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MOLECULAR MARKERS OF GENETIC VARIABILITY IN TRITICALE VARIETIES REGISTERED IN THE CZECH REPUBLIC

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Abstract

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Genetic variability was detected in 15 varieties of triticale (X*Triticosecale* Wittmack., 2n = 6x = 42, BBAARR) registered in the Czech Republic by means of polymorphism of DNA using the RAPD method and the SSR method. For detection we used 80 RAPD primers. The lower reproducibility of the RAPD markers was resolved by means of repeated analyses (3–4 times). On the basis of statistical evaluation a dendrogram was set up, which allows highly significantly to differentiate the varieties Kolor, Modus and Tornado. The remaining 12 analysed varieties formed 4 clusters. In addition to the RAPD markers a protocol of detection of DNA polymorphism was elaborated and optimised with microsatellite (SSR) markers. For the analyses we used 2 SSR markers (1A chromosome [*Xpsp2999*] and 1B chromosome [*Xpsp3000*]), which have been discovered in wheat (*T. aestivum* L.). Basing on these two SSR markers the only variety Triamant was distinguished from the clusters of the other analysed varieties.

triticale, X*Triticosecale* Wittmack., DNA markers, RAPD, SSR

Triticale (X*Triticosecale* Wittmack.) is an autogamous plant with a low share of cross-pollination (4–5%). This means that the majority of varieties are of the line type or a mixture of isogenic lines (Chloupek, 2000). A number of methods are now available for the detection of the genetic variability (diversity), e.g. morphological characteristics; analysis of pedigrees; biochemical markers, particularly proteins and their various isoenzyme variants; molecular (DNA) markers etc.

The aim of this work was to evaluate the genetic diversity within 15 registered varieties of triticale (X*Triticosecale* Wittmack., 2n = 6x = 42) by RAPD and SSR markers.

MATERIAL AND METHODS

The polymorphism of DNA was analysed in 13 winter forms and two spring forms of triticale varieties registered in the Czech Republic (Tab. I). Mixed samples of certified seeds from the 2002 and 2003 harvests were obtained from the Central Institute for Supervising and Testing in Agriculture, testing station in Hradec nad Svitavou.

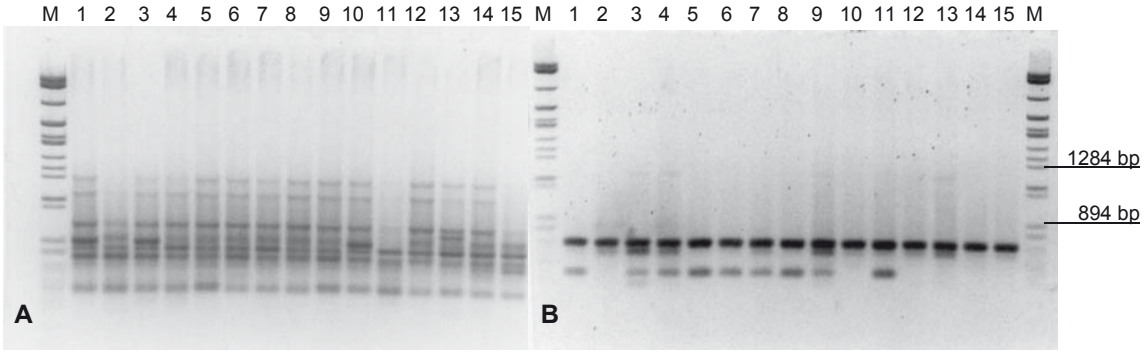
Genomic DNA was isolated from young plants (6 days old) using the Dneasy Plant Mini Kit isolation kit (Qiagen, GE). The DNA concentration was assessed on a spectrophotometer.

I: *Analysed triticale varieties*

Variety		Year of registration	Property
spring	Gabo	1999	Hodowla Roslin Strzelce, Sp. z o. o., Poland
	Legalo	2004	DANKO Hodowla Roslin, Sp. z o. o., Poland
winter	Disco	1997	DANKO Hodowla Roslin, Sp. z o. o., Poland
	Lamberto	2003	DANKO Hodowla Roslin, Sp. z o. o., Poland
	Lupus	2003	NORDSAAT Saatzuchtgesellschaft mbH, Germany
	Kitaro	2003	DANKO Hodowla Roslin, Sp. z o. o., Poland
	Kolor	1996	SELGEN, a. s., Czech Republic
	Marko	2001	Hodowla Roslin Strzelce, Sp. z o. o., Poland
	Modus	1998	NORDSAAT Saatzuchtgesellschaft mbH, Germany
	Presto	1990	DANKO Hodowla Roslin, Sp. z o. o., Poland
	Sekundo	2000	Hodowla Roslin Szelejewo, Sp. z o. o., Poland
	Ticino	2003	Pflanzenzucht Saka GbR, Germany
	Tornado	1996	Hodowla Roslin Strzelce, Sp. z o. o., Poland
	Triamant	2004	Lochow-Petkus GmbH, Germany
	Tricolor	2002	Florimond Desprez, France

RAPD (Random Amplified Polymorphic DNA) analysis:

We analysed 80 RAPD primers chosen in accordance with literature. The reaction mixture for PCR, total volume 25µl contained 0.4 U Taq polymerase (Finnzyme), 1× aliquot buffer (Finnzyme), 0.25 mM of each dNTP (Promega), 20 ng of primer and 25 ng of template DNA. The reaction conditions of PCR in T3 cycler (Biometra) – initial denaturation 1 min. at 94 °C, then 45 cycles – denaturation 1 min. at 94 °C, annealing 2 min. at 35 °C, extension 1 min. at 72 °C. A final extension step at 72 °C for 10 min. followed. Electrophoretic separation was carried out on 1.5% agarose gel and visualization using ethidium bromide (Fig. 1). The lower reproducibility of the RAPD markers was resolved by means of repeated analyses (3–4 times).



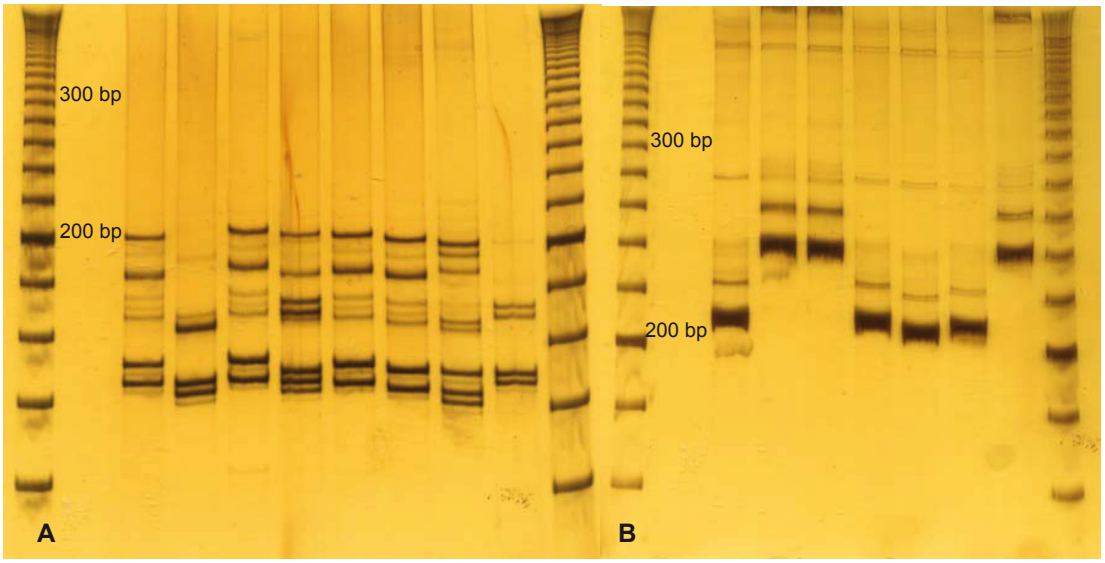
1: *RAPD patterns of 15 triticale genotypes obtained with different primers*
A) *primer OPF13;* B) *primer BC628*
M – DNA size marker (λDNA *Eco*471 /*Ava*II/, MBI Fermentas); 1 – Presto; 2 – Kolor; 3 – Disco; 4 – Modus; 5 – Sekundo; 6 – Marko; 7 – Tricolor; 8 – Kitaro; 9 – Lamberto; 10 – Ticino; 11 – Gabo; 12 – Lupus; 13 – Tornado; 14 – Triamant; 15 – Legalo

SSR (Simple Sequence Repeats) analysis:

We analysed 2 SSR markers (Devos et al., 1995). The reaction mixture for PCR, total volume 25µl contained 0.5 U Taq polymerase (Promega), 1× aliquot buffer, 0.1 mM of each dNTP (Promega), 0.3 M of each primer and 30 ng of template DNA. The reacti-

on conditions of PCR in T3 cycler (Biometra) – initial denaturation 2 min. at 93 °C, then 30 cycles – denaturation 1 min. at 93 °C, annealing 2 min. at 54 °C, extension 2 min. at 72 °C. The amplification SSR products were visualized on 15% non-denaturation polyacrylamid gels in TBE buffer (300 V) followed by colouring with silver (0.2% $AgNO_3$) (Fig. 2).

The resulting electrophoreograms were converted to binary matrices represented by the presence (1) or absence (0) of resulting alleles and then evaluated by means of statistical software FreeTree version 9.1 using the UPGMA construction method and similarity coefficient according to Jaccard. The software TreeView version 1.6 was used for the graphical expression of the matrix.



2: *SSR patterns of 15 triticale genotypes*
A) *Xpsp2999 – (from the left) DNA size marker (20 bp extended DNA ladder), negative control, Kitaro, Lamberto, Ticino, Gabo, Lupus, Tornado, Triamant, Legalo, DNA size marker*; B) *Xpsp3000 – (from the left) DNA size marker, negative control, Presto, Kolor, Disco, Modus, Sekundo, Marko, Tricolor, DNA size marker*

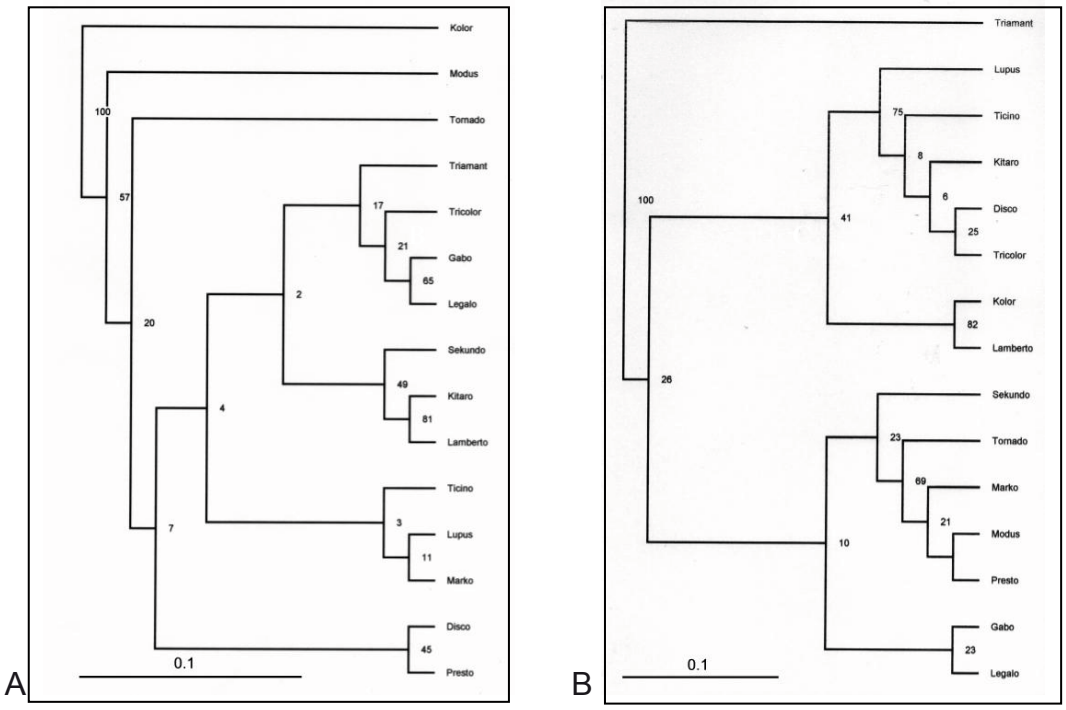
RESULTS AND DISCUSSION

Garg et al. (2001) assessed the RAPD method as the suitable method for mapping the genetic diversity in triticale, second only to the SSR method and better than the AFLP method. In our case the individual RAPD primers resulted in different polymorphism of the obtained DNA products. Out of the 80 RAPD primers used, 8 showed a uniform spectrum of products for all the 15 triticale varieties (Tab. II). The remaining primers highly significantly differentiated the varieties Kolor, Modus and Tornado from the other varieties, which make up four groups (Fig. 3A). One group involves the spring varieties Gabo and Legalo along with the winter varieties Tricolor and Triamant. Since a mixed sample (miniprep) was used, the application of RAPD primers did not make it possible to detect the genetic variability within the individual varieties, unlike prolamine proteins of the triticale kernel (Vyhnánek & Bednář, 2005).

II: *Sequences of the used RAPD primers with a uniform spectrum of products*

Primers	Sequence (5´→3´)
H12	TTC CGA ACC C
H17	GGA AAC CCC T
H39	AGC GTG TCT G
H47	CTC TCC GCC A
H52	GAA ACA CCC C
H61	GTG ACA GGC T
H62	TCG CCG CAA A
H82	GGT GAC GCA G

The methods of detection of SSR markers of wheat in triticale were tested. Kuleung et al. (2004) assessed the possibility of use of wheat and rye SSR markers in triticale and confirmed the transferability of 58% of wheat and 39% of rye SSR markers into the triticale genome. On the basis of a dendrogram and with the aid of 2 SSR markers we managed highly significantly to differentiate the Trimaran variety and the other analysed varieties (Fig. 3B). On the dendrogram we can see a cluster of spring triticale forms (Gabo and Legalo).



3: *Dendrograms of 15 triticale varieties (Jaccard coefficient, P = 99%)*
A) *RAPD primers*; B) *SSR markers*

The SSR markers are localised on the short arm of chromosome 1A [*Xpsp2999*, motive $(CAG)_5$ $(CAA)_8$] and short arm of chromosome 1B [*Xpsp3000*, motive $(CAA)_{15}$]. Bougot et al. (2002) used the SSR marker *Xpsp2999* to distinguish the alleles of *Pm3* resistance to *Blumeria graminis*. Devos et al. (1995) showed the linkage of this marker with LMW glutenins and the gliadin gene *Gli 1-1*. In our case the products ranged between 140 and 160 bp. We detected 4 allele variants. Using this SSR marker in wheat, Manifesto et al. (2001) detected products of similar size (133–157 bp) and described 11 allele variants incl. the zero allele. The zero allele was not detected in the analysed triticale varieties. Linkage with γ-gliadins was described in the SSR marker *Xpsp3000* (Devos et al., 1995); in linkage with the $Yr10_{vav}$ the product of 285 bp is in relation to yellow rust resistance (Bariana et al., 2002). Manifesto et al. (2001) discovered 13 allele variants in wheat (213–285 bp) including the zero allele. In our varieties the product size ranged between 200 and 260 bp. The 260 bp size allele was detected in the Triamant variety only.

In our experiments better resolution of the varieties by the RAPD method was obtained than using SSR markers. The 72 polymorphic RAPD primers highly significantly differentiated 3 varieties while only one was differentiated by 2 SSR markers. The use of a higher number of SSR markers would probably increase the resolution and the results would be in favour of SSR markers similarly as reported by Garg et al. (2001).

SOUHRN

Molekulární markery genetické variability u odrůd tritikale registrovaných v České republice

Genetická variabilita byla detekována metodami molekulární genetiky (RAPD a SSR) u 15 odrůd tritikale (X*Triticosecale* Wittmack., 2n = 6x = 42, BBAARR), které jsou registrovány v České republice.

Pro detekci polymorfizmu DNA bylo využito 80 RAPD primerů. Nižší reprodukovatelnost RAPD analýz byla kompenzována opakováním analýz (3–4×). Na základě statistického vyhodnocení byl sestaven dendrogram, kde je možno vysoce průkazně odlišit odrůdy Kolor, Modus a Tornado. Zbývajících 12 analyzovaných odrůd tvoří čtyři klastery. Byl optimalizován protokol detekce variability DNA pomocí mikrosatelitních markerů u tritikale. Pro analýzy byly využity dva SSR markery (1A chromozom [*Xpsp2999*] a 1B chromozom [*Xpsp3000*]), které byly popsány u pšenice obecné (*T. aestivum* L.). Na základě použitých dvou SSR markerů se podařilo odlišit odrůdu Triamant od klasterů ostatních analyzovaných odrůd.

tritikale, X*Triticosecale* Wittmack., DNA markery, RAPD, SSR

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