

GENETIC RELATIONSHIP BETWEEN SOME BARLEY GENOTYPES (*HORDEUM VULGARE* L.) USING SSR TECHNIQUE (MICROSATELLITE)

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ABSTRACT

The study was carried out in order to determine the degree of genetic kinship among fifteen barley genotypes using SSR technique with 14 pairs of primers. The study showed that, all the primers gave amplification products and showed polymorphism between the studied genotypes. The total number of alleles was 19, with an average of 1.35, and the number of polymorphic alleles was 19, the percentage of polymorphism was 100%. The number of total and polymorphic alleles ranged between one allele as the lowest value for primers (Bmac0209, Bmac0067, Bmag0225, Bmag012, Bmag0394, Bmag0353, Bmag0206, Bmac0031, GBM1362, EBmac0603) and three alleles as the highest value for the primer Bmag0006. The polymorphism information content (PIC) for each primer ranged from 0.32 (Bmac0209, Bmag0225) as the lowest value, to 0.721 (Bmag0385) as the highest value, and average PIC value was 0.446. The highest value of the percent agreement values (PAV) matrix was about 0.909, between Furat 7 and Mohsen White cultivars, which indicate the present of a high degree of genetic kinship, followed by the Furat 5 and Asowad Arabic (0.863), while the lowest value was 0.318 between H10 and Furat 4, indicating a great genetic variation between them. The previous results were in agreement with the genetic kinship analysis and cluster analysis which showed a high degree of genetic similarity between Furat 7 and Mohsen White, followed by Furat 5 and Asowad Arabic.

Key Words: polymorphism, genetic relationship, simple sequences repeats.

الخلف وآخرون

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علاقات القرابة الوراثية بين بعض الطرز الوراثية من الشعير (*Hordeum vulgare* L.) باستخدام تقنية SSR (Microsatellite)

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المستخلص

نُفذ البحث بهدف تحديد درجة القرابة الوراثية بين خمسة عشر طرازاً وراثياً من الشعير، من خلال تقنية التكرارات الترادفية البسيطة SSR، باستعمال 14 زوج من البادئات. أظهرت نتائج الدراسة أن جميع أزواج البادئات أعطت منتجات التضخيم وأظهرت وجود تعددية شكلية بين الطرز الوراثية المدروسة. بلغ عدد الأليلات الكلية قرابة 19 أليلاً، بمتوسط 1.35، كما بلغت عدد الأليلات المتباينة شكلياً نحو 19 أليلاً، وبلغت النسبة المئوية للتعددية الشكلية نحو 100%. تراوح عدد الأليلات الكلية والمتباينة شكلياً بين أليلاً واحداً كأقل قيمة مع كل من أزواج البادئات (Bmac0209، Bmac0067، Bmag0225، Bmag012، Bmag0394، Bmag0353، Bmag0206، Bmac0031، GBM1362، EBmac0603)، و 3 آائل كأعلى قيمة مع زوج البادئة (Bmag0006). وتراوحت قيمة معامل التعددية الشكلية (PIC) لكل بادئة على حده، 0.32 عند أزواج البادئات (Bmac0209، Bmag0225) كأقل قيمة، إلى 0.721 عند زوج البادئة (Bmag0385) كأعلى قيمة، وبلغ متوسط قيمة PIC 0.446. وكانت أعلى قيمة لمصفوفة النسب المئوية للتوافق (PAV) نحو 0.909 بين الصنف فرات 7 والصنف أبيض محسن، ما يدل على أنهما على درجة كبيرة من القرابة الوراثية، تلاهما الصنفين فرات 5 وعربي أسود بنحو 0.863، بينما كانت أقل قيمة للنسب المئوية للتوافق نحو 0.318 بين السلالة H10 والصنف فرات 4، ما يدل على وجود تباين وراثي كبير بينهما. وتفق ذلك مع نتائج التحليل العنقودي وشجرة القرابة الوراثية، حيث كانت أعلى درجة من التشابه الوراثي بين الصنفين فرات 7 وأبيض محسن، تلاهما بين الصنفين فرات 5 وعربي أسود.

الكلمات المفتاحية: التعددية الشكلية، القرابة الوراثية، التكرارات الترادفية البسيطة، جينات، اليلات

INTRODUCTION

Cultivated barley (*Hordeum vulgare* L.) is a member of the Poaceae family, which is the largest family of monocots, and it belongs to the Poales order, and *Hordeum* genus, which includes about 32 species (23). The barley crop ranks fourth in the world list of cereal crops, and comes in terms of economic importance, area, and production after wheat (*Triticum ssp.*), Rice (*Oryza sativa* L.), and Corn (*Zea mays* L.) (8). Barley is a preferred plant in genetic studies, due to its diploid chromosomes number ($2n = 2x = 14$), being self-pollinated, large size of chromosomes, large genetic variation within the genus *Hordeum*, ease of hybridization, wide environmental adaptation ability, and relatively limited water and nutrients requirements. The barley genome has seven pairs of chromosomes that have been identified and characterized (12). In breeding work and programs, genetic variation is important in order to enhance any crop, and therefore, new genetic variances must be constantly detected to follow the improvement process. Introduction, selection, and hybridization are the primary methods for inducing these variations in self-pollinated crops, beside mutations which can play an important role as well (6). Kashif and Khaliq (11) showed that reaching new varieties with high genetic potential for grain has become a primary goal of all breeding and genetic improving programs. To achieve this goal, the plant breeder must know the genotype and the nature of the genes that control the plant's response to different environments. Several chemical and biological techniques have been used to characterize barley (4), but those methods were not effective and adequate enough due to the low allelic variation detection. In general, despite the importance of morphological, physiological, and biochemical characteristics, the need for molecular markers is becoming more important and urgent, as they provide early results, which helps to speed up the selection and breeding processes, as well, shortening the time spent on traditional breeding programs. Molecular markers become more used and common in genetic studies due to the absence of a relationship between the growth stages of the

plant and the molecular markers, therefore, DNA extracted from early stages of the crop life cycle can be used, in addition, the ease of locating the required genetic site for a particular genotype directly, the molecular markers are not affected by the plant's phenotype and the environmental factors which make them more powerful in genetic studies. As will, a large number of markers can be obtained in a relatively short period of time (4). Biotechniques based on molecular markers at the DNA level have been developed for most agricultural crops with the aim of determining genetic diversity (19), such as RAPD, ISSR (9), AFLP (26), STS (14), and an SSR (22, 27). In this area of scientific research, Ramsay et al (18) worked on developing SSR primers for the purpose of molecular characterization and genetic linkage mapping of the barley crop. Simple Sequence Repeats (SSRs) are one of the most important and widespread molecular markers nowadays, as these markers consist of short, tandem repeating sequences, called repeating units, consisting of different combinations of four units, Adenine (A), Cytosine (C), guanine (G) and thiamine (T), the DNA bases, which are abundant in eukaryotic genes, and distributed over all chromosomes, whether in the coding or non-coding regions, and it is one of the most important techniques based on the polymerase chain reaction PCR), and has been applied by Powell et al (17). SSRs, are microsatellite known as simple sequence length polymorphism, which are abundant, spread randomly along the genome, ranges in length from 1-6 bp in Eukaryotes, and they are highly efficient in detecting genetic variations between and within biological populations (16). Assessment of genetic diversity using molecular markers is one of the most basic and important steps in breeding programs. Chaabane et al (5) studied the molecular characterization of some Tunisian barley genotypes (*Hordeum Vulgare* L.) using the 18 SSR primer pairs. Their study showed that a total of 31 alleles were produced, the number of alleles ranged from 1 to 5 with an average of 2.81. The results were analyzed and the genetic kinship tree was plotted, and SSR proved to be effective in distinguishing between genotypes. El-Awady et al (7),

conducted a study to assess genetic variation within local barley strains in Saudi Arabia. 16 pairs of primers (SSRs) were used to characterize six selected barley strains from different cultivated areas. Amplification products were obtained for 15 pairs of SSRs and only seven of them showed patterns with clear polymorphisms. These seven primers gave a total of 16 alleles, and their molecular weight ranged from (275 bp-100), while the number of alleles per primer ranged from 1 to 3, with an average of 2.29 alleles per locus, and the data from these seven primers were sufficient to distinguish between the barley genotypes. Gougerdchi et al. (10) studied and assessed the genetic diversity of 52 barley strains using 68 pairs of SSR primers, of which 47 pairs gave amplification products, and gave a total of 153 alleles with polymorphism. The number of alleles ranged from 2 to 9, with an average of 3.26 alleles per locus. The polymorphism information content (PIC) ranged from 0.07 to 0.81, with a mean of 0.45. The SSR primers also proved their efficiency using cluster analysis as the barley strains were divided into two groups where the genetic diversity was relatively consistent with geographical origins. Al-Naggar et al. (2) studied the genetic diversity of 20 strains of Egyptian wheat using 10 pairs of SSR primers, and the amplification results gave a total of 27 alleles. The number of polymorphic alleles was 23 (85.2%), and PIC ranged from 0.67 to 0.94 indicating the present of genetic diversity between the studied wheat strains using rich informative SSR in this study. Al-Hadeithi (1) studied nine varieties of Iraqi barley, which

were distinguished and characterized using SSRs, to reveal the genetic variation between them. Six pairs of SSRs primers were used in molecular assay of the barley samples. These primers yielded 11 bands that were divided into 8 contrasting and 3 identical bands. The genetic variance reached 80% and ranged between (50-100%). The average number of polymorphic bands for each primer was 1.6. These primers produced amplified bands with molecular weight ranged between 75-900 bp. One unique band with a weight of 200 base pairs appeared and the researchers suggested using this unique band as a genetic marker for further studies. Misganaw et al (15), used 22 pairs of SSR primers to characterize the genetic diversity of 39 barley cultivars collected from the barley breeding program in Ethiopia, SSRs produced a total of 73 alleles at a rate of 5 alleles per locus, and PIC ranged from 0.17 to 0.60 with an average of 0.47, indicating the importance of the primers for future analysis of barley genetic diversity. This research aims to determine the degree of genetic kinship for the studied varieties using the simple sequence repeats (SSR) technique.

MATERIALS AND METHODS

Plant material: The study was carried out using fifteen genotypes of barley, (varieties, strains, wild landraces), obtained from the General Authority for Scientific Agricultural Research, Damascus, Syria. The used varieties are: Asowad Arabic, Mohsen White, Furat 4, Furat 5, Furat 6, Furat 7, Furat 9. While the strains are H1, H6, H10, H13, H18, H19. And the wild landraces were *H. Vulgare* and *H. Spontaneum* (Table 1).

Table 1. Characteristics of studied genotype

Studied genotypes		
Strains	Name	Pedigree
H1	7776 GCSAR	Pamir-147/Sonata/3/Pamir-154//ICB-100709/WB156 ICB03-2240-0AP-10AP-0AP
H6	7771GCSAR	BICHY2000/PRTL
H10	GCSAR7774	CBSS04B00111S-7M-0Y-0M-2Y-0M-0AP
H13	GCSAR7775	6B95.2482//PENCO/CHEVRON-BAR/3/LEGACY CBSS01Y00890T-A-0Y-1M-1M-2Y-0M-0AP
H18	7773GCSAR	Clipper//WI2291*2/WI2269/5/Soufara-02/3/RM1508/Por//WI2269/4/Hml-02/ArabiAbiad//ER/Apm ICB05-0493-10AP-0AP
H19	7770GCSAR	Arabi Abiad X IC-9 H4:013-SAB-4A6 Arabi Abiad X IC-9 H2:013-SAB-4A14/2
Varieties	Description	
Asowad Arabic	The number of days until ripening is estimated at about 130 days, the number of days until maturity is about 160 days, the plant height is 75-85 cm, black seed color, two-row spike, and the average productivity is about 2370 kg. Ha ⁻¹ , and is found in the third stability zone. The variety is sensitive to lodging and frost.	
Mohsen White	The number of days until the spike is estimated at about 130 days, the number of days until maturity is about 167 days, the plant height is about 53 cm, white seed color, two-row spike, and the average productivity is about 2385 kg. Ha ⁻¹ , and is found in the second settlement area. The variety is lodging-sensitive and moderately sensitive to frost.	
Furat 6	The number of days until ripening is estimated at 108 days, the number of days until maturity is about 140 days, the plant height is about 57 cm, white seed color, two-row spike, and the average productivity is about 2435 kg. Ha ⁻¹ , and is found in the second settlement area. The variety is medium sensitive to lodging and frost.	
Furat 9	The number of days until the spike is estimated at about 100 days, the number of days until maturity is about 130 days, the plant height is about 45 cm, black seed color, two-row spike, and the average productivity is about 2630 kg. Ha ⁻¹ , and is found in the third stability zone. And the variety is medium resistance to lodging and frost.	
Furat 7	The number of days until ripening is estimated at 111 days, the number of days until maturity is about 163 days, the plant height is about 63 cm, black seed color, two-row spike, and the average productivity is about 1850 kg. Ha ⁻¹ , and is found in the third stability zone. The variety is lodging-sensitive and frost-resistant.	
Furat 5	The number of days until the ripening is estimated at 127 days, the number of days until maturity is about 171 days, the plant height is about 63 cm, white seed color, six-row spike, and the average productivity is about 2030 kg. Ha ⁻¹ , and is found in the third stability zone. The variety is resistant to lodging and disease.	
Furat 4	The number of days until ripening is estimated at 127 days, the number of days until maturity is about 168 days, the plant height is about 75 cm white seed color, six-row spike, and the average productivity is about 3250 kg. Ha ⁻¹ , and is found in the third stability zone. The variety is resistant to lodging and disease.	
Wild landraces		
<i>H. Vulgare</i>	Wild	
<i>H. Spontaneum</i>	Wild	

Source: General Commission for Scientific Agricultural Research – 2018

Experimental site

The research was carried out in the Biotechnology Laboratory at the Faculty of Agriculture, Damascus university, Syria, during the year 2019-2020.

Experiment procedure

Seed sterilization and planting: The seeds were sterilized before planting, they were soaked with ethanol (70%) for 30 seconds, then transferred into three pitchers, each containing sterile distilled water, and left for 5 minutes, then these seeds were transferred into

Clorox (5%) for 5 minutes, then transferred again and soaked in distilled water three times, every time for 5 minutes, then sterilized seeds of each genotype were taken separately and planted in pots containing sterile turb, and placed in the growth chamber.

DNA extraction: After germination and seedlings emergence, fresh leaves were taken from the seedlings (2-3 weeks old) in order to extract deoxyribonucleic acid (DNA), by grinding 1 gram of green leaves with the help of liquid nitrogen until a fine powder was obtained, which was transferred to 50 ml beaker, and 10 ml of SDS extraction solution were added, then the samples were incubated for 60 minutes with continuous stirring in a water bath at a temperature of 37 ° C, then 10 ml of Chloroform / Iso Amyl alcohol mixture was added in a 1:24 ratio, and the mixture was transferred into 30 mL centrifugation tube, and centrifuged for 10 minutes at 10,000 PRM at 4 ° C, then the aqueous layer (upper layer) containing the required DNA was transferred into a new tube, and Isopropanol solution was added with ratio 2/3 of the aqueous layer's volume, then the precipitated DNA was transferred into a small tube of 2 mL, to which 0.5 mL of cold washing buffer solution (76% ethyl alcohol preserved at -20 ° C) was added, then centrifuged (10,000 rpm) for 10 minutes at 4 ° C. DNA samples were dissolved in 500µl of the TE buffer solution (10 mM Tris-HCl, 1mM EDTA), and the RNA was removed by adding 2µl of RNase (10 mg / ml), then incubated at 37 ° C for half an hour, then the DNA was diluted to 40ng/µl. After that, the quantitative and qualitative assessment of the DNA was done by the means of ultraviolet rays, using a UV-Spectrophotometer to quantify DNA and determine its purity. Electrophoresis was applied at the agarose gel with concentration of 0.8% to know the quality of the used DNA, as the good quality DNA appears as a band, while the poor-quality DNA is scattered and unclear.

Genotyping using SSRs: The SSR technique was used to conduct the molecular study, where 14 pairs of primers (obtained from Fermentas, Germany, with a concentration of 10µM) were tested. Table 2 shows the nucleotide sequences of the primers used in the study. The PCR was performed according to the method of Lawyer et al (13) with some modifications. The final reaction volume was 25 µl using Master mix2X, which was obtained from the same company. The reaction consisted of 2 µl of primers at a concentration of 10 M, 12.5 µl of Master mix, 6.5 µl distilled water, and 40 ng. µl⁻¹ DNA. This reaction was carried out in a thermal recycling device according to the following conditions:

1- Initial denaturation: at a temperature of 94 ° C for 5 minutes, to facilitate the separation of the two DNA strands.

2- 40 cycles, each of which includes the following stages:

a- Denaturation: at 94 °C., for 30 seconds.

b- Annealing: according to the annealing temperature for each primer shown in Table (2), a period of one minute.

c- Extension: at 72 ° C, for one minute.

3- Final extension: at 72 ° C, for ten minutes, then the samples are stored at 4 ° C to be separated afterwards using meta-4-agarose gel. Electrophoresis, was carried out on a 4% meta-4-agarose gel in buffer 1X TBE {(10x TBE buffer = 108 g Tris borate + 55 g Boric acid + 9.2g EDTA, pH = 8)}, and 5µl (5 mg / ml) of Ethidium bromide dye was added, and the DNA samples were loaded by adding 5 µl of special loading fluid (1X Loading buffer Bromophenol blue) consisting of: (15% Ficoll 400 + 1.03% bromophenol Blue + 0.03% xylene cyanol FF + 0.4% Orange G + 10mM Tris-HCl + 50 mM EDTA). A 1Kpb marker ladder (Fermentas, Germany) was injected to determine the molecular weight of the resulting bands. An electric field of 100 volts was used. The gel and the DNA bands were visualized using Image Analyzer (Agle Eye II Staratagene).

Table 2. Nucleotide sequencing of SSRs primers and the annealing temperature

Primer	Nucleotide sequence 3'-5'	Annealing Temp. (°C)
Bmac0209	F- CTAGCAACTTCCCAACCGAC R- ATGCCTGTGTGGACCAT	58□
Bmac0067	F- AACGTACGAGCTCTTTTCTA R- ATGCCAACTGCTTGTTAG	53□
Bmag0225	F-AACACACCAAAAATATTACATCA R- CGAGTAGTTCCCATGTGAC	54□
Bmag0006	F- TTAACCCCCCTCTAG R-TGCAGTTACTATCGCTGATTTAGC	59□
Bmag0125	F- AATTAGCGAGAACAAAATCAC R- AGATAACGATGCACCACC	53□
Bmag0394	F- AATTCATCACAACAAGATAGGA R- AATTGATCTCCCTCTCTATG	54□
GBM1482	F-GAGAGCTAGCCACCATTTC R-GACAGGTCGAGGCTGAGAAG	60□
Bmag0353	F-ACTAGTACCCACTATGCACGA R-ACGTTTCAATAAAATCACAACCTG	56□
Bmag0206	F-TAGAAGTGGGTATTTCTTGA R- TTTTCCCCTATTATAGTGACG	54□
Bmac0031	F-AGAGAAAGAGAAATGTCACCA R-ATACATCCATGTGAGGGC	54□
GBM1362	F-CGCCTCCCTCCTTCTGTGTA R-CCCTTGTGTCTCTTGCAT	59□
EBmac0603	F- ACCGAACTAAATGAACTACTTCG R- TGCAAAGTGTCTATTAAGGG	56□
Bmag0385	F-CTCCACAGAGTCAGAGTTAGA R-CTGACATTAGCTGACTCTCTATC	58□
scssr04056	F- CCCATGAAGCCTCTTTACG R- GGAACGGAGGGAGTATTAAGC	58□

Statistical analysis: The results of the amplification process were collected based on the comparison of the presence and/or absence of DNA bands, and the Dendrogram tree was drawn using Unweighted Pair Group Method (UPGMA) with arithmetic averaging using the statistical program Pop gene 1.31 (21, 25). The values of the polymorphism information content (PIC) were calculated according to the equation:

$$PIC = 1 - \sum (P_{ij})^2$$

where P_{ij} is the frequency of the j th allele for i th primer pair from all studied samples (3, 24). PIC is strong coefficient which give an estimation to discriminate a group of genotypes studied, by taking not only the number of alleles, but also the relative frequencies of each allele (20).

RESULTS AND DISCUSSION

Polymorphism: Simple sequences repeats (SSR) technique was applied, using 14 pairs of

SSR primers, (Figure 1). The results showed that the total number of alleles was 19, with an average of 1.35, and the number of polymorphic alleles was 19 alleles. The number of total and polymorphic alleles ranged between one allele, as the lowest number, for (Bmac0209, Bmac0067, Bmag0225, Bmag012, Bmag0394, Bmag0353, Bmag0206, Bmac0031, GBM1362, EBmac0603) and three alleles per locus, as the highest number, for Bmac0003. The polymorphism percentage was 100% with all pairs of primers. PIC values ranged from 0.32 for (Bmac0209, Bmag0225) as the lowest value, to 0.721 at (Bmag0385) as the highest value, and the general average of the PIC value was 0.446, indicating the ability of using the primers to distinguish between the studied genotypes (Table3, Figure 1). The results are consistent with El-Awady et al. (7), and Gougerdchi et al. (10).

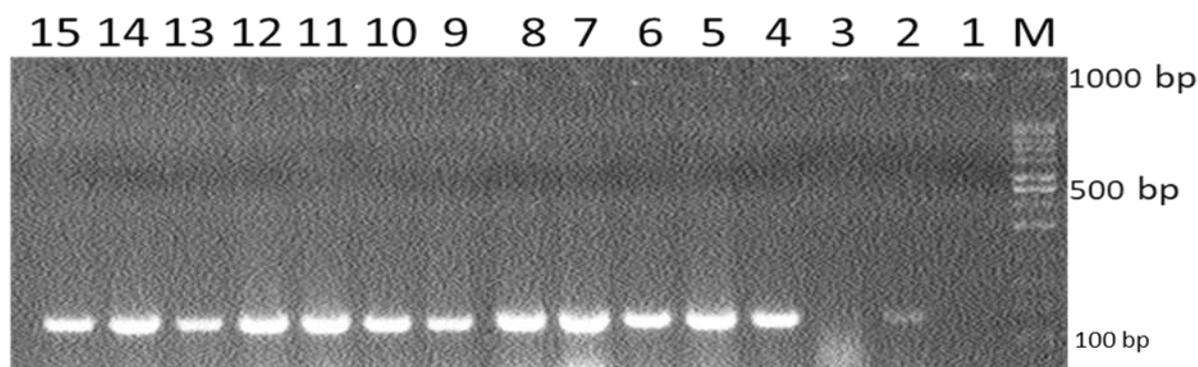


Figure 1. An image of a 2% agarose gel to observe the polymorphism resulting from using (Bmag0394) in all studied genotypes. M represents the marker ladder used to determine the weights and sizes of DNA bundles. The numbers from 1 to 15 stand respectively for: Furat 4, Furat 5, *H. vulgare*, Furat 9, Asowad Arabic, Mohsen White, Furat 6, Furat 7, H1, H6, H18, H19, *H. Spontaneum*, H13, H10

Table 3. used primers codes, total and polymorphic alleles, polymorphisms percentage, and PIC values for wild and cultivated barley

Primer	Total alleles number	Polymorphic alleles number	Polymorphism percentage (%)	PIC
Bmac0209	1	1	%100	0.320
Bmac0067	1	1	%100	0.455
Bmag0225	1	1	%100	0.320
Bmag0006	3	3	%100	0.472
Bmag0125	1	1	%100	0.399
Bmag0394	1	1	%100	0.534
GBM1482	2	2	%100	0.480
Bmag0353	1	1	%100	0.392
Bmag0206	1	1	%100	0.480
Bmac0031	1	1	%100	0.392
GBM1362	1	1	%100	0.392
EBmac0603	1	1	%100	0.480
Bmag0385	2	2	%100	0.721
scssr04056	2	2	%100	0.420
Sum	19	19		6.257
Average	1.35	1.35	%100	0.446

Determining the degree of genetic kinship between the studied genotypes: The genetic relationship between the studied genotypes was determined by applying the PAV (Percent Agreement Values) matrix, where the high values of this matrix indicate the presence of a genetic relationship between two or more genotypes. It is noted from Table 4 that the highest value of PAV is 0.909 between the Furat 7 and Mohsen White, indicating that they have a large degree of genetic kinship, followed by the two genotypes Furat 5 and Asowad Arabic, with a value of 0.863, while the lowest value was 0.318 between H10 and Furat 4, indicating a great genetic variation between them. These results were consistent with the findings of El-Awady et al. (7), where

the highest value of PAV was about 0.9897, but these results did not agree with the lowest value of PVA (0.9897).

Cluster Analysis: Cluster analysis of the studied genotypes resulting from the use of SSR technology allows to divide the studied genotypes into groups reflecting the degree of genetic kinship between them, and genotypes may be grouped into one group based on their original habitat, origin and pedigree. A cluster analysis of the obtained results was conducted with the aim of creating a genetic kinship tree to determine the degree of genetic kinship and drawing a dendrogram between the studied genotypes. The studied genotypes were divided into two main clusters (Figure 2), the first Cluster-1 was divided into two sub-

clusters: the first sub-cluster included the genotype *H. vulgare*, while the second sub-cluster included two groups: the first group included the genotype Furat 4, and the second group branched into two branches, the first branch included two groups of the genotypes, the first included: Furat 5, Asowad Arabic, Furat 9, H19, while the second included H1, H6, and H18. As for the second branch, it was divided into two sub-branches, the first included the genotype *H. vulgare*, and the second included the two genotypes Mohsen

White and Furat 7. The second Cluster-2 split into two sub-clusters: the first sub-cluster contained the H10 and H13 strains, while the second sub-cluster contained the genotype *H. Spontaneum*. In general, Mohsen White and Furat 7 the closest to each other, with a distance of 3,858, followed by Furat 5 and Asowad Arabic with a distance of 4,892, while the genotype *H. Spontaneum* was farthest from the rest of the genotypes, with a distance of 20.844, followed by the Furat 6, with a distance of 18,232.

Table 4. The percent agreement values (PAV) matrix between the studied varieties

Genotype	Furat4	Furat5	H. vulgare	Furat9	Asowad Arabic	Furat6	Mohsen White	Furat7	H1	H6	H18	H19	H. Spontaneum	H13	H10
Furat4	1	0.681	0.636	0.772	0.636	0.727	0.681	0.590	0.772	0.681	0.636	0.636	0.363	0.363	0.318
Furat5		1	0.681	0.818	0.863	0.681	0.818	0.727	0.727	0.818	0.772	0.863	0.590	0.500	0.454
H. Vulgarae			1	0.772	0.545	0.545	0.772	0.681	0.590	0.590	0.636	0.727	0.363	0.636	0.590
Furat9				1	0.772	0.681	0.818	0.727	0.818	0.727	0.772	0.863	0.500	0.500	0.454
Asowad Arabic					1	0.818	0.772	0.843	0.681	0.772	0.818	0.818	0.727	0.545	0.500
Furat6						1	0.772	0.853	0.772	0.681	0.636	0.727	0.545	0.545	0.500
Mohsen White							1	0.909	0.727	0.818	0.772	0.772	0.590	0.500	0.454
Furat7								1	0.636	0.727	0.772	0.772	0.681	0.590	0.545
H1									1	0.818	0.772	0.772	0.500	0.409	0.363
H6										1	0.818	0.681	0.681	0.409	0.454
H18											1	0.818	0.727	0.454	0.409
H19												1	0.545	0.545	0.500
H. Spontaneum													1	0.545	0.681
H13														1	0.772
H10															1

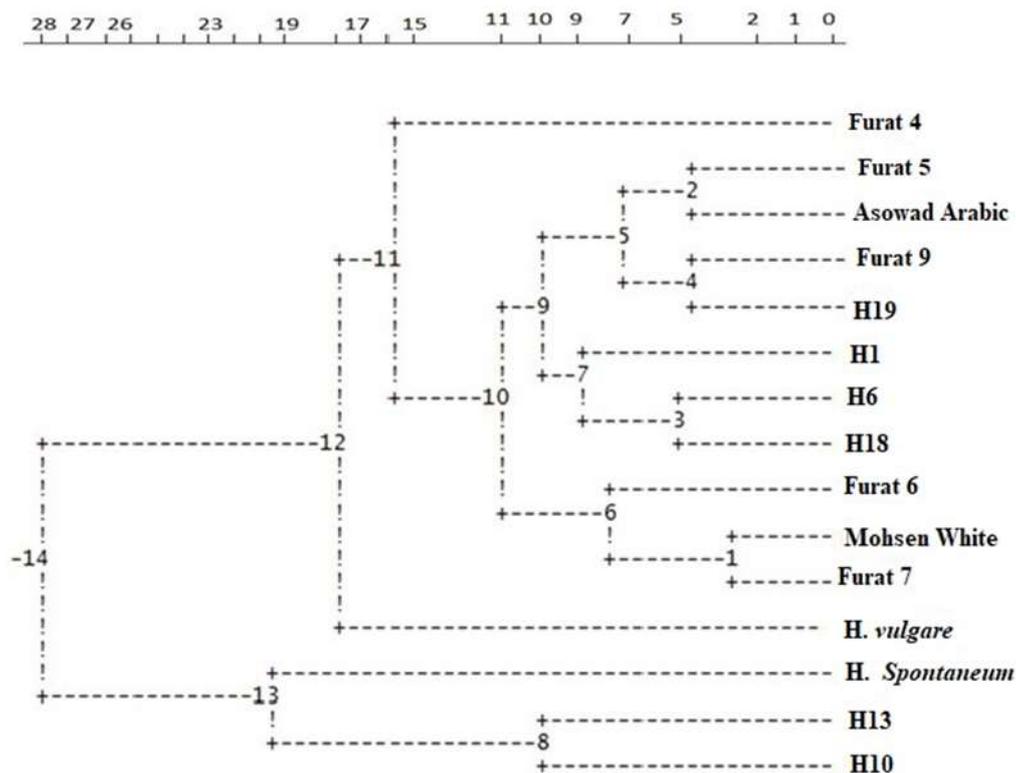


Figure 2. Cluster analysis of studied genotypes using SSR technique

CONCLUSIONS

The used SSR technique was shown to be effective in distinguishing between the studied genotypes of barley. 14 SSRs primers were used and which gave a polymorphism rate of 100%. H10 and H13 strains were associated with the wild landrace *H. Spontaneum*, and it is believed that these strains are wild landraces. The presence of a great genetic diversity among the genotypes used in this study, makes them more suitable for breeding and genetic improvement programs.

RECOMMENDATION

Introducing the genetically diverse genotypes into traditional genetic improvement programs to develop varieties with desirable traits. Studying the morphological, physiological and biochemical characteristics, and determining the locations of the important genes for these genotypes, with aiming of defining their genetic identity, which make it easier for the breeder to choose the appropriate parents according to the aim of breeding.

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