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POLYMORPHISM OF MICROSATELLITE LOCI IN BREAD WHEAT (*Triticum aestivum* L.) AND RELATED SPECIES

ABSTRACT: This study analysed polymorphism of 15 microsatellite loci in the collection comprising of 40 genotypes of bread wheat (*Triticum aestivum* L.), 32 genotypes belonging to other species within *Triticum* genus and 3 genotypes from *Aegilops* genus. The results showed significant differences in the variability of the tested loci in bread wheat and related species. In the collection of bread wheat genotypes, 119 alleles were detected with the average number of 7.9 alleles per locus. In wild and cultivated related species 157 alleles were identified, with the average of 10.5 alleles per locus. All analysed parameters of microsatellite loci variability (PIC value, gene diversity, heterozygosity, etc.) indicated higher level of polymorphism in wild relatives than in the cultivated bread wheat. Analyses of individual genomes indicated that in the bread wheat genetic diversity of the B and D genomes was significantly reduced in relation to the A genome, while the differences in polymorphism between genomes in the wild relatives were significantly lower. The results showed that wild related species can be used as sources for new variability in wheat breeding.

KEYWORDS: *Aegilops* ssp., *Triticum* sp., SSR markers, locus variability

INTRODUCTION

Cultivated wheat is a hexaploid species with genome consisting of three sub-genomes originating from three ancestral species: A genome originates from *Triticum urartu*, B genome originates from *Aegilops speltoides* and D genome originates from *Aegilops tauschii*. The analysis of phylogenetic relations between different *Aegilops* and *Triticum* species, and their relatedness to the cultivated wheat, can contribute to better understanding of the complex wheat genome, as well as the processes that had been carried out throughout the evolution of this important crop.

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One of the preconditions for further improvement of wheat is the assessment of genetic variability of wild relatives for the identification of desirable traits that can be used in breeding. Wild relatives have contributed to the increase of genetic variability in many cultivated species, such as barley, potato, rice, tomato, wheat and many others (Jones *et al.* 2013). It is estimated that the contribution of wild relatives in the genetic improvement of the cultivated species amounts to 1% per year, with a total value of $1 billion in global agriculture (Heywood 2011). Additionally, according to the estimates of Maxted and Kell (2009), 29 cultivated species have benefited from the transfer of useful traits from their wild relatives.

The strategies based on the extensive phenotyping of the germplasm collections, so as to identify wild relatives that can contribute to increased productivity or adaptability of the cultivated plants, are often compared to "finding a needle in a haystack". Molecular evaluation and population genetics were proposed as an alternative approach (Prada 2009). The advantage of using molecular markers and next generation sequencing technologies is the possibility to easily identify the desirable genotypes and alleles that can be used for improvement of certain traits (Kilian and Graner 2012).

The aim of this study was to analyse the genetic variability of microsatellite loci of the bread wheat in comparison to related species from the genera *Triticum* and *Aegilops*. This will give important information on the potential use of wild relatives in wheat breeding.

MATERIAL AND METHODS

In this study, genetic variation of 75 accessions of *Triticum* and *Aegilops* species was evaluated using SSR marker. The collection consists of 40 *T. aestivum* genotypes, 32 accessions of other *Triticum* species and 3 *Aegilops* accessions. Fifteen SSR markers (listed in Table 1) were chosen for the evaluation, 5 for each of the three bread wheat genomes (AA, BB and DD genome).

The genomic DNA was isolated from young leaves using modified CTAB method (Doyle and Doyle 1990). The PCR reactions were carried out according to Röder *et al.* (1998) with specific annealing temperature depending on certain SSR markers. The total amount of PCR reaction was 10 µl, which contained 30 ng of genomic DNA, 1x buffer solution, 2 mM dNTPs, 1.5 mM $MgCl_2$, 10 pmol of each primers and 1 unit of Taq polymerase. The forward primers were labelled with one of the listed fluorescent dyes VIC, 6-FAM, NED or PET. The fragment analysis was performed using capillary electrophoresis at Genetic Analyzer ABI Prism 3130 (Applied Biosystems, Foster City, CA, USA). The electrophoresis data were collected in Data Collection Software v.3.0. and analysed using GeneMapper Software v.4.0 (Applied Biosystems, Foster City, CA, USA).

Descriptive SSR loci parameters, such as number of alleles per locus, allele frequencies, PIC value, heterozygosity, etc., were estimated using GenAlEx 6.5 software (Peakall and Smouse 2012). Significance of the differences in the number of alleles between three wheat genomes was tested with t-test using XLSTAT software (Addinsoft 2007). PowerMarker v.3 software (Liu and Muse 2005) was

used to calculate a shared allele proportion distance matrix among the pairs of genotypes. Visual representation of the distance matrix was performed by UPGMA tree using Dendroscope v.2.7.4 software (Huson *et al.* 2007). Furthermore, principal coordinate analysis (PCoA) was used to depict the relatedness among the genotypes in low-dimensional setting using GenAlEx 6.5. Molecular analysis of variance (AMOVA) was carried out to determine the distribution of the genetic variation among cultivated wheat and related species and within these groups was used Arlequin 3.5 software (Excoffier and Lischer 2010). The significance of φ-statistics was obtained non-parametrically using 1,000 random permutations.

RESULTS AND DISCUSSION

Genetic diversity was investigated in the set of 75 accessions of *Triticum* and *Aegilops* species, using 15 wheat microsatellites. Fragment analyses of PCR products were used for detection of 207 allelic variants in all investigated loci (Table 1). The number of alleles per locus varied from 7 for *Xgwm261* to 21 for *Xbarc12*. The average number of allelic variants in all analysed loci was 13.8, with average PIC (polymorphic information content) value of 0.76. Gene diversity for 15 microsatellite loci varied from 0.69 for *Xgwm261* to 0.91 for *Xbarc12* with an average of 0.79. The gene diversity increased as the number of alleles increased. The obtained results are in agreement with those of Salem et al. (2014) who reported highly significant correlation between gene diversity and the number of alleles per locus ($r = 0.649$, $P<0.01$). They concluded that the number of alleles can be used to evaluate the genetic diversity per loci, genomes and homoeologous group.

Table 1. Polymorphism of 15 microsatellite loci in the collection of 75 *Triticum* and *Aegilops* accessions

Marker	MAF	AN	GD	H	PIC	Genome
cfd65	0.30	17	0.83	0.62	0.81	A
gwm294	0.19	15	0.89	0.00	0.88	A
barc12	0.16	21	0.91	0.09	0.90	A
wmc264	0.19	19	0.89	0.10	0.88	A
cfd71	0.17	19	0.91	0.03	0.90	A
Mean A	0.20	18.2	0.89	0.17	0.88	
taglgap	0.30	15	0.84	0.02	0.83	B
barc164	0.28	10	0.82	0.00	0.79	B
gwm284	0.25	10	0.82	0.06	0.80	B
gpw3071	0.42	17	0.75	0.07	0.73	B
gwm495	0.42	12	0.77	0.08	0.75	B
Mean B	0.33	12.8	0.80	0.05	0.78	
wmc216	0.78	9	0.38	0.00	0.37	D
gwm157	0.46	17	0.71	0.04	0.67	D

gwm261	0.42	7	0.69	0.04	0.64	D
gwm3	0.38	8	0.74	0.26	0.70	D
wmc457	0.32	11	0.82	0.04	0.80	D
Mean D	0.47	10.4	0.67	0.08	0.64	
Total Mean	0.34	13.8	0.79	0.10	0.76	

MAF-Major Allele Frequency, AN – Allele Number, GD – Gene Diversity, H – Heterozygosity, PIC – Polymorphism information content

Analysis of molecular variance (AMOVA) showed that genetic variation was found mainly within populations (89%) while variance among populations of cultivated wheat and wild relatives was only 11%.

In the collection of bread wheat genotypes, 119 alleles were detected with the average number of 7.9 alleles per locus, while in related species 157 alleles were identified with the average of 10.5 alleles per locus (Table 2). The average gene diversity of 0.68 and 0.81 were found in cultivated wheat and relatives, respectively. PIC value ranged in bread wheat from 0.20 (*Xwmc216*) to 0.86 (*Xbarc12* and *Xcfd71*) and in related species from 0.62 (*Xgwm3*) to 0.89 (*Xgwm294* and *Xgwm157*).

Table 2. Polymorphism of 15 microsatellite loci in the collection of 40 *Triticum aestivum* genotypes (cultivated wheat) and 35 related *Triticum* and *Aegilops* species (related species)

Marker	Cultivated wheat					Related species					Genome
	MAF	AN	GD	H	PIC	MAF	AN	GD	H	PIC	
cfd65	0.38	10	0.74	0.90	0.70	0.37	13	0.79	0.38	0.76	A
gwm294	0.31	10	0.84	0.00	0.82	0.18	13	0.89	0.00	0.89	A
barc12	0.23	13	0.87	0.09	0.86	0.25	12	0.87	0.10	0.85	A
wmc264	0.30	10	0.84	0.05	0.82	0.27	14	0.87	0.15	0.86	A
cfd71	0.22	12	0.87	0.07	0.86	0.33	14	0.84	0.00	0.83	A
Mean A	0.29	11.0	0.83	0.22	0.81	0.28	13.2	0.85	0.13	0.84	
taglgap	0.38	7	0.72	0.04	0.67	0.23	12	0.85	0.00	0.84	B
barc164	0.46	7	0.66	0.00	0.61	0.33	8	0.79	0.00	0.76	B
gwm284	0.47	9	0.73	0.10	0.70	0.35	5	0.73	0.00	0.68	B
gpw3071	0.64	6	0.54	0.07	0.50	0.31	15	0.86	0.07	0.85	B
gwm495	0.64	9	0.57	0.07	0.55	0.23	9	0.84	0.10	0.83	B
Mean B	0.52	7.6	0.64	0.06	0.61	0.28	11.7	0.83	0.08	0.82	
wmc216	0.89	5	0.21	0.00	0.20	0.50	6	0.69	0.00	0.66	D
gwm157	0.68	2	0.44	0.00	0.34	0.17	17	0.90	0.10	0.89	D
gwm261	0.54	4	0.61	0.03	0.55	0.34	6	0.74	0.05	0.70	D
gwm3	0.32	6	0.76	0.19	0.72	0.48	6	0.67	0.36	0.62	D
wmc457	0.35	9	0.80	0.03	0.78	0.28	7	0.80	0.06	0.78	D
Mean D	0.55	5.2	0.56	0.05	0.52	0.30	10.7	0.81	0.09	0.79	
Total Mean	0.45	7.9	0.68	0.11	0.65	0.31	10.5	0.81	0.09	0.79	

MAF – Major Allele Frequency, AN – Allele Number, GD – Gene Diversity, H – Heterozygosity, PIC – Polymorphism information content

Different dominant alleles were identified in the collections of bread wheat and related species in 9 out of 15 examined loci. Higher number of alleles in the collection of related species was found in 10 loci (Figure 1), while in three loci (*Xbarc12*, *Xgwm284* and *Xwmc457*) higher number of alleles was found in bread wheat genotypes (Figure 2). The two loci (*Xgwm495* and *Xgwm3*) had the same number of alleles in both groups of genotypes.

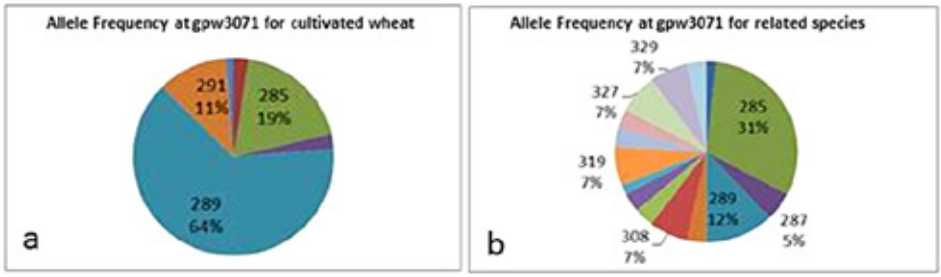

Figure 1. Allele frequencies at the locus Xgpw3071 for cultivated wheat (a) and its relatives (b)

Figure 2. Allele frequencies at the locus Xgwm284 for cultivated wheat (a) and its relatives (b)

All analysed variability parameters showed higher level of polymorphism present in the wild relatives than in the cultivated bread wheat (Table 2). Analyses of individual genomes indicated that in the cultivated wheat genetic diversity of the B and D genomes was significantly reduced in relation to the A genome, while in the related species the differences among the genomes were less pronounced. Thus, in the present study the number of alleles in cultivated wheat for A genome (55) was significantly ($P<0.01$) higher than the B (38) and D (26) genomes. Contrary to our results, some authors found that the B genome chromosomes appear to have high genetic richness compared to the A and D genomes, in various classes of repetitive DNA, particularly microsatellites (Cuadrado and Schwarzacher 1998; Salem *et al.*, 2014). Huang *et al.* (2002) also reported that microsatellite loci of the B genome are more effective than those of the A genome. On the other hand, Roussel *et al.* (2004) concluded that the D genome has a larger number of alleles than the B genome, while the highest number was found in the A genome.

It was possible to distinguish cultivated wheat cultivars from other *Triticum* and *Aegilops* species by using two different methods (PCoA analyses in Figure 3 and UPGMA cluster analyses in Figure 4) for the analysis of molecular data and estimating genetic relationships among genotypes. Similar results were obtained by Yadav *et al.* (2014) in pigeonpea (*Cajanus cajan* (L) Millsp.) and its wild relatives (*C. albicans* and *C. lineatus*). They found that the pattern of genetic divergence obtained by PCA is in close agreement with the results of UPGMA based cluster analysis. In both methods the wild species formed out groups, whereas all the cultivated genotypes showed narrower range of diversity.

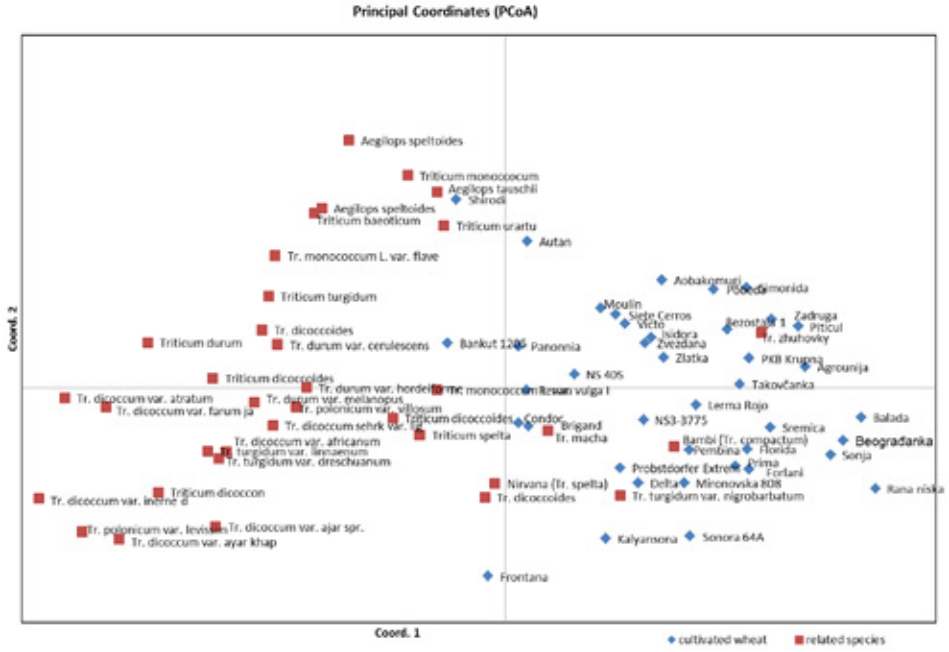

Figure 3. Distribution of 75 accessions of *Triticum* and *Aegilops* species in two-dimensional PCoA system based on molecular data

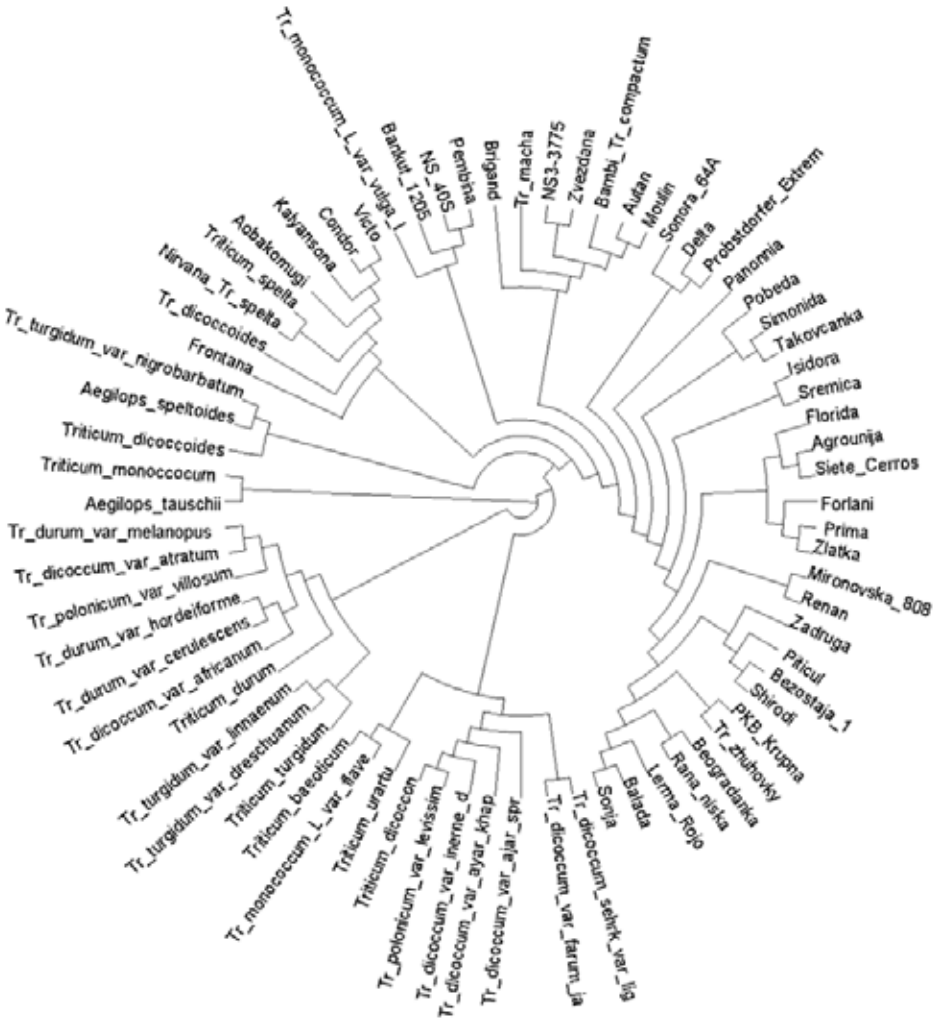

Figure 4. UPGMA tree of 75 accessions of *Triticum* and *Aegilops* species

CONCLUSION

The results showed high diversity present in the collection of cultivated wheat and its relatives. The polymorphism in SSR fragments among different groups of species was shown. It can be concluded that SSR markers can be used in assaying genetic variability of wheat and its wild relatives and establishing of phylogenetic relationships between them. Also, molecular markers can help to organize the genetic variability and expose useful diversity for breeding purposes.

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ПОЛИМОРФНОСТ МИКРОСАТЕЛИТСКИХ ЛОКУСА КОД ГАЈЕНЕ ПШЕНИЦЕ (*Triticum aestivum* L.) И СРОДНИХ ВРСТА

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РЕЗИМЕ: У раду је испитана полиморфност 15 микросателитских локуса у колекцији коју чине 40 генотипова гајене пшенице (*Triticum aestivum* L.), 32 генотипа који припадају другим врстама рода *Triticum* и три генотипа из рода *Aegilops*. Резултати су показали да постоји значајна разлика у варијабилности испитиваних локуса код гајене пшенице и њених сродника. У колекцији генотипова гајене пшенице детектовано је укупно 119 алела, са просеком од 7,9 алела по локусу. Код дивљих и гајених сродника идентификовано је укупно 157 алела, са просеком од 10,5 алела по локусу. Сви израчунати показатељи варијабилности микросателитских локуса (*PIC* вредност, дивергентност гена, хетерозиготност, итд.) указују на већу полиморфност дивљих сродника у односу на гајену пшеницу. Анализа појединачних генома такође је показала да је код гајене пшенице дивергентност Б и Д генома значајно умањена у односу на А геном, док су код дивљих сродника разлике у полиморфности појединачних генома значајно мање. Добијени резултати указују на то да се дивљи сродници могу користити као извори нове варијабилности у оплемењивању пшенице.

КЉУЧНЕ РЕЧИ: *Aegilops* ssp., *Triticum* sp., SSR маркери, варијабилност локуса