World Academy of Science, Engineering and Technology
International Journal of Agricultural and Biosystems Engineering
Vol:8, No:7, 2014

RAPD Analysis of the Genetic Polymorphism in the Collection of Rye Cultivars

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***Abstract*—**In the present study, RAPD-PCR was used to assess genetic diversity of the rye including landrances and new rye cultivars coming from Central Europe and the Union of Soviet Socialist Republics (SUN). Five arbitrary random primers were used to determine RAPD polymorphism in the set of 38 rye genotypes. These primers amplified altogether 43 different DNA fragments with an average number of 8.6 fragments per genotypes. The number of fragments ranged from 7 (RLZ 8, RLZ 9 and RLZ 10) to 12 (RLZ 6). DI and PIC values of all RAPD markers were higher than 0.8 that generally means high level of polymorphism detected between rye genotypes. The dendrogram based on hierarchical cluster analysis using UPGMA algorithm was prepared. The cultivars were grouped into two main clusters. In this experiment, RAPD proved to be a rapid, reliable and practicable method for revealing of polymorphism in the rye cultivars.

***Keywords*—**Genetic diversity, polymorphism, RAPD markers, *Secale cereale* L.

I. Introduction

RYE (*Secale cereale* L.) is a wind pollinated, diploid species with 2n=14 chromosomes [1], [2], and belongs to the grass tribe *Triticeae* like barley and wheat. In the genus *Secale* only *S. cereale* is a cultivated crop, whereas the other species are either wild growing or weeds [3], [4]. Rye is one of the most important cereal crop species of the temperate regions [5], is the second only to wheat among the grains most commonly used in the production of bread. The crop possesses a great number of advantages such as a unique nutritional value, winter hardiness and tolerance to environmental stresses as low temperatures, drought and poor soil conditions, also constitutes a very important gene pool for the breeding of wheat and other related crops [6].

A detailed knowledge and understanding of the genetic relationships among accessions is essential for the success of plant breeding programs as well as for efficient sampling and more informed utilization of available germplasm [7]. Genetic diversity studies on rye will contribute to the maintenance and rational use of germplasm resources in the improvement of rye and related crops [8].

There are various techniques available for evaluation of the crop genetic variability, such as morphological, biochemical and molecular markers [9]. DNA-based molecular markers are the most powerful diagnostic tools to detect DNA polymorphism at the level of specific loci and also at the whole genome level [10]. Molecular methods, such as RAPD (Random Amplified Polymorphic DNA) analysis, offer a valuable opportunity to characterize genetic variation and structure in plant populations [11]-[13]. Molecular (DNA) markers have many advantages over other techniques (independent of environment and plant growth stage, unlimited number, etc.) and they have been increasingly employed for analysis of genetic diversity [9].

RAPD markers are based on amplification of DNA by the polymerase chain reaction (PCR) using primers complementary to random target sites in the genome, a method also known as arbitrarily-primed PCR [14]. These genetic markers have been used to analyze the genetic variability of rye populations, to study the phylogenetic relationships among different rye populations [15], [16], to construct genetic maps [17]-[19], to establish homoeologous relationships among chromosomes of the related *Triticeae* [20], [21], and to detect and to locate genes of interest [22]. The aim of our present study was to evaluate the genetic diversity in the collection of 38 rye genotypes coming from Middle Europe and the Union of Soviet Socialist Republics registered from 1925 to 2006, using 5 RAPD primers.

II. Material and Methods

A. Plant Material

In total, thirty eight cultivars of winter rye 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 were used in that RAPD analyses (Table I). Samples came from Central European countries, fifteen from Czechoslovakia (1925-1991), two from the Czech Republic (1997-2002), fifteen from Poland (1955-2006), one from Hungary (1953) and five from the Union of Soviet Socialist Republics (1977-1986) as a basis for breeding. Seeds of *Secale cereale* L. were grown in pots, in a greenhouse at day/night temperatures of 25/17°C with natural photoperiod and light intensity.

B. Extraction of Genomic DNA

Genomic DNA was isolated from the 14 days leaves with GeneJET Plant Genomic DNA Purification Mini Kit (Thermo

This work was co-funded by European Community under project No. 26220220180: Building Research Centre „AgroBioTech" (50 %) and VEGA project No. 1/0513/13 (50 %).

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Scietific, Gdańsk, Poland) according to the manufacturer's instructions. DNA concentrations were estimated by UV-Vis spectrophotometer Q5000, Quawell. The plant DNA was stored in a freezer at -70°C.

C. RAPD Amplication and Gel Electrophoresis

RAPD analyses were performed using five decamer arbitrary primers (Table II) obtained from Genomed, Warsaw, Poland. Polymerase chain reactions (PCR) were carried out in 25 µl of following mixture: 10.25 µl deionized water, 12.5 µl Master Mix (2x Master Mix, A&A Biotechnology, Gdynia, Poland), 1.25 µl of genomic DNA, 1 µl 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.

PCR products were run on 1 % agarose gels in 1x TBE buffer at 170 V for 1.5 h. 10 µl of DNA samples were loaded into the gels. GeneRuler™ 1kb Plus DNA Ladder (Fermentas, Gdansk, Poland) that gives 15 bands from 75 to 20000 bp, was used as a standard. The gels 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).

D. Data Analysis

The band intensity and presence of RAPD-PCR products, separated on agarose gels, were analyzed by densitometry, using ImageLab™ Software version 4.1 Biorad. Each reproducible band was visually scored for the presence (1) or absence (0) for all genotypes.

Consequently, using binary matice a dendrogram based on hierarchical cluster analysis using UPGMA (Unweighted Pair Group Method using arithmetic Averages) algorithm with the SPSS professional statistics version 17 software package was constructed. Frequencies of incidence of all polymorphic alleles were calculated and used for determination of statistical parameters: diversity index (DI) [23], probability of identity (PI) [24] and polymorphic information content (PIC) [25].
Polymorphic information content (PIC):

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

Diversity index (DI):

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

Probability of identity (PI):

$$PI = \sum p_i^4 + \sum_{i=1}^{i=n-1} \sum_{j=i+1}^{n} \left(2 p_i p_j \right)^2 \quad (3)$$

P_i and p_j are the frequencies of the i^{th} and j^{th} allele in a given genotypes.

TABLE I
LIST OF 38 RYE CULTIVAR, THEIR COUNTRY OF ORIGIN AND BEGINNING OF REGISTRATION USED FOR RAPD ANALYSES

Genotype	Country of origin	Beginning of registration
Valtické	Czechoslovakia	1984
Tešovské	Czechoslovakia	1929
Keřkovské	Czechoslovakia	-
Zenit	Czechoslovakia	1948
Chlumecké	Czechoslovakia	1953
České	Czechoslovakia	1949
Albedo	Czechoslovakia	1991
Židlochovický Panis	Czechoslovakia	1987
Nalžovské	Czechoslovakia	1984
Dobrovické	Czechoslovakia	1984
Vígľašské	Czechoslovakia	1953
Ratbořské	Czechoslovakia	1925
Laznické	Czechoslovakia	1984
Breno	Czechoslovakia	1980
Dobřenické krmné	Czechoslovakia	1958
Aventino	Czech Republic	2002
Selgo	Czech Republic	1997
Warko	Poland	1991
Dankowskie Zlote	Poland	1974
Zduno	Poland	1996
Motto	Poland	1985
Pancerne	Poland	1972
Wojcieszyckie	Poland	1964
Universalne	Poland	1955
Dankowskie Nowe	Poland	1977
Amilo	Poland	1991
Wibro	Poland	1996
Bosmo	Poland	2006
Rostockie	Poland	2006
Hegro	Poland	2006
Walet	Poland	2001
Kier	Poland	2001
Tetra Start	SUN	1977
Čerkascanka tetra	SUN	1985
Voschod 1	SUN	1986
Golubka	SUN	1980
Mnogokoloskaja	SUN	1985
Lovaszpatonai	Hungary	1953

SUN - Union of Soviet Socialist Republics

TABLE II
CHARACTERISTICS OF THE RAPD MARKERS AND THEIR CHROMOSOMAL LOCATION USED IN THIS STUDY

Primer's name	Sequence	Chromosomal location
RLZ 6	5´GTGATCGCAG 3´	7RL
RLZ 7	5´GTCCACACGG 3´	2RL
RLZ 8	5´GTCCCGACGA 3´	7RL
RLZ 9	5´TGCGGCTGAG 3´	2RS
RLZ 10	5´ACGCGCATGT 3´	4RL

III. RESULTS AND DISCUSSION

The selection of the optimal molecular system for identification and analysis of relations between plant accessions is difficult. Attempts to obtain authoritative comparison between marker systems resulted in development of certain parameters describing properties of each technique [26].

RAPDs are effective among the most-widely used markers of economically important traits in cultivated plants [27]. They are applied in plant genome mapping programs [28], [29]. Although the number of RAPD loci that can be mapped is potentially unlimited, the efficiency of searching for strong and reproducible polymorphic fragments may appear rather low [30].

Our study dealt with detection of genetic polymorphism in winter rye cultivars using RAPD markers. For the differentiation of thirty eight rye genotypes, five RAPD markers (Table II) were chosen, as [31].

Five primers generated 43 polymorphic DNA fragments. The number of fragments per primer ranged from 7 to 12 with an average of 8.6. From five RAPD markers chosen, primer RLZ 6 was the most polymorphic, where 12 polymorphic amplification products were detected. The lowest number of different fragments (7) was detected in three primers RLZ 8, RLZ 9 and RLZ 10. To determine the level of polymorphism in the analyzed group of rye, diversity index DI (2), probability of identity PI (3) and polymorphic information content PIC (1) were calculated. All three indicators were calculated for all applied RAPD primers and for their calculation, the individual frequencies of fragments of each marker were used. The diversity index (DI) of the tested RAPD markers ranged from 0.842 (RLZ 8) to 0.907 (RLZ 6) with an average of 0.867. Polymorphic information content ranged from 0.838 to 0.905 with an average of 0.863. The highest value of PIC (0.905) was observed in primer RLZ 6, on the other side the lowest value (0.838) was in primer RLZ 8 (Table III). All PIC and DI values were higher than 0.8 that means high polymorphism detected in the chosen genotypes.

Probability of identity (PI) was low ranged from 0.001 to 0.005 with an average of 0.003 (Table III).

Lower polymorphism using RAPD analysis was detected, as [26], who used 19 RAPD markers that described genetic similarity of 48 inbred lines of rye. Polymorphic information content (PIC) values ranged from 0.31 to 0.50 with an average of 0.46. They used 19 primers that generated only 48 polymorphic bands. The number of fragments per primer ranged from 1 to 4. PIC value, which was calculated for RAPD analysis in other sets of inbred rye lines, was 0.374 [31]. Also low PIC value for RAPD was detected for potato, where reached the level of 0.362 [32], for rice bean (*Vigna umbellata*) 0.243 [33] and for flax 0.32 [34].

On the other hand high polymorphism was detected in the study [35], where 20 microsatellite markers were used to analyse the genetic diversity of 120 individuals of 20 weedy/wild populations of *Secale cereale* collected all around Iran. High levels of diversity, with an average number of 9.4 fragments per locus (ranging up to 11) and high level of PIC value from 0.787 to 0.657 with average of 0.7348 were observed.

TABLE III
STATISTICAL DATA FROM THE ANALYZED RAPD MARKERS OF RYE

Primer´s name	The number of fragments	DI	PIC	PI
RLZ 6	12	0.907	0.905	0.001
RLZ 7	10	0.884	0.883	0.005
RLZ 8	7	0.842	0.838	0.005
RLZ 9	7	0.855	0.850	0.003
RLZ 10	7	0.846	0.840	0.004
Average	8.6	0.867	0.863	0.003

DI – diversity index; PIC – polymorphic information content;
PI – probability of identity.

For the detection of genetic diversity the dendrogram based on hierarchical cluster analysis using UPGMA algorithm was prepared (Fig. 1). Thirty eight varieties of rye were divided into two main clusters. Genotype Keřkovské (Czechoslovakia) separated from other 37 rye genotypes. The second cluster was divided into two groups (2A and 2B). In the subgroup 2A were grouped 5 genotypes which were bred in Czechoslovakia. In the subgroup 2B were included 32 genotypes, of which the genotype Dobrovické separated. A large group of the subcluster 2B formed varieties from Poland (46.87%). Another large subcluster contained varieties from Czechoslovakia (28.12%) as well as all genotypes originating from a Union of Soviet Socialist Republics (15.62%) which were grouped together. Genotypes originating from the Czech Republic (6.25%) were included in the group of genotypes originating from Czechoslovakia and Poland. Using 5 RAPD markers we were able to distinguish 36 rye genotypes. Two genotypes (Golubka and Čerkascanka tetra) from SUN were genetically the closest. They have probably close genetic background (Fig. 1).

Genetic similarities among 20 spring and 22 winter accessions of agronomically different rye varieties from fourteen countries were studied using RAPD techniques, as [8]. Cluster analysis of genetic distance data showed that 42 genotypes were readily classifiable into two main groups: spring and winter groups. Within the spring group, cultivars fell into a North European and an American-Chinese group. Cultivars of winter rye fell into four groups: Northern European, Russian, American and Chinese lines. A UPGMA-dendrogram based on genetic distances of rye cultivars within the winter and spring groups showed that the clusters corresponded well to their geographical locations.

The RAPD technique revealed an extensive amount of variation leading to clear cultivar identification [36]. Surveys of genetic polymorphism in rye have been made, as [17], [31], [37]-[39] using RAPD, RFLP and ISSR markers and isozymes. In these studies, RAPD analysis proved to be a powerful tool with a number of advantages: easy generation of data, requirement of a small amount of DNA and no requirement of previous knowledge of the genome [8].

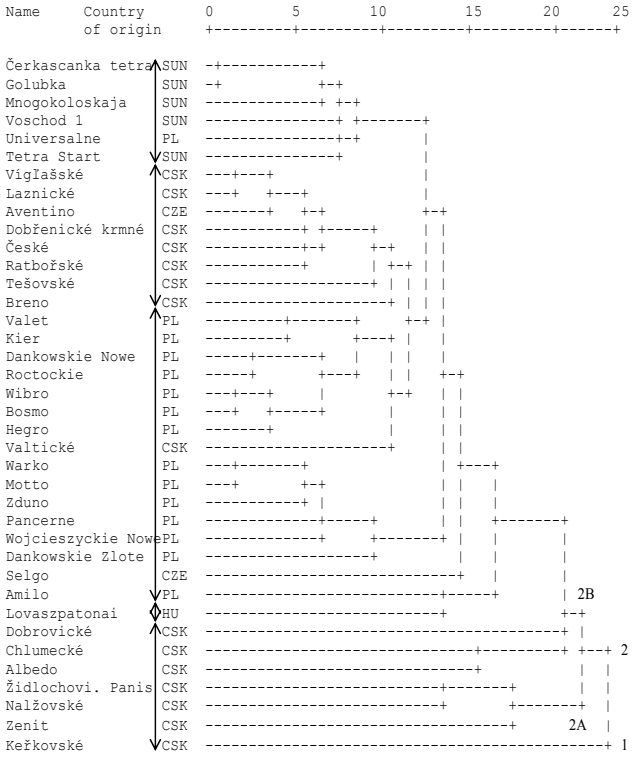

Fig. 1 Dendrogram of 38 rye genotypes prepared based on 5 RAPD markers

CSK - Czechoslovakia, CZ - Czech Republic, HU - Hungary, PL - Poland, SUN - Union of Soviet Socialist Republics.

ACKNOWLEDGMENT

We would like to thank Department of Plant Physiology, Faculty of Agriculture and Economics University of Agriculture in Krakow for realization of this research work.

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