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Detection of Genetic Polymorphism in Iraqi Barley using SSR-PCR Analysis

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Abstract

Nine Iraqi varieties of barley (*Hordeum vulgare* L.) has been differentiated and diagnosed using simple sequence repeat markers to detect their genetic polymorphism. Six SSR primers were used for genetic screening of barley samples (IPA 265, IPA 99, Tuwaitha, Hitra, Rayhan, Shuaa, Bawadi, Samir and Al_khair). These primers generated total PCR product (11) bands divided to 8 polymorphic bands 3 monomorphic bands. the percentage of polymorphism 80% ranged between (50-100%). a mean value of polymorphic band per primer was 1.6 . these primers produced amplification fragment at Molecular weight between 75-900 bp. One unique band was generated at size 200bp, this band can be used as a DNA profiling of all studied genotypes. These results appeared genetic distances ranged between (0.01098-0.99708) among studied varieties. Using the unweighed pair-group method using arithmetic averages (UPGMA) cluster analysis, nine barley landraces were clustered into two main clusters depending on their ancestors and to their spike type (two-row and six-row barley). These results will be useful for barley germplasm management in terms of biodiversity protection and breeder's rights protection.

Keywords: Barley (*Hordeum vulgare* L.), SSR, Genetic Polymorphism, genetic similarity.

الكشف عن تعدد الأشكال الوراثية في الشعير العراقي باستخدام تحليل التكرار التسلسلي البسيط

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الخلاصة

تسعة أصناف من الشعير العراقي تم تمييزها وتشخيصها باستخدام مؤشرات التكرار التسلسلي البسيط للكشف عن التباين الوراثي فيما بينها. استخدمت ستة بادئات خاصة في الفحص الجيني لعينات الشعير (اباء 265، اباء 99، تويثة، الحضر، ريحان، شعاع، بوادي، سمير، الخير). أنتجت تلك البادئات 11 حزمة قسمت الى 8 حزم متباينة و 3 منها حزم متماثلة. وبلغ التباين الوراثي 80% و تراوح بين (50–100%). وكان معدل الحزم المتباينة لكل بادئ 1.6. هذه البادئات أنتجت حزم مضخمه بوزن جزيئي بين 75–900 زوج قاعدي. ظهرت حزمة فريدة واحدة بوزن 200 زوج قاعدي، هذه الحزمة يمكن استخدامها كمؤشر وراثي للأصناف المدروسة. أظهرت هذه النتائج نسبة البعد الوراثي بين (0.01098– 0.99708) للأصناف المدروسة. استخدام التحليل العنقودي جمع الأصناف التسع في مجموعتين رئيسيتين اعتمادا على أسلافهم و نوع السنبلة (2 صف و6 صف) وهذه النتائج تفيد في إدارة الأصول الوراثية من حيث حماية التنوع البيولوجي وحماية حقوق المربي.

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Introduction

Barley is one of the most economic cereal crops tolerant of climatic variations, which is grown in a variety of areas representing a wide range of environmentally [1]. Barley had been subject to considerable genetic studies, it was a diploid (2n-2x-14), largely self fertilizing species with a large genome [2]. Iraq is characterized by a wide diversity of climate varies according to geographical regions accompanied by a large climatic variation and this variation is accompanied by the presence of a genetic base and a wide diversity of genetic large. The morphological variations of the first criteria that have been used in the characterization and classification process and the study of disparities between and within different species. However, in recent times and in the light of rapid development in the science of biotechnology, has discovered criteria and indicators for more precise can achieve this goal and scalability. The development of biotechnology has led to the emergence of a large number of important molecular markers that allow the characterization of organisms and discrimination even among small taxonomic units, including the repeated sections Statistics indicators Simple Sequence Repeats (SSR) which are also called indicators Microsatellite markers.

(SSR) is very important and widespread currently molecular markers. These indicators consist of small clips frequent, called units frequent, found in abundance in the genomes of eukaryotes and are distributed on all chromosomes in both areas genetic code or non code [3]. Repeated units of a number of pairs of nucleotides consist of between (6-1), also surrounded by these units clips located in a single region of the genome members of the same species. Microsatellite indicators differ among themselves in terms of, their locations within the genome and the number of repeated its constituent units, and the quality of nucleotides repeated units. Indicators of SSR intensively spread within the genome of the individual, where said [4] that the presence in the genome barley repeated once every 165 Kpb (kilo a pair of nucleotides Kbp), also found [5] that the repeat units (GA) n and (CA) n within the barley genome is once every 330 and 260 Kpb respectively. In addition, these indicators are characterized by a high level of polymorphism disparities revealed by comparing a number of other technologies [6,7], as well as easily applied and results analysis [8] They used these indicators in many different goals studies such as study of genetic diversity and creating a genetic link maps to a number of important qualities of different plant and types [9]. As well as to distinguish between species and to clarify the evolutionary relationships and rates genetic groups [10].

The current study aimed to assess the genetic polymorphism of barley genotypes using 6 SSR markers.

Materials and methods

Barley Samples

Nine (*H. vulgare* L.) varieties were used in this study. Among these varieties introduced from different regions and currently grow in central and north regions of barley cultivation areas of Iraq Table-1.

DNA Extraction and SSR analysis

Total genomic DNA was extracted from fresh leaves plant by CTAB method for six barley varieties IPA265, IPA99, Tuwaitha, Hirta, Rayhan, Shuaa while DNA extracted from seeds by promega kit for other three barley varieties Bawadi, Samir, Al_khair to produce rapid extraction and high quality extracted DNA. Purity and concentration of DNA was measured by spectrophotometer [11]. Genomic DNA integrity was detected by running on 0.8% agarose gel electrophoresis followed by staining with ethidium bromide and visualized under UV light [12]. DNA samples were diluted to a working concentration of 100 ng/µl in order to be use in the SSR-PCR experiments.

Table 1-Iraqi Barley varieties used in this study from*(SBSTC)and**(UST).

No.	Varieties Names	Pedigree	Breeding Institute	Rows No.
1	IPA 265	Brigs x 9cr.279 (OAP- 2AP-4AP-03355_79/ICARDA)	*(SBSTC)	6 row
2	IPA 99	OAP-4AP-7L,sel /ICARDA	*(SBSTC)	6 row
3	Tuwaitha	Radiation local black x Arevat-IRAQ	*(SBSTC)	2 row
4	Hitra	Radiation local black x Arevat-IRAQ	*(SBSTC)	6 row
5	Rayhan	Radiation Arevat seed-IRAQ	*(SBSTC)	6 row
6	Shuaa	Radiation Arevat seed-IRAQ	*(SBSTC)	6 row
7	Bawadi	Radiation hybrid (F3) local black x Arevat-IRAQ	*(SBSTC)	6 row
8	Samir	Radiation local black x Arevat-IRAQ	**(UST)	6 row
9	Al_khair	Radiation local black x Arevat-IRAQ	**(UST)	6 row

***(SBSTC) State Board of Seeds Testing and Certification. Ministry of Agriculture. Iraq. 2015**

****(UST) Unit of seed technology. Ministry of Science and technology. Iraq. 2015**

Six microsatellite primers pairs were selected based on their chromosomal positions [13,14]. Their names, sequences and chromosomal locations are listed in Table-2. These SSR Primers were synthesis by (Bioneer – Korea) in lyophilized form and dissolved in sterile deionizer distilled water to get final concentrations of (10pmol/ml) Recommended by provider.

Table 2-The primers Names, Sequences, Annealing Temperature (C˚) and Chromosome location were tested in this study [13,14].

No.	Primer Name	Primer Sequences	Annealing Temperature (C˚)	Chromosome Location
1	Bmac0577	F: 5' TCATACAGAAGCCCACACAG 3' R: 5' TGCATGTTCATTCTAGACAGG3'	61 C˚	Bmac0577
2	Ebmac0874	F: 5' AACCATTCCTCACCCAGG 3' R: 5'GTGAATGATGTTGAGGACATTG3'	58 C˚	4hac1g2
3	HVITR1	F:5'-CCACTTgCCAAACACTAgACCC-3' R: 5'-TTCATGCAGATCGGGCCAC-3'	55 C˚	3 (3H)
4	Ebmac0715	F: 5' GCGAACATTGTCATGTTAGTA 3' R: 5' TGTCATGCCAGACCTATG 3'	55 C˚	2hac2b3
5	Bmag13	F: 5'-AAGGGGAATCAAAATGGGAG-3' R: 5'-TCGAATAGGTCTCCgAAgAAA-3'	54 C˚	3 (3H)
6	GMS1	F: 5'-CTGACCCTTTGCTTAACATGC-3' R: 5'TCAGCGTGACAAACAATAAAGG3'	55 C˚	7 (5H)

SSR-PCR amplification were performed in a volume of 20µl containing 5µl of PCR Master Mix (Bioneer-Korea), with concentration (1X) containing, *Taq* DNA polymerase 1U, dNTP (dATP, dCTP, dGTP, dTTP) 250µM, Tris-Hcl (pH9.0) 10mM, KCl 30mM, $MgCl_2$ 30mM, Stabilizer and tracking dye 1.5mM, 10pmol of the primer, and 100 ng of template DNA. Amplification was carried out using a Thermocycler (Applied Biosystems),reactions were submitted to the following PCR parameter: one cycle of 94°C for 5 min, 35 cycles of 1 min denaturing step at 94°C, 1 min annealing temperatures between 54 -- 61°C depending on the different primer combinations and 2 min extension at 72°C, followed by 5 min at 72°C [15]. The SSR products were separated by Electrophoresis 5 Volt/cm for 2 hour using 1.5% agarose gel in 1xTBE buffer (10mM Tris-Borate, 1 mM EDTA) buffer. SSR-PCR products estimated by comparing with the DNA marker ladder (100-1500) bp provided by (Introne Biotechnology-USA).Photo documentation was performed under UV light (365 nm) using a photo imaging system **(**Consort - Belgium).

Data analysis

Molecular weight was estimated by comparing the PCR products with the known size of DNA fragments ladder (bands from 100 to 1500bp). SSR data were scored for the presence (1) or absence (0) of clear bands. Only intense bands were scored visually.

The genetic distance (GD) among cultivars were calculated according to [16]. Based on the similarity matrix, a dendogram showing the genetic relationships between genotypes was constructed using unweighted pair group method with arithmetic mean (UPGMA) [17] by using the software NTSYS-pc version1.80 [18].

Results and Discussion

SSR-PCR analysis

Always using this analysis for Knowledge of the genetic diversity and genetic relationships that considered a basic and essential information for plant breeders in order to be useful in the design of education and improvement programs and in improving the efficiency of operations germplasm management and conservation them from genetic drift [19].

In this study, The genetic polymorphism and relationships among nine barley varieties grown in Iraq were evaluated by SSR-PCR technique by using 6 specific primers. total of PCR product (11) divided to (8) polymorphic bands while other remaining (3) monomorphic bands, all details reported in Table 3. Total polymorphic bands ranging between (1-3), primers (Bmac0577, Bmag13, GMS1) gave the lowest number of polymorphic band but primer (Ebmac0874) gave the highest number of polymorphic band with percentage of polymorphism 80% that ranging between (50-100%), with a mean value alleles per locus was (1.6) as a number of alleles per/ marker. The percentage of polymorphism expressed by SSR primers from this study compared to the results of other researchers such as [15] 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%) with a mean value alleles per locus was (2.29) while the other studies [20, 21] number of bands per primer with an

average of (3.94),(2.81). The molecular weights of amplification bands ranged from (900) bp for pimer Ebmac0874 to (75) bp for primer Bmac0577, the size of these fragments estimated by compared with the size of standard marker ladder fragments used in this study. one unique band produced by primer ($Ebmac0874_{(200)}$) bp, which means the presence of a band in specific variety and the absence of the same band with the same size in all other varieties. Figure-1(1, 2, 3, 4, 5, 6).

Genetic Similarity and Genetic Cluster analysis

According to the SSR data, genetic distance among the nine *H. vulgare* varieties ranged from 0.99708 to 0.01098 Table-4, the highest similarity 0.99708 (99 %) was obtained between IPA 265 and Shuaa and lowest level of similarity 0.01098 (0.1 %) was obtained in Bawadi and Samir. It was shown two main groups in Figure-2, first group divided to two sub clusters, first sub cluster (1 IPA 265) and second sub cluster (7 Bawadi, 8 Samir, 9 Al-Khair). The number of rows is used as a phenotypic diagnosis of barley spike. IPA 265 appeared to be smoothly spike and 6 rows variety, Bawadi, Samir, Al-Khair appeared to be shared with them in row number 6 but differentiated by their spike type, Bawadi and Al_khair smoothly spike while Samir appeared to be roughly spike. Bawadi, Samir and Al-Khair varieties descend from same pedigree was Radiation local black x Arevat-Iraq. Second group divided to two sub clusters, one sub cluster (3Tuwaitha, 4 Hitra), Second sub cluster included (2 IPA 99, 5 Rayhan, 6 shuaa). Tuwaitha shared with Hitra in same pedigree Radiation local black x Arevat-IRAQ but differentiated in row number Tuwaitha 2 row while Hitra 6 row and in spik type Tuwaitha appeared to be roughly spike, Hitra appeared to be smoothly spike . IPA 99 shared with Rayhan, shuaa in same row number 6 but IPA 99 descend from OAP-4AP-7L,sel /ICARDA while Rayhan and shuaa descend from Radiation Arevat seed-IRAQ. Rayhan shared with IPA 99 by roughly spike, shuaa appeared to be smoothly spike. Figure-2.

Results of this investigation of SSR-PCR analysis will benefit barley breeders when selecting potential parents to make choice of the genotypes to be used in new crosses will also facilitate the germplasm management. The best correlation existed among oligonucleotides (Ebmac0874,HVITR1, Bmag13 and GMS1) and were the strongest for differentiated during detection polymorphic bands between barley varieties. It was found that cultivar (IPA265) show unique band and have molecular size range (200 bp) with primer (Ebmac0874) and this band could be used as a DNA marker to identify the barley genotypes.

Table3- Distinct characteristic of SSR primers include in the study: primers name, total number of bands , number of main bands, number of polymorphic bands, number of monomorphic bands, unique bands and percentage of polymorphism

Primer No.	Total Bands	Main Bands	Polymorphic Bands	Monomorphic Bands	Unique Bands	Polymorphism%
1. Bmac0577	8	1	1 (75) bp	--------	--------	100%
2. Embac0874	17	3	3 (900 , 300 , 200)bp	--------	(200) bp	100%
3. HVITR1	10	2	2 (350 , 250) bp	--------	--------	100%
4. Ebmac0715	9	1	---------	1 (150) bp	--------	---------
5. Bmag13	12	2	1 (210) bp	1 (180) bp	--------	50%
6. GMS1	13	2	1 (200) bp	1 (150) bp	--------	50%

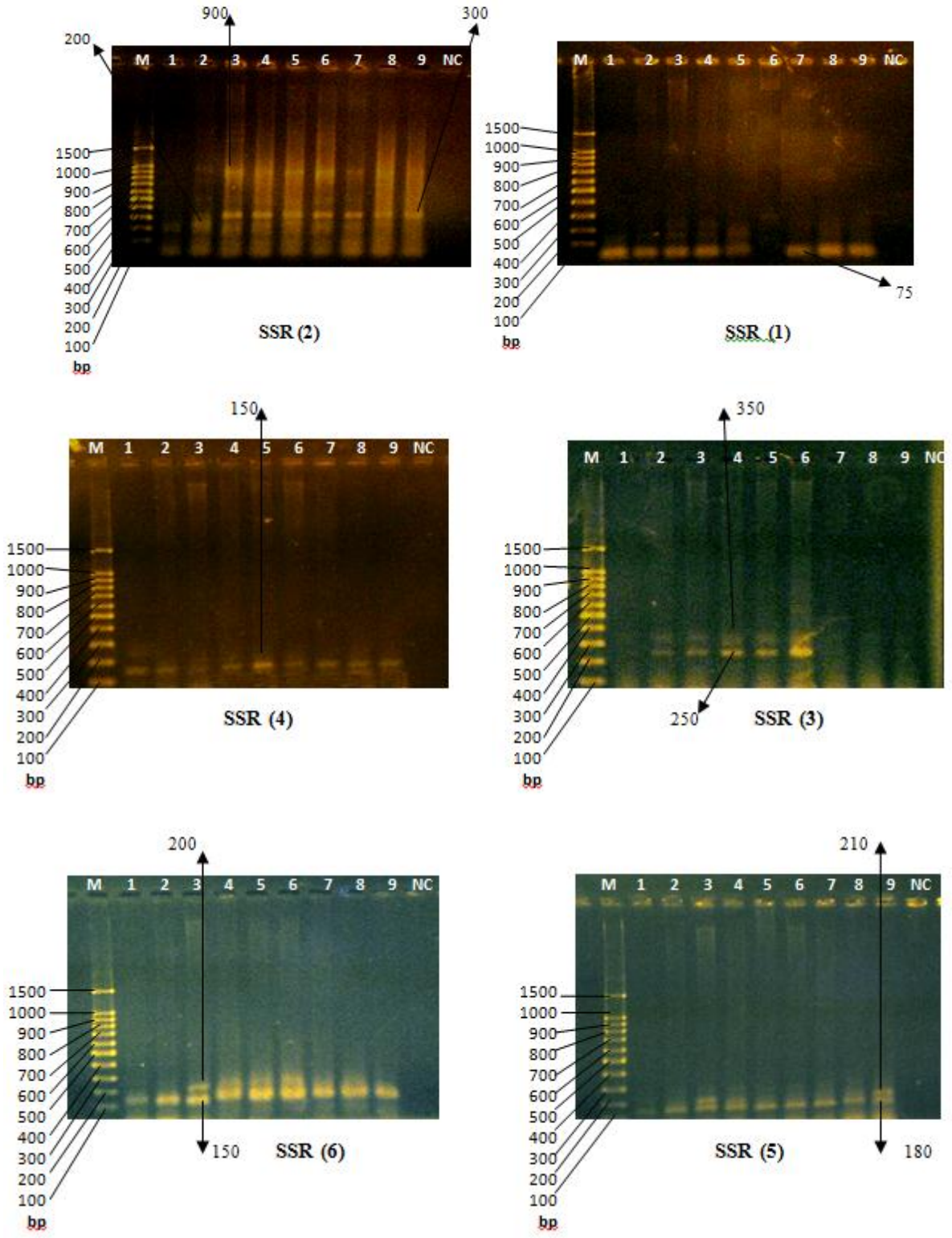

Figure 1-Agarose gel showing the bands of the SSR primers (1,2,3,4,5,6) in barley cultivars. (under a optimum conditions). Lanes 1 to 9 represent 1.(IPA265), 2.(IPA99), 3.(Tuwaitha), 4.(Hitra), 5.(Rayhan), 6.(Shuaa), 7.(Bawadi), 8.(Samir) and 9(Al_khair).

Table 4- Similarity matrix among the analyzed 9 barley genotypes 1.(IPA265),2.(IPA99), 3.(Tuwaitha), 4.(Hitra),5.(Rayhan), 6.(Shuaa), 7.(Bawadi), 8.(Samir) and 9.(Al_khair).

	1	2	3	4	5	6	7	8	9
1	0.00000								
2	0.69226	0.00000							
3	0.85701	0.69857	0.00000						
4	0.64981	0.97773	0.97398	0.00000					
5	0.10389	0.62219	0.65737	0.45770	0.00000				
6	0.99708	0.48562	0.56583	0.87972	0.34852	0.00000			
7	0.74012	0.69881	0.92887	0.67972	0.33367	0.58919	0.00000		
8	0.3687	0.4567	0.546700	0.24567	0.67890	0.4356	0.01098	0.00000	
9	0.456	0.87690	0.76548	0.87972	0.30852	0.30007	0.16700	0.19800	0.00000

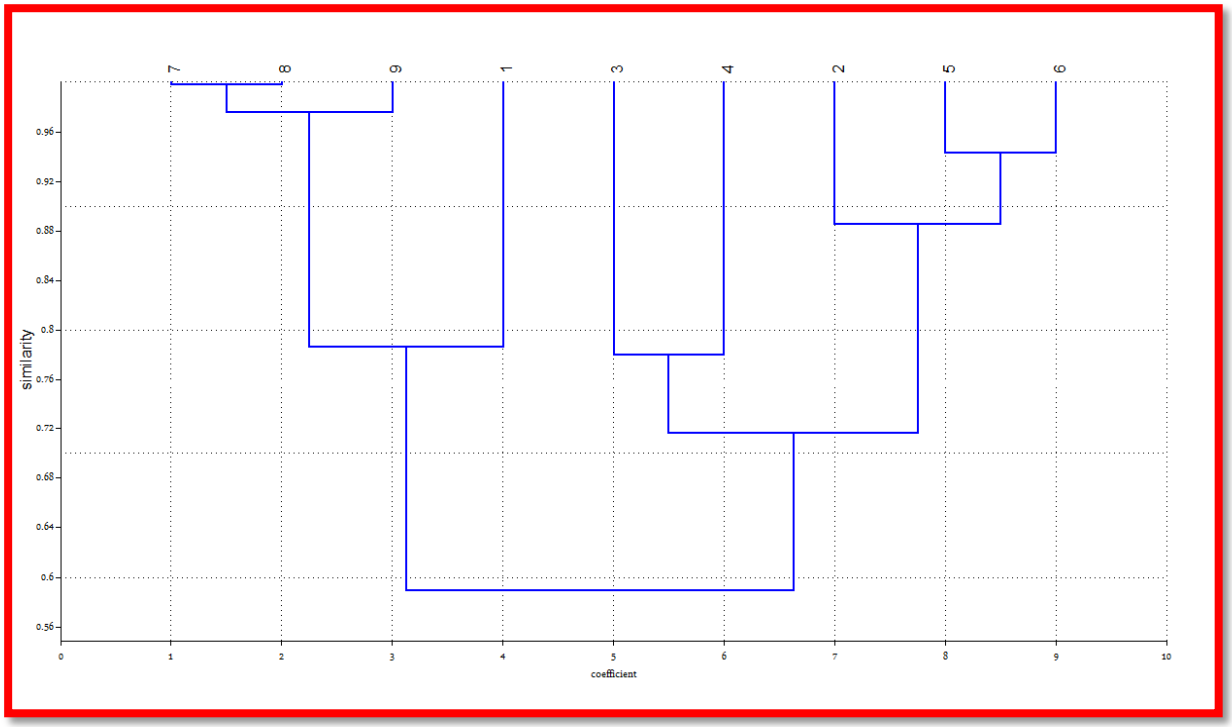

Figure 2-Dendogram constructed by the UPGMA method for clustering 9 barley genotypes included in this study. 1.(IPA265),2.(IPA99), 3.(Tuwaitha), 4.(Hitra),5.(Rayhan), 6.(Shuaa), 7.(Bawadi), 8.(Samir) and 9.(Al_khair).

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