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USING OF SPECIFIC MICROSATELLITES MARKERS FOR CHARACTERIZATION OF WINTER AND SPRING TRITICALE (X *TRITICOSECALE* W.) ACCESSIONS

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Abstract: The aim of this work was to confirm application of 6 wheat SSR markers in the set of 35 winter and 24 spring triticale accessions. We had detected 45 alleles with an average of 7,5 alelles per primer pair. The highest number of alleles was detected for the XBarc 137 (10 alleles). Based on data about frequencies of alleles, the diversity index (DI), the probability of identity (PI) and the polymorphic information content (PIC) were calculated. The average polymorphic information content (PIC) was 0.743 and average DI value was 0.750. The dendrogram separated accessions into two clusters. The first cluster comprised of 58 accessions, but second cluster includes only American accession NE 422-T. Compared with the previous work dealing with the study of protein´s profile, this accession demostrated the highest content of prolamins that are mostly responsible for bread-making quality. Although, the used set of markers were not able to separate 10 cultivars between each other. Therefore, for better differentiation is necessary to use higher number of polymorphic microsatellite markers.
Keywords: triticale; genetic polymorphism; SSR

INTRODUCTION

Hexaploid triticale (X *Triticosecale W.*), a man-made hybrid of wheat (*Triticum spp.*) and rye (*Secale cereale L.*) was created to combine the high yielding capacity of wheat with the stress tolerance of rye (Lukaszewski, 2006). Triticale differs from bread wheat (*Triticum aestivum L.)* (2n= 6x=42, AABBDD) by presence of the R genome of rye that replaces wheat genome D originating from Aegilops tauschii and absence of this genome results in difficulties during the baking process **(Martinek et al., 2008)**. It is becoming increasingly important in agriculture and understanding its genetic diversity is essential for its continued improvement **(Kuleung et al., 2006)**.

Molecular markers such as microsatellites or SSRs (Simple Sequence Repeats) constitute an important tool for studies on genetic diversity, population structure, genetic mapping and crop breeding due to their abundance, codominance, level of polymorphism, reliability and easiness to assay **(Röder at al., 1995; Tesser da Costa et al., 2007)**. These markers are not influenced by environment and reflect genetic similarity without previous knowledge of pedigree information **(Kuleung et al., 2006)**. Microsatellites were used in the study of genetic polymorphism in wheat **(Röder et al., 1995; 1998; Song et al., 2005; Gregáňová et al., 2005)** rye **(Saal and Wricke, 1999)**, corn **(Ražná et al., 2010)** and triticale **(Kuleung et al., 2004; Tams et al., 2004; Kuleung et al., 2006; Tesser da Costa et al., 2007; Vyhnánek et al., 2009, Tyrka et al., 2011).** SSR markers are valuable because of their higher level of transferability to related species, and they can often be used as anchor markers for comparative mapping and evolutionary studies **(Varshney et al., 2005; Vyhnánek et al., 2009).**

The aim of this work was to examine utilization of 6 wheat microsatellites in triticale genome and find genetic relationships within the set of winter and spring triticale accessions.

MATERIAL AND METHODS

The tested set of European and American triticale cultivars (x Triticosecale W.) were provided by Gene Bank of Slovak Republic in Piešťany (34 winter triticale and 25 spring triticale). The one-week old leaves were collected and DNA was immediately isolated by GeneJETTM (Fermentas, USA). The concentration of DNA was checked up on 1.0 % agarose gel and then DNA was diluted to final concentration (25 ng/µl).

PCR conditions: Markers were chosen in accordance with literature resources **(Devos et al., 1995; Kuleung et al., 2006).** The PCR was performed in 20 µl volume. The PCR master mix contained of PCR water, 5 x Green GoTaq®Flexi Buffer, 100 µM dNTP Mix, 0.3 µM primers (Forward and Reverse), 1.5 mM MgCl2, 0.4 U GoTaq® DNA polymerase (Promega, USA). The PCR reaction was performed under these conditions: initial denanuration: 3 min. 94 oC, then 45 cycles – denaturation: 1 min. 94 oC, annealing: 2 min. with different temperature for each primer pair, extension: 2 min. 72 oC and after 45 cycles last extension: 72 oC (10 min.).

PCR products were loaded on 6 % denaturated polyacrylamide gel. The time of electrophoresis was different for each primer pair and depended on estimated size of particular primer. Gels were stained with silver according to **Bassam et al. (1991)**.

The acquired data from electroforeograms were converted to binary matice on the base of presence (1) or absence (0) of particular allele. Consequently, a dendrogram was constructed using UPGMA algorithm with the SPSS professional statistics version 17 software package. According to Rusell et al. (1997), the diversity index (DI), the probability of identity (PI) and the polymorphic information content (PIC) were calculated.

RESULTS AND DISCUSSION

This study deals with the applicability and utilization of 6 wheat SSR (Simple Sequence Repeats) (Table 1) in detection of genetic polymorphism in the set of 59 triticale accessions. Four markers are localized on B wheat genome and two markers on A wheat genome. Globally, 45 alleles (table 1) were detected, with an average of 7.5 allele per primer pair. This number is higher than published others authors **(Kuleung et al., 2006; Tesser da Costa et al., 2007; Vyhnánek et al., 2009)** but roughly correspondents to work of **Tams et al. (2004)**. The number of alleles ranged from 5 (Xbarc 024 - localized on long arm of 6B wheat chromosome) to 10 alelles (XBarc 137 - localized on long arm of 1B wheat chromosome). **Vyhnánek et al. (2009)** detected higher number of alleles for the XBarc 024 (6) but less alleles for the XBarc 137. Based on the frequencies of alleles, statistical indicators of suitability and applicability of particular marker were calculated (DI, PI and PIC).

The index of probability (PI) ranged from 0.008 (Xgwm 46) to 0.083 (XBarc 024). The average PIC value was 0.743 and average DI value was 0.750. Taking into account these parameters (PIC, PI and DI), the best marker from tested set is the Xgwm 46, because its PIC and DI are very close to 1 and its PI value is close to 0. Other markers had also shown a relatively high PIC and DI value. On the other hand, the PI value of all tested markers was very close to 0 so we can recommend all markers in genetic study of triticale.

Manifesto et al. 2001 characterized 105 Argentine bread wheat (Triticum aestivum L.) cultivars by SSR markers. They used 33 pairs of SSR primers. The number of locus ranged from 5 to 13 with an average of 9.4 and the PIC values ranged from 0.40 to 0.84 with an average value of 0.72. **Tams et al. (2004)** investigated European winter triticale using Simple Sequence Repeat (SSR) markers and the coancestry coefficient with regard to genetic diversity and grouping of germplasm. Three to five primer pairs for each of the 42 chromosomes were selected to analyse 128 European winter triticale varietes and breeding

lines. SSR analysis resulted in the identification of 657 alleles with an average of 6.8 alleles per primer pair. The PIC for polymorphic markers was 0.54.

Kuleung at al. (2006) focused on the genetic relationships of 80 hexaploid triticale accessions. They used 43 wheat (Triticum spp.) and 14 rye (Secale cereale L.) SSR markers. Globally, they detected 241 alleles from 57 markers, with an average of 4.2 alleles per locus (ranged from 2 to 11). The average gene diversity was 0.54 and ranged from 0.07 to 0.86.

Tesser da Costa et al. (2007) determined the genetic variability available for triticale (x Triticosecale Wittmack) crop improvement in Brazil. They used 42 wheat genomic microsatellites to estimate the molecular diversity of 54 genotypes, which constitute the base of one the major triticale breeding programs in the country. The effective number of alleles per locus 2.13 and 1.61 with average allelic frequency of 0.34.

Vyhnánek et al. (2009) detected the genetic variability of triticale using wheat and rye SSR markers. They analysed 16 genotypes of triticale using 48 SSR markers (27 wheat and 21 rye SSR markers). They detected 184 alleles with an average of 3.83 alleles pre locus (ranging from 1 to 9). The average polymorphic information content (PIC) was 0,48 ranging from 0.48 ranging between 0.00 and 0.85. Other authors dealt with detection of genetic variability by SSR markers in wheat. **Roussel et al. (2004)** used the set of 41 wheat microsatellite markers, giving 42 polymorphic loci (two loci on each chromosome) to describe genetic diversity in a sample of 559 French bread wheat. A total of 609 alleles were detected. Allele number per locus ranged from 3 to 28 (an average 14.5 alleles. **Salem et al. (2008)** evaluated genetic diversity of the seven wheat (Triticum aestivum L.) varieties using 48 SSR markers and 9 morphological characters. The markers determined 15 loci located on fifteen chromosomes and were capable of detecting 48 alleles with an average of 3.2 alleles per locus. The number of alleles per locus ranged from 2 to 7 and the PIC value ranged from 0.278 for the Xgwm 95 to 0.816 for the Xgwm 437 with an average of 0.548. They also used the Xgwm 46 and detected 4 alleles (0.700), meanwhile **Manifesto et al. (2001)** detected 11 alleles with the PIC value 0.760. In triticale, the Xgwm 46 provided 6 alleles and PIC value was higher (0.809) compared with the results of mentioned authors. The SSR markers were also used in Chinese wheat. **Guo et al. (2011)** detected a total of 809 alleles from 99 wheat accessions using 69 microsatellite markers. They observed an average 11.72 (ranging from 3 to 50) alleles per SSR locus.

Based on the frequencies of alleles from 59 accessions, the dendrogram derived from UPGMA cluster analysis was constructed (Picture 1). Accessions had been separated in two clusters. The first cluster comprised of 58 accessions, but second cluster included only American accession NE 422T. Moreover, in the first cluster 3 smaller clusters were detected. In the previous study of triticale´s protein profile, NE 422-T revealed the highest content of prolamins (41.7 %) **(Trebichalský and Gálová, 2010)**, what means that it could meet the standards of good bread-making quality.

Globally, 10 accessions had not been sufficiently separated. In the set of 16 triticale accessions tested by **Vyhnánek et al. (2009)** one Russian genotype had been significantly differentiated from all set of accessions.

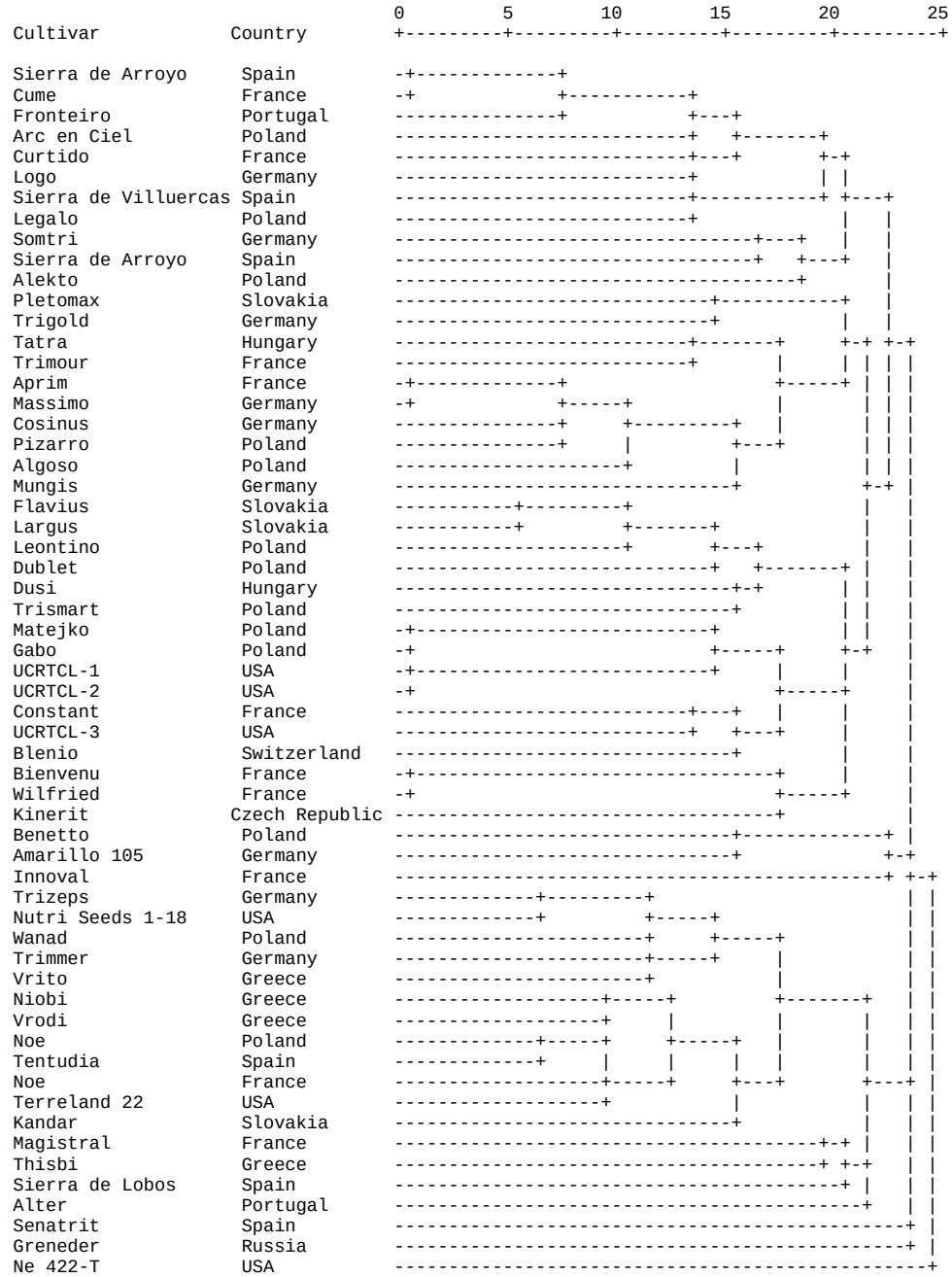
CONCLUSION

This work confirms that SSR wheat markers represent an outstanding way how to detect genetic background not only in wheat accessions but also in triticale accessions. Although, all tested markers were highly polymorphic, we manage not differentiate 10 samples from each other. Therefore, higher number of markers is necessary to differentiate all set of 59 accessions.

Table 1 Description of 6 wheat markers used for analysis of 59 triticale accessions

SSR marker	Localization	[bp]	Motive	Annealing [°C]	Number of alleles	DI	PI	PIC
Xgwm 46	7B	160-188	$(GA)_2GC(GA)_{33}$	60	6	0,814	0,008	0,809
Xgwm234	5B	246-290	$(CT)_{16}(CA)_{20}$	56	8	0,776	0,017	0,766
Xgwm 334	6A	130-150	$(GA)_{19}$	55	8	0,779	0,013	0,765
XBarc 024	6BL	198-238	(TCA)n(TAA)n	53	5	0,606	0,083	0,602
XBarc 137	1BL	274-330	(CT)n	53	10	0,762	0,027	0,761
XBarc 321	3AS	182-216	(CT)n(CA)n	54	8	0,765	0,020	0,756
Average					**7,5**	**0,750**	**0,028**	**0,743**

Picture 1 UPGMA dendrogram of 59 triticale accessions based on 6 SSR markers



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