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GENETIC DIVERSITY OF TRITICALE CULTIVARS BASED ON MICROSATELLITE AND RETROTRANSPOSON-BASED 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 combination of 4 wheat SSR and 4 retrotransposon-based markers. The number of alleles for SSR markers ranged from 8 to 10 with an average number of 8,75 alleles per locus. For IRAP markers the number of alleles ranged from 9 to 10 with an average number of 9,25 alleles per locus Totally, 72 alleles were detected, 37 alleles for IRAP markers and 35 alleles for SSR markers. 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 two major groups. The first group contained all winter triticale varieties and in the second cluster were included all spring triticale varieties. The closest relationship was found out between two Polish winter triticale cultivars, Alekto and Pizarro. Results showed the utility of combination of microsatellite and retrotransposon-based markers for estimation of genetic diversity of triticale genotypes leading to genotype identification.

Keywords: Triticale, SSR, retrotransposon, genetic variability, dendrogram

INTRODUCTION

The triticale (X *Triticosecale* Wittmack) is a synthetic self-polinated crop derived from wheat (*Triticum* sp.) and (*Secale cereale* L.) crossed to bring together in a single species the technological quality and yield potential of wheat with the rye stress resistance and rusticity (**Costa da Tesser *et al.*, 2007**). This cereal is becoming increasingly important in agriculture and understanding its genetic diversity
is essential for its continued improvement (**Kuleung *et al.*, 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**). The major part of cereal genomes is comprised of retrotransposons and microsatellites, and the major factor in the differences between their genome sizes is the varying proportions of these elements (**Schulman et al., 2004**).
Retrotransposons are major generators of genetic diversity and tools for detecting the genomic changes associated with their activity because they create large and stable insertions in the genome (**Kalendar *et al.*, 2011**). They constitute informative molecular markers for plant species as a result of their ability of integrating into a multitude of loci throughout the genome and thereby generating insertional polymorphism between individuals (**Carvalho *et al.*, 2010**). Several studies for detection of genetic diversity in plants by retrotransposon-based markers have been reported (**Žiarovská *et al.*, 2009; Kalendar 2011**).
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**). 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**). Microsatellites are one of the most promising molecular marker types to identify or differentiate genotypes within a species (**Salem *et al.*, 2008**)

The aim of this work was to detect genetic variability among the set of 59 triticale cultivars using 4 microsatellite and 4 retrotransposon-based markers.

MATERIAL AND METHODS

Plant material and DNA isolation

The set of 59 European and American triticale (x *Triticosecale* Witt.) were used for detection. Cultivars were provided by Gene Bank of Slovak Republic in Plant Production Research Center Piešťany, Bratislavská 122, 921 68 Piešťany (34 winter triticale and 25 spring triticale). The 7 day-old leaves were collected and immediately the DNA was isolated by GeneJET™ (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

SSR markers

For the analysis, in accordance with literature (**Devos *et al.*, 1995, Kuleung *et al.* 2006**), 4 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

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)**.

Data analysis

The data from electroforeograms were converted to binary matice on the base of presence (1) or absence (0) of particular allele.

Retrotransposon-based markers (IRAP markers)

IRAP analysis was performed according to **Kalendar and Schulman (2006).** The PCR was performed in a 25 µl reaction mixture containing 25 ng DNA, 1x BioTools buffer (with 2 mM $MgCl_2$), 0.5 µM primer, 200 µM dNTP, and 1U BioTools polymerase (Biotools, B&M Labs, Madrid, Spain). The PCR program consisted of 1 cycle at 95° for 3 min; 32 cycles at 95° for 30 sec and at 60° for 1 min and at 72° for 2 min; and a final extension step of 72° for 5 min.

Electrophoresis conditions

The PCR products were resolved by electrophoresis on 1.5% agarose stained with ethidium bromide during 12 16 hours at 50-60V.

Data analysis

Each band was treated as a single locus. The presence or absence of a fragment of a given length was recorded in binary code.
Consequently, using binary matice a dendrogram based on hierarchical cluster analysis using UPGMA algorithm with the SPSS professional statistics version 17 software package was constructed.

RESULTS AND DISCUSSION

Our study dealt with detection of genetic polymorphism in winter and spring triticale cultivars using combination of microsatellites and retrotransposon 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 4 wheat microsatellite markers described by **Devos *et al.* (1995)** and **Kuleung *et al.* (2006) and** 4 IRAP markers described by **Kalendar and Schulman (2006)** were chosen.
The number of alleles for SSR markers ranged from 8 to 10 with an average number of 8,75 alleles per locus. For IRAP markers the number of alleles ranged from 9 to 10 with an average number of 9,25 alleles per locus.
Overall, 72 alleles were detected, 37 alleles for IRAP markers and 35 alleles for SSR markers with an average 9 alleles per primer pair.
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 9 alleles that corresponds to our detection.
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 utilization of wheat and rye SSR markers for analysis 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).
Carvalho *et al.* (2010) using five IRAP detected 103 polymorphic fragments in a total of 113 bands in a set of 48 old Portuguese bread wheat cultivars. On average, 22.6 bands were amplified per IRAP primer combination.
On the base of the sizes of microsatellite and retrotransposon alleles obtained from 59 accessions, the dendrogram derived from hierarchic cluster analysis using UPGMA algorithm based on Jaccard coefficient was prepared (Figure 1). The genotypes could be grouped into two clusters, cluster I with one unique Greek genotype Vrito and cluster II containing 58 genotypes. Cluster II was further subdivided into three subclusters, the first subcluster containing 23 all spring cultivars that were located close to unique Greek spring triticale cultivar (marked in red). The second cluster containing unique French winter triticale cultivar Noe* was situated close to other 34 winter triticale cultivars (marked in black).
The closest genetic relationship was detected between two Polish cultivars Alekto and Pizarro. We were not able to distinguish only two triticale genotypes based on 4 SSR and 4 retrotransposon-based markers. For differentiation of all triticale genotypes we have to choose more polymorphic markers and do more molecular analysis. At this time we can advise used wheat SSR and retrotransposon-based markers for analysis to produce reproducible polymorphic data.

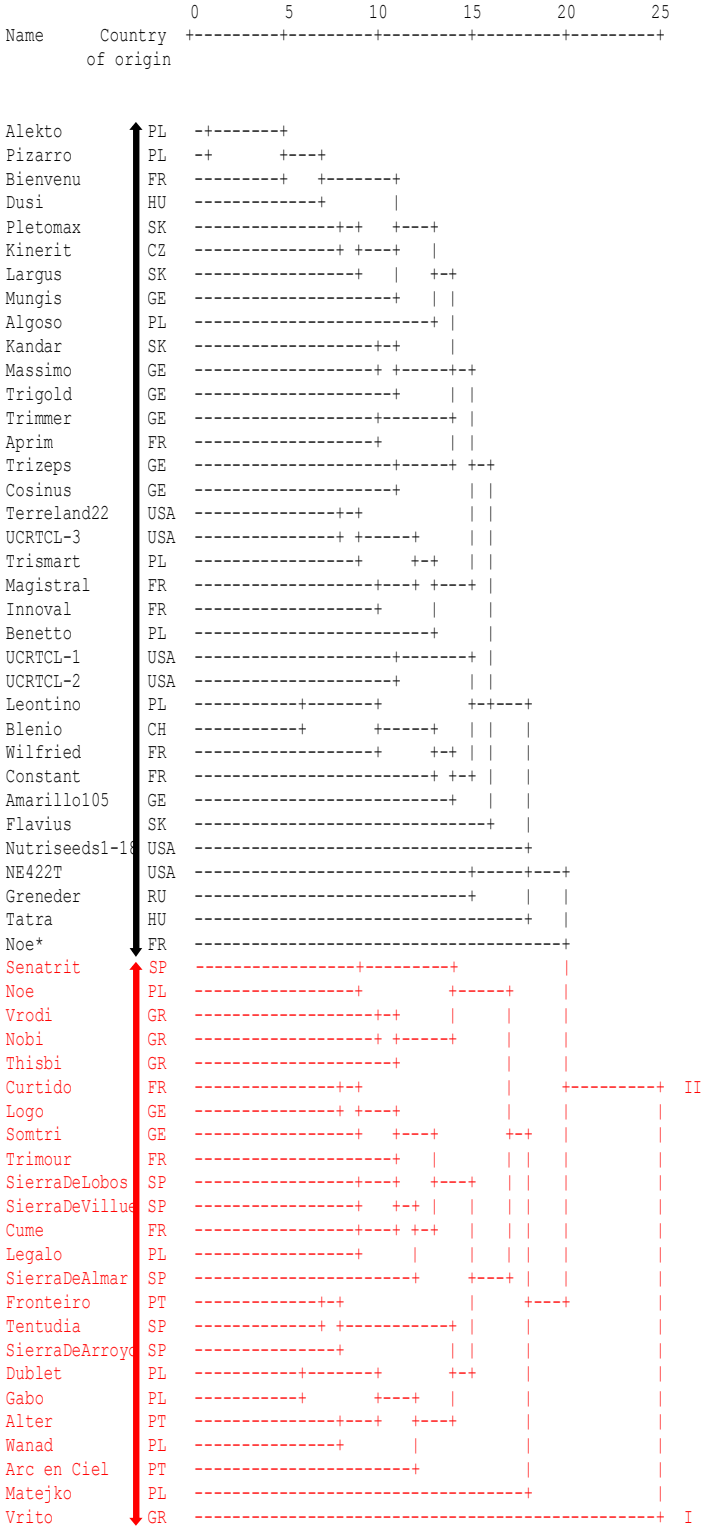

Figure 1 Dendrogram of 59 triticale genotypes prepared based on 4 SSR a 4 IRAP markers (CZ - Czech Republik, CH – Switzerland, FR – France, GE – Germany, GR - Greece, HU – Hungary, PL - Poland, PT - Portugal, SK - Slovakia, SP - Spain)

CONCLUSION

The analysis showed that the microsatellites and retrotransposons are very effective molecular markers for the assessment of the genetic diversity in triticale using wheat SSR markers and retrotransposon-based markers. The dendrogram prepared based on UPGMA algorithm differentiated triticale genotypes into two principal groups of winter and spring form. Using 4 microsatellite and 4 retrotransposon-based markers only two Polish triticale cultivars were not distinguished. Our analysis proved utilization of microsatellites and retrotransposons as polymorphic markers for differentiation of used set of triticale genotypes.

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