SHORT COMMUNICATION

Detection of Genetic Relationships among Spring and Winter Triticale (× *Triticosecale* Witt.) and Rye Cultivars (*Secale cereale* L.) by Using Retrotransposon-based Markers

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Abstract

Trebichalský A., Kalendar R., Schulman A., Stratula O., Gálová Z., Balážová Ž., Chňapek M. (2013): **Detection of genetic relationships among spring and winter triticale (×** *Triticosecale* **Witt.) and rye cultivars (***Secale cereale* **L.) by using retrotransposon-based markers**. Czech J. Genet. Plant Breed., **49**: 171–174.

In the present research, we aimed to detect and evaluate the level of long terminal repeat (LTR) retrotransposons (WIS2, Wilma, Daniela, and Wham) intraspecific variability and intron polymorphism for β-amylase (BAMY) genes in 37 winter and 25 spring triticale cultivars coming from European countries and the USA and 5 Finnish rye cultivars. The triticale and rye genotypes differ significantly with respect to the patterns of the four explored LTR retrotransposons. A neighbour-joining dendrogram has separated all triticale and rye cultivars into three principal clusters: all winter triticale, all spring triticale and all rye cultivars. We have proved that retrotransposon-based markers can be used for differentiation of triticale and rye cultivars.

Keywords: β-amylase (BAMY) genes; long terminal repeat (LTR) genes; genetic diversity; molecular markers; rye; triticale

Triticale (× *Triticosecale* Witt.) is a cereal bred by the crossing of wheat and rye. It has the potential to introduce important economic and environmental benefits to grain production systems. Triticale is a high-quality feedstuff and produces a similar grain yield like other cereal crops, but more biomass, and can cope with a wide range of abiotic stress conditions (Alheit *et al.* 2011). Molecular markers are essential in plant and animal breeding and biodiversity applications, and for the map-based cloning of genes (Kalendar *et al.* 2011). Retrotransposons are widely distributed in higher plants, and make up a large fraction of the genome; in wheat 90% of the genome consists of retrotransposons. Several

studies for detection of genetic diversity in plants by retrotransposon-based markers have been reported (Žiarovská *et al.* 2009; Kalendar 2011). Molecular marker techniques such as SSAP (Sequence-Specific Amplification Polymorphism) and the related IRAP (Inter-Retrotransposons Amplified Polymorphism) and the REMAP (Retrotransposon-Microsatellite Amplified Polymorphism) are based on retrotransposon activity, and are widely used today. The IRAP technique examines the polymorphism of retrotransposons themselves (Todorovska 2007) and largely confirmed absence of any intraspecific variation in wheat, rye and triticale (Bento *et al.* 2008). Smýkal (2006) mentioned as the major advantage of IRAP (over the other methods used in pea) the high information content gained per single PCR analysis, which substantially cuts time and cost. The representatives of the *Triticeae* (wheat, barley and rye) have two distinct forms of β-amylase (BAMY) genes which differ in their expression patterns; one form is specific to the endosperm, while the other has a tissue-ubiquitous pattern of expression (Ziegler 1999). The aim of our study was to identify the degree of genetic diversity between winter and spring cultivars of triticale from different European countries and the USA and Finnish rye cultivars based on retrotransposon-based markers and exon-primed intron-crossing PCR amplification (EPIC) for grass BAMY genes.

Sixty-two triticale cultivars (× *Triticosecale* Witt.) were provided by the Gene Bank of the Slovak Republic in Piešťany. The varieties come from different European countries and some of them from the USA. The five rye varieties were provided by MTT Agrifood Research Finland (Jokioinen). Genomic DNA was manually isolated using the standard CTAB method (Doyle & Doyle 1990) with RNAse A treatment. The sequences of four wheat transposable elements (WIS2, Wilma, Daniela, and Wham) were taken from the TREP database (http://wheat.pw.usda.gov/ggpages/ITMI/Repeats/). Primers (Table 1) for the conserved segments of the long terminal repeat (LTR) and BAMY genes were designed using FastPCR (Kalendar *et al.* 2011). IRAP analysis was conducted according to Kalendar and Schulman (2006). The PCR was performed in a 25 μl reaction mixture containing 25 ng DNA, 1× BioTools buffer, 0.5μM primer, 200μM dNTP, and 1U BioTools polymerase (Biotools, B&M Labs, Madrid, Spain). The PCR program consisted of 1 cycle at 95°C for 3 min; 32 cycles at 95°C for 30 s and at 60°C for 1 min and at 72°C for 2 min; and a final extension step of 72°C for 5 min. PCR amplification was performed in a 25 μl reaction mixture containing 25 ng DNA, 1× BioTools buffer, 0.3μM each primers, 200μM dNTP and 1U BioTools polymerase. The PCR program consisted of 1 cycle at 95°C for 3 min; 32 cycles at 95°C for 20 s and at 68°C for 1 min and at 72°C for 3 min; and a final extension step of 72°C for 5 min. Amplifications were performed in a thermocycler (Biometra GmbH, Goettingen, Germany) in 0.2 ml tubes. The PCR products were resolved by electrophoresis on 1.5% agarose stained with ethidium bromide during 12–16 h at 50–60 V. Each band was treated as a single locus. The presence or absence of a fragment of a given length was recorded in binary code. Based on NJ (Neighbour-Joining) algorithm using PAUP software (Swofford 1998) a dendrogram was constructed (Figure 1). Diversity index (DI), probability of identity (PI) and polymorphism information contents (PIC) were calculated according to Russel *et al.* (1997).

A high level of genetic diversity among the winter and spring triticale cultivars from different European countries and the USA and Finnish rye cultivars was revealed by IRAP analysis and EPIC PCR amplification for BAMY genes. Rye served as a comparative system for detection of common or

Table 1. The list of used long terminal repeat (LTR) retrotransposons and β-amylase (BAMY) primers

Name	LTR retrotransposon source	Sequence	Temperature (°C)
2106	Wis2	TAATTTCTGCAACGTTCCCCAACA	57.1
2107	Wilma	AGCATGATGCAAAATGGACGTATCA	56.8
2108	Wilma	AGAGCCTTCTGCTCCTCGTTGGGT	63.4
2109	Daniela	TACCCCTACTTTAGTACACCGACA	56.0
2123	Wham	GGAAAAGTAGATACGACGGAGACGT	57.9
3162	BAMY	TCCAAGTCTACGTCATGCTCC	56.4
3816	BAMY	GCTGCTGCTGCTTTGAAGTCTGCT	62.3

Table 2. The statistical characteristics of the markers used in triticale

Primer(s)	DI			PIC			PI			No. of polymorphic bands
	winter	spring	rye	winter	spring	rye	winter	spring	rye	
2106 (Wis2)	0.613	0.854	0.874	0.538	0.848	0.870	0.218	0.003	0.002	15
2107 (Wilma)	0.847	0.709	0.851	0.843	0.678	0.845	0.005	0.092	0.015	12
2108 (Wilma)	0.879	0.859	0.886	0.876	0.855	0.884	0.002	0.012	0.002	21
2109 (Daniela)	0.841	0.894	0.855	0.834	0.893	0.851	0.005	0.001	0.004	17
2123 (Wham)	–	0.859	0.831	–	0.855	0.823	–	0.003	0.022	12
BAMY gene	0.818	0.885	0.741	0.816	0.882	0.710	0.008	0.002	0.086	8
Average	0.799	0.844	0.840	0.781	0.835	0.831	0.048	0.019	0.022	14.2

DI – diversity index; PIC – polymorphism information content; PI – probability of identity; BAMY – β-amylase

different bands with triticale cultivars. In triticale and rye, IRAP provided from 12 to 21 polymorphic bands with an average of 15.4 polymorphic bands per primer (Table 2). The frequencies of alleles and the values of DI, PI and PIC were calculated (Table 2). The average PIC and DI values in spring triticale were higher than in winter triticale and rye. DI values for all markers were higher than 0.6, which is generally considered sufficient for this purpose. The average PI values of spring and winter triticale, and rye were low (0.019, 0.048 and 0.022, respectively) and reflect a possibility of differentiating genetically close genotypes. We confirmed that the IRAP technique used was an appropriate way to differentiate between triticale and rye genotypes. The BAMY genes have shown similar results like IRAP markers. The average PIC and DI values of the tested sets of retrotransposon-based markers were higher compared to the average PIC (0.743) and DI (0.750) values obtained by 5 microsatellite SSR markers (Trebichalský *et al.* 2013). Vyhnánek *et al.* (2009) used 48 SSR markers in the study of genetic variability in 16 genotypes of triticale and their average PIC (0.48) and DI (0.52) were lower and PI was higher (0.31) compared to the values of our retrotransposon-based markers. The dendrogram separated all the cultivars in three principal clusters (Figure 1). Whereas the first cluster comprised 37 winter triticale cultivars (marked in blue), the second cluster included 25 spring triticale cultivars (marked in red) and all five rye cultivars (marked in green) were separated in the third cluster. The first cluster was further subdivided into many smaller groups. American cultivar NE 422T and Hungarian cultivar Tatra were significantly separated from all other winter triticale cultivars. In the sub-cluster of winter

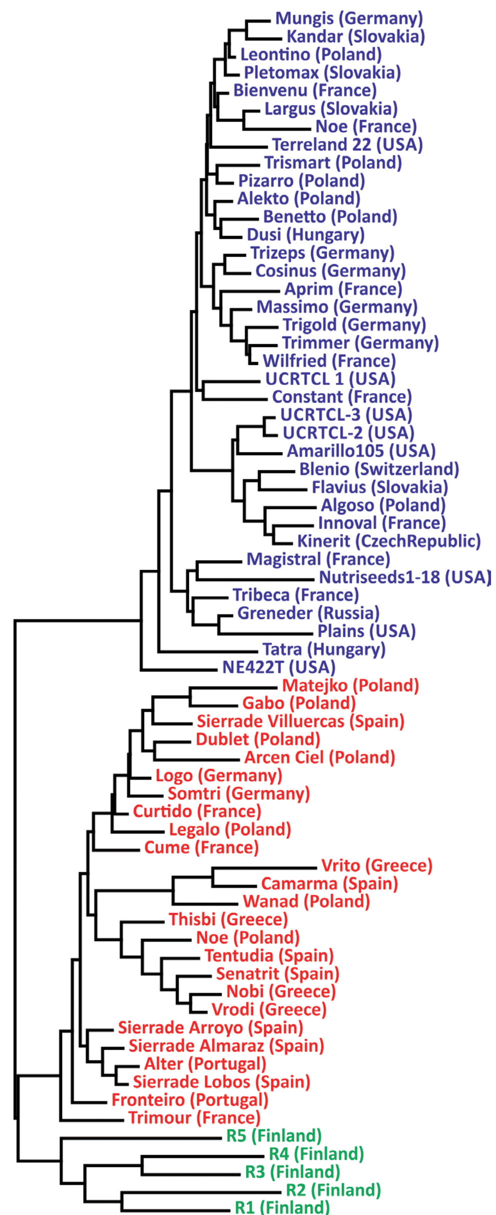

Figure 1. NJ dendrogram (bootstrap replications 1000) for triticale and rye genotypes based on IRAP analysis

triticale cultivars there were many sub-clusters, but the closest genetic relationship was shown by American varieties UCRTLC-2 and UCRTLC-3. The second largest sub-cluster contained spring triticale cultivars. As far as the first cluster is concerned, spring triticale cultivars grouped in many different sub-clusters. Also in the second cluster French cultivar Trimour and Portuguese cultivar Fronteiro separated from other cultivars. The closest genetic relationships were detected between Portuguese cultivar Alter and Spanish cultivar Sierra de Lobos and also between two Greek cultivars Niobi and Vrodi. Trebichalský *et al*. (2013) used 5 SSR markers for detection of genetic polymorphism in 59 triticale cultivars. A constructed dendrogram separated genotypes into two clusters, but the used set of markers was not able to separate 10 cultivars between each other.

It was found that the used retrotransposon-based markers provided enough polymorphism. All cultivars examined were separated from each other. A constructed NJ dendrogram separated all samples into three main clusters: for all winter triticale, for all spring triticale and all rye cultivars. Taking into account all the results (PIC, DI, PI and dendrogram features), we can confirm that this technique of retrotransposon-based markers is highly efficient and a fast way to detect genetic variability between triticale genetically related genotypes.

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