Journal of Microbiology, Biotechnology and Food Sciences

Trebichalský et al. 2013 : 2 (Special issue 1) 1898-1906

REGULAR ARTICLE

DETECTION OF GENETIC DIVERSITY OF TRITICALE BY MICROSATELLITE MARKERS

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ABSTRACT

The aim of our work was to detect genetic variability in the set of 59 winter and spring triticale (x *Triticosecale* Witt.) varieties using 5 wheat SSR markers. Totally, 35 alleles with an average number of 7 alleles per locus were detected. The highest number of alleles showed out *Xbarc 004* (9). Based on the number and frequencies of alleles, the diversity index (DI), the probability of identity (PI) and the polymorphic information content (PIC) of SSR markers were calculated. The polymorphic information content (PIC) ranged from 0.264 to 0.920 with an average of 0.654, which is generally considered sufficient for this purpose. For the assessment of genetic diversity the dendrogram, based on the hierarchical cluster analysis using UPGMA algorithm was prepared. Fifty nine triticale cultivars were grouped into three major clusters. The cultivar Terelland 22 (USA) separated as unique one, second subcluster contained 3 cultivars and third one 55 cultivars. It was not possible to differentiate 15 genotypes between each other. For better differentiation it is necessary to use more polymorphic microsatellite markers. Results showed the utility of microsatellite markers for estimation of genetic diversity of triticale genotypes leading to genotype identification.

Keywords: triticale, SSR, genetic variability, PIC, dendrogram

INTRODUCTION

The hexaploid man-made wheat-rye hybrid triticale (x *Triticosecale* Witt.) is considered a promising crop with a broad genetic potential. It is adapted to a wide range of abiotic stress conditions, is an important high-quality feed stock and produces similar grain yield but more biomass compared to other (**Altheit *et al*., 2011**). Triticale is becoming increasingly important in agriculture and understanding its genetic diversity is essential for its continued improvement (**Kuleung *et al*., 2006).** However, it is rarely used for human consumption because of its poor bread-making quality **(Lukaszewski, 2006)**.

The knowledge of diversity within the triticale gene pool is an important information for today´s line breeding and a basic requirement for future hybrid breeding (**Tams *et al*., 2002**).

Simple Sequence Repeat (SSR) markers are a valuable tool for many purposes, such as mapping, fingerprinting, and breeding in many plant species (**Röder *et al.,* 1998, Gregáňová *et al.,* 2005, Vyhnánek *et al.,* 2009, Ražná *et al.,* 2010, Labajová *et al.,* 2011**). However, they are only available in some economically important crops because of the high cost and labor intensity involved in their development (**Kuleung *et al*., 2006**). In hexaploid wheat, microsatellite DNA markers are consistently found to be more informative than other classes of markers (**Song *et al*., 2005**). 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 our study was to detect genetic relationships in winter and spring triticale cultivars using 5 wheat SSR markers.

MATERIAL AND METHODS

Plant material and DNA isolation

The set of 59 European and American triticale (x *Triticosecale* Witt.) cultivars (from that 35 winter triticale and 24 spring triticale) were used for detection. Cultivars were provided by the Gene bank of Slovak republic in Piešťany. The 7 day-old leaves were collected and immediately the DNA was isolated by GeneJET^{TM} (Fermentas, USA). The

concentration and quality of DNA was checked up on 1.0 % agarose gel coloured by ethidium bromide and detecting by comparing to λ-DNA with known concentration.

PCR conditions

For the analysis, in accordance with literature (**Devos *et al.*, 1995, Kuleung *et al.* 2006**), 5 SSR markers were chosen. The PCR reaction 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 primer), 1.5 mM $MgCl_2$, 0.4 U GoTaq® DNA polymerase (Promega, USA). The PCR reaction was performed under these conditions: initial denaturation: 2 min. at 93 ^{0}C, then 29 cycles – denaturation 1 min. 93 ^{0}C , annealing 2 min. with different temperature at each primer pair (Table 1) and extension 2 min. at 72 ^{0}C.

Electrophoresis conditions and gel resulting

PCR products (5µl) were loaded on 6 % denaturated polyacrylamide gel. The time of electrophoresis differs for each marker, but usually samples ran about 150-200 minutes. Then, gels were stained with silver according to **Bassam *et al.* (1991)**.

Statistical analysis

The 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 Russell et al. (1997) were calculated:
Diversity index (DI):

$$DI = 1 - \sum p_i^2$$

Probability of identity:

$$I = \sum p_i^4 + \sum_{i=1}^{i=n-1} \sum_{j=i+1}^{n} \left(2 p_i p_j\right)^2$$

Polymorphism information contents (PIC):

$$PIC = 1 - \left(\sum_{i=1}^{n} p_i^2 \right) - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2 p_i^2 \cdot p_j^2$$

where p_i and p_j are the frequencies of the *i*th and *j*th allele in given population.

RESULTS AND DISCUSSION

Our study dealt with detection of genetic polymorphism in winter and spring triticale cultivars using microsatellites markers. At the same time, we wanted to verify the transferability and applicability of used wheat markers in triticale cultivars. For the differentiation of 59 triticale genotypes 5 wheat microsatellite markers described by **Devos *et al.* (1995)** and **Kuleung *et al.* (2006)** were chosen. Overall, we detected 35 alleles with an average 7 alleles for primer pair. The number of alleles ranged from five for locus *XBarc* 012 (localized on short arm of chromosome 3A in wheat) to nine for locus *Xbarc 004* (localized on short arm of chromosome 5B in wheat).

The diversity index (DI) of the tested wheat SSR markers ranged from 0.274 to 0.922 with an average of 0.658 and the polymorphic information content (PIC) ranged from 0.264 to 0.920 with an average of 0.654 (Table 1), which is generally considered sufficient for this purpose. The highest DI value was detected for *Xbarc109* and the lowest for *Xbarc012*. Except for *Xbarc012,* four SSR markers used had PIC and DI values higher than 0.6. The average DI value 0.658 is in accordance with previous studies with the similar set of microsatellites (**Manifesto *et al.*, 2001**, **Tams *et al.*, 2004, Vyhnánek *et al.*, 2009**).

Table 1 Characterization of used wheat SSR markers

SSR marker	Localization	[bp]	Motive	Annealing [^{O}C]	Number alleles	DI	PI	PIC
Xpsp 2999	1A	133-157	(CAG)5(CAA)8)n	55	**7**	**0.600**	**0.113**	**0.598**
Xpsp 3000	1B	213-285	(CAA)n	55	**8**	**0.706**	**0.044**	**0.705**
Xbarc 109	5B	232	(ATT)n	55	**6**	**0.922**	**0.084**	**0.920**
Xbarc 004	5B	158	(TTA)n	52	**9**	**0.789**	**0.016**	**0.785**
Xbarc 012	3A	200	(TAA)n	52	**5**	**0.274**	**0.536**	**0.264**
Average					**7**	**0.658**	**0.158**	**0.654**

Vyhnánek *et al.* (2009) studied the genetic variability of 16 genotypes of triticale using the SSR markers. They used 48 SSR markers (27 wheat and 21 rye SSR markers) and

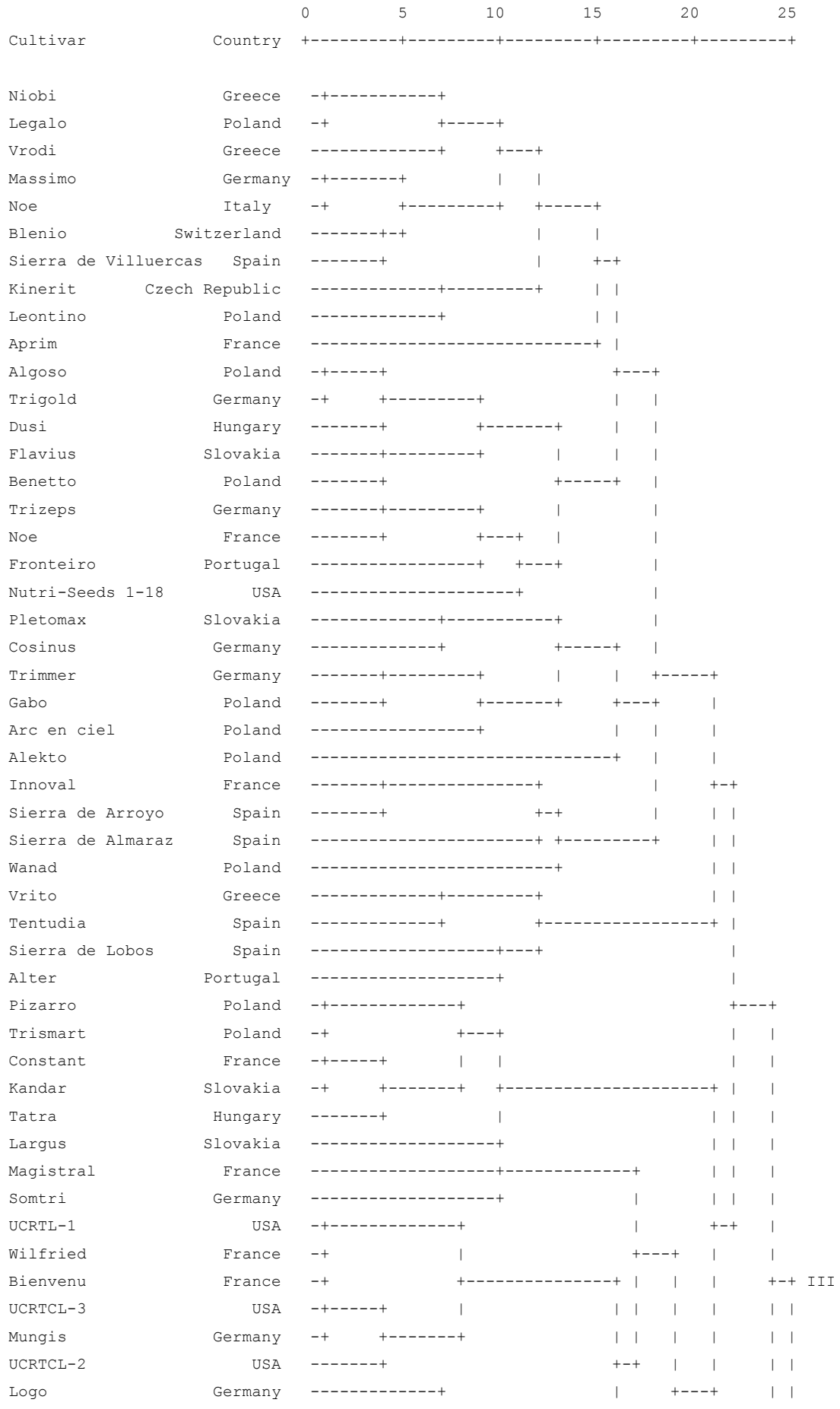
the highest number of alleles detected in *Xpsps 3000* (9) alleles and in *XBarc 004* (7 alleles). The average polymorphic information content of their markers was 0.48 ranging from 0.00 and 0.85. The number of alleles in these two markers (*Xpsps 3000* and *XBarc 004*) responds to our detection. **Manifesto *et al.* (2001)** detected genetic diversity in 105 Argentine bread wheat (*Triticum aestivum* L.) using SSR markers. In *Xpsp 2999* detected 10 + 1 null allele and in *Xpsp 3000* even 12 + 1 null allele. The average PIC value of SSR markers was 0.72, what is higher than the PIC value obtained with RFLP markers.

Tams *et al.* (2004) detected the genetic diversity of 128 European winter triticale using SSR markers. They used 3-5 primer pairs for each of the 42 chromosomes. Their analysis resulted in the identification of 657 alleles with an average of 7 alleles per primer pair, what corresponds to our detection (7.5). The average polymorphism content (PIC) for polymorphic markers was 0.54. They proved that SSR markers which were developed in wheat and rye are suitable for analysing the composite genome of triticale.

Kuleung *et al.* (2006) studied the genetic diversity and relationships of 80 hexaploid triticale accessions representing a more global gene pool using 42 wheat (*Triticum* spp.) and 14 rye (*Secale secale* L.) SSR markers. They detected 141 alleles from 57 markers with an average of 4.2 alleles per locus (ranged from 2 to 11 alleles per locus). The average gene diversity was 0.54 with a range of 0.07 to 0.86.

On the base of the sizes of microsatellite alleles obtained from 59 accessions, the dendrogram derived from UPGMA cluster analysis was prepared (Figure 1). The genotypes could be grouped into three major clusters, cluster I with one unique genotype Terelland originated from the U.S.A., cluster II with three genotypes (Matejko originated from Poland, Thisbi originated from Greece and Senatrit originated from Spain) and cluster III with 55 genotypes. Cluster III was further subdivided into two subclusters, where separated two cultivars Greneder (Russia) and NE 422T (USA).

We were not able to distinguish 15 triticale genotypes. For better differentiation we have to choose more polymorphic markers and do more molecular analysis. At this time we can advise used wheat SSR markers for analysis to produce reproducible polymorphic data.


0 5 10 15 20 25
Cultivar Country
Niobi Greece
Legalo Poland
Vrodi Greece
Massimo Germany
Noe Italy
Blenio Switzerland
Sierra de Villuercas Spain
Kinerit Czech Republic
Leontino Poland
Aprim France
Algoso Poland
Trigold Germany
Dusi Hungary
Flavius Slovakia
Benetto Poland
Trizeps Germany
Noe France
Fronteiro Portugal
Nutri-Seeds 1-18 USA
Pletomax Slovakia
Cosinus Germany
Trimmer Germany
Gabo Poland
Arc en ciel Poland
Alekto Poland
Innoval France
Sierra de Arroyo Spain
Sierra de Almaraz Spain
Wanad Poland
Vrito Greece
Tentudia Spain
Sierra de Lobos Spain
Alter Portugal
Pizarro Poland
Trismart Poland
Constant France
Kandar Slovakia
Tatra Hungary
Largus Slovakia
Magistral France
Somtri Germany
UCRTL-1 USA
Wilfried France
Bienvenu France
UCRTCL-3 USA
Mungis Germany
UCRTCL-2 USA
Logo Germany
III

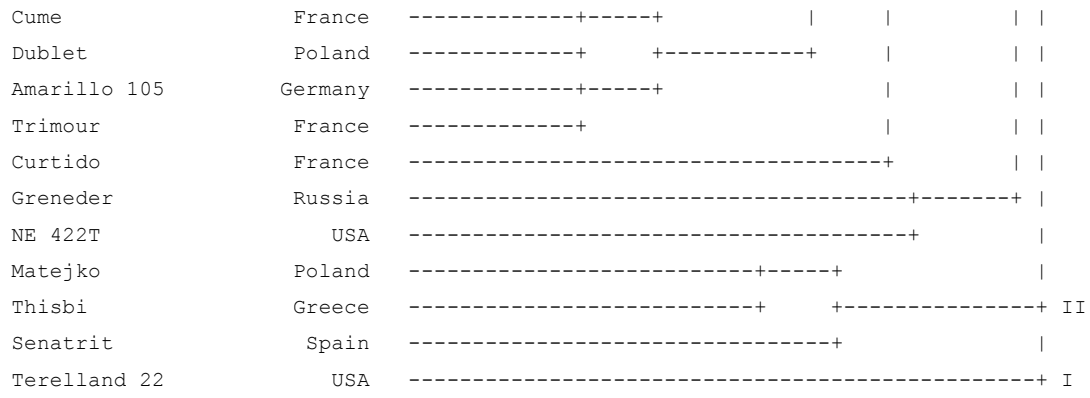


Figure 1 Dendrogram of 59 triticale genotypes prepared based on 5 SSR markers

CONCLUSION

The analysis showed that the microsatellites are very effective molecular markers for the assessment of the genetic diversity in triticale using wheat SSR markers. Dendrogram prepared based on the UPGMA algorithm showed partially clustering of triticale cultivars. For better differentiation of used set of triticale genotypes it is necessary to use more polymorphic microsatellite markers.

Acknowledgments: This contribution is the result of the project implementation: Centre of excellence for white-green biotechnology, ITMS 26220120054, supported by the research Development Operational Programme funded by the ERDF (50%) and KEGA project 034SPU-4/2012 (50%).

REFERENCES

ALTHEIT, K.V. – REIF, J.C. – MAURER, H.P. – HAHN, V. – WEISSMANN, E.A. – MIEDANER, T. – WURSCHUM, T. 2011. Detection of segregation distortion loci in triticale x *Triticosecale* Wittmack based on a high-density DArT marker concensus genetic linkage map. In *BMC Genomics*, vol.12, 2011, p. 1-13.

BASSAM, B. J., CAETANO-ANOLLES, G., GRESSHOFF, P. M. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. In *Analytical Biochemistry*, vol. 196, 1991, p. 80-83.

DEVOS, K.M. - BRYAN, G.J. - COLLINS, A.J. - STEPHENSON, P. - GALE, M.D. 1995. Application of two microsatellite sequences in wheat storage proteins as molecular markers. In *Theoretical and Applied Genetics*, vol. 90, 1995, p. 247–252.

GREGÁŇOVÁ, Ž. - KRAIC, J. - GÁLOVÁ, Z. 2005. Effectiveness of microsatellites in differentiation of elite wheat cultivars. *In* Biologia, vol. 60, 2005, p. 665-670.

KULEUNG, C. – BAENZIGER, P.S. – KACHMAN, S.D. – DWEIKAT, I. 2006. Evaluating the genetic diversity in triticale with wheat and rye SSR markers. In *Crop Science,* vol. 46, 2006, p. 1692-1700.

LABAJOVÁ, M. - SENKOVÁ, S. - ŽIAROVSKÁ, J. - RAŽNÁ, K. - BEŽO, Mi. - ŠTEFÚNOVÁ, V. - ZELEŇÁKOVÁ, L. 2011. The potential of ISSR markers in amaranth gamma-radiance mutants genotypying. In *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 1, 2011, p. 507-521.

LUKASZEWSKI, A. J. 2006. Cytogenetically engineered rye chromosomes 1R to improve bread-making quality of hexaploid triticale. In *Crop Science*, vol. 46, 2006, p. 2183-2194.

MANIFESTO, M.M. – SCHLATTER, A.R. – HOPP, H.E. – SUARÉZ, E.Y. – DUBCOVSKY, J. 2001. Quantitative evaluation of genetic diversity in wheat germplasm using molecular markers. In *Crop Science*, vol. 41., 2001, p. 682-690

RAŽNÁ, K. - BÁTOVSKÁ, A. - BEŽO, M. - MASNICOVÁ, S. - ŽIAROVSKÁ, J. 2010. Analýza DNA polymorfizmu vybraných línií kukurice siatej (Zea mays L.) pomocou PCR-ISSR markérov. In *Acta fytotechnica et zootechnica*, vol. 13, 2010, p. 15-18.

RÖDER, M.S. - KORZUN, V. - WENDEHAKE, K. - PLASCHKE, J. - TIXIER, M.-H. - LEROY, P. - GANAL, M.W. 1998. A microsatellite map of wheat. In *Genetics*, vol. 149, 1998, p. 2007–2023.

RUSSELL, J., FULLER, J., YOUNG, G., THOMAS, B., TARAMINO, G., MACAULAY, M., WAUGH, R., POWELL, W. 1997. Discriminating between barley genotypes using microsatellite markers. In *Genome***,** vol. 40, 1997, p. 442-450.

SONG, Q.L. – SHI, J.R. – SINGH, S. – FICKUS, E.W. – COSTA, J.M. – LEWIS, J. – GILL, B.S. – WARD, R. – CREGAN, P.B. 2005. Development and mapping of microsatellite (SSR) markers in wheat. In *Theoretical and Applied Genetics*, vol. 110, 2005, p. 550-560.

TAMS, S.H. – MELCHINGER, A.E. – OETTLER, G. – BAUER, E. 2002. Assesment of genetic diversity in European winter triticale using molecular markers and pedigree. Proceedings of the 5th International Triticale Symposium, vol. 1, 2002, p. 95-102.

TAMS, S.H. – BAUER, E. – OETTLER, G. – MELCHINGER, A.É. 2004. Genetic diversity in european winter triticale determined with SSR markers and coancestry coefficient. In *Theoretical and Applied Genetics*, vol. 108, 2004, p. 1385-1391.

VARSHNEY, R.J. – GRANER, A. – SORRELLS, M. E. 2005. Genic microsatellite markers in plants: features and applications. In *Elsevier*, vol. 23, 2005, no. 1, p. 48-55

VYHNÁNEK, T. – NEVRTALOVÁ, E. – SLEZÁKOVÁ, K. 2009. Detection of the genetic variability of triticale using wheat and rye SSR markers. In *Cereal Research Communications*, vol. 37, 2009, no. 1, p. 23-29.