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Phenetic and Phylogenetic Relationships of Egyptian *Hordeum vulgare* L. Using morphological Criteria, Molecular Marker and SDS-PAGE

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Abstract

Phenetic and phylogenetic relationships between ten *Hordeum vulgare* L. taxa, seven cultivars (Giza 123, Giza 126, Giza 127, Giza 128, Giza 129, Giza 130 and Giza 2000) and three landraces collected from Sinai (El-Kheroba, El-Sheikh Zuwaid and Wadi Sedr). This study was carried out using morphological criteria, molecular genetic markers (SSRs) and finally soluble protein patterns. The results of morphological traits reflected that Giza 2000 and Wadi Sedr gave superior in vegetative growth and yield productivity in contrast to Giza 129, which showed an inferior vegetative growth and yield. The SSR analyses using 7 primers and revealed detection of a total of 15 bands, among which 8 bands (61.22%) were polymorphic. Finally, we can conclude that genetic dissimilarity tree was produced by the hierarchical cluster analysis based on morphological data stated the pedigree of studied cultivars. However, the genetic similarity tree was produced by UPGMA based on molecular markers (SSRs) and soluble proteins patterns were not in full agreement with the pedigree.

Keywords: *Hordeum vulgare* L., genetic relationships, morphological Criteria, SDS-PAGE Electrophoresis, Molecular markers (SSR-PCR)

Introduction

The genus *Hordeum* L. includes 30 species of annual and perennial grasses, 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)**.
Polyacrylamide gel electrophoresis is considered as a powerful technique for identification process of genotypes depending on biochemical analysis of protein banding patterns. Some protein markers effected by some economic traits that are very impotant in crops breeding. **(Hames, 1990)**. Electrophoretic separation of seed proteins is a powerful and efficient tool in taxonomic evolutionary relationships at both species and subspecies levels **(Badr *et al*., 2000; El-Atroush *et al*., 2015 and Rayan and Osman, 2019).**

Molecular markers are advanced and powerful tools in the assessment of genetic diversity within and between genetic populations **(Russell et al., 1997b and Rayan and Osman, 2019)**. Microsatellite markers have been developed in many crop species, such as soybean, wheat, maize, barley, rice, and potato. In cereals, they show a much higher level of polymorphism than other marker systems. Simple sequence repeats (SSRs) considered a superior marker for assessment of genetic diversity, genetic relationship and phylogenetic development because this marker had high level of polymorphism, codominant inheritance, highly reproducibility, locus specificity and

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random distribution on the genome **(Russell *et al.*, 1997a).** SSRs technique is powerful tool for genetic studies in barely breeding **(Mohamed and Adel, 2012; Nandha and Singh, 2014 and Ferreira *et al.*, 2016).**

The aim of the present study is to detect the genetic relationships between ten Egyptian *Hordeum vulgare* L taxa depending on morphological criteria, molecular genetic markers (SSR-PCR) and proteins banding patterns electrophoresis. Also, determination of the viability and efficiency of SSR as genetic marker in discrimination and identification of the studied taxa (cultivars and landraces).

Materials and Methods

Grains of the seven *Hordeum vulgare* L. cultivars (Giza 123, Giza 126, Giza 127, Giza 128, Giza 129, Giza 130 and Giza 2000) were obtained from Agricultural Research Center, Giza, Egypt, and three landraces were obtained from Egyptian National GenBank (under code number 11557, 11580 and 113737), these landraces collected from Sinai, El-Aresh (El-Kheroba, El-Sheikh Zuwaid) and Ras Sedr (Wadi Sedr) as showed in **Table (1).**

1. Morphological Studies

To diferentiate between studied cultivars depending on morphological criteria (branch number/plant, shoot length, root length, node number/plant, number of grains/spike, grain weight/spike, spike length, spike weight, total grain yield/plant, spike type and grain hallness). The experiment was conducted in a randomized complete block design with three replications in green house. Each pot (30x50 cm) contained ten plants of the studied taxa.

2. Molecular studies

DNA was isolated from ten studied *Hordeum vulgare* L. taxa using Gene Jet Plant Genomic DNA purification Mini Kits (Thermo scientific K0791).

Simple Sequence Repeats of DNA (SSR-DNA):

Table (2) represents seven SSRs primers, their sequences, the annealing temperature used in the PCR reaction, the chromosomal location of derived loci **(Von Korff *et al.*, 2004)**. PCR amplification for different isolated DNA was performed in 0.2 ml PCR eppendorf containing (50 µl) consisted of Dream Taq DNA Polymerase, promega (1 µl), 10x Dream Taq Green Buffer (10µl), MgCl (5µl) dNTP Mix 10mM each (4µl), primer forward (f) and reverse (r), Metabion, German (3 µl) and Template DNA (4µl) then completed to 50µl by Water, nuclease-free. Thermocycler (Bio-Rad) was programed for 35 cycles as follows: 94^{o}C for 3 min (one cycle) then 94^{o}C for 1 min, Tm^{o}C for 1min and 72^{o}C for 2 min (35 cycles) then 72^{o}C for 10 min (one cycle) then held at 4^{o}C.

Agarose was placed in 1X TAE buffer (2%) and boiled in water bath, then ethidium bromide was added to the melted gel after the temperature become 55^{o}C. The melted gel poured in the tray of midi-gel apparatus (horizontal electrophoresis apparatus manufactured by Cleaver, UK) and the comb was inserted immediately, then the comb was removed when the gel become hardened. The electrophoresis buffer (1X TAE) was added and covered the gel. 5 µl of DNA amplified product was loaded in each well and run at 100 V for about 2 hours. The gel was photographed by gel documentation (Bio-Rad) and analyzed by Total Lab program to find out the molecular weight of each band and that to compare the presence and absence of the band among cultivars and these date were imported in MVSP (Multi-Variant Statistical Package) to find the similarity matrix and dendrogram (UPGAMA, suing Jaccard's coefficient) which reflect the relationships among the studied taxa.

Genetic diversity referred to as Polymorphic Information Content (PIC) values were calculated with the following formula **(Anderson *et al.*, 1993)**:

$$\mathrm{PICi} = 1 - \sum_{f=1}^{n} (Pij)^2$$

Where, n is the number of marker alleles for marker i and Pij is the frequency of the jth allele for marker i.

The summary statistics including the number of alleles per locus, major allele frequency, gene diversity, polymorphism information content (PIC) values were determined using Power Marker version 3.25 **(Liu and Muse, 2005)**.

3. Biochemical studies:

- Proteins profile using SDS-PAGE

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of **Laemmli (1970),** as modified by **Studier (1973).** Water-soluble proteins (W.S.P) of studied cultivars were taken from leaves of these plants. Protein fractionations were performed exclusively on vertical slab gel (19.8 cm x 26.8 cm x 0.2 cm) using the electrophoresis apparatus (Cleaver, UK).

The images were captured by Digital camera (Sony, made in japan) and transferred directly to the computer and then the protein

Table (1): The pedigree of studied *Hordeum vulgare* taxa.

No.	Cultivars	Pedigree	origin
1	**Giza 123**	Giza117 / FAO86	Egypt
2	**Giza 126**	Baladi Bahteem/S D729-Por12762-BC	Egypt
3	**Giza 127**	W12291/Bags // Harmal- 02	Egypt
4	**Giza 128**	*WI2291 / 4 /11012-270-22425 / 3 /Apam / IB65 //A 16*	Egypt
5	**Giza 129**	DeirAlla 106/Cel//AS 46/Aths*2	Egypt
6	**Giza 130**	*Comp.cross 229 // Bce Mr /DZ 02391 / 3 / Deir 106A lla106*	Egypt
7	**Giza 2000**	Giza117/Bahteem52// Giza118/ FAO86 / 3/ Baladi16/ Gem.	Egypt
8	**Landraces**	Sinai, El-Aresh, El-Kheroba (Egy., GenBank, code No. 11557)	Egypt
9	**Landraces**	Sinai, El-Aresh, El-Sheikh Zuwaid (Egy., GenBank, code No. 11580)	Egypt
10	**Landraces**	Sinai, Ras Sedr, Wadi Sedr (Egy., GenBank, code No. 113737)	Egypt

Table (2): List of SSR-DNA primers and their nucleotide sequences and its location on chromosome

Primers	Sequence	Annealing Temperature (°C)	Chromosome Location
Bmag13	**Forward** 5'-AAggggAATCAAAATgggAg-3' **Reverse** 5'-TCgAATAggTCTCCgAAgAAA-3'	**54**	**3 (3H)**
MGB391	**Forward** 5'-AgCTCCTTTCCTCCCTTCC-3' **Reverse** 5'-CCAACATCTCCTCCTCCTgA-3'	**54**	**2 (2H)**
GMS1	**Forward** 5'-CTgACCCTTTgCTTAACATgC-3' **Reverse** 5'-TCAgCgTgACAAACAATAAAgg-3'	**55**	**7 (5H)**
EBmac624	**Forward** 5'- AAAAgCATTCAACTTCATAAgA-3' **Reverse** 5'- CAACgCCATCACgTAATA-3'	**54**	**6 (6H)**
Bmag210	**Forward** 5'-ACCTACAgTTCAATAgCTAgTACC-3' **Reverse** 5'-gCACAAAACgATTACATCATA-3'	**54**	**6 (6H)**
Bmag149	**Forward** 5'-CAAgCCAACAgggTAgTC-3' **Reverse** 5'-ATTCggTTTCTAgAggAAgAA-3'	**55**	**5 (1H)**
HVITR1	**Forward** 5'-CCACTTgCCAAACACTAgACCC-3' **Reverse** 5'-TTCATgCAgATCgggCCAC-3'	**55**	**3 (3H)**

bands were analyzed by Total Lab program to find out the molecular weight of each band and that to compare the presence and absence of the band among cultivars and these data were imported in MVSP (Multi-Variant Statistical Package) to find the similarity matrix and dendrogram (UPGMA, suing Jaccard's coefficient) which reflect the relationships among the studied cultivars.

Results

1. Morphological study:

The analysis of variance was calculated for the nine measured morphological traits namely: branch number/plant, shoot length (cm), root length (cm), node number/plant, spike length (cm), spike weight (gm), no. of grains/spike, grain weight /spike (gm) and grain weight/plant (gm) on ten Egyptian barely taxa as shown in **Table (3).** The analysis of variance for studied traits except root length (cm) showed highly significant differences among the studied taxa. This indicated that there are genetic differences between the studied barley taxa.

1.1- Mean agronomic and yield performance

Mean performance values of the all studied traits for the ten Egyptian barely taxa are illustrated in **Table (4)** and **Figure (1)**. The mean values manifested in general that the genotypes Wadi Sedr, Giza 2000 and Giza 123 gave the highest value for all studied traits except branch number/plant, respectively.

According branch number/plant, Giza 128 had the high value (2.65) followed by Giza 127 (2.00) whereas the difference between the rest of genotypes was not significant. Giza 2000, Giza 123 and Wadi Sedr landraces had high shoot length (cm) value as 49.24, 49.17 and 48.78; respectively, whereas the lowest value (38.75) was recorded in Giza 129. In respect to root length (cm) the maximum value (13.54, 13.53 and 12.9) are represented in Wadi Sedr, Giza 2000 and El-Sheikh Zuwaid genotypes, respectively while the minimum value (9.9) was found in Giza 129. Giza 2000, Wadi Sedr and Giza 123 had the high number of node (7.4, 7.32 and 7.22 respectively) while the lowest value was 6.14 in Giza 129. The Wadi Sedr landrace and Giza 2000 cultivar had the highest value for spike length (cm) 4.98 and 4.97 respectively while Giza 129 had the lowest value 2.99 cm. Spike weight (gm) are recorded the maximum value 0.34 and 0.32 in Giza 2000 and Wadi Sedr respectively but minimum value 0.12 in Giza 129. We found that the maximum number of grains per spike 19.65, 19.5 and 19.42 are represented in Wadi Sedr, Giza 2000 and Giza 123 respectively, while the minimum value 11.43 is represented in Giza 129. The highest weight of grain per spike (gm) 0.28 are recorded in both cultivars Giza 2000 and Wadi Sedr but the lowest weight of grain per spike 0.08 are recorded in Giza 129. The grain yield per plant reported the maximum weight (gm) 0.32, 0.31 and 0.3 in Wadi Sedr,

Table (3): Mean squares (MS) of analysis of variance for all studied traits

S.O.V	DF	Branch number / plant	Shoot Length (cm)	Root Length (cm)	Node number/ plant	Spike length (cm)	Spike Weight (gm)	No. of Grains/spike	Grain weight /spike (gm)	Total grain weight/plant (gm)
Replicates	**2**	**0.06**	**9.08****	**23.29****	**1.09****	**2.05****	**0.02**	**3.74**	**0.02**	**0.0297***
Cultivars	**9**	**0.71****	**45.20****	**3.82**	**0.66****	**1.27****	**0.02***	**30.40****	**0.02***	**0.0204***
Error	**18**	**0.08**	**0.95**	**2.61**	**0.10**	**0.29**	**0.01**	**1.15**	**0.01**	**0.0074**

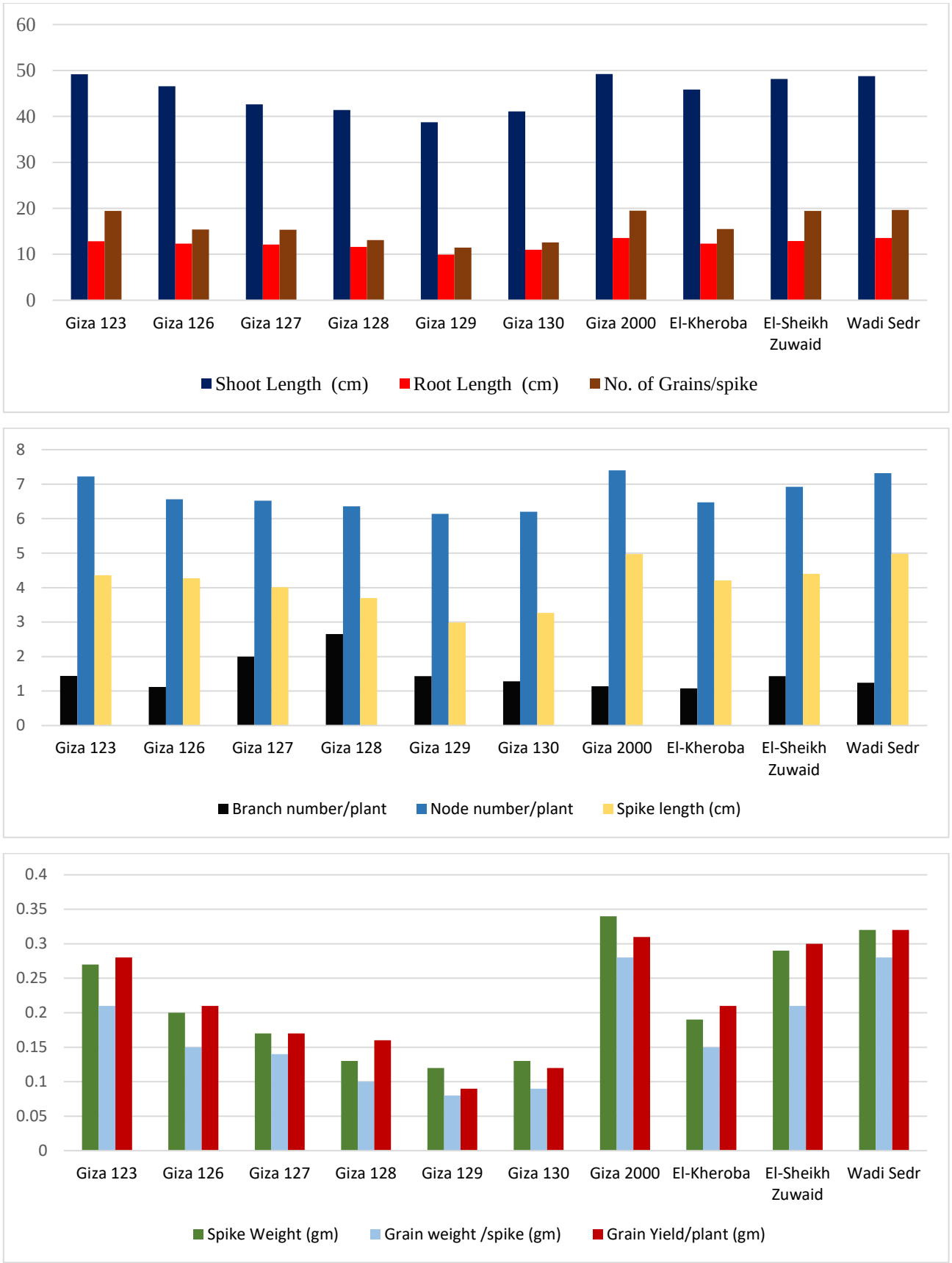

Figure (1): Mean performance values of the all studied traits for the ten Egyptian barely taxa.

Table (4): Mean performance values of the all studied traits for the ten Egyptian *Hordeum vulgare* taxa.

Genotypes	Branch number/plant	Shoot Length (cm)	Root Length (cm)	Node number/plant	Spike length (cm)	Spike Weight (gm)	No. of Grains/spike	Grain weight /spike (gm)	Total grain Yield/plant (gm)	Spike type (number of row)	Grain
Giza 123	1.44^{c}	49.17^{a}	12.86^{a}	7.22^{a}	4.36^{ab}	0.27^{abc}	19.42^{a}	0.21^{ab}	0.28^{ab}	Six Rows	halled
Giza 126	1.12^{c}	46.59^{bc}	12.33^{ab}	6.56^{bc}	4.27^{ab}	0.20^{abcd}	15.37^{b}	0.15^{b}	0.21^{abc}		
Giza 127	2.00^{b}	42.61^{d}	12.10^{ab}	6.52^{bc}	4.01^{bc}	0.17^{bcd}	15.34^{b}	0.14^{b}	0.17^{bc}	Two Rows	
Giza 128	2.65^{a}	41.39^{d}	11.61^{ab}	6.36^{c}	3.70^{bcd}	0.13^{cd}	13.08^{c}	0.10^{b}	0.16^{bc}		
Giza 129	1.43^{c}	38.75^{e}	9.93^{b}	6.14^{c}	2.99^{d}	0.12^{d}	11.43^{c}	0.08^{b}	0.09^{c}	Six Rows	Halless (Naked)
Giza 130	1.28^{c}	41.11^{d}	11.01^{ab}	6.20^{c}	3.27^{cd}	0.13^{cd}	12.56^{c}	0.09^{b}	0.12^{c}		
Giza 2000	1.14^{c}	49.24^{a}	13.53^{a}	7.40^{a}	4.97^{a}	0.34^{a}	19.50^{a}	0.28^{a}	0.31^{ab}		halled
El-Kheroba	1.08^{c}	45.85^{c}	12.33^{ab}	6.47^{bc}	4.21^{ab}	0.19^{bcd}	15.48^{b}	0.15^{b}	0.21^{abc}		
El-Sheikh Zowied	1.43^{c}	48.17^{ab}	12.90^{a}	6.92^{ab}	4.40^{ab}	0.29^{ab}	19.42^{a}	0.21^{ab}	0.30^{ab}		
Wadi Sedr	1.24^{c}	48.78^{a}	13.54^{a}	7.32^{a}	4.98^{a}	0.32^{ab}	19.65^{a}	0.28^{a}	0.32^{a}		
LSD .05	0.49	1.67	2.77	0.53	0.92	0.15	1.84	0.13	0.15		

For all value in such column with the same letter, the difference between the means is not statistically significant.
LSD: Least significant difference at 0.05

Giza 2000 and El-Sheikh Zuwaid respectively while the minimum weight of grain yield per plant (0.09) are reported in Giza 129.

1.2. Genetic relationships among the studied cultivars as revealed by morphological criteria

The greatest distance value (199.136) was recorded between both Giza 129 and Giza 2000, this indicated that these were distantly related variety. On the other hand, the lowest distance value (0.261) was recorded between Giza 2000 cultivar and Wadi Sedr landrace, indicating that both cultivars were closely related to each other as shown in **Table (5) and Figure (2)**. The resulting of morphological phenogram was a completely similar to the pedigree of studied cultivars.

2. Molecular studies:

2.1. Simple Sequence Repeats of DNA (SSR-DNA):

2.1.1. Polymorphism as detected by SSR analysis

Seven microsatellites simple sequence repeats (SSRs) primers were employed to investigate the genetic polymorphism among studied barley cultivars. As shown in **table (6)**, these primers generated a total of 15 bands; 8 of this bands were polymorphic (61.22%). The polymorphic information content (PIC) varied from 0.00 to 0.269 where primers GMS1 and Bmag149 had the highest PIC values while primers EBmac624 and HVITR1 had lowest PIC value. SSR markers characterized four unique markers among the ten studied cultivars including one positive unique SSR markers and three negative unique markers **(Table 6 and Figure 3)**.

2.1.2. Genetic relationships among the studied cultivars as revealed by SSR:

According to similarity matrix of SSR analysis, the highest similarity value (0.923) was recorded between Giza 128 and Giza 129 with Giza 2000 and Wadi Sedr, Giza 2000 with El-Kheroba and El- Sheikh Zuwaid and finally between Wadi Sedr with El-Kheroba and El-Sheikh Zuwaid, this indicating that there were closely relation between them. On the other hand, the lowest similarity value (0.714) was

Table (5): Genetic dissimilarity of ten *Hordeum vulgare* L. cultivars based on morphological criteria.

Matrix File Input										
Case	**Giza 123**	**Giza 126**	**Giza 127**	**Giza 128**	**Giza 129**	**Giza 130**	**Giza 2000**	**El-Kheroba**	**El-Sheikh Zuwaid**	**Wadi Sedr**
Giza 123	**0.000**									
Giza 126	**23.984**	**0.000**								
Giza 127	**61.559**	**16.829**	**0.000**							
Giza 128	**106.178**	**36.166**	**7.644**	**0.000**						
Giza 129	**188.124**	**87.601**	**38.412**	**15.376**	**0.000**					
Giza 130	**119.434**	**41.843**	**12.839**	**2.836**	**8.618**	**0.000**				
Giza 2000	**1.055**	**27.112**	**66.602**	**113.546**	**199.136**	**127.611**	**0.000**			
El-Kheroba	**27.642**	**.573**	**11.550**	**29.563**	**77.232**	**34.750**	**30.938**	**0.000**		
El-Sheikh Zuwaid	**1.104**	**19.518**	**49.106**	**91.168**	**167.690**	**103.783**	**2.355**	**21.646**	**0.000**	
Wadi Sedr	**1.149**	**25.956**	**61.559**	**107.819**	**191.550**	**122.010**	**0.261**	**29.063**	**1.463**	**0.000**

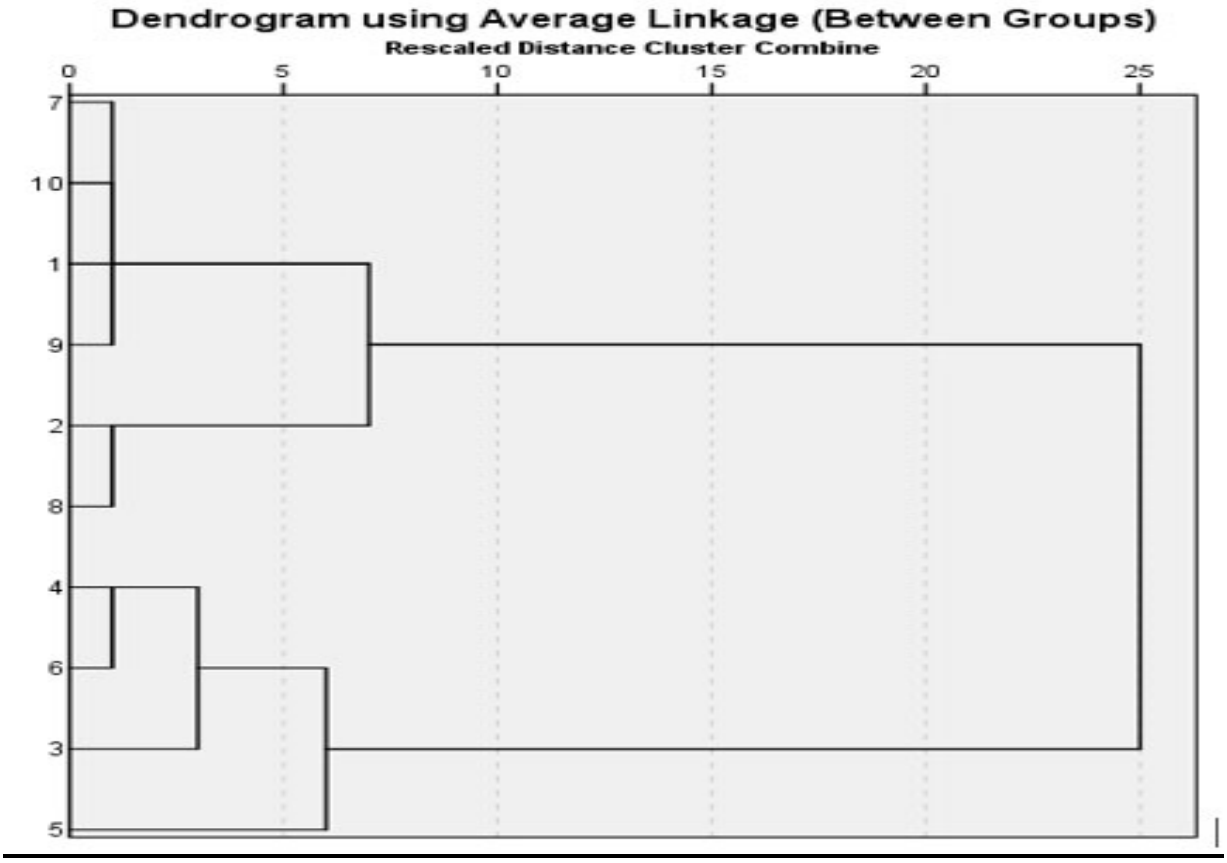

Figure (2): Grouping of barley taxa using hierarchical cluster analysis (average linkage distance) based on morphological traits. Cultivars: 1-Giza 123, 2-Giza 126, 3-Giza 127, 4-Giza 128, 5-Giza 129, 6-Giza 130 and 7- Giza 2000. Landraces cultivars: 8- Sinai, El-Aresh, El-Kheroba, 9- Sinai, El-Aresh, El-Sheikh Zuwaid and 10- Sinai, Ras Sedr, Wadi Sedr.

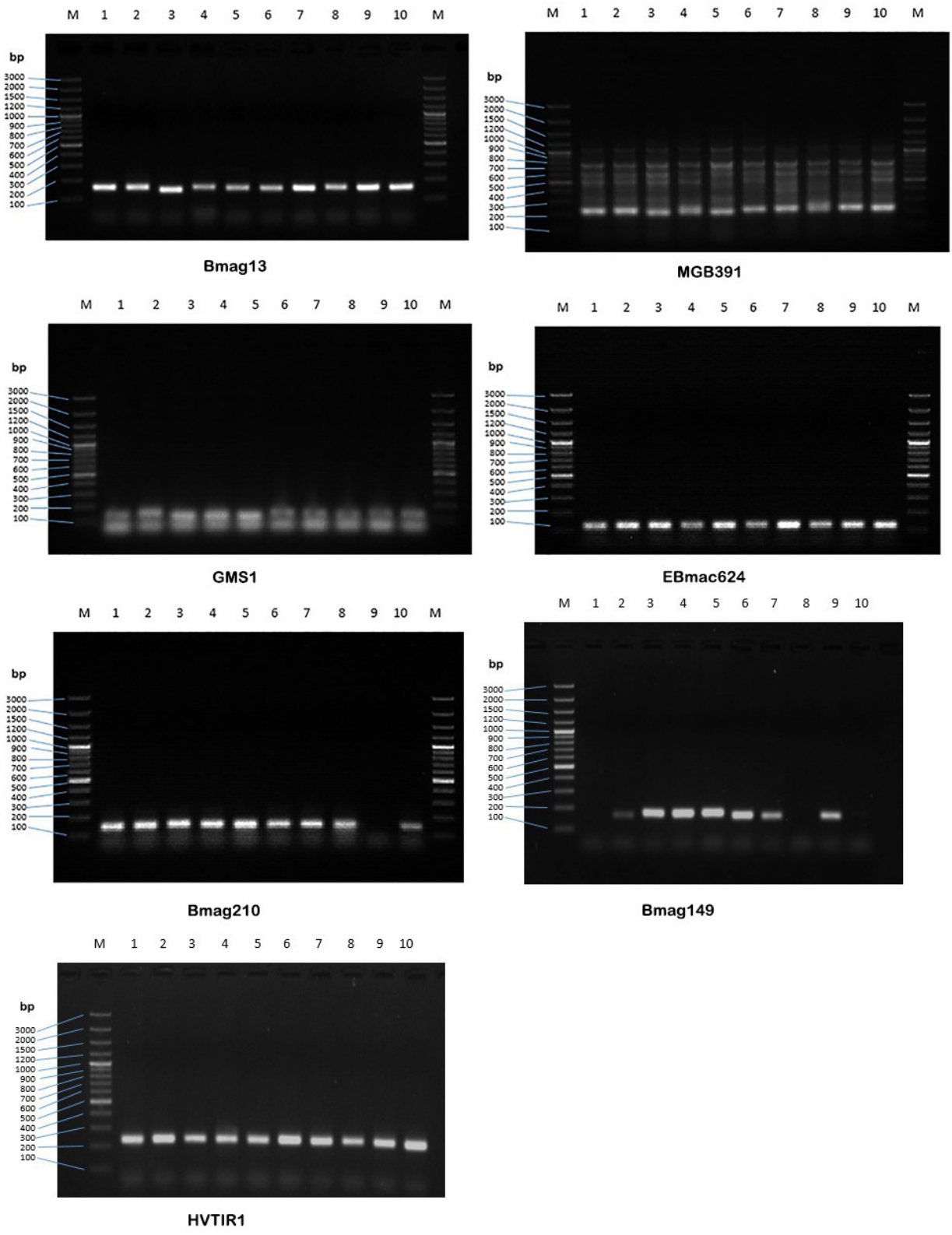

Figure (3): PCR-SSR patern of ten taxa of *Hordeum vulgare* L.

Cultivars: 1-Giza 123, 2-Giza 126, 3-Giza 127, 4-Giza 128, 5-Giza 129, 6-Giza 130 and 7- Giza 2000. Landraces: 8- El-Kheroba, 9- El-Sheikh Zuwaid and 10- Wadi Sedr.

Table (6): Total number of bands, polymorphic bands, percentage of polymorphism, PIC and allele size range as revealed by SSR analysis.

Primer name	Chromo-some Location	Total number of bands	Poly-morphic bands	Mono-morphic bands	Polymor-phism percentage	Positive Unique Marker	Negative Unique Marker	Allele size range (bP)	PIC
Bmag13	**3 (3H)**	**2**	**2**	**0**	**100%**	**1**	**1**	**150&170**	**0.164**
MGB391	**2 (2H)**	**7**	**2**	**5**	**28.57%**	**-**	**1**	**210-900**	**0.075**
GMS1	**7 (5H)**	**2**	**2**	**0**	**100%**	**-**	**-**	**180&200**	**0.269**
EBmac624	**6 (6H)**	**1**	**0**	**1**	**0%**	**-**	**-**	**130**	**0.00**
Bmag210	**6 (6H)**	**1**	**1**	**0**	**100%**	**-**	**1**	**150**	**0.164**
Bmag149	**5 (1H)**	**1**	**1**	**0**	**100%**	**-**	**-**	**180**	**0.269**
HVITR1	**3 (3H)**	**1**	**0**	**1**	**0%**	**-**	**-**	**230**	**0.00**
Total Band		**15**	**8**	**7**	**-**	**1**	**3**	**-**	**-**
Average		**2.14**	**1.14**	**1**	**61.22%**	**-**		**-**	**0.134**

Kheroba and El- Sheikh Zuwaid also between Giza 127 and El-Kheroba and El- Sheikh Zuwaid indicating that these were distantly related cultivars **(Table 7 and Figure 4)**.

3. Biochemical fingerprint (Protein analysis)

3.1. SDS-PAGE of soluble protein analysis

SDS-PAGE is used in the present study to re-assess the phylogenetic relationship between ten Egyptian *Hordeum vulgare* L. cultivars. Leaves soluble protein banding profile is illustrated in figure (5). The total number of bands were 35 bands. The molecular weight of these bands ranged between 12 to 300 kDa as showed in **Figure (5).**

The highest number of bands were 35, detected in Giza 127, Giza 128 and Giza 129 cultivars, while the lowest number of bands were 25 detected in Wadi Sedr landraces. Seven bands were polymorphic giving a 23.33% polymorphism among the cultivars examined. The protein assay permitted the identification of only one variety by unique markers. It showed that Wadi Sedr landrace was characterized by one negative unique protein band with molecular weight of 125 kDa.

3.2. Genetic relationships among the studied cultivars as revealed by protein analysis

According to similarity matrix of protein analysis, the highest similarity value (0.967) was recorded between Giza 2000 and (Giza 127, Giza 128 and Giza 129) indicating that Giza 127, Giza 128 and Giza 129 were closely related to Giza 2000. On the other hand, the lowest similarity value (0.767) was recorded between Giza 126 cultivar and Wadi Sedr landrace indicating that these were distantly related cultivars as shown in **(Table 8 and Figure 6)**.

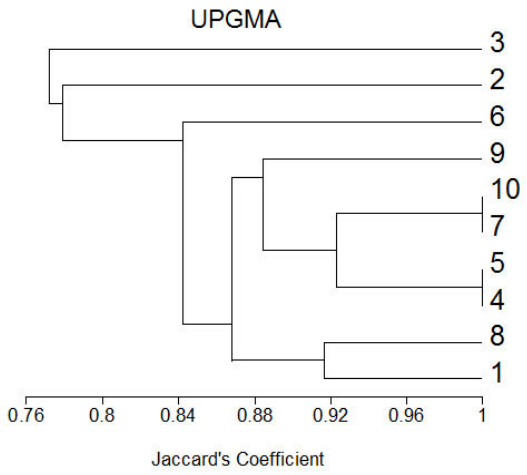

Figure (4): Dendrogram representing the genetic relation-ship among the ten *Hordeum vulgare* L. taxa. using UPGMA cluster analysis of jaccard´s coefficient generated from SSR markers. Cultivars: 1-Giza 123, 2-Giza 126, 3-Giza 127, 4-Giza 128, 5-Giza 129, 6-Giza 130 and 7- Giza 2000. Landraces: 8- El-Kheroba, 9- El-Sheikh Zuwaid and 10- Wadi Sedr.

Table (7): Genetic similarity of the ten *Hordeum vulgare* L. varieties based on SSR banding patterns.

	Giza 123	Giza 126	Giza 127	Giza 128	Giza 129	Giza 130	Giza 2000	El-Kheroba	El-Shikh Zuwaid	Wadi Sedr
Giza 123	1									
Giza 126	0.769	1								
Giza 127	0.769	0.714	1							
Giza 128	0.917	0.846	0.846	1						
Giza 129	0.917	0.846	0.846	1	1					
Giza 130	0.833	0.769	0.769	0.917	0.917	1				
Giza 2000	0.846	0.786	0.786	0.923	0.923	0.846	1			
El-Kheroba	0.917	0.714	0.714	0.846	0.846	0.769	0.923	1		
El-Shikh Zuwaid	0.769	0.714	0.714	0.846	0.846	0.769	0.923	0.846	1	
Wadi Sedr	0.846	0.786	0.786	0.923	0.923	0.846	1	0.923	0.923	1

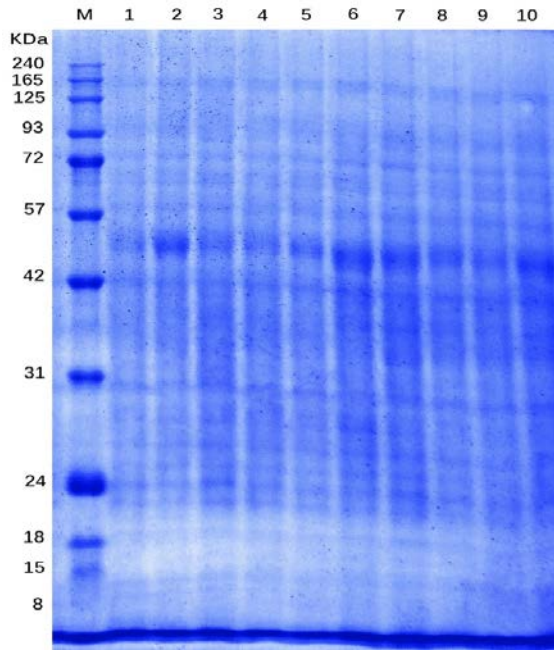

Figure (5): Electrophoretic patterns of ten *Hordeum vulgare* L. taxa for leaves water soluble proteins.
M: Protein marker (245, 165, 125, 93, 72, 57, 42, 31, 24, 18, 15 and 8 KDa)
1- Giza 123, 2- Giza 126, 3- Giza 127, 4- Giza 128, 5- Giza 129, 6- Giza 130, 7- Giza 2000, 8- Sinai, El-Aresh, El-Kheroba, 9- Sinai, El-Aresh, El-Sheikh Zuwaid, 10- Sinai, Ras Sedr, Wadi Sedr.

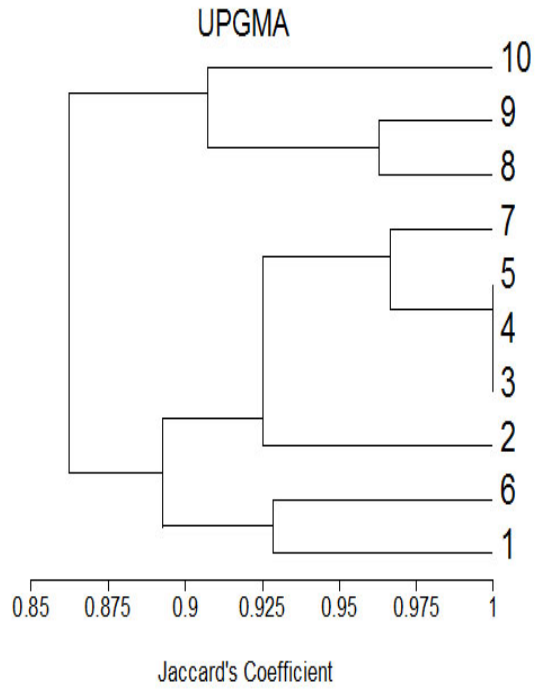

Figure (6): Phenogram of the studied barley taxa using protein analysis.

Cultivars: 1-Giza 123, 2-Giza 126, 3-Giza 127, 4-Giza 128, 5-Giza 129, 6-Giza 130 and 7- Giza 2000.

Landraces: 8- El-Kheroba, 9- El-Sheikh Zuwaid and 10- Wadi Sedr.

Table (8): Genetic similarity matrix of studied *Hordeum vulgare* L. varieties for protein analysis.

	Giza 123	Giza 126	Giza 127	Giza 128	Giza 129	Giza 130	Giza 2000	El-Kheroba	El-Shikh Zuwaid	Wadi Sedr
Giza 123	1									
Giza 126	0.897	1								
Giza 127	0.9	0.933	1							
Giza 128	0.9	0.933	1	1						
Giza 129	0.9	0.933	1	1	1					
Giza 130	0.929	0.897	0.9	0.9	0.9	1				
Giza 2000	0.867	0.9	0.967	0.967	0.967	0.867	1			
El-Kheroba	0.828	0.862	0.867	0.867	0.867	0.893	0.897	1		
El-Shikh Zuwaid	0.862	0.833	0.9	0.9	0.9	0.862	0.931	0.963	1	
Wadi Sedr	0.857	0.767	0.833	0.833	0.833	0.857	0.862	0.889	0.926	1

Discussion

Morphological and physiological traits have been used to measure genetic diversity, but both of these approaches have their limitations, as they are not fully representative of the genetic structure.

Although characterization of cultivars makes it possible to study the level of diversity existing within the cultivars and to establish an index of genetic similarities among different cultivars.

The differentiation between studied *Hordeum vulgare* L. cultivars were depended on some analysis of variance of morphological criteria. We found that, Giza 2000 and Wadi Sedr were considered as the best taxa or genotypes, so we make recommendation to select both cultivars in breeding for enhancement yield productivity through breeding programs where both cultivars gave maximum measurement in most of the studied morphological traits and yield productivity (as outlined in Table 4), while Giza 129 gave the minimum one, so we can excluded it in breeding.

The present study represented that the spike type of all cultivars were six rows except two cultivars Giza 127 and Giza 128 had spike type (two rows) this agrees with **El-Shazly and El-Mutairi (2006)**, while there are two cultivars Giza 129 and Giza 130 were grain Hulless (Naked). The phylogenetic tree was produced by UPGMA based on morphological data stated the pedigree of studied cultivars. Three studied landraces were discriminated according to measurement of different morphological traits and found that they had a similarity with Giza 123, Giza 126 and Giza 2000.

Quantitative estimation of marker viability and the polymorphism can be detected by the mean heterozygosity and the marker index **(Dangi, *et al.*, 2004).**

It has been reported that, the ability to resolve genetic variation may be more directly related to the degree of polymorphism detected by the marker system **(Souframanien and Gopalakrishna, 2004).**

Microsatellites have been successfully applied for detection of genetic diversity **(Korzun *et al.*, 2001 and Russell *et al.*, 1997a)** and genotype differentiation **(Donini *et al.*, 1998 and Virk *et al.*, 1999).**

There are many advantages of SSR markers in genetic studies. They are highly polymorphic, locus specific and abundant. Also, they are distributed over the genome and require only small amounts of genomic DNA for analysis. Microsatellite markers have been developed in many crop species, such as soybean, wheat, maize, barley, rice and potato. In cereals, they show a much higher level of polymorphism than other marker system.

Microsatellites are becoming more widely used for marker assisted breeding and variety

identification due to their high level of polymorphism and ease of use. SSRs become important genetic markers in a wide range of crops species including barley and wheat. They are abundant, dispersed through the genome, and show higher levels of polymorphism than other genetic markers. These features coupled with their ease of detection have made them useful molecular markers. Their potential for automation and their inheritance in a co-dominant manner and additional advantages when compared to other types of molecular markers. SSR loci are believed to evolve in a step-wise manner by the addition or subtraction of single repeat up to 37 different alleles for the one SSR locus have been found in barley **(Maniruzzaman *et al.*, 2014).**

From the present investigation, we found that, seven microsatellites simple sequence repeats (SSRs) primers were employed to investigate the genetic polymorphism among studied barley cultivars, generated a total of 15 bands; 8 of this bands were polymorphic (61.22%). The polymorphic information content (PIC) measure the marker diversity varied from 0.00 to 0.269 where primers GMS1 and Bmag149 had the highest PIC values while primers EBmac624 and HVITR1 had lowest PIC value. SSR markers characterized four unique markers among the ten studied cultivars, including one positive unique SSR markers and three negative unique markers, Bmag13 and GMS1 gave 100% polymorphism while EBmac624 and HVITR1 gave 0% polymorphism, this results agreement with **Chaabane *et al.*, (2009)** and **Mohamed and Adel, (2012)** in the polymorphism percentage with the same primers in barley.

Konarev (1983) stated that, proteins are the primary products in the realization of heredity information and reflect the genetic structure of the organism, SDS-PAGE, which is the most employed techniques for separation and identification of proteins according to their molecular weight **(Haidar *et al.*, 2013; Moradpour *et al.*, 2014 and Natarajan, 2014).**

The use of seed proteins are mainly storage proteins and not likely to be changed in dry mature seed. The use of seed protein electrophoresis is supported by the fact that mature seeds possessed the same protein compounds and this provide valid evidence for relationships of plant **(Khafagi, 2003; Emre *et al.*, 2006; Paragati and sreenath, 2013; Anitalakshmi *et al.*, 2014 and El-Atroush *et al.*, 2015).** Protein patterns of ten cultivars were inspected visually and compared with each other. SDS-PAGE technique of leaf proteins exhibited high similarities between all barley cultivars in their low molecular mass.

UPGMA protein dendrogram is a useful biochemical marker for the identification at the species level **(Khafagi, 2003)** and confirmed the above results obtained. From the results, we observed that the highest similarity value (0.967) was recorded between Giza 2000 and (Giza 127, Giza 128 and Giza 129) indicating that Giza 127, Giza 128 and Giza 129 were closely related to Giza 2000. On the other hand, the lowest similarity value (0.767) was recorded between Giza 126 cultivar and Wadi Sedr landrace indicating that these were distantly related cultivars.

Conclusion

It was found that, the genetic dissimilarity tree was produced by hierarchical cluster analysis based on morphological data stated the pedigree of studied cultivars. However, the genetic similarity tree was produced by UPGMA based on molecular markers (SSRs) and soluble proteins patterns were not in full agreement with the pedigree.

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