NARDI FUNDULEA, ROMANIA
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ROMANIAN AGRICULTURAL RESEARCH, NO. 34, 2017
Print ISSN 1222-4227; Online ISSN 2067-5720

GENETIC SIMILARITY AMONG *AEGILOPS KOTSCHYI* BOISS. WITH *TRITICUM AESTIVUM* L. HYBRID LINES

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ABSTRACT

The University of Life Sciences in Lublin carried out a programme of crossing *Triticum aestivum* L. with wild species of the genus *Aegilops* in order to increase genetic variability in wheat and to improve its quality and resistance to environmental stresses. In this study the ISSR method was used to estimate genetic similarity in F_4 and F_5 *Ae. kotschyi* Boiss. × *T. aestivum* L. cv. Rusałka hybrid lines (KR3, KR4, KR6, KR9) and BC_1F_2 (*Ae. kotschyi* Boiss. × *T. aestivum* L. cv. Rusałka) × *T. aestivum* L. cv. Begra hybrid lines (KRB) and to identify DNA of the wild species in the wheat background. PCR was performed using 16 ISSR primers. A total of 271 bands were obtained. The primers amplified between 7 (ISSR35) and 27 (ISSR16) DNA fragments. The average number of polymorphic products was 15.5 per primer – from 2 (ISSR1) to 27 (ISSR16). Fourteen primers amplified 30 products identifying *Ae. kotschyi* Boiss. chromatin in the wheat background. Polymorphism information content (PIC) values for individual primers ranged from 0.173 to 0.319, with a mean of 0.244. The presence of *Ae. kotschyi* Boiss. - ISSR markers was proved in the case of all the *Ae. kotschyi* Boiss. × *T. aestivum* L. hybrid lines. $ISSR33_{650}$ and $ISSR23_{690}$ markers were detected in the greatest number of hybrids (6 and 8). Unweighted Pair Group Method with Arithmetic Mean (UPGMA) analysis demonstrated that the hybrid lines and parental wheat cultivars had a similarity range from 0.73 to 1.00. *Ae. kotschyi* Boiss. showed 0.19-0.32 similarity to other forms. The hybrid lines were separated into two main clusters: one with two KRB hybrid lines and the Begra cultivar, and the second, more genetically diverse group, comprising the remaining hybrid lines and the Rusałka cultivar. *Ae. kotschyi* Boiss., was not directly linked to any of these groups. Principal Coordinate Analysis was in good agreement with the UPGMA.

Key words: *Aegilops*, germplasm characterization, wheat, ISSR markers.

INTRODUCTION

Genetic diversity is the basis for plant breeding. In the last years, the biodiversity of cultivated wheat varieties significantly decreased (Fu and Somers, 2009). Wild relatives of wheat, such as *Ae. kotschyi* Boiss., can be the source of new resistance genes to abiotic and biotic stresses. Hybrids of wheat with *Ae. kotschyi* Boiss. are more resistant to fungal pathogens, drought and salinity (Shimshi et al., 1982; Thiele et al., 2002; Spetsov, 2004; Marais et al., 2005). In earlier investigations on *Ae. kotschyi* Boiss. × *T. aestivum* L. hybrid lines their quantitative morphological and qualitative features were determined (Prażak and Paczos-Grzęda, 2013). Grains of the hybrid lines had much more protein, and Fe and Zn micronutrients than wheat grains (Rawat et al., 2009; Prażak and Skrzypik, 2010; Prażak and Paczos-Grzęda, 2013).

In practical plant breeding, an important role is played by fast evaluation of breeding materials that determines their genetic diversity, as this makes it possible to select the most genetically diverse breeding materials and to exclude repeating forms from the collection. Due to the very large number of breeding lines and cultivars assembled in gene banks, which in many cases exhibit a high degree of genetic similarity, evaluation of their genetic diversity based on morphological markers has become inadequate. For this reason molecular markers are currently being used on a wide scale. Among the various molecular marker techniques, inter-simple sequence repeat polymorphic DNA (ISSR) has been widely

Received 21 December 2015; accepted 7 February 2017. First online: March, 2017. DII 2067-5720 RAR 2017-169

used for genetic diversity analysis, genetic tagging and phylogenetic studies on wheat species and the closely related *Aegilops* species (Reddy et al., 2000; Ammiraju et al., 2001; Carvalho et al., 2005; Liu et al., 2002; Thuillet et al., 2002; Goryunova et al., 2004; Balyan et al., 2005; Galaev et al., 2006; Landjeva et al., 2006; Hovhannisyan et al., 2011; Deshmukh et al., 2012; Abou-Deif et al., 2013). Due to its good level of repeatability, simplicity of execution and the small amount of DNA required, ISSR-PCR is a suitable technique for analysing plant genotypes. The main aim of the present paper was to estimate genetic similarity to *Ae. kotschyi* Boiss. in the *Ae. kotschyi* Boiss. × *T. aestivum* L. hybrid lines.

MATERIAL AND METHODS

Plant material and DNA extraction

Eight F_4 and F_5 *Ae. kotschyi* Boiss. × *T. aestivum* L. cv. Rusałka hybrid lines (KR3, KR4, KR6, KR9) and two BC_1F_2 (*Ae. kotschyi* Boiss. × *T. aestivum* L. cv. Rusałka) × *T. aestivum* L. cv. Begra hybrid lines (KRB) originated from breeding program for improvement of common wheat *T. aestivum* L. in crossing with *Ae. kotschyi* Boiss. (Prażak and Paczos-Grzęda, 2013). Seeds of the analysed forms were obtained from Faculty of Bioeconomy, University of Life Sciences in Lublin, Poland. DNA was extracted from lyophilized leaves into a microfuge tube (1.5 ml) using a modification of a procedure by Milligan (1992). Genomic DNA was extracted from 15-20 coleoptiles from seedlings in 2 replications.

ISSR analysis

PCR ISSR analysis was performed by a modified procedure based on Ziętkiewicz et al. (1994). The PCR reaction mixture of 15 µl contained 1× PCR buffer (10 mM Tris, pH 8.8; 50 mM KCl; 0.08% Nonidet P40); 160 µM of each dNTP; 470 pM of each primer; 1.5 mM $MgCl_2$; 0.5 units of Taq DNA polymerase; and 60 ng template DNA. Sixteen primers were tested for PCR ISSR analysis: ISSR1: 5'- (AG)8G - 3', ISSR6: 5'- (GT)8C - 3', ISSR11: 5'-(AC)8G - 3', ISSR14: 5'- (GA)7YG - 3', ISSR16: 5'- (GA)8C - 3', ISSR17: 5'- (GA)8YC - 3', ISSR22: 5' - (CA)8G – 3', ISSR23: 5' - (CA)8GC – 3', ISSR27: 5'- (TC)8G - 3', ISSR28: 5'- (TG)8G - 3', ISSR33: 5'- (AG)8T - 3', ISSR34: 5'- (TC)8CC- 3', ISSR35: 5' - (TC)8CG – 3', ISSR36: 5'- (AC)8CG - 3', ISSR37: 5'- (AC)8C - 3', ISSR38: 5'- (CT)8G - 3'.

Amplifications were carried out in a T1 Biometra thermal cycler with an initial denaturation step at 95ºC for 7 minutes followed by amplification for 38 cycles with denaturation at 95ºC for 30 s, annealing for 3 cycles at 54ºC for 45 s, followed by 3 cycles at 53ºC for 45 s and 32 cycles at 52ºC for 45 s, and extension at 72ºC for 2 minutes, with a final extension step at 72ºC for 7 minutes. The amplified products were separated by electrophoresis in 2.5% agarose gel in 1 × TBE buffer containing 0.01% ethidium bromide, in the presence of size markers. The DNA marker GeneRulerTM 100 bp Plus DNA Ladder was used. Separated DNA fragments were visualized under ultraviolet light and photographed.

Data analysis

To estimate the value of the marker system, the PIC (polymorphism information content) value was calculated (Nei, 1973). The results of the molecular analysis were evaluated using a binary matrix in which values of 1 and 0 indicated the presence or absence of the product, respectively. The data matrix was used to calculate the genetic similarity index between pairs of all the genotypes analysed, by means of the Dice formula (Nei and Li, 1979). Genetic relationships were estimated using UPGMA (Unweighted Pair Group Method with Arithmetic Mean) cluster analysis based on genetic similarity indices. NTSYS-pc 2.10q software was used for the calculations (Rholf, 2001). Principal coordinate analysis (PCoA) was calculated by XlStat v. 2014.1.01 Excel add-in software.

RESULTS AND DISCUSSION

The primers used in the ISSR analysis generated a total of 271 bands. Individual oligonucleotides generated from 7 (ISSR35) to 27 (ISSR16) DNA fragments, with a mean of 16.9 per primer. DNA fragment

size ranged between 120 and 2600 bp. The sixteen primers generated 248 polymorphic bands (91.5%), which made it possible to determine the genetic similarity between the tested forms. Individual primers generated from 2 (ISSR1) to 27 (ISSR16) polymorphic bands, with a mean of 15.5 per primer (Table 1).

Table 1. Characteristics of ISSR primers and products

Primer	Sequence	Number of bands				Band size range (bp)	PIC
		Total	Polymorphic	From *Ae. kotschyi*	Specific for *Ae. kotschyi*		
ISSR1	$(AG)_8G$	8	2	1	1	480-1380	0.248
ISSR6	$(GT)_8C$	21	19	3	6	450-1800	0.205
ISSR11	$(AC)_8G$	25	22	4	9	330-1800	0.270
ISSR14	$(GA)_7YG$	18	17	3	0	120-1600	0.319
ISSR16	$(GA)_8C$	27	27	1	9	280-1800	0.232
ISSR17	$(GA)_8YC$	21	19	1	5	220-1250	0.219
ISSR22	$(CA)_8G$	21	21	0	10	430-1800	0.186
ISSR23	$(CA)_8GC$	16	15	2	2	280-1030	0.284
ISSR27	$(TC)_8G$	9	8	1	2	420-1700	0.219
ISSR28	$(TG)_8G$	13	13	1	6	380-1600	0.197
ISSR33	$(AG)_8T$	21	21	3	1	260-1370	0.298
ISSR34	$(TC)_8CC$	17	16	3	4	540-2600	0.291
ISSR35	$(TC)_8CG$	7	7	2	0	680-2600	0.304
ISSR36	$(AC)_8CG$	17	15	0	5	380-2600	0.173
ISSR37	$(AC)_8C$	18	17	3	6	290-1350	0.203
ISSR38	$(CT)_8G$	12	9	2	3	430-1700	0.260
Sum/size range		271	248	30	69	120-2600	0.173-0.319
%		100	91.5	11.1	25.5	-	-
Mean		16.9	15.5	1.9	4.3	-	0.244

Abou-Deif et al. (2013), who analysed genetic diversity and relationships between hexaploid, tetraploid and diploid wheat, reported that eight ISSR primers produced 112 amplified DNA fragments ranging in size from 127 to 1857 base pairs; 17 fragments were monomorphic (15.2%) and 95 were polymorphic (84.8%), with a mean of 11.87 polymorphisms per primer. Kanbar and Kondo (2011), using 29 ISSR markers to analyse genetic distance between twenty cultivars of barley growing in Syria and Japan, obtained 238 bands, of which 177 (74.37%) were polymorphic; on average, the total number of bands generated per primer was 8.20, of which 6.10 were polymorphic. Matos et al. (2001), analysing the phylogenetic relationships of 10 rye landraces and cultivars, used 9 ISSR markers which produced a total of 342 bands, of which 280 were polymorphic (83%). Grądzielewska et al. (2010), in a study on genetic similarity in triticale hybrids with *Aegilops crassa* (4x), identified 220 ISSR products (15.7 fragments per primer), of which 34% were polymorphic. In another study by Grądzielewska et al. (2012), to determine genetic similarity in triticale × *Aegilops juvenalis* (Thell.) Eig hybrids, 14 ISSR primers amplified 240 fragments. A total of 72 fragments were polymorphic (30%), with a mean of 5.1 fragments per primer. Qian et al. (2001), in a study on polymorphism in wild rice *Oryza granulata* Nees & Arn. ex G.Watt, obtained 40% polymorphic ISSR products, while Matos et al. (2001) obtained 82% in rye and Fernández et al. (2002) found 83% in barley. In a study by Paczos-Grzęda and Bednarek (2014) on polymorphism among oat species, the 19 ISSR primers used for DNA profiling

amplified 280 fragments (66.8%). Naghavi et al. (2009) reported that 21 SSR markers detected 273 fragments in *T. aestivum* L. and *Aegilops* L. species. The number of fragments per microsatellite marker varied from 3 to 27. Musilova et al. (2013), using 44 SSR markers, detected a total of 188 alleles in common wheat cultivars. Henkrar et al. (2015) informed that survey with 14 microsatellite loci in 20 Moroccan bread wheat revealed 59 alleles. The number of alleles per locus ranged from 1 for Xbarc263 to 8 for Xgwm577 with an average number of 4.21.

One basis for assessing the usefulness of a primer for revealing polymorphism and differentiating genotypes is polymorphism information content (PIC). In the present study the polymorphism information content (PIC) values for individual primers ranged from 0.173 to 0.319, with a mean value of 0.244 (Table 1). Landjeva et al. (2006), analysing SSR marker polymorphism in varieties of *T. aestivum* L. winter wheat, obtained a wide range of PIC values from 0.10 to 0.81. Musilova et al. (2013), evaluating a collection of wheat genotypes, also obtained a wide range of PIC values for 44 SSR markers, from 0.00 to 0.79 (mean 0.38). Naghavi et al. (2009) obtained PIC values for SSR markers in *T. aestivum* L., *Aegilops crassa* Boiss, *Ae. cylindrica* Host., and *Ae. tauschii* Coss. species ranging from 0.28 to 0.72 (mean 0.58). Vyhnánek et al. (2009) used 48 SSR markers in a study of genetic variability in 16 genotypes of triticale, and found an average PIC of 0.48. Nefzaoui et al. (2014), analysing microsatellite marker polymorphism in durum wheat varieties and landraces, obtained range of PIC values from 0.110 for Xgwm193 marker to 0.556 for Xgwm493 marker, with an average value of 0.363.

In a study by Grądzielewska et al. (2012), PIC values for ISSR markers in triticale × *Aegilops juvenalis* (Thell.) Eig hybrids ranged from 0.39 to 0.66, with a mean of 0.52. Similar mean PIC values for SSR markers in triticale cultivars were obtained by Tams et al. (2004) (0.54) and for rye by Shang et al. (2006) (0.60). Paczos-Grzęda and Bednarek (2014) reported that PIC values in oat species ranged from 0.28 to 0.44 (mean 0.35) for the ISSR method. Sarla et al. (2005), analysing 86 rice forms (cultivars, wild species and landraces), observed that PIC values for the ISSR method ranged from 0.63 to 0.92, on average 0.82. In a study by Grądzielewska et al. (2009), the PIC value estimated using 17 ISSR primers for a group of Greek populations of *Dasypyrum villosum* (L.) Candargy with high genetic similarity ranged from 0.15 to 0.52 (mean 0.33).

Powell et al. (1996), comparing the efficiency of several marker systems in soya (AFLP, RAPD, RFLP and SSR), like many other authors, obtained the highest PIC value for the SSR method (0.60). Li et al. (2000), using SSR primers designed for oat, noted an average PIC value of 0.57 (0.28-0.79) for 12 species of the genus *Avena* and 0.51 (0.10-0.85) for 20 *Avena sativa* L. oat cultivars. When the same authors analysed SSR primers designed for barley, they estimated the PIC value for species of the genus *Avena* at 0.55 (0.31-0.77), and for cultivars at 0.38 (0.15-0.51). An equally high PIC value was obtained by Spada et al. (2004) in rice.

In this study the presence of DNA fragments specific to *Ae. kotschyi* Boiss. in the *Ae. kotschyi* Boiss. × *T. aestivum* L. hybrid lines was confirmed by much more number of ISSR molecular markers (14) than in earlier studies (7) (Prażak and Paczos-Grzęda, 2013). Fourteen primers amplified 30 products identifying genetic material of *Ae. kotschyi* Boiss. in the *Ae. kotschyi* × *T. aestivum* L. hybrid lines (Table 2). The presence of markers specific for *Ae. kotschyi* Boiss. in the genotypes of the *Ae. kotschyi* Boiss. × *T. aestivum aestivum* L. cv. Rusałka and (*Ae. kotschyi* Boiss. × *T. aestivum* L. cv. Rusałka) × L. cv. Begra hybrid lines was confirmed by 2-15 DNA fragments (Table 2). In earlier studies (Prażak and Paczos-Grzęda, 2013) the presence of 1-9 DNA fragments specific to *Ae. kotschyi* Boiss. in the hybrid lines was confirmed by seven ISSR markers. Galaev et al. (2006) reported that SSR-analysis allowed them to characterize genome variability and detect 8 introgressive DNA fragments in *Triticum-Aegilops* Host. hybrid lines.

In our study, the presence of *Ae. kotschyi* Boiss. ISSR markers was proved in the case of

all the *Ae. kotschyi* Boiss. × *T. aestivum* L. hybrids (Table 2). $ISSR33_{650}$ and $ISSR23_{690}$ markers were detected in six and eight hybrid lines. The analysis confirmed earlier studies (Prażak and Paczos-Grzęda, 2013) that showed that $ISSR33_{650}$ and $ISSR23_{690}$ markers were the most effective for germplasm analysis of the hybrid lines. The $ISSR23_{690}$ marker was detected in eight hybrids and $ISSR33_{650}$ in six (Prażak and Paczos-Grzęda, 2013).

Table 2. ISSR markers identifying *Ae. kotschyi* Boiss. chromatin in wheat hybrid lines

Primer	Size of identified polimorphic ISSR fragments												
	Aegilops kotchyi	Rusałka	Begra	F_4 (KR3)	F_5 (KR3)	F_4 (KR4)	F_5 (KR4)	F_4 (KR6)	F_5 (KR6)	F_4 (KR9)	F_5 (KR9)	F_2 (KRB)	F_2 (KRB)
ISSR1	610			610	610								
ISSR 6	520					520							
	990			990		990			990				990
	1290			1290	1290								
ISSR11	620							620		620	620		
	890					890			890			890	890
	930					930		930	930				
	1470					1470	1470					1470	1470
ISSR14	280			280	280								
	340					340			340				
	1010				1010	1010							
ISSR16	555				555								
ISSR17	350				350		350						
ISSR23	455					455							
	690			690	690	690	690	690	690			690	690
ISSR27	690			690									
ISSR28	820							820	820				
ISSR33	380					380							
	650			650	650	650	650					650	650
	1360					1360	1360					1360	1360
ISSR34	1250			1250	1250								
	1400					1400			1400				
	1800			1800						1800	1800	1800	1800
ISSR35	2600			2600	2600				2600				
	2800				2800				2800	2800			
ISSR37	590				590								
	850							850	850				
	1040			1040	1040								
ISSR38	720					720			720				
	1070					1070			1070				1070
Number of products	30			11	13	15	5	5	12	3	2	6	8

In the present study $ISSR34_{1800}$ marker was detected in five hybrid lines. $ISSR6_{990}$, $ISSR11_{890}$, $ISSR11_{1470}$, $ISSR33_{1360}$ polymorphic bands were noted in four hybrids, and $ISSR11_{620}$, $ISSR11_{930}$, $ISSR35_{2600}$, $ISSR35_{2800}$, $ISSR38_{1070}$ in three. The remaining markers were present in one or two hybrid lines. The greatest number of ISSR markers was noted in the F_5 KR3 and F_4 KR4 hybrid lines (13 and 15 markers). Somewhat fewer ISSR polymorphic bands appeared in F_5 KR6 (12 markers), F_4 KR3 (11 markers), and

F_2 KRB (8 markers). Moreover, 2-6 polymorphic bands specific for *Ae. kotschyi* Boiss. were noted in the remaining hybrid lines. No markers were detected in case of the wheat cultivars (Table 2). In earlier studies (Prażak and Paczos-Grzęda, 2013) the $ISSR6_{990}$ polymorphic bands were also observed in four hybrids, and $ISSR35_{2800}$ and $ISSR35_{2600}$ in three. In these investigations the greatest number of ISSR markers was found in the F_5 KR3 and F_4 KR4 hybrid lines (9 markers) (Prażak and Paczos-Grzęda, 2013).

Grądzielewska et al. (2010, 2012) obtained 220 ISSR products, of which 20 were specific for *Aegilops crassa* (4x) Boiss., in triticale × *Ae. crassa* (4x) Boiss. hybrids, and 240 ISSR products, of which 72 were specific for *Aegilops juvenalis* (Thell.) Eig, in triticale × *Ae. juvenalis* (Thell.) Eig hybrids.

Table 3 gives the coefficients of genetic similarity between all analysed forms. Relationships between the hybrid lines and their parental forms are presented in a dendrogram constructed on the basis of similarity indices (Figure 1). The forms studied formed two main groups of similarity. The first group comprised two F_2 KRB hybrid lines, with 1.00 genetic similarity, and the parental wheat Begra cultivar, with 0.95 and 0.96 genetic similarity to these lines. The second, more genetically diverse group, consisted of the remaining hybrid lines and the parental wheat Rusałka cultivar. The second group had four subgroups of similarity. The first subgroup consisted of F_4 KR3 and F_5 KR3 with 0.97 genetic similarity; the second included the F_4 KR6 and F_5 KR6 hybrid lines with 0.93 genetic similarity; the third contained the F_4 KR4 and F_5 KR4 hybrid lines with 0.92 genetic similarity; and the fourth subgroup comprised the F_4 KR9 and F_5 KR9 hybrid lines with 0.98 genetic similarity and the Rusałka cultivar, with 0.92 and 0.90 genetic similarity to these lines. Within the second group, genetic similarity ranged from 0.83 between F_4 KR3 and F_4 KR6 to 0.92 between KR9 and F_5 KR6 (Table 3).

Genetic similarity between the two groups was about 0.77 (Figure 1). *Ae. kotschyi* Boiss. was not directly linked to any of these groups, with 0.19-0.32 similarity to all hybrid lines (Table 3). Principal coordinate analysis based on ISSRs (Figure 2) confirmed the cluster analyses.

Abou-Deif et al. (2013) reported that similarity values showed pronounced differences among the hexaploid, tetraploid and diploid wheat cultivars, ranging from 0.47 to 0.94, with an average of 0.71. Nefzaoui et al. (2014) reported that average genetic diversity among the accessions of durum wheat varieties and landraces was 0.422.

Table 3. Dice coefficient similarity matrix (SI) based on the ISSR markers polymorphism

Form	*Aegilops kotchyi*	Rusałka	Begra	F_4 (KR3)	F_5 (KR3)	F_4 (KR4)	F_5 (KR4)	F_4 (KR6)	F_5 (KR6)	F_4 (KR9)	F_5 (KR9)	F_2 (KRB)	F_2 (KRB)
Aegilops kotschyi	1.00												
Rusałka	0.19	1.00											
Begra	0.19	0.79	1.00										
F_4(KR3)	0.32	0.86	0.74	1.00									
F_5(KR3)	0.30	0.86	0.73	0.97	1.00								
F_4(KR4)	0.31	0.86	0.75	0.84	0.84	1.00							
F_5(KR4)	0.26	0.87	0.75	0.84	0.85	0.92	1.00						
F_4(KR6)	0.27	0.89	0.78	0.83	0.84	0.87	0.88	1.00					
F_5(KR6)	0.30	0.87	0.78	0.87	0.86	0.90	0.88	0.93	1.00				
F_4(KR9)	0.27	0.92	0.77	0.89	0.88	0.90	0.91	0.88	0.92	1.00			
F_5(KR9)	0.26	0.90	0.76	0.90	0.88	0.89	0.91	0.87	0.92	0.98	1.00		
F_2(KRB)	0.27	0.76	0.95	0.75	0.74	0.76	0.76	0.77	0.79	0.81	0.81	1.00	
F_2(KRB)	0.29	0.76	0.96	0.75	0.74	0.76	0.76	0.78	0.80	0.81	0.80	1.00	1.00

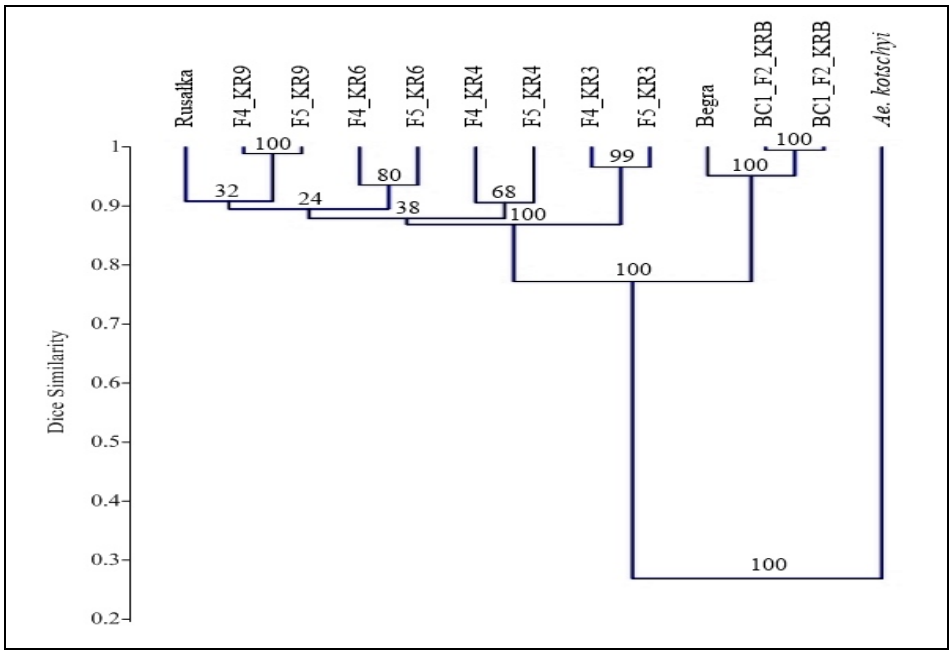

Figure 1. Dendrogram representing the genetic relationship among the 10 wheat hybrid lines and their parental components using UPGMA cluster analysis of Dice similarity coefficient generated from ISSR markers

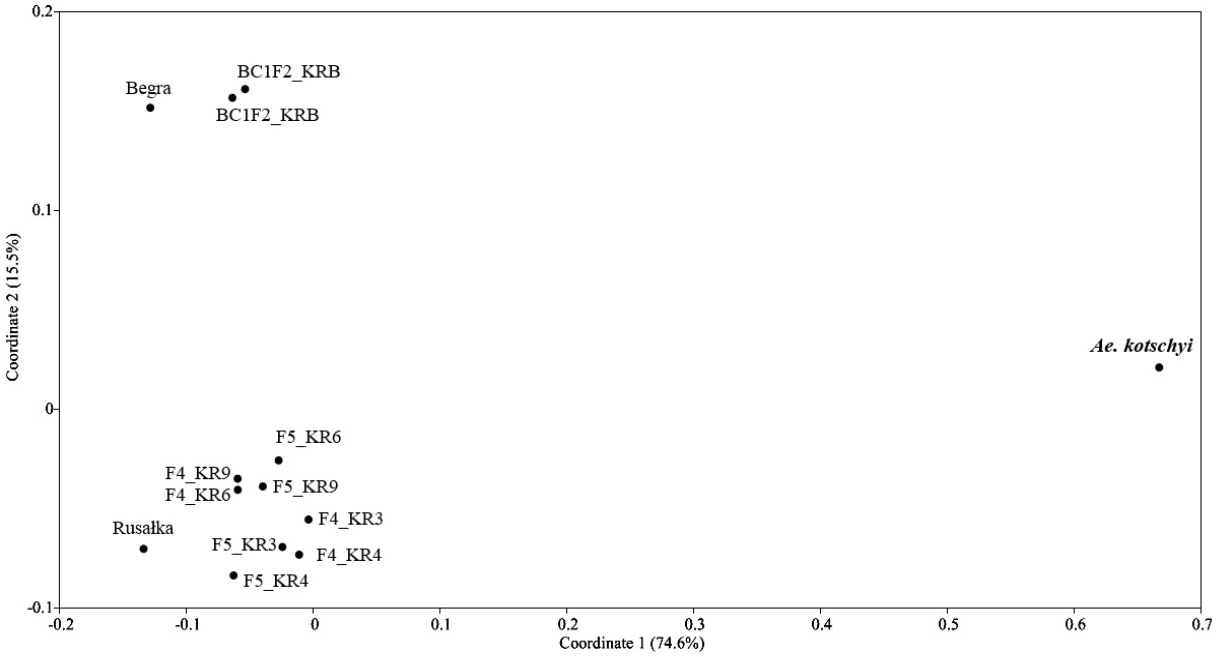

Figure 2. PCoA of *Ae. kotschyi* and wheat hybrids based on ISSR markers

Grądzielewska et al. (2010, 2012) found that Dice genetic similarity values obtained in triticale hybrids and their parental forms using ISSR markers polymorphism ranged from 0.30 to 0.99. The genetic similarity values between the triticale hybrid strains ranged from 0.82 to 0.99, while between hybrids and *Ae. crassa* (4x) Boiss. and *Ae. juvenalis* (Thell.) Eig parental components they ranged from 0.31 to 0.38. Grądzielewska et al. (2009) reported that mean Dice algorithms between pairs of *Dasypyrum villosum* (L.) Candargy populations ranged from 0.82 to 0.93 (mean 0.87). In studies by Tanyolac (2003), Fernández et al. (2002) and Kanbar and Kondo (2011), dendrograms based on the genetic distance derived from ISSR and RAPD markers indicated a very clear pattern of clustering according to the regions in which the barley species and cultivars were grown.

CONCLUSIONS

In conclusion, our results indicate the presence of genetic similarity among the *Ae. kotschyi* Boiss. × *T. aestivum* L. hybrid lines and their parental components. Analysis of ISSR markers can successfully be used to study similarity between different wheat hybrid lines and cultivars. The information regarding genetic similarity makes it possible to remove repeated lines in breeding programs.

The studies confirmed that the ISSR method is highly effective, identifies polymorphism between hybrid lines of wheat with *Ae. kotschyi* Boiss., and can be successfully used for genetic differentiation of wheat breeding materials. The ISSR method correctly reflected the genetic diversity of the hybrid lines of wheat and *Ae. kotschyi* Boiss. and confirmed their hybrid character. The results obtained using the ISSR method confirmed the relatedness of the forms studied.

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