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MOLECULAR CHARACTERIZATION OF RYE CULTIVARS

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ABSTRACT

The results of molecular analysis of 45 rye taxa (*Secale cereale* L.) represented by agricultural varieties originated from Central Europe and the Union of Soviet Socialist Republics (SUN) are presented. The genetic diversity of rye cultivars by 6 SSR markers was evaluated. Six specific microsatellite primer pairs produced 58 polymorphic alleles with an average of 9.7 alleles per locus. The number of alleles ranged from 6 (*SCM2*) to 14 (*SCM86*). Genetic polymorphism was characterized based on diversity index (DI), probability of identity (PI) and polymorphic information content (PIC). The diversity index (DI) of SSR markers ranged from 0.5478 (*SCM2*) to 0.887 (*SCM86*) with an average of 0.778. The lowest value of polymorphic information content was recorded for *SCM2* (0.484) and the highest value for *SCM86* (0.885) of PIC was detected in *SCM86* with an average of 0.760.The dendrogram of genetic similarity was constructed, based on UPGMA algorithm. The hierarchical cluster analysis divided rye genotypes into 4 main clusters. The first cluster of 14 genotypes was subdivided in two subclusters (1a and 1b) where 50% of genotypes were Czechoslovak origin. The second cluster contained four genotypes were three (75%) of them had Czech or Czechoslovak origin. In the third subcluster separated three rye genotypes of different origin. The rest (24) of rye genotypes in the fourth cluster were divided into two subclusters (4a and 4b) where clearly separated group of Polish (4aa) and Czech and Czechoslovak (4ab) genotypes. Two genotypes of 4aa subcluster (Wojcieszyckie and Dankowskie Nowe) from Poland were genetically the closest. In the dendrogram alle genotypes were differentiated and clustering partially reflects geographic origin of studied rye genotypes. In this experiment, SSRs markers proved to be a high informative and usefull tool in genetic diversity research for the distinguishing and characterization of close related varieties.

Keywords: *Secale cereale* L.; polymorphism; microsatellite; PCR; dendrogram

INTRODUCTION

Common rye (*Secale cereale* L.) is one of the most important cereal crops cultivated in Eastern and Northern Europe **(Targońska et al., 2015)**. Rye (*Secale cereale* L.) is a diploid (2n = 2x = 14) annual, cross-pollinated cereal with an effective gametophytic self-incompatibility system. 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 **(Korzun et al., 2001)**. Moreover, rye offers high contents of many nutritionally favorable compounds such as a whole suite of minerals (Zn, Fe, P), beta-glucans, resistant starch, and bioactive compounds. Rye products are characterized by a high level of dietary fiber **(Andersson et al., 2009)** that may contribute to positive health effects **(Rosén et al., 2011)**.

Molecular markers can provide an effective tool for efficcient selection of desired agronomic traits because they are based on the plant genotypes and thus, are independent of environmental variation. Nowadays, several molecular markers are developed, of which simple sequence repeats (SSRs) or microsatellites are the most widely used types **(Jenabi et al., 2011; Maršálková et al., 2014)**.

Simple sequence repeat (SSR) markers show a relatively good transferability between closely related species **(Botes and Bitalo, 2013)** and they are one of the most promising molecular marker types to identify or differentiate genotypes within a species **(Salem et al., 2008)**. They were successfully used in many plant species, e.g. triticale **(Kuleung et al., 2004; Odrouškova and Vyhnanek, 2013)**, **wheat (Röder et al., 1995; Huang et al., 2002)**, rye **(Khlestkina et al., 2004)**, rice **(Jiang et al, 2010)**, maize **(Ignjatovic-Micic et al., 2015)**, and amaranth **(Žiarovská et al., 2013)**.

Rye SSR markers were first developed over 10 years ago **(Saal and Wricke, 1999; Hackauf and Wehling, 2002,)** and have also been used in studies on genetic diversity **(Shang et al. 2006; Bolibok et al., 2005)**.

The aim of our study was to detect genetic variability among the set of 45 rye genotypes using 6 microsatellite markers.

MATERIAL AND METHODS

Forty five 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. Fifteen genotypes of rye came from Czechoslovakia (CSK), another set of fifteen genotypes from Poland (PL), five from Czech Republik (CZ), another five from Hungary (HU) and last five genotypes from Union of Soviet Socialist Republics (SUN). All genotypes are of winter form.

Genomic DNA of rye cultivars was isolated from 100 mg freshly-collected leaf tissue according to GeneJET™ protocol (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.

For analysis, six microsatellite primer pairs were chosen according to the literature **(Saal - Wricke, 1999)**. Used primers were localised on 6R, and 7R chromosomes (Table 1). PCR amplification was performed in 20 μL volume containing 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® polymerase* (Promega, USA). PCR reactions were performed in a thermocykler (Bio-Rad, USA). The PCR program consisted of these steps: an initial denaturation (1 cycle): 2 min. at 93 °C, (29 cycles) denaturation: 1 min. 93 °C, annealing 2 min. with different temperature for each primer pair and extension 2 min. at 72 °C.

The PCR amplicons (5μL) were resolved by electrophoresis on 6.0% denaturating polyacrylamide gel stained with silver according to **Bassam et al., (1991)**. Final PCR amplicons were scanned in UVP PhotoDoc-t® camera system. The size of alleles was determined by comparing with 10 bp standard lenght marker (Invitrogen: 100 – 330 bp). Each band was treated as a single allele.

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 version 17.

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):

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

Polymorphic information content (PIC):

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

RESULTS AND DISCUSSION

The development of molecular markers has opened up numerous possibilities for their application in plant breeding. Suitable markers for detecting polymorphisms at individual and population levels are SSRs **(Shang et al., 2006; Bolibok et al., 2005, Akhavan et al., 2009)**.

Six rye specific microsatellite primer pairs produced 58 polymorphic alleles with an average of 9.7 alleles per locus. The most polymorphic locus was *SCM86* where 14 polymorphic amplification products were detected. On the other hand the lowest polymorphic locus was *SCM2* with 6 polymorphic alleles.

Jenabi et al., (2011) used fifteen wheat and rye derived microsatellite markers to evaluate genetic variation of the mountain rye *Secale strictum* in Iran and to examine the patterns of diversity related to the varieties and geography. They detected high levels of diversity, with an average number of 6.1 alleles per locus (ranging up to 11) and high level polymorphism with polymorphism rate averaging 0.624 (between populations) and 0.357 (within populations) were observed among 125 individuals from 19 populations collected from various regions of Iran. **Gailîte et al., (2013)** analyzed genetic polymorphism of a set of 9 genotypes originated from Latvia using 12 SSR markers. The number of alleles ranged from 1 to 6 with an average number of alleles per locus 3.4. The results from their study indicate that while the Latvian rye collection is small, the genetic and phenotypic diversity contained within and between the accessions is quite high. **Targońska et al., (2015)** studied genetic diversity among 367 Polish rye accessions using 22 previously published simple sequence repeat (SSR) markers.

Table 1 List and characterization of locus specific microsatellite primers used for SSR analysis.

SSR marker	Forward primer (5′ – 3′)	Reverse primer (5′ – 3′)	Chromosomal location	Anealing temperature
SCM 2	GATGACTATGACTACCAGGATGAA	GGAGTGAGAAGGCCGAGAAG	6R	55 °C
SCM 28	CTGGTCCTGGTCTGGTGGGTC	CGCATCGGGTGTGTCGCATAC	6R	60 °C
SCM 40	CGCATCGGGTGTGTCGCATAC	CACATCTTGGGCCTGACACC	7R	60 °C
SCM 86	CAGATAGATGGGTGTTGTGCG	CTCTTCTCGACATCCACACTCC	7R	60 °C
SCM 101	GCCAGCCGCCACCTTAATTG	AGCCCAACTCTTTCGTGCATG	6R	60 °C
SCM 180	GTTTCGTCCCCGTTGCCATC	ACGTGTCGCTTTCCATTGCCC	6R	60 °C

Table 2 Characteristics of used SSR markers in this study.

SSR marker	Number of alleles	DI	PIC	PI
SCM 2	6	0.547	0.484	0.144
SCM 28	11	0.771	0.764	0.027
SCM 40	13	0.867	0.865	0.004
SCM 86	14	0.887	0.885	0.002
SCM 101	9	0.857	0.851	0.004
SCM 180	5	0.740	0.708	0.022
average	**9.7**	**0.778**	**0.760**	**0.034**

Note: DI - diversity index; PI - probability of identity; PIC - polymorphic information content.

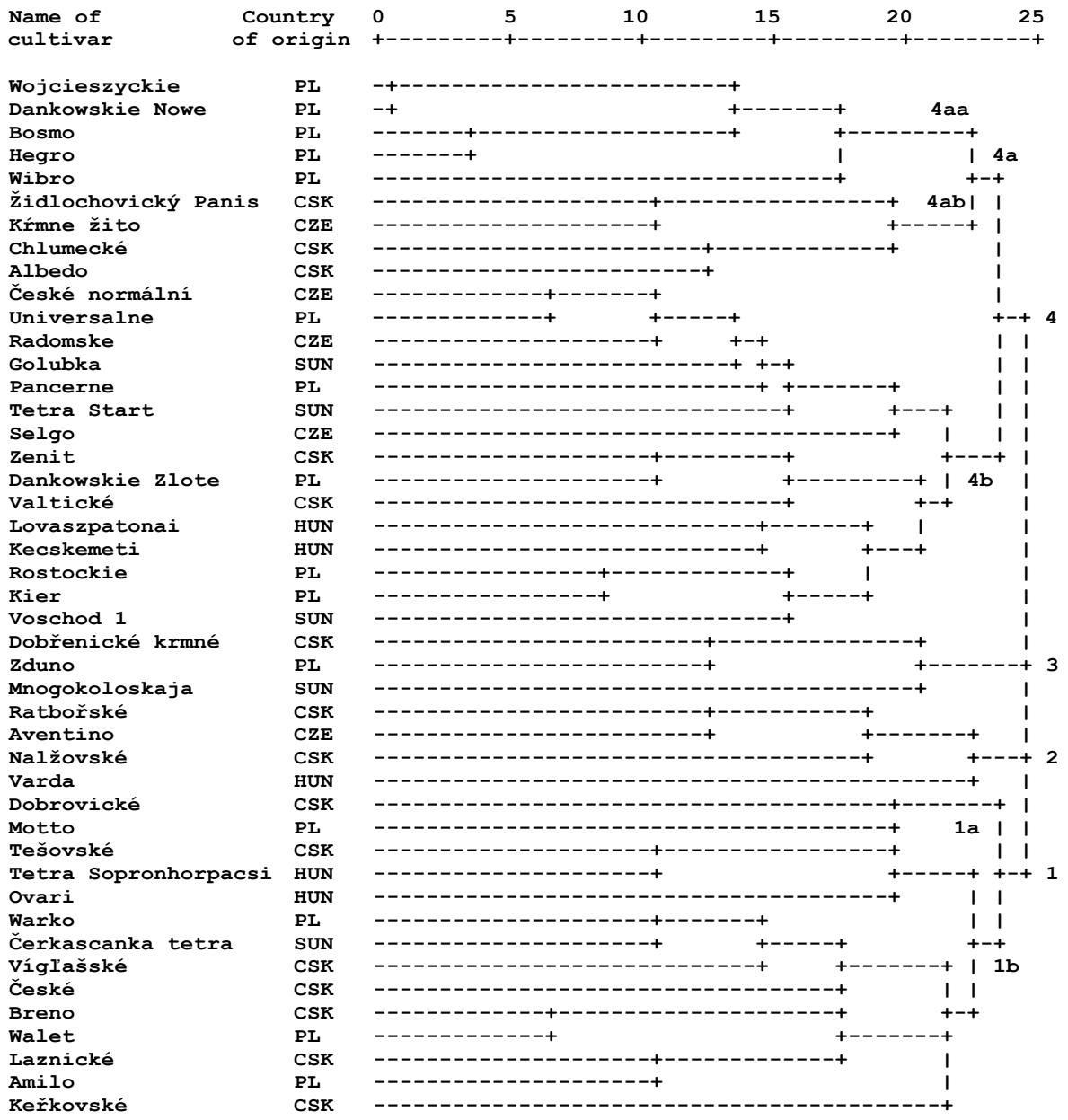

Figure 1 Dendrogram of 45 rye genotypes prepared based on 6 SSR markers.
CSK - Czechoslovakia, CZ - Czech Republic, HU - Hungary, PL - Poland, SUN - Union of Soviet Socialist Republics.

Resulting from the number and frequency of alleles, diversity index (DI), polymorphic information content (PIC) and probabilities of identity (PI) were calculated (Tab. 2). The diversity index (DI) of SSR markers ranged from 0.547 (*SCM2*) to 0.887 (*SCM86*) with an average of 0.778. The lowest value of polymorphic information content was recorded for *SCM2* (0.484) and the highest value for *SCM86* (0.885) of PIC was detected in *SCM86* with an average of 0.760. Only one marker (*SCM2*) reached considerably unfavourable results of DI, PIC, PI and number of alleles compared to average values of tested set. Probability of identity was low ranged from 0.002 (*SCM86*) to 0.144 *(SCM2*) with an average of 0.034 that indicates the possibility to differentiate genetically close genotypes.

Jenabi et al., (2011) found out lower polymorphism in their study. They calculated the within populations PIC value for all microsatellites which ranged from 0.246 to 0.451 with an average of 0.357. **Targońska et al., (2015)** detected the average PIC value for all markers used 0.57. The highest PIC value (0.93) was obtained for SCM152, and the lowest PIC (0.18) was determined for SCM050.

The dendrogram of genetic relationships among 45 rye cultivars based on SSR markers is presented in Figure 1. The hierarchical cluster analysis showed that the rye genotypes were divided into 4 main clusters. The first cluster was divided in two subclusters (1a and 1b). Subcluster 1a contains two genotypes of Czechoslovak and Polish origin. In the subgroup 1b were grouped 12 genotypes which were bred in Czechoslovakia (50%), Poland (25%), Hungary (16.7%) and one coming from Union of Soviet Socialist Republics. The second cluster contained four genotypes were three (75%) of them had Czech or Czechoslovak origin. In the third subcluster separated three rye genotypes of different origin. The rest of rye genotypes in the fourth cluster were divided into two subclusters (4a and 4b). Subcluster 4a was further subdivided into two subclusters, subcluster 4aa with 5 genotypes all coming from Poland and subcluster 4ab with four genotypes of Czech or Czechoslovak origin. Subcluster 4b of 15 genotypes included genotypes of Polish origin (33.3), SUN origin (20%), Czech origin (20%), Czechoslovak origin (13.3) and Hungarian origin (13.3). Two genotypes of 4aa subcluster (Wojcieszyckie and Dankowskie Nowe) from Poland were genetically the closest. We can assume that they have close genetic background (Figure 1).

Targońska et al., (2015) showed that the clustering of rye accessions studied was more weakly correlated with geographic origin than with the source of seeds. **Akhavan et al., (2010)** in the prepared SSR based dendrogram using UPGMA algorithm showed evident broad groupings related to the subspecies. The populations of subsp. *cereale* were mainly grouped but populations belonging to the subsp. *ancestrale* were divided in to two subgroups (groups I and III), indicating higher diversity of the latter subspecies.

CONCLUSION

The objective of this study was to determine the genetic variation among 45 rye varieties using SSR markers. Values of diversity index and polymorphic information content were higher than 0.7 in 83% of SSR markers that means high lever of polymorphism of used markers. WE can recommend them for further analyses. The dendrogram was prepared based on UPGMA algorithm using the Jaccard´s coefficient and divided in to four main clusters. All studied genotypes separated into four clusters. Clustering partially reflected geographic origin of studied rye genotypes. SSR are commonly and extensively used tools for assessment of variability in crops. These marker systems are efficient due to their locus specificity, reproducibility 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. This survey showed the high genetic diversity within the European rye genepool as an important source for crop breeders, and indicated that there is value in sampling for useful genes for crops improvement.

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