Damascus University Journal of Agricultural Sciences
Vo 39 No.2 (2023): 191 - 205

مجلة جامعة دمشق للعلوم الزراعية
المجلد 39 العدد 3 (2023): 191- 205

Efficiency of ISSR markers in genetic divergence analysis among sixteen barley (*Hordeum vulgare* L.) genotypes

Tony Saloom[1]
[1] Research assistant, Department of Biotechnology, General Commission for Scientific Agricultural Research, Damascus, Syria

Abstract:

This research was conducted at labs of the General Commission for Scientific Agricultural Research in Damascus/ Syria during the period of 2021-2022. Inter Simple Sequence Repeats (ISSR) technique was used to determine the genetic relationship among set of 16 barley genotypes (12 lines and 4 Syrian varieties). 14 primers were used, 12 of them gave polymorphism, with 104 bands and a polymorphic percentage of 87.37 %. The number of bands per primer varied from a minimum of 3 bands for the primer P6 to a maximum of 14 bands for the primer P10 with an average of 8.67 bands per primer. The average calculated value of polymorphic information content was (PIC) = 0.2711, effective multiplex ratio was (EMR) = 5.81, marker index was (MI) = 1.56, discriminating power was (DP) = 0.508 and resolving power was (RP) = 3.55, representing the efficiency of used primers for genetic differentiation among the studied genotypes.

Results showed that the genetic distance ranged between 0.189- 0.558 The lowest distance (0.189) was recorded between Bar1 and Bar3, this low value of the genetic distance indicates a great degree of genetic relationship. The largest distance (0.558) was recorded between bar4 and bar7, indicating a far genetic relationship between the two genotypes. It was noted that the dendrogram was divided into two main clusters, separating genotype (bar4) from the rest of studied genotypes which fell with varying degrees of genetic relationship under the second cluster. Our results indicate that the ISSR technique can be used to study genetic relationship among barley genotypes.

Key words: Marker Efficiency, ISSR Technique, Genetic Diversity, Barley Genotypes, *Hordeum Vulgare* L.

Received: 6 /9/2022
Accepted: 15/12/2022

كفاءة معلّمات ISSR (تقنية التكررات الترادفية البسيطة الداخلية) في تحليل التباعد الوراثي بين ستة عشر طرازاً من الشعير (*Hordeum vulgare* L.)

م. طوني سلوم[1]

[1]باحث مساعد في قسم التقانات الحيوية، الهيئة العامة للبحوث العلمية الزراعية، دمشق.

المُلخَّص:

نفذ البحث في مخابر الهيئة العامة للبحوث العلمية الزراعية دمشق/ سورية خلال الفترة 2021–2022. تم استخدام تقنية التكرارات الترادفية الداخلية البسيطة (ISSR) لتحديد درجة القرابة الوراثية بين مجموعة مكونة من 16 نمطًا وراثيًا من الشعير (12 سلالة و4 أصناف سورية). استُخدم لهذا الغرض 14 بادئة أعطت 12 بادئة منها تعددية شكلية polymorphic بين الطرز المدروسة، نجم عن استخدامها ما مجموعه 104 حزمة وبلغت نسبة هذه التعددية 87.37%، تراوح عدد الحزم لكل بادئة بين 3 حزم كأقل عدد مع البادئة P6 و14 حزمة كأعلى عدد مع البادئة P10، بمتوسط عام 8.67 حزمة لكل بادئة. بلغ متوسط كل من: معامل التعددية الشكلية 0.2711 = (PIC)، ونسبة تعدد الإرسال الفعال للمعلم الجزيئي 5.81 = (EMR)، ومؤشر المعلم الجزيئي 1.56 = (MI)، والقدرة التمييزية للمعلم الجزيئي 0.508 = (DP)، وقوة التحليل للمعلم الجزيئي 3.55 = (RP)، وتمثل هذه المؤشرات كفاءة البادئات المستخدمة في تمييز التباين الوراثي بين الطرز المدروسة.

تراوح البعد الوراثي بين 0.189 و0.558، فكانت أدنى قيمة (0.189) بين Bar1 وBar3، وتدل هذه القيمة المنخفضة للتباعد الوراثي على درجة كبيرة من القرابة الوراثية. بينما كانت أعلى قيمة للتباعد الوراثي (0.558) بين bar4 وbar7، ما يدل على وجود تباين وراثي كبير بينهما. ولوحظ أن شجرة القرابة الوراثية انقسمت إلى عنقودين رئيسين، وفصلت الطراز (bar4) عن بقية الطرز الوراثية المدروسة والتي اندرجت تحت العنقود الثاني بدرجات متفاوتة من القرابة. تشير نتائجنا إلى أنه يمكن استخدام تقنية ISSR لدراسة العلاقة الوراثية بين الطرز الوراثية للشعير.

الكلمات المفتاحية: كفاءة المعلم الجزيئي، تقنية ISSR، التنوع الوراثي، الطرز الوراثية للشعير.

تاريخ الايداع: 2022/9/6
تاريخ القبول: 2022/12/15

Introduction:

Barley (*Hordeum vulgare* L.) is a self-pollinating diploid (2n = 2x = 14) cereal crop, which is spread through the world and adapted to various climate conditions (Park *et al*. 2011, 3). Barley is the fourth most important cereal crop after wheat (*Triticum aestivum* L.), maize (*Zea mays* L.) and rice (*Oryza sativa* L.) and plays an important role in human food, livestock feed and malt production (Tanno *et al*. 2002; Molina-Cano *et al*. 2005).

Barley is one of the oldest domesticated crop plants and it probably took place prior to 7000 B.C. in the region of the Near East known as 'Fertile Crescent' including parts of Jordan, Lebanon, Palestine, Syria, Southeastern Turkey, Iraq and Western Iran (Zohary and Hopf 1993; Ivandic *et al*. 2002; Yang *et al*. 2008; Shakhatreh *et al*. 2016).

Barley is highly adaptable to a variety of environments due to its extensive phenotype and genetic diversity (Kumar *et al*., 2014). As a result, its wild progenitor species can be found in western Asia, which provides a very rich genetic diversity resource. Barley has been domesticated and cultivated in highly diverse areas of the world (Stein and Muehlbauer 2018) (Newton *et al*., 2011). Among cereal crops, Barley is considered to be particularly tolerant to drought and salinity. Due to its wide adaptability range, barley is a versatile crop.

There are numerous cultivated collections of germplasm in the barley family (*Hordeum vulgare ssp. vulgare*) and wild barley (*Hordeum vulgare ssp. spontaneum*) (Bockelman and Valkoun, 2010) and in recent years it has been used in different breeding programs. About 141 million tons of barley is grown annually on around 48 million hectares around the world (FAO, 2018). Due to its nutritional value, barley is regarded as an essential food component and is utilized in the food, feed, and beverage sectors (Shaveta *et al*., 2019).

Genetic variation is the key tool for crop development and improved plant resistance to abiotic and biotic stresses. Barley is considered a good genetic model for Triticeae and more genetically complex cereal crops like hexaploid bread wheat (Kleinhofs and Han 2002).

Development of improved cultivars in crop species during last decades resulted in the depletion of genetic diversity and led to vulnerability of these cultivars to most of biotic and abiotic stresses (Ghaffari *et al*. 2014; Nandha and Singh 2014). Study of genetic diversity of landraces, breeding lines and improved cultivars may serve as the source of desirable alleles and can be used in breeding programs for breeding climate resilient varieties (Jannatabadi *et al*. 2014; Bedada *et al*. 2014; Nadeem *et al*. 2018).

Traditionally, morphological traits, cytological characters, biochemical tests, and pedigree information are used to assess genetic diversity and classify barley germplasm. However, these methods are always associated with various limitations and are insufficient to reveal the whole information within barley resources (Matus and Hayes, 2002).

Despite the fact that morphological markers are easily implemented, they do not always reflect the actual genetic relationship, due to the high genotype by location interactions and to limited number of traits studied (Montero-Pau *et al*. 2017). DNA markers have been proved to be more valuable tools used for evaluation of genetic diversity which are not affected by environment, selection, and available in almost unlimited numbers (Wei *et al*. 2014).

DNA markers are usually used to allow cultivar fingerprint and identification of genomes in crops, and they are the best tool to identify the polymorphisms and genetic diversity (Tricase *et al*. 2018).

In the last two decades, different types of molecular markers such as RFLP (Stein *et al*. 2007), RAPD (Albayrak and Gozukirmizi 1999; Tanyolac 2003), AFLP (Assefa *et al*. 2007; El-Esawi *et al*. 2018), ISSR (Tanyolac 2003; Rahimi *et al*. 2014), SSR (Varshney *et al*. 2006; Park *et al*. 2011, 3; Shakhatreh *et al*. 2016; Elakhdar *et al*. 2018) and SNP (Turuspekov *et al*. 2014; Elakhdar *et al*. 2018) have been used as genetic markers for measuring the genetic diversity in various barley collections.

PCR based marker such as random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) have been widely used to study genetic diversity. Genetic adaptability, difference between accessions and between transgenic and non-transgenic plants are being identified by ISSR markers (Cid-Contreras *et al*., 2019).

ISSR is a molecular marker technique, which involve PCR amplifications of DNA using single primer composed of a microsatellite sequence by 2-4 arbitrary which could be used to assess genetic diversity (Yang *et al*. 2020). ISSR is efficient and rapid applications in evaluation, characterization of the genetic material (Tricase *et al*. 2018), and it has been commonly used for genetic diversity in barley (Fernandez *et al*. 2002, Tanyolac *et al*. 2003, Hou *et al*. 2005, Khatab *et al*. 2019).

Objectives:

This study aimed to assess the genetic diversity among 16 barley genotypes using the ISSR markers, and to determine the efficiency of the primers used to separate the studied genotypes.

Materials and Methods:

Plant material:

A set of 16 barley genotypes (12 lines and 4 Syrian varieties: Arabi Abiad, Arabi Aswad, Fourat7, Fourat9) were studied during the period of 2021-2022 at Molecular Genetics Laboratory, Department of Biotechnology, General Commission for Scientific Agricultural Research, Damascus, Syria (Table, 1).

DNA extraction:

Fresh leaves were collected from seedlings and used for genomic DNA isolation using the cetytrimethyl ammonium bromide CTAB method as described by (Lassner *et al*., 1989).

Table 1. Specification of barley genotypes used in the experiment

No.	Genotype	Pedigree
1	Arabi Abiad	local variety
2	Arabi Aswad	local variety
3	Fourat 7	certified variety
4	Fourat 9	certified variety
5	Bar1	AWBlack/Aths//Arar/3/9Cr279-07/Roho/6/Alanda-01/S/CI01021/4/CM67/U.Sask.1800//Pro/CM67/3/DL70
6	Bar2	ARIG8/IMPERIAL//M7/3/RT013/5/ATHS/LIGNEE686/3/DEIRALLA106//SV.ASA/ATTI
7	Bar3	ARIZONA5908/ATHS//AVT/ATTIKI/3/S.T.BARLEY/4/ATHS/LIGNEE686/5/ARBAYAN/ATHS
8	Bar4	RHN-03//LIGNEE527/AS45/4/ABM/11012-2//MBC10593/3/IFB974
9	Bar5	Arda/Moroc9-57/3/Mo.B1337/W12291//Moroc9-75
10	Bar6	RHN-03//LIGNEE527/AS45/4/APM/11012-2//NPCI00593/3/IFB974
11	Bar7	MUNDAH/4/ARAR/H.SPONT.19-15//HML/3/H.SPONT.41-1/TADMOR
12	Bar8	ZANBAKA/5/PYO/CAM//AVT/RM1508/3/PON/4/MONA/BEN//CAM/6/ARAR/H.SPONT.19-15//HML/3/H.SPONT41-1/TADMOR/7/ZANBAKIAN
13	Bar9	CLIPPER//WI2291*2/WI2269/5/SOUFARA-02/3/RM1508/POR//WI2269/4/HML02/ARABYABIAD//ER/APM
14	Bar10	CLIPPER//WI2291*2/WI2269/3/FOURAT2
15	Bar11	MSEL//DEFRA/CLE169
16	Bar12	HMI02/ARABIABIAD/3/API/CM67//NACTA/4/WI2269/ESPE/5/MZQ//GVALLPI002917/3/WI2291/WI2269/6/WI2269
Administration of Field Crops Research - General Commission for Scientific Agricultural Research, Damascus, Syria		

DNA was quantified using spectrophotometer (Bio metra GenRay UV-photometer) by taking absorbance at A260 and A280.

DNA concentration was assessed as follows, (Maniatis,1982)

$$\text{DNA con.}\,(\mu g/\mu l) = \frac{OD260 \times 100\ (\text{Dilution Factor}) \times 50\ \mu g/ml}{1000}$$

DNA quality was checked by agarose gel electrophoresis. DNA bands without smears were considered for PCR amplification.

All genomic DNA samples were uniformed to a final concentration of 50 ng.µl^{-1} and used for PCR amplification reactions.

DNA amplification and visualization by ISSR analysis:

Molecular polymorphism was assessed by a set of 12 ISSR primers (table, 1). The amplification reaction was carried out in thermocycler (Biometra modell T-1 Thermoblock) under the following conditions: initial denaturation at 95°C for 5 minute; 37 cycles of 1 minute at 94 ºC for denaturation, 1 minute for primer annealing at a (Ta) according to the primer (Table, 1), and 1.30 minute at 72 ºC for extension, with a final extension for 10 minutes at 72 ºC. The total reaction volume of PCR amplification was 25 µl containing KAPA Taq ready mix 2X, 20 pM primer and 100 ng of template DNA.

Table 2. ISSR primers profile (name, sequence and annealing temperature (Ta))

Primer No.	Primer Name	Sequences 5'—3'	Ta (C°)
p1	4	(CA)8 AC	48 c°
p2	A830241	(ACTG)5	44 c°
p3	813	(CT)8 T	50 c°
p5	8565	GTC (ACC)6 AC	64 c°
p6	866	C(TCC)5 TC	53 c°
p8	8	(CA)8 GAC	48 c°
p9	862	(AGC)6	53 c°
p10	17899B	(CA)6 GG	46 c°
p12	8082	(CT)9 G	51 c°
p15	5	(CA)8 GT	48 c°
p18	812	(GA)8 A	48 c°
p20	16	CGT (CA)7 C	49 c°

Data analysis:

ISSR bands were scored in a 0-1 binary format and analyzed using the Total Lab 1D software. XLSTAT software was used to build of the cluster dendrogram based upon the UPGMA (unweighted pair group method with arithmetical averages) algorithm.

Marker efficiency analysis

The performance of the primers was measured by calculating different parameters including polymorphic Information Content (PIC), Effective multiplex ratio was calculated (EMR), Resolving Power (RP), and Discriminating Power (DP). The PIC value for each locus was calculated using formula (Roldan-Ruiz *et al*. 2000); $PICi = 2f_i (1 - f_i)$, Where PICi is the polymorphic information content of the locus i, f_i is the frequency of the amplified fragments and $1 - f_i$ is the frequency of nonamplified fragments. The frequency was calculated as the ratio between the number of amplified fragments at each locus and the total number of accessions (excluding missing data). The PIC of each primer was calculated using the average PIC value from all loci of each primer. Effective multiplex ratio was calculated (EMR) was calculated using (Powell *et al*. 1996) formula EMR = n β, where n is the average number of fragments amplified by an individual to a specific system marker (multiplex ratio) and β is estimated from the number of polymorphic loci (n_p) and the number of non-polymorphic loci (n_{np}); $\beta = n_p/(n_p + n_{np})$. The resolving power (RP) of each primer was calculated as (Prevost and Wilkinson, 1999) formula; $R = \Sigma I_b$, where I_b represents the informative fragments. where Ib or band informativeness is represented on a scale of 0–1 and is defined as $Ib = 1 – (2 \times|0.5 – p|)$, where pi is the proportion of individuals containing the ith band. Discriminating Power (DP) estimated by (Tessier *et al*. 1999) as D = 1 – C; where C is the confusion probability is $C = \Sigma ci = \Sigma p_i (N_{pi} – 1) /(N – 1)$, where for N individuals, C is equal to the sum of all ci for all of the patterns generated by the primer. To characterize the capacity of each primer to detect polymorphic loci among the genotypes, we also calculated the Marker Index (MI) for each primer as a product of polymorphic information content (PIC) and effective multiplex ratio (EMR) (Varshney *et al*. 2007); MI = EMR * PIC.

Results

Polymorphism of the ISSR markers

A set of 14 ISSR primers were used to estimate the genetic relationship among 16 Barley genotypes. Only 12 primers amplified successfully (Table, 3). A total of 104 amplification DNA bands, with an average of 8.67 bands/primer, were produced using 12 ISSR primers. The total number of scorable markers produced per individual primer ranged between 3 bands with primer P6 and 14 bands with primers P10 as shown in table (3). 91 bands were polymorphic with an average of 7.58 bands/primer. The highest number of polymorphic bands (13) was observed with primer (p10), while the lowest polymorphic bands (3) were noticed with primers (P6, P9). On the other hand, only 13 bands were monomorphic with an average of 1.08 bands/primer. The highest number of monomorphic bands (5) was observed with primer (P5). A total of 15 unique bands were observed in all 16 tested genotypes (Table, 3).

The polymorphism percentage ranged between 100% in primers (P1, P3, P6, P15 and P20) and 50% in primer (P5) with an average of 87.37%.

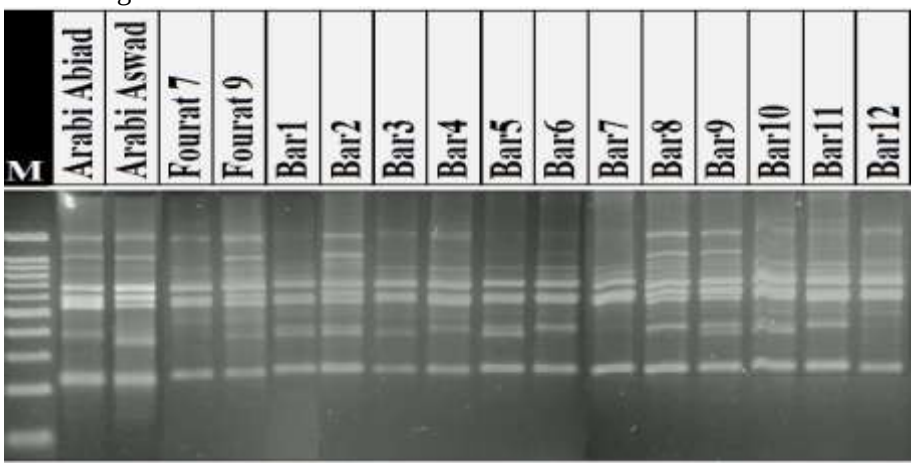

Fig 1. ISSR profile of barley genotypes amplified by ISSR primer P18. M: lader marker.

Table 3. Number of amplified bands and polymorphism percentage.

Primer No.	Number of amplified bands				PPB (%)
	TAB	NPB	NMB	NUB	
p1	9	9	0	1	100
p2	7	5	2	1	71
p3	10	10	0	0	100
p5	10	5	5	1	50
p6	3	3	0	1	100
p8	12	11	1	1	92
p9	4	3	1	1	75
p10	14	13	1	1	93
p12	10	8	2	1	80
p15	9	9	0	3	100
p18	8	7	1	1	88
p20	8	8	0	3	100
Minimum	3	3	0	0	50
Maximum	14	13	5	3	100
SUM	104	91	13	15	-
Average	8.67	7.58	1.08	1.25	87.37

Number of amplified bands (TAB: Total amplified bands, NPB: Number of polymorphic bands, NMB: Number of Monomorphic bands, NUB: Number of Unique bands), PPB (%): Percentage of polymorphic bands (%)

Results in Table (4) revealed that the number of unique bands obtained from ISSR primers ranged from 1 to 3, while primer (P3) did not result any unique band. The highest number of positive bands (1) was obtained with primers (P1, P2, P5), while the highest number of negative bands (3) was obtained with primers (P15 and P20).
The highest number of unique bands (4) were scored in genotype (Bar9). Three out of the 15 unique bands were registered as present bands (positive), while 12 were registered as absent bands (negative). The highest number of positive bands was 2 in genotype Bar4, while the highest number of negative bands was 4 in genotype Bar9 (Table, 4).

Table 4. Number of unique bands generated from 16 barely genotypes based on ISSR markers

	p1	p2	p3	p5	p6	p8	p9	p10	p12	p15	p18	p20	SUM
Arabi Abiad	-	-	-	-	-	-1	-	-1	-	-	-	-	**2**
Arabi Aswad	-	-	-	-	-1	-	-1	-	-	-	-	-	**2**
Fourat 7	-	-	-	-	-	-	-	-	-	-1	-	-1	**2**
Fourat 9	-	-	-	-	-	-	-	-	-	-	-	-	**0**
Bar1	-	-	-	-	-	-	-	-	-	-	-	-	**0**
Bar2	-	-	-	-	-	-	-	-	-	-	-	-	**0**
Bar3	-	-	-	-	-	-	-	-	-	-	-	-	**0**
Bar4	-	1	-	1	-	-	-	-	-	-	-	-	**2**
Bar5	-	-	-	-	-	-	-	-	-	-	-	-	**0**
Bar6	-	-	-	-	-	-	-	-	-	-	-	-	**0**
Bar7	1	-	-	-	-	-	-	-	-1	-	-1	-	**3**
Bar8	-	-	-	-	-	-	-	-	-	-	-	-	**0**
Bar9	-	-	-	-	-	-	-	-	-	-2	-	-2	**4**
Bar10	-	-	-	-	-	-	-	-	-	-	-	-	**0**
Bar11	-	-	-	-	-	-	-	-	-	-	-	-	**0**
Bar12	-	-	-	-	-	-	-	-	-	-	-	-	**0**
SUM	**1**	**1**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**3**	**1**	**3**	**15**

Marker efficiency analysis

To analyze the suitability of the marker to evaluate genetic profiles of Barley, the performance of the marker was measured using three parameters: polymorphic Information Content (PIC), Effective multiplex ratio was calculated (EMR), Marker Index (MI), Resolving Power (RP), and Discriminating Power (DP).
In this study, high PIC value of 0.3545 for primer (P18) and low PIC value of 0.1508 for primer (P5), with an average value of PIC per primer 0.2711 was obtained. The highest effective multiplex ratio (EMR) 8.06 was observed with the primer (P5) and the lowest 2.38 was observed with the primer (P6) with an average EMR of 5.81 per primer. The highest Marker Index (MI) was observed with the primer (P12) (2.33) and lowest in the primer (P6) (0.73) with an average MI of 1.56 per primer was obtained, while the highest Discriminating Power (DP) and Resolving Power (RP) values was observed with the primer (P10) (DP = 0.814, RP = 5.63) and the lowest with the primer (P9) (DP = 0.290, RP = 1.25) with an average of (DP = 0.508, RP = 3.55) per primer was obtained (Table, 5).

Table 5. Primer efficiency parameters.

Primer No.	PIC	EMR	MI	DP	RP
p1	0.3014	7.06	2.13	0.655	5.13
p2	0.2154	5.06	1.09	0.479	2.13
p3	0.3070	6.31	1.94	0.603	4.38
p5	0.1508	8.06	1.22	0.351	2.13
p6	0.3073	2.38	0.73	0.377	1.25
p8	0.2871	5.06	1.45	0.823	5.13
p9	0.2305	3.38	0.78	0.290	1.25
p10	0.2773	6.06	1.68	0.814	5.63
p12	0.3328	7.00	2.33	0.511	5.50
p15	0.2474	7.06	1.75	0.385	2.88
p18	0.3545	5.56	1.97	0.518	4.63
p20	0.2422	6.75	1.63	0.289	2.50
Minimum	0.1508	2.38	0.73	0.290	1.25
Maximum	0.3545	8.06	2.33	0.814	5.63
Average	0.2711	5.81	1.56	0.508	3.55

PIC: Polymorphism information content, EMR: effective multiplex ratio, MI: marker index, DP: Discriminating power, RP: resolving power.

The dissimilarity matrix

Jaccard's dissimilarity coefficient values calculated from ISSR data ranged from 0.189 to 0.558 with mean value of 0.348.

The lowest matrix value (0.189) was observed between genotypes Bar1 and Bar3 suggesting their close relatedness. Whereas genotypes bar4 and bar7 seemed to be the most divergent since they had exhibited the highest genetic distance (0.558).

Table 6. Dissimilarity matrix (Jaccard coefficient):

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	0.000															
2	0.237	0.000														
3	0.330	0.376	0.000													
4	0.323	0.333	0.298	0.000												
5	0.352	0.344	0.244	0.396	0.000											
6	0.411	0.402	0.357	0.385	0.321	0.000										
7	0.452	0.374	0.230	0.427	**0.189**	0.354	0.000									
8	0.543	0.467	0.390	0.453	0.413	0.420	0.365	0.000								
9	0.471	0.443	0.421	0.517	0.338	0.524	0.375	0.451	0.000							
10	0.366	0.375	0.305	0.356	0.247	0.256	0.321	0.367	0.457	0.000						
11	0.310	0.394	0.405	0.356	0.388	0.449	0.459	**0.558**	0.519	0.384	0.000					
12	0.370	0.326	0.349	0.341	0.250	0.321	0.282	0.372	0.481	0.268	0.329	0.000				
13	0.376	0.333	0.395	0.385	0.321	0.268	0.393	0.512	0.524	0.277	0.376	0.238	0.000			
14	0.362	0.264	0.341	0.352	0.265	0.371	0.253	0.363	0.451	0.241	0.360	0.200	0.294	0.000		
15	0.453	0.427	0.442	0.337	0.388	0.484	0.459	0.450	0.400	0.345	0.424	0.407	0.467	0.341	0.000	
16	0.296	0.259	0.229	0.302	0.188	0.294	0.213	0.354	0.375	0.260	0.333	0.245	0.327	0.220	0.265	0.000

1: Arabi Abiad, 2: Arabi Aswad, 3: Fourat 7, 4: Fourat 9, 5: Bar1, 6: Bar2, 7: Bar3, 8: Bar4, 9: Bar5, 10: Bar6, 11: Bar7, 12: Bar8, 13: Bar9, 14: Bar10, 15: Bar11, 16: Bar12.

Cluster analysis as revealed by ISSR

The dendrogram based on Jaccard's dissimilarity coefficient was constructed using the whole ISSR data matrix, ISSR dendrogram obtained from UPGMA cluster analysis was divided into two main clusters at 0.44 genetic distance, separating genotype (bar4) on their own. The other main cluster included the rest of genotypes and was divided into two sub-clusters at 0.43 genetic distance. The first sub-cluster was made up of genotypes (bar5, bar11) with a genetic distance of 0.4. The second sub-cluster was divided into two sub sub-clusters at 0.37 genetic distance; included the rest of genotypes (Fig, 2).

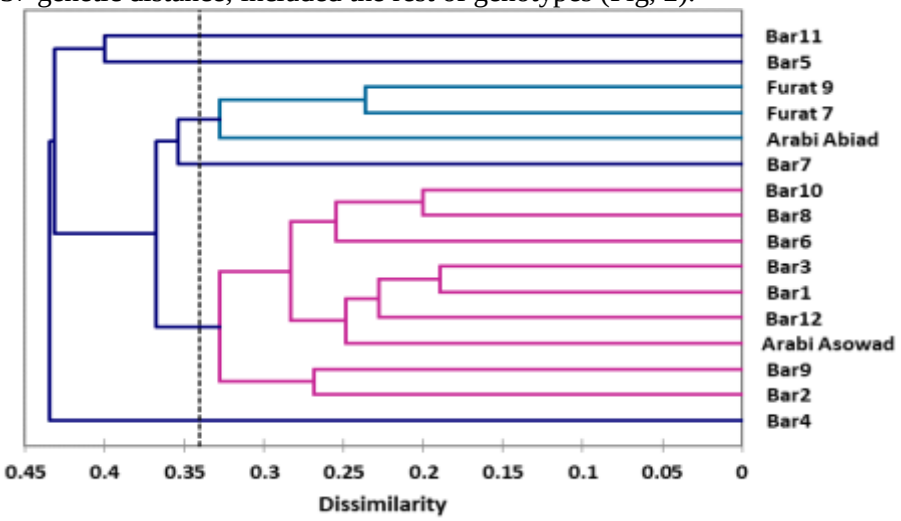

Fig 2. Cluster analysis as revealed by ISSR data.

Discussion:

Evaluation of genetic diversity within and between populations provides useful information for germplasm conservation and design effective breeding strategies (Carvalho 2004; Rubenstein *et al*. 2005). In this study, ISSR marker were employed for genetic diversity analysis within barley genotypes.

ISSR marker showed highly polymorphism rates and out of the 12 ISSR primers, 5 primers (P1, P3, P6, P15 and P20) showed 100% polymorphism.

Results revealed that the average percentage of polymorphism in all genotypes was 87.37 % which is too much close to the results obtained by (Serpoush, 2021) (87.2%) and too far from the results obtained by (Shata *et al.*, 2021) (39.8%) and (Mohamed *et al.*, 2021) (50.07%).

It is expected to obtain high polymorphism using ISSR markers, since microsatellite sequences are highly variable and ubiquitously distributed across the genome (Ng and Tan, 2015).

High percentage of polymorphism in this study confirms the high discriminative power of used ISSR markers in the studied barley genotypes.

In our study, Primers (P15, P20) proved to be the most informative primers based on unique bands, and the average PIC value showed that these markers are effective for genetic diversity analysis in barley.

Results revealed that the average PIC value in all primer was 0.2711 which is too far from the results obtained by (Dhahir Lateef *et al.*, 2021) (0.74), (Serpoush, 2021) (0.714) and (Shata *et al.*, 2021) (0.78); while it is too much close to the PIC values obtained by (Mohamed *et al.*, 2021) which ranged between (0.26-0.37).

In a study by (Shayan *et al.*, 2020), the values of Marker Index (MI) ranged from 3.528 to 29.972 with an average of 9.704, Which does not correspond to the results of Marker Index (MI) that we obtained, as the Marker Index (MI) ranged in our results from 0.73 to 2.33 with an average of 1.56.

In this study, resolving power (Rp) ranged from 1.25 to 5.63, Which is inconsistent with (Guasmi *et al.* 2012) who found that ISSR primers exhibited variations in (Rp) ranged from 0.74 to 1.16.

The higher mean values of the following parameter (EMR, MI, DP and RP) (5.81, 1.56, 0.508 and 3.55 respectively), suggesting that ISSRs are robust molecular markers that can distinguish between barley genotypes.

In earlier studies, researchers observed that barley has a low level of genetic diversity (Hosseini *et al.*, 2022; Serpoush, 2021; Mohamed *et al.*, 2021) which agree with the results of this study, as the Jaccard's dissimilarity coefficient values calculated from ISSR data ranged from
0.189 to 0.558 with mean value of 0.348.

ISSR dendrogram was able to clearly distinguish all barley genotypes. All Syrian varieties except (Arabi Aswad) were placed in the same sub sub sub sub-cluster. The genetically closest relatives were the genotypes Bar1 and Bar3. Whereas genotypes bar4 and bar7 seemed to be the most divergent.

Conclusion

1. ISSR markers have been successfully applied to evaluate genetic relationship among Barley genotypes.
2. ISSR dendrogram was able to discriminate all tested genotypes.
3. The results showed that ISSR primers have the ability to separate barley genotypes from each other.
4. It seems that ISSR markers under study can be used in marker assisted selection of barley genotypes in breeding programs.
5. Our results could be utilized by barley breeders to decide their best choice of parents of their hybrids to reach the best desirable traits.

References:

1. Albayrak G, Gozukirmizi N (1999). **RAPD analysis of genetic variation in barley.** Turk J Agric For 23:627–630
2. Assefa A, Labuschagne MT, Viljoen CD (2007). **AFLP analysis of genetic relationships between barley (Hordeum vulgare L.) landraces from north Shewa in Ethiopia.** Conserv Genet 8:273–280
3. Bedada G, Westerbergh A, Nevo E, Korol A, Schmid KJ (2014). **DNA sequence variation of wild barley Hordeum spontaneum (L.) across environmental gradients in Israel.** Heredity 112:646–655
4. Bockelman HE and Valkoun J (2010). **Barley germplasm conservation and resources.** In: Ullrich, S.E., editor, Barley: Improvement, production, and uses. Wiley-Blackwell, Oxford, UK. p. 144–159.
5. Carvalho MA (2004). **Germplasm characterization of Arachis pintoi Krap. and Greg. (Leguminosae).** PhD Thesis, University of Florida, USA
6. Cid-Contreras RC, Mascorro-Gallardo JO and Valadez-Moctezuma E (2019). **Genotyping and molecular analysis of transgenic sequences in chrysanthemum (Chrysanthemum x morifolium Ramat).** Crop Breeding and Applied Biotechnology.
7. Dhahir Lateef D, Mahmud Mustafa K, and Abdul-razzak Tahir N (2021). **Genomic variation and genetic structure prole of Iraqi barley accessions using ISSR and arbitrary functional gene-based molecular markers.** https://doi.org/10.21203/rs.3.rs-787338/v1
8. Elakhdar A, Kumamaru T, Qualset CO, Brueggeman RS, Amer K, Capo-chichi L (2018). **Assessment of genetic diversity in Egyptian barley (Hordeum vulgar L.) genotypes using SSR and SNP markers.** Genet Resour Crop Evol
9. El-Esawi MA, Alaraidh IA, Alsahli AA, Ali HM, Alayafi AA, Witczak J, Ahmad A (2018). **Genetic variation and alleviation of salinity stress in barley (Hordeum vulgare L.).** Molecules 23:2488
10. FAO (2018). http://www.fao.org/faostat/en/#data/
11. Fernandez M, Figueiras A, Benito C (2002). **The use of ISSR and RAPD markers for detecting DNA polymorphism, genotype identification and genetic diversity among barley cultivars with known origin.** Theoretical and Applied Genetics 104: 845-851
12. Ghaffari P, Talebi R, Keshavarz F (2014). **Genetic diversity and geographical differentiation of Iranian landrace, cultivars and exotic chickpea lines as revealed by morphological and microsatellite markers.** Physiol Mol Biol Plant 20(2):225–233
13. Guasmi F, W Elfalleh, H Hannachi, K Fares, L Touil, N Marzougui, T Triki and A Ferchichi (2012). **The use of ISSR and RAPD markers for genetic diversity among south tunisian barley.** Agronomy: 10. doi:10.5402/2012/952196
14. Hosseini M, Yassaie M, Rashed-Mohassel M. H, Ghorbani R, Niazi A (2022). **Genetic diversity of Iranian wild barley (Hordeum spontaneum Koch.) populations.** Journal of Crop Science and Biotechnology. SN- 2005-8276. https://doi.org/10.1007/s12892-021-00132-2
15. Hou Y. C, Yan Z. H, Wei Y. M, Zheng Y. L (2005). **Genetic diversity in barley from west China based on RAPD and ISSR analysis.** Barley Genetics Newsletter 35: 9-22.
16. Ivandic V, Hackett CA, Nevo E, Keith R, Thomas TB (2002). **Analysis of sequence repeats (SSRs) in wild barley from the Fertile Crescent: associations with ecology, geography and flowering time.** Plant Mole Biol Evol 48:511–527
17. Jannatabadi AA, Talebi R, Armin M, Jamalabadi J, Baghebani N (2014). **Genetic diversity of Iranian landrace chickpea (Cicer arietinum L.) accessions from different geographical origins as revealed by morphological and sequence tagged microsatellite markers.** J Plant Biochem Biotech 23(2):225–229

18. Khatab I. A, El-Mouhamady A. A, Mariey S. A, Elewa T. A (2019). **Assessment of water deficiency tolerance indices and their relation with ISSR markers in barley (Hordeum vulgare L.).** Cu Sci Inte 8: 83-100
19. Kleinhofs A and F Han (2002). **Molecular mapping of the barley genome. In: Slafer G, Molina-Cano JS, Savin R, Araus JL, Romagosa I, editors, Barley Science Recent Advances from Molecular Biology to Agronomy of Yield and Quality.** Food Products Press, New York pp 665.
20. Kumar V, Khippal A, Singh J, Selvakumar R, Malik R, Kumar D, Kharub AS, Verma RPS and Sharma I (2014). **Barley research in India: Retrospect and Prospects.** Journal of Wheat Research 6(1):1-20
21. Lassner M.W, Peterson P, and Yoder J.I (1989). **Simultaneous amplification of multiple DNA fragments by polymerase chain reaction in the analysis of transgenic plants and their progeny.** Plant Mol. Biol. Rep., 7:116–128.
22. Maniatis T, Fritsch E.F, and Sambrook J (1982). **Molecular cloning:Laboratory manual.** Cold Spring Harbor Laboratory Press, ColdSpringHarbor/ NY.
23. Matus IA and PM Hayes (2002). **Genetic diversity in three groups of barley germplasm assessed by simple sequence repeats.** Genome 45(6):1095-1106. https://doi.org/10.1139/g02-071
24. Mohamed A. H, Omar A. A, Attya A. M, Elashtokhy M. M. A, Zayed E. M, and Rizk R. M (2021). **Morphological and molecular characterization of some egyptian six-rowed barley (Hordeum vulgare l.).** Plants, 10(11). https://doi.org/10.3390/plants10112527
25. Molina-Cano J, Russell J, Moralejo M, Escacena J, Arias G, Powell W (2005). **Chloroplast DNA microsatellite analysis supports a polyphyletic origin for barley.** Theor Appl Genet 110(4):613–619
26. Montero-Pau J, Blanca J, Esteras C, Martínez-Pérez E. M, Gómez P, Monforte A. J, Picó B (2017). **An SNP-based saturated genetic map and QTL analysis of fruit-related traits in Zucchini using Genotyping-by-sequencing.** BMC genomics 18: 1-21
27. Nadeem MA, Nawaz MA, Shahid MQ, Dog˘an Y, Comertpay G, Yıldız M, Hatipog˘lu R, Ahmad F, Alsaleh A, Labhane N, O¨ zkan H, Chung G, Baloch FS (2018). **DNA molecular markers in plant breeding: current status and recent advancements in genomic selection and genome editing.** Biotechnol Biotechnol Equip 32:261–285
28. Nandha PS, Singh S (2014). **Comparative assessment of genetic diversity between wild and cultivated barley using gSSR and EST-SSR markers.** Plant Breed 133:28–35
29. Newton AC, Flavell AJ, George TS, Leat P, Mullholland B, Ramsay L, Revoredo-Giha C, Russell J, Steffenson BJ, Swanston JS, and Thomas WT (2011). **Crops that feed the world 4. Barley: a resilient crop? Strengths and weaknesses in the context of food security.** Food Security 3: 141-178.
30. Ng W.L, and Tan S.G (2015). **Inter-simple sequence Repeat (ISSR) Markers: Are we doing it right? ASM.** Sci. J., 9: 30- 39.
31. Park SK, Lee DJ, Baek HJ, Lee J, Farooq M (2011). **Study of the genetic diversity of Korean, Chinese and Japanese landraces of barley (Hordeum vulgare L.) using microsatellites.** Biodiv Res Conserv 23:3–13
32. Powell W, Morgante M, and Andre C (1996). **The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis.** Mol. Breed. 2(3), 225–238. http://dx.doi.org/10.1007/BF00564200
33. Prevost A, Wilkinson MJ (1999). **A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars.** Theor Appl Genetic 98:107–112

34. Rahimi M, Majidi Hervan I, Valizadeh M, Darvish Kajori F, Ebrahimpour F (2014). **Genetic diversity among wild and cultivated barley by ISSR marker.** Bull Env Pharmacol Life Sci 3(10):57–62
35. Roldan-Ruiz I, Dendauw J, Vanbockstaele E, Depicker A, De Loose M (2000). **AFLP markers reveal high polymorphic rates in ryegrasses (Lolium spp.).** Mol Breed 6:125–134
36. Rubenstein DK, Heisey P, Shoemaker R, Sullivan J, Frisvold G (2005). **Crop genetic resources: an economic appraisal.** United States Department of Agriculture (USDA). Econ Info Bull; No:2. (www.ers.usda.gov)
37. Serpoush M (2021). **Molecular diversity of Azerbaijani wild and cultivated barley genotypes.** Journal of Cereal Research, 13(Spl1), 37–42. https://doi.org/10.25174/2582-2675/2021/115268
38. Shakhatreh Y, Baum M, Haddad N, Alrababah M, Ceccarelli S (2016). **Assessment of genetic diversity among Jordanian wild barley (Hordeum spontaneum) genotypes revealed by SSR markers.** Genetic Resources and Crop Evolution 63: 813822
39. Shata S. M, Said W. M, Abdel-Tawab F. M, Kamal L. M (2021). **Morphological and Quantitative traits of phylogenetic relationships of some barley (Hordeum vulgare L.) accessions in Egypt.** Journal of Scientific Research in Science, 38, (1):16-35.
40. Shaveta, Kaur H, and Kaur S (2019). **Hulless Barley: A new era of research for food purposes.** Journal of Cereal Research 11(2): 114-124 doi.org/10.25174/2249-4065/2019/83719
41. Shayan S, Vahed M. M, and Mohammadi S. A (2020). **Genetic Diversity and Grouping of Winter Barley Genotypes for Root Characteristics and ISSR Markers Abstract Background and Objectives.** 43(3), 323–336. https://doi.org/10.22055/ppd.2019.27840.1684
42. Stein N and Muehlbauer GJ (2018). **The Barley Genome, Compendium of Plant Genomes.** doi:10.1007/978-3-319-92528-8_8.
43. Stein N, Prasad M, Scholz U, Thiel T, Zhang H, Wolf M, Kota R, Varshney RK, Perovic D, Grosse I, Graner A (2007). **A 1,000-loci transcript map of the barley genome: new anchoring points for integrative grass genomics.** Theor Appl Genet 114(5):823–839
44. Tanno K, Taketa K, Komatsuda T (2002). **A DNA marker closely linked to the vrs1 locus (row-typed gene) indicates multiple origins of six-rowed cultivated barley (Hordeum vulgare L.).** Theor Appl Genet 104:54–60
45. Tanyolac B (2003). **Inter-simple sequence repeat (ISSR) and RAPD variation among wild barley (Hordeum. vulgare subsp. spontaneum) populations from west Turkey**. Genetic Resources and Crop Evolution 50: 611-614
46. Tessier C, David J, This P, Boursiquot J. M and Charrier A (1999). **Optimization of the choice of molecular markers for varietal identification in Vitis vinifera L.** Theor. Appl. Genet. 98(1), 171–177.
47. Tricase C, Amicarelli V, Lamonaca E, Rana R. L (2018). **Economic analysis of the barley market and related uses, Grasses as food and feed.** IntechOpen.
48. Turuspekov Y, Abugalieva S, Ermekbayev K, Sato K (2014). **Genetic characterization of wild barley populations (Hordeum vulgare ssp. spontaneum) from Kazakhstan based on genome wide SNP analysis.** Breed Sci 64(4):399–403
49. Varshney RV, Grosse I, Hahnel U, Siefken R, Prasad M, Stein N, Langridge P, Altschmied L, Graner A (2006). **Genetic mapping and BAC assignment of EST-derived SSR markers shows non-uniform distribution of genes in the barley genome.** Theor Appl Genet 113:239–250
50. Wei Q, Wang Y, Qin X, Zhang Y, Zhang Z, Wang J, Chen J (2014). **An SNP-based saturated genetic map and QTL analysis of fruit-related traits in cucumber using specific-length amplified fragment (SLAF) sequencing.** BMC genomics 15: 1-10
51. Yang M, Abdalrahman H, Sonia U, Mohammed A. I, Vestine U, Wang M, Toughani M (2020). **The application of DNA molecular markers in the study of Codonopsis species genetic variation, a review.** Cellular and Molecular Biology 66: 23-30

52. Yang S, Wei Y, Qi P, Zheng Y (2008). **Sequence polymorphisms and phylogenetic relationships of hina gene in wild barley from Tibet, China.** Agric Sci China 7(7):796–803
53. Zohary D, Hopf M (1993). **Domestication of plants in the old world: the origin and spread of cultivated plants in West Asia.** Europe and the Nile Valley, 2nd edn. Clarendon Press, Oxford