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APPLICATION OF SIMPLE SEQUENCE REPEAT (SSR) MARKER TO DIFFERENTIATE AMONG EIGHT BARLEY VARIETIES

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ABSTRACT

The present investigation was carried out to identity eight barley varieties using DNA fingerprinting detected by (SSR)-PCR molecular markers and SDS- PAGA protein electrophoresis. Twelve anchored SSR primers were used for fingerprinting of the eight barley varieties and 76 bands were detected, from which 56 bands (74%) were polymorphic. Giza 125 variety produced the largest number of markers. It produced 14 band markers in seven primers (SSR 1, SSR 4, SSR 5, SSR 6, SSR 7, SSR 11 and SSR 12). Giza 123 variety produced five bands in three primers (SSR 5, SSR 8 and SSR 12). Giza 127 produced four bands in three primers (SSR 5, SSR 8 and SSR 9). Giza 124 produced two bands in two primers (SSR 2 and SSR 8). Also Giza 132 variety produced two bands in two primers (SSR 9 and SSR 11). Giza 129 variety has one band marker in primer SSR 8. On the other hand, the results of protein electrophoresis revealed that, total number of bands was 13, out of which 7 bands (54%) were polymorphic. Protein electrophoresis produced four marker bands, three of them were found in variety Giza 124 and one in variety Giza 129. Existing genetic differences between varieties are of great importance from the point of view of the breeder to take advantage in barley improvement programs.

Key words: Barley, *Hordeum vulgare*, L. polyacrylamide gel electrophoresiss (SDS-PAGE), simple sequence repeat (DNA-SSR)

INTRODUCTION

Barely, *Hordeum vulgare* L. is recognized as one of the most economic and important cereals in the world, by area and production ranked as the fourth most important cultivated crop. Following wheat, rice and maize, it can be grown in a wide range of environmental conditions. Barley, is one of the principal cereal crops in the world and is cultivated in all temperate areas (Von Bothmer *et al.,* 1995). Sodium dodecyl sulphate polyacrylamide gel electrophorsiss (SDS-PAGE) is most economical simple and extensively used biochemical technique for analysis of genetic structure of germplasm. Gel electrophoresis can directly equate variation in protein banding patterns to gene coding various proteins and proved to be useful in revealing polymorphic loci that encode isozymes or proteins (Masoje *et al.,* 2001). Seed storage protein markers and molecular markers have been used as tools to enhance barley variety identification capabilities for several years. Among different methods of molecular markers, SSR markers have proved to be markers of choice for several applications in breeding because of their multi-allelic nature for having Codominant inheritance reproducibility, abundance and wide genomic distribution (Gupta and Varshney, 2000).

Multilocus fingerprinting methods based on the polymerase chain reaction (PCR) have been extensively used to study the relationships among varieties and varieties of many different plants. The most informative polymorphic marker system currently available is microsatellite or sample sequence repeat (SSRs) (Tantz and Renz, 1984). Various molecular marker techniques have been developed into powerful tools for diversity analysis and

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establishing relationships between varieties. Co-dominant markers have become important genetic markers in a wide range of crop species, including barley (Struss and Plieske, 1988). SSR marker is abundant dispersed throughout the genome and show higher levels of polymorphism than other genetic markers (Russel *et al.,* 1997). Their feature coupled with their ease of detection have made them useful molecular markers for this reason SSR markers are ideal for distinguishing and identification of accessions that are genetically very similar (Saker *et al.,* 2005). Their potential for automation and their inheritance in a codominant manner are additional advantages when compared to other types of molecular markers. Up to 37 different alleles for one SSR locus have been found in barley (Saghai-Maroof *et al*., 1994).

MATERIALS AND METHODS

The present study was carried out at the Department of Seed Technology Research, Field Crops Research Institute, ARC during 2014. Barley genotypes included eight varieties as shown in Table 1.

Electrophoresis of Total Proteins

Total proteins were extracted from seeds and SDS-PAGE was performed according to the protocol described by Laemmli (1970).

Molecular Markers

SSR-PCR analyses: Twelve SSR primers were used to evaluate the eight barley varieties as shown in Table 2.

Plant Material and DNA Extraction

For genomic DNA isolation, seeds of each barely variety were germinated and grown to the four-leaf stage. The seedlings were used for DNA extraction by DNeasy plant minikit (Quigen Inc., Cat.no.69104, and USA). The DNA concentration of the final samples was measured by ultraviolet (UV) spectrophotometer at 260 nm. The integrity of the DNA was checked out by electrophoresis in a 1% agarose gel in TAE buffer.

Polymerase Chain Reaction (PCR) Conditions

DNA amplification was carried out in PCR tubes containing 25 µL reaction mixture, having 1 µL template DNA, 1 µL SSR primer, 15 µL of dd H_2O and 7 µL PCR mix. Amplification was carried out in a PTC- 200 thermal cycler (MJ Research, Watertown, USA) programmed as follows: The temperature profile consisted of an initial denaturation step of DNA at 94°C for 2 min, followed by 35 cycles: 94°C for 45 sec., 57–65°C for 1 min, and 72°C for 1 min 30 sec. Annealing temperatures were optimized individually for each SSR (listed in Table 1). After the final cycle, samples were incubated at 72°C for 10 min to ensure complete extension.

Gel Electrophoresis

Gel electrophoresis was applied according to Sambrook *et al.* (1989). The run was performed for one hour at 80 volt in pharmacia submarine (20 × 20 cm). Bands were detected on UV–transilluminator and photographed by Gel documentation 2000, Bio- Rad. Fragment size of SSR was estimated from the gel by comparison with the 100 + 1.5 kb ladder marker. The bands were recorded as either present or absent into a database of "1"and "0".

RESULTS AND DISCUSSION

Biochemical Analysis by Protein Electrophoresis

The SDS-PAGE patterns for total soluble proteins in the eight barley varieties are shown in Fig. 1 and Table 3. All markers were scored for presence/absence (+/-). A maximum number of 13 bands, were detected with molecular weights (mw) ranged from 380 to 80 KDa and 7 of them were polymorphic (54% polymorphism). Band with MW of about 380, 330 and 299 KDa could be considered as a positive marker of Giza 124 variety, which was present only in this variety but absent in all other varieties under study, while band on MW 190 KDa was present only in barley variety Giza 129. This band is considered positive specific marker for this variety. The genetic diversity in barley varieties have been addressed by using biochemical evidence derived by the electrophoresis separation of the seed protein (Facciol *et al.,* 1999). Karimzadeh *et al*. (2006) observed changes in the electrophoresis pattern of water soluble proteins from barley varieties, also Ali *et al*. (2007) identified 12 polymorphic bands with different expressions in six barley genotypes.

Table 1. Number and name of eight barley varieties

No.	Variety	Row type	Pedigree
1	Giza 123	6R	Giza 117/FAO 86
2	Giza 124	6R	Giza 117//Bahteem 52// Giza 118/FAO 86
3	Giza 125	6R	Sisterr line to Giza 124
4	Giza 126	6R	Baladi Bathteem/SD 729-Por 12762-BC
5	Giza 127	2R	WI2291"/Bags"//Harm/al-02"
6	Giza 129	6R	Deir Alla 106/Cel//As46/Aths*2
7	Giza 132	6R	Rihane-05//As 46/Aths/Lignee 686
8	Giza 2000	6R	Giza 121/Giza 124

Table 2. Name and sequence of SSR primers which were used for SSR-PCR analyses

Primer name	Sequence
SCindo2587- SSR 1	Forward 5'GGT GAC CCA GCC AAA TTT TA3'
	Reverse5' GCA GCT GCT AGT TGG TTC ATC3'
SCind60002- SSR 2	Forward 5'CAT CAT CAC GCC ACC ATA CT3'
	Reverse 5'CGA ATG CAG TAC AGC CTC AG3'
Bmag0211- SSR 3	Forward 5'ACA TCA TGT CGA TCA AAG C3'
	Reverse 5' ATT CAT CGA TCT TGT ATT AGT CC3'
HVm4- SSR 4	Forward 5'GCA AAG TCG TCG AAG GAG3'
	Reverse 5'CCA GTC CAA TGG CAT CTA CA3'
HVLTPP- SSR 5	Forward 5'CAA AGT ACA ACA AAC TCA CGA3'
	Reverse 5'AGA CGC TGA GTA CGT TGA G3'
HVHVA1- SSR 6	Forward 5' CGA CCA AAC ACG ACG ACT AAA GGA3'
	Reverse 5' CAT GGG AGG GGA CAA CAC3'
Bmac0018-SSR 7	Forward 5' GTC CTT TAC GCA TGA ACC GT3'
	Reverse 5' ACA TAC GCC AGA CTC GTG TG3'
EBmac070- SSR 8	Forward 5'TGG CAC TAA AGC AAA AGA C3'
	Reverse 5' ATG ATG AGA ACT CTT CAC CC3'
Scind16991-SSR 9	Forward 5' GGG CTT CCC CTC CTT TGT AT3'
	Reverse 5'CGC CGT TCC AGT TTA ACT TC3'
HVm40- SSR10	Forward 5'CGA TTC CCC TTT TCC CAC3'
	Reverse 5' ATT CTC CGC CGT CCA CTC3'
EBmag041-SSR 11	Forward 5' AAA CAG CAG CAA GAG GAG3'
	Reverse 5'GAA ACC CAT CAT AGC AGC3'
HVm36-SSR 12	Forward 5' TCC AGC CGA CAA TTT CTT G3'
	Reverse 5' AGT ACT CCG ACA CCA CGT CC3'

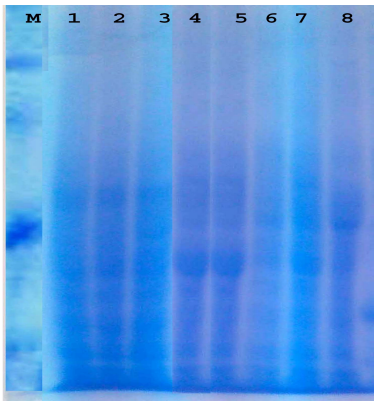

Fig. 1. SDS-PAGE profiles of eight barley varieties

Table 3. SDS-PAGE of total seed protein in eight barley varieties

Band number	MW	Giza 123	Giza 124	Giza 125	Giza 126	Giza 127	Giza 129	Giza 132	Giza 2000
1	380	-	+	-	-	-	-	-	-
2	330	-	+	-	-	-	-	-	-
3	299	-	+	-	-	-	-	-	-
4	265	+	+	+	+	+	+	+	+
5	240	-	-	-	-	+	+	+	+
6	230	+	+	+	+	-	-	-	-
7	200	+	+	+	+	+	+	+	+
8	190	-	-	-	-	-	+	-	-
9	170	+	+	+	+	+	+	+	+
10	140	+	+	+	+	+	+	+	+
11	110	+	-	+	+	+	+	-	+
12	90	+	+	+	+	+	+	+	+
13	80	+	+	+	+	+	+	+	+

Molecular analysis of eight barley varieties using Simple Sequence Repeat (SSR) markers

For SSR analysis, DNA of eight barley varieties were subjected to PCR against 12 specific primers of SSR as described in Fig. 2 and illustrated in Table 4. Total number of bands were 76 bands, 56 of them were polymorphic (74% polymorphism). The highest number of bands were shown in primers SSR 5, SSR 8 and SSR 9 (9 bands) while, the lowest one was obtained in primer SSR 3 (2 bands) and SSR 7 (3 bands). On the other hand, only primer SSR 9 generated no monomorphic bands (100% polymorphism), while the lowest polymorphism was 0% detected in primer SSR 3 as shown in Table 4.

Primer SSR1 produced 8 bands 6 of them were polymorphic (75% polymorphism). This analysis indicated the presence of six positive markers for Giza 125 variety. Primer SSR2 showed 4 bands; three of them were polymorphic (75% polymorphism), this primer (SSR2) indicated the presence of one marker for Giza 124 variety. Primer SSR3 yielded 2 monomorphic bands (0% polymorphism). Primer SSR4 revealed 6 bands; 4 of them were polymorphic (67% polymorphism) indicated the

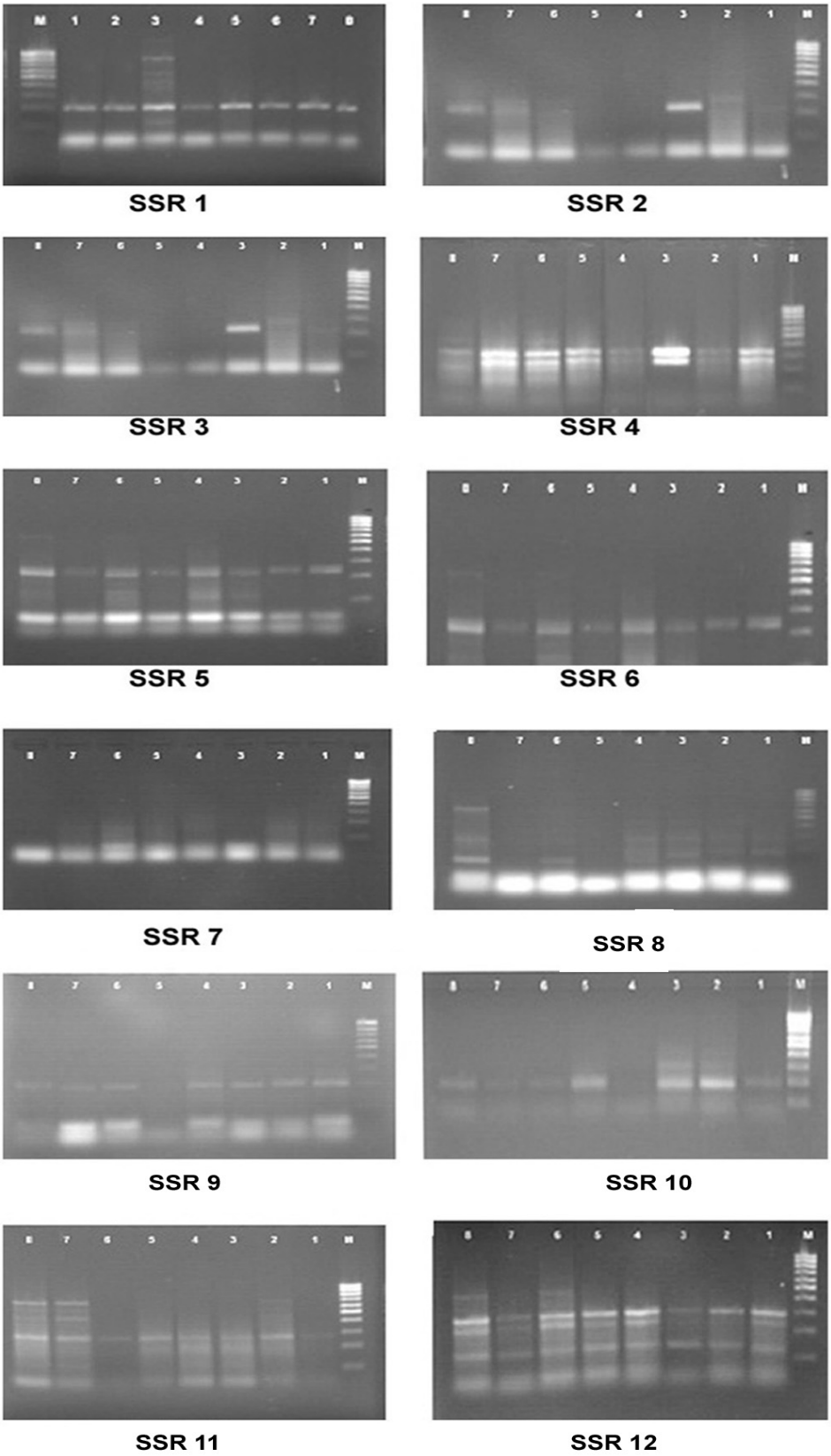

Fig. 2. SSR banding patterns amplified with 12 primers

Table 4. Levels of polymorphism and unique genotypes specific bands for eight barley varieties by 12 SSR primers

Band / Primer	Total bands	Polymorphic bands	Monomorphic Bands	Polymorphism (%)	Unique bands	
					Genotype	MW
SSR 1	8	6	2	75	Giza 125	1470 bp
						870 bp
						515bp
						405 bp
						355 bp
						115 bp
SSR 2	4	3	1	75	Giza 124	250 bp
SSR 3	2	0	2	0	0	0
SSR 4	6	4	2	67	Giza 125	525 bp
SSR 5	9	7	2	78	Giza 123	550 bp
						220 bp
					Giza 125	530 bp
					Giza 127	102 bp
SSR 6	7	5	2	71	Giza 125	725 bp
						560 bp
						460 bp
SSR 7	3	1	2	33	Giza 125	230 bp
SSR 8	9	8	1	89	Giza 123	580 bp
						265 bp
					Giza 124	120 bp
					Giza 127	190 bp
						180 bp
					Giza 129	170 bp
SSR 9	9	9	0	100	Giza 127	255 bp
					Giza 132	205 bp
					Giza 2000	200 bp
SSR 10	4	2	2	50	0	0
SSR 11	8	7	1	88	Giza 125	65 bp
					Giza 132	560 bp
SSR 12	7	4	3	57	Giza 123	415 bp
					Giza 125	450 bp
Total	76	56	20	74		

presence of one marker in variety Giza 125. Primer SSR5 revealed 9 bands 7 of them were polymorphic (78% polymorphism) This primer has four markers for three different varieties two of them in Giza 123, one marker for Giza 125 and also one marker in Giza 127. Primer SSR6 produced 7 bands; 5 of them were polymorphic (71% polymorphism) indicated the presence of three markers for variety Giza 125. Primer SSR7 produced 3 bands 1 of them was polymorphic (33% polymorphism); it has one marker for Giza 125. Primer SSR 8 revealed 9 bands 8 of them were polymorphic (89% polymorphism) it has six markers in four different varieties two of them in Giza 123 and two in Giza 127, while one marker in each of Giza 124 and Giza 129 varieties. Primer SSR 9 produced 9 polymorphic bands (100% polymorphism) indicated three markers for three different varieties Giza 127, Giza 132 and Giza 2000. Primer SSR 10 produced 4 bands 2 of them were polymorphic (50% polymorphism). Primer SSR 11 produced 8 bands 7 of them was polymorphic (88% polymorphism) this primer has two markers in two different varieties Giza 125 and Giza 132. Primer SSR12 produced 7 bands 4 of them were polymorphic (57% polymorphism), it produced 2 marker bands with two different varieties Giza 123 and Giza 125.

SSR markers can produce a large number of markers as observed in Giza 125 which was identified by 7 primers at different molecular weight (MW), it produced 14 bands; six of them in primer SSR 1 (1470, 870, 515, 405, 355 and 115 bp) , three of them in primer SSR 6 (725, 560 and 460 bp) and one band in primers SSR 4 (525 bp), SSR 5 (530 bp), SSR 7 (230 bp), SSR 11 (65 bp) and SSR 12 (450 bp). Giza 123 showed five band markers, two of them in primer SSR 5 (550 and 220 bp), two in primer SSR 8 (580 and 265 bp) and one band in primer SSR 12 (415 bp). Giza 127 has four marker bands, two of them in primer SSR 8 (190 and 180 bp), one band in primer SSR 5 (102 bp) also one band in primer SSR 9 (255 bp). Giza 124 recorded two bands in two different primers, SSR 2 (250 bp) and SSR 8 (120 bp). Also Giza132 has two band markers, one of them in primer SSR 9 (205 bp) and the other in primer SSR 11 (560 bp). Giza 129 produced one marker at primer SSR 8 (170bp) as well as Giza 2000 has one marker at primer SSR 9 (200 bp), while no marker could be observed for Giza 126. The previous genetic variability among barely varieties is important to plant breeder in barley breeding programs. Abdellaoue *et al.* (2007) studied the genetic variation and relationships among 13 local barley accession and the variety Martin using simple sequence repeat (SSR) markers and physiological traits. They showed that a total of 43 bands were generated by 15 SSR primers with an average of 2.6 easily detectable bands per primer. Ramzi *et al.* (2009) found high level of polymorphism make simple sequence repeats (SSR) the molecular marker of choice for diversity analysis in plant species by using 18 simple sequences repeats (SSR) marker to characterize six Tunisian barley varieties. At the same time, Kadri *et al.* (2009) studied the genetic variation and relationships among12 local barley genotypes and the varieties Martin and Manel using random amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR), high level of polymorphism was found with both RAPD and SSR markers. El-Awady and EI-Tarras, (2012) indicated that genetic variability within barley landraces is fundamental for barley breeding, they used 16 simple sequence repeats (SSR) markers to characterize six barley landraces from different cultivated regions in Kingdom of Saudi Arabia. A variety of polymerase chain reaction (PCR), based molecular markers is useful tools for the study of genetic diversity. For detection of genetic variation in barley, different classed of molecular markers were used. However, among these classes, the simple sequence repeats (SSR) or microsatellites derived from genomic DNA and amplified fragment length polymorphism (AFLP) have been used separately as well as in combination in many studies (Maeatri *et al.,* 2002, Matus and Hayes, 2002, Turpeinen *et al.,* 2003, Nevo *et al.,* 2005, Chaabance *et al.,* 2009).

Combined analyses for eight barley varieties

Similarity index and dendrogram across the eight barley varieties under investigation based on SSR analyses are shown in Table 5 and Fig. 3, respectively. The comparison revealed that the

Table 5. Similarity matrix among the eight barley varieties using SSR analyses

Variety	Giza 123	Giza 124	Giza 125	Giza 126	Giza 127	Giza 129	Giza 132
Giza 124	0.780						
Giza 125	0.622	0.698					
Giza 126	0.712	0.841	0.727				
Giza 127	0.692	0.730	0.634	0.800			
Giza 129	0.703	0.686	0.538	0.721	0.788		
Giza 132	0.685	0.696	0.545	0.767	0.738	0.852	
Giza2000	0.722	0.765	0.632	0.814	0.750	0.800	0.814

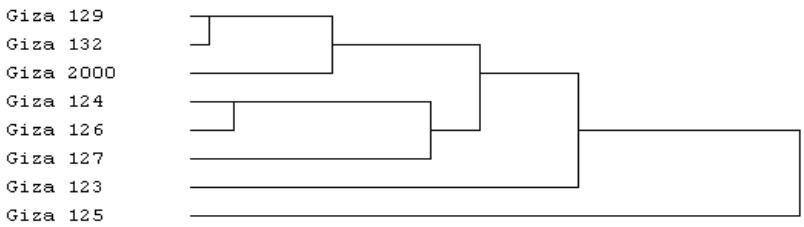

Fig. 3. Dendrogram of the genetic distances among the eight barley varieties based on SSR analyses

most closely related varieties were Giza 129 and Giza 132 (similarity matrix of 0.852). The lowest relationships were recorded for varieties Giza 125/ Giza 129 (similarity matrix of .538), followed by varieties Giza 125/ Giza 132 (similarity matrix of 0.545). These varieties resulted in two main clusters. One of them involved the variety Giza 125, while the second cluster involved the rest of varieties. The second cluster was divided into two subclusters, one included Giza 123, while the other subcluster involved Giza 127, Giza 126, Giza 124, Giza 2000, Giza 132 and Giza 129. Yang and Ding (2003) found that the dendrogram generated by the SSR matrix agrees better with the genealogy and known pedigree of the barley varieties result. The dendorgrem of genetic distances was constructed based on UPGMA method using midpoint joining procedure of Nei and Li (1979) dissimilarity matrix.

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استخدام معلمات SSR للتمييز بين ثمانية أصناف من الشعير

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أجريت هذه الدراسة للتمييز بين ثمانية أصناف من الشعير باستخدام تكنيك SSR-PCR والتفريد الكهربى للبروتين وكانت النتائج المتحصل عليها من الدراسة باستخدام 12 بادئات من SSR-PCR قد أظهرت 76 علامة جزيئية منهم 56 مختلفة بنسبة 74% كما وجد أن الصنف جيزة 125 به أكبر علامات جزيئية مميزة له (14 علامة جزيئية) فى سبع بادئات مختلفة (SSR11, SSR 7, SSR 6, SSR 5, SSR4, SSR 1 SSR12)، يليه الصنف جيزة 123 به خمس علامات جزيئية في ثلاث بادئات (SSR12, SSR 8, SSR 5)، يليه الصنف جيزة 127 به أربع علامات جزيئية في ثلاث بادئات (SSR 9 ,SSR 8, SSR5)، يليه الصنف جيزة 124 به علامتين جزيئيتين في بادئين (SSR8, SSR2)، يليه الصنف جيزة 132 به علامتين جزيئيتين فى بادئين (SSR 11, SSR 9)، كما وجد أن في صنف جيزة 129 علامة جزيئية واحدة في البادىء (SSR8)، بالإضافة إلى ما سبق فقد أوضحت نتائج التفريد الكهربي للبروتين وجود 13 علامة جزيئية منها 7 علامات مختلفة بنسبة 54% كما أظهرت النتائج أربع علامات جزيئية مختلفة ثلاثة منها في الصنف جيزة 124 وعلامة جزيئية واحدة في الصنف جيزة 129، وتمثل هذه الاختلافات أهميه كبيرة من وجهة نظر مربى النبات للاستفادة بها في برنامج تحسين الشعير.

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