جامعة عين شمس
Faculty of Women for, Arts, Science, and Education

Scientific Publishing Unit

Journal of Scientific Research in Science

Biological Sciences

Volume 38, Issue 1, 2021

ISSN 2356-8372 (Online) \ ISSN 2356-8364 (print)

Journal of Scientific Research in Science 2021, 38, (1): 16-35

Contents lists available at EKB

Journal of Scientific Research in Science

Journal homepage: https://jsrs.journals.ekb.eg/

Morphological and Quantitative traits of phylogenetic relationships of some barley (Hordeum vulgare L.) accessions in Egypt

S. M. Shata [1,*], W. M. Said [1], F. M. Abdel-Tawab [2], L. M. Kamal [2].

[1] Botany Department, Faculty of Women for Arts, Science and Education, Ain-Shams University, Cairo, Egypt .

[2] Genetics Department, Faculty of Agriculture, Ain-Shams University, Giza, Egypt.

Abstract

phylogenetic relationships of eleven accessions of (*Hordeum vulgare* L.) collected from different region of Egypt were assessed. Fifteen quantitative morphological traits were used, the measured data were evaluated statistically using ANOVA, phylogenetic tree were constructed using UPGMA. Also Inter simple sequence repeat (ISSR) molecular marker technique were used for DNA fingerprinting and assessing genetic diversity and phylogenetic relationships in barley germplasm. The results showed that ISSR primers produced 140 bands their size ranged between 110-1600 bp with 39.8% polymorphism percentage. Polymorphic information content PIC was 0.74 for ISSR. UPGMA dendrogram was divided into two clusters by morphological traits and ISSR analysis. Genetic similarity matrix was examined with Jacard's coefficient, maximum similarity was found between B8 and B7 (98%) with morphological analysis both from (North Sinai) and between B9 and B10 (96%) with ISSR analysis. Determination of genetic diversity between barley is of major importance for characterization of barley germplasm, breeding programs and conservation purposes. Morphological traits and ISSR analysis are effective tools for detecting genetic variations. The results showed that *H. vulgare* have high ratio of variation. This study may be considered as reference study for further studies on *H. vulgare* and may contribute to species concept and breeding programs.

Keywords: Quantitative morphological characterization, Genetic diversity, ISSR, *Hordeum vulgare*.

1. Introduction:

Barley (*Hordeum vulgare* L.) is the fourth most important cereal crop after wheat, maize and rice. it was one of the first domesticated cereals, It is also one of the founder crops of old world agriculture. Barley is a model experimental system because of its short life cycle, morphological, physiological and genetic characteristics [1]. Cultivated barley (*Hordeum vulgare* L.) was domesticated from its wild relative (*Hordeum spontaneum* C. Koch) as early as 7.000 BC [2]. It has been

*Corresponding author: S. M. Shata, Botany Department, Faculty of Women for .Arts, Science and Education, Ain-Shams University, Cairo, Egypt
E-mail: Sara.M.ahmed@women.asu.edu.eg

used for human food and animal feed in the world since ancient times, The importance of barley production is extremely increasing with increasing need to feed animal production and industrial purpose [3]. A lot of studies have been conducted to reveal the substantial level of genetic diversity in barley using morphological markers [4], and DNA markers are usually used to allow cultivar fingerprint and identification of genomes in crops, Inter Simple Sequence Repeat Polymorphic (ISSR) is efficient and rapid applications in evaluation, characterization of the genetic material [3].

Despite the fact that morphological markers are easily implemented and economical, they do not always reflect the actual genetic relationship, due to the high genotype by location interactions and to limited number of traits studied [5]. DNA markers have been proved to be more valuable tools used for evaluation of genetic diversity which are not affected by environment, selection, and available in almost unlimited numbers [6]. ISSR is a molecular marker technique, which involve PCR amplifications of DNA using single primer composed of a microsatellite sequence by 2-4 arbitrary which could be used to assess genetic diversity [7]. ISSR has been commonly used for genetic diversity in barley [8, 9, 10, 11]. In this study, we aimed to assess the phylogenetic relationships and genetic diversity among 11 barley using quantitative morphological traits and ISSR marker as efficient DNA marker tools.

2. Materials and Methods:

2.1. Plant materials:

Eleven Egyptian barley accessions were used in this investegation, which were kindly provided by the Egyptian National Gene Bank (NGB) Giza, Egypt. Their codes, gene bank numbers and regions are recorded in Table (1). Which were used in field experiment and DNA analysis.

Table (1): Accession of barley *Hordeum vulgare L*. with their Gene bank bar code number and regions of collection.

no	Accession code	Gene bank number	Regions
1-	**B1**	11418	Sharqia
2-	**B2**	11514	Dakahlia

no	Accession code	Gene bank number	Regions
3-	**B3**	11717	Marsa Matruh
4-	**B4**	11721	Marsa Matruh
5-	**B5**	11726	Minya
6-	**B6**	11727	Red sea
7-	**B7**	11773	North Sinai
8-	**B8**	11782	North Sinai
9-	**B9**	11820	Sharqia
10-	**B10**	11917	Alexandria
11-	**B11**	11937	Red sea

2.2. Morphological characterization:

A field experiment was conducted at the research farm of Faculty of Agricultural, Ain Shams University, Shubra El-Khima, Egypt. During the growing season 2016/2017. Based on randomized complete design (RCD) with three replications [12]. Plot sizes were 11 rows, 20 cm apart, 1 m long. Sowings were done at the growing season of 2016/2017. A total of 15 quantitative morphological descriptor traits measured for all accessions at maturity (after 100 days of swing) which were: root length, plant height, stem length, number of nodes, leaf base length, flag leaf length, flag leaf width, spike length, spike axis length, awn of central lemma length, number of spikelets/spikes, leaf area and the weight of 1000 grains. Also, days of heading were recorded. These characters are mainly recognized by International Plant Genetic Resources Institute [13].

2.3. DNA isolation and ISSR-PCR Amplification:

Genomic DNA of the 11 barley accessions under investigation was extracted from grains using (DNeasy plant Mini Kit, QIAGEN) according to the supplier's instructions. Polymerase chain reaction (PCR) amplification was prepared in a volume

of 25 µl using (25 ng) of genomic DNA, (2.5 µl) of dNTP, (2.5 µl) of $MgCl_2$, (10 pmol) of each primer (2.5 µl), and a (0.2 µl) of Taq polymerase, (2.5 µl) of 10X PCR buffer and (12.5 µl) d.w. H_2O . PCR was carried out as the following program; one cycle at (95°C for 5 min.), then 35 cycles were performed as follow: (1 min. at 95°C) for denaturation, (45 sec. at 55°C) for annealing and (30 sec. at 72°C) for extension, then incubated at (72°C for 7 min.) using ten ISSR primers which were used for this study as listed in Table (2). Amplified products were separated using agarose gel electrophoresis (2%) in 1 x TBE buffer against 100 bp DNA Ladder as a size marker. Fragments were detected with ethidium bromide and documented on Gel Documentation UVITEC, UK.

Table (2): ISSR primers with their sequence.

Name Primer	Primer Sequence 5'´→ 3'
INC1	5'- AGA GAG AGA GAG AGA GYC -3'
INC2	5'- AGA GAG AGA GAG AGA GYG -3'
INC3	5'- ACA CAC ACA CAC ACA CYT -3'
INC4	5'- ACA CAC ACA CAC ACA CYG -3'
INC5	5'- GTG TGT GTG TGT GTG TYG -3'
INC6	5'- CGC GAT AGA TAG ATA GATA-3'
INC7	5'- GAC GAT AGA TAG ATA GATA -3'
INC8	5'-AGA CAG ACA GAC AGA CGC -3'
INC9	5'- GAT AGA TAG ATA GAT AGC -3'
INC10	5'- GAC AGA CAG ACA GAC AAT -3'

A: Adenine, T: Thymine, G: Guanine, C: Cytosine and Y: (C or T)

2.4. Data Analysis:

Data were recorded on 10 randomly selected plants per plot. Analysis of variance was performed for all measured traits (quantitative morphological traits) to test the significance of variance among genotypes. Two sets of data were generated, morphological measurements and molecular markers, and used to evaluate genetic variability.

ISSR bands were scored either as present (1) or as absent (0). Then, scores were entered into a binary matrix on the program PAST (free programs on web) software [14]. Similarity of quantitative morphological data and ISSR molecular marker was calculated using the Nei and Li/Dice similarity index [15], and similarity estimates were analyzed using UPGMA (unweighted pair group method using arithmetic averages) clustering algorithm [16]. The resulting clusters were expressed as dendrogram. The polymorphic information content (PIC) of each marker was calculated using the formula $PIC = 1 - \Sigma P_i^2$ where Pi is the band frequency of the allele [17].

3. Results:

3.1. Morphological traits:

3.1.1. Agro-morphological variation:

To investigate the biodiversity among 11 barley accessions, fifteen quantitative morphological traits were recorded in Table (3). Our results showed a wide polymorphism for all quantitative morphological traits. Mean of root length clearly indicated that the Egyptian barley B1 recorded the highest value (13cm), while the lowest value was (9.8cm) recorded by accession B11. The tallest plants were observed in accession B4 (106cm) from Marsa Matrouh while the shortest was recorded in accession B1 (85 cm) in from Sharqia.The number of tillers also varied from (9.3) in B1 to (8) in B11 from Red Sea. The highest value of stem length were (79cm) in accession B11, on the other hand the lowest value were (63.3cm) recorded by accession B2 from Dakahlia. Number of nodes was highest in accession B1 and B4 (6.3) and lowest in accession B11 (5) from Red sea. Data showed that sheathing leaf base length in accession B1 and B4 recorded the highest value (13.5cm), while the lowest values was recorded in accession B2 (9.3 cm). The highest values of flag leaf length (22.2 cm) in accession B4, while the lowest values were (16.8cm) in accession

B1. Flag leaf width ranged from (1.3 cm) in accession B5 and B6 to (1 cm) in accession B2, B4 and B9. For spike length the tallest was recorded in accession B7 (19.3 cm), while the shortest were recorded in B8 (16 cm). Spike axis length was the longest in accession B11 (10.2 cm) and the shortest in accession B3 (5.2 cm).The highest values of awn of lemma of central spikelet were in accession B3 and B4 (14.3 cm), while the lowest values were recorded in accession B8 (11.7 cm). Number of spikelets per spike was highest (72) in accession B 11 and lowest (52.8) in accession B8. Leaf area was highest (25 cm^2) in accession B5 and lowest (16.2cm^2) in accession B1. Weight of 1000 grain (TGW) were highest (54.2 gm) in accession B4 and lowest (28.9 gm) in accession B2. Days to heading were highest (72 days) in accession B2 and B 11 and lowest in accession B3 (66 days).

Analysis of variance (ANOVA) indicates the presence of significant differences in several traits such as awn of central lemma length, number of spikelets per spike, days of heading and leaf area. Also some highly significant traits such as leaf base length, leaf width, weight of 1000 grain and spike axis length.

3.1.2. Cluster analysis of morphological traits:

The dendrogram obtained from UPGMA cluster analysis of 15 quantitative morphological traits of 11 *Hordeum* accessions illustrated in (Figure 1) divided the accessions into two distinct clusters with genetic similarity ranging from 0.936 to 1.00. Cluster I contains three accessions, accession B4 (six-rowed) was separated at taxonomic distance of 0.952 from accessions B5and B11 (tow rowed) which grouped together at taxonomic distance of 0.965. Cluster II contains 8 accessions all of six-rowed except B2 with two-rowed but it was integrated with the second cluster. This cluster was further subdivided into two sub-clusters at taxonomic distance of 0.944, sub-cluster A included six accessions, accessions B3 separate at taxonomic level 0.956, while accession B6 was separated at taxonomic distance of 0.965, on the other hand accessions B7 and B8 were closly related (both from North Sinai) grouped together at 0.978. As for accessions B9 and B10 they were grouped together at a taxonomic distance of 0.971. Sub-cluster B contains accession B2 and accesion B1at taxonomic level of 0.949. The similarity coefficient of 15 quantitative morphological traits are shown in Table (4), maximum ratio of similarity coefficient was observed between accessions (B7& B8) 98 % both from North Sinai and (B9& B10) 98 % both six-rowed from Sharkia and Alexandria, respectively, indicating an important genetic

similarity between them. Whereas the lowest similarity ratio was 0.91% between accessions B2 and B11 both tow rowed from Dakahlia and Red sea, respectively.

3.2. Molecular results:

3.2.1. Assessment of ISSR markers:

PCR amplification of genomic DNA isolated from the 11 barley accessions yielded a total of 140 bands, of which 57 were polymorphic (39.8%) and 83 were monomorphic. Overall size of the PCR amplified fragments ranged from 110 to 1600 bp (Table 5) . The banding patterns of barley genotypes are shown for the primers INC1, INC2, INC3, INC4, INC5, INC16, INC7, INC8, INC9 and INC10 (Fig. 2). Marker INC1 gave 12 bands with molecular size ranged from 160-830 bp, three bands were polymorphic representing 25 % polymorphic percentage. Whereas, the remaining 9 bands were monomorphic. Accession B1 gave the highest number of bands (11) and reveled specific positive marker at (830 bp), with PIC of 0.49. INC2 primer produced 14 bands, with a molecular size ranging from 140-1300 bp, eleven of them were monomorphic and 3 bands were polymorphic representing polymorphism percentage of 21 %, accessions B3 and B4 gave the highest number of bands (14) while accessions B9 and B10 gave the lowest number of bands (11), with PIC of 0.80. INC3 primer produced 16 bands with molecular sizes ranging from 250-1100 bp, only one band appeared to be polymorphic at 510 bp, representing 6.25 % polymorphic percentage, the remaining 15 band were monomorphic, with PIC of 0.36. INC4 primer generated 16 bands with molecular size ranging from 220-1300 bp. The number of polymorphic bands were 4 representing 25 % polymorphic percentage. Two accessions exhibited specific positive markers in accession B1 at (1300 bp) and in accession B11 at (370 bp), accession B8 gave the lowest number of bands(13), with PIC of 0.73. INC5 primer showed the amplification of 14 bands with molecular size ranging between 140-1050 bp, nine bands were polymorphic representing 64 % polymorphism percentage. Whereas, the remaining bands were monomorphic. Two accessions with specific negative marker were detected; accession B1 at (600 bp) and for accession B2 at (490 bp). The lowest number of bands were (8) observed in accession B11, with PIC of 0.88. INC6 primer generated 13 bands ranging in size from 180-1550 bp, eight bands were polymorphic representing 62 % polymorphism percentage, whereas, the remaining five bands were monomorphic. Two specific positive markers were detected in accession B1 at (350 bp) and at (1100). The lowest

number of bands was (6) were generated by accession B6, with PIC of 0.83. INC7 total number of bands generated by this primer were (8) which was the lowest number of bands in all 10 ISSR primers, ranging in sizes between 210-1600 bp. Five bands were polymorphic representing 63 % polymorphism. The remaining 3 were monomorphic. One accession specific positive marker was detected for accession B5 at (850 bp), with PIC of 0.78. INC8 number of bands produced were 17, with molecular size ranging from 110 bp to 1150 bp, 11 bands were polymorphic bands representing 65 %. Whereas, the remaining 6 bands were monomorphic. Two specific positive marker were detected for accession B8 at (580 bp) and for accession B11 at (530 bp) and 3 negative markers for B8 at (580bp), for B11at (530bp) and (350bp), respectively, with PIC of 0.88. INC9 primer showed the amplification of 15 bands with molecular size ranging between 150-1050 bp, there were six polymorphic bands representing 40 % polymorphism percentage. The remainig 9 bands were monomorphic, with PIC of 0.88. INC10 primer showed the amplification of 15 DNA bands with molecular sizes ranging between 110-1100 bp. There were four polymorphic bands representing 27 % polymorphism percentage. One specific negative marker detected in accession B4 at (190 bp), and one unique positive band at 140bp in accession B3. The remainig 11 bands were monomorphic, with PIC of 0.73.

3.2.2. Genetic diversity and relationships:

The dendrogram obtained from UPGMA cluster analysis of ten ISSR markers of 11 *Hordeum* accessions illustrated in (Fig. 3). Phenogram revealed two main clusters with genetic similarity ranging from 0.88 to 0.98. Cluster I separated at genetic distance of 0.88, it contains 5 accessions and was divided into two subclusters, subcluster A included accessions B7 and B8 both from North Sinai were grouped together at genetic distance of 0.938, while accessions B9 and B10 were grouped together at genetic distance of 0.96. On the other hand accession B11 was separated in single group at genetic distance of 0.91. Cluster II contains 6 accessions, in two subslusters, subcluster C included accessions B2 and B3 were grouped together at genetic distance of 0.938, while accession B4 was separated at 0.93, as for accessions B5 and B6 were groped together at 0.938. On the other hand subcluster D included single accession B1only separated at a genetic distance of 0.89. Table (5) showed the genetic similarity index among the11 accessions of barley based on banding patterns

of 10 ISSR markers similarity index ranged from 96% to 86%. The maximum values of genetic similarity (96%) were detected between accessions B9 and B10, whereas the minimum value was (86 %) detected between accessions B2 and B11.

4. Discussion:

Genetic diversity between barley accessions collected from Egypt was evaluated with the association of quantitative morphological traits with molecular marker data which act as a beneficial tool in varietal development [18, 19, 20]. The characterization revealed extensive variation between barley accessions in almost all quantitative morphological traits studied as indicated in (Table 3). This can be explained by the evolution of these accessions in distinct agroclimatic areas, suggesting significant levels of variation in response to selection pressure as reported by numerous authors [21, 22]. Also, a small relationship between the geographic origin of accessions and their separation was showed by cluster analysis as illustrated in the morphological dendrogram (Fig. 1) where accession B7 & B8 both from North Sinai clustered together. Genetic similarity between barley accessions through cluster analysis was described previously [23, 24, 25] suggesting that variability can be to some extent explained by microclimatic differences within regions and geographic isolation.

Morphological markers are affected by environmental conditions, not always available for analysis, time-consuming and are disposed to uncertain interpretations to assess. Therefore, PCR-based molecular marker technology is receiving more attention and has numerous advantages over the single use of standard markers in breeding programs and cultivar identification. Here, Inter-simple sequence repeat (ISSR) technique was used to study genetic diversity and DNA fingerprinting to detect phylogenetic relashoinships between barley [8, 9, 26].

ISSR based checking of genetic diversity established high polymorphism between barley accessions of Egyptian barley collection, this finding was also noted in rice [27] and orange [28]. ISSR markers were commonly used to differentiate between accessions, at times even more efficiently than SSR markers. Many studies emphasized that ISSR technique was recommended in previous and recent studies as an effective tool for genotypic evaluation in various plant species [29, 30, 31, 32, 33, 34].

In this study, dendrogram made using UPGMA from ISSR based on genetic similarity matrix showed that group structure is partially dependent on geographical distribution. Clustering accessions with the same geographic origin together such as B7 & B8 from North Sinai also B3 & B4 from Mersa Matruh as indicated in (figure 3). This pattern of geographically-related grouping by molecular markers has also been described in wheat [35]. But, our data do not agree with previous studies on barley and Aegilops [36, 37, 38] which state that barley does not cluster molecularly in ways that reflect geographic origin.

Moreover, results gave highly polymorphic profiles showing 140 polymorphic bands detected by 10 ISSR primers, showing high percentage of polymorphism (39.8%). Higher level of polymorphism (62%) has been found in Moroccan barley [19]. Our data displayed 57 of polymorphic bands and 83 monomorphic bands as indicated in (Table 5).

5. Conclusion:

Molecular marker based and morphological clusters and their accompanying analysis displayed different hierarchical patterns of genetic diversity between the accessions. But despite their disparity, the two approaches were found to be independently useful for assessing degree of relatedness and the overall genetic variation patterns among the analyzed barley accessions. However, molecular marker offer powerful tools in the evaluation of intra-specific relationships and were more effective and informative in grouping barley accessions. Investigating the genetic structure of barley accessions of Egypt remains of significant importance for elite gene exploitation and genetic improvement.

Table (3): Quantitative morphological traits of 11 *Hordeum vulgare* L. accessions.

Trait	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	F value
1-Root length	13^{a} ±1	10^{b} ±2	11.7^{ab} ±1.5	11.7^{ab} ±1.5	12^{ab} ±1	10^{b} ±1	10^{b} ±2	10^{b} ±1	10.3^{ab} ±2.1	10^{b} ±1	9.8^{b} ±1.3	1.69
2-Plant height	85^{c} ±5	98.3^{abc} ±10.4	105^{ab} ±13.2	106^{a} ±15.3	95.3^{abc} ±4.5	95^{abc} ±5	96^{abc} ±5.8	96^{abc} ±5.8	91^{abc} ±10.4	96^{abc} ±11.5	86^{bc} ±11.5	1.37
3-No. of tillers	9.3^{a} ±1.5	9^{a} ±1	9.3^{a} ±0.6	8.7^{a} ±1.2	9^{a} ±1	7.3^{a} ±0.6	8^{a} ±1	7.3^{a} ±2.1	9^{a} ±1	9^{a} ±1	8^{a} ±1	1.26
4-Stem length	64.7^{b} ±11.2	63.3^{b} ±1.2	70.7^{a} ±1.2	77.3^{a} ±14.2	73.7^{a} ±11.7	79^{a} ±7.9	76^{a} ±16.8	72.3^{a} ±6.4	67.3^{a} ±4	68.3^{a} ±7.6	79^{a} ±5.3	1.07
5-No. of nodes	6.3^{a} ±1.2	5.7^{a} ±0.6	5.7^{a} ±1.2	6.3^{a} ±1.2	5.7^{a} ±0.6	5.7^{a} ±0.6	5.3^{a} ±0.6	5.3^{a} ±0.6	5.3^{a} ±0.6	5.7^{a} ±0.6	5^{a} ±0	0.90
6-Leaf base length	13.5^{a} ±0.5	9.3^{de} ±0.6	12.7^{abc} ±0.6	13.5^{a} ±0.5	13.2^{ab} ±0.8	10.8^{ab} ±1	12.7^{cde} ±1.5	11.3^{abc} ±0.6	10.3^{bcd} ±0.6	12.3^{de} ±2.1	11.7^{abc} ±0.6	5.85**
7-Leaf length	16.8^{b} ±0.8	18.3^{ab} ±2.3	19.3^{ab} ±1.2	22.2^{a} ±1.9	21.7^{a} ±5.1	21.3^{a} ±1.5	20.3^{ab} ±2.5	20.7^{ab} ±1.5	20.7^{ab} ±1.5	20.3^{ab} ±0.6	22^{a} ±1	1.68
8-Leaf width	1.1^{ab} ±0.1	1^{c} ±0.1	1.1^{bc} ±0.1	1^{c} ±0.1	1.3^{a} ±0.1	1.3^{a} ±0.1	1.1^{bc} ±0.1	1.1^{bc} ±0.1	1^{c} ±0.1	1.2^{ab} ±0.1	1.1^{bc} ±0.1	5.60**
9-Spike length	16.8^{ab} ±2.5	17^{ab} ±1	17.7^{ab} ±0.6	17.7^{ab} ±0.6	19^{ab} ±2.6	16.2^{b} ±0.8	19.3^{a} ±2.1	16^{b} ±1	16.5^{ab} ±0.9	16.7^{ab} ±1.5	19.3^{a} ±2.1	1.80
10-Spike axis length	6.7^{bcd} ±0.6	6.3^{cd} ±0.3	5.2^{d} ±0.3	5.5^{cd} 0.5±	9.8^{a} ±1.6	5.2^{d} ±0.3	7.9^{b} ±1.0	6.8^{bc} ±0.6	6.5^{bcd} ±0.5	7.0^{bc} ±1.0	10.2^{a} ±1.0	13.3**

Trait	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	F value
11-Awn of central lemma length	14.0^{a} ±0.9	14.2^{a} ±0.8	14.3^{a} ±0.6	14.3^{a} ±1.0	13.2^{ab} ±1.0	11.8^{b} ±0.3	14.2^{a} ±0.8	11.7^{b} ±1.5	13.2^{ab} ±1.0	12.8^{ab} ±0.8	12.7^{ab} ±0.8	3.4*
12-No. of spikelets	54.9^{bcd} ±3.5	49.8^{d} ± 0.6	54^{cd} ±1.5	68.1^{abc} ±3.5	69^{ab} ±3.0	57^{bcd} ± 1.0	56.1^{bcd} ± 0.6	52.8^{d} ± 2.3	57.9^{abcd} ±2.5	63^{abcd} ±4.4	72 ^{a} ±1.7	2.8*
13- Days of heading	70.3^{ab} ±1.5	72.0^{a} ±2	66.0^{b} ±1	70.7^{ab} ±2.5	71.0^{ab} ±2	68.0^{b} ±1	67.3^{b} ±2.1	67.0^{b} ±2	70.3^{ab} ±1.5	67.0^{b} ±2	72.0^{a} ±2	4.9*
14- Leaf area	16.2^{b} ±1.1	16.6^{b} ±2.1	19.2^{ab} ±1	20.8^{ab} ±2.4	25.0^{a} ±6.8	24.5^{a} ±2.3	19.5^{ab} ±1.5	21.2^{ab} ±2.5	19.4^{ab} ±2.4	21.5^{ab} ±1.5	22.6^{ab} ±1.5	3.1*
15- Wt of 1000 grain	38.6^{b} ±1.2	28.9^{c} ±1.2	38.4^{b} ±2	54.2^{a} ±1.7	46.0^{a} ±1.3	33.5^{b} ±1.6	40.8^{a} ±1.6	41.5^{a} ±1.1	33.5^{b} ±1.1	34.4^{ab} ±1	45.3^{a} ±1	11.5**

Mean ± Standard deviation, Means with the same litters was not significant difference, *, ** Significant at 0.05 and 0.01 levels, respectively.

Table (4): Similarity index among 11 accessions of *Hordeum vulgare* L. based on 15 qualitative morphological traits.

	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11
B1	1										
B2	0.95	1									
B3	0.95	0.95	1								
B4	0.92	0.92	0.96	1							

B5	**0.94**	**0.93**	**0.95**	**0.96**	**1**						
B6	**0.93**	**0.94**	**0.95**	**0.94**	**0.96**	**1**					
B7	**0.94**	**0.95**	**0.96**	**0.95**	**0.96**	**0.96**	**1**				
B8	**0.94**	**0.94**	**0.96**	**0.94**	**0.96**	**0.97**	**0.98**	**1**			
B9	**0.96**	**0.96**	**0.95**	**0.93**	**0.95**	**0.96**	**0.96**	**0.96**	**1**		
B10	**0.94**	**0.95**	**0.95**	**0.93**	**0.95**	**0.96**	**0.97**	**0.97**	**0.98**	**1**	
B11	**0.93**	**0.91**	**0.92**	**0.94**	**0.97**	**0.95**	**0.95**	**0.94**	**0.94**	**0.94**	**1**

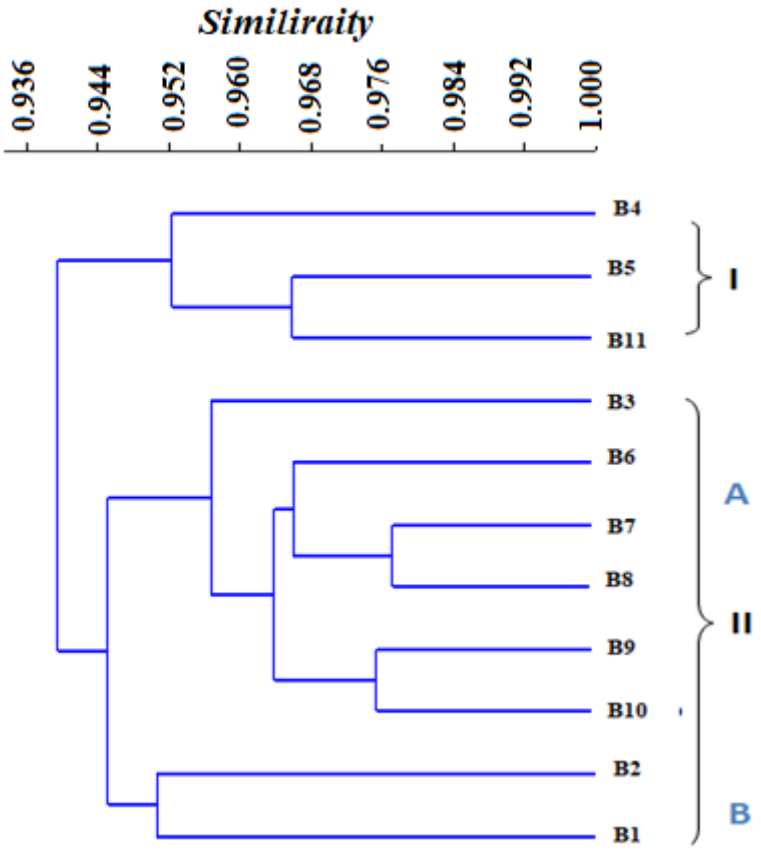

Figure (1): Dendrogram using UPGMA cluster for 15 quantitative morphological traits of 11 *Hordeum vulgare* L.

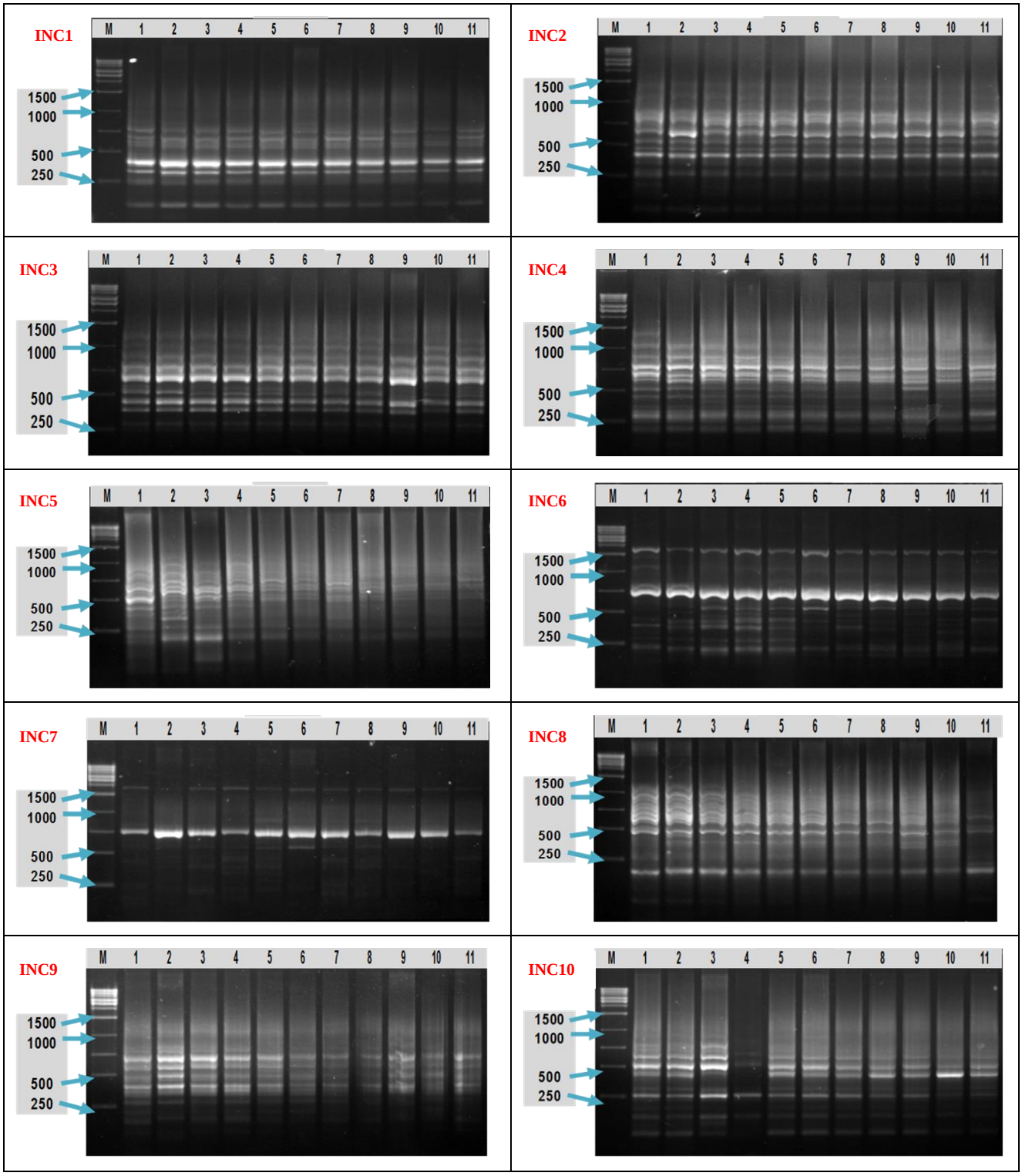
INC1
INC2
INC3
INC4
INC5
INC6
INC7
INC8
INC9
INC10
M 1 2 3 4 5 6 7 8 9 10 11
1500
1000
500
250

Figure (2): Banding patterns of 11 *Hordeum vulgare* L. accessions using 10 ISSR primers.

Table (5): List of ISSR primers, number of total bands(TB), polymorphic band(PB), monomorphic band (MB),the percentage of polymorphism(PP), specific band (SB) and Polymorphic information content (PIC).

Primer	MW	TB	PB	MP	PP	SB	PIC
INC1	160-830	12	3	9	25%	+(1)	0.49
INC2	140 - 1300	14	3	11	21%	0	0.80
INC3	250 - 1100	16	1	15	6%	0	0.36
INC4	220 - 1300	16	4	12	25%	+(2)	0.73
INC5	140 - 1050	14	9	5	64%	-(2)	0.88
INC6	180 - 1550	13	8	5	62%	+(2)	0.83
INC7	210 - 1600	8	5	3	63%	+(1)	0.78
INC8	110 - 1150	17	11	6	65%	+(3),+(2)	0.88
INC9	150 - 1150	15	9	6	40%	0	0.88
INC10	110 - 1100	15	4	11	27%	+(1),-(1)	0.73
Total	----	140	57	83	----	15	----
Mean	----	14	5.7	8.3	39.8	1.5	0.74

Table (6): Similarity index among 11 accessions of barley based on banding patterns of 10 ISSR primers.

	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11
B1	**100**										

B2	**91**	**100**									
B3	**89**	**94**	**100**								
B4	**90**	**93**	**94**	**100**							
B5	**89**	**91**	**92**	**94**	**100**						
B6	**90**	**91**	**90**	**91**	**94**	**100**					
B7	**87**	**89**	**90**	**91**	**90**	**92**	**100**				
B8	**87**	**87**	**89**	**88**	**89**	**92**	**94**	**100**			
B9	**90**	**89**	**87**	**88**	**91**	**92**	**91**	**94**	**100**		
B10	**90**	**88**	**87**	**90**	**91**	**94**	**92**	**95**	**96**	**100**	
B11	**87**	**86**	**88**	**89**	**88**	**90**	**92**	**91**	**91**	**90**	**100**

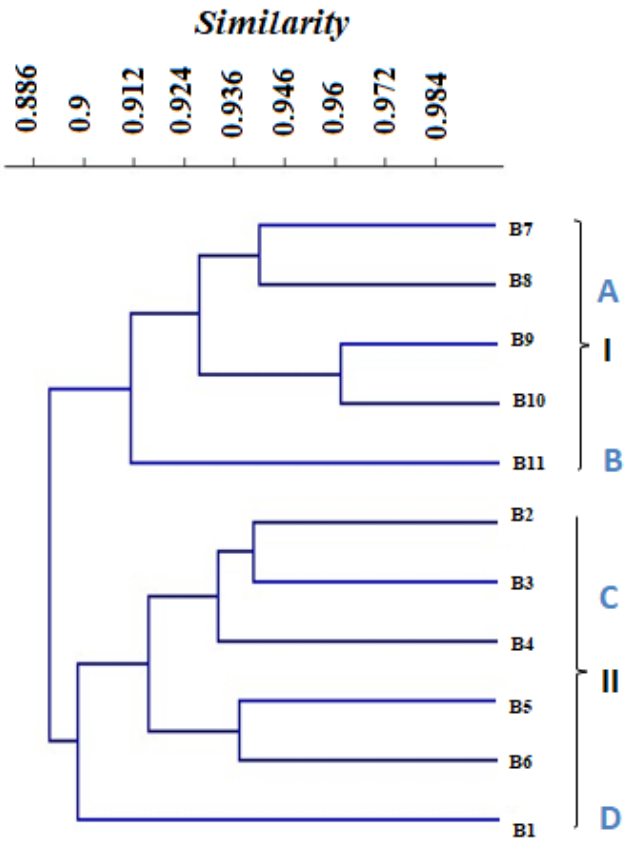

Figure (3): Dendrogram using UPGMA cluster analysis of 10 ISSR markers

References:

[1] E. F. El-Hashash, K. M. El-Absy, Barley (Hordeum vulgare L.) Breeding, Advances in Plant Breeding Strategies: Cereals. Springer, Cham: (2019) 1-45

[2] D. Allel, A. Ben-Amar, M. Lamine, C. Abdelly, Relationships and genetic structure of North African barley (Hordeum vulgare L.) germplasm revealed by morphological and molecular markers: Biogeographical considerations, South African Journal of Botany 112 : (2017) 1-10

[3] C. Tricase, V. Amicarelli, E. Lamonaca, R. L. Rana, Economic analysis of the barley market and related uses, Grasses as food and feed. IntechOpen: (2018)

[4] Y. Shakhatreh, M. Baum, N. Haddad, M. Alrababah, S. Ceccarelli, Assessment of genetic diversity among Jordanian wild barley (Hordeum spontaneum) genotypes revealed by SSR markers, Genetic Resources and Crop Evolution 63: (2016) 813-822

[5] J. Montero-Pau, J. Blanca, C. Esteras, E. M., Martínez-Pérez, P. Gómez, A. J. Monforte, B. Picó, An SNP-based saturated genetic map and QTL analysis of fruit-related traits in Zucchini using Genotyping-by-sequencing, BMC genomics 18: (2017) 1-21

[6] Q. Wei, Y. Wang, X. Qin, Y. Zhang, Z. Zhang, J. Wang, J. Chen, An SNP-based saturated genetic map and QTL analysis of fruit-related traits in cucumber using specific-length amplified fragment (SLAF) sequencing, BMC genomics 15: (2014) 1-10

[7] M. Yang, H. Abdalrahman, U. Sonia, A. I. Mohammed, U. Vestine, M. Wang, M. Toughani, The application of DNA molecular markers in the study of Codonopsis species genetic variation, a review, Cellular and Molecular Biology 66: (2020) 23-30

[8] M. Fernandez, A. Figueiras, C. Benito, The use of ISSR and RAPD markers for detecting DNA polymorphism, genotype identification and genetic diversity among barley cultivars with known origin, Theoretical and Applied Genetics 104:(2002) 845-851

[9] B. Tanyolac, Inter-simple sequence repeat (ISSR) and RAPD variation among wild barley (Hordeum. vulgare subsp. spontaneum) populations from west Turkey, Genetic Resources and Crop Evolution 50: (2003) 611-614

[10] Y. C. Hou, Z. H. Yan, Y. M. Wei, Y. L. Zheng, Genetic diversity in barley from west China based on RAPD and ISSR analysis, Barley Genetics Newsletter 35: (2005) 9-22.

[11] I. A. Khatab, A. A. El-Mouhamady, S. A. Mariey, T. A. Elewa, Assessment of water deficiency tolerance indices and their relation with ISSR markers in barley (Hordeum vulgare L.), Cu Sci Inte 8: (2019) 83-100

[12] W. T. Federer, D. Raghavarao, On augmented designs, Biometrics: (1975) 29-35

[13] A., Addisu, T. Shumet, Variability, heritability and genetic advance for some yield and yield related traits in barley (Hordeum vulgare L.) landraces in Ethiopia, International Journal of Plant Breeding and Genetics 9: (2015) 68-76.

[14] V. Hampl, A. Pavlícek, J. Flegr, Construction and bootstrap analysis of DNA fingerprinting-based phylogenetic trees with the freeware program FreeTree: application to trichomonad parasites, International journal of systematic and evolutionary microbiology 51: (2001) 731-735

[15] M. Nei, W. H. Li, Mathematical model for studying genetic variation in terms of restriction endonucleases, Proceedings of the National Academy of Sciences 76: (1979) 5269-5273

[16] R. R. Sokal, A statistical method for evaluating systematic relationships, Univ. Kansas, Sci. Bull. 38: (1958) 1409-1438

[17] J. S. C. Smith, E. C. L. Chin, H. Shu, O. S. Smith, S. J. Wall, M. L. Senior, J. Ziegle, An evaluation of the utility of SSR loci as molecular markers in maize (Zea mays L.): comparisons with data from RFLPs and pedigree, Theoretical and Applied Genetics 95: (1997) 163-173

[18] S. Yasuda, J. Hayashi, I. Moriya, Genetic constitution for spring growth habit and some other characters in barley cultivars in the Mediterranean coastal regions, Euphytica 70: (1993) 77-83

[19] E. H. Dakir, M. L. Ruiz, P. García, M. P. de la Vega, Genetic variability evaluation in a Moroccan collection of barley, Hordeum vulgare L., by means of storage proteins and RAPDs, Genetic Resources and Crop Evolution 49: (2002) 619-631

[20] D. Allel, A. Ben-Amar, M. Lamine, C. Abdelly, Relationships and genetic structure of North African barley (Hordeum vulgare L.) germplasm revealed by morphological and molecular markers: Biogeographical considerations. South African Journal of Botany 112: (2017) 1-10.

[21] A. K. Singh, J. Smartt, C. E. Simpson, S. N. Raina, Genetic variation vis-a-vis molecular polymorphism in groundnut, Arachis hypogaea L., Genetic Resources and Crop Evolution 45: (1998) 119-126

[22] J. Souframanien, T. Gopalakrishna, A comparative analysis of genetic diversity in blackgram genotypes using RAPD and ISSR markers, Theoretical and Applied Genetics 109: (2004) 1687-1693

[23] N. Bahrman, J. Le Gouis, D., Hariri, L. Guilbaud, L. Jestin, Genetic diversity of old French six-rowed winter barley varieties assessed with molecular, biochemical and morphological markers and its relation to BaMMV resistance, Heredity 83: (1999) 568-574

[24] S. Hamza, W. B. Hamida, A. Rebaï, M. Harrabi, SSR-based genetic diversity assessment among Tunisian winter barley and relationship with morphological traits, Euphytica 135: (2004) 107-118

[25] R. Eshghi, E. Akhundova, Genetic diversity in hulless barley based on agromorphological traits and RAPD markers and comparison with storage protein analysis, African Journal of Agricultural Research 5: (2010) 97-107

[26] F. Guasmi, W. Elfalleh, H. Hannachi, K. Feres, L. Touil, N. Marzougui, A. Ferchichi, The use of ISSR and RAPD markers for genetic diversity among south tunisian barley, International Scholarly Research Notices 2012: (2012).

[27] M. M. Saker, S. S. Youssef, N. A. Abdallah, H. S. Bashandy, A. M. El Sharkawy, Genetic analysis of some Egyptian rice genotypes using RAPD, SSR and AFLP, African Journal of Biotechnology 4: (2005).

[28] M. Lamine, A. Mliki, Elucidating genetic diversity among sour orange rootstocks: a comparative study of the efficiency of RAPD and SSR markers, Applied biochemistry and biotechnology 175: (2015) 2996-3013

[29] C. E. McGregor, C. A. Lambert, M. M. Greyling, J. H. Louw, L. Warnich, A comparative assessment of DNA fingerprinting techniques (RAPD, ISSR, AFLP and SSR) in tetraploid potato (Solanum tuberosum L.) germplasm, Euphytica 113: (2000) 135-144

[30] M. Arif, N. W., Zaidi, Y. P. Singh, Q. M. R. Haq, U. S. Singh, A comparative analysis of ISSR and RAPD markers for study of genetic diversity in Shisham (Dalbergia sissoo), Plant Molecular Biology Reporter 27: (2009) 488-495

[31] A. M. Gorji, P. Poczai, Z. Polgar, J. Taller, Efficiency of arbitrarily amplified dominant markers (SCoT, ISSR and RAPD) for diagnostic fingerprinting in tetraploid potato, American journal of potato research 88: (2011) 226-237

[32] V. Izzatullayeva, Z. Akparov, S. Babayeva, J. Ojaghi, M. Abbasov, Efficiency of using RAPD and ISSR markers in evaluation of genetic diversity in sugar beet, Turkish Journal of Biology 38: (2014) 429-438

[33] M. Lamine, A. Mliki, Elucidating genetic diversity among sour orange rootstocks: a comparative study of the efficiency of RAPD and SSR markers, Applied biochemistry and biotechnology 175: (2015) 2996-3013

[34] K. N. Vaja, H. P. Gajera, Z. A. Katakpara, S. V. Patel, B. A. Golakiya, Biochemical indices and RAPD markers for salt tolerance in wheat genotypes, Indian Journal of Plant Physiology 21: (2016) 143-150

[35] Ö. A. Sonmezoglu, B. Bozmaz, A. Yildirim, N. Kandemir, N. Aydin, Genetic characterization of Turkish bread wheat landraces based on microsatellite markers and morphological characters, Turkish Journal of Biology 36: (2012) 589-597

[36] P. Strelchenko, O. Kovalyova, K. Okuno. "Genetic differentiation and geographical distribution of barley germplasm based on RAPD markers." *Genetic resources and crop evolution* 46: (1999) 193-205.

[37] E. D. Owuor, A. Beharav, T. Fahima, V. M. Kirzhner, A. B. Korol, E. Nevo, Microscale ecological stress causes RAPD molecular selection in wild barley, Neve Yaar microsite, Israel, Genetic Resources and Crop Evolution 50: (2003) 213-224

[38] A. Mahjoub, M. S. El Gharbi, K. Mguis, M. El Gazzah, N. B. Brahim, Evaluation of genetic diversity in Aegilops geniculata Roth accessions using morphological and RAPD markers, Pakistan journal of biological sciences: PJBS 12: (2009) 994-1003

الملخص العربي

الصفات المورفولوجيه و الكميه للعلاقات التطوريه للعلاقات التطوريه لبعض انواع الشعير (*Hordeum vulgare* L.) في مصر

ساره محمود شطا -وفاء مرسي سعيد -فتحي محمد عبد التواب- لمياء مصطفى كمال

الملخص

في هذه الدراسة ، تم اختبار العلاقات التطوريه لإحدى عشر صنف من الشعير (*Hordeum vulgare* L) التي تم جمعها من مناطق مختلفة في مصر باستخدام 15 صفه مورفولوجية كمية ، و تم تقييم البيانات المقاسة إحصائيًا باستخدام ANOVA ، ثم إنشاء شجرة وراثية باستخدام UPGMA. كما تم استخدام تقنية (ISSR) لبصمات الحمض النووي لتقييم الاختلاف الوراثي والعلاقات التطورية في الأصول الوراثية للشعير. و أظهرت النتائج أن بادئات (ISSR) أنتجت 140 شريط يتراوح حجمها بين 110-1600 bp مع نسبة تعدد الأشكال الجينية 39.8 ٪. كان محتوى معلومات تعدد الشكل الجيني (PIC) هو 0.74 لـ ISSR. تم تقسيم الشجرة الوراثية بإستخدام UPGMA إلى مجموعتين حسب الصفات المورفولوجية و ايضا حسب تحليل ISSR. ثم تم فحص مصفوفة التشابه الوراثي باستخدام معامل جاكارد ، و تم العثور على أكبر درجه تشابه بين الصنفB8 و الصنف B7 و هوا (96%) في التحليل المورفولوجي وبين الصنف B9 و الصنف B10 و هو (96%) في تحليل ISSR. إن تحديد التنوع الجيني بين الشعير له أهمية كبيرة في توصيف المادة الوراثية للشعير وبرامج التربية وأغراض الحفظ. الصفات المورفولوجية و ISSR فعالة للكشف عن الاختلافات الجينية. و أظهرت النتائج أن الشعير *H. vulgare* لديه نسبة عالية من التباين. قد تعتبر هذه الدراسة بمثابة دراسة مرجعية لدراسه الشعير و العلاقات التطورية داخل هذا النوع وقد تساهم في مفهوم النوع وبرامج التربية.