة حبة ووزن المحصول الكلى ودليل الحصاد، تفوق الصنف جيزة ١٢٦ فى خمس صفات وهى طول السنبلة وعدد السنابل وعدد الحبوب فى السنبلة ووزن المائة حبة ودليل الحصاد، تفوق الصنف جيزة ٢٠٠٠ فى اربع صفات وهى طول النبات وطول السنبلة ووزن المائة حبة ودليل الحصاد، تفوق الصنف جيزة ١٢٤ فى اربع صفات وهى طول السنبلة وعدد الحبوب فى السنبلة ومحصول الحبوب ودليل الحصاد، تفوق الصنف الثنائى جيزة ١٢٧ فى صفتين هما المحصول البيولوجى ومحصول الحبوب، تفوق الصنف جيزة ١٣٢ فى صفة واحدة فقط وهى دليل الحصاد، تفوقت الاصناف التجارية جيزة ١١٧ وجيزة ١١٨ فى صفة المحصول البيولوجى فقط، سجلت الأصناف جيزة ١١٨، جيزة ١٢٤، جيزة ١٢٦، جيزة ١٣٢ اعلى نسبة فى خمسة صفات وهى (نسبة الانبات، طول الريشة، طول الجذير، دليل حيوية البذرة، دليل حيوية البادرة) فى كلا الموسمين، سجل الصنف جيزة ١٢٤ اعلى نسبة انبات لاختبار الشيخوخة فى كلا الموسمين وكذلك اعطى اقل قراءة فى درجة التوصيل الكهربى مما يدل على ان هذا الصنف يتحمل الحراره وذو حيويه عالية. بينما سجل الصنف جيزة ١٢٩ اعلى قراءة فى درجة التوصيل الكهربى مما يدل على انخفاض حيوية هذا الصنف وبالتالى انخفاض نسبة الانبات، امكن تمييز مجموعة من البروتينات المختلفة فى البذور تحت الدراسة باستخدام التفريد الكهربى للبروتين فوجد ان هناك اختلافات فى الاوزان الجزيئية

لبروتينات. تم إجراء تحليل RAPD باستخدام خمسة بادئات تم اختيارهم وفقا لنتائج سابقة. تم الحصول على تباين وراثى معنوى بين الأصناف على المستويين المورفولوجى والجزيئي. تمكنت بادئات الـــ RAPD التي تم استخدامها من تقدير تعدد الأشكال للحمض النووي للأصناف. وتم تقدير دليل التشابه بين أصناف الشعير العشرة على المستوى المورفولوجي، وأنماط البروتين والتي أشارت إلى أن الصنف جيزة ١١٧ والصنف جيزة ١١٨ هي أكثر الأصناف تقاربا. تم مقارنة شجرة القرابة المرسومة على اساس نتائج الصفات المورفولوجية و المحصول ومكوناته، وانماط البروتين وايضا نتائج ال RAPD و النتائج المدمجة لكل ما سبق والتي أكدت أن الصنف جيزة ١١٧ والصنف جيزة ١١٨ تقعان فى في نفس المجموعة.