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DIVERSITY MAINTENANCE OF SOME BARLEY (*HORDEUM* SPP) GENETIC RESOURCES USING SSR-BASED MARKER

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ABSTRACT

Barley is an important cereal crop cultivated in many parts of the world since ancient times. Genetic diversity was studied in a set of 16 barley (*Hordeum* spp) genotypes using 25 microsatellite (SSR) loci. A total of 94 different alleles distributed over all the seven chromosomes of barley. Out of these, 76 alleles were polymorphic with an average of 3.04 alleles/primer. Major allele frequency, gene diversity and PIC were estimated based on allele frequencies, having the mean values of 0.543, 0.571 and 0.513, respectively. Principal component analysis indicated the presence of high genetic variability among barley genotypes. ATACO, Canela, Local Abiad Sulaimani and Amal2 were identified to be highly distanced from other genotypes. The dendrogram created by hierarchical cluster analysis using the UPGMA algorithm was able to discriminate the genotypes into 7 groups according to their genetic distance. Both analyses parallel to principal coordinate analysis are indicating the close relatedness between Quinn, Zanbaka, Al-khair and Arta/3/Avar genotypes that exchange genetic material through continuous previous breeding programs. These values indicate high differentiation ability of SSR markers for genetic studies in barley and their utility for barley genetics and breeding.

Keywords: Allele, Barley, Genetic diversity, Polymorphic Information Content, Simple Sequence Repeat.

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INTRODUCTION

Barley (*Hordeum vulgare* L.) is considered to be one of the ancient cereals crops that was domesticated around 8000 BC from its wild relatives in Fertile Crescent of the Middle East (Kling *et al*., 2004; Dai *et al*., 2012). Later studies also hypothesized the occurrence of barley domestication in a wide region of the Eastern Mediterranean region of Western Asia (Poets *et al*., 2015). In addition to its commercial value as a feed or malt grain crop, barley is regaining popularity in human diet due to the rich content of antioxidant and b-glucan (Newman and Newman, 2008). Barley is one of the cereal crops that played a significant role in the development of agriculture. It represents the fourth most important cereal crops globally, after wheat, maize and rice (Tricase *et al*., 2018; FAOSTAT, 2019).

Barley is an annual autogamous grain crop that has early maturation and high drought tolerance (Sallam *et al*., 2019), grown in varied and marginal environments (Lister *et al*., 2018), making the crop to be widely distributed around the world (Garstang *et al*., 2011; Carter *et al*., 2019), tolerating wide range of biotic and abiotic stresses (Dawson *et al*., 2015).

It is second to wheat as the most important nutritional grain crops grown in low rainfall environments (Mohamed and Adel, 2012), providing a reasonable yield in marginal and drought areas (Baum *et al*., 2004).

There are over 32 species for *Hordeum* genus throughout the world to include diploid, polyploid, perennial, and annual types (Bothmer *et al*., 2009). Both six-rowed (*Hordeum vulgare* L. *hexastichum*) and two-rowed barley (*Hordeum vulgare* L. *distichum*) emerged during the process of their domestication and selection (Kilian *et al*., 2006), It is also reported that the six-rowed barley is derived from the two-rowed wild barley as a result of mutations and selection pressure to give different spikelets morphologically (Komatsuda *et al*., 2007). The cultivated barley diploid species (2n=2x=14) is a self-pollinating with a genome size of approximately 5100 Mbp of a haploid nucleus (Wicker *et al*., 2017). It is realized that in barley genetic bottlenecks occurred during domestication and modern improvement programs. as current elite varieties hold less diversity compared to their wild relatives or landraces (Kilian *et al*., 2006; Prada, 2009).

Genetic diversity among and within plant species is in danger of being reduced. In wild species, genetic diversity might be lost because of severe reduction in population size, whereas in domesticated crops genetic diversity may be lost due to narrowing of genetic base during consequent breeding programs to produce commercial varieties (Govindaraj *et al*., 2015).

Regularly, using elite crop germplasm as parents for breeding programs along with farmer performance creates genetic erosion and reducing genetic diversity (Duvick, 2005; Chaudhary, 2013), leading to more genetic uniformity.

The availability of genetic diversity among genotypes is a prerequisite and important step in the development of new and elite cultivars (Singh *et al*., 2014; Govindaraj *et al*., 2015). Diversity study is particularly useful in the characterization of cultivars for choosing right parent for breeding hybrids (Jaiswal *et al*., 2010). The efficient evaluation of barley's genetic diversity is necessary for efficient development in breeding programs for creation of superior cultivars, pattern of population structure (Amezrou *et al*., 2018) and effective conservation of this plant (Malysheva-Otto *et al*., 2006).

Traditionally, morphological characters have been used to evaluate the diversity and their relatedness. Besides been labor intensive, this method is often thought to be influenced by environmental conditions (Bosch *et al*., 2014). Rapid molecular marker application has been developed to eliminate crop-stage dependency and extensive field trials. Techniques of DNA fingerprinting especially those based on polymerase chain reaction have been established, and they are currently available (Jaiswal *et al*., 2010). Advances in DNA technology have increased the number and types of molecular marker for studying the genetic diversity of plant species. DNA markers are highly reproducible, independent of environmental factors, and are considered to be a valuable tool to provide precise insights into the diversity available for crop species (Nadeem *et al*., 2018). Different molecular markers (AFLP, ISSR, S-SAP, SSR and SNPs) have been used to characterize germplasm and investigate genetic diversity (Sorkheh *et al*., 2017; Jasim Aljumaili *et al*., 2018). Due to the high level of polymorphism and ease of use, SSR markers are widely used in crops breeding, accelerating the efficiency of cereals improvement (Hayden *et al*., 2008; Lenaerts *et al*., 2019). They are also abundant, dispersed throughout the genome, having the advantages of high variability from co-dominant and multi-allelic polymorphisms, and accurate and rapid detection (Ahmad *et al*., 2018; Liu *et al*., 2018; Viruel *et al*., 2018; Curto *et al*., 2019).

SSR markers have been extensively used in barley genetic diversity (Elakhdar *et al*., 2016a; Ferreira *et al*., 2016; Elakhdar *et al*., 2018; Arya *et al*., 2019), Association mapping (Jamali *et al*., 2017) linkage mapping and phylogenetic analysis (Lakew *et al*., 2013; Zhang *et al*., 2014). Among different DNA marker types used in barley, microsatellites have proven to be the markers of choice for genetic diversity studies for this plant species (Wang *et al*., 2010; Hua *et al*., 2015; Todorovska *et al*., 2019).

Although numerous studies have been reported on the diversity of barley using different molecular markers (Al-Hadeithi *et al*., 2012; Rahimi *et al*., 2014; Hussain and Adeel, 2017), limited evidence is available about the diversity of the usable barley cultivars in Iraq. To make optimal use of Iraqi germplasm for effective breeding and conservation, evaluation of genetic diversity appears to be essential.

The objective of this research was, therefore, to estimate the extend genetic distance among barley cultivars using SSR markers. It aimed at generating the baseline of useful genetic information to facilitate the germplasm evaluation for the future improvement and conservation of barley genetic resources.

MATERIALS AND METHODS

Plant Materials: A total of sixteen barley genotypes were used in this study. The genotypes were obtained from different sources of local research centers and international organizations (Table1).

Table 1. Name, pedigree and origin of the sixteen barley genotypes involved in the current study.

Genotype	Row-type	Pedigree	Origin
Clipper	2 row	Clipper/3/JLB37-74	Agriculture Research Center-Erbil
Bohoth H1	2 row	-	
Local Abiad Sulaimani	2 row	-	Agricultural Research Station of Bakrajo
ACSAD 157	2 row	-	
Amal 2	6 row	-	
IBA 99	6 row	OAP-4AP-7L,sel /ICARDA	ICARDA
Furat 2	6 row	SLB34-65/Arar//Furat-2 ICB04-0787-0AP-20AP-0AP-0TR-0TR-0AREC	
MORA	2 row	Mora/NB1054/3/Mola/SHYRI/ABUPO*2/JET/4	Agricultural Research Station of Bakrajo
COB	2 row	ALELI/CANELA/3/BICHY2000/4/GUENZA	

Canela	2 row	CANELA/ZEDAR#2/LIMON/3/MSE	CIMMYT
ATACO	6 row	ATACO/ Bermejo/Higo/3/ Cln-B/80	
ABN-B	2 row	KA-B/RAISA/3/ALELI/4/LEMON/5	
Quinn	6 row	Quinn/Aloe/Cardo/3/Ciru	
Zanbaka	6 row	Zanbaka/JLB37-064	State Board of Seeds Testing and Certification. Ministry of Agriculture. Iraq
Al-khair	6 row	Radiation local black x Arevat-IRAQ	Unit of seed technology / Iraq
Arta/3/Avar	6 row	Arta/3/Hml- 02//Esp/1808-4L	State Board of Seeds Testing and Certification. Ministry of Agriculture. Iraq

DNA Isolation: The study was conducted at the Central Laboratory and the Animal Science laboratory of the College of Agricultural Engineering Sciences, University of Sulaimani. DNA was extracted from fresh leaf tissue of the barley genotypes were grown in pots for three weeks, using a modified cetyltrimethylammonium bromide (CTAB) method described by Stein *et al*. (2001).

After extraction the DNA quality was checked for all the genotypes using 1% agarose gel. The extracted genomic DNA was kept in the freezer (-20ºC) until used in a polymerase chain reaction. The concentration of each DNA sample was diluted to 20-30 ng/µl by comparing the fluorescence of the genotypes' samples with DNA ladder bands of the same agarose gel under UV fluorescent.

SSR Primers for PCR reaction: Twenty-five PCR-based SSR primers were used in the current study. Twenty-four primers were from the barley genome and selected from previous genetic diversity studies of barley, while one primer was derived from a gene conferring resistance to barley stem gall midge ((*Mayetiola hordei*), and used in screening the current genotypes. The oligonucleotide primers were ordered from Sinaclon company (Iran). Detailed information about the name and sequences of the primers used is shown in Table 2.

SSR positions were amplified using polymerase chain reaction thermocycler (MultiGene OptiMax Thermal Cycler, Labnet Company). PCR reaction mix was purchased from Sinaclon company (Iran) and contained 10× assay buffer, 2.5 mM $MgCl_2$, 400 µM dNTP's and 1U of *Taq* DNA Polymerase. Each amplification contained 10µ of reaction master mix (Sinaclon), 2-3µl of genomic DNA (20-30ng in total), 2µl (20ng/µl) of each forward and reverse primers, then completed to the final volume of 20µl with de-ionized water (dH_2O).

Table 2. List of SSR primers, sequences, annealing temperature and their sources used to screen the barley genotypes.

No.	Primer name	Forward sequence (F: 5'-3')	Reverse sequence (R: 3'-5')	Annealing temp. (ºC)	Sources
1	19RTCT	GCATATACAATAGCAAAATTAAGC	GGAGGAGAGGAGAAGAAGAGG	60	(Tyrka *et al*., 2008)
2	1LATC	CTTGGTGATGTGGTTCTCGTT	CTCACCCAAAAGGAATGGTG	60	
3	25RACT	CCATACAACTTCAGGTGAGGG	AACTGTTCCAAGGGTCTCGAT	60	
4	Bmac0134	CCAACTGAGTCGATCTCG	CTTCGTTGCTTCTCTACCTT	53	(Blori-Moghadam *et al*., 2011)
5	Bmac0163	TTTCCAACAGAGGGTATTTACG	GCAAAGCCCATGATACATACA	55	(Massadeh *et al*., 2015)
6	BMAC624	AAAAGCATTCAACTTCATAAGA	CAACGCCATCACGTAATA	50	(Naceur *et al*., 2012)
7	EBmac701	ATGATGAGAACTCTTCACCC	TGGCACTAAAGCAAAAGAC	55	(Oliver *et al*., 2009)
8	GB318	CGGCTCAAGGTCTCTTCTTC	TATCTCAGATGCCCCTTTCC	62	(Raoudha *et al*., 2007)
9	GB357	GCTCCAGGGCTCCTCTTC	AGCTCTCTCTGCACGTCCTT	60	
10	GB371	CACCAAGTTCACCTCGTCCT	TTATTCAGGCAGCACCATTG	62	
11	GB384	CTGCTGTTGCTGTTGTCGTT	ACTCGGGGTCCTTGAGTATG	60	
12	GB391	AGCTCCTTTCCTCCCTTCC	CCAACATCTCCTCCTCCTGA	60	
13	GB402	CAAGCAAGCAAGCAGAGAGA	AACTTGTGGCTCTGCGACTC	60	
14	HVCMA	GCCTCGGTTTGGACATATAAAG	GTAAAGCAAATGTTGAGCAACG	55	(Blori-Moghadam *et al*., 2011)

15	MAG13	AAGGGGAATCAAAATGGGAG	TCGAATAGGTCTCCGAAGAAA	56	(Raoudha *et al*., 2007)
16	MAG149	CAAGCCAACAGGGTAGTC	ATTCGGTTTCTAGAGGAAGAA	56	
17	MAG210	ACCTACAGTTCAATAGCTAGTACC	GCACAAAACGATTACATCATA	50	
18	MS02	AGAGTAGTGGAAAGAAGTT	TGGTAGTGAGATGAGGTGAC	52	(Raoudha *et al*., 2007)
19	MS1	CTGACCCTTTGCTTAACATGC	TCAGCGTGACAAACAATAAAGG	52	
20	QLB1	CCAAGAACAACACAAGGGAAA	CCCAACAACTTGTGCCTAAAA	60	(Tyrka *et al*., 2008)
21	V13GEIII	AGGAACCCTACGCCTTACGAG	AGGACCGAGAGTGGTGGTGG	54	
22	VB23D	GGTAGCAGACCGATGGATGT	ACTCTGACACGCACGAACAC	62	(Raoudha *et al*., 2007)
23	VGLUEND	TTCGCCTCCATCCCACAAAG	GCAGAACGAAAGCGACATGC	62	
24	VITR1	CCACTTGCCAAACACTAGACCC	TTCATGCAGATCGGGCCAC	55	
25	MhA6*	AATTATGTAAACCGAACCGAAC	CGAATCCAAAGAGGAAGTGG	54	(Mezghani-Khemakhem *et al*., 2012)

*; from Barley Stem Gall Midge (*Mayetiola hordei*).

The PCR program was set up for one initial denaturation cycle at 94°C for 4 minutes, followed by 40 cycles of 60 seconds at 94°C, and then 60 seconds at the appropriate annealing temperature (adjusted previously between 50-65°C), followed by 2 minutes of extension step at 72°C. Termination stage was set up with a final extension of 72°C for 10 minutes. From each DNA sample, a total of 10µl aliquot of the PCR product was mixed with 3µl of electrophoresis 6x loading buffer. Then, the samples were loaded onto 1% agarose gel in TBE buffer (1%). The amount of 3µl ethidium bromide (0.5 µg/mL) was added to the gel when cooled down, and mixed before pouring into the tray. After loading the samples, the gels were run on 80V for 120 minutes. The fragments were separated by electrophoresis and visualized under UV Transilluminators.

Data analysis: The amplified fragments of the primers' alleles were scored as "1" and "0" for the presence and absence of alleles, respectively. Gene diversity and polymorphic information content were estimated on the basis of frequencies of identified alleles. The polymorphism percentage was calculated for all polymorphic SSR markers according to the method of Blair *et al*. (1999).

$$\text{Polymorphism } \% = \frac{\text{total number of bands} - \text{number of monomorphic bands}}{\text{total number of bands}} \text{X100}$$

The polymorphic information content (PIC) of each microsatellite locus was evaluated through allelic frequency (Anderson *et al*., 1993):

$$\text{PIC} = 1 - \sum_{i=1}^{k} pi^2$$

Where *k* is the total number of alleles detected for a microsatellite and p*i* the frequency of the *i*th allele in germplasm.

Binary matrix data was applied for the calculation of Jaccard's similarity coefficient using the XLSTAT 2017 software. Jaccard's coefficient was converted to dissimilarity matrix to create a dendrogram using the unweighted pair-group method with arithmetic averages (UPGMA). To determine the relationship between different genotypes, the principal component analysis (PCA) was conducted using the same software. DARwin software (version 6) was used to analyze Principal Coordinate Analysis and bootstrap values at the nodes of dendrogram after 1000 permutation to estimate the reliability of tree topology.

RESULTS AND DISCUSSION

Due to limited genetic variation among modern crop species, efficient use of the available genetic variation of local and non-adapted modern cultivars is necessary for germplasm maintenance, and for improving different crops (Russell *et al*., 1997; Tanksley and McCouch, 1997; Frankham *et al*., 2010; Chen *et al*., 2020), since the development of new improved genotypes requires the exploration and characterization of genetic diversity in available germplasm (Elakhdar *et al*., 2018). This diversity study may improve the efficiency of germplasm management operations, preventing the loss of allelic diversity through random genetic drift (Russell *et al*., 1997; Frankham *et al*., 2010).

Studying genetic distance at molecular level in barley germplasm is an important prerequisite for maximum exploitation of barley genotypes in breeding programs and also for the conservation of barley genetic diversity (Malysheva-Otto *et al*., 2006). Since Iraq is part of Fertile Crescent which is the center of barley domestication, exploring the local germplasm with the elite varieties could maximize the extent of genetic diversity in breeding programs to develop new cultivars with higher adapted to local biotic and biotic stress.

Due to the advantages of microsatellite markers over other classes of molecular markers, they were employed to study the genetic diversity of the available barley genotypes (including the domestics and introduced genotypes). The SSRs used in the current investigation are distributed across all seven barley chromosomes and generated clear patterns of polymorphism. A total of 25

SSR markers were polymorphic and able to detect 94 alleles when applied to the 16 barley genotypes,

In genetic diversity analysis, the number of alleles per locus is counted to be a significant indicator for diversity and it depends on the genotypes, the loci investigated and the marker type (Pasam *et al*., 2014) . In the current study, about 2-8 alleles were detected per a locus with an average of 3.76 alleles (Table 3).

Table 3. Amplification information, chromosomal location, polymorphism, alleles and gene diversity, and PIC values for 25 SSR primers applied on 16 barley genotypes.

No.	Primer	Chr. No.	Total allele	Polymorphic allele	Polymorphism %	Size of amplified bands	Major allele frequency	Gene Diversity	PIC
1	19RTCT	3H	6	1	16.667	80-650	0.750	0.375	0.305
2	1LATC	3H	2	2	100	160-180	0.500	0.555	0.456
3	25RACT	3H	2	2	100	190-210	0.813	0.320	0.294
4	Bmac0134	2 (2H)	2	2	100	100-140	0.438	0.672	0.612
5	Bmac0163	5H	2	2	100	150-160	0.688	0.461	0.398
6	Bmac624	6 (6H)	3	3	100	120-150	0.688	0.492	0.458
7	EBmac0701	4H	2	2	100	100-150	0.750	0.398	0.354
8	GB318	7 (5H)	4	4	100	150-600	0.438	0.695	0.649
9	GB357	7 (5H)	6	6	100	220-1200	0.188	0.883	0.871
10	GB371	6 (6H)	8	8	100	150-1200	0.375	0.742	0.701
11	GB384	5H	2	2	100	190-220	0.625	0.469	0.359
12	GB391	2H	3	3	100	510-550	0.750	0.414	0.387
13	GB402	5 (1H)	2	1	50	80-250	0.625	0.469	0.359
14	HVCMA	7H	3	3	100	150-300	0.500	0.648	0.592
15	MAG13	3 (3H)	3	3	100	150-190	0.438	0.602	0.516
16	MAG149	5 (1H)	8	8	100	150-700	0.313	0.828	0.810
17	MAG210	6 (6H)	2	1	50	120-170	0.813	0.305	0.258
18	MS02	7 (5H)	5	1	20	150-1200	0.688	0.430	0.337
19	MS1	5H	2	2	100	120-150	0.500	0.500	0.375
20	QLB1	5H	4	4	100	250-900	0.438	0.703	0.657
21	V13GEIII	3 (3H)	3	3	100	100-500	0.625	0.563	0.524
22	VB23D	4 (4H)	8	7	87.500	100-1200	0.125	0.898	0.889
23	VGLUEND	5 (1H)	2	1	50	280-350	0.688	0.430	0.337
24	VITR1	3 (3H)	6	3	50	210-550	0.438	0.695	0.645
25	MhA6	-	4	2	50	150-1000	0.375	0.727	0.682
	Total		94	76	80.851				
	Average		3.760	3.04			0.543	0.571	0.513

This result is greater than 2.6/locus reported by Elakhdar *et al*. (2016b), but lesser than 4 alleles/locus that found by Elakhdar *et al*. (2018), and 6.29 alleles/locus found by Todorovska *et al*. (2019). Size of amplified bands (from 80 to 1200bp) was estimated by comparing the PCR products with the known size of DNA fragments ladder. SSR alleles were manually scored as 1 or 0 (the presence and absence, respectively) only for clear bands. Out of this number, a total of 76 alleles were polymorphic and had an average of 3.04 alleles/primer. This result was higher than the average of 2.4 alleles/locus obtained by Wang *et al*. (2010) in a collection of 40 different genotypes from different origins. Our results indicating high genetic distance between the barley genotypes under study. Polymorphic alleles were ranged from 1 to 8 alleles for different primers used. A lower average was also (1.6 alleles per locus) obtained by Al-Hadeithi (2016) who used six SSR markers to screen nine barley varieties including IPA99 and Al-khair.

Primers GB371 and MAG149 had the highest polymorphic value of eight alleles/primer. Polymorphism varied for different markers, ranged from 16.667% to 100%, however the majority of primers were highly polymorphic. This divergence may be due to the diversification of varieties and the choice of SSR primers with high allele number per locus, from previous investigations of barley genotypes (Table 2). The considerable number of the detectable and polymorphic fragments might be expected to the number of GC of the primers utilized in this research. The distinction in the number of bands amplified by different primers is influenced by several factors such as primer sequence and

less number of annealing sites in the genome (Tahir, 2014).

The amplified alleles had different sizes, ranging from 80bp-1200bp. The highest major allele frequency (0.813) was obtained from SSR markers 25RACT and MAG210, while the minimum value of 0.125 was obtained from SSR marker VB23D. Gene diversity was expressed reversely to the allele frequency. It means VB23D marker is more specified with common alleles rather than rare alleles in the population of barley under study and the population has not reached the allelic saturation yet (Greenbaum *et al*., 2014). High values of gene diversity and PIC rate for this marker indicating the informative power of VB23D marker for studying the genetic diversity and population structure of barley crop. Same pattern was realized for the gene diversity of this DNA marker, giving less differentiation power (Gougerdchi *et al*., 2014). Genetic diversity was estimated, using SSR markers for barley genotypes. The average gene diversity for the entire samples set was found to be 0.571, with a range of 0.305 for marker MAG210 to 0.828 for MAG149 (Table 3). The broad range of gene diversity among barley genotypes, based on the SSR markers used, might be a source of the different genetic resources in barley, such as landraces, advanced breeding lines, cultivars. PIC of SSR markers is able to discriminate genotypes and evaluate the level of gene variation, which mostly depend on the allelic diversity. PIC values of > 0.889 and < 0.258 here indicate the loci with high and low diversity, respectively. Number of genotypes and the efficiency of markers for detecting alleles are relatively determine the PIC values. The PIC mean value was 0.513 which slightly higher than PIC value of 0.36 by Elakhdar *et al*. (2016b), and lower than PIC value of 0.54 by Pasam *et al*. (2014).

Transferability of SSR markers has been reported previously across species and genera of the insects (Weng *et al*., 2007) and plant species themselves (Ahmad, 2013), while according to our knowledge no transferable common marker has been realized between insect and plant species before, since we have assured non contamination of barley genotypes with any genomic DNA of *Mayetiola hordei*. This attempt had a reasonable result of amplification and polymorphism detection in the current study by using the SSR primer (MhA6) from barley stem gall midge (*Mayetiola hordei*). Four alleles were amplified having 50% of polymorphism among the barley genotypes.

The marker gave reasonable gene diversity and PIC values of 0.727 and 0.682, respectively. This compatibility between the host and its pathogen alleles might be referring to the emphasized relation through gene-for-gene interaction to shape the mechanism of plant immune system against its pathogen (Chisholm *et al*., 2006)

Principal companion analysis was performed to estimate the relative importance and contribution of each genotype to the total variance and illustrate the genetic diversity among the 16 genotypes (Table 3).

Table 3. First and second components scores of the 16 barley genotypes and their Egin-values.

Component	PC1	PC2
Clipper	0.015	0.079
Bohoth H1	0.155	0.001
Local Abiad Sulaimani	0.127	**0.364**
ACSAD 157	0.129	0.012
Amal 2	0.000	**0.384**
IBA 99	0.017	0.047
Furat 2	0.002	0.170
MORA	0.049	0.015
COB	0.038	0.028
Canela	**0.630**	0.069
ATACO	**0.661**	0.058
ABN-B	0.001	0.200
Quinn	0.075	0.016
Zanbaka	0.174	**0.264**
Al-khair	0.132	**0.213**
Arta/3/Avar	0.140	**0.347**
Eigenvalue	14.090	10.062
Variability (%)	18.540	13.240

Note: The important contributions are represented in bold.

The first two Principal Components (PCs) with Eigen-value >10 accounted for 31.78% of the total genetic variation (Table 3 and Figure 1), while the others, with the Eigen-values above one decimal number from PC3-PC15, were explained the remaining percent of total genetic variation. The score of the first component (PC1) was high and significant for ATACO and Canela giving the squared value of 0.661 and 0.630, respectively. While numerous genotypes showed the highest and significant value of 0.384 for Amal2 in PC2. The significance of the components values here shows the importance of the above genotypes as principal discriminatory factors for the barley genotypes under study.

A principal component analysis was performed to interpret the distance between the barley genotypes, as the first and second principal components (PC1 and PC2) were used to express the biplot diagram. The Scree plot indicates the most of the variation is derived the first and second factors (F1 and F2) in the Eigen-value of the genotypes data. The results obtained from Biplot-PCA indicated the presence of high genetic variations among barley genotypes based on SSR marker data (Figure 1). ATACO and Canela were also found to be very distanced genetically from other genotypes, followed by Local Abiad Sulaimani and Amal2 that distanced from the rest of genotypes under study.

Barley breeding institutes and research centers should take consideration of the necessity for the collection, conservation, and utilization of the local cultivars and landraces. The importance of this action has been emphasized in this investigation by giving a reasonable extension to barley gene pool via the contribution of indigenous genetic resources such as Local Abiad Sulaimani. The other 12 barley genotypes were dispersed on the plot with less genetic distance. The presence of common ancestors might be the reason for the close distance between these genotypes, framing them within a group corporation (Mohamed and Adel, 2012).

Presence of high levels of genetic diversity among the barley genotypes lead to the allelic richness that may represent their characteristics in the population. This level of diversity might be due to their geographical pattern, different pedigree, characteristics and growth behavior.

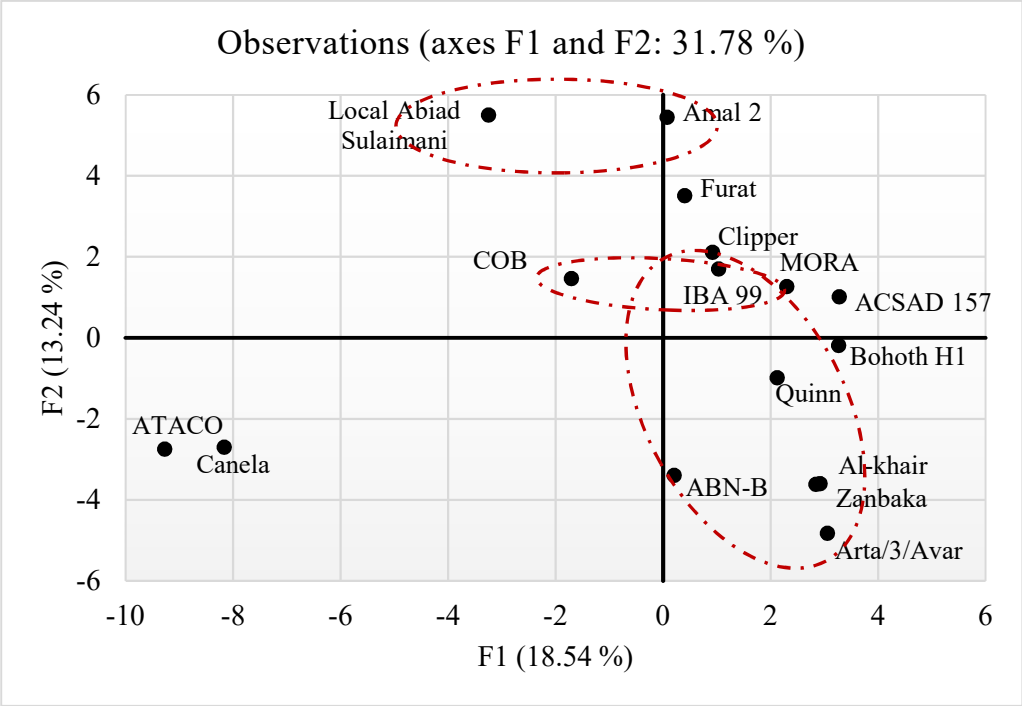

Figure 1. Biplot diagram of principal component analysis of the first and second components for the distribution of 16 barley genotypes, based on SSR DNA marker

Agglomerative Hierarchical Clustering Using SSR Marker Data: Pairwise comparisons were made between all genotypes and the average dissimilarity values were calculated based on the microsatellite-derived data. The distance between all 16 barley genotypes was evaluated using the alleles derived from applying 25 SSR primers. All 76 polymorphic alleles across the genotypes were utilized to evaluate the relationship between the genotypes. Dissimilarity values were ranged from 0.333 (between Zanbaka and Arta/3/Avar) to 0.849 (between ACSAD 157 and ATACO), as given in Table 4. This range of dissimilarity indicated the presence of reasonable variability among the genotypes under study. Hybridization program might be reasonable if conducted between ACSAD 157 and ATACO, due to the high dissimilarity observed to obtain higher heterosis value for important traits, and also mitigating the acceleration of extinction of primitive and adaptive genes among the studied genotypes (Govindaraj *et al*., 2015). The calculated small distances between Zanbaka and Arta/3/Avar could have corresponded to their origination from a common ancestor or some genetic materials might be exchanged among parental roots of these genotypes, making them be incorporated in one main group.

Table 4. Dissimilarity matrix among the 16 barley genotypes based on SSR marker data, following Jaccard's coefficient analysis.

Genotype	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16
V1	0.000															
V2	0.526	0.000														
V3	0.481	0.582	0.000													
V4	0.404	0.451	0.625	0.000												
V5	0.455	0.554	0.451	0.545	0.000											
V6	0.455	0.412	0.451	0.431	0.481	0.000										
V7	0.554	0.519	0.558	0.480	0.556	0.471	0.000									
V8	0.492	0.396	0.569	0.500	0.491	0.491	0.453	0.000								
V9	0.453	0.582	0.538	0.600	0.509	0.589	0.558	0.373	0.000							
V10	0.623	0.768	0.633	0.741	0.727	0.679	0.755	0.630	0.574	0.000						
V11	0.755	0.808	0.750	0.849	0.811	0.740	0.820	0.782	0.723	0.622	0.000					
V12	0.607	0.490	0.714	0.569	0.636	0.611	0.608	0.451	0.588	0.600	0.756	0.000				
V13	0.500	0.491	0.632	0.638	0.527	0.554	0.547	0.455	0.500	0.698	0.808	0.520	0.000			
V14	0.518	0.420	0.649	0.500	0.621	0.462	0.593	0.444	0.490	0.667	0.827	0.479	0.420	0.000		
V15	0.444	0.545	0.632	0.509	0.627	0.412	0.649	0.534	0.582	0.698	0.784	0.549	0.431	0.354	0.000	
V16	0.519	0.449	0.702	0.500	0.672	0.547	0.648	0.500	0.577	0.725	0.816	0.478	0.480	0.333	0.348	0.000

Note: V1: Clipper, V2: Bohoth H1, V3: Local Abiad Sulaimani , V4: ACSAD 157, V5: Amal2, V6: IBA 99, V7: Furat 2, V8: MORA, V9: COB, V10: Canela, V11: ATACO, V12: ABN, V13: Quinn, V14: Zanbaka, V15: Al-khair and V16: Arta/3/Avar

Success in breeding purposes, utilization and effective conservation of genetic resources depends on correct estimations of these parameters. To avoid the potential risks associated with bottlenecking the genetic diversity in barley, the adoption of genotypes with wide diversities have to be involved as parental lines in barley improving programs.

The amplified loci arranged in matrix data were utilized to evaluate the relationship between the genotypes under study by creating a dendrogram using the Jaccard's similarity coefficient. All the 16 genotypes were discriminated into seven main clusters at 0.505 threshold value of dissimilarity (Figure 2). The available genetic distance among the barley genotypes, based on SSR data, shows a wide distribution tendency of the barley genotypes in this region.

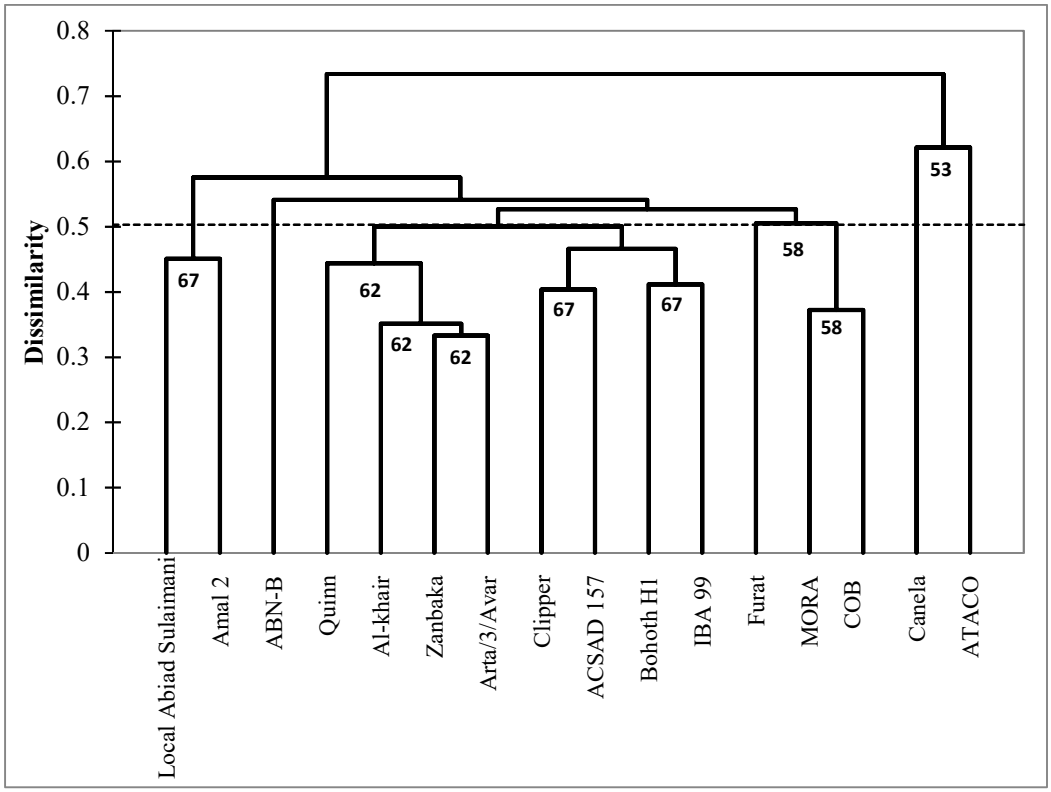

Figure 2. Dendrogram generated using Unweighted pair-group average of Jaccard's coefficient analysis dissimilarity, showing the distance between the barley genotypes using SSR marker data. Dissimilarity values are present at left side of the dendrogram. Bootstrap values are shown at the nodes

Despite the presence of higher genetic distance within the classes (78.60%) compared to the distances between the classes (21.4%), agglomeration method of unweighted pair-group average was able to cluster the genotypes into seven groups. The first main cluster was comprised of eight genotypes (Clipper, Bohoth H1, ACSAD 157, IPA99, Quinn, Zanbaka, Al-khair and Arta/3/Avar), having 12.232 within-class variances. However, this cluster has been split into two main sub-groups. The genotypes Quinn, Zanbaka, Al-khair and Arta/3/Avar were incorporated into the first sub-group while the rest genotype of this cluster incorporated into the second sub-group. Same results of clustering Quinn, Zanbaka, Al-khair and Arta/3/Avar genotypes in the first sub-group has been obtained by Tahir (2014) using RAPD DNA markers, confirming their strong genetic relationship compared to other genotypes. Care should be taken in involving these genotypes into hybridization programs, trying to include those clustered separately.

The second cluster included two genotypes of Local Abiad Sulaimani and Amal2. They implicate narrow genetic diversity with each other. Exchanged genetic material between these two genotypes might be occurred through previous breeding programs in Iraq, also they could be descent from the same origin (Hamza *et al*., 2004).

No large differences in genetic diversity between the clustered groups have extinguished based on row-types in the current study, however different grouping structures corresponding to row-type been reported earlier in of in American barley germplasm (Hamblin *et al*., 2010). However two-rowed barley are

specified with the dried or rainfed condition while six-rowed are more specified with the irrigated regions in Iraq, admixed ancestry of both barley types has been realized in the analysis of the genotypes under study. In order to assess the clustering of barley accessions based on SSR polymorphism, we conducted a principal coordinate analysis (PCoA). The scatter plots for the two first axes showed that accessions formed two main principal groups (Figure 3). The first group includes nine barley genotypes (Clipper, Bohoth H1, ACSAD 157, IPA99, Quinn, Zanbaka, MORA, Al-khair and Arta/3/Avar), while the second is only two barley involved (Amal2 and Local Abid Sulaimani). PCoA results are in agreement with the UPGMA and PCA analysis in terms of clustering and genotypes involved.

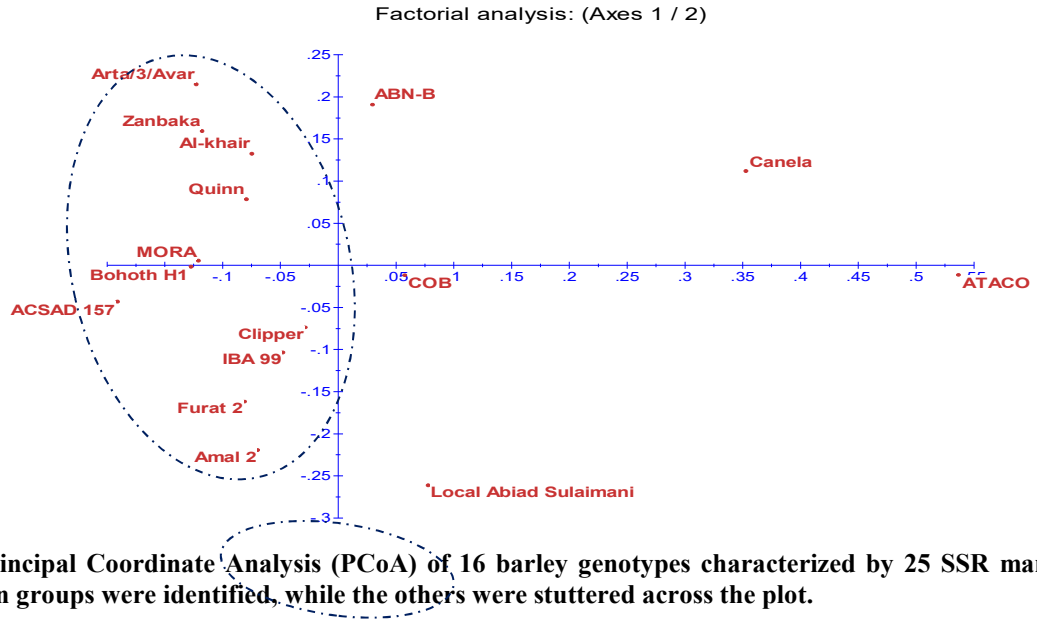

Figure 3. Principal Coordinate Analysis (PCoA) of 16 barley genotypes characterized by 25 SSR marker. Two main groups were identified, while the others were stuttered across the plot.

However the characterizations in barley genotypes are largely focused on the distributions of diversity based on eco-geographical distribution (Yahiaoui *et al*., 2008; Hübner *et al*., 2009; Russell *et al*., 2016), this characterization is not emphasized directly on the current barley genotypes, behaving pattern for genotypes of different geographical location are not always different genetically (Evanno *et al*., 2005; Yahiaoui *et al*., 2008). No distinct regional grouping patterns of barley genotypes from same or adjacent regions appeared in same groups clusters by Yadav *et al*. (2018) and Mekonnon *et al*. (2015) based on agronomic characters.

While posing reasonable distance between this group and the others (having 2.398 value as a minimum distance to dendrogram centroid), will assure a reasonable contribution of these two genotypes as a potential gene source to improve the adaptation of barley for the future hybridization program in the region. The fourth cluster also had involved both MORA and GOB with less within-class distance (9.5). Other genotypes of Furat2, Canela, ATACO and ABN-B were individually distributed on clusters 3, 5, 6 and 7, respectively. The results here indicate the efficiency of SSR markers as powerful tools in detecting co-dominant single locus to examine the genetic relationship between barley genotypes. Bootstrap values were ranged from 0.53-0.67, however the values are not very high, they may support the relations identified between the studied genotypes. It has been indicated that over 30% of bootstrap value suggests good robustness of the dendrogram (Kahodariya *et al*., 2015). The genetic relationship of the barley genotypes was clearly identified by cluster analysis identifying the potential and ability of microsatellite markers for genome analysis in barley, however monitoring the extent of genetic diversity of the barley genotypes with higher precision can be achieved by using more DNA markers. Both PCA and Dendrogram analysis methods showed a high degree of variation among analyzed genotypes. The results would be valuable for the breeders in such a way that the most promising genotypes in the population maybe selected from different clusters for crop improvement. Estimating the genetic diversity at the DNA level within the current barley genotypes could provide a clear figure of the genetic diversity of this crop in Kurdistan-Iraq to exploit parent selection, the identification of sub-genomic regions for the targeted increase in variability, and identification of new distinct gene pools in barley to be involved in the future improvement programs of this crop.

Conclusion: Molecular diversity analysis with SSR markers was used successfully as a tool for the genetic diversity of barley. The current results demonstrate that the data generated from a set of 25 SSR markers were highly informative. They were able to distinguish all 16 genotypes successfully with 76 polymorphic alleles (3.04 alleles/primer in average), however, further SSR markers combining with different DNA based techniques are more valuable for prominent discrimination among the barley genotypes. The transferability of MhA6 as a common marker from Stem Gall Midge *Mayetiola hordei* to *Hordeum* was identified. The markers identified with high polymorphism allele diversity and PIC will be useful for the conservation of poorly characterized barley genetic resources and determining the extent of the available barley gene pool. Results of this diversity analysis with PCA, AHC and PCoA will assist the potential parent selection to be used in improving programs and germplasm management of barley crop.

The present diversity available in barley genotypes is the collection from local and international researched centers for the current exotic and indigenous germplasm available in the region. This finding would be new sources of variation for beneficial traits such as early maturity, resistance to biotic and abiotic stresses, to meet the instant challenges of global food security. In the upcoming period, there is a need to further strengthen the genetic bases for barley germplasm to assist varietal development in the future.

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