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Cytogenetical and Biochemical Characterization of Some Egyptian Barley (*Hordeum Vulgare* L.) Cultivars

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Abstract: Cytogenetical and biochemical investigations of seven Egyptian barley cultivars were performed using karyotype analysis and SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of both water soluble and water-non soluble seed storage proteins under reducing conditions. The karyotype analysis was performed according to feulgen squash technique, photographed, and then analyzed using video test karyotyping software. According to the caryotype analysis, the fifth chromosome pair in Line 2 cultivar is characterized by the presence of satellite on its short arms. Twenty-four protein bands were obtained from the SDS-PAGE of the water soluble seed storage protein fraction, three out of them were common while the other 21 were polymorphic. The protein profile of the buffer soluble seed storage protein fraction showed 28 protein bands, four out of them were common and the other 24 were polymorphic. The genetic relationships of the 7 Egyptian barley cultivars were investigated based on similarity of karyotypes and seed storage protein profiles using the NTSYS-pc2 (Numerical Taxonomy and Multivariate Analysis System). The two resulting dendrograms were highly similar and divided the seven genotypes into two main clusters. The first cluster consisted of Line 1, Line 2 and Line 3 and the other consisted of GIZA 123, GIZA 125, GIZA 127 and Line 35. It can be concluded that Both karyotype analysis and seed storage protein electrophoresis (SDS-PAGE) succeeded either together or separately to identify barley cultivars.

Key words: barley cultivars, karyotype analysis, seed storage proteins, SDS-PAGE.

INTRODUCTION

The cultivated barley (*Hordeum vulgare* L.) is one of the main cereals of the belt of the Mediterranean agriculture and a founder crop of Old World Neolithic food production (Harlan and Zohary, 1966). It withstands drier condition, poorer soils, and some salinity compared. So it has been the principal grain produced in numerous areas and an important element of the human diet, brewing malts, and the most important feed supplement for domestic animals. It is characterized by the three one-flowered spikelets at each rachis node, the two lateral ones are either rudimentary or sterile and the central one is fertile in the two-rowed barley or both of them are fertile in the six-rowed barley. Barley is extensively studied as a favorite genetic experimental plant species, mainly owing to its diploid nature (2n=2x=14), self fertility, large chromosomes (6-8 µm), moderate genome size (5.3x109 bp), high degree of natural and easily inducible variation, ease of hybridization, wide adaptability, and relatively limited space requirements(Zohary and Hopf, 1993).

Proteins account for about 10 % of the dry weight of mature barley grains. They are classified into storage proteins (prolamins, that is soluble in alchol/water mixtures) and non-storage proteins i.e. structural and metabolic proteins such as albumins (water soluble), globulins (soluble in dilute saline), and glutelins (soluble only in dilute acid or alkali). The major storage proteins in barley (prolamins) are called hordeins. They account for 35-50 % of the total grain nitrogen (Kirkman *et al.*, 1982 and Kreis and Shewry, 1992) and classified into four groups or families of polypeptides called B, C, D and γ-hordeins. The electrophoretic separation of wild and cultivated barley seed storage proteins has been also used as a powerful evidence for cultivar identification and wild barley biodiversity and phylogeny studies (Giles, 1984; Gebre *et al.* 1986; Heisel *et al.* 1986, El Rabey *et al.*, 2002, El Rabey, 2004, El Rabey and Zayed, 2005 and El Rabey, 2008).

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In the present study, karyotype analysis using the Video test software and seed storage proteins electrophoresis (SDS –PAGE) have been used for Egyptian barley cultivars characterization.

MATERIALS AND METHODS

Grains of seven barley cultivars (Table 1) were kindly supplied by the Egyptian Agricultural Research Center (ARC) and the gene bank of the Egyptian Desert Research Center (DRI). Names of the cultivars, number of rows and their sources are illustrated in Table 1.

Table 1: Name and source of the studied Egyptian barley cultivars.

Serial No.	Cultivar name	Cultivar source	Number of rows	Remarks
1	GIZA 123	ARC	6	Salt tolerant
2	GIZA 125	ARC	6	Salt tolerant
3	GIZA 127	ARC	2	Salt tolerant
4	Line 1	DRI	6	Salt tolerant
5	Line 2	DRI	6	Salt tolerant
6	Line 3	DRI	6	Salt tolerant
7	Line 35	DRI	6	Salt tolerant

ARC = Agricultural Research Center, GIZA, Egypt.   DRC = Desert Research Center, Cairo, Egypt.

Cytogenetics:

Cytolgenetical studies were performed using the Feulgen squash technique (Feulgen and Rossenbeck, 1924). The most spread C-metaphase chromosomes of the seven germplasms under study were photographed using Carl Zeiss photo-microscope. Photographs were scanned and then analyzed using video test karyotyping software. The chromosome length, chromosome area, middle line length and centrometry index characteristics were scored to characterize the selected cultivars.

SDS–polyacrylamide Gel Electrophoresis (Sds-page) of Seed Storage Proteins:

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

Statistical Analysis:

Protein gels were scored as 0/1 for absence/presence of the bands, respectively and the resulting 52 protein bands were analyzed using the NTSYS-pc2.0 software (Rohlf, 1998).The karyotypes were analyzed using video test karyotyping software. The scored data were standardized and de-centered before using in the statistical analysis. Similarity coefficient matrices were calculated using simple matching similarity algorithm (Sokal and Sneath, 1963). Phylogenetic dendrograms were constructed using the UPGMA method (Unweighted Pair-Group Method with arithmetical algorithms Averages; Sneath and Sokal, 1973) and the correlation cophenetic coefficients were calculated using Mantel's test (Mantel, 1967). For the above mentioned analyses, the NTSYS PC2.0 software was used.

RESULTS AND DISCUSSION

Karyotype Analysis:

Figure (1) shows the karyotypes of the 7 Egyptian barley germplasms under study, Table 2 illustrates statistics of these karyotypes and Figure 2 shows histograms representing the mean of chromosome length, chromosome area, middle line length and centrometry index of the 7 cultivars. The shortest chromosome in GIZA 123 was 0.66 μ and the longest one was 1.16 μ with a ratio of shortest / longest chromosome of 0.57, a mean chromosome length of 0.9 μ and a mean centromeric index (short arm / long arm) of 37.34% (Table 2). The shortest chromosome in GIZA 125 was 0.65 mkm and the longest one was 0.94 μ with a ratio of shortest / longest chromosome of 0.69 and a mean chromosome length of 0.82 μ and a mean centromeric index of 43.15%. In GIZA 127, the shortest chromosome was 0.64 μ and the longest was 1.32 μ with a ratio of shortest / longest chromosome of 0.48 and a mean chromosome length of 0.95 and a mean centromeric index of 40.88%. The shortest chromosome in Line1 was 0.98 μ and the longest one was 1.41 μ with a ratio of shortest / longest chromosome of 0.7, a mean chromosome length of 1.23

Table 2: Summary of the karyotype analysis of the 7 Egyptian barley cultivars under study.

Cultivar	Short Chrom. (S) μ	Long Chrom. (L) μ	S/L	Mean L	Mean of Centromery Index
GIZA 123	0.66	1.16	0.57	0.9	37.34
GIZA 125	0.65	0.94	0.69	0.82	43.15
GIZA 127	0.64	1.32	0.48	0.95	40.88
Line 1	0.98	1.41	0.7	1.23	45.6
Line 2	0.77	1.56	0.49	1.03	34.23
Line 3	0.9	1.48	0.61	1.2	37.67
Line 35	0.68	1.36	0.51	0.94	30.3

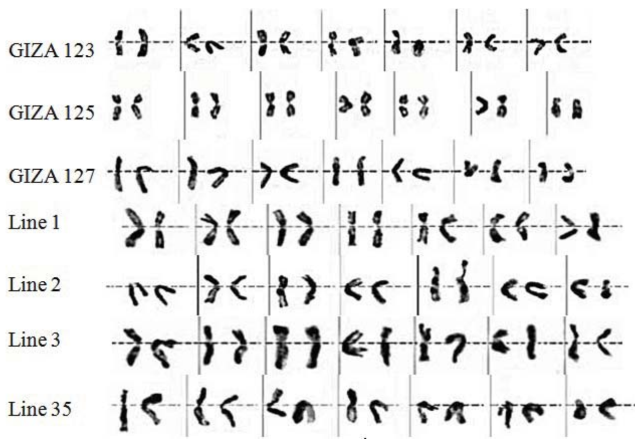

Fig. 1: Karyotype of the 7 Egyptian barley cultivars under study.

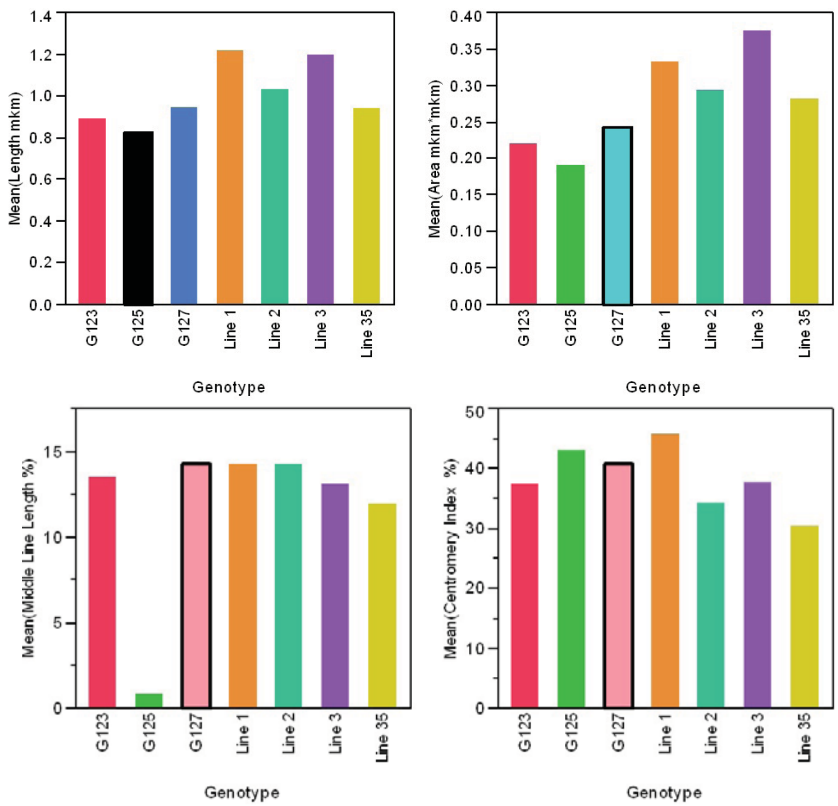

Fig. 2: Histograms showing the mean chromosome length, the mean chromosome area, mean middle line length and the mean centrometry index of the 7 Egyptian barley cultivars under study.

and a mean centromeric index of 45.6%. The shortest chromosome in Line 2 cultivar was 0.77 μ and the longest one was 1.56 μ with a ratio of shortest / longest chromosome of 0.49, a mean chromosome length of 1.03 and a mean centromeric index of 34.23%. In Line 3 cultivar, the shortest chromosome was 0.9 μ and the longest one was 1.48 with a ratio of shortest / longest chromosome of 0.61, a mean chromosome length of 1.2 and a mean centromeric index of 37.67%. In Line 35 cultivar, the shortest chromosome was 0.68 μ and the longest one was 1.36 μ with a ratio of shortest / longest chromosome of 0.51, a mean chromosome length of 0.94 and a mean centromeric index of 30.3%.

Briefly, the shortest chromosome across all samples was scored in the cultivar GIZA 127 (0.64 μ) and the longest one was scored in Line 2 (1.56 μ). The highest ratio of shortest / longest chromosome was obtained in Line (1 0.7) and lowest value was obtained in GIZA 127 (0.48), Table 2. The highest mean chromosome length value across all samples was scored from Line 1 (1.23 μ) and the lowest value was scored in GIZA 127 (0.82 μ). The highest mean centromeric index value was scored in Line 1, whereas the lowest value was scored in Line 35. It was also noticed that the fifth chromosome pair in Line 2 is characterized by the presence of satellite on its short arms.

Polyacrylamide Gel Electrophoresis (SDS-PAGE) of Storage Seed Proteins:

The SDS polyacrylamide gel electrophoresis of the water soluble and water-non soluble seed storage proteins of the 7 Egyptian barley cultivars is shown in Figure 4 (A, B), respectively. The distribution of the water soluble and water-non soluble protein bands throughout the 7 barley cultivars and their molecular weights are illustrated in Tables 3 and Table 4, respectively.

Table 3: Protein score and molecular weight of the water soluble seed storage proteins of the 7 Egyptian barley cultivars under study.

	kDa	GIZA123	GIZA 125	GIZA127	Line1	Line2	Line3	Line35
1	212	1	1	1	0	1	1	1
2	130	0	0	1	0	1	0	0
3	120	0	0	1	1	1	0	0
4	116	0	0	0	0	0	1	1
5	110	0	0	1	0	1	0	0
6	105	1	1	1	1	1	1	1
7	102	0	0	1	1	1	1	0
8	100	0	0	0	1	1	0	0
9	80	0	0	0	0	0	1	0
10	77.4	1	1	1	0	1	0	1
11	69	1	0	1	1	1	1	0
12	66.4	1	0	1	1	1	1	1
13	63	0	0	1	1	1	0	0
14	59	0	0	1	1	1	0	0
15	50.5	0	0	1	0	0	0	0
16	49.3	1	0	0	1	1	1	0
17	42.7	1	1	1	1	1	1	1
18	40.7	1	0	0	0	0	0	0
19	39.8	0	1	1	1	0	0	0
20	36.9	1	1	1	1	1	1	1
21	27	1	1	1	1	1	0	0
22	25.5	0	0	1	1	1	1	1
23	24	0	0	0	0	0	0	1
24	22	0	0	1	1	1	0	0

Twenty-four protein bands were scored in the water soluble seed storage protein fraction, three out of them (105, 42.7 and 36.9 kDa) were common (monomorphic) and the other 21 (212, 130, 120, 116, 110, 102, 100, 80, 77.4, 69, 66.4, 63, 59, 50.5, 49.3, 40.7, 39.8, 27, 25.5, 24, 22 kDa) were polymorphic. The 24 kDa and the 80 kDa bands are present only in Line 3, the 50.5 kDa band is characteristic to GIZA 127 and the 40.7 kDa band is characteristic to GIZA 123.

The protein profile of the water-non soluble seed storage protein fraction showed 28 protein bands, four out of them (212, 130, 97.2 and 90 kDa) were common (monomorphic) and the other 24 (158, 116, 110, 107, 80.4, 69.5, 66.4, 62, 55.6, 52.2, 51.8, 48.7, 45, 42.7, 40, 38, 36.7, 36.35, 34.6, 30.7, 29.5, 27 and 25) were polymorphic. The 27 kDa band is present only in Line 35.

The Genetic Relationship Based on Karyotype Analysis Measurements:

The measurements of the seven cultivars karyotypes were used as databases for generating the genetic relationships between the studied germplasms using NTSYS-pc program (Figure 3). The studied cultivars were

Table 4: Protein score and molecular weight of the buffer soluble seed storage proteins of the 7 Egyptian barley cultivars under study.

	kD	GIZA 123	GIZA 125	GIZA 127	Line1	Line2	Line3	Line35
1	212	1	1	1	1	1	1	1
2	158	0	1	1	1	1	0	0
3	130	1	1	1	1	1	1	1
4	116	0	1	0	0	1	1	0
5	110	1	0	1	0	0	0	0
6	107	0	0	0	1	1	1	1
7	97.2	1	1	1	1	1	1	1
8	90	1	1	1	1	1	1	1
9	80.4	0	1	1	1	1	1	0
10	69.5	0	0	0	0	1	1	0
11	66.4	1	0	0	0	0	0	1
12	62	0	1	1	1	1	0	0
13	55.6	0	1	1	0	0	1	0
14	52.2	1	0	0	0	0	1	1
15	51.8	0	0	0	1	1	0	1
16	48.7	0	0	0	0	1	0	1
17	45	1	1	1	0	0	1	0
18	42.7	0	0	0	1	1	1	1
19	40	1	1	1	0	0	0	0
20	38	0	0	0	1	1	1	1
21	36.7	1	1	1	0	0	0	1
22	36	0	0	0	1	1	1	0
23	35	1	1	1	0	0	0	1
24	34.6	0	0	0	1	1	1	0
25	30.7	1	1	1	0	0	0	1
26	29.5	0	0	0	1	1	1	0
27	27	0	0	0	0	0	0	1
28	25	0	0	0	1	1	1	0

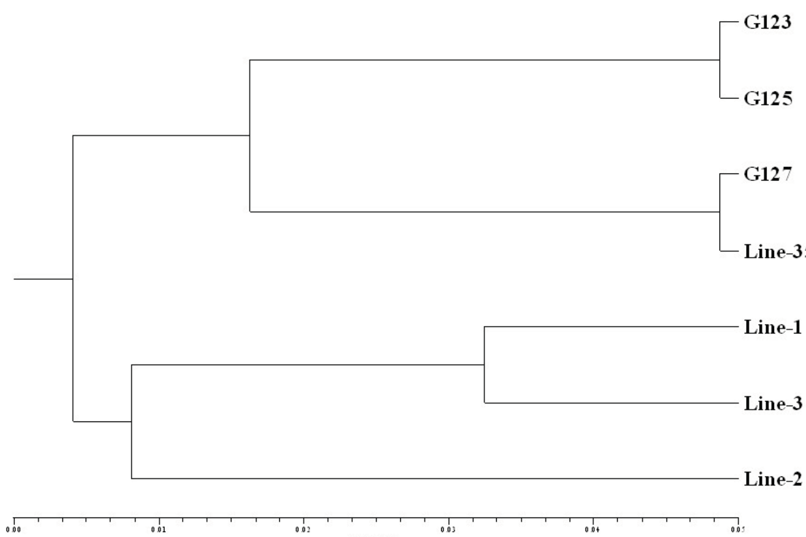

Fig. 3: The genetic relationships among the studied barley cultivars based on karyotype measurements using NTSYS-pc2 program.

clustered into two main clusters. The first cluster contains Line 1, Line 2 and Line 3. Line 1 and Line 3 were closely related to each other than Line 2 which was relatively distant from both of them. The other cluster contains two subclusters each consists of two cultivars. GIZA 123 and GIZA 125 were clustered together in the first subcluster, whereas Line 35 and GIZA 127 were clustered together in the second subcluster.

The Genetic Relationship Based on Seed Storage Protein Electrophoresis Data:

Both water soluble and water-non soluble seed storage protein bands (in total 24+28=52 band) were used to address the genetic relationship between the studied cultivars using NTSYS-pc2 program software (Figure 5). The studied germplasms were clustered in two main clusters, the first one consists of three

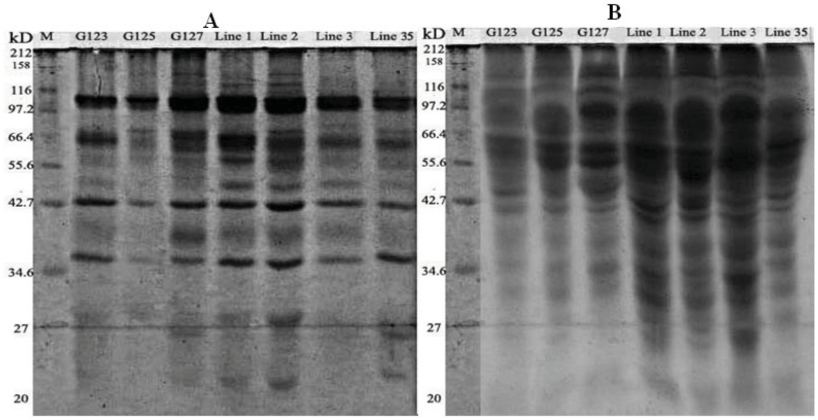

Fig. 4: SDS-PAGE of the water soluble (A) and the buffer soluble (B) seed storage proteins of the studied 7 Egyptian barley cultivars.

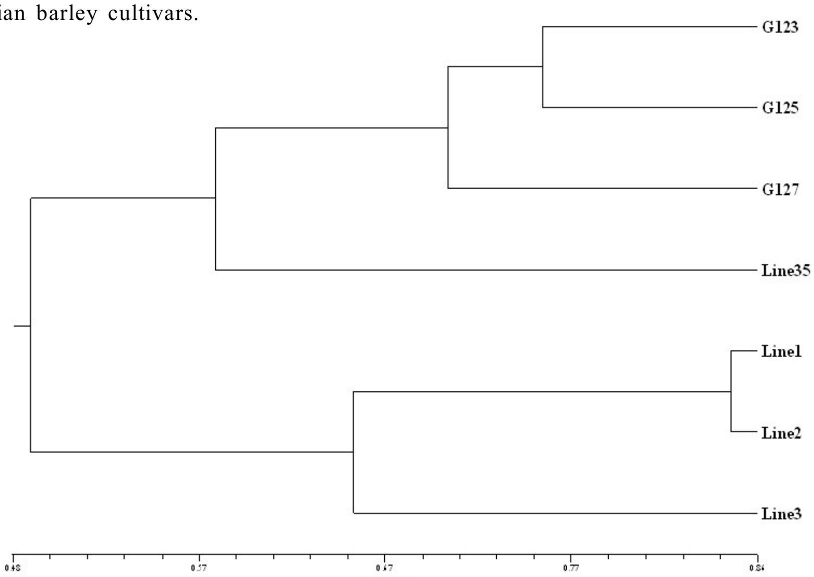

Fig. 5: Dendrogram showing the genetic relationships among the seven barley germplasms based on both water and buffer soluble seed storage protein data using NTSYS-pc2 program.

cultivars; Line 1, Line 2 and Line 3. Line 1 and Line 2 were closely related to each other than line 3 which was relatively distant from both of them. The second cluster consists of four cultivars; GIZA 123, GIZA 125, GIZA 127 and Line 35. GIZA 123 and GIZA 125 are closely related to each other. GIZA 127 was relatively distant from both of them followed by Line 35. This result is nearly similar to that obtained based on the karyotype analysis measurements. Very high percentage of correlation was obtained between the two dendrograms through Mantel's test (r = 0.99).

Discussion:

Karyotype analysis has been successfully used for species speciation and cultivar identification (Bothmer *et al.*, 1981; Shao, 1982; Shao *et al.*, 1987, Badr *et al.*, 1996 and El Rabey *et al.*, 2002). In the current study, the karyotype analysis revealed that the shortest chromosome across all samples was 0.64 μ which was scored in GIZA 127 cultivar and the longest one was 1.56 μ and scored in Line 2 cultivar. The highest ratio of shortest / longest chromosome was 0.7 and scored in Line 1 and lowest value was 0.48 and scored in GIZA 127. The highest mean chromosome length value across all samples was 1.23 μ and scored in Line 1 and the lowest value was o.82 μ and scored in GIZA 127. The highest mean centromeric index value was scored in Line 1, whereas the lowest value was scored in Line 35.

GIZA 127, which represents the two rowed cultivar in this study, is characterized by its containing the shortest chromosome across all samples (0.64 μ), the lowest short/long chromosome ratio (48%) and the lowest chromosome mean length (0.82 μ). It was also noticed that a 50.5 kDa protein marker is characterized cultivar GIZA 127 in the water soluble seed storage protein fraction. It was also worthy to note that the fifth chromosome pair in Line 2 is characterized by the presence of satellite on its short arms.

Seed storage protein electrophoresis are also a valuable evidence in cultivar identification and wild species phylogeny studies (Gebre *et al.* 1986; Heisel *et al.* 1986, El Rabey *et al.*, 2002 , El Rabey, 2004 and El Rabey and Zayed, 2005 and El Rabey, 2008). Generally, Twenty-four protein bands were obtained from the SDS-PAGE of the water soluble seed storage protein fraction, three out of them were common while the other 21 were polymorphic. The 24 kDa and the 80 kDa bands 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 seed 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.

The genetic relationship among the studied barley cultivars was addressed in the light of kayotype analysis and the storage seed protein electrophoresis using the NTSYS-pc2 program (Rohlf, 1998). The studied cultivars were clustered in two highly similar clusters based on both karyotype analysis and seed storage protein electrophoresis. The first cluster consists of Line 1, Line 2 and Line 3 and the other cluster contains GIZA 123, GIZA 125, Line 35 and GIZA 127. The two dendrograms based on both karyotype analysis measurements and seed storage protein data are highly consistent with each other with a Mantel test value of 0.99 according to the method of Mantel (1967). Highly significant positive correlation between the two analysis (r = 0.99) was revealed through the Mantel's test which reflect the consistence of the analyses. Thus, it could be said that either of the two types of analyses could be used to characterize the Egyptian barley germplasms. More studies are required on the Egyptian germplasms to characterize them not only on the basis of cytogenetical, molecular and biochemical markers, but also based on the specific characters of their genome.

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REFERENCES

Abo-Elkhier, A., 1989. Physiological genetic studies in barley (*Hordeum* sp.). Master degree thesis, Genetic department, Faculty of Agriculture Ain Shams University, Cairo, Egypt.

Badr,A., M.A. Hammoud and H.A. El Rabey, 1996. Chromosomal studies in the Egyptian flora V. Chromosomal relationships in the genus *Astragalus* L. (*Fabaceae*) and their taxonomic inferences. Cytologia, 61: 105-111.

Bothmer, R., von, N. Jacobsen and R.B. Jorgensen, 1981. Phylogeny and Taxonomy in the genus *Hordeum*. Barley Genetics IV Proc. 4th Int. Barley Genet. Symp., Edinburgh: 13- 21.

El Rabey, H., 2008. Molecular taxonomy of Egyptian *Hordeum murinum* L. complex as revealed by RAPD-PCR and seed storage protein electrophoresis. Taeckholmia, 28: 57–76.

El Rabey, H. and M. Zayed, 2005. The role of molecular evidences in justifying the response of barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) seedlings to lead ion treatment. The Egyptian Journal of Biotechnology, 20: 107-120.

El Rabey, H., A. Ibrahim, A. Badr, K. El Halafawy and F. Salamini, 2002. DNA and seed protein fingerprinting of Egyptian crop plant . I. The relationships of 15 barley cultivars (*Hordeum vulgare* L.). The second international conference on biological sciences. 27-28 April, (2): 79-93.

Feulgen, R. and H. Rossenbeck, 1924. Mikroskopisch-chemischer Nachweis einer Nucleinsaure vom Typus der Thymonucleinsäure und die darauf beruhende elektive Färbung vom Zellkernen in Mikroskopischen praparaten. Hoppe Seyler Z Physiol Chem., 203-248.

Gebre, H., K. Khan and A. Foster, 1986. Barley cultivar identification by polyacrylamide electrophoresis of hordein proteins: Catalogue of cultivars. Crop Science, 26: 454-460.

Giles, E., 1984. A comparison between quantitative and biochemical variation in the wild barley *Hordeum murinum*. Evolution, 38: 34-41.

Harlan, J.R. and D. Zohary, 1966. Distribution of Wild Wheats and Barley. Science 153: 1074- 1080.

Heisel, S., D. Peterson and B. Jones, 1986. Identification of United States barley cultivars by sodium dodecyl sulfate polyacrylamide gel electrophoresis of hordeins. Cereal Chemistry, 63(6): 500-505.

Kirkman, M.A., P.R. Shewry and B.J. Miflin, 1982. The effect of nitrogen nutrition on the lysine content and protein composition of barley seeds. Journal of the Science of Food and Agriculture, 33: 115-127.

Kreis, M. and P.R. Shewry, 1992. The control of protein synthesis in developing barley seeds. Barley Genetics, Biochemistry, Molecular Biology and Biotechnology. Edited by Peter Shewry, Long Ashton, Bristol BS 18 9AF, UK.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 15: (227-259): 680-685.

Mantel, N., 1967. The detection of disease clustering and a generalized regression approach. Cancer Res., 27: 209-220.

Rohlf, F.J., 1998. NTSYSpc. Numerical taxonomy and multivariate analysis system, version 2.02c. Exeter Software, New York.

Shao, Q., 1982. The evolution of cultivated barley. Barley Genetic IV: 22-24.

Shao, Q., J. Zhou and A. Li, 1987. Genetic distance between cultivated barley and its close-related wild relative. Barley Genetics V: 83-86.

Sneath, P.H.A. and R.R. Sokal, 1973. Numerical taxonomy. Freeman, San Francisco.

Sokal, R.R. and P.H.A. Sneath, 1963. Principles of Numerical Taxonomy. Freeman. San Francisco, pp: 359.

Zohary, D. and M. Hopf, 1993. Domestication of Plants in the Old World. Oxford, Clarendon Press: 54-64.