Enhancement of some barley (*Hordeum vulgare L.*) resistance for nematode (*Heterdra avanae*) using DNA fingerprinting analysis

Shimaa E. Rashad[a], Ibthal S. Eldemerdash[b], Hamdy M. Hamed[b], Magda A.M. El-Enany[b], Samy A.A. Heiba[b]

[a]Microbial Genetics Department, Biotechnology Research Institute, National Research Centre, [b]Genetics and Cytology, Department, Biotechnology Research Institute, National Research Center, Giza, Egypt

Correspondence to Shimaa E. Rashad, Microbial Genetics Department, Biotechnology Research Institute, National Research Centre, Giza, Egypt. Tel: 01095324626/01274047773; E-mail: Se.rashad@nrc.sci.eg, Shimaar30@yahoo,com

Received: 18 February 2023
Revised: 28 April 2023
Accepted: 10 May 2023
Published: 20 November 2023

Egyptian Pharmaceutical Journal 2023, 22:573–581

Background
Heterodera avenae, the cereal cyst nematode or European cyst nematode, is a plant pathogen and an obligate parasite of cereal crops including barley, oats, wheat, and rye. Cereal crops infected with this nematode are more susceptible to infection by fungal diseases such as Rhizoctonia root rot.

Objective
The main objective of the present study was to map *Heterodera avenae* resistance and to compare seven different species. This study also aimed to increase the efficiency and precision of standard procedures for testing the resistance of barley toward root-lesion nematodes.

Materials and methods
The genetic variability analysis of seven barley genotypes, of Egypt was achieved using 7 Inter simple sequence repeat (ISSR) primers. Phylogenetic relationships of seven accessions of (*Hordeum vulgare L.*) collected from different region of Egypt were assessed. The soil was sterilized in an autoclave of each pot was planted in the comparison experiment, as well as in the treatment experiment with nematode *Heterdra avanae* infected soil brought from an infected field, and this soil was added to the treated pots. Also (ISSR) molecular marker technique was used for DNA fingerprinting and assessing genetic diversity and phylogenetic relationships in barley germplasm.

Results and conclusion
A significant correlation was observed between the Jaccard's dissimilarity matrices based on ISSR markers, as revealed by Mantel test using the Pearson correlation coefficient ($r=0.69$; $P<0.05$). The results showed that ISSR primers produced 37 bands their size ranged between 100-2000 bp with (87.5%). polymorphism percentage. Polymorphic information content PIC was 0.74 for ISSR. Unweighted Pair Group Method with Arithmetic (UPGMA), Dendrogram was divided into two clusters by morphological traits and ISSR analysis. Genetic similarity matrix was examined with Jaccard's coefficient, maximum similarity was found between Giza126 and Giza127 (99%) with morphological analysis both and lowest similarity between Giza123 and Giza126 (6%) with ISSR analysis. Determination of genetic diversity between barley is of major importance for characterization of barley germplasm, breeding programs and conservation purposes. Morphological traits and ISSR analysis are effective tools for detecting genetic variations. The results showed that *H. vulgare* have high ratio of variation. This study may be considered as reference study for further studies on H. vulgare and may contribute to species concept and breeding programs.

Keywords:
barley (*Hordeum vulgare* L.), DNA fingerprinting, ISSR, nematode (*Heterdra avanae*)

Egypt Pharmaceut J 22:573–581
© 2023 Egyptian Pharmaceutical Journal
1687-4315

Introduction

One of the first crops to be grown was barley (*Hordeum vulgare L.*). It is still one of the most widely grown cereal crops in the globe. Around the globe, there are many different areas where barley is grown. Compared with other cereals, it is more drought and salinity-tolerant due to its brief growing season Newton and colleagues [1].

Due to its simplified genomic makeup, the genus *Hordeum* is regarded as a premier plant for experimental genetics. Barley is regarded as a suitable genetic model for Triticeae and more genetically complex cereal crops like hexaploidy bread wheat due to its true diploid nature and genome's similarity to that of other grain cereals Kleinhofs and colleagues [2].

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

 DOI: 10.4103/epj.epj_36_23

The determination of the impact of various media compositions on the embryogenic reaction and regrowth of various barley genotypes. The three barley genotypes El-kasr, G126, and G130 were successfully detected for the presence of somaclonal variation using biochemical and molecular genetic studies of protein, isozymes, and Random Amplified Polymorphic DNA (RAPD) Rashad and colleagues [3]. The success of RAPD markers in identifying relationships between these genotypes and demonstrating the connection between molecular level yield characteristics. To ensure that barley can be produced sustainably and used to its full potential by barley breeders working with gene banks, it is essential that its diversity is identified. In-depth phenotyping and genotyping of the barley collections employing cutting-edge molecular, biochemical, and physiological techniques can accomplish this Merwad and colleagues [4]. Inter simple sequence repeat (ISSR) analysis and morphological characteristics are useful methods for identifying genetic variations Shata and colleagues [5].

As an early check on the effectiveness of transformation, the transient expression of the alpha-fetoprotein (AFP) gene was examined. With a variety of parameters and the barley genotypes El-Kasr, Giza126, and Giza130, transformation tests were conducted. The type of explants and mature embryos based on the Beta-glucuronidase gene (GUS) transient expression findings were among the transformation parameters that were evaluated. The Alpha-Fetoprotein (AFP) and Beta-glucuronidase (GUS) gene' particular primers and probes were used in PCR analysis Rashad and colleagues [6].

In comparison to simple sequence repeat (SSR) marker analysis, ISSR markers are more effective at detecting genetic diversity among the examined cultivars of Alfalfa. Compared with the SSR marker, the ISSR marker is more selective and yields more useful data Heiba and colleagues [7].

Barley genotypes are a valuable resource as possible gene donors for the creation and upkeep of contemporary crop varieties due to their genetic diversity. Therefore, choices relating to the conservation and use of the germplasm collection in genetic improvement are based on information and understanding of this genetic diversity. Plant breeders may find it easier to assess genetic variation directly between relatives without the influence of environmental variables with the aid of DNA-based molecular markers. Additionally, a potentially infinite number of polymorphic marker loci can be evaluated using DNA techniques Jones and colleagues [8].

Plant genetic variety can be estimated using ISSR markers. motif (di-, tri-, tetra-, or penta nucleotides) and primers anchored at the 3' or 5' end by two to four arbitrary, frequently degenerate nucleotides, ISSR analysis involves the polymerase chain reaction (PCR) amplification of regions between adjacent regions Zietkiewicz and colleagues [9].

This research used ISSR markers to compare the biological processes of seven different species and map the resistance of *Heterodera avenae*.

Materials and methods

Plant materials

In this study, seven barley genotypes (*Hordeum vulgare L.*) with various genetic origins were used; Table lists their code numbers, names, and pedigrees (Table 1). Field Crops Research Center, ARC, and Giza, Egypt all contributed barley grains for this study.

Using ISSR-PCR technique to determine phylogenetic tree for seven varieties of barley in steps as follow:

The experimental cultivars were planted in pots

Each pot contained 50 barley seeds from one of the seven different types of barley: Giza123, Giza124, Giza126, Giza127, Giza128, Giza130, and Giza 2000. An autoclave was used to sterilize the earth. In both the comparison trial and the treatment experiment, 3 duplicates of each pot were planted. The treated pots also received soil that had been

Table 1 Code number, names, abbreviations and pedigrees of the seven barley genotypes

Code number	Genotype name	Abbreviations	Pedigrees	Origin
1	Giza 123	(G 123)	*Giza 117/FAO 86*	Egypt
2	Giza 124	(G 124)	*Giza 117/ Bahteem 52// Giza 118/ FAO 86*	Egypt
3	Giza 126	(G 126)	*Baled Bahteem/SD 729-POR12762-BC*	Egypt
4	Giza 127	(G 127)	*W12291/B0gs/Hamal-02*	Egypt
5	Giza 128	(G 128)	*W12291/4/11012-2170-22425/3/Apam/B65/A16*	Egypt
6	Giza 130	(G 130)	*Comp.cross 229//Bco.Mr.DZ02391/3/Deir all 106*	Egypt
7	Giza 2000	(G 2000)	*Giza 117/ Bahteem 52// Giza 118/ FAO 86 /3/ Giza 121*	Egypt

contaminated with the nematode *Heterdra avanae* and was taken from an infected field in the Abusweri zone of the Ismailia Governorate. In order to check for nematode infestation, the area of the greenhouse side at the National Research Centre Dokki in the Giza Governorate of Egypt was irrigated, followed up on for two months, and samples were collected from the fresh leaves of plants in the two trials with barley.

DNA extraction

Genomic total for 9 alleles following a biokits procedure, DNA was extracted from seedlings that were 6 days old and weighed about 1 g fresh weight. The DNA quality was assessed by staining the DNA with ethidium bromide (0.1 g/ml) following electrophoresis in a 1% agarose gel at voltage of 100 volt for 1 h in 1xTBE buffer.

ISSR analysis

In the reaction combination, which had a total volume of 25 l, there were the following components: 12.5 µl Master Mixes, 3 µl of each primer, and 100 ng template DNA. Using a Thermolcycler (gen-Amp PCR system 9700), DNA amplification was performed. Reactions were subjected to the following PCR programmed: pre-denature at 94°C for 1 min, followed by 35 cycles of primer annealing at Tm (°C) for 1 min, extension at 72°C for 1 min, and final extension at 72°C for 5 min Perez de la Torre and colleagues [10]. Table 2 contains a summary of the ISSRs primers used in PCR reactions, along with their names, sequences, and annealing temperatures.

The PCR

(1) Ethidium bromide was used to stain the PCR products after loading them onto a 2% agarose gel. The run was conducted with an 80 volt constant voltage, and amplified products were identified using a UVP Gel documentation device under ultraviolet light. Only after witnessing and contrasting the bands in three different amplifications for each primer and each cultivar were, they deemed reproducible and scorable.
(2) Gel electrophoresis The ISSR amplification products will be separated on 1.5% agarose gels in 1X TAE solution using DNA ladders (1Kb for ISSR analysis). According to Hou and colleagues [11], the polymers will then be visible after being stained with ethidium bromide. The gel documentation method, Biometra-Bio Doc. Analyze, will be used to photograph and document the PCR products.
(3) Data analysis For data processing, only distinct, unambiguous, and reproducible bands will be taken into account. Every band will be treated as a separate location. To identify positive and negative markers, data will be scored as (1) for existence and (0) for absence for each cultivar in accordance with Khatab and colleagues [12]. The unweighted pair group method with an arithmetical average will be used to create a Dendrogram using the similarity coefficients produced by the SPSS programme version 10 Federer and Raghavarao [13]. Unweighted Pair Group Method with Arithmetic (UPGMA).

Statistical analyses

All statistics were presented as means with standard errors. The *T*-test was used to gauge the statistical importance. When *P* less than or equal to 0.05, values were deemed statistically significant.

ISSR bands were given a present (1) or absent number (0). The PAST (free programs on the web) software was then used to input the scores into a binary matrix Hampl and colleagues [14]. The Nei and Li/Dice similarity index was used to determine the quantitative morphological data's similarity to the ISSR molecular marker, and the UPGMA (unweighted pair group method using arithmetic averages) clustering algorithm was used to evaluate the similarity estimates Nei and Li, Sokal [15,16].

Table 2 ISSR markers, sequence of primer, total number (TB) and number of polymorphic bands (PB), percentage of polymorphism (PP)

Name primer	Primer sequence 5′→ 3′	Tm (°C)	TB	PB	PP
A1	5′- AGAGAGAGAGAGAGAGC-3′	53	4	3	75
Am1	5′-AGAGAGAGAGAGAGAGT-3′	53	6	5	83.3
Am2	5′-TCTCTCTCTCTCTCTCC-3′	53	8	7	87.5
UBC888	5′-BDBCACACACACACACA-3′	55	5	4	80
UBC890	5′-VHVGTGTGTGTGTGTGT-3′	56	4	3	75
Am3	5′-GAGAGAGAGAGAGAGAT-3′	45	5	4	80
Am9	5′-ACACACACACACACACYA-3′	53	5	3	60
Average of value			5.29	4.14	77.26

The dendrogram used to represent the resulting groups. The formula PIC = 1 Pi 2 was used to determine the polymorphic information content (PIC) of each marker, where Pi is the band frequency of the gene Smith and colleagues [17].

Results and discussion

Morphological traits

Agro-morphological variation

Six morphological traits from 7 barley accessions were documented in Table to examine the biodiversity among them. According to our findings, all quantitative morphological characteristics are highly polymorphic. The plant height value for Egyptian barley treatments Giza 2000 was 71.87 cm, while accession Giza 126 got the lowest value at 54.17 cm. The Flag leaf area was seen in accession Giza 2000 (40.78 centimeters), while accession Giza 128 had the shortest length (24.76 cm). The quantity of leaves was also 17.78 in Giza 2000, while the accession Giza 127 record for the lowest value was 8.43 cm. In accession Giza 124, the number of tillers was (4.6 cm), while accession Giza 128 recorded the lowest figure (2.65 cm).

The numbers of spikes measuring 14.05 centimeters were most numerous in accession Giza 124 and least numerous in accession Giza 130. (10.09 cm). According to the data, Grain Yield in Accession Giza 130 had the greatest value (1.99 cm), while Accession Giza 123 had the lowest values (0.96 cm). Using quantitative morphological characteristics and molecular marker data, which are useful tools in varietal development, genetic diversity between barley genotypes gathered from Egypt was assessed Singh and colleagues [18]. Nearly all quantitative morphological traits examined showed significant variation between barley genotypes according to the characterization, as shown in (Table 3).

Numerous traits, including plant height, flag leaf area, number of leaves, number of tillers, spike length, and grain yield, show substantial differences according to

Table 3 Morphological traits of 7 barley accessions

Genotype and treatment	Plant height (cm)	Flag leaf area cm^3	Number of leaves	Number of tillers	Spike length (cm)	Grain Yield
Giza123						
Cont.1	75.34^a	30.13^b	12.44c	3.12^c	11.12^c	1.66
Treat1	66.43^a	30.55^b	12.22^c	3.18^c	10.98^c	0.96
Treat 2.	65.4^a	28.76^b	11.18^c	3.55^c	9.43^c	0.8
Treat3	62.32^a	26.78^b	10.22^c	3.76^c	9.25^c	0.77
Average	64.72^a	28.7^b	11.21^c	3.5^c	9.89^c	0.84
Mean	67.3725	29.055	11.515	3.4025	10.195	1.0475
Std. Dev.	5.5913	1.6984	1.0234	0.3049	0.9916	0.4168
Std. Error	2.7956	0.8492	0.5117	0.1524	0.4958	0.2084
LSD	At 5%			2.29**		
Giza 124						
Cont.2	66.76^a	37.23^b	15.87^c	4.74^c	14.54^c	1.1
Treat1	65.7^a	35.43^b	15.67^c	4.6^c	14.05^c	1.09
Treat2	63.77^a	33.45^b	12.89^c	3.98^c	13.6^c	0.99
Treat3	61.06^a	31.56^b	11.56^c	3.45^c	12.76^c	0.95
Average	63.51^a	33.48^b	13.37^c	4.01^c	13.47^c	1.01
Mean	64.3225	34.4175	13.9975	4.1925	13.7375	1.0325
Std. Dev.	2.5025	2.452	2.1191	0.5951	0.7563	0.0741
Std. Error	1.2513	1.226	1.0595	0.2975	0.3782	0.0371
LSD	At 5%			2**		
Giza 126						
Cont.3	55.12^{ab}	33.54^b	9.55^c	3.45^c	11.56^c	$.1.56^c$
Treat1	54.17^{ab}	32.87^b	9.44^c	3.23^c	11.32^c	1.23^c
Treat2	53.14^{ab}	31.76^b	9.56^c	3.12^c	10.89^c	1.21^c
Treat3	52.66^{ab}	30.55^b	9.33^c	3.06^c	10.21^c	1.09^c
Average	53.32^{ab}	31.72^b	9.44^c	3.14^c	10.81^c	1.18^c
Mean	53.7725	32.18	9.47	3.215	10.995	1.2725
Std. Dev.	1.0972	1.3114	0.108	0.1718	0.5922	0.2014
Std. Error	0.5486	0.6557	0.054	0.0859	0.2961	0.1007
LSD	At 5%			0.87*		
Giza 127						
Cont.4	56.96^{ab}	29.76^b	8.89^c	3.87^c	12.76^c	1.8^c
Treat1	55.18^{ab}	28.99^b	8.43^c	3.9^c	12.09^c	1.67^c

(*Continued*)

Table 3 (*Continued*)

Genotype and treatment	Plant height (cm)	Flag leaf area cm^3	Number of leaves	Number of tillers	Spike length (cm)	Grain Yield
Treat2	52.16^{ab}	27.36^{b}	7.89^{c}	3.66^{c}	11.65^{c}	1.66^{c}
Treat3	51.67^{ab}	28.04^{b}	7.09^{c}	3.72^{c}	11.52^{c}	1.33^{c}
Average	53	28.13^{b}	7.8^{c}	3.76^{c}	11.75^{c}	1.55^{c}
Mean	53.9925^{ab}	28.5375	8.075	3.7875	12.005	1.615
Std. Dev.	2.5145	1.0541	0.7735	0.1159	0.5593	0.2004
Std. Error	1.2573	0.527	0.3867	0.0579	0.2797	0.1002
LSD	At 5%			0.87*		
Giza 128						
Cont.5	70.12^{a}	25.56^{bc}	9.13^{c}	2.77^{c}	10.87^{c}	1.8^{c}
Treat1	68.1^{a}	24.76^{bc}	9.05^{c}	2.65^{c}	10.72^{c}	1.43^{c}
Treat2	66.67^{a}	23.6^{bc}	8.09^{c}	2.43^{c}	8.65^{c}	1.2^{c}
Treat3	65.23^{ab}	21.23^{bc}	7.78^{c}	2.87^{c}	8.9^{c}	1.35^{c}
Average	66.67^{a}	23.2^{bc}	8.31^{c}	2.65^{c}	9.42^{c}	1.33^{c}
Mean	67.53^{a}	23.7875	8.5125	2.68	9.785	1.445
Std. Dev.	2.0867	1.8853	0.6795	0.1894	1.1723	0.2551
Std. Error	1.0433	0.9427	0.3398	0.0947	0.5862	0.1276
LSD	At 5%			1.48*		
G1za 130						
Cont.6	66.65^{a}	33.76^{b}	15.96^{c}	3.87^{c}	10.16^{c}	2.1^{c}
Treat1	64.76^{a}	32.16^{b}	15.9^{c}	3.8^{c}	10.09^{c}	1.99^{c}
Treat2	61.43^{a}	31.78^{b}	13.65^{c}	3.55^{c}	9.45^{c}	1.73^{c}
Treat3	60.64^{a}	30.25^{b}	13.07^{c}	3.21^{c}	9.02^{c}	1.6^{c}
Average	62.28^{a}	31.4^{b}	14.21^{c}	3.52^{c}	9.52^{c}	1.77^{c}
Mean	63.37	31.9875	14.645	3.6075	9.68	1.855
Std. Dev.	2.8229	1.4415	1.5028	0.2985	0.5438	0.2301
Std. Error	1.4115	0.7207	0.7514	0.1492	0.2719	0.1151
LSD	At 5%			1.7*		
Giza2000						
Cont.7	72.54^{a}	41.65^{b}	17.43^{c}	4.06^{c}	13.23^{c}	1.67^{c}
Treat1	71.87^{a}	40.78^{b}	17.78^{c}	4.11^{c}	13.21^{c}	1.56^{c}
Treat2	67.55^{a}	39.45^{b}	14.67^{c}	3.78^{c}	11.45^{c}	1.44^{c}
Treat3	66.43^{a}	37.67^{b}	14.18^{c}	3.63^{c}	12.44^{c}	1.33^{c}
Average	68.62^{a}	39.3^{b}	15.54^{c}	3.84^{c}	12.37^{c}	1.44^{c}
Mean	69.5975	39.8875	16.015	3.895	12.5825	1.5
Std. Dev.	3.0577	1.7332	1.8524	0.2287	0.8398	0.1472
Std. Error	1.5288	0.8666	0.9262	0.1143	0.4199	0.0736
LSD	At 5%			1.94*		

Mean±Standard deviation, Means with the same litters was not significant difference. *, **Significant at 0.05 and 0.01 levels, respectively.

analysis of variance (ANOVA). In conclusion, the morphological traits are affected by the concentrations of nematodes used under the study, where the lower in the nematode *Heterdra avanae* will result in a direct improvement.

Cluster analysis of morphological traits

The number of spikes measuring 14.05 cm was most numerous in accession Giza 124 and least numerous in accessions Giza 130. (10.09 cm). According to the data, Grain Yield in Accession Giza 130 had the greatest value (1.99 cm), while Accession Giza 123 had the lowest values (0.96 cm).

Numerous traits, show substantial differences according to analysis of variance (ANOVA). In conclusion, the rise in the nematode *Heterdra avanae* will result in a direct improvement.

On the other hand, Giza2000 and Giza123 were separated at a phylogenetic distance of 0.524 and 0.524, respectively. Maximum genetic similarity coefficient values of 70% and 20%, respectively, were found for Giza2000 and Giza124 genotypes, suggesting a significant degree of genetic similarity. The accessions Giza128 and Giza123, both two rowed, had the lowest similarity ratio of 0.03% are shown in (Table 4 and Fig. 1). The evolution of these genotypes in various agroclimatic regions, which suggests significant levels of variation in response to selection pressure as described by many authors Souframanien and Gopalakrishna, Allel and colleagues [19,20], can

Table 4 Similarity index among 7 accessions of *Hordeum vulgare* L. based on 6 qualitative morphological traits

Case	Giza123	Giza124	Giza126	Giza127	Giza128	Giza130	Giza2000
Giza123	1						
Giza124	0.67	1					
Giza126	0.29	0.54	1				
Giza127	0.11	530.	0.29	1			
Giza128	0.03	0.68	230.	80.0	1		
Giza130	0.10	630.	310.	140.	30.0	1	
Giza2000	0.01	0.70	0.39	0.16	0.03	0.12	1

be used to explain this. Additionally, cluster analysis demonstrated a weak correlation between the regional origin of genotypes and their separation, as shown in the morphological dendrogram (Fig. 1).

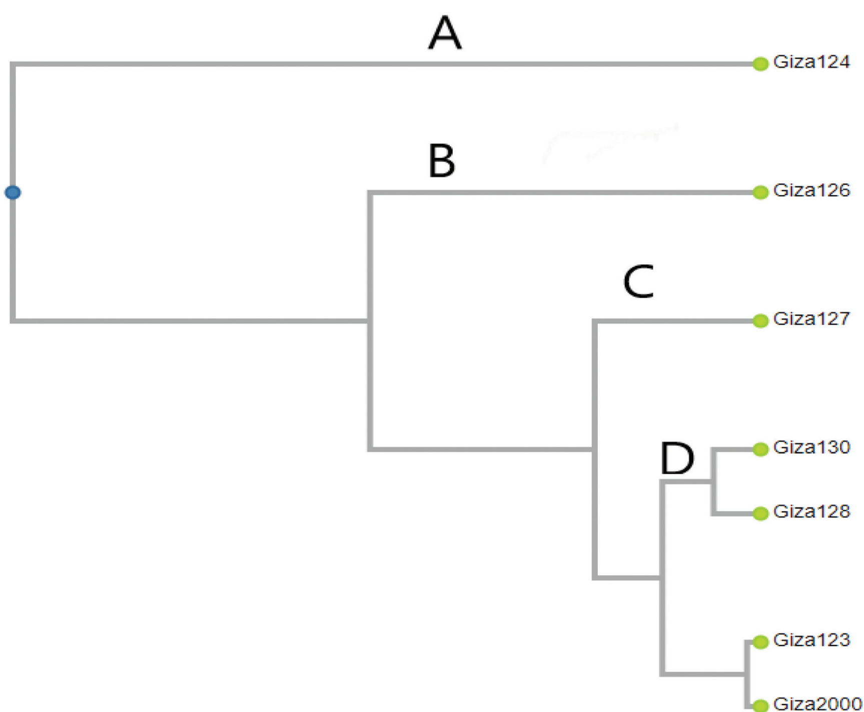
Molecular results

Assessment of ISSR markers

A total of 37 bands were produced by PCR amplification of genomic DNA separated from the 7 barley genotypes, of which 57 (or 29%) were polymorphic. The primer Am2 produced the highest score (8 TB), whereas the primer A1 and UBC890 produced the lowest score (4 TB) (2). The PCR-amplified fragments' overall sizes varied from 100 to 2000 bp (Table 5). Barley gene banding patterns for primers A1, Am1, Am2, UBC888, UBC890, Am3, Am9 are displayed (Fig. 2). With a molecular size range of 200–900 bp, marker A1 produced four bands, four of which were polymorphic, or 75% of the sample was polymorphic. The surviving 3 bands, however, were polymorphic bands (PB). Genotype Giza 130 revealed the most bands (2) and a unique positive marker at (400 bp). Giza 127, Giza 128 and Giza 2000 gave the greatest number of bands (5), while accessions Giza 123 gave the lowest number of bands. Am1 primer generated 6 bands with a molecular size ranging from 300-900 bp, 3 bands were (PB) indicating polymorphism percentage of 83.3%. (1). Am2 primer generated 8 bands, indicating 87.5% polymorphism, with molecular sizes ranging from 150 to 1000 bp; the remaining 7 bands were PB.

The molecule sizes of the 5 bands produced by the UBC888 primer ranged from 300 to 1100 bp. There were four PB bands total, which represents an 80% polymorphic rate. In the accession Giza 127 and Giza

Figure 1

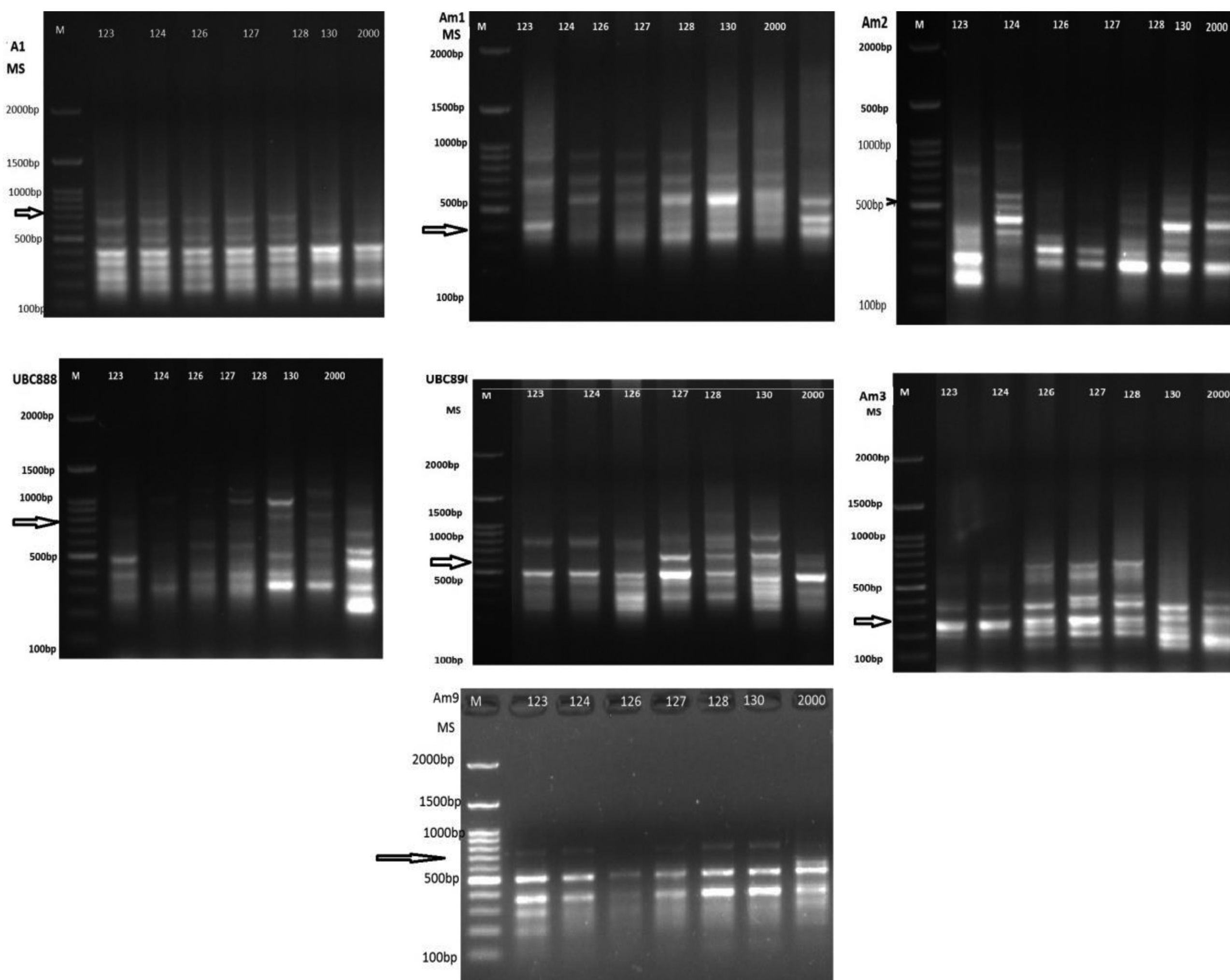


Dendrogram using UPGMA cluster for 6 quantitative morphological traits of 7 *Hordeum vulgare L.*

Table 5 Similarity index among 7 genotypes of barley based on banding patterns of 7 ISSR primers

Case	Giza123	Giza124	Giza126	Giza127	Giza128	Giza130	Giza2000
Giza123	100	.					
Giza124	43	100					
Giza126	6	43	100				
Giza127	29	43	99	100			
Giza128	66	34	43	43	100		
Giza130	9	39	98	97	15	100	
Giza2000	21	34	88	88	78	63	100

Figure 2



Seven barley varieties' ISSR profiles, for instance the Am1 primer. Seven ISSR primers were used to detect a total of 37 bands, of which 29 were polymorphic bands (PB). Primer Am2 had the highest score (8 TB), while primer UBC890 had the lowest score (4 TB), as shown in Table 1.

2000 at (700 bp) and in the Giza 126 at, two genotypes displayed particular favorable markers (300 bp). Three of the four bands amplified by the UBC890 primer were polymorphic, accounting for 75% of the total polymorphism rate. The bands' molecular sizes ranged from 100 to 1000 bp. Four of the five bands produced by the Am3 primer—representing an 80% polymorphism percentage—were polymorphic, with sizes varying from 100 to 900 bp. Three of the five bands amplified by the Am9 primer were polymorphic, indicating a 60% polymorphism percentage, and the remaining two bands ranged in size from 200 to 800 bp.

Prior research on the genetic similarity of barley genotypes using cluster analysis Bahrman and colleagues, Hamza and colleagues, Eshghi and Akhundova [21–23] suggests that geographic

isolation and regional microclimate variations can partially account for diversity. Environmental factors influence morphological markers, they are not always accessible for study, they take time to complete, and they are prone to ambiguous interpretations.

Seven barley varieties' ISSR profiles, for instance the Am1 primer. Seven ISSR primers were used to detect a total of 37 bands, of which 29 were polymorphic bands (PB). Primer Am2 had the highest score (8 TB), while primer UBC890 had the lowest score (4 TB), as shown in (Table 1).

Genetic diversity and relationships

The Dendrogram from the UPGMA cluster analysis of seven ISSR markers from seven *Hordeum* varieties is shown in (Figure 1). Three distinct clusters were visible on the phenogram, with genetic similarity values varying from 0.03 to 1.00. One genotype, Giza124, is present in Cluster A and was isolated at a phylogenetic distance of 0.29. One genotype is present in Cluster B; Giza126 was isolated from the rest at a taxonomic distance of 0.15. Cluster C only includes one genotype, Giza127, which was separated from Cluster C by 0.063 taxonomic units. Four Genotypes make up the two sub-clusters D of the Genotypes; They were grouped together at a taxonomic distance. Giza128 and Giza130 were divided at a taxonomic distance of 1.804 and 1.804, respectively. Maximum genetic similarity coefficient values of 99 and 99%, respectively, were found for the Giza126 and Giza127 genotypes, suggesting a significant degree of genetic similarity. The accessions Giza126 and Giza123, which were both towed, had the lowest closeness ratio of 6%, as shown in (Table 5).

The use of PCR-based molecular marker technology in breeding programmes and cultivar identification is therefore growing in popularity because it has many benefits over using only conventional markers. DNA fingerprinting was used in this instance to identify phylogenetic relationships between the barley species and the Inter-simple sequence repeat (ISSR) method was used to investigate genetic diversity Guasmi and colleagues, Fernandez and colleagues, Tanyolac [24–26]. High polymorphism was found between Egyptian barley collection accessions using ISSR-based genetic diversity analysis; this result was also observed for rice Saker and colleagues [27] and orange Lamine and Mliki [28]. It was standard practice to distinguish between accessions using ISSR markers, which were occasionally even more effective than SSR markers. Numerous studies Lamine and Mliki, Gorji and colleagues, Izzatullayeva and colleagues, Vaja and colleagues [28–31] stressed that the ISSR method was suggested in earlier and more recent studies as an efficient tool for genotypic evaluation in a variety of plant species.

The dendrogram generated in this research using UPGMA from ISSR based on the genetic similarity matrix demonstrated that group structure is somewhat influenced by geographic distribution. Combining genes from the same region, such as Giza 128 and Giza 130, or Giza 123 and Giza 2000, as shown in (Fig. 1). Wheat has also been characterized as following this pattern of geographically-related grouping by molecular markers Sonmezoglu and colleagues [32]. Our results, however, contradict earlier research on barley and Aegilops Strelchenko and colleagues, Owuor and colleagues, Mahjoub and colleagues [33–35] that claim that the molecular clustering of barley does not correspond to its place of origin. Additionally, the findings revealed highly polymorphic profiles with 37 polymorphic bands found by 7 ISSR primers, demonstrating a high level of polymorphism (87.5%). Moroccan barley has a lower amount of polymorphism (60%) Dakir and colleagues [36]. 37 polymorphic bands were visible in our data, as noted in (Table 2).

Conclusion

Different hierarchical patterns of genetic diversity between the genotypes were revealed by molecular marker-based and morphological clusters, as well as the research that went along with them. However, despite their differences, the two methods were found to be equally helpful for determining the degree of relatedness and the general patterns of genetic diversity among the examined barley genotypes. However, molecular markers were more efficient and illuminating in classifying barley genotypes and provide strong instruments for the assessment of intra-specific relationships. For top gene exploitation and genetic advancement, understanding the genetic makeup of Egyptian barley genotypes is still very important.

Financial support and sponsorship

Nil.

Conflicts of interest

The authors declare there are no conflicts of interest.

References

1 Newton AC, Flavell AJ, George TS, Leat P, Mullholland B, Ramsay L, *et al.* Crops that feed the world 4. Barley: a resilient crop? Strengths and

weaknesses in the context of food security. Food Security 2011; 3:141–178.

2 Kleinhofs A, Han F. 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. New York: Food Progucts Press 2002.

3 Rashad Sh E, Abdel-Tawab FM, Fahmy EM, Saker MM. Somaclonal variation from mature embryo explants of some Egyptian barley genotypes. Egypt J Genet Cytol 2020; 49:103–121.

4 Merwad MA, Mostafa EAM, Ashour NE, Saleh MMS, El- Demerdash IS, Rashad SE. Horticultural Studies And Genetic Relationship Via Dna Fingerprinting Using RAPD Markers Between Sewi Date Palm And Two Superior Seeded Females. Plant Cell Biotechnology And Molecular Biology 2020; 22:56–66.

5 Shata SM, Said WM, Abdel-Tawab FM, Kamal LM. Morphological and Quantitative traits of phylogenetic relationships of some barley (*Hordeum vulgare L.*) accessions in Egypt. Journal of Scientific Research in Science 2021; 38:16–35.

6 Rashad ShE, Abdel-Tawab FM, Fahmy EM, Saker MM. Transformation system of mature embryo of some Egyptian barley genotypes. Egypt J Genet Cytol 2020; 49:89–102.

7 Heiba SAA, Ali RT, Abdel-Rahman HM, Rashad SE. Detected molecular markers for Alfalfa (Medicago sativa) using ISSR and SSR under Egyptian conditions. International Journal of Latest Technology in Engineering, Management & Applied Science (IJLTEMAS) 2022; XI:2278–2540.

8 Jones CJ, Edwards KJ, Castaglione S, Winfield MO, Sala F, van de Wiel C, *et al.* Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. Molecular Breeding 1997; 3:381–390.

9 Zietkiewicz E, Rafalski A, Labuda D. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 1994; 20:176–183.

10 Pérez de la Torre M, García M, Heinz R, Escandón A. Analysis of genetic variability by ISSR markers in Calibrachoacaesia. Electronic Journal of Biotechnology 2012; 15:8–8.

11 Hou YC, Yan ZH, Wei YM, Zheng YL. Genetic diversity in barley from west China based on RAPD and ISSR analysis. Barley Genetics Newsletter 2005; 35:9–22.

12 Khatab A, El-Mouhamady AA, Mariey SA, Elewa TA. Assessment of water deficiency tolerance indices and their relation with ISSR markers in barley (*Hordeum vulgare L.*). Cu Sci Inte 2019; 8:83–100.

13 Federer WT, Raghavarao D. On augmented designs. Biometrics 1975; 31:29–35.

14 Hampl V, Pavlícek A, Flegr J. Construction and bootstrap analysis of DNA fingerprinting-based phylogenetic trees with the freeware program FreeTree: application to trichomonad parasites. Int J Syst Evol Microbiol 2001; 51:731–735.

15 Nei M, Li WH. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences 1979; 76:5269–5273.

16 Sokal RR. A statistical method for evaluating systematic relationships. Univ Kansas, Sci Bull 1958; 38:1409–1438.

17 Smith JSC, Chin ECL, Shu H, Smith OS, Wall SJ, Senior ML, Ziegle J. An evaluation of the utility of SSR loci as molecular markers in maize (Zea mays L.): comparisons with data from RFLPs and pedigree. Theoretical and Applied Genetics 1997; 95:163–173.

18 Singh K, Smartt J, Simpson CE, Raina SN. Genetic variation vis-a-vis molecular polymorphism in groundnut, Arachis hypogaea L. Genetic Resources and Crop Evolution 1998; 45:119–126.

19 Souframanien J, Gopalakrishna T. A comparative analysis of genetic diversity in blackgram genotypes using RAPD and ISSR markers. Theoretical and Applied Genetics 2004; 109:1687–1693.

20 Allel D, Ben-Amar A, Lamine M, Abdelly C. Relationships and genetic structure of North African barley (*Hordeum vulgare L.*) germplasm revealed by morphological and molecular markers: Biogeographical considerations. South African Journal of Botany 2017; 112:1–10.

21 Bahrman N, Le Gouis J, Hariri D, Guilbaud L, Jestin L. Genetic diversity of old French six-rowed winter barley varieties assessed with molecular, biochemical and morphological markers and its relation to BaMMV resistance. Heredity 1999; 83:568–574.

22 Hamza S, Hamida WB, Rebaï A, Harrabi M. SSR-based genetic diversity assessment among Tunisian winter barley and relationship with morphological traits. Euphytica 2004; 135:107–118.

23 Eshghi R, Akhundova E. Genetic diversity in hulless barley based on agromorphological traits and RAPD markers and comparison with storage protein analysis. African Journal of Agricultural Research 2010; 5:97–107.

24 Guasmi F, Elfalleh W, Hannachi H, Feres K, Touil L, Marzougui N, Ferchichi A. The use of ISSR and RAPD markers for genetic diversity among south tunisian barley. Int Sch Res Notices 2012; Article ID 952196, 10 pages, 2012. https://doi.org/10.5402/2012/952196.

25 Fernandez M, Figueiras A, Benito C. 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 2002; 104:845–851.

26 Tanyolac B. Inter-simple sequence repeat (ISSR) and RAPD variation among wild barley (*Hordeum. vulgare* subsp. spontaneum) populations from west Turkey. Genetic Resources and Crop Evolution 2003; 50:611–614.

27 Saker MM, Youssef SS, Abdallah NA, Bashandy HS, El Sharkawy AM. Genetic analysis of some Egyptian rice genotypes using RAPD, SSR and AFLP. African Journal of Biotechnology 2005; 4:882–890.

28 Lamine M, Mliki A. Elucidating genetic diversity among sour orange rootstocks: a comparative study of the efficiency of RAPD and SSR markers, Appl Biochem Biotechnol 2015; 175:2996–3013.

29 Gorji AM, Poczai P, Polgar Z, Taller J. Efficiency of arbitrarily amplified dominant markers (SCoT, ISSR and RAPD) for diagnostic fingerprinting in tetraploid potato. American journal of potato research 2011; 88: 226–237.

30 Izzatullayeva V, Akparov Z, Babayeva S, Ojaghi J, Abbasov M. Efficiency of using RAPD and ISSR markers in evaluation of genetic diversity in sugar beet. Turkish Journal of Biology 2014; 38:429–438.

31 Vaja KN, Gajera HP, Katakpara ZA, Patel SV, Golakiya BA. Biochemical indices and RAPD markers for salt tolerance in wheat genotypes. Indian Journal of Plant Physiology 2016; 21:143–150.

32 Sonmezoglu ÖA, Bozmaz B, Yildirim A, Kandemir N, Aydin N. Genetic characterization of Turkish bread wheat landraces based on microsatellite markers and morphological characters. Turkish Journal of Biology 2012; 36:589–597.

33 Strelchenko P, Kovalyova O, Okuno K. ‘Genetic differentiation and geographical distribution of barley germplasm based on RAPD markers. Genetic resources and crop evolution 1999; 46:193–205.

34 Owuor ED, Beharav A, Fahima T, Kirzhner VM, Korol AB, Nevo E. Microscale ecological stress causes RAPD molecular selection in wild barley, Neve Yaar microsite, Israel. Genetic Resources and Crop Evolution 2003; 50:213–224.

35 Mahjoub A, El Gharbi MS, Mguis K, El Gazzah M, Brahim NB. Evaluation of genetic diversity in Aegilops geniculata Roth accessions using morphological and RAPD markers. Pakistan journal of biological sciences: PJBS 2009; 12:994–1003.

36 Dakir EH, Ruiz ML, García P, de la Vega MP. Genetic variability evaluation in a Moroccan collection of barley, *Hordeum vulgare L.*, by means of storage proteins and RAPDs. Genetic Resources and Crop Evolution 2002; 49:619–631.