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REGULAR ARTICLE

EXPLORATION OF GENETIC RELATIONS BETWEEN WINTER TRITICALE (X *Triticosecale* Witt.) AND RYE (*Secale cereale* L.) CULTIVARS USING RETROTRANSPOSON-BASED MARKERS

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ABSTRACT

Retrotransposons can be used for markers because their integration creates new joints between genomic DNA and their conserved ends. In our work, we had focused on finding of genetic relationships between winter triticale (X *Triticosecale* Witt.) and rye (*Secale cereale* L.) using retrotransposon-based markers. Together, five IRAP primers combinations and β-amylase (BAMY) genes were used for polymorphism detection of 37 cultivars of winter triticale and 5 cultivars of rye. From the obtained data, we had constructed UPGMA dendrogram, which differentiate samples on the basis of genetic relationships in two principal cluster and several subclusters. We had confirmed close relationships between cultivars Leontino and Pletomax, Trimmer and Wilfried or between cultivars Sorento and Amarillo 105.

Keywords: retrotransposon-based markers, genetic relationships, triticale, rye

INTRODUCTION

Triticale (X *Triticosecale* Wittmack), a man-made hybrid of wheat (*Triticum* spp.) and rye were created to combine the high yielding capacity of wheat with the stress tolerance of rye (**Lukaszewski, 2006**). Retrotransposon are an evolutionarily ancient class of mobile genetic elements that transpose replicatively within their host genomes via RNA intermediates (**Flavell, 1999**). They can be used as markers because their integration creates new joints between genomic DNA and their conserved ends (**Kalendar and Schulman, 2006)**. Retrotransposons can be divided into two main categories, those with long terminal repeats LTRs and those that lack such repeats, for example, long interspersed nuclear elements (LINEs) **(McCarthy and McDonald 2004)**. Retrotransposons have several advantages as molecular markers over the other systems described above. Their abundance and dispersion can yield many marker bands, the pattern possessing a high degree of polymorphism due to transpositional activity. **Kalendar *et al.* (1999)** developed the IRAP (Inter-Retrotransposon Amplified Polymorphism), method based on the position of given LTRs within the genome. These markers are generated by the proximity of two LTRs using outward-facing primers annealing to LTR target sequences.

The representatives of the *Triticeae* (wheat, barley, rye) have two distinct forms of β-amylase (1,4-α-glucan maltohydrase; E.C.3.2.1.2), 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 objective of our study was to reveal genetic relationships between 37 genotypes of winter triticale and 5 cultivars of rye using by retrotransposon-based markers IRAP and β-amylase (BAMY) genes.

MATERIAL AND METHODS

Plant material and DNA isolation

Collection of the 37 genotypes of winter triticale (X *Triticosecale* Witt.) was provided by the Gene bank of Slovak republic in Piešťany. The 5 cultivars of rye (*Secale cereale* L.) were obtained from MTT (Jokioinen, Finland). The 7 day-old leaves were collected and prepared for a straight DNA isolation. We have used the CTAB method (**Ausubel *et al.*,**

1995) with RNaseA for quick DNA extraction. Concentration of DNA was measured by Nanodrop.

Table 1 List of samples and their origin

Country of origin	Cultivars
Poland	Sorento, Leontino, Pizarro, Algoso, Alekto, Trismart
USA	NE422T, UCRTCL1-2001, UCRTCL3-2001, UCRTCL2-2001, Terrelland 22, Nutri Seeds I-18, Plains
Russia	Greneder
Germany	Mungis, Trimmer, Trizeps, Trigold, Amarillo 105, Massimo, Cosinus
France	Aprim, Bienvenu, Constant, Wilfried, Tribeca, Innoval, Magistral, Noe
Hungary	Dusi, Tatra
Switzerland	Blenio
Czech republic	Kinerit
Slovakia	Flavius, Kandar, Largus, Pletomax

PCR conditions

The PCR reaction was performed in 25 µl mixture (PCR premix + DNA). PCR premix (24 µl) comprised of Milli-Q water, 10 x BioTools buffer, 10 mM dNTP, 10 µM primer and 5 U/µl BioTools polymerase. The PCR reaction ran in the Biometra thermocycler under these conditions: 2 min denaturation step at 98°C; 29-32 cycles of 10s at 98°C, 60s at 60°C and 60s at 72°C; 5 min a final extension at 72°C. The number of cycles and an overall time of PCR reaction depended strongly on the used primer (29x – 32x). After PCR, 40 µl of 1 x loading buffer was added to tubes with PCR products and than the mixture was loaded on the prepared gels.

Electrophoresis conditions

There were prepared 1.5 % agarose gel with ethidium bromide and loaded 8 µl of each sample on the gel. Generally, voltage at intervals 50-60 V for 16-19 hours was used. The actual position of DNA in gels was checked with UV transilluminator. Gels were scanned by FUJIFILM FLA-5100 (Fuji Photo Film (Europe) GmbH., Germany) imaging system with a resolution of 50 µm. Dendrogram was constructed by MEGA 5 software.

RESULTS AND DISCUSSION

Retrotransposons are ubiquitous in plants and play a major role in plant gene and genome evolution. In many cases, retrotransposons comprise over 50% of nuclear DNA content (**Kumar and Bennetzen, 1999**). These markers have been developed for studing of bread wheat (*Triticum aestivum*) genome and its wild relatives (**Queen *et al.*, 2004**).

Aim of our work was to use retrotransposon-based markers IRAP and β-amylase (BAMY) genes for purpose of genetic relationships detection between winter triticale and rye. Gels were evaluated on the basis of presence or absence of band in each line. Obtained data from gels (**Fig 1**) were used for construction of UPGMA dendrogram (**Fig 2**), which differentiated samples in two principal clusters.

IRAP used **Bento *et al.* (2010)** to asses genome rearrangements in wheat-rye addition lines obtained by the controlled backcrossing of octoploid tritcale to hexaploid wheat followed by self-fertilization. The comparative analysis revealed in those lines the presence of wheat-origin bands absent in triticale, and the absence of rye-origin and triticale-specific bands.

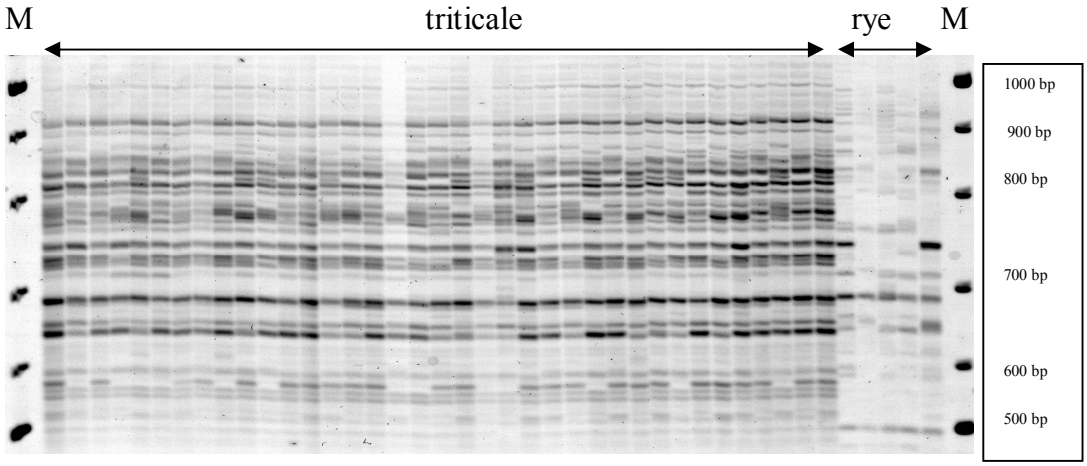

Figure 1 IRAP banding profiles of triticale and rye (M -size marker).

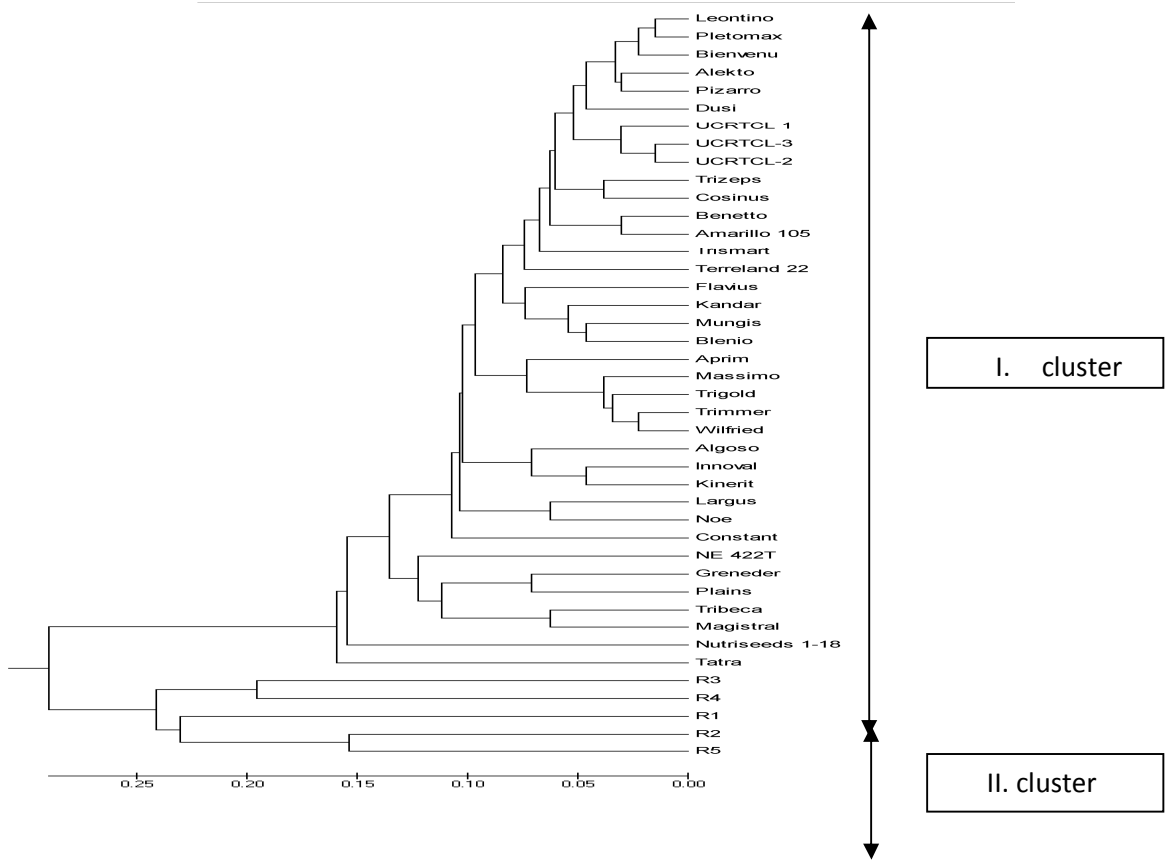

Figure 2 UPGMA dendrogram

The first cluster involved samples of triticale and comprised of several sub-cluster. The first subcluster is divided into two groups, with evident close relationships between Polish cultivar Leontino and Slovak Pletomax, Polish cultivars Alekto and Pizarro and American UCRTCL-1, UCRTCL-2 and UCRTCL-3. In the second subcluster, close genetic relationships between German cultivars Trizeps and Cosinus, Polish cultivar Sorento and German Amarillo 105 and between German cultivar Mungis and Swiss cultivar Blenio were proved. The third subcluster comprised of two French (Aprim, Wilfried) and three German cultivars (Massimo, Trigold and Trimmer).

In the last subclusters genetic proximity between cultivars Innoval (France) and Kinerit (Czech republic), Largus (Slovakia) and Noe (France), Greneder (Russia) and Plains (USA), Tribeca and Magistral (both France) was revealed.

CONCLUSION

The used markers allowed us to differentiate samples of triticale and rye in two groups. Dendrogram arranged samples of triticale in several groups, such that we can better define genetic background of these crops.

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