PHYLOGENETIC RELATIONSHIPS OF SOME BARLEY (*Hordeum vulgare* L.) ACCESSIONS IN EGYPT

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Barley (*Hordeum vulgare* L.) is one of the founder crops of Old-World agriculture, it was one of the first domesticated cereals. It is also a model experimental system because of its short life cycle and morphological, physiological, and genetic characteristics. Barley is used for, in order of importance, animal feed, brewing malts and human food. Barley is found in widely varying environments globally. It has wide adaptation ability to different climatic conditions and various environments comprising drought and irrigated environments (Nevo, 1992; Gomez-Macpherson, 2001).

Kianoosh *et al.,* (2017) stated that barley, is one of the oldest cultivated crops all over the world. The study of origin, genetic variation and evolutionary relationships in barley is important in the conservation and restoration of biodiversity of wild germplasm. Genetic diversity in domesticated crop species is crucial to fight new pests and diseases and to produce better-adapted varieties for the changing environments.

Besides morphological markers, DNA markers are ordinarily used to allow cultivar identification and fingerprint of genomes in crops; SSR and SCoT are rapid and efficient applications in evaluation, characterization of genetic material (Olgun *et al.*, 2015).

Microsatellites, or simple sequence repeats (SSRs), are stretches of DNA consisting of tandemly repeated short units of 1–6 base pairs in length. The value and uniqueness of microsatellites arise from their codominant inheritance, multiallelic nature, extensive genome coverage, relative abundance, and simple detection by PCR using two unique primers, that flank the microsatellite and hence define the microsatellite locus (Thiel *et al.,* 2003).

Start codon targeted (SCoT) is a novel method for generating plant DNA

markers. Developed based on the short conserved region flanking the ATG start codon in plant genes. This method uses single primers 18-mer in single primer polymerase chain reaction (PCR) and an annealing temperature of 50ºC. PCR amplicons are resolved using standard agarose gel electrophoresis (Collard and Mackill, 2009).

The present study aims to derive data from morphological and molecular attributes to assess the phylogenetic relationships of a collection of barley (*Hordeum vulgare* L.) taxa, based on some morphological, SSR and SCoT analysis

MATERIALS AND METHODS

This study includes eleven accessions of barley *Hordeum vulgare* L.*,* Morphological study was carried out using quantitative traits and molecular study was carried out using SSR and SCoT techniques and further for developing new crop accessions.

1. Plant materials

Eleven Egyptian barley accessions *Hordeum vulgare* L. seeds were kindly provided by Gene Bank of Egyptian National Gene Bank (NGB) Giza, Egypt. Accessions were collected from different regions of Egypt, Their code numbers and regions are recorded in Table (1)

2. Field experiment

This experiment was carried out at the research farm of Faculty of Agricultural, Ain Shams University, Shubra El-Khima, Egypt, during the growing season of 2016. Based on randomized complete block design (RCD) with three replications, to reduce the variance introduced into the data arising from location during the growth seeds were planted by hand in 1 x 3 m plots spacing 20 cm between rows. Ten seeds of each accession were planted in 5 hills and maintained until the end of the experiment. Plots were kept free from weeds. Irrigation was conducted at weekly intervals.

3. Morphological Characterization

Fifteen quantitative morphological traits of *Hordeum* accessions were recorded (Table 2). Ten randomly selected individual plants were used for recording charactrers.

4. Molecular analysis

4.1 DNA extraction and purification

DNA extraction was performed using DNeasy plant Mini Kit (QIAGEN).

PCR was performed in 25µl volume tubes according to Williams *et al.,* (1990).

4.1.1 Polymerase Chain Reaction (PCR) conditions for Simple Sequence Repeats (SSRs) and Start Codon Targeted (SCoT)

The DNA amplifications were performed in an automated thermal cycle (model Techno 512) programmed for one cycle at 94ºC for 4 min followed by 45 cycles of 1 min at 94ºC, 1 min at 37ºC, and 2 min at 72ºC, reaction was finally stored at 72ºC for 10 min. 10 used primers and their sequences were recorded in Tables (3 & 4)

4.2 Molecular data Analysis

The banding patterns generated by PCR marker analyses were compared to determine the genetic relatedness of the samples under study. Clear and distinct amplification products were scored as '1' for presence and '0' for absence of bands. Bands of the same mobility were scored as identical. The genetic similarity coefficient (GS) between two genotypes was estimated according to Dice coefficient (Sneath and Sokal, 1973).

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.

The similarity matrix was used in the cluster analysis. The cluster analysis was employed to organize the observed data into meaningful structures. At the first step, when each accession represents its own cluster, the distances between these accessions are defined by the chosen distance measure (Dice coefficient). However, once several accessions have been linked together, the distance between two clusters is calculated as the average distance between all pairs of accessions in the two different clusters. This method is called Unweighted Pair Group Method using Arithmetic Average (UPGMA) (Sneath and Sokal, 1973). The polymorphism information content (PIC) was calculated for each marker according to a simplified version of *Botstein et al.*, (1980) as follows:

PIC=1-Σ(Pij)2

Where: Pij is the frequency of the i alelle of the locus in 11 accessions revealed by the j primer.

RESULTS AND DISCUSSIONS

1. Quantitative morphological traits of the 11 *Hordeum vulgare* L. accessions

Fifteen quantitative morphological traits of 11 *Hordeum vulgare* L. accessions were recorded in Table (5). The results indicate the existence of high morphological variations in barley accessions. Mean of root length clearly indicated that the Egyptian barley B1 recorded the highest value (13 cm), while the lowest value was 9.8cm recorded by accession B 11. The tallest plants were observed in accession B4 with mean of 106cm from Marsa Matrouh, while the shortest was recorded

in accession B1 (85 cm) from Sharkia. Number of tillers also varied from 10 in accession B1 to 5.5 in accession B11 from Red sea. The highest of stem length was 79 cm recorded in accession B11, on the other hand, the lowest value of stem length was 63.3cm recorded by accession B2 from Dakahlia. Number of nodes was highest in accession B1 and B4 (6.3) and lowest in accession B10 and B11 from Alexandria and Red sea respectively. Data showed that sheathing leaf base length in accession B1, B4 and B5 recorded the highest value (13.5 cm), while the lowest values were 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 (16.8 cm) in accession B1. Flag leaf width ranges from 1.27 cm in accession B5 and B6 to 1.3 cm in accession B2, B4 and B9. For spike length, the tallest was recorded in accession B6 (19 cm), while the shortest recorded in accession B9 (16cm). Spike axis length was the longest in accession B11 (10.2 cm) and shortest in accession B3 (5.2 cm) . Highest values of central awn length in accession B3 and B4 (14.3cm), while the lowest values were recorded in accession B8 (11.7 cm). Number of spikelets was highest (72) in accession B11 and lowest (52.8) in accession B8. Days of heading were highest (72 days) in accession B2 and B11 and lowest in accession B3 (66 days). Leaf area was highest (25 cm^2) in accession B5 and lowest (16.2 cm^2) in accession B1. Weight of 1000 grain was highest (54.2 g) in accession B4 and lowest (28.9 g) in accession B2.

Analysis of variance (ANOVA) indicate 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, and some high significant traits such as leaf base length, leaf width, weight of 1000 grain and spike axis length.

1.1. Cluster analysis of 15 Quantitative morphological traits of 11 *Hordeum valgare* L. accessions

The similarity coefficient of 15 quantitative morphological traits shown in Table (6), 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, whereas the lowest similarity ratio was 0.91% between accessions B2 and B11 both tow- rowed from Dakahlia and Red sea, respectively.

The dendrogram obtained from UPGMA cluster analysis of 15 quantitative morphological characteristics of 11 *Hordeum* accessions is illustrated in Fig. (1). It showed that the accessions were divided into two distinct clusters with genetic similarity rang from 0.936 to 1.00.

Cluster I contain three accessions, accession B4 was separated at taxonomic distance of 0.952 from accessions B5 and B11 both were two-rowed and grouped together at taxonomic distance of 0.965.

Cluster II contain 8 accessions; all of them were six-rowed except B2 with two rowed but it was integrated with the second cluster. This cluster was further subdivided into two subclusters at taxonomic distance of 0.944, subcluster 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 closely 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. Subcluster B contain accession B2 and B1 at taxonomic level 0.949.

Variation in morphological traits based on geographic origin has been established before, where differences are probably related to agronomic adaptation to environments (Bothmer *et al*., 1992). *H. vulgare* cultivated though many phytogeographical regions in Egypt (Boulos, 1995; 2005; 2009)

The most important characters for determination and recognizing of different species number of spikes and spikelets but should always use combination of different characters (von Bothmer and Jacobson, 1985).

Phenotypic heterogeneity in different species is reported because of phenotypic plasticity, which is defined as the flexibility of individual genotypes to grow and develop alternation in response to changing the environmental factors, also somatic polymorphism related to several important characteristics, particularly during their reproductive period (Booy *et al*., 2000; Dekker, 2003).

Hagenblad *et al.,* (2019) studied morphological and genetic characterization of barley accessions in the Canary Islands using 57 accessions collected from Gene-banks. they found that although accessions from the same island tended to be similar, the results showed morphological and genetic diversity both within and among islands

2. Molecular analysis

DNA markers have numerous applications in plant molecular genetic research. One of the most common uses of DNA markers have been the assessment of genetic diversity within crop germplasm (Collard and Mackill, 2009)

2.1. SSR analysis

PCR amplification of the genomic DNA isolated from the 11 barley accessions yielded a total of 50 bands, of which 26 were polymorphic (64 %) and 24 were monomorphic, overall size of the PCR amplified fragments ranged from 50 to 722 bp. (Table 7) shows list of SSR 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). The banding patterns of barley genotypes are shown in Fig. (2).

2.1.1. Cluster analysis as revealed by 10 SSR primers for 11 *Hordeum* accessions

Table (8) shows the genetic similarity indexes among 11 accessions of barley based on banding patterns of 10 SSR markers, detected the maximum value of genetic similarity was 94% between B3 and B4, whereas the minimum value was 70 % between accessions B1 and B5.

The dendrogram obtained from UPGMA cluster analysis of the ten SSR markers of 11 *Hordeum* accessions is illustrated in Fig. (3). It was divided into two distinct clusters with genetic similarity ranged from 0.75 to 0.99.

Cluster I contain the 10 accessions in four subclusters, subcluster A included three accessions, accessions B2 and B9 were grouped together at genetic level of 0.95 and were closely related, while accession B6 was separated from them at genetic distance of 0.91. Subcluster B also include three accessions, accessions B3 and B4 were grouped together at genetic similarity of 0.93 separated from accession B10 at genetic distance of 0.92. As for subcluster C, it included three accessions; accession B5 was separated at single distance from the closely related B7, B8. On the other hand, subcluster D included single accession, accession 11 separated at genetic distance 0.84. Cluster II contained only one accession B1 separated from the rest at genetic distance 0.75.

SSR have become important molecular markers for a wide range of applications, such as phenotype mapping, marker assisted selection of crop plants, genome mapping and characterization and a range of molecular ecology and diversity studies (Robinson *et al.*, 2004).

SSR amplification products (alleles) are amplified using PCR so that only a small amount of starting DNA is required. Alleles vary according to the number of repeat units present, but other mutations have also been shown to be responsible for allele length variation (Kumar *et al*., 2009).

Naceur *et al.*, (2012) studied the phylogenetic relationships of 31 barley accessions from North Africa (Algeria, Tunisia, and Egypt) using 11 SSR primers. A total of 478 reproducible bands were scored, Nandha and Singh, (2014) stated that species specific markers have a great potential for introgression of important traits from wild to cultivated barley, tomato, potato, rice, maize and barley. An overview of the reported results for barley indicated that diversity parameters varied significantly among studies (Matus and Hayes, 2002).

It is noteworthy that cluster analysis is a valuable tool for subdividing genotypes into groups including similar and dissimilar accessions and has a great value from the breeder's point of view for initiating barley hybrid programs.

2.2. SCoT analysis

PCR amplification of the genomic DNA isolated from the 11 barley accessions yielded a total of 143 bands, of which 108 were polymorphic (72%) and 35 were monomorphic (28 %). Overall size of the PCR amplified fragments ranged from 125 to 2060 bp . List of SCoT 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) are shown in Table (9). The Scot banding patterns of barley genotypes are shown in Fig. (4).

2.2.1. Cluster analysis as revealed by 10 SCoT primers for 11 *Hordeum* accessions

Table (10) show the genetic similarity indexes among the 11 accessions of barley based on banding patterns of 10 SCoT markers and detected the maximum value of genetic similarity was 80 % between B9 and B11, on the other hand the minimum value was 50 % detected between B3 and B10, and also between B4 and B8.

The dendrogram obtained from UPGMA cluster analysis of ten SCoT markers of 11 *Hordeum* accessions is illustrated in Fig. (5). The results showed that the dendrogram was divided into two distinct clusters with genetic similarity range from 0.55 to 0.95.

Cluster I was divided into two subclusters at genetic distance 0.95; subcluster A contains four accessions, accession B7 separated at 0.71, accessions B9 and B11 were closely related grouped in a single sub-cluster at genetic distance of 0.80, while accession B8 was separated at genetic distance of 0.67. Sub-cluster B contained only accession B10 in a separate sub-cluster at 0.65.

On the other hand, cluster II was divided into sub-cluster C which included three accessions; accession B6 was separated at genetic distance of 0.66, while accessions B1 and B2 were grouped together at 0.71. Subcluster D also included three accessions, accession B3 was separated at genetic distance 0.69, while accessions B4 and B5 were grouped together at genetic distance of 0.76.

SCoT is based on PCR technology, as a new molecular marker method, it has several advantages: simple, low-cost, high polymorphic, extensive genetic information and its primers are universal in plants. These advantages have been validated through studies on genetic diversity in rice (Collard and Mackill, 2009), peanut (Xiong *et al.*, 2011) and potato (Gorji *et al*., 2011).

SCoT markers are multilocus which are helpful in obtaining high genetic polymorphism. Besides, SCoT markers are expected to be linked to functional genes and corresponding traits, thus the amplicons can be converted to

gene targeted marker systems (Xiong *et al*., 2011).

Guo *et al*., (2012) used SCoT technique to assess genetic relationships among 64 grape varieties. Seventeen informative primers were selected from 36 SCoT primers based on their ability to produce repeatable and clear polymorphic and unambiguous bands among the varieties.

3. Combined data of quantitative morphological traits with 10 SSR and 10 SCoT primers for 11 *Hordeum* accessions

Combination of the data obtained from the two molecular marker systems along with quantitative morphological traits could give more accurate estimation to the genetic relations among the studied accessions, as illusterated in Table (11) and (Fig. 6). This agreed with the results reported by Abdel-Tawab *et al*., (2008).

SUMMARY

phylogenetic relationships of eleven accessions of *Hordeum vulgare* L. collected from different region of Egypt were assessed. Fifteen quantitative morphologcal traits were used, the measured data were evaleuated statistically using ANOVA, phylogenetic tree were constructed using UPGMA. Also simple sequance repeats (SSRs) and start codon targeted (SCoT) mulecular marker techniques were used for DNA fingerprinting and assessing genetic divesity and phylogenetic relationships in barley germplasm. The results showed that SSR primers produced 50 bands ranged in size between 50-722 bp with 64% polymorphism percentage and SCoT primers produced 143 bands, ranged in size between 165-2060 bp with 72% polymorphism percentage. Polymorphic information content PIC was 0.44 and 0.82 for SSRs and SCoT, respectively. UPGMA dendrogram was divided into two clusters by each of morphological traits, SSRs and SCoT analysis. Genetic similarity matrix was examined with Jacard's coefficient, maximum similarity was found between B7 and B8 (0.98%) with quantitative morphological analysis, between B3 and B4 (94%) with SSRs analysis and between B9 and B11 (80%) with SCoT analysis. However, combined cluster analysis between the three components together revealed better resolution for distinction between these taxa.

REFERENCES

Abdel-Tawab F. M., Rashed M. A., Azer S. A., El-Metabteb G. M. and khafaga F., (2008). Molecular fingerprinting and marker-assisted selection in citrus species. Egypt. J. Genet. & Cytol., 37: 335-358.

Booy G., Hendriks R. J. J., Sulderns M.J.M., Van Groenendeal J.M. and Vosman B., (2000). Genetic diversity and the survival of populations. Plan Biol., 2: 379-395.

Bothmer R. V., (1992). The wild species of Hordeum: relationships and potential use for improvement of cultivated barley. Barley: genetics, biochemistry, molecular biology and biotechnology., 3-18.

Botstein D., White R. L., Skolnick M., Davis R. W., (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet., 32: 314-331.

Boulos L., (1995). Flora of Egypt checklist. Al Hadara Publishing Cairo Egypt, 1-283.

Boulos L., (2005). Flora of Egypt Vol.VI. Al Hadara Publishing Cairo Egypt, 1-617.

Boulos L., (2009). Flora of Egypt checklist. Revised annotated edition. Al Hadara Publishing Cairo Egypt, 292.

Collard B. C. , and Mackill D. J., (2009). Start codon targeted (SCoT) polymorphism: a simple, novel DNA marker technique for generating gene-targeted markers in plants. Plant Molecular Biology Reporter, 27: 86.

Dekker J., (2003). The foxtail (Setaria) species-group. Weed Science, 51: 641-656.

Gomez-Macpherson H., (2001). *Hordeum vulgare*. http://ecoport.org/ep?Plant=1232&entityType=PL****&entityDisplayCategory=full

Gorji A. M., Poczai P., Polgar Z. and Taller J., (2011). Efficiency of arbitrarily amplified dominant markers (SCoT, ISSR and RAPD) for diagnostic fingerprinting in tetraploid potato. Am. J. Potato Res., 88: 226-237.

Guo D. L., Zhang J. Y., and Liu C. H., (2012). Genetic diversity in some grape varieties revealed by SCoT analyses. Molecular Biology Reports, 39: 5307-5313.

Hagenblad J., Leino M. W., Afonso G. H., and Morales D. A., (2019). Morphological and genetic characterization of barley (*Hordeum vulgare* L.) landraces in the Canary Islands. Genetic Resources and Crop Evolution, 66: 465-480.

Kianoosh C., Leila Z., Reza Z. A. and Saeid J. H., (2017). Study of genetic variation among diverse barley (*Hordeum vulgare* L.) genotypes using agro-morphological traits and RAPD, ISSR, AFLP markers. Research Journal of Biotechnology, 12: 6.

Kumar P., Gupta V. K., Misra A. K., Modi D. R., and Pandey B. K., (2009). Potential of molecular

markers in plant biotechnology. Plant omics, 2: 141.

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

Naceur A. B., Chaabane R., El-Faleh M., Abdelly C., Ramla D., Nada A. and Sakr M., (2012). Genetic diversity analysis of North Africa's barley using SSR markers. Journal of Genetic Engineering and Biotechnology, 10: 13-21.

Nandha P. S. and Singh J., (2014). Comparative assessment of genetic diversity between wild and cultivated

barley using g SSR and EST-SSR markers. Plant breeding, 133: 28-35.

Nevo E., (1992). Barley: Genetics, Biochemistry, Molecular Biology and Biotechnology. University of Haifa: 19-43.

Olgun M., Budak Başçiftçi Z., Ayter N. G., Turan M., Koyuncu O., Ardıç M., Ağar G., and Takıl E., (2015). Genetik divergence in some barley (*Hordeum vulgare* L.) genotypes by RAPD and ISSR Analyses. Süleyman Demirel Üniversitesi Ziraat Fakültesi Dergisi, 10:102-109.

Robinson A. J., Love C. G., Batley J., Barker G. and Edwards D., (2004). Simple sequence repeats marker loci discovery using SSR primer. Bioinformatics, 20: 1475-1476.

Sneath P. H. A. and Sokal R. R., (1973). Numerical Taxonomy. Freeman, San Francisco, California.

Thiel T., Michalek W., Varshney R., and Graner A., (2003). Exploiting EST databases for the development and characterization of gene-derived SSR-markers in barley (*Hordeum vulgare* L.). Theoretical and Applied Genetics, 106: 411-422.

Von Bothmer R. and Jacobson N., (1985). Barley origin, taxonomy, and related species. Barley Agronomy, 26: 19-51.

Williams J. G. K., Kubelike A. R., Livake K. T., Rafalski J. A. and Tingoy S., V. (1990). DNA polymorphism amplified by random primers are useful as genetic markers. Nucleic Acid Research, 18: 6531-6539.

√Xiong F., Zhong R., Han Z., Jiang J., He L., Zhuang W. and Tang R., (2011). Start codon targeted polymorphism for evaluation of functional genetic variation and relationships in cultivated peanut (*Arachis hypogaea* L.) varieties. Mol. Biol. Rep., 38: 3487-3494.

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

no	Accession	Gene bank bar code	Regions
1-	B1	11418	Sharkia
2-	B2	11514	Dakahlia
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	Sharkia
10-	B10	11917	Alexandria
11-	B11	11937	Red sea

Table (2): Quantitative morphological traits of *Hordeum vulgare* L. accessions.

Trait	Trait definition
1-Root length	Measured at maturity from the ground to the tip of the longest fibrous root (pulling carefully to avoid rupture).
2-Plant height	Length of randomly selected plants measured from the ground to the tip of the spike excluding awns at maturity.
3-No. of tillers	Number of fertile tillers (spike bearing) of randomly selected plants counted at maturity.
4-Stem length	Length of the main stem by cm, measured from the cotyledonary node up to the shoot apex on the base of terminal spike.
5-No. of nodes	Number of internodes of the main stem.
6-Leaf base length	Sheathing base of flag leaf.
7-Leaf length	Length of flag leaf by cm.
8-Leaf width	At the widest part of flag leaf by cm.
9-Spike length	From the base of the spike to the top of the awn.
10-Spike axis length	From the base of the spike to the top of the awn excluding awns.
11-Awn of central lemma length	Distance from tip of the spike to the end of the awn (cm).
12-No. of spikelets	Number of all spikelets per spike on randomly selected plants, counted at maturity.
13- Days of heading	Number of days from planting to the day when 50% of the heads fully flower (heading) emerge of flag leaf.
14- Leaf area	Leaf length X leaf width X 0.905 (Kemp, C. D. 1959).
15- Wt 1000 grain	As the weight of 200 kernels taken from the bulk seed of the plot and multiplied by 5.

Table (3): SSR primers with their sequence and chromosome number.

No	Primer name	Sequence 5'-3' forward	Sequence 5'-3' revers	Chromosome no.
1	MGB391	AGCTCCTTTCCTCCCTTCC	CCAACATCTCCTCCTCCTGA	2H
2	HVITR1	CCACTTGCCAAACACTAGACCC	ATTCATGCAGATCGGGCCAC	3H
3	HV13GEIII	AGGAACCCTACGCCTTACGAG	AGGACCGAGAGTGGTGGTGG	3H
4	MGB396	CGCTAGCTTGTTTCTCGTTTG	TCGCATGGCATCAACTACAG	4H
5	MGB402	CAAGCAAGCAAGCAGAGAGA	AACTTGTGGCTCTGCGACTC	1H
6	Bmag149	CAAGCCAACAGGGTAGTC	ATTCGGTTTCTAGAGGAAGAA	1H
7	HVGLUEND	TTCGCCTCCATCCCACAAAG	GCAGAACGAAAGCGACATGC	1H
8	MGB371	CACCAAGTTCACCTCGTCCT	TTATTCAGGCAGCACCATTG	6H
9	MGB356	TGGTCTGGAGCTCTCAACAG	AAGCCACATTGAAGGAGCAC	6H
10	EBmac624	AAAAGCATTCAACTTCATAAGA	CAACGCCATCACGTAATA	6H

Table (4): SCoT primers with their sequence.

No	Primer	Primer sequence 5´→ 3´
1	**SCoT 1**	5'- ACG ACA TGG CGA CCA CGC -3'
2	**SCoT 2**	5'- ACC ATG GCT ACC ACC GGC -3'
3	**SCoT 3**	5'- ACG ACA TGG CGA CCC ACA -3'
4	**SCoT 4**	5'- ACC ATG GCT ACC ACC GCA -3'
5	**SCoT 9**	5'- ACA ATG GCT ACC ACT GCC -3'
6	**SCoT 10**	5'- ACA ATG GCT ACC ACC AGC -3'
7	**SCoT 12**	5'- CAA CAA TGG CTA CCA CCG -3'
8	**SCoT 13**	5'- ACC ATG GCT ACC ACG GCA -3'
9	**SCoT 14**	5'- ACC ATG GCT ACC AGC GCG -3'
10	**SCoT 15**	5'- CCA TGG CTA CCA CCG GCT -3'

Table (5): 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**
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- 1000 grain wt	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 letters was not significant differe. * P<0.01, **P<0.001

Table (6): Similarity indexes among 11 accessions of *Hordeum vulgare* L. based on 15 quantitative 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

Table (7): List of SSR 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	MS	TB	PB	MP	PP	SB	PIC
MGB391	90-450	8	4	4	50%	+(2), -(2)	0.29
HVITR1	90-110	3	2	1	67%	+(1), -(1)	0.15
HV13GEIII	55-390	7	0	7	0%	0	0.00
MGB396	50-170	4	3	1	75%	-(1)	0.55
MGB402	175-510	6	1	5	83%	+(1)	0.80
Bmag149	60-300	4	2	2	50%	0	0.32
HVGLUEND	400-100	4	3	1	75%	+(1)	0.66
MGB371	65-320	7	7	0	100%	+(3), -(1)	0.83
MGB356	60-722	5	2	3	40%	0	0.25
EBmac624	52-57	2	2	0	100%	0	0.57
Total	----	50	26	24	----	13	----
Mean	----	5	2.6	2.4	64%	----	0.44

Table (8): Similarity indices among 11 accessions of barley based on banding patterns of 10 SSR markers.

	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11
B1	**100**										
B2	**72**	**100**									
B3	**84**	**86**	**100**								
B4	**84**	**86**	**94**	**100**							
B5	**70**	**83**	**82**	**82**	**100**						
B6	**74**	**91**	**87**	**87**	**84**	**100**					
B7	**74**	**84**	**90**	**87**	**93**	**82**	**100**				
B8	**78**	**86**	**89**	**89**	**91**	**84**	**93**	**100**			
B9	**74**	**85**	**88**	**88**	**84**	**89**	**86**	**85**	**100**		
B10	**79**	**91**	**93**	**93**	**87**	**88**	**91**	**93**	**92**	**100**	
B11	**75**	**86**	**82**	**85**	**79**	**84**	**81**	**85**	**91**	**87**	**100**

Table (9): List of SCoT 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	MS	TB	PB	MP	PP	SB	PIC
SCoT 1	240 - 745	9	6	3	66%	+(2), -(1)	0.88
SCoT 2	165 - 1160	14	8	6	57%	+(3)	0.70
SCoT 3	125 - 1245	14	7	**7**	50%	+(1)	0.80
SCoT 4	310 - 1870	15	12	3	80%	+(6)	0.83
SCoT 9	270 - 1920	15	13	2	86%	+(4)	0.88
SCoT 10	350 - 1790	16	15	1	93%	+(1), -(3)	0.90
SCoT 12	275 – 1935	**18**	**18**	**0**	**100%**	+(3), -(1)	0.90
SCoT 13	385 – 2060	16	11	5	68%	-(4)	0.90
SCoT 14	335 – 1050	**8**	**3**	5	**37%**	+(1)	0.88
SCoT 15	220 - 1335	**18**	15	3	83%	+(4)	0.48
Total	----	143	108	35	----	34	
Mean	----	14.3	10.8	3.5	72%	3.4	0.82

Table (10): Similarity index among 11 accessions of barley based on banding patterns of 10 SCoT markers.

	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11
B1	**1.00**										
B2	**0.71**	**1.00**									
B3	**0.65**	**0.66**	**1.00**								
B4	**0.63**	**0.61**	**0.70**	**1.00**							
B5	**0.61**	**0.63**	**0.68**	**0.78**	**1.00**						
B6	**0.63**	**0.69**	**0.59**	**0.62**	**0.62**	**1.00**					
B7	**0.63**	**0.66**	**0.57**	**0.63**	**0.59**	**0.69**	**1.00**				
B8	**0.59**	**0.58**	**0.54**	**0.50**	**0.55**	**0.60**	**0.67**	**1.00**			
B9	**0.64**	**0.61**	**0.52**	**0.59**	**0.59**	**0.63**	**0.74**	**0.67**	**1.00**		
B10	**0.59**	**0.54**	**0.50**	**0.56**	**0.54**	**0.55**	**0.61**	**0.61**	**0.69**	**1.00**	
B11	**0.63**	**0.59**	**0.53**	**0.58**	**0.59**	**0.59**	**0.70**	**0.68**	**0.80**	**0.69**	**1.00**

Table (11): Similarity index among 11 accessions of barley based on combined quantitative morphological traits, 10 SSR markers and 10 SCoT markers.

	B_1	B_2	B_3	B_4	B_5	B_6	B_7	B_8	B_9	B_10	B_11
B_1	1										
B_2	0.70	1									
B_3	0.70	0.72	1								
B_4	0.69	0.69	0.79	1							
B_5	0.62	0.68	0.72	0.78	1						
B_6	0.65	0.75	0.68	0.70	0.68	1					
B_7	0.65	0.71	0.68	0.70	0.70	0.72	1				
B_8	0.64	0.66	0.65	0.62	0.67	0.67	0.75	1			
B_9	0.66	0.71	0.63	0.68	0.66	0.71	0.77	0.72	1		
B_10	0.64	0.65	0.64	0.68	0.64	0.65	0.70	0.71	0.76	1	
B_11	0.65	0.67	**0.61**	0.66	0.64	0.66	0.72	0.73	**0.83**	0.73	1

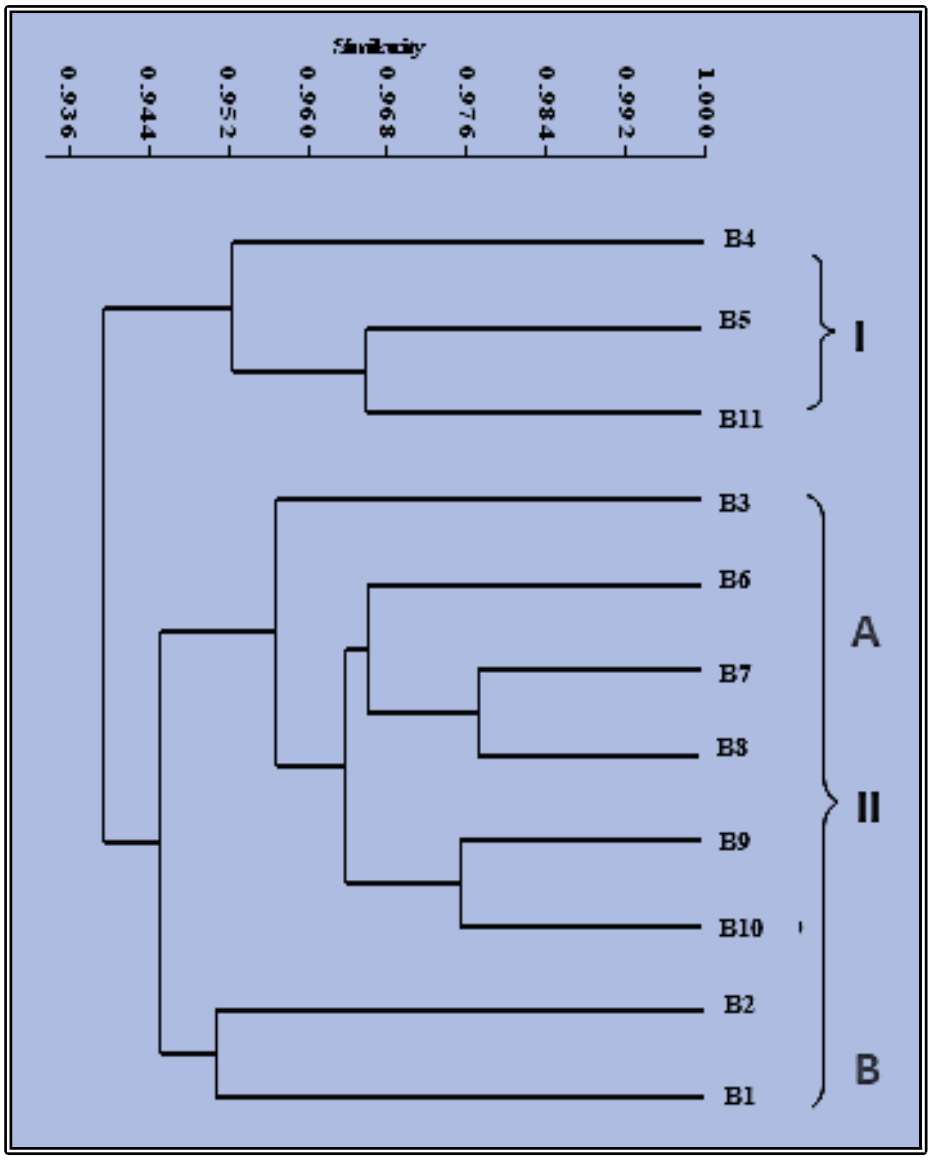

Fig. (1): Dendrogram representing the quantitative morphological traits of 11 *Hordeum vulgare* L. accessions using UPGMA cluster analysis generated from 15 quantitative morphological traits.

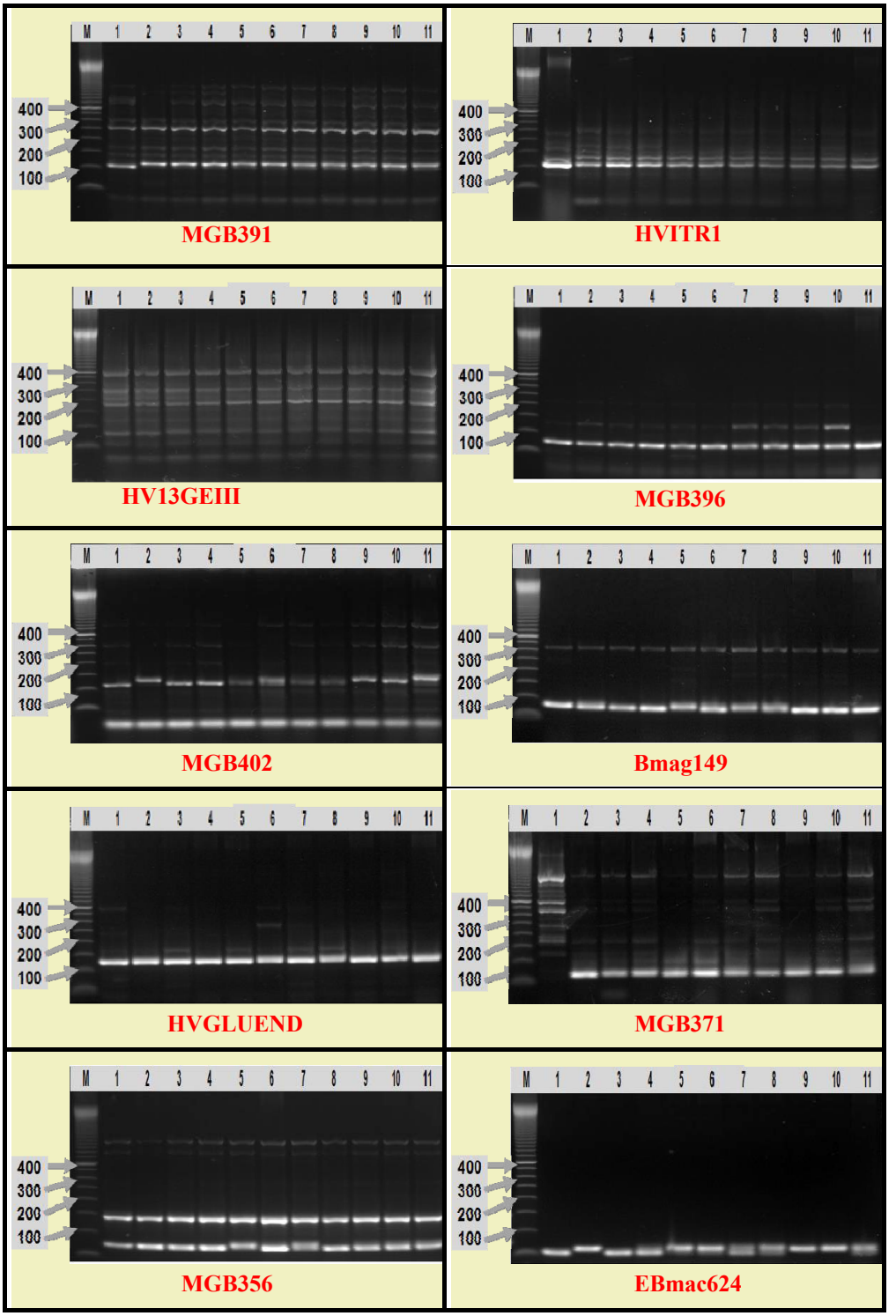

Fig. (2): Banding patterns of 11 *Hordeum vulgare* L. accessions using 10 SSR primers.

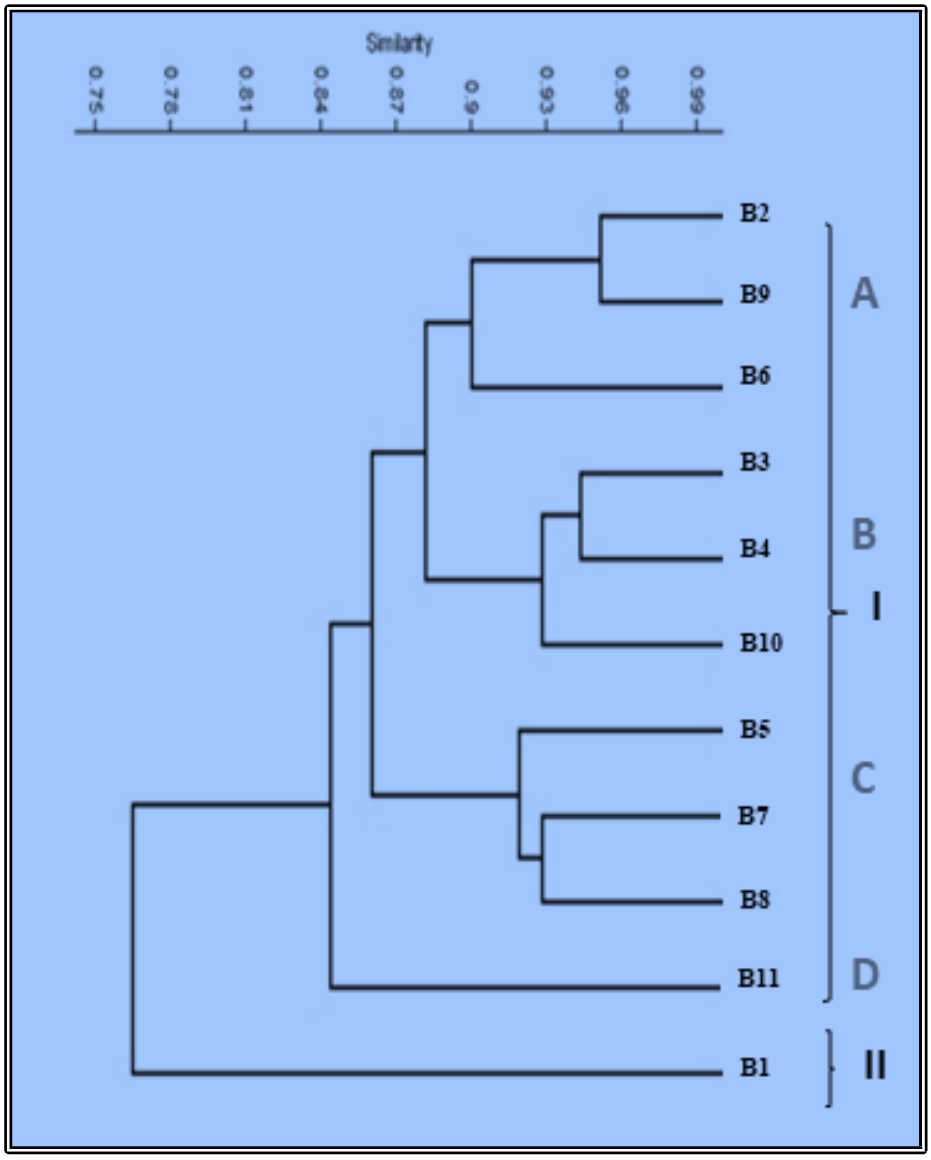

Fig. (3): Dendrogram using UPGMA cluster analysis of ten SSR markers.

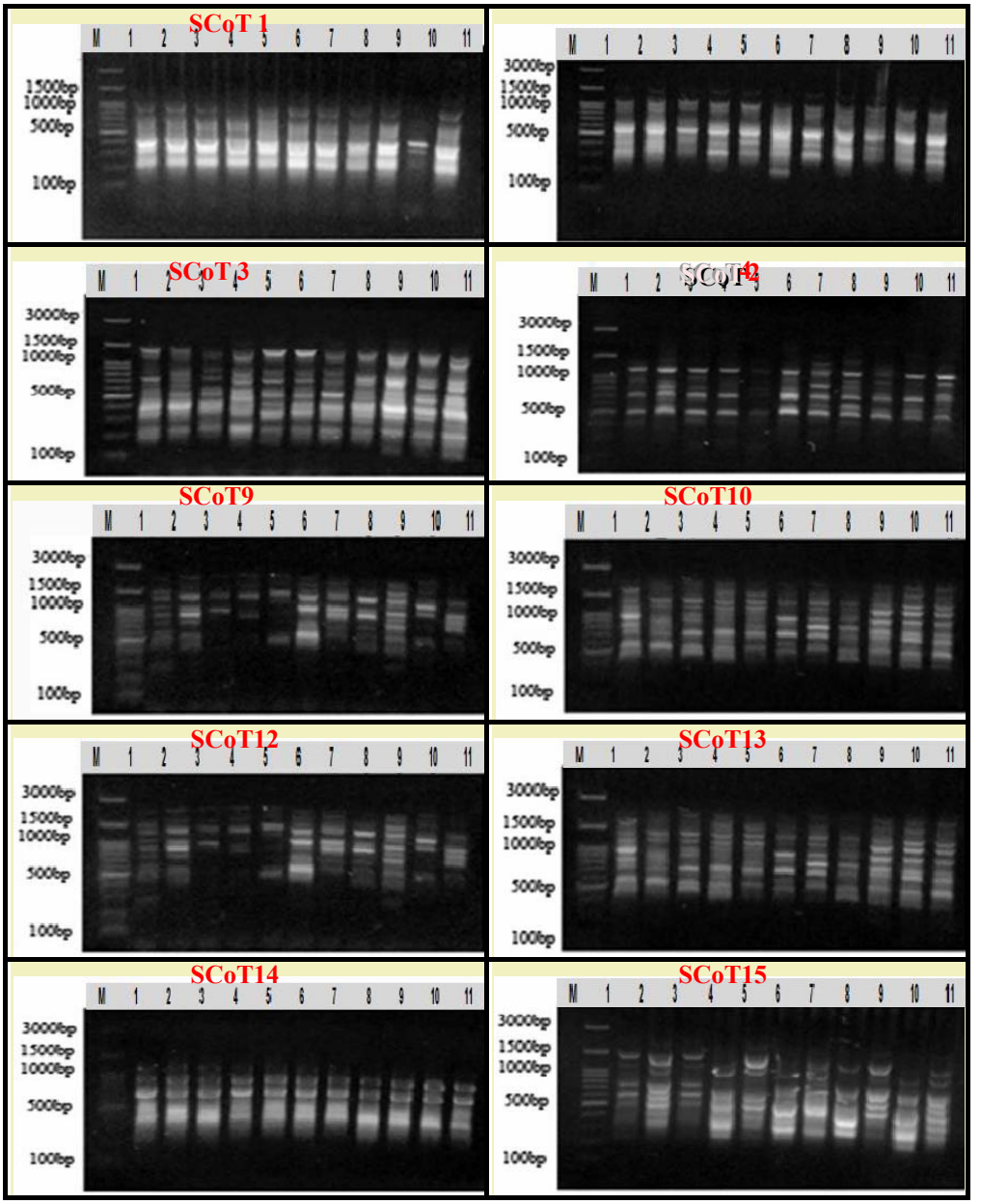

Fig. (4): Banding patterns of 11 *Hordeum vulgare* L. Accessions using 10 SCoT primers.

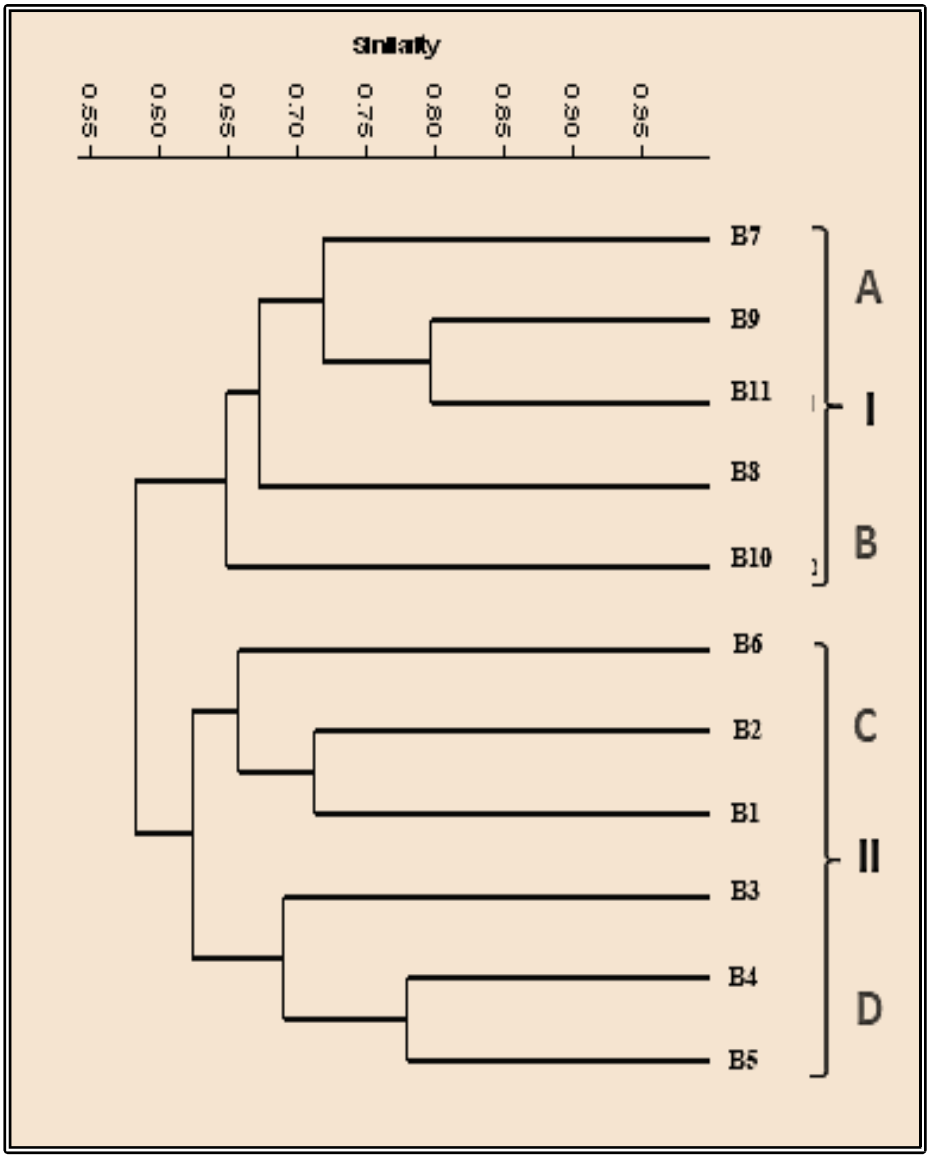

Fig. (5): Dendrogram using UPGMA cluster analysis of ten SCoT markers.

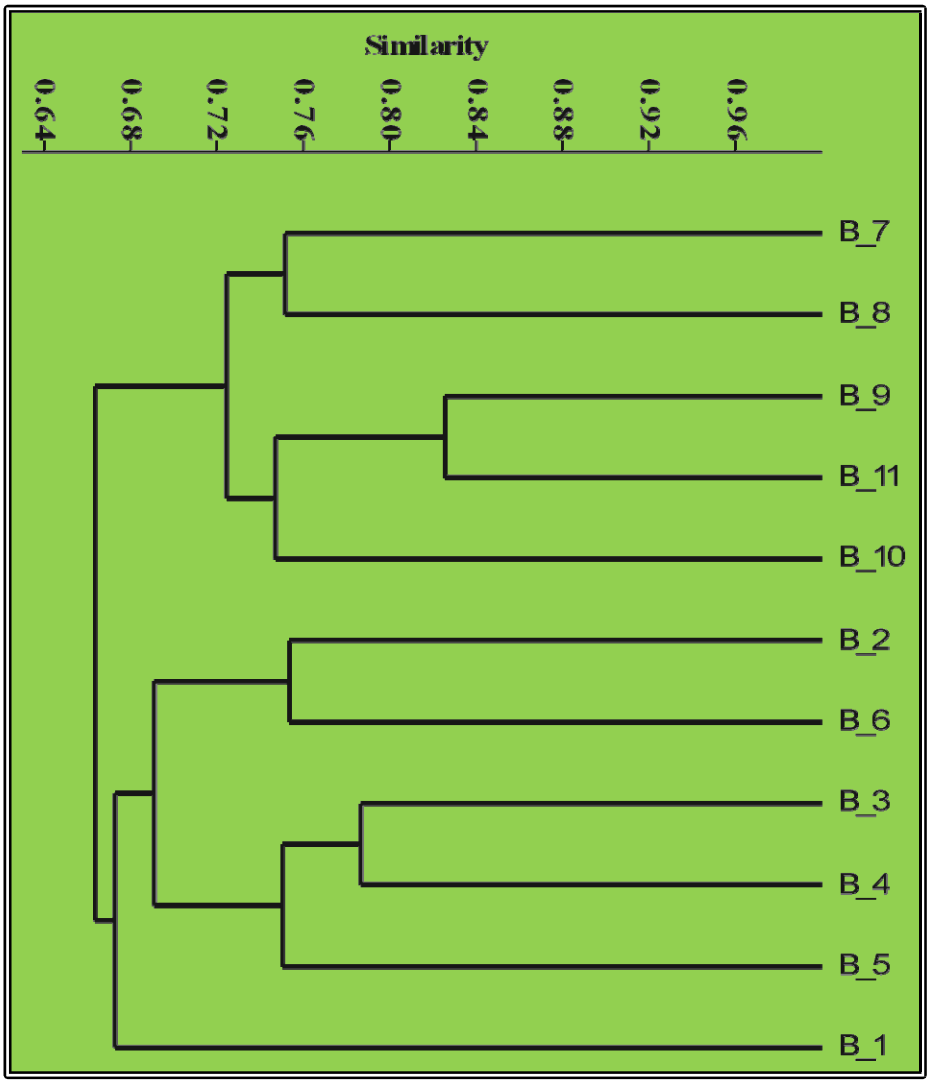

Fig. (6): Dendrogram using UPGMA cluster analysis of combined qualitative morphological traits with 10 SSR markers and 10 SCoT markers.