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GENETIC DIVERSITY AMONG EGYPTIAN BARLEY CULTIVARS USING MULTIVARIABLE AND SRAP MARKER ANALYSES

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ABSTRACT

A two-year field screening analysis was carried out in of Nubaria Agricultural Research Station during the two growing seasons 2015/ 2016 and 2016/2017 to determine the genetic diversity and relationships among 19 Egyptian barley cultivars using multivariable analysis for some physiological, quality, agronomic characters, grain yield and Sequence-Related Amplified Polymorphism (SRAP) marker analysis. Variance analysis of the traits showed that there are significant differences among the genotypes under study with respect to all traits. Principal component analysis was carried out for all traits under study clarifying about 53.6% of total variance. Cluster analysis revealed four distinct groups which were clustered according to their morphological traits Similarity levels among the pairs of cultivars ranged between 39.77 and 98.36% .Seven SRAP combination primers were used to assess the genetic variation among all cultivars. The primers showed that the average percentage of polymorphic loci was 65.2% and the average band number amplified from each pair of primers was 5.14 bands, of which included 6.2 polymorphic bands. Highest PIC was related to primer me6+em5 was (0.94) indicating that this primer is highly informative. The average percentage of polymorphic loci for all cultivars was 79.3%. The average of both genetic diversity indicters such as effective number of alleles and Shannon's diversity index were (1.205 and 3.802) respectively. The dendrogram of SRAP markers had clustered all the Egyptian cultivars into four groups each group include the most closed cultivars together with genetic similarity coefficients (GSC) ranging from (0.66) to (1.00). The results of the present study showed that there were high genetic differences among Egyptian barley cultivars which provide new information about the relationships among Egyptian barley cultivars which are useful for cultivar identification and for their utilization in further barley breeding programs.

Key words: ***Hordeum vulgar, Agro- morphological traits, Multivariable analysis, SRAP markers, PIC, UPGMA cluster analysis***

INTRODUCTION

Barley, (*Hordeum vulgare* L.) is documented as one of the most economic and important cereals in the world. On behalf of the area and production, barley is the fourth most important cultivated crop, following, wheat, rice and maize. It can be grown in a wide range of environmental conditions and gives acceptable yields in areas that are not suitable for growing most of the other cereals crops due to problems of abiotic and biotic stress (Katja *et al* 2009).

Assessment of the genetic diversity in a crop species is fundamental to its improvement (Cao *et al* 1998). The estimation of genetic diversity can beanalyzes by a specific method or a combination of methods such as pedigree records, morphological traits, biochemical markers and molecular

markers. Pedigree analysis, which was the most widely used for estimating the degree of similarity between cultivars or populations, but the necessary information on pedigree is not always available or accurate (Guasmi *et al* 2012).Morphological and physiological analysis methods also are commonly used to study the genetic diversity within barley germpalsm. However these methods are limited for some stages of plant growth and might be affected by environment (Massood *et al* 2003). Principal Component Analysis (PCA) have been used in barley as a powerful tool for grouping and screening of huge number of cultivars based on morphological traits (Sharafi *et al* 2014). Cluster analysis can assist as a tool of selection and data reduction *via* similarity coefficient based on morphological traits. Also, it provides useful information about genetic diversity in crops, such as barley (Ibrahim *et al* 2011)

Molecular markers are an important tool to evaluate the genetic variation among relatives without effect of environment and considered as a tool with conventional breeding for crops improvement. In barley different molecular markers were used for genetic diversity such as RFLPs (Noli *et al* 1997), RAPD (Guasmi *et al* 2012), SSR (Varshney *et al* 2008), ESTs (Salem *et al* 2010) and SNP (Varshney *et al* 2008).Sequence-Related Amplified Polymorphism (SRAP) as a new markers has been established to be a suitable tool for genetic diversity studies more than other markers because of its simplicity, reproducibility, discloses numerous, and co-dominant markers (Li and Quiros 2001).SRAP marker is becoming the marker of choice for classification and genetic diversity studies of many cereal crops such as barley (Yang *et al* 2008 and 2010 , and Samah *et al* 2017a and 2018b). This study aimed to evaluate genetic diversity and relationships among Egyptian barely cultivars for physiological, grain protein percentage, agronomic characters and grain yield, using Multivariable and SRAP marker, in order to classify them for use in barley breeding programmers

MATERIALS AND METHODS

Nineteen barley cultivars (*Hordeum vulgare* L.*)* were used in this study (Table 1).The cultivars were grown at Nubaria Agricultural Research Station during the two growing seasons 2015/2016 and 2017/2018. The experiment was conducted in a Randomized Complete Block Design (RCBD) with three replicates. Each plot consisted plant with one cultivar, which content four rows 2.0-m long and 20-cm apart (plot area = 1.6 m^2) with three replications. The recorded characters included; grain protein content (GPC), total chlorophyll content, plant height, days to heading, number of spikes m^{-2}, number of grains spike-^{1}, 1000 grain weight, and grain yield (ardfed^{-1}).

Table 1. Name, type, row number type and pedigree of 19 barley cultivars used in the field experiment.

No.	Name	Type	Row	Pedigree	Year of released
1	Giza 117	Hulled	Six	Baladi 16/Palestine 10	
2	Giza 118	Hulled	Six	Beecher (Introduced from USA)	
3	Giza 119	Hulled	Six	Baladi16/Gem(G.I. 7243)	
4	Giza 121	Hulled	Six	Baladi16/Gem.	
5	Giza 123	Hulled	Six	Giza 117/FAO 86	1988
6	Giza 124	Hulled	Six	Giza 117/Bahteem 52// Giza 118/FAO 86	
7	Giza 125	Hulled	Six	Giza117 / Bahteem52// Giza118 /FAO86(sister line to G.124	1995
8	Giza 126	Hulled	Six	BaladiBahteem/S D729-Por12762-BC.	1995
9	Giza 127	Hulled	Two	W12291/B0gs//Hamal-02	1996
10	Giza 128	Hulled	Two	W12291/4/11012-2170-22425/3/"Apam"/"B65"//"A16"	1996
11	Giza 129	Hulless	six	DeirAlla 106/Cel//As46/Aths*2''	2001
12	Giza 130	Hulless	six	Comp.cross"229//Bco.Mr./DZ02391/3/Deir Alla 106	2001
13	Giza 131	Hulless	six	CM67B/CENTENO//CAMB/3/ROW906.73/4/GLORIABAR/ COME-B/5/FALCONBAR/6/LINO	2001
14	Giza 132	Hulled	Six	Rihane-05//AS 46/Aths*2Athe/ Lignee 686	2006
15	Giza 133	Hulled	Six	ICB91-0343-0AP-0AP-0AP-281AP-0AP	2011
16	Giza 134	Hulled	Six	ICB91-0343-0AP-0AP-0AP-289AP-0AP	2011
17	Giza 135	Hulless	six	ZARZA/BERMEJO/4/DS4931//GLORIABAR/COPAL/3/SEN/5/AYAROS	2011
18	Giza 136	Hulless	six	PLAISANT/7/CLN-B/LIGEE640/3/S.P-B//GLORIAAR/ COME B/5/FALCONBAR/6/LINOCLN-B/A/S.P-/LIGNEE640/3/S.P-B//GLORIA-BAR/COME B/5/FALCONBAR/6/LINO	2011
19	Giza2000	Hulled	Six	Giza117/Bahteem52// Giza118/ FAO86 / 3/Baladi16/ Gem	2003

Molecular Marker*s*

DNA Extraction and SRAP – PCR Amplification

Genomic DNA of the 19 barley cultivars under investigation was extracted from leaves using CTAB method according to Doyle and Doyle (1990). DNA con-centration was measured using Nanodrop (ND-1000 Spectrophotometer). PCR cycling was carried out as the following program; initial denaturation at 94 °C for 4 min, followed by five cycles comprising for 1-min denaturation at 94 °C, 1-min annealing at 35 °C, and 30 s of

elongation at 72 °C. In the following 30 cycles, denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, and elongation at 72 °C for 30 s were carried out, ending with an elongation step for 10 min at 72 °C. Seven SRAP primer combinations were used their names and sequencing are listed in Table 2. The PCR products were separated by electrophoresis using 2% agarose gel in 1 x TAE buffer against 100 bp DNA Ladder as a size marker. Bands were detected with ethidium bromide staining and visualized under UV light, then photographed on Gel Documentation.

Table 2. Seven SRAP primer combinations their names and sequences

Name	primer sequences	Name	primer sequences
me2	**F: TGAGTCCAAACCGGAGC**	**em3**	**R:GACTGCGTACGAATTAAT**
me2	**F:TGAGTCCAAACCGGAG**	**em4**	**R:GACTGCGTACGAATTTGC**
me4	**F:TGAGTCCAAACCGGAGC**	**em6**	**R:GACTGCGTACGAATTGAC**
me5	**F:GAGTCCAAACCGGAAG**	**em4**	**R:GACTGCGTACGAATTTGC**
me5	**F:GAGTCCAAACCGGAAG**	**em6**	**R:GACTGCGTACGAATTGAC**
me6	**F: TGA GTC CAA ACC GGA CA**	**em5**	**R: GACTGCGTACGAATTTGC**
me6	**F: TGA GTC CAA ACC GGA CA**	**em6**	**R:GACTGCGTACGAATTGAC**

Data analysis

Agro-morphological traits analysis

Analysis of variance for combined analysis for all traits of 19 barley cultivars across two studied seasons, revealed accepted homogeneity of errors (Bartlett 1937). All statistical analyses were performed using the computer software MSTAT-C Computer Program according to Steel *et al* (1997) and, using Duncan's New Multiple Range Test (Duncan 1955).Principal component and Cluster analysis using Euclidian coefficient average linkage method was performed to get the cluster and genetic similarity based on the morphological traits using a computer software program Minitab v.12 (1996) according to Kovach (1995).

Molecular markers analysis.

The amplified bands from SRAP were scored as a binary data under the heading of total scorable bands determined for each cultivar. The data were used to estimate the genetic similarity on the basis of number of shared amplification products Nei and Li (1979). Polymorphism information content (PIC) values were done to distinguish between cultivars for each primer according to Anderson *et al* (1993). Cluster analysis was performed to produce a denderogram using un-weighted pair-group method with arithmetical average (UPGMA) based on Jaccard's similarity coefficient using PAST program adapted by Hammer *et al*. (2001).

RESULTS AND DISCUSSION

Agro- morphological traits analysis

Analysis of variance of physiological (total chlorophyll content SPAD),quality (grain protein content) and agronomic characters (days to heading, plant height), and grain yieldand its components(number of grains spike^{-1},number of spikes m^2 and 1000 grain weight) showed that there existed significant differences among the genotypes under study with respect to all traits as shown in Table (3). Total Chlorophyll content is one of the major factors affecting photosynthesis; the results clearly indicated that the cultivars differed significantly in total chlorophyll content. Highest total chlorophyll content was found in Giza 131 with value of 55.1 SPAD and the lowest total chlorophyll content found in Giza 121 (40.6 SPAD).

Barley grain is used primarily as an energy and protein source for animal feed. High protein content is desirable for feed production. In this study, protein content ranged from 8.8% for Giza 117 to 15.2% for Giza135 (Table 3). Earliness is other important and favorable characters in barley, due to farmers need to produce second crops especially for animal feed in the same growing cycle. Days of heading ranged from 90.3 (Giza 123) to 102.2 days (Giza 121) among all cultivars with an average 96.7 days; it means that there was significant differences among genotypes.Plant height is one of the most important early selection criteria in barley breeding, being a direct component of lodging resistance and an indirect component of both yield and quality .In this study the shortest cultivar was Giza 133 (87.0 cm). The tallest cultivar was Giza 2000 with value of 120.8 cm.

There was a highly significant variation in 1000-grain weight of barley cultivars based on their spike type and other traits. The highest 1000-grain weight was measured in Giza 127 and Giza 128 which they have two-row spike types. 1000-grain weight ranged from 46.1 g (Giza 132) to 61.6 g (Giza 128) among all cultivars an average of 55.4 g. Concerning No .of spikes m $^{-2}$, the results in Table (3) showed that Giza 2000 gave the highest number of tillers m^{-2}with values of 663.5 but Giza 135 and Giza 126 showed the lowest number of tillers m^{-2} (340.0 and 364.2) respectively. Regarding, No. of grains spike^{-1}, the data in Table (3) showed that Giza 131 (six row) had the highest number of grains spike^{-1} with mean of 72.7 and the lowest number of grains (28.3) was detected by Giza 127(two row).

Grain yield is a complex trait depending upon a large number of environmental, agronomical and physiological factors. In this study a significant differences were found among all cultivars with mean yield of 15.3 ard fed^{-1}. According to mean value of the cultivars the highest grain yield was determined by Giza 130 with (20.2 ardfed^{-1}) followed by Giza 2000 (19.6 ard fed^{-1}) and the lowest value was 14.1ard fed^{-1} in Giza 118. Genetic diversity in barley breeding program based on morphological traits and pedigree information was measured by Chand *et al* (2008), Eshghi and

Akhundova (2010) and Samah *et al* (2017). They showed that grain yield is the final product of the action and interaction of number of components such as number of tillers, number of grains spike^{-1}, 1000-grain weight, plant height and harvest index.

Table 3. Combined means analysis of physiological, quality, agronomic characters and grain yield, of 19 tested Egyptian barley cultivars across two growing seasons.

Cultivars	Total Chlorophyll SPAD	Protein content %	Heading data (days)	Plant height (cm)	Grain Yield (ard fed^{-1})	1000 grain weight (g)	No. spike m^{-2}	No. grains spike^{-1}
Giza 117	45.6	8.8	96.7	100.0	15.1	51.6	442.1	66.0
Giza 118	46.6	9.0	97.2	96.3	14.1	58.5	495.8	64.0
Giza 119	41.1	10.0	95.7	107.7	14.7	55.1	430.8	61.5
Giza 121	40.6	10.1	102.2	109.5	18.0	56.8	505.0	64.0
Giza 123	47.0	10.9	90.3	110.0	19.5	60.8	655.8	66.0
Giza 124	46.6	11.7	100.5	102.3	17.9	51.7	554.2	64.0
Giza125	48.7	10.8	101.3	109.7	15.7	54.3	365.8	62.0
Giza126	49.3	10.6	99.7	108.8	16.7	58.3	364.2	72.0
Giza127	43.1	10.7	94.5	113.5	17.7	61.4	619.2	28.3
Giza128	48.0	11.1	95.8	112.2	18.8	61.6	613.8	29.0
Giza 129	43.2	11.8	92.5	111.0	15.1	56.4	466.7	66.0
Giza 130	47.5	11.9	91.7	112.5	20..2	52.9	643.8	63.0
Giza 131	55.1	12.1	94.8	99.2	17.7	60.2	549.2	72.7
Giza 132	47.1	11.1	102.0	91.3	14.6	46.1	468.3	61.0
Giza 133	45.6	11.4	101.7	87.0	15.4	54.6	603.3	64.0
Giza 134	47.0	10.2	98.8	113.7	15.2	55.1	557.5	66.0
Giza 135	45.5	15.2	92.2	110.5	16.5	51.9	340.0	66.7
Giza 136	50.1	12.2	93.5	116.2	18.2	48.7	486.7	72.0
Giza 2000	51.3	10.1	94.0	120.8	19.6	56.3	663.5	71.3
Average	96.4	10.9	96.7	106.9	15.3	55.4	521.8	62.1
LSD 0.05	1.56	0.35	1.5	2.3	0.46	1.56	24.9	1.99
C.V%	2.82	2.25	1.25	1.87	5.7	2.5	4.1	2.7
F test	**	**	*	**	**	**	*	*

Multivariable analysis

Principal component analysis (PCA)

The PCA analysis was applied to identify the traits that were the main source of the variability and to illustrate the genetic diversity among the 19 Egyptian barley germplasm. The first and two principal components accounted for 53.6% (PCA1=30.4% +PCA2 =23.2%) of the total variability among the 19 cultivars for all the investigated traits as shown in (Table 4 & Fig. 1). The first principal component (PCA1) could justify the most amount of variance among genotypes (30.4%). Positive correlation was found between this component of the variation and grain protein content, 1000 grain weight, no. grains spike, plant height, no. spikesm^{-2} and grain yield. Regarding high correlation between first component and yield and other traits associated with that, this component can be called yield component. BiPlot analysis as shown in (Fig. 1), grouped all cultivars according the PCA1 and PCA2 values.

Table 4. Estimates of the first two principal components for all characters evaluated on 19 barley cultivars across two growing seasons (2015/2016 and 2016/2017).

Parameters	PCA1	PCA2
Eigenvalue	2.42	1.85
Percentage variance (%)	30.4	23.2
Cumulative variance (%)	30.4	53.6
Traits		
Total Chlorophyll content SPAD	-0.185	0.27
Protein ratio % PR	0.10	-0.52
Heading data (day)HD	-0.38	0.49
Plant height (cm) PH	0.33	-0.32
Grain yield(ardfad^{-1}) GR	0.33	-0.29
1000 grain weight	0.42	-0.22
No spikes m^{-2} SP/M	0.46	-0.31
No grain spike $^{-1}$ (g) K/SP	0.401	-0.273
Cultivars		
Giza 117	-1.05	0.86
Giza 118	-0.44	1.51
Giza 119	-0.03	0.51
Giza 121	1.45	-1.96
Giza 123	2.22	-0.11
Giza 124	-0.60	0.09
Giza 125	0.12	-0.67
Giza 126	1.86	-0.47
Giza 127	3.28	-1.22
Giza 128	0.96	-0.78
Giza 129	-0.42	0.97
Giza 130	0.18	-0.30
Giza 131	1.20	-0.13
Giza 132	-2.39	0.67
Giza 133	-1.22	-1.54
Giza 134	-0.01	-0.31
Giza 135	0.48	-0.49
Giza 136	-0.38	1.47
Giza 2000	1.28	-0.93

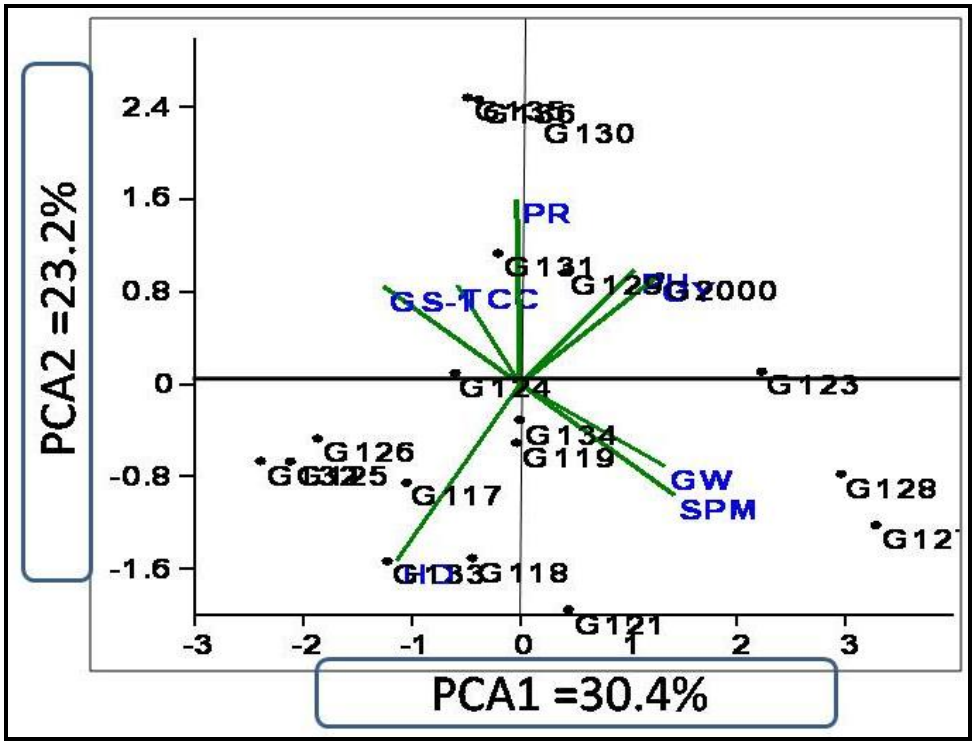

Fig. 1. Biplot analysis of 19 barley cultivars based on morphological and PCA values.

Cultivars that were selected by PCA1 were grouped into groups according their values, group 1 included the cultivars that had high positive PCA1 values more than one such as Giza 121, Giza 123, Giza 126,Giza 127, Giza 131 and Giza 2000. The second group II includes the cultivars which had positive PCA1 and values less one such as Giza 125, Giza 128, Giza 130 and Giza135. The second principal component PCA2 clarified 23.2 % of the total variability subjective by total chlorophyll content , number of grains spike^{-1} and days to heading**.**

Cluster analysis and genetic similarity

likewise to PCA analysis, the cluster analysis of 19 barley cultivars based on the average of all studied characters across the two seasons constructed a distance matrix using the Euclidian coefficient average linkage method is displayed in Table (5) and graphically illustrated in dendrogram showing similarity among all the cultivars (Fig. 2). The 19 cultivars were divided into four groups. The cultivars in each group were selected in Principal component analysis in the same discrete group in cluster analysis. The first and second group includes the cultivars that had the high performance of studied traits, high grain yield, high and moderate

values of PCA1 such as first group (Giza Giza 121, Giza 123, Giza 126, Giza 127, Giza 131 and Giza 2000) and second group (Giza 125, Giza 128 and Giza 135). The similarity levels among the pairs of cultivars ranged between 39.77 and 98.36%. The highest similarity level was recorded between two cultivars Giza 130 and Giza 135 equaled 98.36% with distance coefficient of 6.25 followed by two cultivars Giza 127 and Giza 128 with 98.02% and between Giza 133 and Giza 134 recorded 97.10 and 96.66% similarity level respectively.

Table 5. Similarity levels for 19 barley calculate by cluster analysis based on agro–morphological traits.

Step	Number of cluster	Similarity level	Distance level	Clusters joined		New cluster	Number of new entries
1	18	98.36	6.251	12	17	12	2
2	17	98.02	7.566	9	10	9	2
3	16	97.10	11.060	7	8	7	2
4	15	96.66	12.717	6	16	6	2
5	14	95.92	15.562	1	3	1	2
6	13	95.04	18.909	6	13	6	3
7	12	94.98	19.139	5	19	5	2
8	11	93.73	23.898	11	18	11	2
9	10	93.17	26.057	7	12	7	4
10	9	92.22	29.670	2	11	2	3
11	8	92.02	30.414	2	14	2	4
12	7	89.77	39.019	4	5	4	3
13	6	8.5	45.579	1	2	1	6
14	5	85.76	54.278	6	15	6	4
15	4	83.95	61.195	4	9	4	5
16	3	73.10	102.565	1	6	1	10
17	2	59.90	152.882	1	7	1	14
18	1	39.77	229.647	1	4	1	19

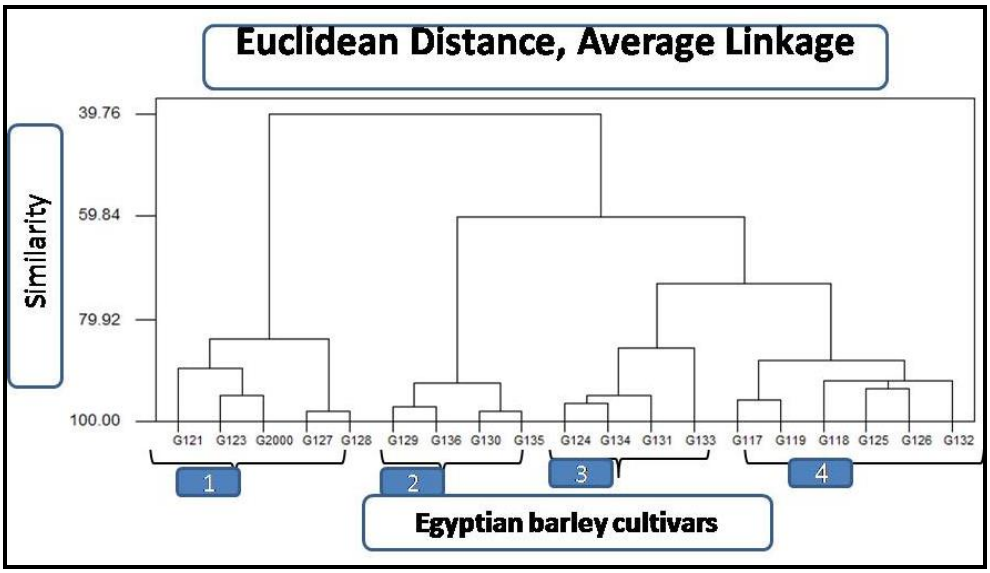

Fig. 2. Cluster analysis based on agro– morphological traits to classify 19 barley cultivars.

Lowest similarity level was obtained between Giza 117 and Giza 121 (39.77%). There were 13 couples of cultivars that were closely related to each other where the similarity level among them was more than 80% Meanwhile the results showed that cultivars Giza 124, Giza 125, and Giza 121 had low similarity levels (dissimilarity) with (Giza 117) and may produce good results if they are crossed together. It is notable that cluster analysis considered a valuable tool for subdividing number of genotypes in groups including similarity and dissimilarity genotypes which would help the breeder to plan an effective breeding program These results were in a good harmony with results of Žáková, and Benková (2006), Eshghi and Akhundova (2010), Meng *et al* (2016), Samah and Rania (2017), Arshadi (2018) and Samah *et al* (2018a).

Molecular marker analysis

Amplification results of SRAP-PCR marker analysis

In total, 59 bands were amplified with seven SRAP primer combinations. The number of amplified bands ranged from 6 to 10, with the molecular size between 100 to 1,300 bp. Results in Table (6) showed that the average percentage of polymorphic loci for all primer combinations was 67.7% and the average band number amplified from each pair of primers was 8.42% bands, of which included 6.0 % polymorphic bands. The highest polymorphism (90.0%) (Fig. 3) was found by primer me6+em6. The lowest polymorphism (16.6%) were found by primer me5+em4 (Fig. 3). Polymorphic information content (PIC) values, used to measure the genetic

diversity ranged from 0.18 to 0.94 with an average of 0.69%. The highest PIC was related to primer combination me6+em6 was (0.94), indicating that this primer combination is highly informative (PIC >0.5 Botstein *et al* 1980).

Table 6. List of used SRAP primer names, sequences, No. of total fragments, No. of polymorphic bands, polymorphism% and polymorphism information contents (PIC).

	Name	No. of Total band	No. of polymorphic bands	Polymorphism %	polymorphic information content PIC
1	me2+em3	9	7	77.7	0.78
2	me2+em4	8	6	75.0	0.76
3	me4+em6	7	4	57.1	0.58
4	me5+em4	6	1	16.6	0.18
5	me5+em6	9	7	77.7	0.78
6	me6+em5	10	8	80.0	0.82
7	me6+em6	10	9	90.0	0.94
Average		8.42	6.0	67.7	0.69
Total		59	42		

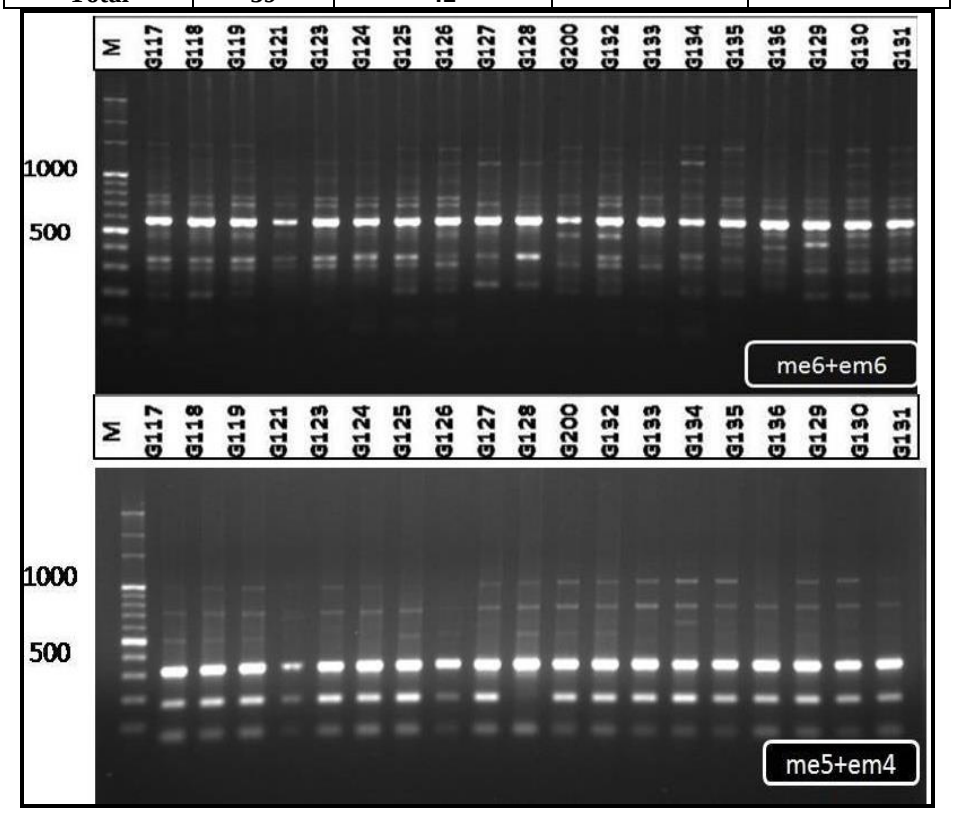

Fig. 3.Agarose gel electrophoresis of SRAP amplification products of 19 different Egyptian barley cultivars.

Genetic diversity among the 19 cultivars

Results in Table 7 showed genetic diversity among 19 Egyptian barley cultivars, the percentage of polymorphic loci ranged from 59.1% (Giza 121) to 89.5% for (Giza 127, Giza 2000, Giza 136 and Giza 133). The average percentage of polymorphic loci for all cultivars was 79.3%. Polymorphic information content (PIC) values, used to measure the genetic diversity ranged from 60.2% to 92.1% with an average of 80.8%. High genetic diversity among all cultivars was found for Giza 127 (92.4%) and Giza 2000, Giza 136 and Giza 133 with a value of 92.1%.

The effective number of alleles and Shannon's diversity index both were valuable indexes for estimation of genetic diversity level are shown in Table (7).The obtained effective number of alleles ranged from ranged from 0.975 for Giza 117 to 1.311 for Giza 126 with an average of 1.205. The obtained Shannon's information index ranged from 3.466 (Giza 121) to 3.932 (Giza 127, Giza 2000, Giza 136 and Giza 133) with average of (3.802). Moreover, the changes of these indexes were consistent with the percentage of polymorphic loci.

Table 7. Genetic diversity among 15 barley cultivars using seven SRAP primer combinations.

Cultivars	Total polymorphic band	Total monographic band	Percentage of polymorphic bands	Shannon's information index	Effective number of alleles	polymorphic information content PIC
Giza 117	44	13	77.2	3.784	0.975	0.781
Giza118	34	23	59.6	3.526	1.06	0.601
Giza 119	45	12	78.9	3.807	1.151	0.812
Giza 121	32	25	59.1	3.466	1.172	0.602
Giza123	38	19	66.7	3.638	1.069	0.657
Giza124	42	15	73.7	3.738	1.273	0.745
Giza 125	50	7	87.7	3.912	1.237	0.897
Giza 126	41	16	71.9	3.714	1.311	0.725
Giza 127	51	6	89.5	3.932	1.219	0.924
Giza 128	48	9	84.2	3.871	1.286	0.852
Giza 2000	51	6	89.5	3.932	1.287	0.921
Giza 136	51	6	89.5	3.932	1.238	0.921
Giza 133	51	6	89.5	3.932	1.109	0.921
Giza 134	43	14	75.4	3.761	1.241	0.768
Giza 135	45	12	78.9	3.807	1.289	0.791
Giza 136	50	7	87.7	3.912	1.283	0.881

Giza 129	**48**	**9**	**84.2**	**3.871**	**1.266**	**0.851**
Giza 130	**46**	**11**	**80.7**	**3.829**	**1.219**	**0.813**
Giza 131	**49**	**8**	**86.0**	**3.892**	**1.219**	**0.893**
Average	**45.2**	**11.8**	**79.3**	**3.802**	**1.205**	**0.808**

UPGMA Cluster analysis

Cluster analysis shaped a dendrogam among the 19 Egyptian barley cultivars based on seven SRAP fragments using Jaccard's genetic similarity coefficient and outlined by the Un-weighted Pair-Group Method (UPGMA) (Fig4). The dendrogram of SRAP markers had clustered all the Egyptian cultivars into four groups, each group include the most close cultivars together. Group I consisted of three Egyptian barley cultivars (Giza 121, Giza 124 and Giza 133) .Group II consisted of four Egyptian barley cultivar (Giza 136, Giza 117, Giza 132 and Giza 2000). However, Group III consisted of six Egyptian barley cultivar (Giza 126, Giza 125, Giza 127, Giza 129, Giza 135 and Giza 118). Group IV consisted of six Egyptian barley cultivar (Giza 119, Giza 123, Giza 131, Giza 134, Giza 128 and Giza 130), indicting the close relationship within each of pair of barley cultivars.

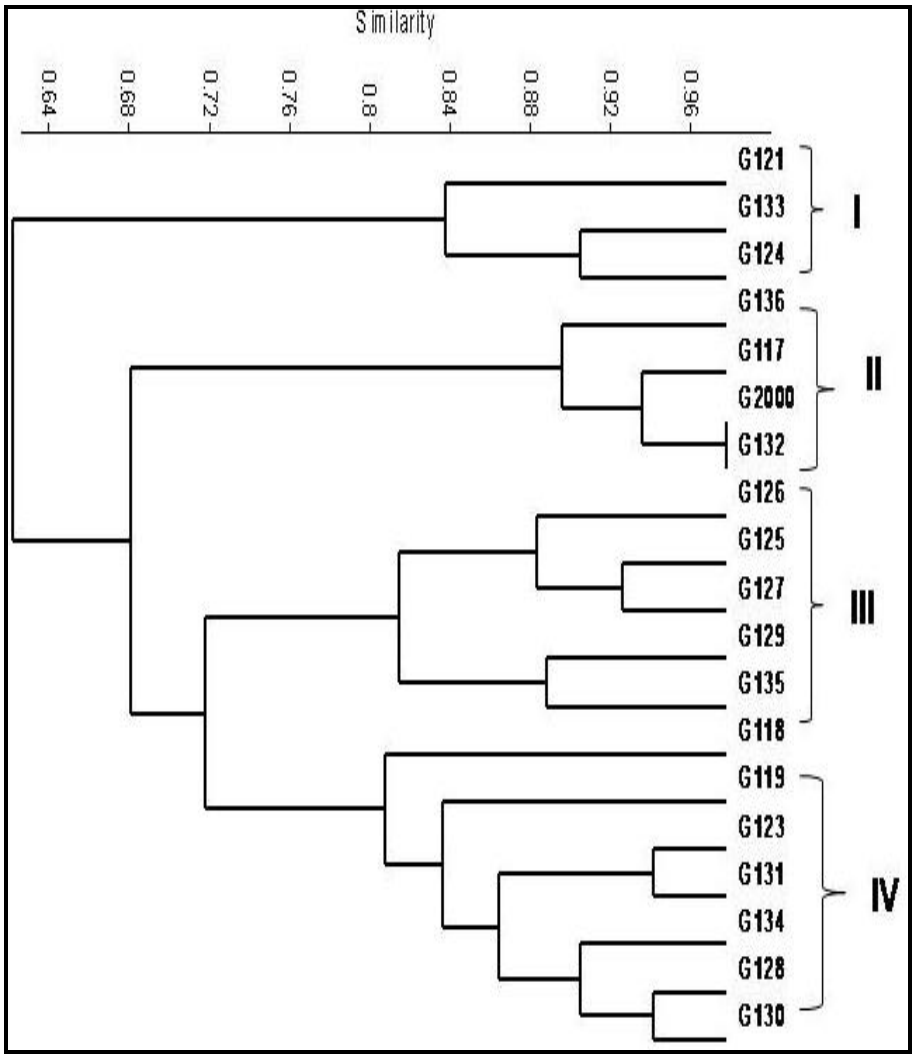

Fig. 4. Dendrogram obtained from UPGMA cluster based on SRAP data.

Genetic Similarity based on SRAP markers

The genetic similarity is an important index for estimation of the genetic differentiation among Egyptian barley cultivars using Jaccard's similarity coefficients (Table 8).The genetic similarity coefficient (GSC) ranged from low similarity (0.66) (between Giza124 and Giza118) which proposes that these were the least-related cultivars to high similarity (1.00) between(Giza2000 and Giza132). Also High similarity (0.96%) were observed between Giza117and both of Giza2000 and Giza132 and Giza 130 and Giza 131, indicating that was a very close relationship among these cultivars.

Table 8. Genetic distance estimates for 19 barley cultivars based on seven SRAP primers markers analysis.

CUL.	G117	G118	G119	G121	G123	G124	G125	G126	G127	G128	G2000	G132	G133	G134	G135	G136	G129	G130
G118	0.81																	
G119	0.88	0.77																
G121	0.65	0.60	0.62															
G123	0.88	0.84	0.85	0.75														
G124	0.76	0.66	0.72	0.69	0.79													
G125	0.83	0.79	0.79	0.64	0.86	0.86												
G126	0.79	0.74	0.75	0.72	0.81	0.76	0.89											
G127	0.82	0.78	0.79	0.69	0.85	0.79	0.93	0.82										
G128	0.85	0.81	0.88	0.65	0.88	0.76	0.89	0.85	0.89									
G2000	0.96	0.84	0.85	0.68	0.92	0.72	0.86	0.81	0.85	0.88								
G132	0.96	0.84	0.85	0.68	0.92	0.72	0.86	0.81	0.85	0.88	1.00							
G133	0.82	0.71	0.79	0.63	0.85	0.86	0.86	0.82	0.79	0.82	0.79	0.79						
G134	0.79	0.75	0.82	0.61	0.82	0.71	0.83	0.79	0.83	0.93	0.82	0.82	0.77					
G135	0.81	0.70	0.78	0.62	0.78	0.67	0.79	0.75	0.85	0.81	0.85	0.85	0.72	0.76				
G136	0.93	0.75	0.82	0.61	0.82	0.77	0.83	0.79	0.77	0.79	0.89	0.89	0.83	0.74	0.76			
G129	0.82	0.71	0.72	0.63	0.79	0.68	0.80	0.82	0.79	0.82	0.85	0.85	0.73	0.77	0.79	0.77		
G130	0.89	0.85	0.85	0.69	0.92	0.79	0.93	0.89	0.93	0.96	0.92	0.92	0.86	0.89	0.85	0.83	0.86	
G131	0.85	0.81	0.81	0.72	0.96	0.82	0.89	0.85	0.89	0.92	0.88	0.88	0.89	0.86	0.81	0.79	0.82	0.96

In this study, SRAP marker gave 57 alleles which were amplified by seven primer combinations in 19 cultivars, it was higher in alleles number than other DNA markers in the genetic diversity in barley such

RAPD (Guasmi *et al* 2012), SSR (Varshney *et al* 2007) and ESTs (Salem *et al* 2010). Different polymorphism and number of amplified band has been detected in barley using SRAP markers. Yang *et al* (2010) reported 86.6% polymorphic bands in hulless barley in China with 7.35 average number of bands per primer. Mariey *et al* (2017) found 100% polymorphic bands with 6.4 average number of bands per primer in Egyptian barley for salinity stress. Samah *et al* (2018b) found 100% polymorphic bands with 6.4 average number of bands per primer in Egyptian for water stress. The high polymorphic percentage (94%) and PIC value (0.96), together with a moderate genetic similarity (0.96) observed among 19 cultivars in this study suggests a high level of heterogeneity. The high polymorphism percentage in this study agree with those obtained by (Yang *et al* (2008 and 2010), Said *et al* (2015) and Mariey *et al* (2017) and Samah *et al* (2018b) who used SRAP marker to evaluate the genetic diversity in barley and suggested that SRAP technology is useful for genetic diversity and relationship analyses, marker assisted selection and genetic map construction in barley. From the data, it is clear that there was a wide genetic diversity among 19 Egyptian barley cultivars based on the seven SRAP markers analysis.

The association of molecular markers with phenotypic evaluation is one of important factors to understand and investigate the genetic role of tolerance by prediction the genomic regions that affect the plant's response (*Roy et al 2011*). In the present study, morphological characters analysis (physiological, quality, agronomic characters and grain yield) of the Egyptian barley cultivars was used with molecular analyses (SRAP marker) to investigate the genetic relationships among 19 Egyptian barley cultivars. SRAP marker was able to differentiate among different DNA of high and low performance in all agronomic traits evaluated. Dendrogram based on SRAP rather than agree with morphological characters distance. Also, the range of genetic distance based on morphological characters was on average near to SRAP markers. From these results it is noted that 19 Egyptian barley cultivars showed a significant variation in agronomic traits and SRAP polymorphisms. This study using SRAP markers and agronomic traits revealed considerable amount ofgenetic diversity among 19 barley cultivars. The SRAP data can be used in selectingdiverse parents in breeding program and in maintaining genetic variation in the germplasm. Also, this study shows that analyzing higher numbers of genotypes may not add much practical value to a general plant improvement program, unless a specific crossing program is aimed towards the improvement of specific traits. It is therefore suggested that a focused breeding scheme should be adopted while analyzing genome diversity estimates for parent selection to gain maximum value and practical impact on a breeding program. The results provide new information about the relationships between Egyptian barley cultivars which are useful for cultivar identification and their utilization in further barley breeding programs in Egypt.

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دراسة التنوع الوراثي لأصناف الشعير المصرية باستخدام التحليل متعدد المتغيرات وواسمات SRAP

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تم إجراء تجربتين في محطة البحوث الزراعية بالنوبارية خلال موسمين متتاليين ٢٠١٤/٢٠١٥ و ٢٠١٥/٢٠١٦ ، لتحديد التنوع الوراثي والعلاقات بين أصناف الشعير المصرية باستخدام تحليل متعدد المتغيرات لبعض الصفات الفسيولوجية و الجودة و الصفات الحقلية ومحصول الحبوب ودراسة علاقات التتابع لتعدد الأشكال المكبرة (SRAP). أظهر تحليل التباين للصفات وجود فروق كبير بين التراكيب الوراثية لجميع الصفات. تم إجراء تحليل مكون رئيسي لجميع الصفات موضحًا حوالي ٥٣.٦٪ من التباين الكلي. كشف شجرة النسب الوراثية عن أربع مجموعات متميزة تم تجميعها وفقًا لصفاتها المورفولوجية والتي تراوحت مستويات التشابه بين أزواج الأصناف من ٣٩.٧٧ الى ٩٨.٣٦٪. استخدمت سبعة البادئات لتقييم الاختلاف الوراثي بين جميع الأصناف. أظهرت النتائج SRAPأن متوسط النسبة المئوية للمواقع متعددة الأشكال لكل البادئات كانت ٦٥.٢٪ وكان متوسط متعدد الأشكال من كل زوج من البرايمرات ٥.١٤٪، بما في ذلك ٦.٢٪ من متعددة الأشكال. أعلى (PIC) ، كان موجود فى البريمر em5 + me6كان (٠.٩٤) مشيرا إلى أن هذا البريمر هو غني بالمعلومات. كان متوسط النسبة المئوية للمواقع متعددة الأشكال لجميع الأصناف ٧٩.٣٪. وكان المتوسط لكل من عدد مؤشرات للأليل ومؤشر التنوع في شانون هما (١.٢٠٥ و ٣.٨٠٢). وقد جمَّعت شجرة النسب الوراثية بناء على الدليل الجزئي SRAP جميع الأصناف المصرية في أربع مجموعات تضم كل مجموعة الأصناف الأكثر قرابة مع معامل التشابه الوراثي (GSC) من ٠.٦٦ إلى ١.٠٠ . أظهرت نتائج الدراسة الحالية وجود فروق وراثية عالية بين أصناف الشعير المصرية التي تقدم معلومات جديدة عن العلاقة بين أصناف الشعير المصرية القديمة والحديثة التي تفيد في تحديد الأصناف واستخدامها في برامج الشعير الأخرى.

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