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Use of SSR marker to assessment the Genetic Diversity of some *Hordeum vulgare* Genotypes

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Abstract: The present study was carried out to evaluate the genetic diversity in barley (*Hordeumvulgare* L.) using SSR markers. Thirty two simple sequence repeats (SSRs) markers were used to characterize fifty five barley genotypes. Five markers showed monomorphic band profiles, while 27 discriminatory markers, generated clear patterns with high polymorphism markers produced a total of 203 alleles. The number of alleles per marker ranged from 2 to 36 with a mean of 7.52 alleles per locus. Eleven unique alleles were observed in nine genotypes. The Polymorphic information content (PIC) values ranged from 0.28 to 0.96 with a mean value of 0.65, and the gene diversity ranged from 0.31 to 0.96 with a mean value of 0.70.Our results showed that the gene diversity values were very similar to the PIC values and the number of alleles detected by SSR marker correlated positively with the genetic diversity value (r= 0.67**). The dendrogram discriminated all the genotypes and clustered them separately in two major groups according to their pedigree, and showed high levels of polymorphism among the genotypes included in this study.
Key words:Hordeum, SSR markers, genetic diversity, genotype, unique alleles, PIC, GD.

Introduction

Cultivated barley (*Hordeumvulgare subsp. vulgare*) is one of the most important crop cereals in the tribe Poaceae that cultivated over the temperate regions[1]. Based on several reports it has been originated from Fertile Crescent in Near East or from Tibetan in the west China[2,3]. Many studies have demonstrated that Tibetan wild barley populations were clearly different from the Fertile Crescent wild barley in respect to their distribution, ecology; morphology, archaeology, cytogenetics and isozyme complement[4,5]. Barley is widespread in various temperate climate regions all over the world. Within the cereals, barley ranks fourth in terms of total world production. It is an important crop for direct human consumption and for animal feed, and considered as a model species for genetic analysis[6].

Molecular markers have been proved to be valuable tools in the characterization and evaluation of genetic diversity within and between species and populations. Many types of molecular markers have been used to characterize germplasm, with each method differing in principle, application, type and amount of polymorphism detected, cost and time requirement. SSR markers are tandem, 1 to 5, nucleotide repeats found in

eukaryotic genomes. SSRs are abundant, co dominant, and informative and their detection can be automated. This makes them excellent molecular marker system for many types of genetic analyses, including linkage mapping, germplasm surveys, and phylogenetic studies[7]. SSR are widely used in cereal research and their exploitation in breeding has increased the speed and efficiency of genotypes improvement [8].SSR markers are highly informative because their polymorphism rates are high. They have the advantage of being a PCR marker, i.e. they are fast and relatively cheap to analyze. In addition, SSR markers are distributed all over the genomes[9]. All these factors make them the marker of choice for genetic research.

Polymorphic information content (PIC) is a measure of a marker's informativeness. Different PIC values were obtained from marker studies using different genetic materials in barley.[10]reportedPIC values ranging from 0.01 to 0.92. [11]reported PIC values ranging from 0.8 to 0.88 with mean 0.84.[12]reported PIC values ranging from 0.29 to 0.89 with mean 0.64.[13] reported PIC values ranging from 0.068 to 0.78 with mean 0.45.

The number of alleles per locus is a significant indicator of genetic diversity [14]. And they have identified a total of 55 alleles at 10 microsatellite loci, and in the individual loci they have detected from 3 to 9 alleles with an average of 5.5 alleles per locus on his study on 30 barley genotypes.[15] recorded 67 alleles ranging from two to six alleles per locus with a mean value of 3.94 alleles per locus in his study on Thirty four Turkish barley genotypes using 17 SSR primers.[16] recorded 31 alleles ranging from 1 to 5 with an average of 2.81 alleles per locusin their study on 12 Tunisian barley genotypes.

Material and methods:

Plant material: A total of 55 barley genotypes were used for this study 10 of which are local varieties obtained from General Commission for Scientific Agricultural Research (GCSAR) while the rest 45 are new lines obtained from the international center for agricultural research in the dry areas (ICARDA**)** for this study**.** The accession code and pedigree data of genotypes used in this study are shown in Table 1.

Table 1: Accession code and pedigree data of genotypes used in this study.

Pedigree	Genotype	Accession code
Variety	Arabiabiad	Arabiabiad
Variety	Arabiaswad	Arabiaswad
Variety	Arta	Arta
Variety	$Forat_2$	$Forat_2$
Variety	$Forat_3$	$Forat_3$
Variety	$Forat_4$	$Forat_4$
Variety	$Forat_5$	$Forat_5$
Variety	$Forat_6$	$Forat_6$
Variety	$Forat_7$	$Forat_7$
Variety	$Forat_9$	$Forat_9$
Harmal	Harmal	Harmal
CM67/3/Apro//Sv02109/Mari/	ICB99-0703-9AP-0AP	$Line_{12}$
Hma-02//11012-2/CM67/3/Alanda/5/Rhn 03// Lignee527/NK1272/4/Lignee527/Chn01/3/Alanda	ICB99-0728-6AP-0AP	$Line_{13}$
Arta/KEEL	ICB00-0842-154AP-0AP	$Line_{14}$
Arta/KEEL	ICB00-0842-179AP-0AP	$Line_{15}$
Arta/KEEL	ICB00-0842-196AP-0AP	$Line_{17}$
Arta/KEEL	ICB00-0842-212AP-0AP	$Line_{18}$

Pedigree	Genotype	Accession code
Arta/KEEL	ICB00-0842-216AP-0AP	$Line_{19}$
Arta/KEEL	ICB00-0842-298AP-0AP	$Line_{21}$
Arta/KEEL	ICB00-0842-412AP-0AP	$Line_{22}$
Cerise/Lignee1479//Morac9-75/pmB/3/Moroc9-75/Hmi-02	ICB01-0090-3TR-0AP	$Line_{23}$
Moroc9-75/Hml-02/3/ER/Apm//Akrash	ICB01-0183-4AP-0AP	$Line_{24}$
As46/Rhn-05/3/Arbyan-01//M6/Robur-35-6-3	ICB01-0282-13TR-0AP	$Line_{26}$
Rhn//Bc/Coho/3/DeirAlla106//Api/EB89-8-2-15-4/4/Arbayan-01//M6/Robur-35-6-3	ICB01-0284-4TR-0AP	$Line_{27}$
Rhn//Bc/Coho/3/DeirAlla106//Api/EB89-8-2-15-4/4/Fassa-01	ICB01-0399-7TR-0AP	$Line_{28}$
QB813-2/3/As46//Giza121/pue	ICB01-0716-1TR-0AP	$Line_{29}$
Roho/4/Zanbaka/3/ER/Apm//Lignee131/5/Arta/3/Hml-02//Esp/1808-4L	ICB01-0509-17AP-0AP	$Line_{30}$
ICB-118540/3/Alanda//Lignee527/Arar	ICB01-1000-1TR-0AP	$Line_{31}$
Rt071/3/Alanda//Lignee527/Arar	ICB01-1001-13TR-0AP	$Line_{32}$
Alanda/HAMRA/5/U.Sask.1766/Api//Cel/3/Weeah/4/Arar	ICB01-1057-11TR-0AP	$Line_{33}$
Onslow/Arda	ICB92-0926-0AP-3AP-0AP-8TR-2TR-0AP	$Line_{34}$
Soufara-02/3/RM1508/Por//W12269/4/Hml-02/ArabiAbiad//ER/Apm	ICB92-0926-0AP-3AP-0AP-8TR-2TR-0AP	$Line_{36}$
Soufara-02/3/RM1508/Por//W12269/4/Hml-02/ArabiAbiad//ER/Apm	ICB92-0926-0AP-7AP-0AP-5TR-5TR-0AP	$Line_{37}$
Soufara-02/3/RM1508/Por//W12269/4/Hml-02/ArabiAbiad//ER/Apm	ICB92-0926-0AP-7AP-0AP-16TR-1TR-0AP	$Line_{39}$
Soufara-02/3/RM1508/Por//W12269/4/Hml-02/ArabiAbiad//ER/Apm	ICB92-0926-0AP-7AP-0AP-17TR- 9TR-0AP	$Line_{40}$
Soufara-02/3/RM1508/Por//W12269/4/Hml-02/ArabiAbiad//ER/Apm	ICB92-0926-0AP-9AP-0AP-7TR-5TR-0AP	$Line_{41}$
Soufara-02/3/RM1508/Por//W12269/4/Hml-02/ArabiAbiad//ER/Apm	ICB92-0926-0AP-26AP-0AP-5TR-5TR-0AP	$Line_{42}$
Soufara-02/3/RM1508/Por//W12269/4/Hml-02/ArabiAbiad//ER/Apm	ICB92-0922-0AP-38AP-0AP-16TR-1TR-0AP	$Line_{43}$
Soufara-02/3/RM1508/Por//W12269/4/Hml-02/ArabiAbiad//ER/Apm	ICB92-0922-0AP-38AP-0AP-7TR-2TR-0AP	$Line_{44}$
Lignee527/NK1272/3/Nacha2//Lignee640/Hma-01	ICB95-0281-0AP-6AP-0AP-7TR-4TR-0AP	$Line_{46}$
Lignee527/NK1272/3/Nacha2//Lignee640/Hma-01	ICB95-0281-0AP-6AP-0AP-7TR-6TR-0AP	$Line_{47}$
Lignee527/NK1272/3/Nacha2//Lignee640/Hma-01	ICB95-0281-0AP-6AP-0AP-9TR-1TR-0AP	$Line_{48}$
Lignee527/NK1272/Nacha2//Lignee640/Hma-01	ICB95-0281-0AP-6AP-0AP-17TR-7TR-0AP	$Line_{50}$
Rihane-03/3/As46/Aths*2//Aths/Lignee686	ICB95-0602-0AP-1AP-0AP-9TR-3TR-0AP	$Line_{52}$
Rihane-03/3/As46/Aths*2//Aths/Lignee686	ICB95-0602-0AP-5AP-0AP-9TR-3TR-0AP	$Line_{53}$
Rihane-03/3/As46/Aths*2//Aths/Lignee686	ICB95-0602-0AP-5AP-0AP-14TR-7TR-0AP	$Line_{54}$
Rihane-03/3/As46/Aths*2//Aths/Lignee686	ICB95-0602-0AP-5AP-0AP-13TR-1TR-0AP	$Line_{55}$
Rihane-03/3/As46/Aths*2//Aths/Lignee686	ICB95-0602-0AP-10AP-0AP-5TR-1TR-0AP	$Line_{58}$
Rihane-03/3/As46/Aths*2//Aths/Lignee686	ICB95-0602-0AP-10AP-0AP-11TR-6TR-0AP	$Line_{59}$
Rihane-03/3/As46/Aths*2//Aths/Lignee686	ICB95-0602-0AP-10AP-0AP-15TR-1TR-0AP	$Line_{60}$

Pedigree	Genotype	Accession code
Rihane-03/3/As46/Aths*2//Aths/Lignee686	ICB95-0602-0AP-10AP-0AP-15TR-4TR-0AP	$Line_{61}$
Rihane-03/3/As46/Aths*2//Aths/Lignee686	ICB95-0602-0AP-10AP-0AP-15TR-9TR-0AP	$Line_{63}$
Rihane-03/3/As46/Aths*2//Aths/Lignee686	ICB95-0602-0AP-16AP-0AP-20TR-4TR-0AP	$Line_{64}$
Rihane-03///Lignee527/Aths	ICB95-0611-0AP-2AP-0AP-4TR-3TR-0AP	$Line_{65}$
Rihane-03///Lignee527/Aths	ICB95-0611-0AP-2AP-0AP-4TR-7TR-0AP	$Line_{66}$

DNA extraction and PCR amplification:

Seeds were grown in a growth chamber at 27°C under a 12/12 h day/night photoperiod. Total DNA was extracted from young leaves ofcollected from 10 to 15-day old seedlings using CTAB method of[17]. The extraction buffer (pH 8) was composed of10 mM EDTA, 1.4M NaCl, 50mM Tris-HCl (pH8),3% (w/v) CTAB ,1% PVP (w/v), 1% 2-mercaptoethanol.DNA quality was determined using 1% agarose gel and then quantified by spectrophotometer. DNA concentration was adjusted to 50 ng μL^{-1} to be used in the SSR reactions.

Genomic regions flanking SSR sequences were amplified using specific primers via polymerase chain reaction (PCR). Thirty two SSR markers were selected on the basis of their chromosomal locations[7,18-24]. The markers names, primer sequences and chromosomal locations are listed in Table 2.

Table 2: Marker names, primer sequences and chromosomal locations of the thirty two SSR markers used for genetic diversity analysis of barley genotypes.

SSR Marker	Primer sequence	Chromosome Location	Refferance
Bmag0378	CTTTTGTTTCCGTAGCATCTA ATCCAACTATAGTAGCAAAGCC	2H	(20)
Bmag0749	CGGATTCTTGAGTAGTCTCTG GATCTGTTTTTGTAGAACATGC	2H	(20)
GMS0001	CTGACCCTTTGCTTAACATGC TCAGCGTGACAAACAATAAAGG	5H-7H	(22)
HVM54	AACCCAGTAACACCGTCCTG AGTTCCCTGACCCGATGTC	2H	(7)
HvLOX	CAGCATATCCATCTGATCTG CACCCTTATTTATTGCCTTAA	5H	(23)
scssr05939	TCATTGGGCTCTTCTACGG GCAAACCGGACTAAGTATGC	5H	(18)
scssr02306	TGCCTTGTTTATGTAATATCTTGTG GGCGTAAATAAGAGTGTCTTCAG	5H	(18)
scssr03907	CTCCCATCACACCATCTGTC GACATGGTTCCCTTCTTCTTC	5H	(18)
scssr04056	CCCATGAAGCCTCTTTACG GGAACGGAGGGAGTATTAAGC	7H	(18)
scssr07759	GCAACTCCTCATCATCTCAGG CAACAGCCAGAAGGTCTACG	2H	(18)
scssr07970	TGCATTGGGAGTGCTAGG TGCAAGAAGCCAAGAATACC	7H	(18)
scssr10148	AAGCAGCAAAGCAAAGTACC TCATCAGCATCTGATCATCC	5H	(18)
scssr15864	GCATAAACGGGTGTAAGAGC CATCCAGTTCAGAGGATAGAGC	7H	(18)
HvXan	CAGCCACCTCCATAGTACTT CTGCTCTAGGCTCGTGTT	2H	(20)
GMS003	TTTCAGCATCACACGAAAGC TTGCATGCATGCATACCC	2H	(22)
EBmac854	GTCGATATAACGCACTTTCA ATTCCCTCTAACACACCAAC	5H	(20)

SSR Marker	Primer sequence	Chromosome Location	Refferance
Bmag0125	AATTAGCGAGAACAAAATCAC AGATAACGATGCACCACC	2H	(20)
EBmatc0039	TAGTCTCTTCATTTATACCATCACC CATGCTGATCCCCCTTCT	2H	(20)
GBM1309	ATAACTCTTCGTGCCGAACC ACGTAGACCTCCACCACCTG	2H	(19)
Bmag0223	TTAGTCACCCTCAACGGT CCCCTAACTGCTGTGATG	5H	(20)
GBM1231	CTGCAGACCCTGAAATTGGT GGATCCCTTTTGACGTGCTA	5H	(19)
GBM1295	GAGCATCCCGTTTGCATAGT GTTCGACGGCAGTGATGAGT	5H	(18)
GBM5008	CAGCATCCATCAGCAATGAA ATGTTTGGCTTCTTCGTCCA	5H	(18)
GMS061	CACCTGTTCCGTCCCGTC AACCTCTTTTTTATCCCTCGC	5H	(22)
EBmatc0003	AATTTTGCAAAGCTGGAGG CATTATGGTGGGGTTCATGT	5H-7H	(19)
GBM1463	GCTTCTGACCGAGAACGAAC CATAGCCAGAGGCCATGAAT	5H	(19)
HVCMA	GCCTCGGTTTGGACATATAAAG GTAAAGCAAATGTTGAGCAACG	7H	(23)
Bmag0385	CTCCACAGAGTCAGAGTTAGA CTGACATTAGCTGACTCTCTATC	7H	(20)
Bmac0064	CTGCAGGTTTCAGGAAGG AGATGCCCGCAAAGAGTT	7H	(20)
Bmag0482b	TATATGTCGGGAGAGATCAAG ATAGTTTAGCCCTCCACTAGC	7H	(20)
GBM1362	CGCCTCCCTCCTTCCTGTA CCCTTGTTGTCCTCTTGCAT	7H	(18)
HVM11b	CCGGTCGGTGCAGAAGAG AAATGAAAGCTAAATGGCGATAT	7H	(24)

PCR reactions was performed in a total volume of 25 µl containing 200-250 ng DNA, 12.5 µl of GoTaq Green Master Mix (Promega) and 0.25 µM of each primer. The amplifications were carried out using APOLLO Thermocycler (USA). PCR amplification procedure of SSR markers was performed by an initial denaturation step of 5 min at 94 °C followed by 30 cycles of three steps: denaturation for 1 min at 94°C, annealing for 1 min at 58 or 60 °C, extension for 1 min at 72 °C with a final extension for 10 min at 72 °C. Amplified PCR products were separated using 8% non-denaturing polyacrylamide gel electrophoresis in 1x TBE buffer (107.8 g Tris, 7.44 g EDTA and 55 g boric acid in 1 liter of distilled H_2O) containing 31.5 g urea, 50 µl of TEMED and 400 µl ammonium persulfate, and then the gels were stained by ethidium bromide and visualized under UV light. A 50bp and 100bp DNA Ladder was used as a molecular size standard.

Analysis of SSR data:

Molecular weights for microsatellite products, in base pairs, were estimated using TotalLab software (Ultra· Lum Inc., Claremont, Calif.). The summary statistics including the number of alleles per locus, genetic diversity and polymorphism information content (PIC) values were determined usingPOWER MARKER version 3.25 Software [25](http://statgen.ncsu.edu/powermarker). The allele frequency data was used to export the data in binary format (allele presence = "1" and allele absence = "0"). The 0/1 matrix was used to calculate genetic distance using Nei coefficient [26].

And the resultant dissimilarity matrix was employed to construct dendrogram using Unweighted Pair Group Method of Arithmetic Means (UPGMA) as implemented in POWER MARKER with the tree viewed using TREEVIEW Version 0.5.0 (By Roderic DM, Page, Build 7600).

Table 3: SSR marker, allele number, polymorphic information content (PIC) and genetic diversity (GD) for the thirty two SSR markers used for genetic diversity analysis of barley genotypes.

SSR marker	Allele number	PIC	GD
Bmag0378	5	0.71	0.75
Bmag0749	6	0.46	0.51
GMS0001	9	0.77	0.79
HVM54	6	0.67	0.71
scssr05939	3	0.59	0.67
scssr02306	5	0.67	0.72
scssr03907	13	0.83	0.84
scssr04056	5	0.61	0.67
scssr07759	11	0.62	0.67
scssr07970	36	0.96	0.96
scssr08447	5	0.58	0.62
scssr10148	13	0.86	0.88
scssr15864	3	0.43	0.52
GMS003	5	0.57	0.64
EBmac854	4	0.54	0.59
Bmag0125	13	0.86	0.87
GBM1309	7	0.71	0.75
Bmag0223	8	0.60	0.65
GBM1231	4	0.64	0.70
GBM1295	8	0.74	0.78
GBM5008	6	0.73	0.76
GMS061	5	0.69	0.73
EBmatc0003	2	0.37	0.50
GBM1463	4	0.54	0.60
HVCMA	7	0.67	0.71
Bmac0064	2	0.36	0.47
GBM1362	5	0.56	0.59
HVM11b	3	0.28	0.31
Sum	7.52	0.65	0.70
Mean	203		

Results and Discussion:

The results of molecular assay in studying 55 barley genotypes using 32 SSR markers are to Determining the efficiency of using markers and the genetic relationships among the studying genotypes.

All the used SSR marker produced amplification product, but five of them showed monomorphic band profiles (HVM54, HvXan, EBmatc0039, Bmag0385 and Bmag0482b). while The 27 discriminatory markers, generated clear patterns with high polymorphism, so they used to evaluate the genetic diversity of the 55 barley genotypes. And they revealed a total of 203polymorphic alleles, The number of alleles varied from 2 (EBmatc0003, Bmac0064) to 36 (scssr07970) alleles per locus with a mean of 7.52 alleles per locus.[10]Recorded 138 alleles and the number of alleles ranged from 2 to 16 alleles with an average value of 8.11 alleles per locus, in collection of 32 barley genotypes from Iran,[27]recorded 85 alleles. The alleles per locus ranged from 1 to 5 alleles with an average of 2.4 alleles per locus detected from 40 China barley accessions.[28]recorded 43 alleles and the number of alleles per primer ranged from 2 to 4 with an average of 2.87 alleles per SSR primer in collection of 14 Tunisian barley genotypes.

Five unique alleles were observed, The marker GBM1309provides a unique marker for the variety ArabiAbiad (142bp). The marker GMS0001provides a unique marker for the $Line_{33}$(115 bp). The marker scssr03907 provides a unique marker for the $Line_{33}$ (140 bp). The marker scssr04056 provides a unique marker for the $Line_{34}$ (111 bp). the marker scssr04056 provides a unique marker for the $Line_{22}$ (104 bp).The unique alleles are important because they may be diagnostic of a particular inbred line or for regions of the genome specific to a particular type of genotype [29]. Moreover, the occurrence of the unique allele is an indication of the diversity present in a germplasm and its potential as a reservoir of novel alleles for crop improvement [30].

The polymorphism information content (PIC) is a measure of allelic variability and evenness at a particular locus. In this study the PIC values ranged from 0.28 (HVM11b) to 0.96 (scssr07970) with a mean value of 0.65 (Table 3). In previous studies, different PIC values were obtained using different genetic materials in barley.[11] reported PIC values ranging from 0.8 to 0.88 with mean 0.84.[12]reported PIC values ranging from 0.29 to 0.89 with mean 0.64.[16] reported that PIC values ranged from 0.28 to 0.60 with an average value of 0.50. [13] reported PIC values ranging from 0.068 to 0.78 with mean 0.45.

The gene diversity (GD) ranged from 0.31 (HVM11b) to 0.96 (scssr07970) with an average value of 0.70 (Table 3). Our results showed that the genetic diversity values were very similar to the PIC values, and that agree with the results of[29]. and [31]who reported that PIC is synonymous with the term "gene diversity".

The number of alleles detected by SSR marker correlated positively with the gene diversity value (r= 0.67**), Similar results confirming the positive correlation between the number of alleles detected by SSR marker and the genetic diversity value were also recorded by many researches such as[12], in their study of 30 genotypes of Iranian barley. and[22], in their study of 163 genotypes of barley.

The genetic dissimilarity matrix was established by Nei coefficient[26] and it show the genetic dissimilarity between the studying genotypes, the result showed the highest genetic similarity was between $line_{65}$ and $line_{64}$ (0.09), followed between $line_{43}$ and $line_{42}$ (0.12), and the lowest genetic similarity was between $line_{60}$ and $line_{27}$ (0.79).

A dendrogram showing the genetic relationships between genotypes was constructed by the UPGMA method to express the results of cluster analysis based on data obtained by SSR amplification products. The dendrogram discriminated all the genotypes and clustered them separately in two major groups (Fig. 1).The first group clustered into two subgroups, the first subgroups contains ($line_{61}$, $line_{66}$, $line_{63}$, $line_{64}$, $line_{65}$, $line_{27}$, $line_{22}$, and $line_{23}$), The second subgroups containes all the variaties and ($line_{26}$, $line_{12}$, $line_{13}$, $line_{21}$, $line_{14}$, $line_{15}$, $line_{24}$, $line_{17}$, $line_{19}$, $line_{18}$,)

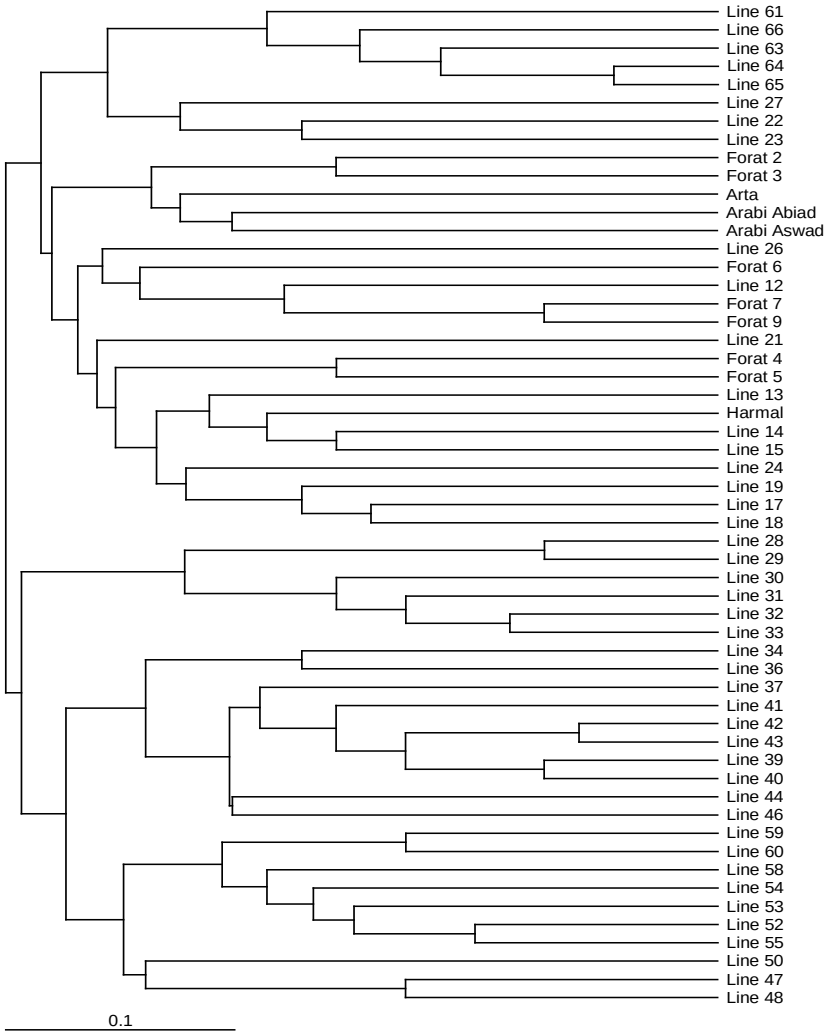
The second group clustered into two subgroups, the first subgroups contains ($line_{28}$, $line_{29}$, $line_{30}$, $line_{31}$, $line_{32}$, and $line_{33}$), The second subgroups containes the remaining lines (Fig. 1).

Our results showed that the studied barley genotypes were clustered according to their pedigree, while [32]reported in their study that barley genotypes clustered according to their their geographical locationin the Kingdom of Saudi Arabia. but the Tunisian barley genotypes clustered according to their spike types[16]. And the barley genotypes clustered according to salt tolerance[33,34].

It was reported that SSRs are highly variable and therefore able to distinguish closely genetically related plant genotypes [35]. Based on these results, high level of genetic diversity was observed in studied barley genotypes. The smallest genetic distance was observed between $line_{65}$ and $line_{64}$ (0.09), that indicate their genetical kinship. However, the biggest genetic distance was observed between$line_{60}$ and $line_{27}$ (0.79) indicates their genetical differences that might be due to mutation and/or selection[11].

In general, the results showed high levels of polymorphism among the genotypes included in this study. Which refers to the high ability of SSR markers to reveal most of the information in a single locus and can be used for genetic analysis at molecular level determination of genetic similarity and clustering barley cultivars. However, the relationship observed using molecular markers may provide information on the history and biology of genotypes, but it does not necessarily reflect what may be observed with respect to agronomic traits[36]. Further morphological and physiological investigations as well as genetic approaches will help to find the exact nature of these genetic variations.

Figure 1: The Nei`s genetic distance (Nei 1973) based dendrogram generated using SSR data and UPGMA clustering method, showing relationships among 40 barley genotypes.



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