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Assessment of Genetic Diversity in Triticum spp., Secale Cereale And Triticale Using Issr Markers And Sds-Page

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Genetic diversity among some wild close relatives of wheat was estimated using inter simple sequence repeats (ISSR) and SDS-PAGE markers. Four Triticum, Secale cereale and Triticale genotypes including seven Triticum, Secale and Triticale accessions belonging to one diploid (*Secale cereale*), three tetraploid (Triticum durum (4x)) and two hexaploid (Triticale (6x)) in addition to one hexaploid wheat (*Triticum aestivum*) species sampled from different ecogeographical regions of Egypt; a *durum* wheat cultivars 'Baniswef 1 and 3 and Sohag 3', a local wheat cultivars growing in upper Egypt 'Giza 168', a bread wheat cultivar and two triticale strains beside one rye strain were evaluated. Genetic diversity among wheat accessions was estimated using 11 ISSR primers. Of the approximately 153 detected ISSR markers, 65 (42%) were polymorphic with 4 bands per used primer pair. Cluster analysis of 7 accessions belonging to the four species by UPGMA cluster analysis based on Jaccard's similarity estimates for ISSR data divided all accessions into one major cluster and Secale cereale separated from the major cluster. two major sub-clusters reflecting almost their genome composition. The first one included wheat species having AB genomes ('Baniswef 1 and 3 and Sohag 3'), while second cluster included wheat species having ABD and ABR genomes. The genetic similarity coefficients ranged from 0.36 between Triticale 1 and rye accession and 0.84 between Triticale 1 (6x) and Triticale 2 (6x). Two Triticum durum species of were ranked as the second most related species (0.81). The results of SDS-PAGE revealed a total number of 145 bands with molecular weights (MW) ranging from about 17 kDa to 158 kDa, which were not necessarily present in the 7 genotypes. Data revealed seven common bands (monomorphic), six unique bands while the remaining 90 band were polymorphic with 62% polymorphism. The unique Bands at the molecular weight 158 and 41 kd were the distinguishable band of Baniswef 3. The unique Band at the molecular weight 64 kd was the distinguishable band detected of Sohag 3. The unique Bands at the molecular weight 62 and 55 kd were detected only in Rye. The unique Band at the molecular weight 17 kd was detected only in Baniswef 1.

Key words: Inter simple sequence repeats (ISSR), genetic diversity, wheat, Triticum spp., Rye, Triticale, SDS-PAGE.

Introduction

Bread wheat (*Triticum aestivum* L.) is the most widely grown plant in the world due to its physiological characteristics that adapt wheat cultivars for production in a wide range of eco-geographical conditions and the chemical and physical properties of the wheat gluten that contribute to the wide use of wheat grain for many different food products. It is the staple food for 35% of the world's population, and is becoming increasingly important in the developing world (**CIMMYT 2018**). To meet the demand for developing high yielding and stress-resistant wheat cultivars, it is desirable to increase the genetic base of this crop. There has been a growing concern about the remaining variability in the bread wheat gene pool which is grossly insufficient to address current and future breeding objectives (**Rejesus et al., 1996**). In last decades, the narrow genetic basis of modern wheat cultivars is well evident, as breeders prefer using either improved cultivars as parents or advanced breeding materials to accelerate the development of new cultivars. While in the beginning, selection was utilized to isolate pure lines from heterogeneous landraces or natural populations, today improved cultivars were used as parents in wheat breeding programs. It is therefore necessary to broaden the genetic base of wheat. The wild relatives of bread wheat, *T. aestivum* L., are considered as potential sources of useful alleles for bread- wheat improvement. The genus *Secale* L. and *Triticum* L. have contributed two of the three (A, B and D and R) and one of the three (A, B and R) Triticale genomes, respectively. The genus Triticum contains many species comprising diploids, Tetraploid and hexaploids, that originated from center of origin (**van Slageren, 1994**). Consequently, study of the genetic diversity of the genetic resources of such species may provide significant information regarding their potential for breeding purposes. Genetic erosion caused by modern cultivation procedure has narrowed the genetic base of many crops, including bread wheat. Wild relatives and related species can be successfully crossed with bread wheat (**Jiang et al., 1993; Sharma, 1995; Arzani et al., 2000**). Consequently, genes from the wild relatives can be introgressed in the cultivated wheats through recombination of the homologous chromosomes, and undesirable gene linkages can be mostly broken by repeated backcrossing to cultivated

wheat (**Friebe et al., 1996**). Amphiploids from interspecific crosses between tetraploid wheats and Aegilops species, as their close relatives, are useful bridging germplasm for introduction of desirable alien characters to bread wheats (**Friebe et al., 1996**). Egypt is very rich in habitat diversity due to the diversity in its geomorphology, topography and climate. This has aided the survival of a diverse plant species in the wild. Amongst the Egyptian flora, there are some of the most important food crops such as wheat and its wild close relatives. These species represent a large reservoir of useful characteristics that can be exploited for wheat improvement. Many agronomically interesting characterisics, comprising resistance to biotic and abiotic stresses have been transferred from these species to wheat (**Jiang et al., 1993; Friebe et al., 1996**). Comparative study of microsatellite diversity in wheat germplasm among a wide range of world areas indicated that the greatest genetic diversity originated from Iran (**Huang et al., 2002**). Traditionally, germplasm has been characterized based on agronomic and morphological studies, but recently use of molecular markers to study diversity of crop species has become common. DNA markers have the advantage of directly detecting sequence variation among cultivars. The use of Inter simple sequence repeats, ISSRs and is routine method for quickly and efficiently estimating relationships between lines and populations of many plant species. ISSR is an efficient, reproducible technique (**Vos et al., 1995 and Sofallan et al., 2009**).

ISSR has been widely used to discriminate between different accessions of a number of plants species including *T. aestivum* L. (**Barrett and Kidwell 1998; Barrett et al., 1998; Bohn and Melchinger, 1999; Ridout and Donini,1999; Soleimani et al., 2002; Almanza Pinzon et al.,2003**).

Quantification of genetic diversity in cultivated and wild crops has important implications for breeding programs and for the conservation of genetic resources. The primary objective of this study was to understand the extent and pattern of genetic diversity among diploid and polyploid wild species of wheat using ISSR marker.

Materials and Methods

Plant materials

A collection of seven Triticum, Rye and Triticale accessions belonging to one diploid Rye (*Secale cereale*), three belonging to *T. durum*, one belonging to *Triticum aestivum* and two belonging to Triticale were also used in this study, Table (1).

Sodium dodecyl sulfate (SDS) - Polyacrylamide gel electrophoresis analysis

The extraction of proteins was carried out in accordance with **Galli and Feldman (1983)**. The relative mobilities of the subunits were obtained by SDS-PAGE according to **Laemmli (1970).** Low molecular weight standard proteins (97.000, 66.000, 43.000, 30.000 and 20.100) were used. Gels with 10 per cent acrylamide, were stained in mixture of methanol: acetic acid: water (5:1:5) containing 0.2% of Comassie Brilliant Blue R250 (Sigma), and destained in methanol: acetic acid: water (5:1:5). HMW glutenins were designated according to **Payne and Lawrence (1983)** and LMW glutenins according to **Nieto-Taladriz et al. (1997).**

ISSR analysis

The experiment was conducted in the Biotechnology Laboratory, Department of Genetics and genetic engineering, Faculty of Agriculture, Benha University. Seven wheat and close relatives' accessions were used in this study (Table 1). Coding numbers are used according to the order of collection.

Table 1. Wild wheat relatives (Triticum and Secale accessions), synthetic wheat lines, durum and bread wheat cultivars used for diversity study.

S/n	Species	Accessions	Genome	Growing status
1	Secale cereale	Petikus	R	Experimental
2	Triticum durum	Baniswef 1	AB	commercial
3	Triticum durum	Baniswef 3	AB	commercial
4	Triticum durum	Sohag 3	AB	commercial
5	Triticum aestivum	Giza 168	ABD	commercial
6	Triticale	Triticale 1	ABR	Experimental
7	Triticale	Triticale 3	ABR	Experimental

Total Genomic DNA Extraction

DNA was extracted from wheat young leaves (**Saghai-Maroof et al., 1984**). The extracted DNA was diluted to obtain a final concentration of 25 ng/µL in order to use it in the PCR amplification.

PCR amplification

The ISSR amplification was carried out in a 25µL volume, according to Hoisington et al., 1994. The amplifications were performed in a BioRad thermocycler. The PCR products were detected by 1.6% agarose gel electrophoresis that was stained with

ethidium bromide. Then, the PCR products were visualized in a ultraviolet light using transluminator. In order to better distinguish the bands we used the molecular ladder contained known fragments.

Data analysis

The PCR product bands were scored as [1] for the presence and [0] for absence. The obtained data were used for analyses of genetic associations in the examined wheat material. A similarity matrix was constructed using the NTSYS-pc (Numerical Taxonomy and Multivariate Analysis for personal computers) software, version 2.1 (**Rohlf, 2000**). For all pairs, wise comparisons were done, according to Jaccard's similarity coefficient. A dendrogram was constructed from the similarity matrix using the UPGMA method (Unweighted Pair-Group Method with Arithmetical Averages) and the SAHN subprogram (Sequential, Agglomerative, and Hierarchical and Nested clustering).

Table 2. ISSR primers (and their sequences) which produced polymorphisms across three durum wheat cultivars, one bread wheat cultivar, two triticale cultivars and one rye cultivar, respectively.

Primer	Sequence
ISSR- 1	5'-AGAGAGAGAGAGAGAGTC-3'
ISSR- 2	5'-AGAGAGAGAGAGAGAGTG-3'
ISSR- 4	5'-ACACACACACACACACTG-3'
ISSR- 5	5'-GTGTGTGTGTGTGTGTAG-3'
ISSR- 11	5'-ACACACACACACACACTA-3'
ISSR- 12	5'-ACACACACACACACACTC-3'
ISSR- 13	5'-AGAGAGAGAGAGAGAGTT-3'
ISSR- 14	5'-CTCCTCCTCCTCCTCTT-3'
ISSR- 15	5'-CTCTCTCTCTCTCTCTRG-3'
ISSR- 18	5'-GAGCACACACACACACAT-3'
ISSR- 20	5'-GAGTGTGTGTGTGTGTGT-3'

Molecular fingerprints based on SDS- proteins:

SDS-PAGE: Data presente1d in Fig. 1 and Table 2 summarized the SDS-PAGE of soluble proteins for the seven accessions of wheat. Analysis of gel revealed that molecular weight of protein subunits ranged between 11.99 to 72.00 kDa.

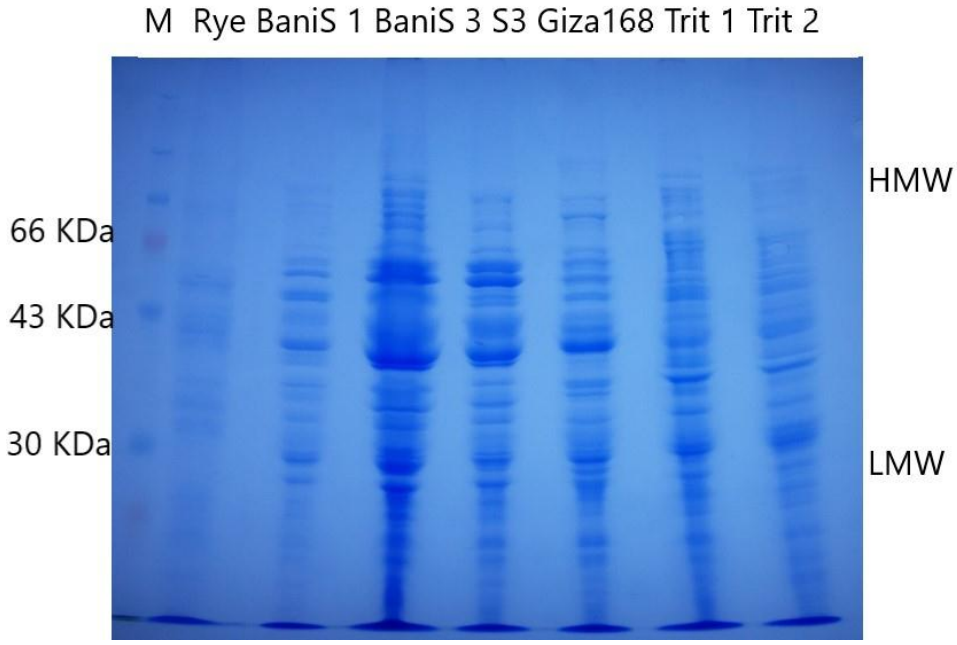


Fig. (1): SDS-PAGE profiles of soluble proteins extracted from seven genotypes of wheat and close relatives.

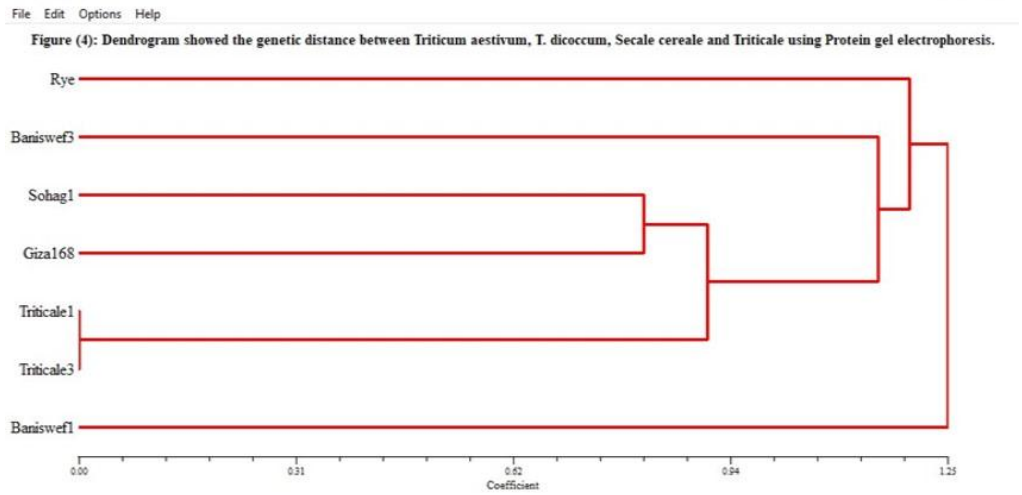


Fig. 2. Dendrogram based on UPGMA of Jaccard's similarity matrix represents the genetic relatedness among *T aestivum, T. dicoccum, Secale cereale and Triticale* cultivars based on the analysis of Protein electrophoresis banding patterns.

Table 3. Revealed the molecular weights of the soluble proteins extracted from seven genotypes of wheat and close relatives.

M W.	Rye	B 1	B 3	S 3	G 168	T 1	T 2	
158	0	0	1	0	0	0	0	Unique band
137	0	1	0	0	1	1	1	Polymorphic bands
129	0	0	1	0	1	0	0	Polymorphic bands
126	0	0	1	0	0	1	1	Polymorphic bands
124	0	0	1	0	0	1	1	Polymorphic bands
116	0	0	0	0	0	1	1	Polymorphic bands
114	0	1	1	0	0	0	0	Polymorphic bands
110	0	0	0	1	1	1	1	Polymorphic bands
107	1	1	1	0	0	0	0	Polymorphic bands
102	1	1	1	1	1	1	1	Common bands
98	0	1	1	1	1	0	0	Polymorphic bands
94	0	0	0	0	0	1	1	Polymorphic bands
86	0	1	1	1	1	1	1	Polymorphic bands
79	0	1	1	1	1	1	1	Polymorphic bands
74	1	1	1	1	1	1	1	Common bands
70	1	0	0	0	0	1	1	Polymorphic bands
67	0	1	1	1	1	1	1	Polymorphic bands
64	0	0	0	1	0	0	0	Unique band
62	1	0	0	0	0	0	0	Unique band
58	1	1	1	1	1	1	1	Common bands
55	1	0	0	0	0	0	0	Unique band
52	1	1	1	1	1	1	1	Common bands
49	1	1	1	1	1	0	0	Polymorphic bands
46	0	1	1	1	1	1	1	Polymorphic bands
42	1	1	1	1	1	1	1	Common bands
41	0	0	1	0	0	0	0	Unique band
39	0	0	0	0	1	1	0	Polymorphic bands
37	1	1	1	1	1	1	1	Common bands
34	1	1	1	1	1	1	1	Common bands
32	1	0	0	1	1	0	0	Polymorphic bands
30	0	1	1	1	1	1	1	Polymorphic bands
28	0	1	1	1	1	1	0	Polymorphic bands
25	0	1	1	1	1	1	1	Polymorphic bands
21	0	1	0	0	0	1	1	Polymorphic bands
19	0	0	0	1	1	1	1	Polymorphic bands
17	0	1	0	0	0	0	0	Unique band
Total	13	21	23	20	22	24	22	145

Table 4. Nei's genetic identity between Wheat species, Secale cereale and Triticale for SDS-PAGE patterns of soluble proteins extracted from seven accessions.

	Rye	Banyswef 1	Banyswef 3	Sohag 1	Giza 168	Triticale1	Triticale2
Rye	1.00						
Baniswef 1	0.38	1.00					
Baniswef 3	0.38	0.57	1.00				
Sohag 1	0.50	0.52	0.61	1.00			
Giza 168	0.47	0.50	0.50	0.81	1.00		
Triticale1	0.41	0.52	0.61	0.75	0.61	1.00	
Triticale2	0.41	0.52	0.61	0.75	0.61	1.00	1.00

Thirteen bands were obtained in rye. Twenty-one bands were detected in Baniswef. Twenty third bands were found in Baniswef 3. Sohag 1 revealed twenty bands. Giza 168 and Triticale 2 showed 22 bands. The highest number of bands (24) was observed in Triticale 1. Gliadin and glutenin, two storage protein groups, have been recognized in the endosperm (**Wall, 1979 and Rodriguez-Quijano et al.,(2010)**. Gliadin and glutenin are synthesized on the endoplasmic reteculum in the developing endosperm. Both of them are deposited in the developing endosperm. Both of them are deposited in protein bodies (**Shewry and Niflin, 1985**). Glutenins are considered as the major determinants of elesticity (**Tatham et. Al., 1985**). The analysis revealed that higher variability between the electrophoretic bands in the 7 wheat accessions. The study showed an electrophoretic variability of the proteins in the three original tetraploid parents; Baniswaf 1 and 3 in addition to Sohag 3 and one hexaploid bread wheat variety. Geat variation between Petkus and the hexaploid triticale 1 and 2. Data resulted from this study can be useful for the breeding of wheat including one or more rye chromosomes. Moreover, it is very important to predict the quality of the resulted proteins in breeding programes (**Mahgoub, 1988; Bakheet, 1990 and Demais et al., 2018**).

Results

All 11 primers generated 153 bands of different sizes for 7 accessions of the genus Wheat and its close relatives with an average of 14 bands per primer, (Table 2). ISSR pattern obtained with the eleven primers are shown in Fig. 4. Maximum number of fragments (25 bands) was found with primer ISSR 15, whereas the smallest number (8 bands) was generated by primer ISSR 11. The sum of 65 out of 153 fragments was polymorphic, with 42.84 % of mean percentage of polymorphic bands (PPB). The number of polymorphic bands varied from 13 (for primer ISSR 15) to 1 (for primer ISSR 18). The potential of ISSR markers to generate genetic information through polymorphic fragments depends on the microsatellite frequency and their distribution in the genome wide scale of the species **(Morgante et al., 1993, Bekhit 2007a and b and Bekhit et al., 2007c).** In agreement of this result, in the study of microsatellite primers in wheat it was assumed that the polymorphism rate would be higher when the motifs are composed of three or four nucleotides (**Song et al., 2002**).

Table 5. List of ISSR primers including nucleotide sequences, number of monomorphic and polymorphic bands, total bands and percentages of polymorphism for ISSR primers among seven accessions of wheat species, rye and triticales.

No.	*ISSR primers*	Total bands	No. of polymorphic bands	% of polymorphism	Monomorphic bands	Unique bands	Mean of band frequency
1	***ISSR- 1***	14	9	86 %	2	3	0.6
2	***ISSR- 2***	13	4	77 %	3	6	0.5
3	***ISSR- 4***	12	5	83 %	2	5	0.5
4	***ISSR- 5***	12	7	92 %	1	4	0.5
5	***ISSR- 11***	8	1	38 %	5	2	0.7
6	**ISSR- 12**	11	1	36 %	7	3	0.7
7	***ISSR- 13***	18	11	94 %	1	6	0.4
8	***ISSR- 14***	17	9	82 %	3	5	0.5
9	***ISSR- 15***	25	13	84 %	4	8	0.5
10	***ISSR- 18***	10	1	20 %	8	1	0.9
11	***ISSR- 20***	12	4	58 %	5	3	0.6
	Total	153	65		41	46	

The highest number of ISSR fragments was obtained for Secale cereale (92), whereas the lowest (79) was for Triticum durum (Sohag 3). At the species level, the highest percentage of polymorphism loci (92 %) was observed in Triticale while the lowest (54 %) was observed in Rye (Table 3).

Table 6. Genetic distances, as the total number of ISSR band differences, among the studied *Triticum aestivum, T. dicoccum, Secale cereale and Triticale* genotypes after using twelve primers.

	Rye	Banyswef 1	Banyswef 3	Sohag 1	Giza 168	Triticale1	Triticale2
Rye	1.00						
Banyswef 1	0.61	1.00					
Banyswef 3	0.60	0.90	1.00				
Sohag 1	0.58	0.80	0.83	1.00			
Giza 168	0.59	0.81	0.80	0.80	1.00		
Triticale1	0.54	0.75	0.75	0.75	0.83	1.00	
Triticale2	0.55	0.78	0.75	0.74	0.87	0.92	1.00

The cophenetic coefficient of ISSR clustering from Jaccard's similarity matrix was equal to 0.9968 (p = 0.002). Therefore, to infer phylogenetic relationships, the 0/1 matrix was subjected to the construction of similarity matrix using Jaccard's coefficients. The unweighted pair group method with arithmetic mean (UPGMA) dendrogram based on Jaccard's coefficients indicated that 7 accessions were divided into one major cluster with a separated rye. The major cluster was divided into two main clusters. The first major cluster was divided into 2 subclusters (A and B). The subcluster A was composed of three accessions of Triticum durum (Beniswaf 1 and Baniswef 3 in a subcluster and Sohag 3 separated in a subcluster). the subcluster B having 3 accessions of tritcum aestivum (Giza 168 separated in a subcluster) and Triticale (Triticale 1 and 2 in a subcluster), (Fig. 2). Similarity of *T. durum* and *T. aestivum* accessions was from 0.21 (between 3 and 3 accessions) to 0.12 (between accessions 1 and 2).

Our results confirmed that the ISSR markers had the potential to detect genetic variability in Tetraploid wheat, hexaploid wheat, Rye and triticale varieties (Fig. 1). According to these results, it was confirmed that the ISSR molecular markers could properly differentiate wheat varieties with genomes A, B and D from Genome R in rye and triticale and could be used in variety identification purposes.

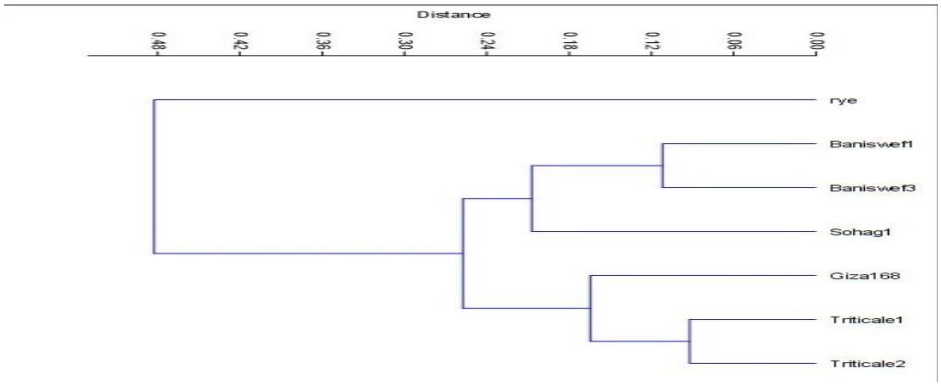


Fig. 2. Dendrogram of seven wheat species, rye and triticale based on Jaccard genetic similarity coefficients using 11 ISSR polymorphisms.

The molecular markers are commonly used in studies of population structure, genetic diversity for pre-breeding and breeding germplasms, and in distinguishing one individual genotype to preserve the property of breeding rights. ISSR technique has been proven to be useful in population genetic diversity studies (**Lage et al., 2003; Arzani et al., 2005 and Reif et al., 2005 and Shoaib and Arabi (2006))**.

In this study, we found a high degree of polymorphism in the Triticum accessions studied (with values in range of 20–94 and a mean of 42.48 %) (Table 2). These primers also were showed high polymorphism in other species. Therefore, these primers may be useful to investigate the genetic diversity of other members of poaceae or Gramineae family.

The UPGMA dendrogram based on Jaccard's coefficients indicated that 7 accessions were divided into two major clusters (Fig. 1). All species in first major cluster have tetraploid wheat with the two genomes, while the species of second cluster have hexaploid wheat and triticale with the three genomes.

Conclusion

In conclusion, ISSR is an effective and promising molecular marker system for detecting genetic variation in wheat species. Our data provide evidence

of a genetic diversity between the tested wheat, rye and triticale spp. accessions. The results obtained in this study indicated the highest identity between Triticale 1 and Triticale 2 and the lowest identity between Triticum durum and Secale cereale. Furthermore, the results showed an increased distance between the accession 1 and other accessions of Triticum species. Overall, this study demonstrated that the ISSR is a useful tool in Wheat genomic diversity studies and to detect their relationships.

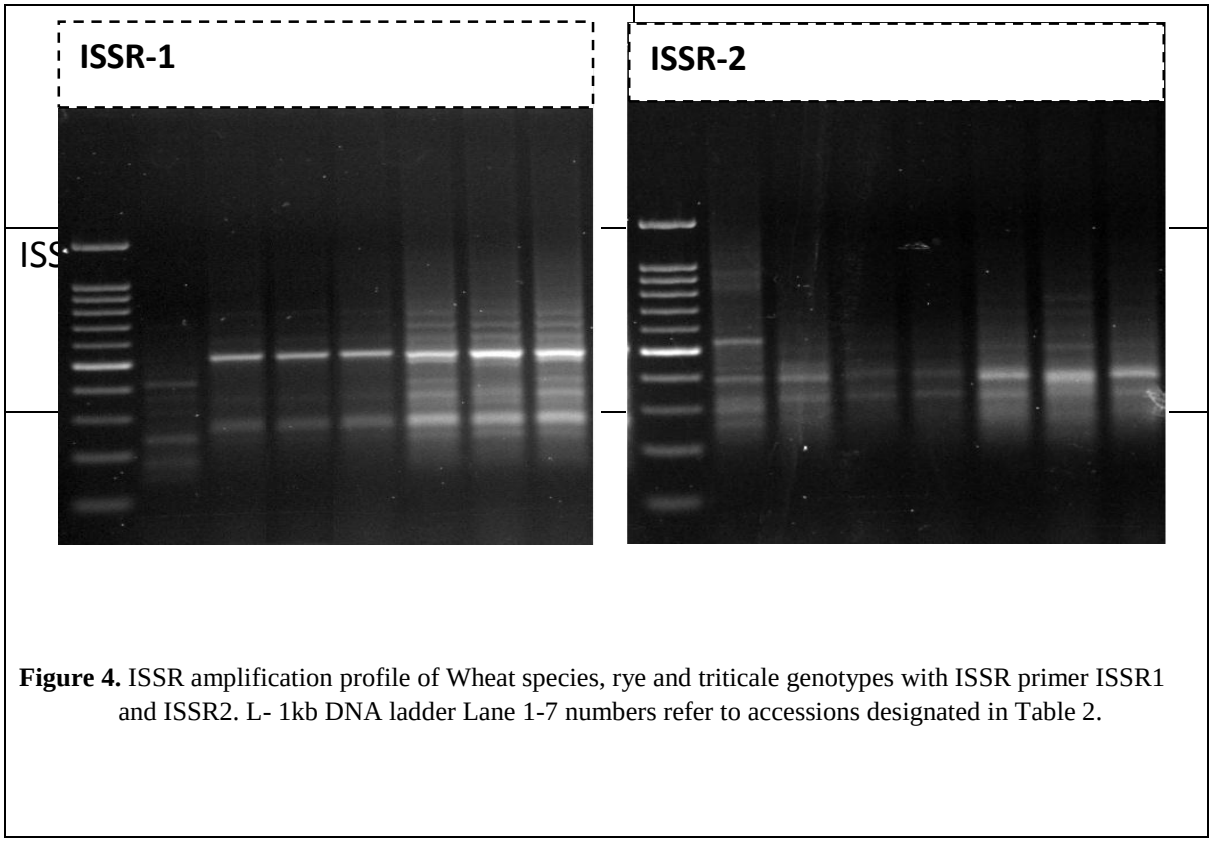
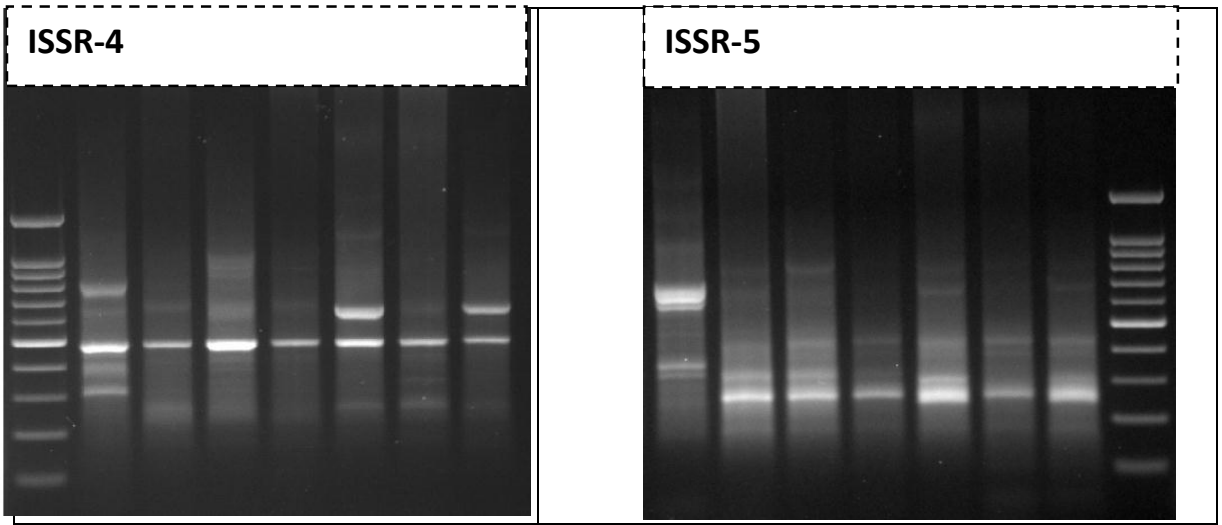
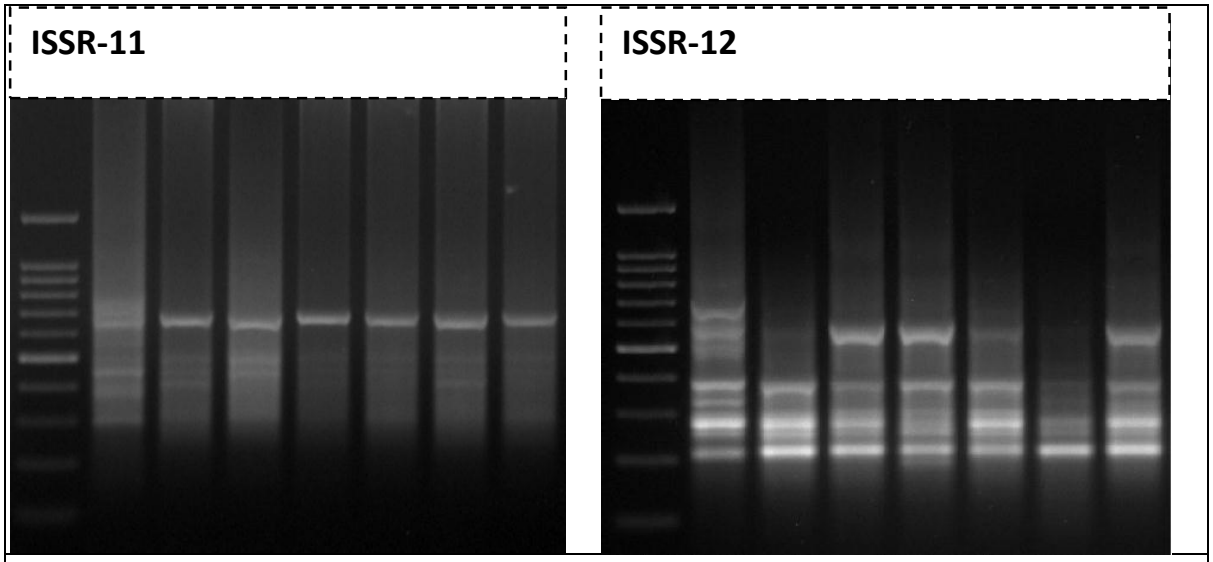


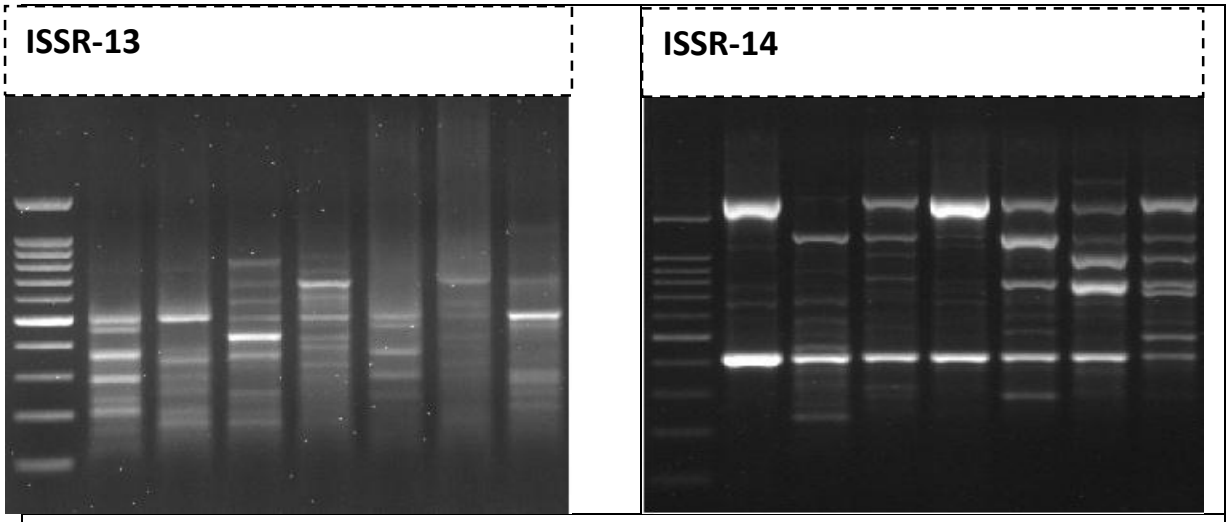
Figure 4. ISSR amplification profile of Wheat species, rye and triticale genotypes with ISSR primer ISSR1 and ISSR2. L- 1kb DNA ladder Lane 1-7 numbers refer to accessions designated in Table 2.



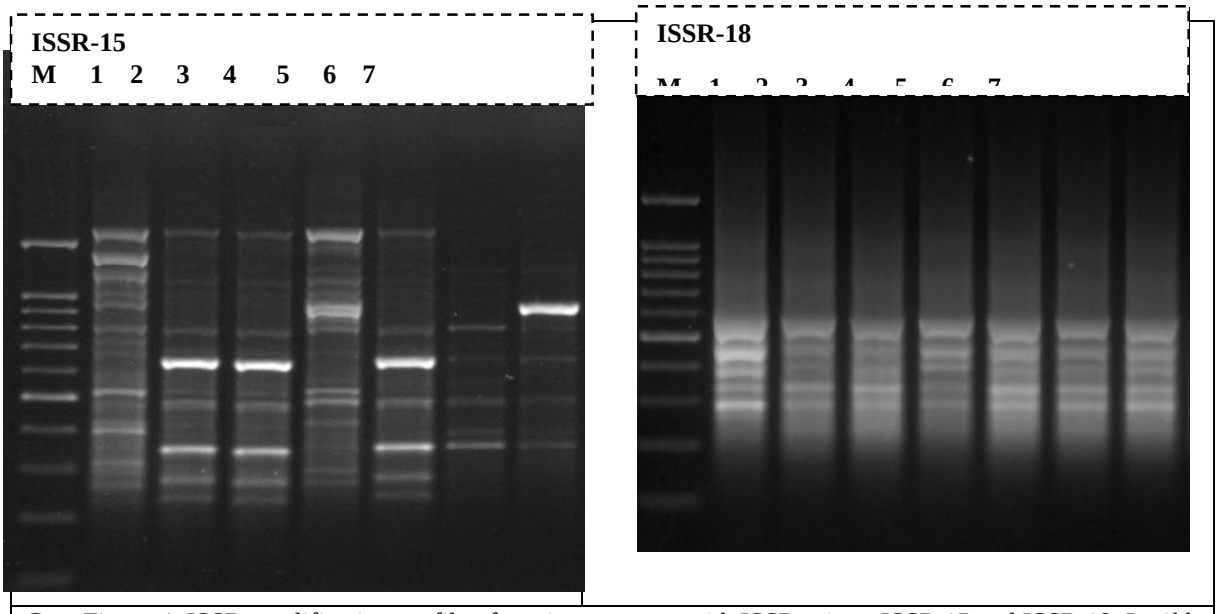
Cont Figure 4. ISSR amplification profile of Wheat species, rye and triticale genotypes with ISSR primer ISSR5 and ISSR6. L- 1kb DNA ladder Lane 1-7 numbers refer to accessions designated in Table 2.



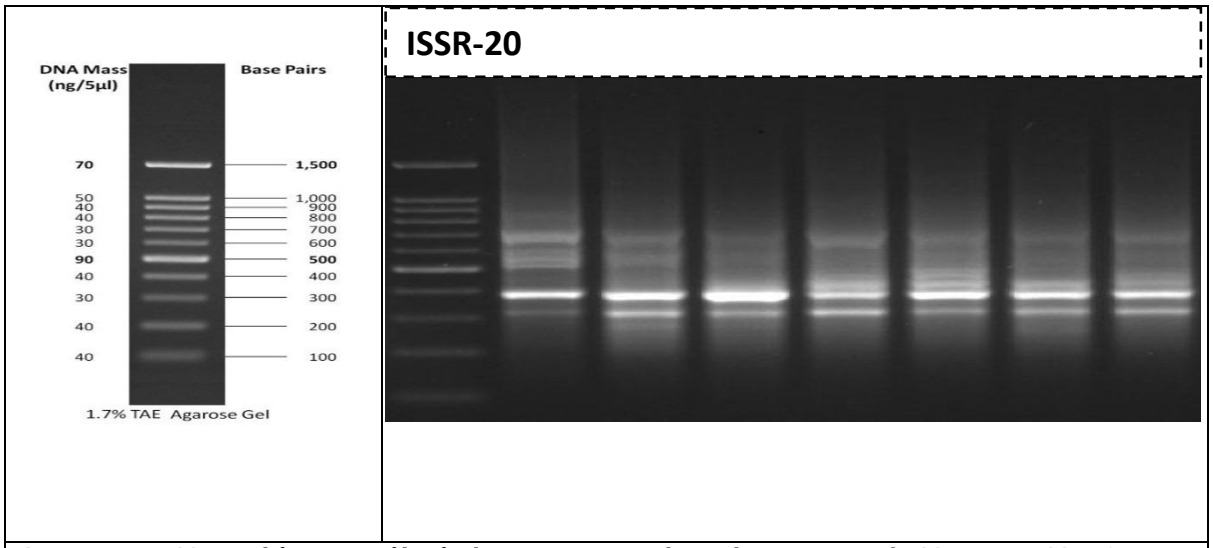
Cont' Figure 4. ISSR amplification profile of Wheat species, rye and triticale genotypes with ISSR primer ISSR11 and ISSR 12. L- 1kb DNA ladder Lane 1-8 numbers refer to accessions designated in Table 2.



Cont Figure 4. ISSR amplification profile of Wheat species, rye and triticale genotypes with ISSR primer ISSR 13 and ISSR 14. L- 1kb DNA ladder Lane 1-7 numbers refer to accessions designated in Table 2.



Cont Figure 4. ISSR amplification profile of stevia genotypes with ISSR primer ISSR 15 and ISSR 18. L- 1kb DNA ladder Lane 1-7 numbers refer to Wheat species, rye and triticale designated in Table 2.



Cont Figure 4. ISSR amplification profile of Wheat species, rye and triticale genotypes with ISSR primer ISSR 20.

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تقدير التنوع الوراثي بين بعض أصناف القمح السداسى و الرباعى و الراى و التريتيكال باستخدام تكنيك تكرارات التتابعات البينية البسيطة (ISSR) وتفريد البروتين SDS–PAGE.

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تم تقدير التنوع الوراثي بين بعض التراكيب الوراثية للقمح و الاجناس القريبة (الراى و التريتيكال) باستخدام تكنيك تكرارات التتابعات البينية البسيطة (ISSR) وتفريد البروتين SDS–PAGE. تم تقييم ثلاثة أصناف من القمح الرباعى "بنى سويف ١ و ٣ وسوهاج ٣" ، وهى أصناف قمح محلية تنمو في صعيد مصر بالإضافة الى الصنف "جيزة ١٦٨" و هو صنف قمح خبز و سلالتان من التريتيكال بجانب سلالة الراى بيتيكا. تم تقدير التنوع الوراثي بين هذه التراكيب الوراثية باستخدام ١١ واسم ISSR. من حوالي ١٥٣ حزمة ISSR تم الكشف عنها ، كانت هناك ٦٥ حزمة (٤٢٪) متعددة الأشكال بمتوسط ٤ حزم لكل واسم. تم تحليل الشجرة الوراثية للتراكيب الوراثية السبعة التابعة لنوعى القمح و اجناس الراى و التريتيكال بواسطة تحليل مجموعة UPGMA بالاستناد إلى تقديرات درجة تشابه Jaccard لبيانات ISSR بتقسيم جميع التراكيب الوراثية إلى مجموعة رئيسية واحدة بينما انفصلت سلالة الراى Secale عن المجموعة الرئيسية. عكست تحت المجموعتان الرئيسيتان تقريبا التكوين الجينومى لكل منهما حيث تضمنت تحت المجموعة الأولى أنواع القمح التي تحتوي على الجينومات AB 1 (بنى سويف ١ و ٣ و سوهاج ٣) ، بينما تضمنت تحت المجموعة الثانية أنواع القمح التي تحتوي على جينومات ABD و ABR. تراوحت معاملات التشابه الوراثي من ٠.٣٦ بين Triticale 1 وسلالة الراى و ٠.٨٤ بين (Triticale 1 و (Triticale 2). كشفت نتائج SDS–PAGE عن عدد إجمالي يبلغ ١٤٥ حزمة بأوزان جزيئية تتراوح ما بين حوالي ١٧ كيلو دالتون و ١٥٨ كيلو دالتون ، والتي لم تكن موجودة بالضرورة في التراكيب الوراثية السبعة. كشفت المعطيات عن وجود سبعة حزم شائعة (monomorphic) ، وستة حزم متفردة بينما كان الحزم ال ٩٠ الباقية متعددة الأشكال بنسبة ٦٢٪ تعدد الأشكال. الحزم المتفردة من نوعها في الوزن الجزيئي ١٥٨ و ٤١ كيلو دالتون كانت الحزمة المميزة لبني سويف ٣. كانت الحزمة المتفردة من نوعها في الوزن الجزيئي ٦٤ كيلو دالتون هي الحزمة المميزة في سوهاج ٣. تم اكتشاف الحزم المتفردة من نوعها في الوزن الجزيئي ٦٢ و ٥٥ كيلو دالتون فقط في الراي. تم اكتشاف الحزمة المتفردة عند الوزن الجزيئي ١٧ كيلو دالتون فقط في التركيب الوراثى بنى سويف ١.