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ASSESSMENT OF RAPD POLYMORPHISM IN RYE (*SECALE CEREALE* L.) GENOTYPES

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ABSTRACT

The results of genetic analysis of 38 rye taxa (*Secale cereale* L.) represented by agricultural varieties originating from Central Europe and the Union of Soviet Socialist Republics (SUN) are presented. The genetic diversity of rye cultivars by 5 RAPD markers was evaluated. Five primers gave 42 polymorphic fragments (99.52 %) with an average of 8.4 bands per primer. The most polymorphic primer was RLZ12, where 10 polymorphic amplification products were detected. Overleaf the lowest polymorphic primer was RLZ5 with 7 polymorphic products. Genetic polymorphism was characterized based on diversity index (DI), probability of identity (PI) and polymorphic information content (PIC). The dendrogram of genetic similarity was constructed, based on the Jaccard´s coefficient. In dendrogram three clusters were differentiated. The first cluster contained genotypes from Czechoslovakia, Poland and Czech Republic. The second cluster contained cultivars coming from Union of Soviet Socialist Republics and Hungary. In the next cluster Poland, Czech Republic and Czechoslovakia genotypes were situated. Two genotypes Bosmo and Wibro have not been distinguished. For better distinction of the analysed rye genotypes, it is necessary to use a higher number of RAPD markers. In this experiment, RAPD proved to be a rapid, reliable and practicable method for revealing of polymorphism in the rye cultivars.

Keywords: Rye (*Secale cereale* L.), polymorphism, RAPD, dendrogram

INTRODUCTION

Rye (*Secale cereale* L.) is a diploid (2n = 2x = 14) annual, cross-pollinated cereal with an effective gametophytic self-incompatibility system. Similar to many crops of the old World, S. *cereale* evolved of the Near East. Main regions of diversity are Turkey, Libanon, Syria, Iran, Iraq, and Afghanistan. Rye was, however, never cultivated as a crop there but grew and still grows as a weed within the stands of barley and wheat **(Carena, 2009)**. On a global scale rye (*Secale cereale* L.) is a minor crop, its production being about 5 % that of wheat or rice. However, in northern European countries with extreme climatic and poor soil conditions, rye may occupy up to 30 % of the acreage **(Altpeter and Konzun, 2007)**. The main advantages of rye over other winter cereals are its excellent tolerance to low temperatures and the ability to realize relatively high grain yields under environmental conditions in which other crops perform poorly. Rye is also known to have the lowest requirements for chemical treatments like fertilizers or pesticides, which makes it an ecologically and economically sound crop for specific regions **(Konzun *et al*., 2001)**.
Since 1990, random amplified polymorphic DNA (RAPD) markers have been successfully applied for identification of DNA polymorphism in various plant species **(Williams *et al*., 1990)**. They are often used for screening of a wide range of genetic stocks in order to find linkage with traits of agronomic significance **(Masojć *et al*., 2001)**.
Suitability of RAPD markers for the construction of genetic maps, fingerprinting and phylogenetic studies has been proved by many authors **(Yang and Quiros, 1995; Nilsson *et al*., 1997; Divaret *et al*., 1999)**. In cereal crops such as wheat **(Saleh, 2012; Bibi *et al*., 2012)**, peach **(Bakht *et al*., 2013)**, barley **(Bakht *et al*., 2011)**, the technique has been applied to identifying cultivars and revealing phylogenetic relationships among them. In the case of rye, there are a few papers **(Iqbal and Rayburn, 1994; Matos *et al*., 2001; Persson *et al*., 2002; Petrovičová *et al*., 2014)** that have reported the application of the RAPD marker technique to rye molecular identification, and the technique was proved to be effective for characterizing the genetic background of rye **(Ma *et al*., 2004)**. The aim of our study was to detect genetic variability among the set of 38 rye genotypes using 5 RAPD primers.

MATERIAL AND METHODS

Plant Material

Thirty eight rye (*Secale cereale* L.) genotypes were used in the present study. Seeds of rye were obtained from the Gene Bank of the Slovak Republic of the Plant Production Research Center in Piešťany and Gene Bank of the Czech Republic of the Crop Research Institute in Prague (Tab 1).

DNA Isolation

Genomic DNA of rye cultivars was extracted from 14 days leaves with GeneJET Plant Genomic DNA Purification Mini Kit (Thermo Scietific, Gdańsk, Poland) according to the manufacturer's instructions. DNA concentrations were estimated by UV-Vis spectrophotometer Q5000, Quawell.

Polymerase Chain Reaction (PCR) and Gel Electrophoresis

RAPD analyses were performed using five random 10mer arbitrary primers (Tab 2) obtained from Genomed, Warsaw, Poland. Amplification DNA was conducted in 25µl reaction volume containing the following reagents: 10.25µl deionized water, 12.5µl Master Mix (2x Master Mix, A&A Biotechnology, Gdynia, Poland), 1.25µl of genomic DNA, 1µl of primer. PCR amplifications were performed on a labcycler (Sencoquest, Göttingen, Germany) following amplification profile: An initial denaturation step at 94°C for 1 min, followed by 10 cycles of amplification 5s at 94°C, 30 s at 37°C and 30 s at 72 °C and next 35 cycles of 5 s at 94°C, 30 s at 37°C and 1 min at 72 °C.
Amplified products were size-fractioned using by electrophoresis in 1% agarose gels in 1 x TBE buffer at 170 V for 1.5 h. GeneRulerTM 1kb Plus DNA Ladder (Fermentas, Gdansk, Poland) that gives 15 bands from 75 to 20000 bp, was used as standard. The bands were visualized by Midori Green staining (Nippon Genetics Europe GmbH, Düren, Germany) and photographed under UV light using a ChemiDoc™ MP System (Biorad, Warszawa, Poland).

Data Analysis

The band intensity and presence of RAPD-PCR products, were analysed by densitometry, using ImageLabTM Software version 4.1 Biorad. Each

reproducible band was visually scored for the presence (1) or absence (0) for all genotypes. For determination of the genetic relationships between rye genotypes a dendrogram was used. The dendrogram was constructed based on principle of hierarchical cluster analysis using UPGMA (Unweighted Pair Group Method using arithmetic Averages) algorithm on the basis of Jaccard's coefficient in statistical program SPSS.

Frequencies of incidence of all polymorphic alleles were calculated and used for determination of statistical parameters: diversity index (DI) **(Weir, 1990)**, probability of identity (PI) **(Paetkau *et al*., 1995)** and polymorphic information content (PIC) **(Weber, 1990)**.

Diversity index (DI) $DI = 1 - \sum p_i^2$

Probability of identity (PI) $PI = \sum p_i^4 + \sum_{i=1}^{i=n-1} \sum_{j=i+1}^{n} (2 p_i p_j)^2$

Polymorphic information content (PIC): $PIC = 1 - \left(\sum_{i=1}^{n} p_i^2 \right) - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2 p_i^2 \cdot p_j^2$

P_i and p_j are the frequencies of the ith and jth allele in a given genotypes.

Table 1 List of 38 rye cultivars their taxon and country of origin used in this study

Genotype	Taxon	Country of origin
Valtické	*S. cereale* L. subsp. *cereale* var. *cereale*	Czechoslovakia
Tešovské	*S. cereale* L. subsp. *cereale* var. *cereale*	Czechoslovakia
Keřkovské	*S. cereale* L. subsp. *cereale* var. *cereale*	Czechoslovakia
Zenit	*S. cereale* L. subsp. *cereale* var. *cereale*	Czechoslovakia
Chlumecké	*S. cereale* L. subsp. *cereale* var. *cereale*	Czechoslovakia
České	*S. cereale* L. subsp. *cereale* var. *cereale*	Czechoslovakia
Albedo	*S. cereale* L. subsp. *cereale* var. *cereale*	Czechoslovakia
Židlochovický Panis	*S. cereale* L. subsp. *cereale* var. *cereale*	Czechoslovakia
Nalžovské	*S. cereale* L. subsp. *cereale* var. *cereale*	Czechoslovakia
Dobrovické	*S. cereale* L. subsp. *cereale* var. *cereale*	Czechoslovakia
Vígľašské	*S. cereale* L. subsp. *cereale* var. *cereale*	Czechoslovakia
Ratbořské	*S. cereale* L. subsp. *cereale* var. *cereale*	Czechoslovakia
Laznické	*S. cereale* L. subsp. *cereale* var. *cereale*	Czechoslovakia
Breno	*S. cereale* L. subsp. *cereale* var. *cereale*	Czechoslovakia
Dobřenické krmné	*S. cereale* L. var. *h*	Czechoslovakia
Aventino	*S. cereale* L	Czech Republic
Selgo	*S. cereale* L. subsp. *tetraploidum* KOBYL	Czech Republic
Warko	*S. cereale* L. subsp. *cereale* var. *cereale*	Poland
Dankowskie Zlote	*S. cereale* L. subsp. *cereale* var. *cereale*	Poland
Zduno	*S. cereale* L. subsp. *cereale* var. *cereale*	Poland
Motto	*S. cereale* L. subsp. *cereale* var. *cereale*	Poland
Pancerne	*S. cereale* L. subsp. *cereale* var. *cereale*	Poland
Wojcieszyckie	*S. cereale* L. subsp. *cereale* var. *cereale*	Poland
Univerzalne	*S. cereale* L. subsp. *cereale* var. *cereale*	Poland
Dankowskie Nowe	*S. cereale* L. subsp. *cereale* var. *cereale*	Poland
Amilo	*S. cereale* L. subsp. *cereale* var. *cereale*	Poland
Wibro	*S. cereale* L. subsp. *cereale*	Poland
Bosmo	*S. cereale* L.	Poland
Rostockie	*S. cereale* L.	Poland
Hegro	*S. cereale* L.	Poland
Walet	*S. cereale* L.	Poland
Kier	*S. cereale* L.	Poland
Tetra Start	*S. cereale* L. subsp. *tetraploidum* KOBYL	SUN
Cerkascanka tetra	*S. cereale* L. subsp. *tetraploidum* KOBYL	SUN
Voschod 1	*S. cereale* L. subsp. *cereale* var. *cereale*	SUN
Golubka	*S. cereale* L. subsp. *cereale* var. *cereale*	SUN
Mnogokoloskaja	*S. cereale* L. subsp. *cereale* var. *cereale*	SUN
Lovaszpatonai	*S. cereale* L. subsp. *cereale* var. *cereale*	HUN

Legend: SUN - Union of Soviet Socialist Republics

Table 2 List of random primers their sequences and chromosomal location used for RAPD analysis.

Primer's name	Sequence	Chromosomal location
RLZ1	5´AAGCACCGGC3´	3RS
RLZ5	5´CGTCGTGGAA3´	4RL
RLZ11	5´TCCGCGGTCT3´	6RS
RLZ12	5´TGCCGCTAAG3´	7RL
RLZ13	5´TCGCGCTGTC3´	7RL

RESULTS AND DISCUSSION

The development of molecular markers has opened up numerous possibilities for their application in plant breeding. PCR-based markers, including random amplified polymorphic DNA (RAPD), have been developed to effectively analyse of genetic polymorphism **(Ko *et al*., 2002)**.

Five 10 mer arbitrary primers produced 42 DNA fragments with an average of 8.4 per primer. The size of the amplified products ranged from 350 to 20000 bp. Of the total 42 bands obtained, 41 were polymorphic. Percent of polymorphism ranged from 97.61 % (RLZ1) to 100 % (RLZ5, RLZ11, RLZ12, RLZ13). The most polymorphic primer was RLZ12, where 10 polymorphic amplification products were detected. Overleaf the lowest polymorphic primer was RLZ5 with 7 polymorphic products.

The frequencies of alleles and the values of DI, PI and PIC were calculated (Tab 3). All three features were calculated for all used RAPD primers by using individual frequencies of the fragments for each marker. The diversity index (DI) of RAPD markers ranged from 0.818 (RLZ5) to 0.862 (RLZ11) with an average of 0.843. The lowest values of polymorphic information content were recorded for RLZ11 (0.859) and the lowest PIC values were detected for RLZ5 (0.814) with an average of 0.839. Probability of identity was low ranged from 0.003 to 0.007 with an average of 0.007 that indicates the possibility to differentiate genetically close genotypes.

Persson *et al.* (2002) detected the amount and distribution of genetic variation within and between accessions of 9 landraces and 3 cultivars of cultivated rye from Northern Europe. They tested total of 100 primers, of which 15 were used. Of the 60 amplification products (bands) that were scored (an average of 3.9 bands/primers), 58 bands (97 %) found to be polymorphic. The average number of bands per primer for each accession was 2.75 the average percent of polymorphic loci was 68.9 %.

Previous studies reported that levels of polymorphism in rye detected by RAPD technique were 9 – 72 % for various primers **(Iqbal and Rayburn, 1994)** and 45 % **(Loarce *et al*., 1996)**. PIC values were determined 0.374 **(Myśkow *et al*., 2001)** 0.863 in rye **(Petrovičová *et al*., 2014)**, in coffee 0.78 **(Mishra *et al*., 2011)** and in iris 0.178 **(Azimi *et al*., 2012)**.

RAPD analysis is widely used for the study plant genetic polymorphism in wheat **(Abd-El-Haleem *et al*., 2009; Cifci and Yagdy 2012)** barley **(Abdellaoui *et al*., 2010; Guasmi *et al*., 2012)** triticale **(Orlovskaya *et al*., 2012)** and maize **(Okumus, 2007; Mukharib *et al*., 2010)**.

Table 3 Characteristics of RAPD markers used in this study

RAPD Primers	Number of fragments	Polymorphism (%)	DI	PIC	PI
RLZ1	8	97.61	0.855	0.849	0.003
RLZ5	7	100	0.818	0.814	0.016
RLZ11	8	100	0.862	0.859	0.003
RLZ12	10	100	0.852	0.851	0.005
RLZ13	9	100	0.830	0.823	0.007
Average	8.4	99.52	0.843	0.839	0.007

Legend: DI - diversity index; PI - probability of identity (PI); PIC - polymorphic information content

The dendrogram of genetic relationships among 38 rye cultivars based on RAPD primers is presented in Figure 1. The cluster tree analysis showed that the rye genotypes were divided into 3 main clusters.

The group of RAPD primers were divided during the basic screening of 38 analysed cultivars. The first cluster was divided in two subclustres (1A and 1B). Subcluster 1A contains three genotypes of Czechoslovak origin. In the subgroup 1B were grouped 13 genotypes which were bred in Poland (53.8 %), Czechoslovakia (38.5 %), Czech Republic (7.7 %). The second cluster was divided into two groups (2A and 2B). In cluster 2A two rye genotypes were separated - Mnogokoloskaja (SUN) and Lovaszpatonai (HUN). Subcluster 2B included 4 genotypes of Union of Soviet Socialist Republics origin. The other rye varieties in the third cluster were divided into two subclusters (3A and 3B). Seven varieties of rye coming from Czechoslovakia and one genotype from Czech Republic formed subcluster 3A. Subcluster 3B contained eight rye genotypes originating from the Poland. We could not distinguish 2 genotypes, Wibro and Bosmo grouped in 3B subcluster, which can be caused due the same genetic background (Fig 1).

Persson *et al.* (2002), constructed dendrogram using UPGMA algorithm among the 12 rye accessions. The genetic distance value average among the accessions was 0.066 and the cophenetic correlation at the dendrogram was 0.907. The final dendrogram showed six clusters. The clusters I, IV, V and VI each include a single accession; one landrace from Sweden, one from Germany, one from Norway and one from Finland, respectively. In cluster II, two Swedish landraces were grouped together. The cluster III showed six accessions and it could be divided into two subclusters: the first one with three landraces from Sweden and the second one with three improved cultivars.

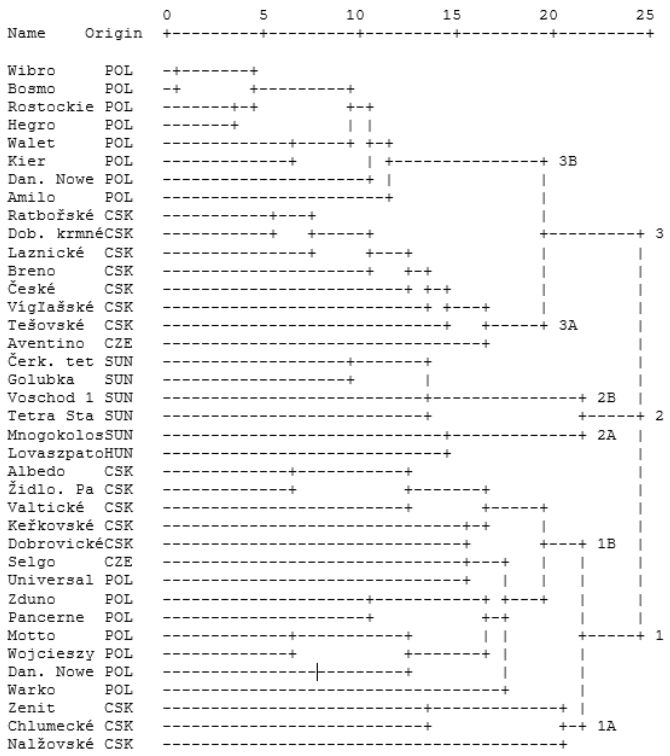

Figure 1 Dendrogram of 38 rye genotypes prepared based on 5 RAPD markers CSK - Czechoslovakia, CZ - Czech Republic, HU - Hungary, PL - Poland, SUN - Union of Soviet Socialist Republics.

CONCLUSION

The objective of this study was to determine the genetic variation among 38 rye varieties using RAPD markers. Genetic polymorphism was characterized based on diversity index (DI), probability of identity (PI) and polymorphic information content (PIC). Values of the polymorphic information content value ranged from 0.814 to 0.859 with an average of 0.839 and diversity index value ranged from 0.818 to 0.862 with an average of 0.843. The dendrogram was prepared based on the Jaccard´s coefficient and divided in to three clusters. RAPD are commonly and extensively used tools for assessment of variability in crops. These marker systems are efficient due to their ease, rapidity and reliability, for analysis of molecular differentiation and for resolving taxonomic problems in plants. Our result showed appreciably high genetic diversity among the rye genotypes studied.

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