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Genetic Diversity Among Some Libyan Barley Genotypes Based on the Morphometric and Molecular Levels

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Abstract

One of the main crops with major global economic and agricultural importance is barley (*Hordeum vulgare* L.). For the purpose of effectively conserving, using, and improving this essential crop, it is imperative to comprehend the genetic diversity and relatedness among barley landraces. Furthermore, the genetic variability of twelve Libyan barley genotypes was assessed based on different morphometric traits and molecular analysis using eleven Inter Simple Repeat (ISSR) and seven Sequence-Related Amplified Polymorphisms (SRAP) primers. The results of ISSR primers revealed 55 bands while the SRAP primers generated 46 bands. The primers used in the two approaches revealed that12 unique bands among the 12 barley genotypes. The molecular analysis of ISSR and SRAP revealed 76.36% and 45.65% polymorphisms, respectively. A total of 62.38% of both methods' polymorphism was found. Due to their shared heritage, the 12 barley genotypes under investigation showed a notable degree of genetic diversity, as indicated by the cluster analysis. In the regard, the principal component analysis (PCA) and heat map results validated the cluster analysis constructed on the two molecular techniques. Finally, the ISSR and SRAP patterns' similarities are congruent with the morphological traits of these accessions, as well as their relatedness and geographic distribution.

Keywords: Genetic diversity; PCA; ISSR; SRAP; Barley (*Hordeum vulgare* L.).

1. Introduction

One of the cereal crops cultivated most widely in the world; barley (*Hordeum vulgare* L.) is also the oldest and fourth largest cereal plant. Furthermore, the genome of barley, which belongs to the genus Hordeum, is diploid (2n) and has fourteen chromosomes. This crop requires minimal inputs from the agricultural sector, such as insecticides, fertilizer, and irrigation, and is abundant in dry and semi-arid regions of the Middle East and North Africa. According to recent studies, the ability to withstand abiotic stress and the presence of the glutenin protein are also essential **[1- 3].** In this sense, barley has traditionally been a vital and important economic crop in Libya. Bread and the well-known traditional Libyan dish Bazine are both made using barley flour. Furthermore, barley grains and hay are widely utilized for livestock feed and malting **[4].**

In the same context, Barley, *Hordeum vulgare* L., has a short growth season and a broad range of adaptability. It has fourteen chromosomes and is a member of the grass family. Barley ranks as the fourth most essential crop in terms of agricultural production worldwide, behind wheat, rice, and corn **[5].** Also, Barley is a cereal crop that ranks fourth and is widely farmed in poor nations as one of the most important necessary cereals **[6].**

Nowadays, genetic markers are the accepted and commonly used technique for recording uncommon plants **[7].** Essentially, genetic diversity was estimated using molecular markers, which was very helpful in the propagation of important domestication genes in genotypes of barley and wheat **[8].** Additionally, it can't be denied that improvements of genomic resources of both crops are owed to advances in sequencing technologies. Furthermore, reference genomes are accessible for barley, wheat, and a few of their progenitors paves the method for addressing unanswered problems regarding the domestication genomics of these two crops. Moreover, there are variations in the molecular markers' technological prerequisites, financial investment, repeatability, polymorphism, locus specificity, dominance or co-dominance, and genome abundance. All molecular markers are categorized as PCR-based markers Random Amplified Polymorphic DNA, Amplified Fragment Length Polymorphism, Simple Sequence Repeat, Inter simple Sequence Repeat, and Single Nucleotide or non-polymerase chain reaction (PCR)-based markers Restriction Fragment Length Polymorphism **[9].** Molecular markers were primarily used to measure genetic variety, which was very helpful in the genotypes of barley, and wheat for the propagation of important domesticated genes **[10].** However, using PCR-based markers called SRAP, with every Open Reading Frame (ORF) amplification, many co-dominant markers are produced. By utilizing forward and reverse primers with 17–18 nucleotides apiece and a main sequence with 13–14 bases, SRAPs target open reading frames in genomic sequences **[11].**

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Likewise, it is discovered that SRAP markers are equally powerful and varied as AFLP, but collecting them involves a far less complicated technological process. To assess large-scale germplasm collections' genetic diversity and improve Quantitative Trait Loci (QTL) in sophisticated hybrids, SRAP markers are mostly used in horticultural and agronomic research **[12].** Research on biodiversity may be able to assist breeders in defining genetic variability and making significant selections for important economic features in various crops. Additionally, it has been demonstrated that SRAPs is a useful and effective marker for identifying genetic connections and polymorphisms **[13- 15].**

Among the most effective marker systems currently in use are ISSRs markers, which produce a variety of informative bands **[16].** Because ISSRs molecular markers are believed to be able to amplify DNA regions between two microsatellites, they are widely utilized **[17].** Furthermore, ISSRs are an excellent illustration of the selectivity of microsatellite markers since they use random markers and may be created without exact sequence information **[18],** when it comes to polymorphism, resolving power (Rp), and band informativeness (Ib), ISSR primers are powerful molecular markers capable of differentiating between genotypes. **[19].** In this sense, the ISSR marker can also be used to accurately evaluate genetic variation and population structure in barley genotypes **[20, 21].**

Using some morphometric parameters associated with overall growth and productivity by ISSR and SRAP molecular markers, the study aims to evaluate the genetic diversity among various barley genotypes obtained from the Gene Bank of Libya on the phenotypic and molecular levels.

2. Materials and Methods

2.1. Plant material

Twelve barley genotypes namely, Ebrwan ganub, Thwath ganub, Bargug ganub, kammasy ganub, Ganub maklusa, Ganub Alaryl, Elalab wady hay, Ganub taredaa hamraa, 126, 123, Mumyazah 130 and Mumyazah 2000 which were obtained from Libyan central gene bank. The aforementioned genotypes were grown up to 90 days after germination. Some yield related traits were measured and used as morphometric parameters to assess the biodiversity on the phenotypic level among these genotypes; these characters were No. of leaves /plant, No. of tellers/plant, No. of spikes/plant, plant height, and yield index.

3. Methods

3.1. DNA Extraction

Green young leaves of the twelve barley genotypes were collected in liquid nitrogen for DNA extraction. DNA extraction has been performed using DNeasy plant Mini Kit Isolation protocol (Bio-basic.com).

3.2. ISSR-PCR Amplification

Sequences of eleven ISSR primers **(Table 1)** were used according to **[22, 4, and 23].** The polymerase chain reaction (PCR) has been conducted in automated thermal cycle (model Techno 512) apparatus. Total reaction volume of the PCR was adjusted in 25 µl. The PCR program was as follows: 94 ˚C for 4 min followed by 40 cycles of 1 min at 94 ˚C, 1 min at 57 ˚C, and 2 min at 72 ˚C. The reaction has been finally kept at 72 ˚C for 10 min. Agarose gel of 2% concentration was prepared for the electrophoresis of the PCR amplicons, in 1X TBE buffer, the molecular standard employed was a 100 bp DNA ladder. The produced bands were visualized photographed using UV transilluminator and subjected to further analysis.

Table 1: ISSR primers names and sequences

ID	Sequence	ID	Sequence
807	5’ AGA GAG AGA GAG AGA GT 3`	HB-10	5` GAG AGA GAG AGA CC 3`
98A	5` CA CA CA CA CA CA AC 3`	HB-11	5` GTG TGT GTG TGT CC 3`
49B	5` CAC ACA CAC ACA GG 3`	HB-12	5`CAC CAC CAC GC 3`
HB-1	5` CAA CAA CAA CAA CAA 3`	HB-13	5´ GAG GAG GAG GC 3`
HB-4	5` GAC AGA CAG ACA GACA 3`	HB-15	5´ GTG GTG GTG GC 3`
HB-8	5`GAG AGA GAG AGA GG 3`		
ID	**Sequence**	**ID**	**Sequence**
807	5’ AGA GAG AGA GAG AGA GT 3`	HB-10	5` GAG AGA GAG AGA CC 3`
98A	5` CA CA CA CA CA CA AC 3`	HB-11	5` GTG TGT GTG TGT CC 3`
49B	5` CAC ACA CAC ACA GG 3`	HB-12	5`CAC CAC CAC GC 3`
HB-1	5` CAA CAA CAA CAA CAA 3`	HB-13	5´ GAG GAG GAG GC 3`
HB-4	5` GAC AGA CAG ACA GACA 3`	HB-15	5´ GTG GTG GTG GC 3`
HB-8	5`GAG AGA GAG AGA GG 3`		

3.3. SRAP-PCR Amplification

Seven SRAP primer pairs sequences **(Table 2)** have been used to determine the polymorphism between the twelve barley genotypes under investigation according to **[24, 13- 15].** The PCR was conducted in Thermal Cycler apparatus (Techno 512, UK. The PCR program was as follows: 2 min at 94 ˚C, then 35 cycles of three steps: 1 min at 94 ˚C, 30 Sec at 35 ˚C and 90 Sec at 72 ˚C. The temperature was gradually raised to 50 ˚C for the next 35 cycles of annealing, and then it was held at 72 ˚C for one cycle of five minutes. For the electrophoresis of the PCR amplicons, a 2% concentration agarose gel was generated in 1X TBE buffer. The molecular standard utilized in this process was a 100 bp DNA ladder. The resultant bands were captured on camera with a UV transilluminator and then subjected to additional examination.

3.4. Statistical analysis

Morphological and yield-related traits were analyzed statistically according to **[25].**

Table 2: Sequences of the SRAP combinations used in the PCR reaction

No.	Primer combination	Forward primers	Reverse primers
1	Me-1xEM5	5-GA CTGCGTACGAATTAAT 3`	5-GACTGCGTACGAATTAAC 3`
2	Me-2xEM5	5-TGA GTC CAA ACC GGA GC 3`	5-GACTGCGTACGAATTAAC 3`
3	Me-2xEM6	5-TGA GTC CAA ACC GGA GC 3`	5-GACTGCGTACGAATT GCA 3`
4	Me-2xEM7	5-TGA GTC CAA ACC GGA GC 3`	5-GACTGCGTACGAATTCAA 3`
5	Me-6xEM7	5-GAC TGC GTA CGA ATT GCA 3`	5-GACTGCGTACGAATTCAA 3`
6	Me-9xEM3	5-TGAGTCCAAACCGGAGG 3`	5-TGAGTCCAAACCGGAAT 3`
7	Me-9xEM10	5-TGAGTCCAAACCGGAGG 3`	5-GACTGCGTACGAATTCAT 3`

3.5. Molecular Data Analysis

The DNA-banding patterns' resolved PCR products, referred to as ISSR and SRAP, were given a present (1) or absent (0) score. The Program of SPSS version 22 was used to estimate the dendrogram, and Dice was used to estimate the genetic similarities **[26].** Clusters Vis https://biit.cs.ut.ee/clu were used to create principle component analysis (PCA) and heat map diagrams by combined molecular, and morphological data **[25, 35].**

4. Results

Analyses of variance among the 12 selected Barley genotypes were investigated to assess the variation of some yield related traits. The results demonstrated considerable differences between studied genotypes, as indicated in (**Table 3).** The results indicated that genotypes Ebrwan ganub and Thwath ganub showed the greatest plant height (130 cm), while genotype 123 was the lowest one (114 cm).

Table 3: Mean values of some morphological traits of the 12 barley genotypes

Genotypes	Plant height	No. Leaves/plant	No. Tillers/plant	No . Spick/plant	Yield Index
Ebrwan ganub	130	7	26	23	88.46
Thwath ganub	130	6	26	23	88.46
Bargug ganub	128	8	25	21	24.00
kammasy ganub	128	7	24	22	91.67
Ganub maklusa	124	6	24	21	87.50
Ganub Alaryl	128	6	26	21	80.77
Elalab wady hay	127	7	24	21	87.50
Ganub taredaa hamraa	119	6	23	21	91.30
126	122	7	21	17	80.95
123	114	6	26	21	80.77
Mumyazah 130	125	5	19	13	68.42
Mumyazah 2000	126	6	19	13	68.42

= lowest mean values and = Highest mean values

The No. of leaves/plant genotype Bargug ganub was the highest with (8) leaves/plant, while genotype Mumyazah 130 was the lowest in this trait with (5) leaves/plant. Genotypes Ebrwan ganub, Thwath ganub, Ganub Alaryl and 123 recorded the highest in number of tillers/plant (26), while Mumyazah 130 and Mumyazah 2000 were the lowest in the trait with 19 tillers/plant. On the other hand, genotypes Ebrwan ganub and Thwath ganub scored the highest number of spikes/plant (23), while genotypes Mumyazah 130 and Mumyazah 2000 were the lowest in this trait with (13) spikes/plant. Moreover, in yield index, genotype kammasy ganub was found to be the highest in the trait with ratio of (91.67) followed by genotype Ganub taredaa hamraa with ratio of (91.30), while genotypes Mumyazah 130 and Mumyazah 2000 were the lowest in this trait with ratio of (68.42).

4.1. Molecular identification

Biodiversity among the 12 barley genotypes was assessed on the molecular level using the combined data of ISSR and SRAP. The results showed that, overall, the 15 used primers produced a total number of 101 bands, out of these; there were 63 polymorphic bands and 38 monomorphic bands with polymorphism ratio of 62.38% as shown in **(Table 4).**

Table 4: Total No. of alleles, monomorphic and polymorphic bands, polymorphism percentage, and unique bands generated with eleven ISSR and seven SRAP markers for the 12 barley genotypes

Technique	Total no. of Bands	Monomorphic Bands	Polymorphic Bands	Polymorphism %	Unique Bands
ISSR	**55**	**13**	**42**	**76.36**	**9 (8 P +1N)**
SRAP	**46**	**25**	**21**	**45.65**	**4 (2P +2N)**
Combined	**101**	**38**	**63**	**62.38**	**13 (10P+3N)**

According to the results of UPGMA cluster analysis, the consensus tree and similarity index constructed from the combined data of ISSR and SRAP were evaluated as well **(Table 5)** and **(Figure 1).** The findings indicated that, Dice similarity coefficient ranged from 0.66 (1, 11) (Ganub taredaa hamraa, Ganub Alaryl) to 0.91 (8, 10) (Thawath ganub, Kammasy ganub). The consensus tree includes the 12 barley genotypes under consideration, produced from binary data from the DNA profiles utilizing the combined data of the eleven ISSR primers and seven SRAP primer pairs. Two distinct clusters were

created from the analysis of these combined markers data. The first cluster included (Ganub taredaa hamraa) genotype only, whereas the second cluster separated into two sub-clusters, the first sub-cluster included (Ganub Alaryl), and (Elalab wady hay) genotypes in a division of this sub-cluster, the other division of the same sub-cluster included Bargug ganub, Kammasy ganub, Thawath ganub and Ebrwan ganub. The second sub-cluster was separated into two divisions the first division included genotypes 126 and Ganub maklusa. Mumyazah2000 and 123, also, Mumyazah130, made up the second division of the second sub-cluster.

Table 5: The Dice similarity coefficient was calculated according to combined ISSR and SRAP data analysis for each of the twelve barley genotypes under investigation

	1	2	3	4	5	6	7	8	9	10	11	12
1	1											
2	0.78	1										
3	0.75	0.83	1									
4	0.74	0.84	0.85	1								
5	0.79	0.83	0.78	0.81	1							
6	0.73	0.81	0.76	0.75	0.82	1						
7	0.73	0.79	0.78	0.77	0.78	0.86	1					
8	0.75	0.81	0.8	0.79	0.8	0.84	0.9	1				
9	0.75	0.75	0.74	0.79	0.76	0.80	0.82	0.86	1			
10	0.74	0.76	0.79	0.76	0.75	0.79	0.87	0.91↑	0.89	1		
11	0.66↓	0.74	0.71	0.70	0.71	0.79	0.81	0.81	0.83	0.88	1	
12	0.72	0.76	0.71	0.72	0.77	0.81	0.83	0.83	0.77	0.82	0.86	1

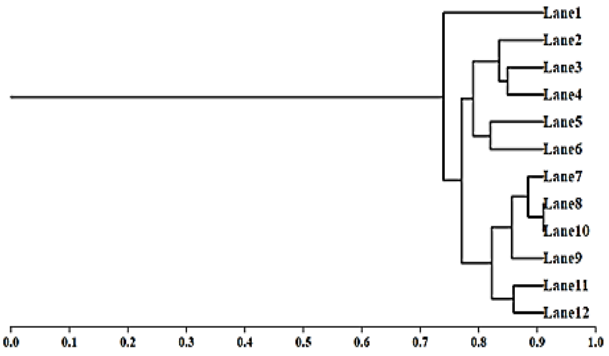

Figure (1): Dendrogram tree presented the genetic distance between the 12 studied barley genotypes constructed on the combined data of ISSR and SRAP where (1) Ganub taredaa hamraa, (2) Mumyazah 130, (3) 123, (4) Mumyazah2000, (5) 126, (6) Ganub maklusa, (7) Ebrwan ganub, (8) Thwath ganub, (9) Bargug ganub, (10) kammasy ganub, (11) Ganub Alaryl and (12) Elalab wady hay

4.2. Heat map of the 12 selected barley genotypes

The consensus tree grouped the 12 studied barley genotypes founded on the measured morphometric traits data as well as the combined data of ISSR and SRAP included the 12 barley genotypes under investigation as shown in **(Figure 2).** The results demonstrated that, the 12 barley genotypes were grouped into two main clusters, A and B. cluster A included 7 genotypes and classified into four sub clusters. Sub cluster1 contains genotypes Ebrwan ganub and Thwath ganub while sub cluster2 contains genotypes Bargug ganub and kammasy ganub, moreover, sub cluser3 contains genotypes Ganub Alaryl and Elalab wady hay, finally, and the fourth sub cluster contained genotype Ganub maklusa, Cluster B had five genotypes and was divided into three sub-clusters. Sub cluster1 contains genotypes Mumyazah 2000 and 123, while sub cluster2 contains genotypes Mumyazah 130 and 126; finally, the third sub cluster contains genotype Ganub taredaa hamraa.

4.3. The principal component analysis (PCA)

The 12 barley genotypes under study were distributed into four groups by the (PCA) founded on ISSR and SRAP data, as shown in **(Figure 2).** The first cluster included seven genotypes which classified into four sub clusters, the first sub cluster included genotypes Thwath ganub, Ebrwan ganub while the second sub cluster comprised genotypes Kammasy ganub, Bargug ganub. The third sub cluster included genotypes Ganub alaryl, Elalab wady hay, finally the fourth sub cluster included genotype Ganub Maklusa. The second main cluster comprised genotypes Mumayazah130 and 126. The third main cluster included genotypes 123, Mumyazah2000 the fourth main cluster included genotype Ganub taredaa hamraa. These results were similar in agreement to the results which were found from the heat map.

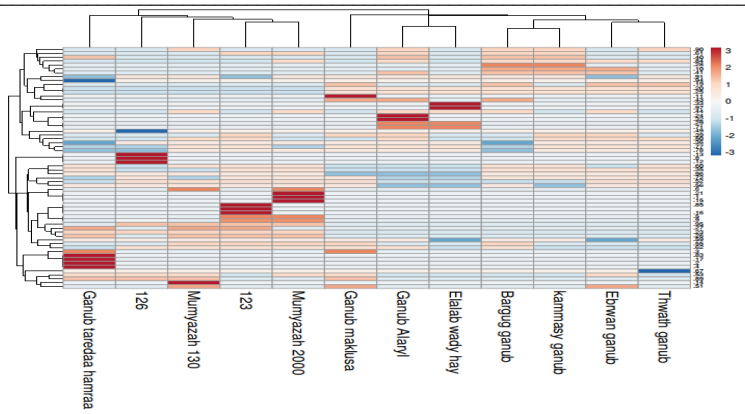

Figure (2): Heat map relationships among the 12 selected Barley genotypes

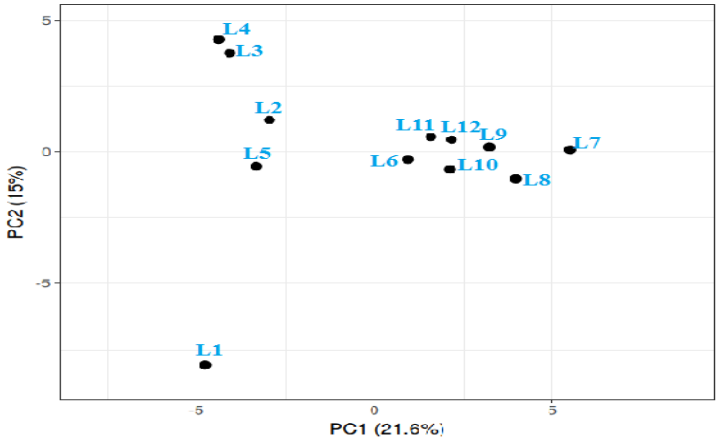

Figure (3): The genetic relationships between the 12 barley genotypes under investigation were displayed by PCA analysis (L1) Ganub taredaa hamraa, (L2) Mumyazah 130, (L3) 123, (L4) Mumyazah2000, (L5) 126, (L6) Ganub maklusa, (L7) Ebrwan ganub, (L8) Thwath ganub, (L9) Bargug ganub, (L10) kammasy ganub, (L11) Ganub Alaryl and (L12) Elalab wady hay

5. Discussion

Understanding ancestral relations and managing genetic resources for agriculture effectively to enhance breeding programs can be accomplished through the investigation of germplasm genetic diversity **[27, 28].** This result agreed with the results which obtained from the UPGMA clustering analysis that shows the diversions of the 12 barley species under investigation, in contrast to a phylogenetic tree that was produced using a Heat Map and displayed graphically **(Figure 2).** In summary, the gathered data revealed that the species were split into three distinct branches (clades) among the twelve barley genotypes. Out of the 12 barley genotypes, 11 were sorted into three clades, while one genotype (Ganub taredaa hamraa) was distributed jointly as an out-group in the basal position of the tree. Finally, the current study was aimed to explore molecular characterization, marker polymorphisms, morphological, and genetic diversity analysis among 12 different barley genotypes belonging to the well-profiled geographical zone. Moreover, ISSR and SRAP fingerprinting molecular markers were utilized to assess the genetic study, demonstrating that they are a powerful tool for developing possible diagnostic markers for genotype analysis. Seven ISSR markers and eight SRAP markers indicated clear amplification in the 12 barley cultivars under investigation, with a polymorphism rate of 62.38%. Additionally, genetic diversity was established by creating dendrogram of all the distinct barley genotypes utilizing ISSR, SRAP, and their combined data, PCA, and heat map technologies. Despite discrepancies in PCA results based on genomic data, genotypes were classified into four groups that were more closely related to their use in Egypt. Heat-maps provide a wealth of information about the genetic diversity of various plant breeds **[29].** Also, the markers indicated a great amount of genetic differences among the 12 barley genotypes under inquiry related to their distinct geographical distributions, and some genetic differences were conducted using altered types of marker technologies. Therefore, these results have shown successful sources of diversity which will help plant breeders to assessment the genetic diversity and successfully select traits of economic importance such as yield index. These results proved that ISSR and SRAP techniques are reliable and powerful tools for assessing genetic polymorphisms among the 12 barley genotypes under investigation. Two field trials were conducted to examine biodiversity among 50 barley landraces. The results showed that these genotypes reflect a sort of diversity in the assessed morphometric features and yield components, particularly the number of grains per spike and weights per thousand grains. Principal component analysis differentiated many landraces that were gathered beside the checks and shared similar characteristics such as plant height and spike length **[30].** The results agreed with the genetic diversity and genetic relationships among 59 Turkish barley accessions using sequence-related amplified polymorphism (SRAP) markers **[31]**. However, low variation values in some quantitative traits and the qualitative traits were estimated **[32].** Overall, the results indicated that some traits could be used as potential germplasm preservation

and development programs. The genetic diversity between 260 accessions, 239 landraces and 21 barley breeding lines, were estimated using single nucleotide polymorphism (SNP) array **[33]**. The results revealed a total of 983 highly informative SNP markers were used for diversity analysis. The molecular results corroborated those obtained by evaluating the genetic diversity and relationships between 105 barley genotypes using high-density single nucleotide polymorphisms (SNPs) markers **[34]**. Their findings demonstrated a significant level of genetic diversity in barley genotypes, with an average of 0.253, polymorphism information content (PIC) of 0.216, and minor allelic frequency (MAF) of 0.118. Additionally, genetic differentiation indicated variances amongst barley populations that ranged from 0.019 to 0.117, indicating a moderate level of genetic divergence. According to analysis of molecular variance (AMOVA), accessions and populations accounted for 46.43% and 52.85%, respectively, of all genetic variation. The heat-map, principal components, and population structure analysis all support the existence of four separate clusters. It is conceivable to establish that there is significant genetic variation among barley genotypes.

6. Conclusion

Based on several genotypes, this study showed significant variation in morphological and molecular profiles. High variety was observed for several morphological parameters, including plant height and the number of leaves per plant, among the 12 Libyan barley genotypes that were estimated in this study No. Pick/ plant, and No. Tillers / plant, and Yield Index. Furthermore, variations in the level of genetic variation across all genotypes were shown by ISSR and SRAP analysis. In plant breeding efforts, the high genetic variety discovered may be utilized to develop novel cultivars and supply relevant data for the conservation of biodiversity. The results of the phylogenetic tree, the heat map created using the ISSR-revealed Dice similarity coefficient, and the SRAP analysis show the genetic relationship between the most productive barley genotypes. Lastly, these findings might help plant breeding programs differentiate between bottle gourd genotypes.

7. Conflicts of interest

There are no conflicts to declare.

8. References

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