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THE GENETIC DIVERSITY OF COMPONENTS OF RYE HYBRIDS

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Abstract: The genetic variability between 5 open-pollinated varieties of rye (*Secale cereale* L) and between the components of rye hybrids was estimated using PCR-based marker analysis. The 22 maternal single crosses and 11 restorers were the components of hybrids investigated in the preofficial trials at the Plant Breeding and Acclimatization Institute in Radzików (PBAI), the Danko Breeding Co. Ltd (DBC) and in the Poznań Breeding Co. Ltd (PBC) during the 2001 growing season. The PCR system using semispecific primers targeting the intron-exon junction sequences of plant genes was applied for the evaluation of the genetic diversity of rye breeding materials. The genetic distances between varieties were relatively low; the coefficients of dissimilarity did not exceed 0.15. The highest average distance between maternal hybrids and restorers (0.28) was found in materials from PBAI, whereas in materials from PBC and DBC the average distances were lower and reached 0.21 and 0.20 respectively. The cluster analysis based on PCR data indicated that hybrid components that originated from different breeding centres exhibited different genetic characters.

Key Words: Genetic Diversity, Hybrid Breeding, PCR, Semi-Specific Primers, Winter Rye

INTRODUCTION

The assessment of genetic diversity existing in available germplasm is fundamental for the improvement of agricultural plants. This is of considerable importance in breeding programs exploiting the effect of heterosis, where the genetic distances between components affect hybrid performance.

In rye, as in maize, selfing results in severe inbreeding depression and hybrids display strong heterosis. Hybrid breeding in rye is based on "Pampa" type cytoplasmic-genic male sterility as a hybridizing mechanism. In hybrid varieties

of rye, the heterosis effect depends on genetic distances between inbred lines created by a male sterile single cross and pollinator. The substantial progress achieved in hybrid rye breeding in Germany has proved that to make maximum use of heterosis, the parental components should be developed from genetically divergent gene pools [1]. Molecular techniques allow a precise measurement of the genetic divergence between and among different groups of germplasm. The abundance of information on DNA sequences of plant genomes permits the application of PCR primers targeting known sequences of the genome. Semi-specific PCR and the use of primers with partial homology to sequences of intron-exon junctions seems to be an alternative to RAPD and other tedious and expensive methods such as RFLP and AFLP. In contrast to the RAPD system, the semi-specific primers used generated more complex and much more polymorphic band patterns. The usefulness of this system in the evaluation of the diversity between inbred lines of maize and rye was documented in our previous study [2, 3]. The other important advantage is that using these semi-specific primers, one avoids targeting the heterochromatic regions of the plant genome. This is of particular importance in such cereals as rye, where repeat sequence regions are very abundant.

MATERIAL AND METHODS

The material consisted of the following open-pollinated varieties: Walet, Kier, Amilo and Dańkowskie Żłote were obtained from DBC and Adar from PBC. Eleven single crosses (SC1-SC11) and 6 restorers (R1-R6) originated from PBAI. The seeds of 6 single crosses (SC12-SC17) and 2 restorers (R7 and R8) were obtained from DBC and the seeds of 5 single crosses (SC18-SC22) and 2 restorers (R9 and R10) from PBC. The restorer R11 resulted from co-operation between PBAI and DBC.

The DNA was isolated from bulks containing equal quantities of the leaf tissue of 15-25 plants. The DNA was prepared according to the procedure of Davis *et al.* [4]. The quantity of DNA was evaluated fluorometrically according to the procedure described in the TKO fluorometer booklet (Hofer Sci., San Francisco, USA).

The polymerase chain reactions were carried out using a Uno II thermocycler (Biometra, Göttingen, Germany). The 20 µl reaction mixture contained: 15 ng of genomic DNA template, 1.0-1.2 µM of primer, 200 µM each of dATP, dCTP, dGTP and dTTP, 2 mM MgCl₂, 1.0 unit of Taq polymerase (MBI, Fermentas, Vilnius, Lithuania) and an appropriate reaction buffer. Amplification was carried out in two steps: in the first seven cycles the annealing temperature was 50°C for 15 base primers and 60°C for 18 base primers. This was followed by a further 33 cycles in which the annealing temperature was respectively 54°C or 64°C for 15 or 18 base primers. In all the cycles, the denaturation was carried out for 40 sec. at 95°C, annealing for 1 min. and amplification for 2 min. at 72°C. The

amplification products were separated by electrophoresis on 1.5% agarose gels, stained with ethidium bromide and photographed under UV light.

The photographs were analysed using "Fragment NT" (Molecular Dynamics, Sunnyvale, CA, USA). For each genotype, the presence of a band was marked as 1 or its absence as 0. The data were inputted as a binary matrix. The genetic distances between pairs of accessions, as per Nei and Li [5], were calculated using "Microsoft Excel" by the following equation:

$$GD_{(i,j)} = 1 - [(2N_{(i,j)} / (N_{(i)} + N_{(j)}))]$$

Where $N_{(i,j)}$ is the total number of bands common to genotypes i and j and $N_{(i)}$ and $N_{(j)}$ is the total number of bands present in i and j respectively. The UPGMA cluster analysis was performed using "Statistica 5" (StatSoft, Kraków, Poland).

RESULTS AND DISCUSSION

Genetic diversity between population varieties and between components of rye hybrids was investigated using exon targeting (ET) and intron targeting (IT) primers, 15 and 18 bases in length [6]. The genetic distances between open-pollinated varieties were relatively low. The indices of dissimilarity ranged from 0.11 to 0.15 (Tab.1). These data suggest that the breeding programs of open-pollinated varieties have reached their limit of genetic variability.

The evaluation of diversity between the components of rye hybrids was based on the analysis of 293 DNA fragments amplified with the use of 12 primers. Sufficiently clear and polymorphic banding patterns of amplified DNA fragments were obtained using some primers 15-18 bases in length, e.g. IT34 (Fig. 1).

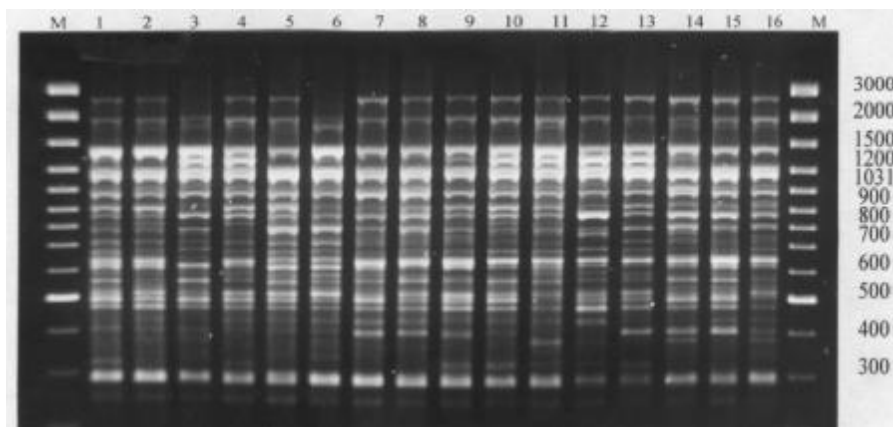


Fig. 1. Amplification of the DNA of 16 components of rye hybrids using primer IT34/15 5'(ACTTACCTGGCCGAG)3'. M-DNA size standard, lanes 1-6 (single crosses) and 7-8 (restorers) from Danko Breeding Company, lanes 9-13 (single crosses) and 14-16 (restorers) from Poznań Breeding Company.

Tab. 1. Indices of dissimilarity of rye varieties calculated from an analysis of 654 DNA fragments.

Variety	Amilo	Kier	Walet	Adar
Dańkowskie Złote	0.13	0.12	0.13	0.15
Amilo		0.13	0.13	0.15
Kier			0.12	0.13
Walet				0.11

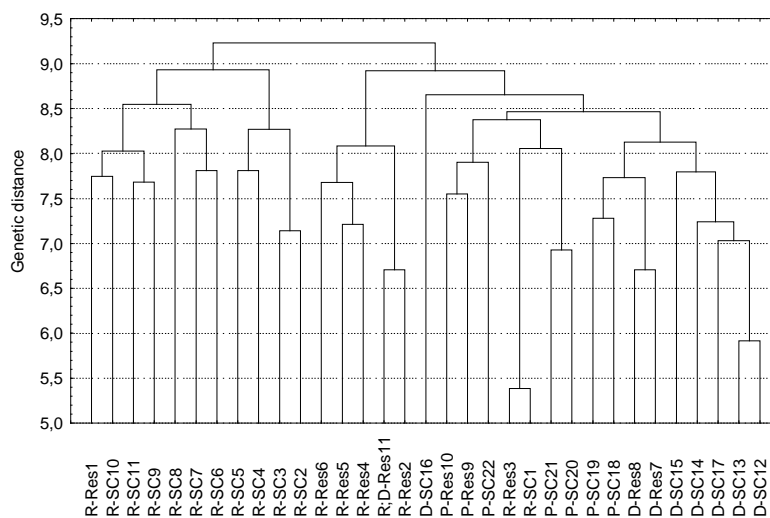


Fig. 2. Association among single crosses (SC) and restorers (Res) of rye revealed by UPGMA cluster analysis of 293 DNA fragments. R - Plant Breeding and Acclimatization Institute in Radzików, D - Danko Breeding Company, P - Poznań Breeding Company.

The highest average distance between maternal single crosses and restorers was found for materials from PBAI. These distances ranged from 0.21 to 0.36, with an average of 0.28. In the materials from DBC, these distances were lower and varied from 0.18 to 0.24, with an average of 0.20. Similar results were obtained for materials from PBC where the indices of dissimilarity between single crosses and restorers ranged from 0.17 to 0.27, with an average of 0.21.

The results of the analysis also indicated that maternal single crosses originating from different breeding centres exhibit differences in their patterns of amplified DNA fragments. The average index of dissimilarity between single crosses from PBAI and those from DBC or PBC was 0.28 and the average distance between single crosses from DBC and PBC was 0.21. The separation of materials from three breeding centres into somewhat distinct gene pools was confirmed by the

cluster analysis based on the PCR data (Fig. 2). The results of the grouping are consistent with the origin of accessions from particular breeding centres, with only few exceptions.

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