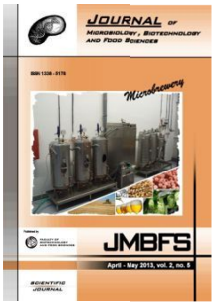

JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

Ondroušková and Vyhnánek 2013 : 2 (5) 2366-2368

STUDY OF GENETIC VARIABILITY OF TRITICALE VARIETIES BY SSR MARKERS

*Jana Ondroušková *[1], Tomáš Vyhnánek[1,2]*

Address(es): MSc. et MSc. Jana Ondroušková
[1]Mendel University in Brno, Faculty of Agronomy, Department of Plant Biology, Zemědělská 1, 61300 Brno, Czech Republic, +420 545 133 014.
[2]Mendel University in Brno, Faculty of Agronomy, CEITEC MENDELU, Zemědělská 1, 61300 Brno, Czech Republic.

*Corresponding author: jana.ondrouskova@mendelu.cz

ARTICLE INFO

Received 28. 2. 2013
Revised 27. 3. 2013
Accepted 29. 3. 2013
Published 1. 4. 2013

Short communication

OPEN ACCESS

ABSTRACT

For the detection of genetic variability ten genotypes of winter triticale (×*Triticosecale* Wittmack, 2n = 6x = 42; BBAARR) were selected: nine varieties and one breeding line with good bread-making quality KM 4-09 with the chromosome translocation 1R.1D $_{5+10}$-2. 25 microsatellites markers located in the genome A, B, D and R were chosen for analysis. Eighty-four alleles were detected with an average of 3.36 alleles per locus were detected. For each microsatellite statistical values were calculated diversity index (DI), probabilities of identity (PI) and polymorphic information content (PIC) were calculated and averages statistical values are: DI 0.55, PI 0.27 and 0.5 PIC. Overall dendrogram based on the UPGMA method (Jaccards similarity coefficient) significantly distinguished two groups of genotypes and these groups were divided into sub-clusters. A set of 5 SSR markers (*Xwms0752*, *Xbarc128*, *Xrems1237*, *Xwms0861* and *Xbarc170*) which have the calculated PIC value higher than 0.68 that are sufficient for the identification of the analyzed genotypes was described.

Keywords: Triticale, ×*Triticosecale* Wittmack, SSR, microsatellites, genetic variability

INTRODUCTION

Triticale (×*Triticosecale* Wittmack, 2n = 6x = 42; BBAARR) is an interspecific hybrid produced by crossing tetraploid wheat and rye. As the maternal plant was used wheat, rye was the paternal plant. Thanks to that triticale has high feeding value, usability in less favorable conditions, better resistance to cold and relatively higher resistance against diseases. The absence of D genome causes inappropriate bread-making quality. This deficiency can be eliminated by various substitutions and translocations of chromosomes (**Martinek *et al.*, 2008**).

With the increasing interest about triticale rises also the interest in breeding programs. The main prerequisite for successful breeding and utilization of specific genes for the desired properties is the knowledge of appropriate markers. Initially morphological and yield characteristics, monogenic resistance, etc. were used for indirect selection and subsequently molecular markers – at first proteins and then isoenzymes were used. Useful tool for detection of variation on the level of genetic information are techniques based on DNA markers. One of these methods is a technique based on the detection of genetic variability of microsatellites, which are short nucleotide repeats which differ in the number of repetitions, with are known since 1989 **(Tautz, 1989)**. In plants they have been used since 1993 **(Morgante and Olivieri, 1993)**. Easily detectable microsatellites repetition polymorphism allows the monitoring of genetic variability between species, within species or within individuals in a population. Evaluation of genetic similarities and differences between plant species provide important information for the protection of plants and breeding of new varieties. In recent years, microsatellite markers due to the high polymorphism and co-dominant character proved to be an effective tool for estimating genetic diversity and phylogenetic relations. They are widely used in many species such as wheat **(Gregáňová *et al.*, 2005; Kuleung *et al.*, 2006)**, rye **(Khlestkina *et al.*, 2004)** and triticale **(Da Costa *et al.*, 2007)**.

MATERIAL AND METHODS

The used ten genotypes of winter triticale (×*Triticosecale* Wittmeck, 2n = 6x = 42, BBAARR) included eight varieties (Hortenso, Inpetto, Pawo, SW Talentro, Tulus, Mungis, Agrano and Cando) that are registered in the Czech Republic, further Nazareth which was in 2012 restricted and a breeding line KM 4-09 with the chromosome translocation 1R.1D $_{5+10}$-2 (Tab 1). Is a result of the breeding program for improved bred-making quality from Agrotest fyto, Ltd. Kroměříž. Mixed samples of certified seeds were obtained from the Center Institute for Central and Testing in Agriculture in Brno and from Agrotest fyto, Ltd. Kroměříž (genotype KM 4-09).

Genomic DNA was isolated from young plants (6 days old) using the isolation kit DNeasy Plant Mini Kit (Qiagen, GE). The DNA concentration was assessed on a spectrophotometrically. 25 markers which were described in wheat **(Kuleung *et al.*, 2006; Röder *et al.*, 1998; Stephenson *et al.*, 1998)** and rye **(Khlestkina *et al.*, 2004)** were used (Tab 2). The reaction mixture for PCR of a total volume 25 µl contained 0.5 U *Taq* polymerase (Promega), 1× aliquot buffer, 0.1mM of each dNTP (Promega), 0.3 M of each primer and 30 ng of template DNA; the reaction conditions of PCR in T3 cycler (Biometra) – initial denaturation 2 min. at 93 ºC, then 30 cycles – denaturation 1 min. at 93 C, annealing 2 min. at 54 ºC, extension 2 min. at 72 ºC. Useful step seems to be control electrophoresis on 1.5% agarose gel (stained with ethidium bromide) with a fraction of the sample after amplification, which makes it possible to select usable samples for the separation on polyacrylamide gels. The amplification of SSR products was than visualized on 8% non-denaturating polyacrylamid (PAA) gels in TBE buffer (300 V) followed by staining with silver (0.2% $AgNO_3$). The resulting electrophoretograms were converted to binary matrices represented by the presence (1) or absence (0) of the alleles and then evaluated by means of the statistical software FreeTree version 9.1 (**Hampl *et al.*, 2001**) using the UPGMA (Unweight Pair Group Method with Arithemetic Mean) construction method and similarity coefficient according to Jaccard **(Jaccard, 1908)**. The software TreeView version 1.6 (**Page, 1996**) was used for the graphic visualization of the matrix. The diversity index (DI), the probabilities of identity (PI) and the polymorphic information contents (PIC) of SSR markers were calculated according to **Russell *et al.* (1997)**.

Table 1 Analyzed genotypes of winter triticale

Genotypes	Property
Agrano	Pflanzenzucht Saka GbR, GER
Cando	SW Seed Hadmersleben GmbH, GER
Hortenso	Hodowla Roslin Szelejewo Sp. z o.o, PL
Inpetto	SW Seed BV, GER
KM 4-09	Agrotest fyto, s. r.o, CZ
Mungis	Lochow-Petkus GmbH, GER
Nazaret	Selgen a.s., CZ
Pawo	Hodowla Roslin Strzelce, Sp. z o.o., PL
SW Talentro	SW Seed BV, GER
Tulus	NORDSAAT Saatzucht GmbH, GEER

Legend: CZ – Czech Republic, GER – Germany, PL – Poland

Table 2 Characterization of the SSR markers and their statistical analysis

SSR marker	Chromosomal location	Number of alleles	DI	PI	PIC
XBarc003	6A	2	0.42	0.42	0.33
Xbarc004	5BS	4	0.66	0.17	0.6
Xbarc012	3AS	4	0.64	0.16	0.59
Xbarc018	2BS	3	0.67	0.14	0.62
Xbarc024	3BL	3	0.62	0.18	0.57
Xbarc077	3BL	3	0.54	0.28	0.47
Xbarc109	5B	4	0.64	0.17	0.59
Xbarc128	2BS	4	0.74	0.06	0.72
Xbarc137	1BL	4	0.58	0.19	0.55
Xbarc 165	5AL	3	0.58	0.25	0.5
Xbarc170	4AL	4	0.72	0.09	0.69
Xbarc195	6AS	4	0.55	0.22	0.52
Xbarc321	3AS	3	0.59	0.24	0.52
Xgwm445	2A	2	0.42	0.42	0.33
Xpsp2999	1A	4	0.66	0.16	0.61
Xpsp3000	1B	4	0.61	0.19	0.57
Xrems1135	7R	1	0	1	0
Xrems1186	7R	2	0.48	0.39	0.36
Xrems1187	5R	2	0.32	0.51	0.27
Xrems1237	5R	4	0.72	0.07	0.7
Xrems1303	1R	3	0.46	0.34	0.41
Xwms0752	1R, 1A	4	0.7	0.08	0.68
Xwms0861	1R(2B)	7	0.73	0.1	0.69
Xwms1300	1R (7A,7B)	4	0.45	0.3	0.44
Xwms642	1DL	2	0.32	0.51	0.27
Average		**3.36**	**0.55**	**0.27**	**0.5**

Legend: DI – diversity index, PI – probabilities of identity, PIC – polymorphic information content

RESULTS AND DISCUSSION

Genetic variability of ten secondary hexaploid triticale genotypes (×*Triticosecale* Wittmack, 2n = 6x = 42; BBAARR) was assed using 25 SSR markers. Despite, that the D genome is not present in secondary hexaploid triticale (BBAARR) SSR marker *Xwms642*, which is located in the D genome of wheat, was successfully amplified. **Kuleung *et al.* (2006)** report that some of the D genome loci of chromosomes were derived from the genome of octoploid triticale (BBAADDRR) and were introduced into homeologous chromosomes, what was confirmed by several authors **(Tams *et al.*, 2004; Dou *et al.*, 2006)**. **Vyhnánek *et al.* (2009)** also successfully used in triticale six SSR markers from the D genome. Four SSRs showed polymorphic spectrum and one of them was also able to distinguish one of the genotypes from the others. Location of these sequences in the genome of triticale is unknown.

In our set of ten triticale genotypes 84 alleles were detected (Table 2), i.e. on average 3.36 alleles per locus. One SSR marker (*Xrems1135)* out of the 25 analyzed gave a uniform spectrum, in most cases from 2 to 4 alleles, and also a zero allele in SSR marker *Xrems1303* were detected. The null allele of *Xrems1303* was also identified in a larger set of triticale genotypes by **Vyhnánek *et al.* (2009)**. The highest number of 7 alleles was detected in *Wms861*. For the analyzed set the average values were: DI 0.55 (0.0 – 0.74), PI 0.27 (0.06 – 1.0) and PIC 0.5 (0.0 – 0.72). Average number of detected alleles and the PIC value were lower than reported by **Tams *et al.* (2004)** and **Kuleung *et al.* (2006)**; probably caused by a lower number of analyzed genotypes. Also localization of used microsatellites could influence the value, because if the SSR markers are derived from exon (EST-SSR), the variability is lower **(Tóth *et al.*, 2000)**. On the basis of the DI, PI and PIC values we can recommend 5 SSR markers (*Xwms0752*, *Xbarc128*, *Xrems1237*, *Xwms0861* and *Xbarc170*) which have the calculated PIC value higher than 0.68 that are sufficient for the identification of the analyzed genotypes.

Two alleles in microsatellites in varieties Agrano (*Xbarc109, Xbarc018* and *Xpsp3000*) and Hortenso (*Xbarc018* and *Xwms1300*) were detected. Triticale is a self-pollinated crop with a 19% tendency to cross-pollination **(Malik *et al.*, 1984)**. Because DNA was isolated from a mixed sample it was not possible to distinguish if of two sister lines were the detection in the seed sample or if the varieties had heterozygous constitution. Only analysis of individual plants could answer the question.

Amplicons ranged in size from 70 to 340 bp with an average size about 200 bp, which were similar to **Röder *et al.* (1998)**, **Khlestkina *et al.* (2004)** and **Kuelung *et al.* (2006)**.

On the basis of statistical elaboration we set up a dendrogram of similarities (Jaccard's coefficient) of the analysed genotypes (Figure 1). Dendrogram is consisting of two significantly separated clusters. Cluster number one is divided into two sub-clusters. The first sub-cluster consists of the German variety Tulus, which is separated from the two German varieties Agrano and Cando. The second sub-cluster consists of the breeding line KM 4-09 separated from a group composed of the Polish variety Pawo and the German variety SW Talentro. The cluster number two includes four varieties, which are divided into sub-groups, in descending order: the Polish variety Hortenso, the German variety Inpetto, the German variety Mungis and along with Czech variety Nazaret. We didn't detect influence of provenance on the formations of clusters. Only in the case of the variety Mungis and Inpetto was found common progenitor, variety Modus, was found in Plant Genetic Resources Documentation in the Czech Republic. The other varieties did not have a common progenitor in their pedigree nor their had origin from the same breeding station.

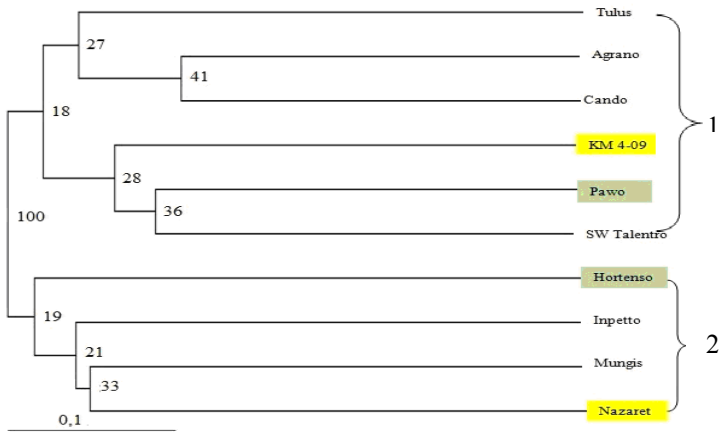

Figure 1 Dendrogram characterizing the genetic relations of triticale genotypes tested by 25 SSR markers (yellow – Czech varieties, grey – Polish varieties, without color – German varieties, 1 – cluster one, 2 – cluster two)

CONCLUSION

The results demonstrate the practical application and usability of SSR markers described in wheat and rye for determination of genetic variability in triticale. In this study a set of 5 SSR markers sufficient to distinguish all analyzed varieties and breeding lines was found. These results can be used in breeding programs aimed at triticale, e.g. in Agrotest fyto, Ltd. Kroměříž, the Czech Republic.

Acknowledgments: Our thanks are due to Dr. P. Martinek (Agrotest fyto, Ltd.) for his cooperation. This work was supported by a project GA CR no. 204/09/H002 from the Czech Scientific Foundation.

REFERENCES

Da COSTA, C. T., ALBUQUERQUE, A. CH. S., NASCIMENTO, A., ARCELINO, F. C., PEREIRA, J. F. 2007. Genetic diversity of Brazilian triticales evaluated with genomic wheat microsatellites. *Pesquisa Agropecuária Brasileira*, 40(11), 1576-1585.

DOU, Q. W., TANAKA, H., NAKATA, N., TSUJIMOTO, H. 2006 Molecular cytogenetic analyses of hexaploid lines spontaneously appearing in octoploid triticale. *Theoretical and Applied Genetics*, 114(1), 41-47.

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

HAMPL, V., PAVLÍČEK, A., FLEGR, J. 2001. Construction and bootstrap analysis of DNA fingerpriting-based phylogenetic trees with a freeware program FreeTree: Application to Trichomonad parasites. *International Journal of Systematic and Evolutionary Microbiology*, 51(4), 731-735.

JACCARD, P. 1908. Nouvelles recherches sur la distribution florale. *Bulletin del la Société Vaudoise des Sciences Naturelles,* 44, 223–270.

KHLESTKINA, E. K., PESTSOVA, E. G., RÖDER, M. S., MALYSHEV, S. V., KORZUN, V., BÖRNER, A. 2004. Mapping of 99 new microsatellite-derived loci rye (*Secale cereale* L.) including 39 expressed sequence tags. *Theoretical and Applied Genetics*, 109(4), 725-732.

KULEUNG, C., BAENZIGER, P. S., KACHMAN, S. D., DWEIKAT, I. 2006. Evaluating the genetic diversity of triticale with wheat and rye SSR markers. *Crop Science Society of America,* 108(6), 1692-1700.

MALIK, K. P. S. 1984. Seed set by spontaneous cross-pollination in triticale. *Cereal Research Communications,* 12(12), 111-112.

MARTINEK, P., VINTEROVÁ, M., BUREŠOVÁ, I., VYHNÁNEK, T. 2008 Agronomic and quality characteristics of triticale (×Triticosecale Wittmack) with HMW glutenin subunits 5+10. *Journal of Cereal Science*, 47(1), 68-78.

MORGANTE, M., OLIVIERI, A. M. 1993. PCR-amplified microsatellites as markers in plant genetics. *Plant Journal*, 3(1), 175-182.

PAGE, R. D. M. 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences*, 12(3), 357-358.

RÖDER, M. S., KORZUM, V., WENDEHAKE, K., PLASCHKE, J., TISIER, M. H., LEROY, P., GANAL, M. W. 2007. A microsatellite map of wheat. In *Genetics Society of America*, 149(4), 2007 - 2023.

RUSSELL, K., FULLER J., YOUNG G., THOMAS B., TARAMINO G., MACULAY M., WAUGH R., POWELL W. 1997. Discriminating between barley genotypes using microsatellite markers. *Genome,* 40(4), 442-450.

STEPHENSON, P., BRYAN, G., KIRBY, J., COLINS, A., DEVOS, K., BUSSO, C., GALE, M. 1998. Fifty new microsatellite loci for the wheat genetic map. *Theoretical and Applied Genetics*, 97(5), 946 - 949.

TAMS, S. H., BAUER, E., OETTLER, G., MELCHINGER, A. E. 2004. Genetic diversity in European winter triticale determined with SSR markers and coancestry coefficient. *Theoretical and Applied Genetics*, 108(7), 1385-1391.

TAUTZ, D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Research*, 17, 6436-6471.

TÓTH, G., GÁSPÁRI, Z., JURKA, J. 2000. Microsatellites in different eukaryotic genomes: survey and analysis. *Genome Research*, 10(7), 967-981.

VYHNÁNEK, T., NEVRTALOVÁ, E., SLEZÁKOVÁ, K. 2009. Detection of the genetic variability of triticale using wheat and rye SSR markers. *Cereal Research Communications*, 37(1), 23 – 29.