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DETECTION GENETIC VARIABILITY OF *SECALE CEREALE* L. BY SCOT MARKERS

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ABSTRACT

Rye (*Secale cereale* L.) is our traditional cereal used for baking. The genetic variability of grown rye has been reduced by modern agronomic practices, which subsequently prompted the importance of search for species that could be useful as a gene pool for the improving of flour quality for human consumption or for other industrial uses. Therefore, the aim of this study was to detect genetic variability among the set of 45 rye genotypes using 8 SCoT markers. Amplification of genomic DNA of 45 genotypes, using SCoT analysis, yielded 114 fragments, with an average of 14.25 polymorphic fragments per primer. The most polymorphic primer was SCoT 36, where 21 polymorphic amplification products were detected. In contract the lowest polymorphic primer was SCoT 45 with 5 polymorphic products. Genetic polymorphism was characterized based on diversity index (DI), probability of identity (PI) and polymorphic information content (PIC). The hierarchical cluster analysis showed that the rye genotypes were divided into 2 main clusters. One rye genotype Motto, origin from Poland formed a separate subcluster (1b). Subscluster 2a included only genotype Valtické (CSK). In this experiment, SCoT proved to be a rapid, reliable and practicable method for revealing of polymorphism in the rye cultivars.

Keywords: *Secale cereal*; SCoT markers; genetic diversity

INTRODUCTION

Rye (*Secale cereale* L.) is a member of the *Triticeae* tribe of the grass family *Poaceae* and related to bread wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.). It has the largest genome ~7.9 Gbp **(Bartoš et al., 2008)** among all diploid *Triticeae* with more than 90% repetitive sequences **(Bauer et al., 2016)**.

Secale cereale L. has been studied by morphological **(Persson et al., 2006)**, cytological **(Schlegel et al., 1987; Alkimova et al., 2004)**, isozymes **(Vence et al., 1987)**, ribosomal DNA spacer lengths **(Reddy et al., 1990)**, restriction fragment length polymorphisms (RFLPs) **(Saal and Wricke, 1999)**, amplified fragment length polymorphisms (AFLPs) **(Chikmawati et al., 2005)** and microsatellite **(Bolibok et al., 2005; Shang et al., 2006)** analyses **(Akhavan et al., 2010)**. In recent years, a number of molecular markers have been employed for genetic diversity evaluation, genetic mapping, and quantitative trait locus analysis **(Vivodík et al., 2016)**. Molecular markers are useful for cultivar identification, biodiversity analyses, for phylogenetic studies and other applications **(Semagn et al., 2006)**. The choice of the marker system to use for a particular application depends on its ease of use and the particular objectives of the investigation. It has been suggested that the measure of genetic diversity by molecular markers for breeding purposes should be based on functionally characterized genes, or targeted genes, as these may reflect functional polymorphisms **(Andersen and Lübberstedt, 2003; Guo et al., 2012)**.

SCoT is a simple, novel and gene-targeted DNA marker based on the short conserved region in plant genes **(Collard and Mackill, 2009)**. Primers for SCoT marker analysis were designed from the conserved region surrounding the translation initiation codon, ATG **(Joshi et al., 1997; Sawant et al., 1999)**. A single 18-mer oligonucleotides is used as both forward and reverse primer for PCR, and the annealing temperature is set at 50 °C **(Gao et al., 2014)**.

SCoT markers are more reproducible than RAPD and ISSR, and it is suggested that primer length and annealing temperature are not the sole factors determining reproducibility **(Gorji et al., 2011)**. The utility of this primer in genetic diversity analysis has been reported in a number of plant species **(Collard and Mackill, 2009; Gorji et al., 2011; Xiong et al., 2009; Amirmoradi et al., 2012; Guo et al., 2012; Luo et al., 2012; Sujatha et al., 2013; Rathore et al., 2014)**.

The present study is focused on estimation of genetic distance between 45 rye genotypes, based on 8 SCoT markers. Although the information gathered here would be helpful in future for genomic mapping studies leading to development of rye cultivars with broader genetic background to obtain improved crop productivity.

MATERIAL AND METHODOLOGY

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 (Table 1).

Genomic DNA of rye cultivars was extracted from leaves of 14-day old plantlets 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.

A total of 8 SCoT primers developed by Collard and Mackill (2009) were selected for the present study (Table 2). Each 15 μL amplification reaction consisted of 1.5 μL (100 ng) template DNA, 7.5 μL Master Mix (Genei, Bangalore, India), 1.5 μL 10 pmol primer, and 4.5 μL distilled water. Amplification was performed in a programmed thermocycler (Biometra, Germany) using the following program: 94 °C for 3 min; 35 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 2 min; a final extension at 72 °C for 5 min. Amplified products were separated in 1.5% agarose in 1× TBE buffer. The gels were stained with ethidium bromide and documented using gel documentation system UVP PhotoDoc-t® camera system. A dendrogram was constructed based on hierarchical cluster analysis using the unweighted pair group method with arithmetic average (UPGMA).

Table 1 List of *Secale cereale* cultivars, their country of origin and taxa used in this study.

	Genotype	Country of origin	Taxa
1	Valtické	CSK	*S. cereale* L. subsp. *cereale* var. *cereale*
2	Tešovské	CSK	*S. cereale* L. subsp. *cereale* var. *cereale*
3	Keřkovské	CSK	*S. cereale* L. subsp. *cereale* var. *cereale*
4	Zenit	CSK	*S. cereale* L. subsp. *cereale* var. *cereale*
5	Chlumecké	CSK	*S. cereale* L. subsp. *cereale* var. *cereale*
6	České	CSK	*S. cereale* L. subsp. *cereale* var. *cereale*
7	Albedo	CSK	*S. cereale* L. subsp. *cereale* var. *cereale*
8	Židlochovický Panis	CSK	*S. cereale* L. subsp. *cereale* var. *cereale*
9	Nalžovské	CSK	*S. cereale* L. subsp. *cereale* var. *cereale*
10	Dobrovické	CSK	*S. cereale* L. subsp. *cereale* var. *cereale*
11	Vígľašské	CSK	*S. cereale* L. subsp. *cereale* var. *cereale*
12	Ratbořské	CSK	*S. cereale* L. subsp. *cereale* var. *cereale*
13	Laznické	CSK	*S. cereale* L. subsp. *cereale* var. *cereale*
14	Breno	CSK	*S. cereale* L. subsp. *cereale* var. *cereale*
15	Dobřenické krmné	CSK	*S. cereale* L. var. *multicaule*
16	Aventino	CZE	*S. cereale* L.
17	Selgo	CZE	*S. cereale* L. subsp. *tetraploidum* KOBYL
18	Radomske	CZE	*S. cereale* L
19	České normální	CZE	*S. cereale* L
20	Kŕmne žito	CZE	*S. cereale* L
21	Warko	POL	*S. cereale*
22	Dankowskie Zlote	POL	*S. cereale* L. subsp. *cereale* var. *cereale*
23	Zduno	POL	*S. cereale* L. subsp. *cereale* var. *cereale*
24	Motto	POL	*S. cereale* L. subsp. *cereale* var. *cereale*
25	Pancerne	POL	*S. cereale* L. subsp. *cereale* var. *cereale*
26	Wojcieszyckie	POL	*S. cereale* L. subsp. *cereale* var. *cereale*
27	Universalne	POL	*S. cereale* L. subsp. *cereale* var. *cereale*
28	Dankowskie Nowe	POL	*S. cereale* L. subsp. *cereale* var. *cereale*
29	Amilo	POL	*S. cereale* L. subsp. *cereale* var. *cereale*
30	Wibro	POL	*S. cereale* L. subsp. *cereale*
31	Bosmo	POL	*S. cereale* L.
32	Rostockie	POL	*S. cereale* L.
33	Hegro	POL	*S. cereale* L.
34	Walet	POL	*S. cereale* L.
35	Kier	POL	*S. cereale* L.
36	Tetra Start	SUN	*S. cereale* L. subsp. *tetraploidum* KOBYL
37	Čerkascanka tetra	SUN	*S. cereale* L. subsp. *tetraploidum* KOBYL
38	Voschod 1	SUN	*S. cereale* L. subsp. *cereale* var. *cereale*
39	Golubka	SUN	*S. cereale* L. subsp. *cereale* var. *cereale*
40	Mnogokoloskaja	SUN	*S. cereale* L. subsp. *cereale* var. *cereale*
41	Lovaszpatonai	HUN	*S. cereale* L. subsp. *cereale* var. *cereale*
42	Ovari	HUN	*S. cereale* L. subsp. *cereale* var. *cereale*
43	Kecskemeti	HUN	*S. cereale* L. subsp. *cereale* var. *cereale*
44	Tetra Sopronhorpacsi	HUN	*S. cereale* L. subsp. *tetraploidum* KOBYL
45	Varda	HUN	*S. cereale* L.

Note: CSK - Czechoslovakia, CZ - Czech Republic, HUN - Hungary, PL - Poland, SUN - Union of former Soviet Socialist Republic.

For the assessment of the polymorphism between rye genotypes and usability SCoT markers in their differentiation we used polymorphic information content (PIC) **(Weber, 1990)**, diversity index (DI) **(Weir, 1990)** and the probability of identity (PI), **(Paetkau et al., 1995)**.

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

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

$$DI = 1 - \sum p_i^2$$

where p^i and p^j are frequencies of i^{th} and j^{th} fragment of given genotype.

RESULTS AND DISCUSSION

The development of molecular markers has opened up numerous possibilities for their application in plant breeding. PCR-based markers, including Start Codon Targeted (SCoT) polymorphism, have been developed to analyse genetic polymorphism effectively.

In the present study, the representatives of the genus *Secale cereale* collected from different parts of central Europe and from the Union of Soviet Socialist Republics were differentiated by the DNA fingerprinting patterns using 8 SCoT primers. The efficacy of the SCoT technique in this study is further supported by the obtained PIC and DI and PI values of the primers used in the analysis. The PIC value of the SCoT marker system was found to be 0.78 which are at par with the optimal PIC.

PCR amplification of DNA using 8 primers (Table 2) for SCoT analysis produced 123 DNA fragments that could be scored in all 45 genotypes of rye. The number of amplified fragments varied from 5 (SCoT 45) to 21 (SCoT 36), and the amplicon size ranged from 400 to 3000 bp. Of the 123 amplified bands 114 were polymorphic, with an average of 14.25 polymorphic bands per primer. Results indicated the presence of wide genetic variability among different genotypes of rye. From these eight primers, primer SCoT 36 was the most polymorphic, where 21 polymorphic amplification products were detected. The lowest number of amplified polymorphic fragments (5) was detected by primer SCoT 45. The percentage of polymorphism ranged from 73.7% to 100%.

To determine the level of polymorphism in the analysed group of rye genotypes, diversity index DI, probability of identity PI and polymorphic information content PIC were calculated. All three indicators were applied for all eight SCoT primers and for their calculation, the individual frequences of fragments of each marker were used. The diversity index (DI) of SCoT markers ranged from 0.433 (SCoT 45) to 0.36 (SCoT 26) with an average of 0.834. The lowest values of polymorphic information content were recorded for SCoT 45 (0.418) and the the highest PIC values were detected for SCoT 26 (0.936) with an average of 0.835. Probability of identity was low ranged from 0.0003 to 0.032 with an average of 0.007 that indicates the possibility to differentiate genetically close genotypes.

Bhattacharyya et al. (2013) detected genetic variability in the wild genotypes of *Dendrobium nobile* Lindl. collected from different parts of Northeast India and they using a Start Codon Targeted (SCoT) marker system. A total of sixty individuals comprising of six natural populations were investigated for the existing natural genetic diversity. One hundred and thirty two (132) amplicons were produced by SCoT marker generating 96.21% polymorphism. The PIC value of the SCoT marker system was 0.78 which is lower than in our study. In study **Luo et al. (2012)**, start codon targeted (SCoT) markers were employed to investigate the genetic diversity of 73 mango accessions obtained from Guangxi province, China. A total of 275 bands were amplified by thirty-four SCoT primers, of which 203 (73.82%) were polymorphic. **Luo et al. (2012)** detected lower percentage of polymorphism in comparison to our study.

Start codon-targeted markers were utilized by **Gajera et al. (2014)** who used 19 SCoT markers for characterization and genetic comparison among 20 mango cultivars. These primers produced total 117 loci across 20 cultivars, of which 96 (79.57%) were polymorphic.

A set of 18 primers SCoT primers were used to fingerprint 20 peanut accessions. 18 primers generated a total of 157 fragments with a mean of 8.72, ranging from 4 (SCoT 25) to 17 (SCoT 6) per primer. Of 157 bands, 97 (61.78%) fragments were present in all the 20 accessions and 60 bands (38.22%) were polymorphic. Polymorphic index (PI) per primer ranged from 0.09 (SCoT 19) to 1.65 (SCoT 15), with an average of 0.82 **(Xiong et al., 2011)**. Lower PIC using SCoT analysis was detected, **Arya et al. (2014)** in *Morinda tomentosa* and that 0.189 ±0.103.

Table 2 Statistical characteristics of the SCoT markers used in rye.

SCoT primer	Primer sequence (5´ – 3´)	TNoB	NoPB	PIC	DI	PI
SCoT 6	CAACAATGGCTACCACGC	18	18	0.93	0.93	0.0004
SCoT 9	CAACAATGGCTACCAGCA	12	10	0.905	0.907	0.0009
SCoT 26	ACCATGGCTACCACCGTC	19	19	0.936	0.936	0.0003
SCoT 28	CCATGGCTACCACCGCC	18	17	0.934	0.934	0.0003
SCoT 36	GCAACAATGGCTACCACC	21	21	0.934	0.934	0.0003
SCoT 45	ACAATGGCTACCACTGAC	5	4	0.418	0.433	0.032
SCoT 54	ACAATGGCTACCACCAGC	11	11	0.883	0.886	0.001
SCoT 59	ACAATGGCTACCACCATC	19	14	0.741	0.712	0.022
Average		**15.37**	**14.25**	**0.35**	**0.834**	**0.007**
Total		**123**	**114**	**-**	**-**	**-**

Note: TNoB – Total number of bands, NoPB – Number of polymorphic bands, PIC- polymorphic information content, DI - diversity index, PI - probability of identity.

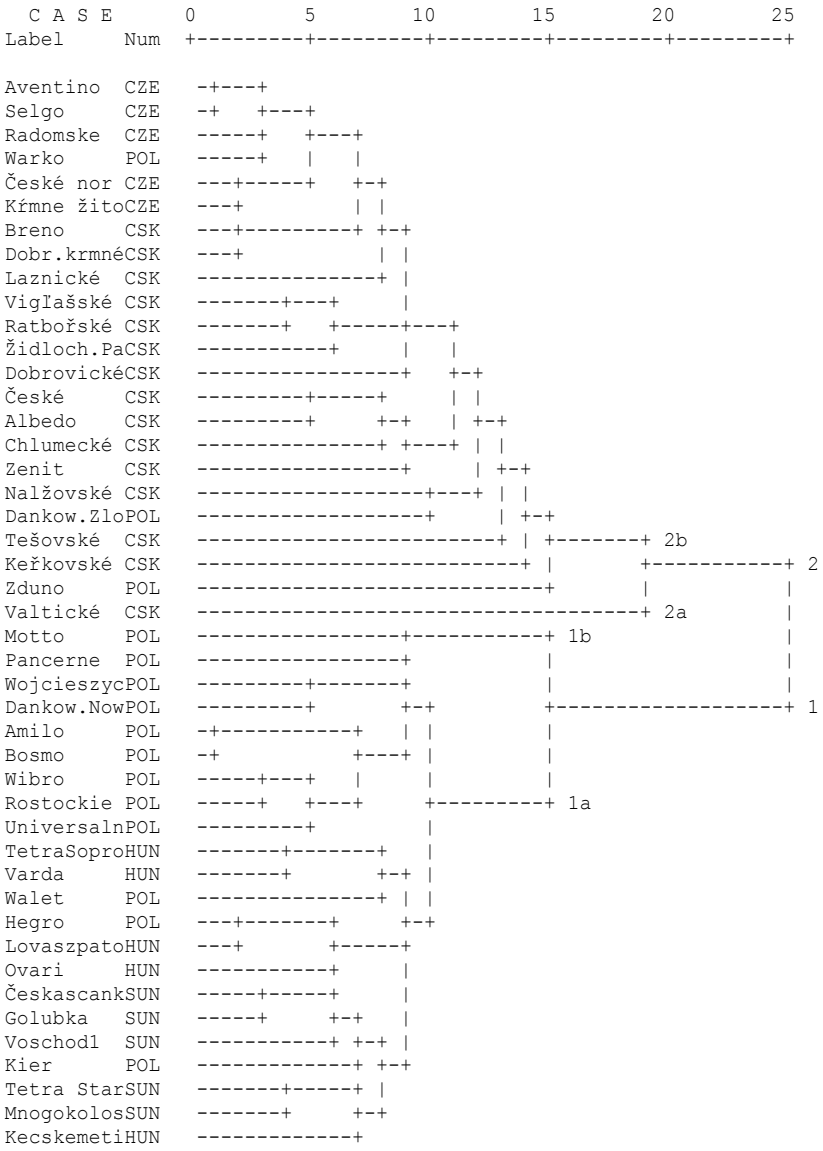

Figure 1 Dendrogram of 45 rye genotypes prepared based on 8 SCoT markers.
Note: CSK - Czechoslovakia, CZ - Czech Republic, HUN - Hungary, PL - Poland, SUN - Union of former Soviet Socialist Republic.

Luo et al. (2010) evaluated genetic variation and relationships among 47 mango germplasm and 3 relative species from Guangxi province in China, using Start Codon Targeted (SCoT) markers. 33 SCoT primers yielded a total of 273 clear and bright bands and their sizes ranged between 250 bp and 4000 bp. The number of bands varied from 3 (SCoT 9) to 15 (SCoT 70) with an average of 8.27 bands per primer. Of 273 bands, 208 bands (76.19%) were found to be polymorphic, the number of polymorphic bands varied from 2 (SCoT 9 and SCoT 54) to 14 (SCoT 70) with an average of 6.3 bands per primer. The detected polymorphism per primer among the tested accessions ranged from 40% (SCoT54) to 100% (SCoT3 and SCoT61).

The SCoT polymorphism marker technique has been successfully applied in rice **(Collard and Mackill, 2009)** peanut **(Xiong et al., 2011)**, cicer **(Pakseresht et al., 2013)**, oak **(Alikhani et al., 2014)** and potato **(Gorji et al., 2011)**.

For determination of the genetic relationships among rye genotypes a dendrogram was used. The dendrogram was constructed based on principle of hierarchical cluster analysis using UPGMA algorithm in statistical program SPSS. Analyzed rye genotypes were divided into two major clusters (1 and 2). The first cluster was divided in two subclustres (1A and 1B). Subcluster 1A contains 11 genotypes which were bred in Poland (52.4%), and group of genotypes coming from Union of Soviet Socialist Republics (23.8%) and Hungary (23.8%). Subscluster 1B included only Polish genotype Motto. The second cluster was divided into two groups (2A and 2B). In cluster 2A one rye genotype was separated – Valtické (CSK). Three varieties of rye coming from Poland, fourteen genotypes from Czechoslovakia and five genotypes from Czech Republic formed subcluster 2B. We could not distinguish 2 genotypes, Aventino and Selgo grouped in 2B subcluster, which can be caused due the same genetic background (Figure 1).

CONCLUSION

Overall, the 8 SCoT markers were fairly successful at specifically fingerprinting the Secale accessions. The primer sets scored amplified clear, well-resolved fragments with little stutter. The SCoT markers could distinguish between the various *Secale* species. The SCoT marker technique may be most correlated to the functional gene and their corresponding traits because its primers were designed according to the short conserved region surrounding the ATG translation start codon, where the ATG context is a part of the functional gene.

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