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Detection of Genetic Polymorphism in Seven Barley *Hordeum vulgare* L. Varieties Using SSR

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Abstract: Investigation was carried out at the laboratory of Biotechnology, Faculty of Agriculture, Damascus University, during the season 2017-2018. Seven varieties were planted to determine the degree of genetic similarity using SSR-technique (Simple Sequence Repeats), and 14 double primers were used for this purpose. The analysis results revealed that all primers showed polymorphism among the evaluated varieties, except Bmag0385. primers produced a total of 42 alleles with a polymorphic percentage of 88.27%. The number of alleles for each primer varied from 1 allele for the primer (Bmac0067) to 7 alleles for the primer (Bmag0006) in average of 3 alleles per primer. Cluster analysis and Dendrogram showed the highest degree of genetic similarity between variety Arabi asuad and variety Arabi abiad (0.7619). While it was low between variety Fourat4 and variety Arabi abiad (0.3571), and varieties Fourat4 and Fourat3 (0.3571) which indicated wide genetic diversity among them.

Keywords: Barley, *Hordeum vulgare* L., SSR marker, polymorphic, Dendrogram.

Introduction

Barley (*Hordeum vulgare* L.) is an important cereal, which evolves through domestication to use not only as a food grain but also as malting and a feed grain (Pourkheirandish & Komatsuda, 2007). It is of high nutritional value, as it can be used with wheat in the bread industry (Mishra & Shivakumar, 2000; Al-Zergawy, 2016). Malt can be used by bakers to encourage the degradation of starch in wheat flour to increase the efficiency of the fermentation process and produce coal gas (CO2), due to its high content of starch-degrading enzymes, such as α-amylase and ß-amylase (OECD, 2004).

Barley occupies the fourth rank in the cereal crops in the world after wheat, rice, and maize (FAOSTAT, 2018). The total area planted with the barley in the Arab countries is estimated at 4.2 million hectares, and the productivity is about 868.2 kg. Ha^{-1}(AOAD, 2017). Syria also ranks first in the Arab world in terms of the area planted with barley 1187.2 thousand hectares, and the productivity is about 244 kg. Ha^{-1} (SASG, 2018).

SSR (Simple Sequence Repeats) markers are simple, consist of one-to-six base pairs, repeated motifs, which can be used genetic

markers based on polymerase chain reaction (PCR) to produce codominant markers (Morgante *et al*., 2002).

A number of studies and researches had been done to explore the development of SSR markers in barley, which led to discover hundreds of SSR markers which are available for further researches (Von Korff *et al*., 2004; Beaubien & Smith, 2006).

Microsatellite -based genetic maps of barley was developed by Ramsay *et al*. (2000) and Li *et al*. (2003) using 242 and 127 SSR primers, respectively. Furthermore, Varshney *et al*. (2007) produced a high-density map for barley using 775 simple sequence repeat (SSR) markers.

Perry *et al*. (2014) studied the genetic polymorphism of 48 malting and feed barley varieties grown in Canada using 10 pairs of SSR primers. The number of alleles for each primer varied from 3 to 10 alleles, with average of 5.6 alleles per primer.

Al-Hadeithi. (2016) studied nine Iraqi varieties of barley (*Hordeum vulgare* L.) that has been differentiated and diagnosed using simple sequence repeat markers to detect their genetic polymorphism, using 6 pairs of SSR primers. These primers generated total PCR product 11 bands, in average of 1.6 alleles per primer, with a polymorphic percentage of 80%.

Arya *et al*. (2019) used 14 pairs of SSR primers to study genetic diversity in fifty varieties of barley. The results showed that fifty-five alleles were recorded and the alleles number ranging between 2 and 7 with a mean of 3.93 alleles per primer. The polymorphism information content (PIC) value ranged between 0.084 and 0.740.

The current study aimed to identify the efficiency of using SSR markers and to assess the genetic polymorphism of barley varieties. In addition to determine which varieties are genetically more distant for using in crossbreeding programs.

Materials & Methods:

Barley Samples: Seven Barley (*Hordeum vulgare* L.) varieties were used in this study, which are local varieties obtained from General Commission for Scientific Agricultural Research (GCSAR) in Syria, and the Arab Center for the Studies of Arid Zones and Dry Lands (ACSAD) for this study.(Two varieties are landraces: Arabe abiad and Arabe asuad, while the remaining were breeding varieties. All the varieties are growing widely in Syria. They are having enormous morphological variability like number of tillers, number of rows, productivity, resistance to abiotic and biotic stresses, etc. (Table 1).

DNA Extraction and SSR analysis:

We extracted DNA genome from fresh leaves that collected from 2 to 3 weeks old seedlings by using SDS method. Concentration and Purity of DNA were conducted by spectrophotometer. Using a 0.8% agarose gel electrophoresis followed by staining with ethidium bromide and photographed under UV light to determine the genomic DNA integrity. DNA samples and were extended to a working concentration of 40 ng.μl^{-1} in order to be use in the SSR-reactions.

Fourteen microsatellite primers pairs were selected based on their chromosomal locations. Their names, sequences and chromosomal locations were listed in table (2).

Table (1): Barley varieties in this study.

No.	Varieties Name	Pedigree	Rows No.
1	Arabe abiad	Local Variety	2 row
2	Arabe asuad	Local Variety	2 row
3	Fourat3	Arabe abiad -10Kr M4 – Krb-1982-2	2 row
4	Fourat4	Baladi16/ Api// Dir Alla 106	6 row
5	Fourat6	EBC(A) // WI2291//Harmal- 03 KRB 87-5-E0-E0-E1-E0	2 row
6	Fourat7	Arabe aswad – Sel –Has 87-1	2 row
7	Acsad1713	ACSAD1468/5/ARIZONA5908/ATHS//AVT/ATTIKI/3/ S.T BARLEY/4/ATHS/LIGNEE686 ICB 89-0321-8BO-1AP-1AP-0TR-0AP-34AP-0AP-0AP	6 row

PCR reactions was done according to (Lawyer *et al*., 1993) with some adjustment, each 25µL PCR reaction solution contained 1µL template DNA (40 ng μL^{-1}), 12.5 µl of Master Mix obtained from company (Fermentas, Germany), 20 pmol of each primer and complete the volume with distilled water .The reaction procedure was carried out with the program set to an initial denaturation at 94 °C for 5 min; 40 cycles of the following three steps: (1) denaturation at 94°C for 30 sec, (2) annealing at (53 -60) °C (depending on primer used) for 1 min, (3) extension at 72 °C for 1 min with a final extension at 72 °C for 10 min (El-Awady & El-Tarras, 2012).

Amplified PCR products were run on 4% MetaPhor agarose gel in 1xTBE buffer [Tris (108 g), EDTA (9.2 g) and boric acid(55 g) in 1 liter of distilled H2O], and after that the gel was stained by ethidium bromide. A DNA marker ladder with a gradient of 50-1000 bp providing by (Fermentas, Germany) was used as the control. Photo documentation was performed under UV light (365 nm) using an Image Analyzer (Vilber Lourmat).

Analysis of SSR data:

Molecular weight was estimated by the means of a known size of DNA fragments ladder (bands from 50 to 1000bp). Only intense clear bands were scored visually for the absence (0) or presence (1). Dendrogram was constructed by employing the resultant dissimilarity matrix using Unweighted Pair Group Method of Arithmetic Means (UPGMA) (Yeh *et al*., 1999) as implemented in POWER MARKER with the tree viewed using the software pop gene 32.1.

We calculated the polymorphism information content (PIC) for every SSR by using the method of Smith *et al*. (1997) as follows:

$PIC = 1-\Sigma(Pij)^2$ where Pij is the frequency of the i^{th} allele revealed by the j^{th} SSR locus.

Table (2): primer names and sequences, chromosome locations of the fourteen SSR primers.

SSR primer	Primer sequence	Chromosome location
Bmac0209	f- CTAGCAACTTCCCAACCGAC r- ATGCCTGTGTGTGGACCAT	3H
Bmac0067	f- AACGTACGAGCTCTTTTTCTA r- ATGCCAACTGCTTGTTTAG	3H
Bmag0225	f-AACACACCAAAAATATTACATCA r- CGAGTAGTTCCCATGTGAC	3H
Bmag0006	f- TTAAACCCCCCCCCTCTAG r-TGCAGTTACTATCGCTGATTTAGC	3H
Bmag0125	f- AATTAGCGAGAACAAAATCAC r- AGATAACGATGCACCACC	2H
Bmag0394	f- AATTCATCACAACAAGATAGGA r- AATTGATCTCCCTCTCTCTATG	5H
GBM1482	f-GAGAGCTAGCCACCATTTGC r-GACAGGTCGAGGCTGAGAAG	4H
Bmag0353	f-ACTAGTACCCACTATGCACGA r-ACGTTCATTAAAATCACAACTG	4H
Bmag0206	f-TAGAACTGGGTATTTCCTTGA r- TTTTCCCCTATTATAGTGACG	7H
Bmac0031	f-AGAGAAAGAGAAATGTCACCA r-ATACATCCATGTGAGGGC	7H
GBM1362	f-CGCCTCCCTCCTTCCTGTA r-CCCTTGTTGTCCTCTTGCAT	7H
EBmac0603	f- ACCGAAACTAAATGAACTACTTCG r- TGCAAACTGTGCTATTAAGGG	7H
Bmag0385	f-CTCCACAGAGTCAGAGTTAGA r-CTGACATTAGCTGACTCTCTATC	7H
scssr04056	f- CCCATGAAGCCTCTTTACG r- GGAACGGAGGGAGTATTAAGC	7H

Results & Discussion

The genetic diversity of 7 barley varieties was evaluated using 14 SSR determine the markers efficiency and the genetic relationships among the studied varieties.

Different sizes of DNA fragments amplified by the same pair of SSR prime except Bmag0385 which showed monomorphic band profiles, and that agree with the results of Monawekh *et al.* (2015). The use of these primers resulted a total of 42 alleles, the average number of alleles per SSR locus was 3. The variation ranges were from 1 (Bmac0067) to 7 (Bmag0006) alleles per primer (Plate 1).

In study conducted by Ferreira *et al.* (2016) using 64 Brazilian barley genotypes, 280 alleles were recorded and the number of alleles per locus ranged between 1 and 18 alleles with a mean of 8 allele per primer. Shuorvazdi *et al.* (2014) detected 225 alleles using 144 barley genotypes with number of alleles per locus ranged between 2 and 14 alleles with an a mean of 5 allele per primer. Al- Hadeithi (2016) performed a study on 9 Iraqi varieties of barley, the results showed 11 alleles, ranged between 1 and 3 alleles per primer with an average of 1.6 allele per SSR locus.

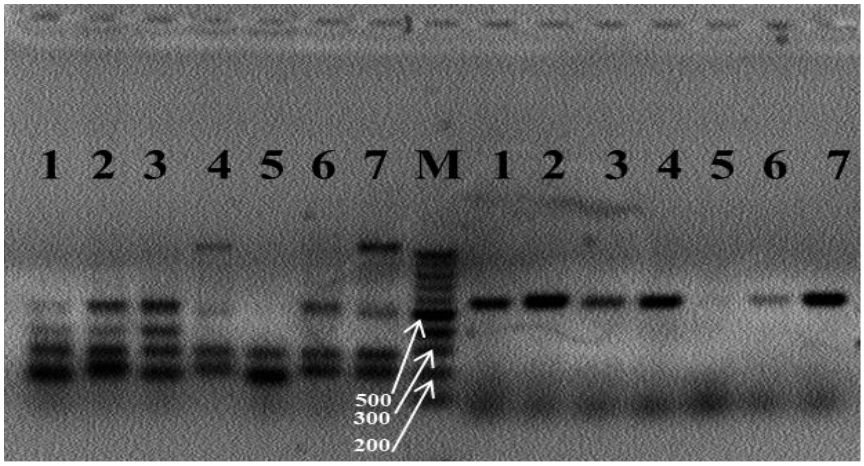

Plate (1): MetaPhor Agarose gel display SSR profiles amplified by Bmag0006, GBM1362 in 7 barley varieties. (under a optimum conditions). Lanes 1 to 7 represent 1: Arabe abiad, 2: Arabe asuad, 3: Fourat3, 4: Fourat4, 5: Fourat6, 6: Fourat7 and 7: Acsad1713.

The percentage of polymorphism expressed by SSR primers from this study (88.27%) compared to the results of other researchers, such as (El-Awady & El-Tarras, 2012) It was reported that microsatellites are typically markers used to assessment of the genetic variability in Saudi Arabia barley registered polymorphism ranging between (50-100%), while the other study (Al- Hadeithi, 2016) registered that polymorphism ranging between (50-100%) with an average 80%. PIC is an index of microsatellite DNA variation degree, reflecting the level of microsatellite DNA polymorphism. In our study the PIC values ranged between 0.41 (Bmag0125) and 0.5 (Bmac0031, Bmag0353, Bmag0006) with an average 0.44 (Table 3). Other studies showed different PIC values obtained using different SSR primers in barley. Shuorvazdi *et al.* (2014) registered PIC values ranging between 0.05 and 0.90 with a mean 0.51. Sardou *et al.* (2011)

recorded PIC values between 0.29 and 0.89 with an average of 0.64. While Monawekh *et al.* (2015) studied PIC values that ranged from 0.21 (Bmag0749) to 0.88 (scssr07970) with an average value of 0.49.

Table (3): Distinct characteristic of SSR primers include in the study: Primers name, total number of bands , number of main bands, percentage of polymorphism and PIC.

Primer	Total Bands	Main Bands	Polymorphism%	PIC
Bmag0225	3	3	100	0.49
Bmag0394	3	3	100	0.45
Bmag0206	2	1	50	0.49
Bmac0031	3	3	100	0.50
Bmag0385	2	0	0	0
Scssr04056	2	2	100	0.46
Bmag0006	7	6	85.71	0.50
GBM1362	2	2	100	0.49
EBmac0603	5	5	100	0.43
Bmag0125	2	2	100	0.41
GBM1482	3	3	100	0.45
Bmag0353	2	2	100	0.50
Bmac0067	1	1	100	0.49
Bmac0209	5	5	100	0.45
Sum	42	37	-	
Mean	3	2.71	88.27	**0.44**

Genetic Similarity and Genetic Cluster analysis

According to the SSR data, genetic distance among the seven barley varieties ranged between 0.7619 and 0.3571. The highest similarity (0.7619) was registered between Arabe asuad and Arabe abiad and lowest (0.3571) was registered between Fourat4 and Arabe abiad, as well between Fourat4 and Fourat3. (Table 4). It was shown two main groups in fig. (1), first group divided to two sub clusters, first sub cluster (Fourat3) and second sub cluster (Arabe asuad, Arabe abiad). Second group divided to two sub clusters, one sub cluster (Fourat4), Second

sub cluster included (Fourat6, Fourat7, Acsad1713).

Table (4): Percent Agreement (PAV) between the studied varieties resulting from the application of unweighted pair group method with arithmetic mean (UPGMA).

	Arabe abiad	Arabe asuad	Fourat3	Fourat4	Fourat6	Fourat7	Acsad1713
Arabe abiad	**	0.7619	0.7143	0.3571	0.5952	0.5714	0.4048
Arabe asuad		**	0.7143	0.4048	0. 5952	0.5714	0.5476
Fourat3			**	0.3571	0.5000	0.5714	0.4048
Fourat4				**	0.4762	0.5952	0.6190
Fourat6					**	0.7381	0.6190
Fourat7						**	0.6905
Acsad1713							**

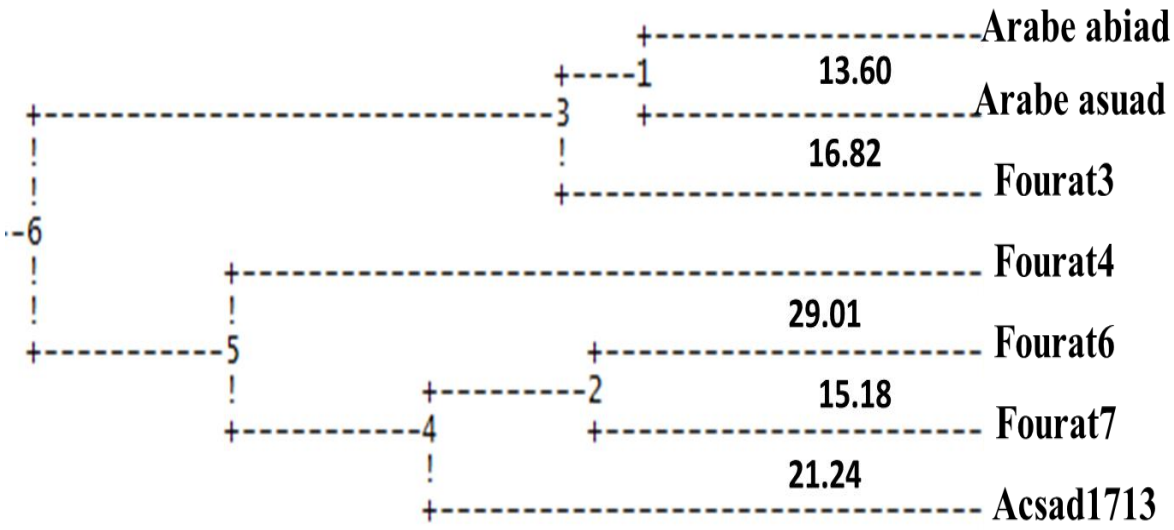

Fig. (1):- Dendrogram for clustering 7 barley varieties included in this study.

This study results stated that the used barley varieties were clustered based on their lodging resistance. Meanwhile, it was reported that barley genotypes clustered accordance with their pedigree in study conducted by Monawekh *et al.* (2015). Furthermore, Mohamed & Adel (2012) noticed that barley genotypes clustered in groups in accordance with the geographical location in the Kingdom of Saudi Arabia. However, the Tunisian barley genotypes clustered depending on their spike types (Chaabane *et al.*, 2009). As well, barley genotypes clustered in accordance with salt tolerance (Khatab & Samah, 2013; Mariey *et al.*, 2013).

Conclusions and Recommendations

1-The SSR technique showed a polymorphism of 87.67%, resulting from the use of 14 pairs of primers, which indicates the effectiveness of the technique in distinguishing between the studied varieties.

2-The selection of genetically separated fathers and their inclusion in crossbreeding programs, where the dendrogram was divided into two clusters, the first cluster included varieties of Fourat4 and Fourat7 and Acsad1713 and Fourat6, while the second cluster included varieties of Arabe abiad and Arabe asuad and Fourat3 with varying the degree of genetic similarity.

3-In the future, it is possible to locate the genes responsible for important traits by using and isolating QTLs.

4-Determine the sequencing of morphological patterns of lodging genes.

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Conflicts of interest

The authors declare that they have no conflict of interests.

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الكشف عن التعددية الشكلية بين سبعة أصناف من الشعير (*Hordeum vulgare* L.) باستخدام تقنية التكرار التسلسلي البسيط

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المستخلص: نُفذ البحث في مخبر التقانات الحيوية التابع لكلية الزراعة، جامعة دمشق للعام 2017-2018. حيث تمت زراعة سبعة أصناف من الشعير بهدف تحديد درجة القرابة الوراثية فيما بينها باستعمال تقنية SSR (Simple Sequence Repeats) واستخدم لهذا الغرض 14 زوج من البادئات. أظهرت نتائج التحليل فعالية البادئات المستخدمة في إعطاء تعددية شكلية polymorphic بين الأصناف المدروسة باستثناء البادئة Bmag0385، ونجم عن استخدامها ما مجموعه 42 أليل، وبلغت نسبة هذه التعددية 88.27%، كما تراوح عدد الأليلات لكل بادئة بين أليل واحد للبادئة (Bmac0067) وسبعة أليلات للبادئة (Bmag0006) بمتوسط قدره 3. أظهر كلاً من التحليل العنقودي وشجرة القرابة الوراثية أن أعلى درجة قرابة وراثية كانت بين الصنفين عربي أبيض وعربي أسود بمسافة (0.7619)، في حين كانت أقل درجة قرابة وراثية بين الصنف فرات4 والصنف عربي أبيض (0.3571) والصنفين فرات4 وفرات3 بمسافة (0.3571) مما يدل على وجود تباين وراثي كبير بينها.

الكلمات المفتاحية: الشعير، مؤشرات SSR، التعددية الشكلية، شجرة القرابة الوراثية.