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Biodiversity of some Landraces in Barley (*Hordeum vulgar* L.) Germplasm based on Qualitative, Quantities Traits and Molecular Markers

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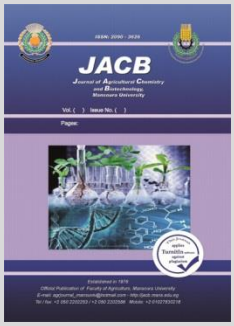

ABSTRACT

The present experiment was conducted in Genetic Resources Research Dept., at Bahteem Research Station during 2017/2018 and 2018/2019 seasons, to estimate genetic biodiversity among barley accessions using various methods. The results showed that the flag leaf anthocyanin coloration and awn color in anthocyanin were classified into two groups, present or absent. Also chlorophyll color and type of leaves were classified into two groups, density and wild linear, light green and linear. In addition, growth habit in awns was classified into two groups, erect and semi erect. On the other hand, wax in flag leaf in first awn and spike were classified into three groups, very weak, weak and medium. Moreover, terminal color in anthocyanin was classified into three groups, very weak, medium and strong. As well as, density of awns was classified into three groups, very density, dense and medium. Otherwise, wax of flag leaves was classified into four groups, very weak, medium, strong and very strong. The results in quantity accession no. 38 gave the highest 1000-kernel weight and kernel weight. The correlation coefficient positive significant between awnless and 1000-kernel weight, also 50% heading date and plant height. Molecular markers analysis evaluated using Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeat (ISSR). A high level of polymorphism was found with both RAPD and ISSR markers. In RAPD analysis total 111 bands out of 59 polymorphic bands and 52 monomorphic, while ISSR analysis total 76 bands out of 37 polymorphic bands and 39 monomorphic bands.

Keywords: Biodiversity, landraces, qualitative, quantities and molecular markers.

INTRODUCTION

Barley (*Hordeum vulgar* L.) is an important crop used for animal feed, malt manufactures and human food. Its importance derives from the ability to grow and produce in marginal environments (Baum *et al.*, 2004). Barley is cultivated in different geographical regions and considered the most important source for animal feeding, local barley landraces were used by farmers in most of the geographical regions of the Egypt. Because of its high adaptability, farmers still cultivate the traditional barley landraces in most of barley cultivation regions. The landraces are the most diverse populations of cultivated plants (Franke *et al.*, 1995). Effective management and utilization of these resources depends to a large extent on appropriate characterization of their genetic diversity. The genetic diversity among and within landraces makes them a valuable resources as potential donors of genes for the development and maintenance of modern crop varieties and for direct use by farmers (Soleri and Smith 1995). Assessment of the extent of genetic variability within barley, including the wild relatives, is fundamental for barley breeding and the conservation of genetic resources, and is particularly useful as a general guide in the choice of parents for breeding hybrids (Hou *et al.*, 2005). Morphological variation for barley collected from different parts of Egypt (Marsa- Matrouh, Sinai and Assiut). A variety of polymerase chain reaction (PCR) based on molecular markers are useful tools for the study of genetic diversity. For detection of genetic variation in barley, different classes of molecular markers were used. However, among these classes, ISSR or microsatellite are short (mostly 2 to 4bp) tandem repeats of DNA sequence, their polymorphism originates from a different number of repetitive core motifs present at one locus (Ellegren, 2004; Matus and Hayes, 2002; Nevo *et al* ., 2005 and Chaabane *et al.*, 2009). ISSRs are co-dominant, abundant and informative and their detection can be automated. This makes them an excellent molecular markers system for many types of genetic analysis including linkage mapping germplasm surveys, and phylogenetic studies (Liu *et al.*, 1996). Some studies used only the random amplified polymorphic DNA (RAPD) markers (Hussein *et al.*, 2005) or RAPD and morphological markers (El-Shazly and El-Mutairi, 2006), to analyze the pattern of genetic diversity within barley accession grown in the Egypt.

Thus, the objective of this study was to investigate the diversity among thirty accession of barley landraces which classified into both two and six rows types. Biodiversity investigation included morphological and agronomic traits determinations and comparing RAPD and ISSR analysis as molecular markers.

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MATERIALS AND METHODS

The thirty accessions of barley obtained from the Genetic Resources Research Dept. Field Crops Research Institute (FCRI) - Agriculture Research Center (ARC) were used in the present study. Planting was done in two seasons (2017/2018 and 2018/2019), at Bahteem Research Station in a randomized complete block design with three replications. Each experiment plot consisted of two rows 4-m long and 20 cm wide. Spacing between plants in the rows was kept at 5cm in environmental condition. At maturity ten plants were randomly selected from each plot for subsequent measurements. Quantities characters included flag leaf anthocyanin coloration, awns color in anthocyanin , wax in flag leaf in first awn and spike, length of a wingless at tip relative to length of ear, chlorophyll color and type of leaves, terminal color in anthocyanin, wax of leaves flag, flag leaf anthocyanin coloration of auricles, density of a wings, growth habit in a wings and degree of wax in a wing, as following in quantities: 50% heading date, physiological maturity date, plant length, length of awn, awnless, No. of tellers, 1000 kernel weight and kernel weight in plant. The analysis of variance was done according to (Snedecor and Cochran 1980).The description of studied material was made according to IPGRI and NBGR, descriptor (Tiegist *et al.*, 2010), using qualitative and quantitative characters species study Morphological variation for barley collected from different parts of Egypt (Marsa- Matrouh, Sinai and Assiut) as shown in table (1).

Table 1. Collection area, accession and types of barley.

Collection area	Accession	type	Collection area	Accession	type
Marsa- Matrouh	4-1 collection	Two rows	Sinai	25-1-8	Six rows
Assiut7895	28	Two rows	Assiut7896	29	Six rows
Assiut7940	46	Two rows	Assiut7918	38	Six rows
Sinai	125	Two rows	Assiut7935	44	Six rows
Sinai	1-37	Six rows	Assiut7945	47	Six rows
Assiut7787	3	Six rows	Assiut8047	48	Two rows
Assiut7809	4	Six rows	Assiut7049	50	Six rows
Assiut7810	5	Six rows	Sinai	107	Six rows
Assiut7832	13	Six rows	Local variety	124Giza	Six rows
Assiut7852	15	Six rows	Local variety	132	Six rows
Assiut7854	17	Six rows	Sinai	163	Six rows
Assiut7856	19	Six rows	Sinai	175	Six rows
Assiut7888	21	Six rows	Sinai	208	Six rows
Assiut7889	22	Six rows	Sinai	335	Six rows
Assiut7891	24	Six rows	Marsa-Matrouh	351	Six rows

DNA extraction:

Genomic DNA was extracted and purified from young leaves of the samples by using 2% CTAB extraction buffer according to (Doyle and Doyle, 1987). DNA concentrations of total genomic DNA in each sample was estimated using a spectrophotometer (TU 1880 Double Beam UV-VIS). All the DNA samples were stored at −20 °C

RAPD-and ISSR PCR analysis:

RAPD and ISSR assays were performed as described in (Adawy *et al.,* 2005), for biodiversity screening. The analysis was carried out using 9 RAPD primers and 5 ISSR primers (Table 2). RAPD and ISSR PCR reactions were conducted using anchored primers, which were synthesized by Eurofins, Germany. Amplification was performed in a Gene Amp® PCR System 9700 thermal cycler (Applied Biosystems). The general program was started with initial denaturation step at 94 ºC for 5 min followed by 40 cycles of denaturation step at 94 ºC for 5 min, annealing step at 36 ºC for RAPD or 45 ºC for ISSR for 5 min and extension step at 72 ºC for 1.5 min. The final extension step was at 72 ºC for 7 min. Electrophoresis of PCR products were performed on 1.7 % agarose gel stained with 0.5 mg/ml ethidium bromide. DNA bands were visualized on a UV transilluminator at 302 nm and photographed by Molecular Imager® Gel Doc™ XR+ System.

Table 2. Names, sequences and annealing temperature of RABD and ISSR primers used for barley accessions analysis

No.	Primer Name	Sequence of primer (5´ –3´)	Annealing temperature
RAPD-1	OP-A02	TGCCGAGCTG	36ºC
RAPD-2	OP-A05	AGGGGTCTTG	36ºC
RAPD-3	OP-A16	GGGTAACGCC	36ºC
RAPD-4	OP-A18	CAATCGCCGT	36ºC
RAPD-5	OP-B15	AGCCAGCGAA	36ºC
RAPD-6	OP-B18	GACCAATGCC	36ºC
RAPD-7	RMn-P1	CAGAAGCGGA	36ºC
RAPD-8	RMn-P2	GGGTAACGCC '	36ºC
RAPD-9	RMn-P3	TGTCATCCCC	36ºC
ISSR-1	UBC-810	GAGAGAGAGAGAGAGAT	45ºC
ISSR-2	UBC-812	GAGAGAGAGAGAGAGAA	45ºC
ISSR-3	UBC-814	CTCTCTCTCTCTCTCTA	45ºC
ISSR-4	UBC-402	CCCGCCGTTG	45ºC
ISSR-5	UBC- 45	AGAGAGAGAGAGAGAGT	45ºC

Genetic Data analysis:

The scored binary data generated by RAPD and ISSR markers were compared to determine the genetic relatedness of the 30 barely accession similarity matrices and cluster analyzing for RAPD and ISSR markers were performed individually as well as collectively. The RAPD and ISSR binary matrices were processed using the Bio-Rad diversity database software package and converted into similarity matrices according to Dice coefficient (Sneth and Sokal, 1973). The formula used by Dice to estimate the genetic similarity coefficient (GS) between two accessions was as follows:

Dice formula: **GSij = 2a/ (2a+b+c)**

Where

GSij is the measure of genetic similarity between individuals i and j, (a) is the number of bands shared by i and j, (b) is the number of bands present in i and absent in j, and (c) is the number of bands present in j and absent in i.

RESULTS AND DISCUSSION

Qualitative characters:

The present results revealed a significant variation among most morphological characters in barley germplasm. The ranges of variation observed among the germplasm for eleven morphological characters are presented in Table (3). Variations among germplasm were observed for flag leaf of anthocyanin in coloration, awns color in anthocyanin, wax in flag leaf in first awn and spike, length of a wingless at tip relative to length of ear, chlorophyll color and type of leaves, terminal color in anthocyanin, wax of flag leaves, flag leaf anthocyanin coloration of auricles, density of awns, growth habit in awns and degree of wax in awns. Beside the previous mentioned traits, germplasm of barley was morphologically diversified. These results are in agreement with (Tiegist *et al.*, 2010). The flag leaf anthocyanin of auricles was classified into two groups, present and absent, as well as awns color in anthocyanin. Also, chlorophyll color and type of leaves were classified into two groups, density and wild linear, light green and linear. Moreover, degree of wax in awns was classified into two groups, medium and very weak. As well as, growth habit in awns was classified into two groups, erect and semi erect. On the other hand, wax in flag leaf in first awn and spike were classified into three groups, medium, weak and very weak. In addition, large variation was observed in length of a wings at tip relative to length of ear was classified into three groups, long in spike, equal and short. Also, terminal color in anthocyanin was classified into three groups, very weak, medium and strong. Furthermore, density of awns was classified into three groups, very dense, dense and medium. Otherwise, wax of flag leaves was classified into four groups, weak, medium, strong and very strong. Also, flag leaf anthocyanin coloration of auricles was classified into four groups, weak, medium, strong and very strong. These results agree with (IPGR 1996).

Morphological diversity of different traits relates to the different regions and agro ecological zone for a total of thirty accessions in barley, these accessions differ in phenotype as a result of obtaining them from different places.

Table 3. Description of some agronomic characters in 30 accessions in barley.

Code . no	Collection area	Flag Leaf of anthocyanin Coloration	Awns Color in anthocyanin	Wax in Flag Leaf in first awn and spike	Length of a wingless at tip relative to length of ear	Chlorophyll color and type of leaves	Terminal color in anthocyanin	Wax of leaves flag	Flag leaf anthocyanin coloration of auricles
1	4-1	present	present	Medium	Longer in spike	Density and wild linear	Very weak	Medium	Weak
2	28	present	present	Medium	Longer in spike	Light green and linear	Very weak	Weak	Strong
3	46	present	present	Very weak	Longer in spike	Light green and linear	Strong	Very strong	Strong
4	125	present	present	Weak	Longer in spike	Density and wild linear	Strong	Strong	Strong
6	1-37	absent	present	Very weak	Equal	Light green and linear	Strong	Weak	Weak
7	3	absent	present	Very weak	Equal	Density and wild linear	Medium	Strong	Weak
8	4	absent	present	Weak	Equal	Density and wild linear	Strong	Weak	Weak
9	5	absent	present	Very weak	Equal	Density and wild linear	Very Strong	Strong	Weak
10	13	absent	present	Weak	Shorter	Density and wild linear	Strong	Medium	Weak
11	15	absent	present	Very weak	Equal	Light green and linear	Very Strong	Strong	Weak
12	17	absent	absent	Very weak	Longer	Density and wild linear	Strong	Strong	Weak
13	19	absent	present	Very weak	Longer	Light green and linear	Very Strong	Medium	Weak
14	21	absent	present	Very weak	Longer	Density and wild linear	Medium	Weak	Weak
15	22	absent	present	Very weak	Equal	Light green and linear	Strong	Weak	Weak
16	24	present	present	Very weak	Longer	Light green and linear	Very Strong	Medium	Medium
17	25-1-8	absent	present	Very weak	Shorter	Light green and linear	Strong	Strong	Weak
18	29	absent	present	Weak	Shorter	Density and wild linear	Very Strong	Strong	Medium
20	38	absent	present	Medium	Shorter	Light green and linear	Strong	Weak	Weak
21	44	absent	present	Medium	Shorter in spike	Light green and linear	Very Strong	Strong	Weak
22	47	absent	present	Medium	Shorter	Light green and linear	Strong	Strong	Medium
23	48	present	present	Medium	Longer	Light green and linear	Very Strong	Strong	Weak
24	50	absent	present	Medium	Longer	Light green and linear	Very Strong	Strong	Weak
25	107	absent	present	Weak	Longer	Light green and linear	Very Strong	Strong	Weak
26	G124cv	absent	present	Weak	Longer	Light green and linear	Very Strong	Strong	Weak
27	G132CV	absent	present	Weak	Equal	Light green and linear	Very Strong	Strong	Very Strong
29	136	absent	present	Very weak	Longer	Density and wild linear	Very Strong	Strong	Weak
30	175	absent	present	Weak	Longer	Light green and linear	Very Strong	Very Strong	Weak
31	208	absent	present	Medium	Longer	Light green and linear	Strong	Strong	Strong
32	355	absent	present	Medium	Shorter	Light green and linear	Very Strong	Very Strong	Weak
33	351	absent	present	Medium	Longer	Light green and linear	Very Strong	Strong	Weak

Table 3. cont.

Code .no	Collection area	Density of a wings	Growth habit in a wings	Degree of wax in awns
1	1-4	Very dense	Erect	Medium
2	28	Dense	Erect	Very weak
3	46	Dense	Erect	Very weak
4	125	Dense	Erect	Very weak
6	1-37	Very dense	Erect	Medium
7	3	Dense	Erect	Medium
8	4	Dense	Erect	Very weak
9	5	Dense	Erect	Very weak
10	13	Medium	Erect	Very weak
11	15	Dense	Erect	Very weak
12	17	Dense	Erect	Very weak
13	19	Medium	Erect	Very weak
14	21	Very dense	Erect	Very weak
15	22	Dense	Erect	Very weak
16	24	Dense	Erect	Very weak
17	25-1-8	Dense	Erect	Very weak
18	29	Dense	Semi Erect	Very weak
20	38	Dense	Erect	Very weak
21	44	Dense	Erect	Very weak
22	47	Dense	Semi Erect	Very weak
23	48	Medium	Semi Erect	Very weak
24	50	Very dense	Erect	Very weak
25	107	Dense	Erect	Very weak
26	G124cv	Dense	Erect	Very weak
27	G132cv	Medium	Semi Erect	Very weak
29	136	Very dense	Semi Erect	Very weak
30	175	Dense	Erect	Very weak
31	208	Dense	Semi Erect	Very weak
32	335	Dense	Semi Erect	Very weak
33	351	Very dense	Erect	Very weak

Quantitative characters.

The results of analysis of variance (ANOVA) for all studied traits across the two seasons are presented in Table (4). Data revealed highly significant difference among accessions for all studied traits. Heading date, physiological maturity, plant height, No. of tellers, 1000-kernel weight and kernel weight, except length of awn, awnless, out of the eight traits studied assuming leads effects of changes caused by different, years on the genetic performance of the entries.

Table 4. Analysis of variance of thirty accessions for eight characters.

s.o.v	d.f	Heading date	Physiological maturity	Plant height	Length of awn	awnless	No. of tellers	1000-kernel weight	Kernel weight
Replications	2	0.6478	5.0188	656.715	0.00516	0.0027	0.0077	3.216	1345.217
genotype	29	63.122**	20.046**	980.066**	3.0784	5.2642	13.544**	92.879**	70.1045**
error	58	0.6617	2.702	49.663	0.089	0.095	0.139	2.139	7.9446

Results in Table (5) revealed that in the combined means over the two seasons, the accession number 4-1 collection in Marsa Matrouh were the earliest in days to 50 % heading date (80.50) , but accession No. 351 collection in Marsa Matrouh was the latest (95.50 days). These results are smaller in the combined means in the two seasons due to the Less influence of environmental effect. Also early date in physiological maturity was in accession No. (22) that was collected in Assiut reached 118.50 days of maturity, while accession (38) that was collected in Assiut also was late in physiological maturity reached 131 days. The early physiological maturity character is dependent on a miner gene complex (Suddihiyam *et al.*, 1992). This germplasm showed early heading date and maturity with low kernel yield potential as compared to other barley accession. Both early and late maturity accession are important for plant breeding programs for adaptation of barley accession to various ecological regions as well as for research on photoperiod and thermo sensitivity.

Barley was shown to be high sensitive to day length as a short day's plant (Suddihiyam *et al.*, 1992). Who also reported significant interactions between temperature and day length for heading date. Meanwhile combined means for plant height revealed that accession No. 17 had the tallest plants (139.45cm), while the shortest plants were shown by accession No. 29 recorded (74.17cm).

Table 5a. Mean of days to heading date and physiological maturity of 30 accessions in barley in two seasons.

	50% heading date			Physiological maturity		
Accession No.	**2015/2016**	**2016/ 2017**	**Combined**	**2015/2016**	**2016/2017**	**Combined**
4-1	80.67	80.33	80.50	128.67	126.00	127.33
28	82.00	85.00	83.50	128.00	126.67	127.33
46	83.67	90.67	87.17	127.00	125.67	126.33
125	86.33	90.33	88.33	123.33	123.00	123.17
1-37	89.67	93.33	91.50	127.33	126.67	127.00
3	92.00	93.67	92.83	124.33	126.33	125.33
4	88.17	91.83	90.00	128.33	129.33	128.83
5	90.00	92.67	91.33	125.33	126.00	125.67
13	89.00	90.67	89.83	128.67	129.00	128.83
15	88.00	90.67	89.33	128.00	127.67	127.83
17	94.00	93.33	93.67	124.33	123.00	123.67
19	88.33	90.33	89.33	128.67	127.00	127.83
21	86.67	85.00	85.83	126.67	125.33	126.00
22	94.00	92.67	93.33	118.33	118.67	118.50
24	92.00	92.00	92.00	131.67	130.00	130.83
25-1-8	95.00	90.67	92.83	129.33	129.33	129.33
29	86.00	92.33	89.17	124.33	126.00	125.17
38	91.00	94.33	92.67	131.00	131.00	131.00
44	91.33	99.33	95.33	126.00	128.33	127.17
47	94.33	94.67	94.50	125.00	127.67	126.33
48	84.00	82.33	83.17	128.67	127.67	128.17
50	82.00	89.33	85.67	128.00	129.67	128.83
107	87.33	90.67	89.00	125.00	124.33	124.67
G124 CV	87.00	91.00	89.00	128.00	127.33	127.67
G132CV	86.33	92.00	89.17	126.00	127.33	126.67
136	91.33	91.33	91.33	129.00	127.33	128.17
173	85.67	84.00	84.33	122.67	123.67	123.17
208	93.33	90.67	92.00	125.00	123.00	124.00
335	93.67	94.00	93.83	124.67	122.67	123.67
351	97.00	94.00	95.50	129.00	127.33	128.17
Mean	89.06	90.86	89.96	126.70	126.57	126.63
LSD%	1.69	1.94	1.33	3.23	3.37	2.68
CV	1.16	1.31	0.90	1.56	1.63	1.30

Table 5b. Mean of plant height and length of awn of 30 accessions in barley in two seasons. .

	Plant length (cm)			Length of awn (cm)		Combined
Accession No.	**2015/2016**	**2016/ 2017**	**Combined**	**2015/2016**	**2016/2017**	
4-1	111.00	122.17	116.58	9.92	10.73	10.33
28	79.33	97.67	88.50	6.17	5.50	5.83
46	106.67	117.67	112.17	6.92	6.33	6.63
125	135.33	131.67	133.50	9.43	6.78	8.11
1-37	110.33	118.33	114.33	6.00	5.40	5.70
3	129.33	130.90	130.12	7.50	6.00	6.75
4	81.83	102.50	92.17	6.00	5.70	5.85
5	97.92	112.23	105.08	6.00	5.43	5.72
13	112.67	120.00	116.33	6.50	6.76	6.63
15	96.83	104.50	100.67	7.33	6.15	6.74
17	145.57	133.33	139.45	5.83	5.69	5.76
19	78.77	85.50	82.13	6.58	7.13	6.86
21	122.17	124.17	123.17	7.75	6.73	7.24
22	111.83	119.33	115.58	5.87	5.70	5.78
24	139.23	133.33	136.28	6.13	5.68	5.91
25-1-8	138.33	136.33	137.33	6.00	5.47	5.73
29	61.33	87.00	74.17	7.42	6.61	7.01
38	136.67	130.67	133.67	7.00	6.45	6.73
44	138.20	137.00	137.60	6.67	6.10	6.38
47	98.63	113.33	105.98	6.50	5.93	6.22
48	97.67	105.50	101.58	7.08	6.50	6.79
50	94.33	109.83	102.06	7.75	8.13	7.94
107	99.42	113.17	106.29	8.90	7.73	8.32
G124CV	102.47	118.33	110.40	6.17	6.10	6.13
G132CV	104.83	117.00	110.92	7.67	6.14	6.91
136	86.47	97.33	91.90	6.74	7.13	6.94
175	77.17	93.67	85.42	6.50	6.33	6.42
208	123.67	128.33	126.00	8.17	7.70	7.93
355	125.93	124.63	125.28	5.83	5.41	5.62
351	120.28	125.67	122.98	6.00	6.33	6.17
Mean	107.94	115.93	111.93	6.92	6.44	6.68
LSD%	4.90	22.11	11.51	0.76	0.59	0.49
CV	2.78	11.68	6.30	6.69	5.60	4.46

Table 5c. Mean of awnless and number of taller of 30 accessions in barley in two seasons.

	awnless (cm)			No. of tellers		Combined
Accession No.	**2015/2016**	**2016/ 2017**	**Combined**	**2015/2016**	**2016/2017**	
4-1	7.42	6.75	7.08	6.50	7.00	6.75
28	10.17	9.25	9.83	7.67	8.85	7.95
46	7.83	6.83	7.33	4.17	4.00	4.17
125	6.67	6.37	6.52	10.17	9.00	9.58
1-37	7.50	6.64	7.07	10.33	10.33	10.33
3	7.17	7.07	7.12	7.33	7.60	7.47
4	7.50	6.13	6.82	9.33	9.50	9.42
5	7.67	6.67	7.17	10.83	9.67	10.25
13	7.17	7.13	7.15	5.67	5.70	5.68
15	10.00	8.47	9.23	10.33	9.79	10.06
17	8.17	7.77	7.97	9.67	8.93	9.30
19	7.71	7.77	7.74	11.58	10.77	11.18
21	7.33	6.97	7.15	3.67	4.50	4.08
22	8.83	8.37	8.60	6.67	6.20	6.43
24	12.40	11.07	11.73	10.17	9.31	9.74
25-1-8	6.50	6.66	6.58	11.33	10.66	11.00
29	9.92	9.67	9.79	6.63	6.88	6.76
38	8.00	7.54	7.77	10.00	9.14	9.57
44	7.50	7.28	7.39	3.83	4.02	3.92
47	6.00	5.27	5.64	4.67	4.90	4.72
48	9.08	8.95	9.02	7.33	7.13	7.23
49	7.08	6.42	6.75	7.50	7.28	7.39
107	8.08	7.34	7.71	10.17	9.57	9.87
G124 CV	8.67	7.03	7.85	6.83	7.53	7.18
G132CV	9.00	8.50	8.75	9.63	8.67	9.15
136	10.00	11.18	10.59	5.90	6.35	6.02
175	8.17	8.00	8.08	9.83	7.99	8.91
208	6.50	8.83	6.67	7.33	8.09	7.70
335	6.30	6.67	6.48	11.83	10.00	10.92
351	7.50	8.50	8.00	7.83	8.80	8.32
Mean	8.04	7.64	7.84	8.21	7.97	8.07
LSD%	0.83	2.35	0.50	0.82	1.64	0.61
CV	6.36	18.86	3.93	6.12	12.41	4.60

Table 5d. Mean of 1000- kernel weight , kernel weight of 30 accessions in barley in two seasons

	1000-kernel weight			Kernel weight		
Accession No.	**2015/2016**	**2016/ 2017**	**Combined**	**2015/2016**	**2016/2017**	**Combined**
4-1	43.64	49.47	46.56	17.97	31.60	24.79
28	30.80	53.33	42.07	29.87	36.61	33.24
46	41.09	46.93	44.01	23.00	31.78	27.39
125	40.35	47.03	43.69	22.05	32.66	27.45
1-37	38.07	44.90	41.48	33.47	34.54	34.00
3	39.94	26.63	33.29	36.65	29.24	32.94
4	28.28	41.03	34.66	28.88	33.35	31.11
5	40.26	46.03	43.15	24.85	32.64	28.74
13	42.06	63.67	52.66	21.93	38.38	30.16
15	44.31	43.30	43.80	22.53	29.85	26.19
17	29.61	48.97	39.29	26.93	30.01	28.47
19	32.94	63.70	48.32	28.00	39.73	33.86
21	36.19	45.00	40.60	41.50	36.04	38.77
22	51.49	43.10	47.30	29.25	33.06	31.16
24	48.96	47.03	48.00	32.97	35.74	34.35
25-1-8	50.69	47.00	48.84	23.29	33.41	28.35
29	51.94	51.17	51.55	25.17	32.87	29.02
38	36.90	67.13	52.02	35.01	42.33	38.67
44	45.85	48.33	47.09	21.24	31.12	26.18
47	33.86	38.67	36.27	15.37	22.26	18.81
48	48.06	51.27	49.67	18.00	29.48	23.74
50	34.39	47.17	40.78	18.61	27.69	23.15
107	49.90	49.46	49.68	17.70	29.83	23.77
G124CV	46.19	37.47	41.83	35.64	29.31	32.47
G132CV	53.71	47.61	50.66	32.87	36.01	34.44
136	51.54	54.35	52.95	34.47	36.02	35.25
175	36.76	39.23	38.00	27.24	33.76	30.50
208	44.35	50.87	47.61	35.01	38.35	36.68
355	39.44	33.72	36.58	24.51	24.74	24.63
351	35.93	42.63	39.28	27.30	29.92	28.61
Mean	41.59	47.42	44.51	26.76	32.73	29.74
LSD%	3.83	3.08	2.39	2.82	9.18	4.60
CV	5.64	3.98	3.29	6.46	17.18	9.48

Regarding to length of awn, our results revealed that accession no. 4-1 had the tallest awns (10.33 cm), while the shortest awn shown by accession no. 355 recorded was (5.62cm). Meanwhile the combined means for awnless revealed that accession no. 24 had the tallest awnless (11.73 cm) while, the shortest awn was shown by accession no. 47 recorded (5.64 cm). Concerning of the no. of tellers in the combined analysis data of accessions numbers (19 and 25-1-

8) reached (11.18 and 11.00cm) respectively, while accession number 44 had the lowest (3.92cm). The combined analysis for 1000- kernel weight showed that accession numbers (136, 13 and 38) had he heaviest kernel (52.95, 52.66 and 52.02gm), while accession numbers (3 and 4) had the lowest kernel (33.29 and 34.66 gm). With respect to the combined analysis of kernel weight accessions (38 and 21) gave the highest kernel weight (38.67 and 38.77 gm.) respectively, while the lowest kernel weight accession no. 50 record 23.15. The data showed that smaller between results of the two seasons may be due the less influences in environmental interaction. Studied characters also revealed large genetic diversity. The germplasm with a wide range of variation for agronomic characters had potential to determine the best germplasm for different environments. The results supported that the accession no. (136) the highest 1000-kernel weight and kernel weight. The potentiality suited for different ecological conditions. These results are in agreement with (Tiegist *et al.*, 2010).

Data in Table (6) indicated that genotypic and phenotypic variances were found to be higher for plant height and kernel weight in two seasons. There were differences among genotypic and phenotypic variances for two characters indicating the less influence of environmental effects. These results are in harmony with those obtained by (El-Refaey *et al.*, 2017). Meanwhile moderate genotypic and phenotypic variance in 1000 kernel weight, and kernel weight in two seasons. There was difference among genotypic and phenotypic variance environmental effects. Although significant results have been experienced the variation between the genotypes were not broad for awnless (2.00 and 11.14) and kernel weight (7.5 and 39.71) in second season, hereby selection was less effective.

The phenotypic coefficient of variability (PCV) was generally higher than the genotypic (GCV) for all characters, but in many cases the two values differed only slightly .The highest values of GCV and PCV were shown for , plant height and kernel weight in first season .These results are greed with those obtained by (El-Refaey *et al.*, 2017).

Meanwhile, moderate estimate (GCV) and (PCV) for no. of tellers. (22.46, 23.49 and 7.39, 11.79) in two seasons, 1000-kernel weight in two seasons (38.91, 43.31 and 47.70, 50.21) and plant height in second season (42.13, 94.84). Whereas lowest values were scored for physiological maturity in two seasons (1.56, 2.58 and 1.57, 2.69), length of awn in two seasons (5.18, 6.21 and 5.70, 6.38) and awnless in second season (2.00, 11.14) .These results are agreed with (El-Refaey *et al.*, 2017). Heritability estimates and genetic advance would give better idea about the possible gain of selection.

High heritability in broad since (70%) has been observed in respect to all the studied traits except for awnless in the second season. High heritability has been recorded for plant height, 1000 kernel weight and kernel weight per plant, coupled with high genetic advance in two seasons. Meanwhile high heritability for 50% flowering date and length of awn associated with moderate genetic advance in two seasons, respectively as shown in Table (6), indicating the possibility of the improvement of these characters through simples selection. Similar results reported by (El-Refaey *et al.*, 2017). Meanwhile high estimates of high heritability in two seasons recorded for physiological maturity, coupled with low genetic advance. These results indicated were unsuitable for improvement through conventional selection and confirmed that higher heritability alone was not enough. These results are in an agreement with those (El-Refaey *et al.*, 2017). Whereas broad sense heritability estimates were relatively low for awnless in second season with value of 0.40% coupled with moderate genetic advance (27.01) indicated greater influence of environmental in the expression of the character.

Table 6. Genotypic coefficient of variance (GCV), phenotypic coefficient of variance (PCV), broad sense heritability (h^2_b) and genetic advance (GA) from selection for the studied traits barley in two seasons.

Traits	Seasons	6G	6P	6E	GCV	PCV	h^2	GA	GA%	SI%
50% heading date	First	17.23	13.23	0.96	6.45	6.85	0.98	31.55	41.96	7.53
	Second	14.86	16.27	1.41	5.45	5.97	0.97	27.76	35.92	7.10
Physiological maturity	First	5.92	9.82	3.90	1.56	2.58	0.82	14.17	13.11	5.52
	Second	5.95	10.20	4.25	1.57	2.69	0.81	14.50	13.42	5.62
Plant height	First	48.74	491.75	9.10	149..08	151.86	0.99	860.13	431.07	39.03
	Second	146.52	329.48	182.96	42.13	94.84	0.71	409.64	414.22	31.46
Length of awn	First	1.07	1.29	0.22	5.18	6.21	0.94	2.13	36.05	2.00
	Second	1.10	1.23	0.13	5.70	6.38	0.96	2.19	37.97	1.95
Awnless	First	1.81	2.007	0.26	7.51	8.59	0.95	3.48	50.72	2.53
	Second	0.45	2.52	2.07	2.00	11.14	0.40	1.76	27.01	2.79
No. of tellers	First	5.53	5,78	0.25	22.46	23.49	0.99	10.03	143.20	4.23
	Second	1.70	2.71	1.01	7.39	11.79	0.83	3.98	38.57	2.90
1000-kernel weight	First	48.55	54.04	5.49	38.91	43.31	0.96	91.66	258.33	91.66
	Second	67.86	71.43	3.57	47.70	50.21	0.98	123.55	305.38	14.87
kernel weight	First	44.53	47.54	2.98	55.48	59.18	0.98	81.80	358.24	81.80
	Second	7.37	38.99	31.62	7.51	39.71	0.91	28.24	101.14	10.99

Correlation coefficient yield is a complex quantitative trait, greatly influenced by environmental fluctuations. Hence, selection based on yield performance alone may indicate a based results and leads to ambiguity. A study of nature and degree of association of component characteristics with yield assume greater importance for fixing up characteristics that play a decisive role influencing yield. Selection would therefore be more effective, if it is based on component characteristics rather than directly on yield.

Correlation coefficient analysis measures the mutual relationship among various characteristics and is used determine the component character on which indirect selection can be done for improvement in yield. The characteristics, heading date showed that significant and negative in plant height, and highly significant in length of awn as showen in table (7). Although awnless showed negative and significant 1000-kernel weight. Meanwhile the character length of awn showed highly significant correlation with kernel weight these results are agreement with (Tiegist *et al.*, 2010).

Table 7. Simple correlation coefficients of eight studied traits on barley accessions combined across two seasons.

Characters		Physiological maturity	Plant height	Length of awn	Awnless	No. of tellers	1000-kernel	Kernel weight
50% heading date	P	-0.063	0.405*	-0.530**	0.128	0.134	-0.081	0.031
	G	-0.061	0.434*	-0.565**	-0.137	0.139	-0.087	0.047
Physiological maturity	P		0.000	-0.040	0.163	0.073	0.200	0.083
	G		-0.050	-0.033	0.193	0.103	0.267	0.103
Plant height	P			-0.009	-0.264	-0.019	-0.073	0.125
	G			-0.013	-0.271	-0.008	-0.076	0.097
Length of awn	P				-0.138	-0.199	0.236	-0.114
	G				-0.145	-0.204	0.263	-0.136
Awnless	P					0.027	0.407*	0.280
	G					0.042	0.433*	0.344
No. of tellers	P						0.033	0.045
	G						-0.38	0.085
1000-kernel weight	P							0.166
	G							0.202

Polymorphism analysis detected by RAPD and ISSR markers

In this study, nine RAPD and five ISSR primers which used for analysis of thirty barley accession produced amplification products and all resulted in polymorphic fingerprint patterns as shown (figure 1, 2) and (Table 8). Nine RAPD primers produced 111 bands with an average of 12.3 bands per primer. Out of the total of 111 amplified fragments, 59 were polymorphic, with an average of 6.6 polymorphic bands per primer.

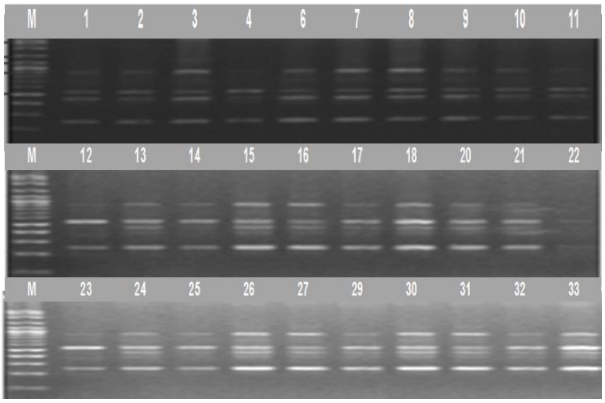

Figure 1. RAPD profiles of the thirty barley accession using primer (OP-B18). 1 to 33 samples accession, M: DNA molecular weight marker (1kb DNA ladder).

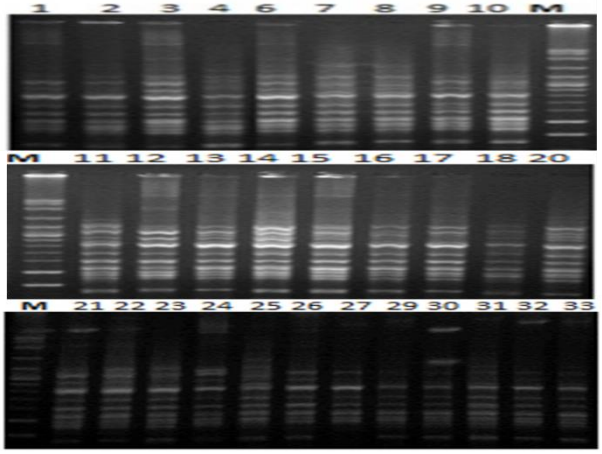

Figure 2. ISSR profiles of the thirty barley accession using primer (OP-B18). 1 to 33 samples accession, M: DNA molecular weight marker (1kb DNA ladder).

Table 8. Nine RAPD and five ISSR used in this study, the total bands (TB), monomorphic bands (MB), polymorphic bands (PB), percentage of polymorphic bands (%PB)

Primer	TB	MB	PB	%PB
OP-A02	14	7	7	50
OP-A05	13	5	8	62
OP-A16	10	5	5	50
OP-A18	9	4	5	56
OP-B15	12	5	7	58
OP-B18	15	7	8	53
RMn-P1	14	7	7	50
RMn-P2	11	5	6	55
RMn-P3	13	7	6	46
Total	111	52	59	
Average	12.3	5.8	6.6	53
UBC-810	18	9	9	50
UBC-812	14	8	6	43
UBC-814	12	6	6	50
UBC-402	17	9	8	47
UBC- 45	15	7	8	53
Total	76	39	37	
Average	15.2	7.8	7.4	49

This represented a level of polymorphism of 53% from these nine primers. Primer OP-A05 was the most polymorphic bands, where 8 polymorphic amplification products were detected. The lowest number of amplified polymorphic fragments (5) was detected by primer OP-A16 and OP-A18. The polymorphism percentage ranged from 46% (RMn-P3) to 62% (OP-A05). Five ISSR primers produced 76 bands with an average of 15.2 bands per primer. Out of the total of 76 amplified fragments, 37 were polymorphic, with an average of 7.4 polymorphic bands per primer. This represented a level of polymorphism of 49% from these nine primers. Primer UBC-810 was the most polymorphic bands, where 9 polymorphic amplification products were detected. The lowest number of amplified polymorphic fragments (6) was detected by primer UBC-812 and UBC-814 the polymorphism percentage ranged from 47% (UBC-402) to 53% (UBC- 45).

Cluster analysis based on SCoT marker

The dendrogram of thirty barley accessions based on RAPD and ISSR markers using UPGMA and similarity matrix computed according to Dice coefficient (Figure 3). The dendrogram comprised two main clusters; the first cluster including two sub-clusters, the first sub-cluster contains four accessions (1, 2, 3, and 4) while second sub-cluster contains thirteen accessions (18, 24, 30, 19, 25, 22, 28, 20, 26, 21, 27, 23 and 29). The second cluster divided into two sub clusters; first sub-cluster contains five accessions (5, 11, 17, 9 and 15), while the another sub-cluster contains eight accessions (6, 7, 8, 10, 12, 13, 14 and 16).

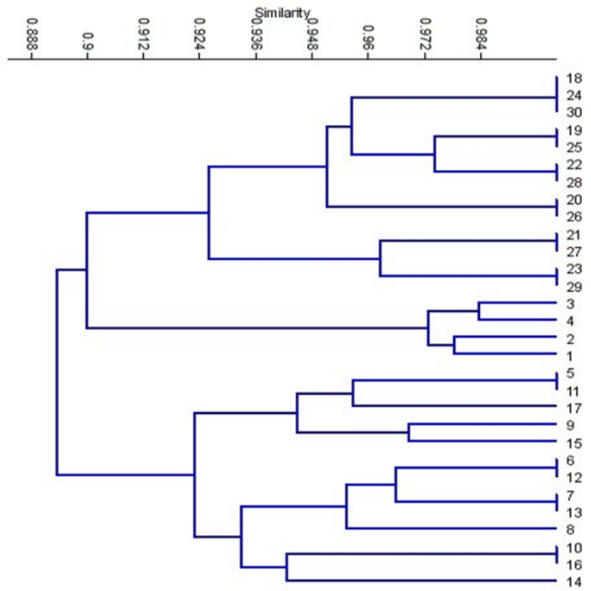

Figure 3. Dendrogram for the thirty accessions in barley constructed from RAPD and ISSR data using UPGMA and similarity matrix computed according to Dice coefficient.

REFERENCES

Adawy, S. S.; Hussein Ebtissam, H. A.; S. E. M. E. ismail and H. A. El-Itriby (2005). Genomic diversity in date palm (*Phoenix dactylifera* L.) as revealed by AFLP's in comparison to RAPD's and ISSR's. Arab J. of Biotech. Vol. 8 (1), 99-114.

Baum M; Grano S. and Ceccarell S. (2004). Localization of quantitative trait loci for dry land characters in barley by linkage mapping. Challenges and strategies for Dry land Agriculture CSSA special publication, 32: 191-202.

Chaabane R.; El-Felah M.; Ben Salah H.; Ben Naceur M.; Abdelly S.; Ramla D.; Nada A. and Saker M. (2009). Molecular characterization of Tunisian Barley (*Hordeum vulgare* L.) Genotypes using Microsatellite (SSRs) Markers. EUR. .J. Sci. Res. 36: 6-15.

Doyle JG. and Doyle J.L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue .Phytochemical Bulletin 19: 11-15.

Ellegren H. (2004). microsatellite :Simple Sequences with complex evaluation. Nat. Rev. Genetic. 5: 435-445.

El-Refaey R. A.; El-Gammaal A. A.; El-Shawy E. E. And Mansour R. E. A. (2017). Estimation of some genetic parameters in some barely genotypes under normal and saline soil conditions . Egypt Plant Breed. 21 (5) : 396-415.

El-Shazly H. and El-mutairi Z. (2006). Genetic relationships of some barley cultivars, Based on morphological criteria and RAPD finger printing. International J. Bot. 2: 252 -260.

Franke, O. H.; Brown, A.H.D. and Burdon, J.J. (1995). The genetic diversity of cultivated plants. In: The conservation of plant biodiversity .Cambridge Univ. press, Cambridge .UK. pp. 39-74.

Hou Y.C.; Yan Z.H.; Weiy M. And Zheng Y.L. (2005). Genetic diversity in barley from west china based on RAPD and ISSR analysis.Barley Gent. News. 35: 9-22.

Hussein, M. H.; Saker, M. M.; Moghaieb, R. E. and Hussein, H. A. (2005). Molecular characterization of salt tolerance in the genomes of some Egyptian and Saudi Arabian barley genotypes. Arab j. Biotech. 8: 241-252.

IPGR (1996). Ethiopia : Country reported to the FAO International Technical conference on Plant Genetic Resources .Leipzig 1996.

Liu ZW.; Biyashev RM. and Saghai Maroof MA. (1996). Development of Simple Sequence Repeat DNA markers and their integration into Barley linkage map .Theor. Appl. Genet. 93: 869-876.

Matus IA. and Hayes PM. (2002). Genetic diversity in three groups of barley germplasm assessed by simple sequence repeats . Genome, 45: 1095-1106.

Nevo E.; Beharev A.; Meyer RC.; Hackett CA. And Foster BP. (2005). Genomic, microsatellite adaptive divergence of wild barley by microclimatic stress in evaluation canyon .Israel Biol. J. Linn. soc. 84: 205-224.

Snedecor G.W. and W.G. Cochran (1980). Statistical Methods 17thed . Iowa state univ, Ames, USA.

Sneth P.H.A. and Sokal R.R. (1973). Numerical Taxonomy –The principles and practice of Numerical Classification .W.H. Freeman and Company San Francisco.

Soleri D. and Smith SE. (1995). Morphological and phonological comparisons of two Hopi maize varieties conserved in situ and exsiu .Econ .Bot .49: 56-77.

Suddihiyam .P.B.T.; Steer and D.W. Tumer (1992). The flowering of sesame (Sesamum indicum L.) in response to temperature and photo-period .Austral. J. Agr. Res. 34-1101-1116.

Tiegist DA.; Andrea MB. and Jens T. (2010). Morphological diversity of Ethiopian barley (Hordeumvulagare L.) in relation to geographic regions and altitudes. Hereditias. 147.

التنوع الحيوي لبعض السلالات في الشعيرعلى أساس الصفات الوصفية والكمية والمعلمات الجزيئية

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أجريت هذه التجربة بقسم بحوث الأصول الوراثية بمحطة بحوث بهتيم فى الفترة فى موسمين زراعيين 2017/ 2018و 2018/2019 لتقييم التنوع الوراثى فى سلالات الشعير بطرق متنوعة. وأوضحت النتائج أختلافات كثيرة فى بعض الصفات الزراعية حيث أن الأنثوسيانين فى ورقة العلم والسفا انقسم لمجموعتين (موجود أوغائب) وكذلك الكلوروفيل ونوع الأوراق (أخضر كثيف وورقة رمحية أو أخضر خفيف وورقة شريطية) وبالنسبة لنمو السفا أما (قائم أو نصف قائم). أيضا وجود الشمع على ورقة العلم والسنبلة أما ضعيف جدا أو ضعيف أو متوسط بالأضافة لذلك اللون النهائى للأنثوسيانين قسم لثلاث مجموعات (ضعيف جداً ومتوسط وقوى) أما كثافة السفا فكانت كثيفة جداً أو كثيفة أو متوسطة. أيضاً كانت كمية الشمع على الأوراق كانت أما (ضعيفة جداً أو متوسطة أو قوية أو قوية جداً). وبالنسبة للصفات الكمية كانت السلالة (38) مرتفعة فى وزن الألف حبة (52.02) وكذلك وزن الحبة (38.67). وأيضاً كان هناك ارتباط عالى المعنوية وموجب بين وزن الألف حبة وقلة السفا – وطول النبات و50% طرد السنابل. وتم عمل التحاليل الجزيئية لتقدير التنوع الوراثى ودرجة القرابة الوراثية باستخدام تحاليل الـ RAPD و ISSR ووجد أن هناك مستوى عالى من التعددية المظهرية حيث استخدم 14 بادئ 9 منهم خاص بالـ RAPD و 5 بادئ خاص ISSR وكان عدد الحزم الكلية 111حزمة مع تحاليل الـ RAPD منهم 59 حزم متعددة و52 حزم وحيدة . أيضا عدد الحزم الكلية باستخدام ISSR كان 76 منهم 37 حزم متعددة و39 حزم وحيدة.