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ORIGINAL RESEARCH PAPER

The genetic polymorphism between the wild species and cultivars of rye *Secale cereale* L.

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Abstract
The aim of this research was to analyze genetic polymorphism between nine wild species and three cultivars of rye (genus *Secale* L.). The genetic polymorphism was assessed by means of the RAPD method (random amplified polymorphic DNA). The coefficients of genetic similarity between the species were calculated on the basis of amplified products and they were presented in a dendrogram. The highest genetic similarity was found between the *Secale cereale* 'Amilo' and *S. cereale* ssp. *ancestrale* ecotype 30226 (70%), whereas the lowest genetic similarity was observed between *S. sylvestre* and *S. cereale* ssp. *dighoricum* (33%). The results indicate considerable usefulness of the wild species for crossbreeding with the cultivars of the genus *Secale* and as genetic resources for breeding programs.

Keywords
genus *Secale*; RAPD; polymorphism of DNA; genetic similarity

Introduction

The main goal of research in evolutionary biology and plant breeding programs is to improve functional traits and to assess and increase genetic diversity between the populations or within species. It is necessary to know the genetic variability within a particular species to specify the available gene pool and in consequence, the characteristics of varieties or individual plants [1–3]. The search for the genes which determine functional traits in rye is a key issue necessary to specify for the aim of breeding and especially to make an optimal selection of parental germplasm for crossbreeding in plant breeding programs, for example: cytoplasmic male sterility, sprouting resistance genes, *Sec* genes on rye chromosomes, quantitative trait loci in rye, and male fertility restoration locus in rye [4–8]. The plant material selected for crossbreeding should be strongly genetically diversified so that the probable result of crossbreeding can lead to the development of the greatest possible genetic variability in offspring plants.

In recent years there has been stagnation in the progress in rye breeding because of the absence of highly diversified input material for crossbreeding. The problem of limited genetic variation in rye has been discussed for over 30 years [9]. Due to the fact that gene pools of European provenance have been extensively used in breeding, it is necessary to expand genetic variability in further improvement of productive populations, i.e., cultivars. Dopierała et al. [10] informed that intensive selection in the process of hybrid rye breeding narrows genetic diversity and KWS Lochow Polska studied collections of inbred lines from ancient Polish varieties (over 70 years old), old Polish and Russian varieties (over 40 years old), and local populations. Identical

conclusions were made by Targońska et al. [11] who suggest that the genetic pool of current rye cultivars is becoming narrower during breeding processes. The reconstruction of progress in the breeding of rye, which is a common cereal in Poland, will largely depend on the enrichment of domestic cultivars with the genes from specimens bred as a result of interspecies crossbreeding between cultivars and wild species [12,13].

The selection of varieties or species for distant crossbreeding, which is based on the analysis of genetic polymorphism, may enable transfer of economically useful traits from the species where so far the attempts of distant crossbreeding have been unsuccessful and considerably increase the efficiency of regeneration of hybrid plants. As far as breeding work on rye is concerned, it is possible to enrich new cultivars with the functional traits derived from the wild species of rye, e.g., resistance to downy mildew and brown rust, resistance to lodging, and sprouting in the ear and male sterility [14,15].

The method of random amplified polymorphic DNA (RAPD) markers is simple to use and it relatively precisely determines the genetic distance [2,16]. It is helpful for further selection, because it is possible to initially qualify or reject the crossbreeding combination. The method is often used in the analysis of genetic similarity of numerous plant species and it is not limited to cereals only but it is also used for soybean or winter oilseed rape [17,18]. The RAPD method is often used in the search for the markers which are strongly coupled with the genes responsible for functional traits. It is also used for the construction of genetic maps of the species which have not been described yet, for the assessment and analysis of variability as well as for the assessment of genetic similarity between cultivated varieties and species [19]. Gradzielewska et al. [20] pointed out that RAPD is a technique using short (8–12 base pairs) arbitrary primers to amplify random segments of DNA along the whole genome. This method has a low cost, requires small amounts of DNA and does not need prior knowledge of the genome sequences. The data received in a short time and the number of markers obtained is sufficient to discriminate species and genera. Similar results were obtained by Ćwiklińska et al. [21] and the authors suggest that RAPD and AFLP markers are equally suited for studying the genetic similarity of wild species and subspecies.

Material and methods

Nine wild species and three cultivars of *Secale cereale* L. plants were the objects of the research (Tab. 1). The research material was 25 seedlings of plants grown from grains from the collection of the Department of Genetics and Plant Breeding, Poznań University of Life Sciences, Poland. The seeds were sown onto Petri dish lined with filter paper. The genomic DNA was isolated with the column-based method from the leaves of two-week-old seedlings by means of a Genomic Mini AX Plant kit (A&A BIOTECHNOLOGY) according to the manufacturer's protocol. After the isolation, the DNA concentration was measured with a NanoDropLite spectrophotometer (ThermoScientific) and then all the samples were diluted to a concentration of 50 ng/μL. The material was stored at a temperature of –20°C.

The RAPD was carried out in 12.5 μL of the mixture which was composed of deionized water (5 μL), DreamTaq PCR Master Mix (2×) – 6.25 μL (ThermoScientific), primer – 20 pm/μL, and 25 ng/μL DNA extract. A Professional Basic Gradient thermocycler (Biometra) was used for amplification. The conditions of the PCR reaction were programmed for initial denaturation of 60 s at 94°C, followed by 10 cycles (denaturation at 94°C for 30 s, primer binding at 35°C for 30 s, synthesis at 72°C for 30 s). For the remaining 30 cycles, the following PCR profile was applied: 30 s at 94°C, 30 s at 37°C, and 60 s at 72°C, with a final synthesis step of 180 s at 72°C. The amplification was repeated 2 or 3 times for each sample, so the summary of the results shows only repetitive analyses. Of 38 primers tested, thirteen 10-nucleotide primers (Operon Technologies) were selected for the analyses. The selection criteria included the level of polymorphism detected and other studies on the analysis of similarity in the genus *Secale* where RAPD markers were used [2,21,22]. Tab. 2 is a summary of the primers used and their nucleotide sequence.

Tab. 1 The species and subspecies of *Secale cereale* analyzed for genetic similarity.

Species	Subspecies (ssp.)	Cultivar (cv.)	Life-form†	Type‡
Secale cereale L.	*cereale*	Dańkowskie Złote	A	C
Secale cereale L.	*cereale*	Amilo	A	C
Secale cereale L.	*cereale*	Skat	A	C
Secale cereale L.	*afghanicum*		A	W
Secale cereale L.	*ancestrale*	ecotype 17791 (Sweden)	A	W
Secale cereale L.	*ancestrale*	ecotype 30226 (Turkey)	A	W
Secal ecereale L.	*segetale*		A	W
Secale cereale L.	*dighoricum*		A	W
Secale strictum	*africanum*		P	W
Secale strictum	*ciliatoglume*		P	W
Secale sylvestre			A	W
Secale vavilovi			A	W

† A – annual life-form; P – perennial life-form. ‡ C – cultivar; W – wild.

Tab. 2 The primers selected for amplification, their sequence and degree of polymorphism are revealed.

Primer	Sequence (5′–3′)	Level of polymorphism generated (%)	Range of molecular weights of products (bp)
OPA-04	AATCGGGCTG	85	2100–273
OPA-07	GAAACGGGTG	57	1540–203
OPA-09	GGGTAACGCC	89	1027–266
OPA-12	TCGGCGATAG	90	1250–144
OPA-16	AGCCAGCGAA	80	1200–230
OPA-17	GACCGCTTGT	73	1920–238
OPA-20	GTTGCGATCC	69	1560–240
OPB-08	GTCCACACGG	77	1272–220
OPB-10	CTGCTGGGAC	80	1669–243
OPB-17	AGGGAACGAG	58	1305–140
OPB-20	GGACCCTTAC	80	2200–428
OPC-01	TTCGAGCCAG	75	2050–207
OPI-12	AGAGGGCACA	71	1915–246

The electrophoresis of RAPD products was carried out in 1.5% agarose gel with 1 µL solution of ethidium bromide for 2 hours at a voltage of 100 V. An O'RangeRuler 100 bp size marker (Fermentas) with the range of identification of molecular weights from 100 to 1500 base pairs was used for the identification of molecular weights. The isolated DNA fragments were visualized in UV light and recorded in images. The images were analyzed and the molecular weights of RAPD amplification products were determined by means of UVI Band v. 12.14 software.

The genetic similarity was estimated by means of genetic similarity coefficients, which were derived as a result of comparison of the number of RAPD products between the species, by means of the DICE algorithm and with the following formula:

$$GS_{xy} = \frac{2n_{xy}}{n_x + n_y}$$

where n_{xy} refers to the number of amplicons present both in species x and species y, n_x – the number of amplicons present in species x, n_y – the number of amplicons present in species y [23].

The matrixes containing the genetic similarity coefficients were used for the construction of dendrograms, the analysis of which enabled graphic presentation of the genetic similarity between the objects under investigation.

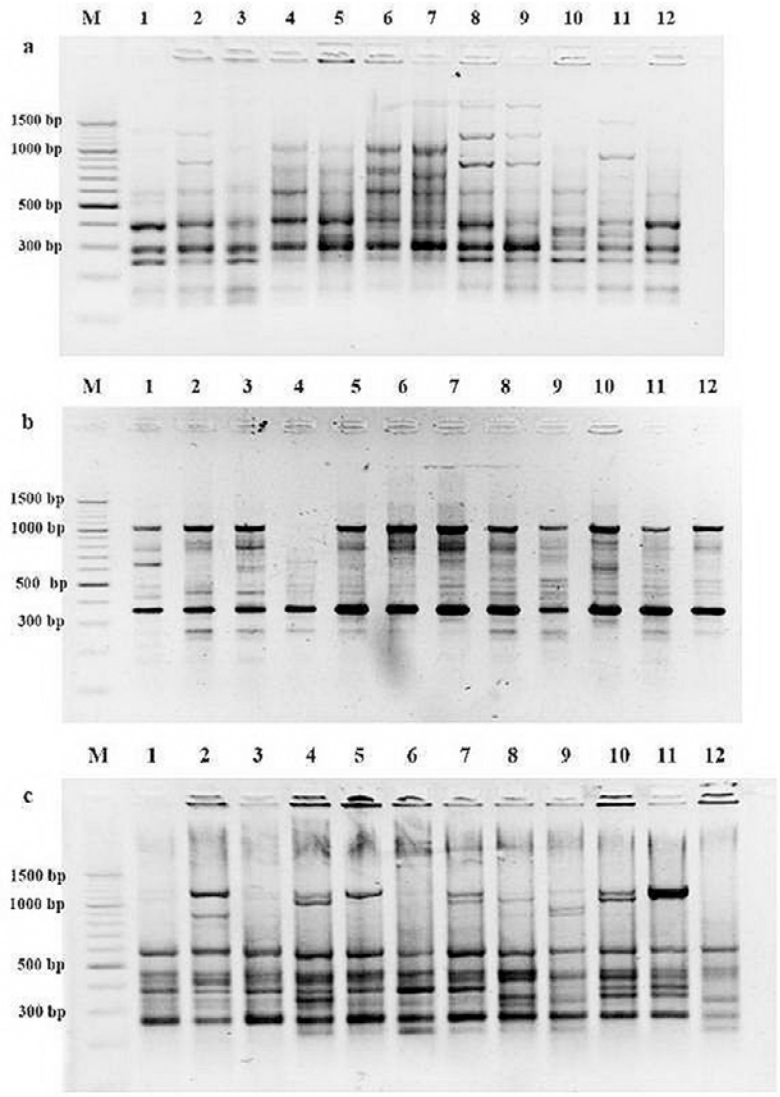


Fig. 1 The electrophoretic distribution of DNA amplification products obtained by means of the RAPD method with the OPA-17 (**a**), OPA-16 (**b**), and OPC-1 (**c**) primers. The paths are marked in the following manner: 1 – *S. cereale* ssp. *dighoricum*; 2 – *S. cereale* ssp. *segetale*; 3 – *S. vavilovi*; 4 – *S. cereale* ssp. *ancestrale* 17791; 5 – *S. cereale* 'Amilo'; 6 – *S. cereale* ssp. *ancestrale* 30226; 7 – *S. strictum* ssp. *africanum*; 8 – *S. strictum* ssp. *ciliatioglume*; 9 – *S. cereale* 'Dańkowskie Złote'; 10 – *S. sylvestre*; 11 – *S. cereale* ssp. *afghanicum*; 12 – *S. cereale* 'Skat'; M – O'RangeRuler 100 bp marker.

Results

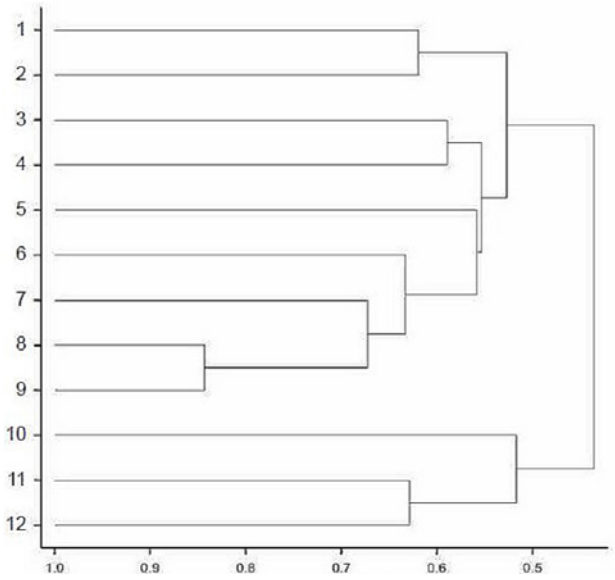
In order to analyze the genetic polymorphism between the cultivars and wild species of *Secale cereale*, 38 primers were tested, 13 of which revealed a high level of polymorphism, which was revealed by RAPD analysis.

On average, the primers used for amplification generated 12 band products with different molecular weights, including nine polymorphic products. The highest number of products (15) was obtained with the OPA-17 primer (Fig. 1a); the lowest number (8) – with the OPC-1 primer (Fig. 1c). A total of 151 amplicons was generated, including 113 polymorphic amplicons.

The analysis of the amplified products by means of the DICE algorithm enabled the determination of the coefficients of genetic similarity between the species and cultivars of *S. cereale* under investigation. The table of genetic similarity coefficients was used for the construction of a dendrogram (Fig. 2). The genetic correlations between the objects are shown.

The greatest genetic similarity was observed between *S. cereale* ssp. *ancestrale* with the ecotypes 17791 and 30226; the similarity coefficient was 84%. *Secale sylvestre* and *S. cereale* ssp. *dighoricum* were the least similar to each other; the similarity coefficient was only 33%. The values of similarity between the cultivars ranged from 56% to 62%. As far as the wild species are concerned, the highest values of the coefficient of similarity to the cultivars was observed in the following species: *S. cereale* ssp. *africanum* (58% on average), *S. cereale* ssp. *afghanicum* (56% on average), and two ecotypes of *S. cereale* ssp. *ancestrale* (60% on average). The greatest similarity to the cultivars was observed in the wild species of *S. cereale* ssp. *ancestrale* ecotype 30226, as it showed 70% of genetic similarity to *S. cereale* 'Amilo'. The wild species that was least similar to *S. cereale* ssp. *cereale* was *S. cereale* ssp. *dighoricum*, as its similarity to the cultivars 'Scat' and 'Amilo' was only 38%. As far as the perennial species are concerned, *S. strictum* ssp. *africanum* proved to be the most genetically similar to *S. cereale* 'Amilo' (63 %).

The dendrogram distinguished two cluster groups of the wild species and *S. cereale* cultivars. The first group contains *S. vavilovii* and *S. cereale* ssp. *dighoricum* (with the similarity coefficient 63%), and *S. cereale* ssp. *segetale* (50% of similarity to the other two). The other group consists of the perennial *S. strictum* ssp. *africanum* and *S. strictum* ssp. *ciliatoglume*. Two subgroups can be distinguished in group 2. The first subgroup contains *S. cereale* 'Dańkowskie Złote' and *S. cereale* 'Skat' (59% of similarity to each other). The similarity in the other subgroup ranges from 53% between *S. strictum* ssp. *africanum* and *S. cereale* ssp. *afghanicum*, to 70% between *S. cereale* 'Amilo' and two *S. cereale* ssp. *ancestrale* ecotypes. The dendrogram illustrates the high similarity between the cultivars and wild species, i.e., *S. strictum* ssp. *africanum*,



Genetic similarity (%)

1 – *Secale strictum* ssp. *ciliatoglume*

2 – *Secale sylvestre*

3 – *Secale cereale* ssp. *cereale* cv. Dańkowskie Złote

4 – *Secale cereale* ssp. *cereale* cv. Skat

5 – Secale cereale ssp. afghanicum

6 – Secale strictum ssp. africanum

7 – *Secale cereale* ssp. *cereale* cv. Amilo

8 – *Secale cereale* ssp. *ancestrale* ecotype 17791 (Sweden)

9 – *Secale cereale* ssp. *ancestrale* ecotype 30226 (Turkey)

10 – *Secale cereale* ssp. *segetale*

11 – *Secale cereale* ssp. *dighoricum*

12 – *Secale vavilovi*

Fig. 2 A dendrogram of genetic similarity between the wild species and cultivars of the *Secale* genus.

S. cereale ssp. *afghanicum*, and *S. cereale* ssp. *ancestrale*. It also shows the distinctness of *S. sylvestre* and *S. strictum* ssp. *ciliatoglume* and their high genetic distance from the other species, especially from *S. cereale* ssp. *dighoricum* and *S. vavilovi*.

Discussion

The search for new genetic variability and enrichment of the existing variability are important approaches in modern cereal breeding [12,24]. Traditional breeding methods were based on strong selection pressure and impoverished the diversity and caused genetic uniformity of cultivars. The limitation of diversity directly impact on the lack of input materials for new breeding programs. Modern breeding programs are developed in order to improve and/or create desired characteristics (e.g., high yield, resistant to pests and diseases, drought-resistant). New varieties of rye may become the main source of high quality nutrient- rich grains, especially in the regions at high altitudes or in the regions with poor soils and a short vegetation period.

The low variability within *S. cereale* ssp. *cereale* causes considerable difficulties in the achievement of the breeding goals. Therefore, the breeding of new cultivars that yield better, are resistant to diseases and pests or with the increased tolerance of environmental pressures is very difficult. However, the variability can be increased by distant crossbreeding.

A wide range of research projects led to the conclusion that the wild species of the *Secale* genus have desirable characteristics and represent a considerable source of genetic diversity. Therefore, wild species of the genus *Secale* may be used for the expansion of the genetic pool and acceleration of breeding progress [25]. The wild species of the genus *Secale* subjected to the analysis of similarities proved to be particularly interesting for the application in the growing of cereals, because they contain the genes that contribute to increased accumulation of proteins in caryopses and the accumulation of amino acid lysine, exogenous to humans. Wild species of the genus *Secale* are also resistant to numerous plant diseases and have many important morphological and biochemical traits. These traits may be transferred both to rye cultivars and to other related species of cereals [26,27].

Descriptions of the genes transferred from wild species to cultivars usually concern those responsible for resistance to brown rust and downy mildew, which are transferred from *S. strictum* and *S. kuprijanovi* [28,29], or those responsible for resistance to lodging and sprouting, which are transferred from *S. sylvestre*, *S. anatolicum*, and *S. vavilovi* [30]. Examples of other breeding successes with the use of the wild species of the *Secale* genus include the transfer of self-pollination genes from *S. vavilovi* [31] and perenniality genes from *S. strictum* to the *S. cereal* cultivars [32].

In practice, crossbreeding between cultivars and wild species is very difficult. This is due to the frequent occurrence of chromosomal aberrations or other cytogenetic disorders during cell divisions. As a result, caryopses may not develop or the fertility of hybrids in the F_1 generation decreased. Another problem is the low heritability of the transferred traits and poor quality and quantity of the yield [33]. In recent years, most crossbreeding programs based on wild species and cultivars have been

successful. This has been due to the continuous progress in in vitro techniques, which overcome the barriers to distant crossbreeding, and by the higher proportion of molecular techniques in the selection process [30,34,35].

The right selection of material for crossbreeding of wild species with cultivars may be decisive to the success of breeding programs. Therefore, modern plant breeding, often uses efficient tools provided by biotechnology, especially molecular markers [36–38].

Due to the low cost and low laboriousness, most often the techniques of obtaining molecular markers for plant breeding are based on polymerase chain reaction (PCR). RAPD is the simplest method of using PCR for analysis of genome sequence variation. The method uses random amplified polymorphic DNA fragments (RAPD). It is a marker system which uses a random but known sequence of 9–11 nucleotides in length [39]. Primers, which hybridize during a PCR to a DNA matrix in many regions of the genome, start amplification simultaneously. Electrophoresis is applied to isolate amplification products in agarose gel with staining by means of ethidium bromide or silver. The application of this method does not require knowledge of the DNA sequence under analysis, a large amount of genetic material or outstanding workload. Unfortunately, the dominant character of markers makes identification of non-dominant and codominant alleles in a given locus impossible [40].

In spite of undoubted advantages, the application of this method is not free from limitations. Due to the character of application of arbitrary primers RAPD is a method of low specificity and repeatability. Unfortunately, usually the longest stage of RAPD analyses is PCR optimization of test selection of appropriate primers which generate high polymorphism. Therefore, the system of RAPD molecular markers is usually applied in preliminary investigations which are supposed to narrow down the search for appropriate crossbreeding material [2,16] At later stages of selection based on molecular analysis, markers with high specificity for a particular sequence are applied, such as AFLP and/or SNP, SSR DArT [21,41,42]. The determination of genetic similarity between the species and subspecies, wild forms and cultivars may also answer the questions related to phylogenesis, diversification of the primary pool of genes, and evolution of the genus *Secale* [1,3,42,43].

Research [3,44] unequivocally points to the fact that the *S. sylvestre* is the most distant from other species of the genus *Secale* and it shows particularly low similarity to *S. cereale*. This fact may point to different phylogenesis; *S. sylvestre* is a form which evolved earliest, whereas *S. cereale* evolved a relatively short time ago. A similar thesis may be put forward on the basis of our own research findings. *Secale sylvestre* was observed to be in a separate group of similarity, together with the perennial *S. strictum* ssp. *ciliatoglume*, i.e., outside the rest of the species. The genetic similarity between these two species was as high as 62%, whereas *S. sylvestre* showed only 42% similarity to the other wild species. The genetic separateness of *S. sylvestre* and *S. strictum* ssp. *ciliatoglume* has been described in other studies [3,45], which is in accordance with our findings.

A close phylogenetic relationship between *S. vavilovi* and *S. cereale* ssp. *dighoricum*, which come from Eastern Europe and the Middle East, have been revealed [22,44]. In our analysis, the species were classified in the same group (63% of similarity), which confirms the common origin of the species.

It is generally accepted that perennial species are genetically different from annual ones. However, *S. strictum* ssp. *africanum* revealed a relatively high similarity to the *S. cereale* subspecies (43% compared with *S. cereale* ssp. *dighoricum* and 64% compared with *S. cereale* ssp. *ancestrale*) [45]. *Secale strictum* ssp. *africanum* was also highly genetically related to all the cultivars under analysis (58% similarity, on average). On the basis of these findings and due to the presence of the genes of resistance to plant diseases, we can assume that this perennial species is very useful as input material for immunity breeding and crossbreeding with high-yield rye cultivars.

The aim of this study was also to assess the relationship between genetic polymorphism and the potential for crossbreeding between wild species and cultivars. Studies on plant breeding generally prove that the effectiveness of crossbreeding and the efficiency of obtaining caryopses from the hybrids between the cultivars and wild species are very low. It ranges from 1.58% for the hybrids between the cultivars and *S. sylvestre* to 15.53% for the hybrids with *S. africanum* [13].

However, in spite of the unfavorable effect of higher temperature on breeding there was relatively high effectiveness observed for crossbreeding between the cultivars and *S. africanum*. Although *S. africanum* is phylogenetically older than *S. cereale*, it is genetically similar to a great extent. Presumably, genetic similarity may have considerably influenced the higher efficiency (15.53%) than the average value obtained in crossbreeding with the other wild species and with *S. cereale* ssp. As far as other crossbreeding combinations are concerned (the use of *S. cereale* ssp. *cereale* as the paternal form), the obtained effectiveness values were even lower, i.e., 7% for crossbreeding with *S. cereal* ssp. *ancestrale* and only 0.43% for *S. sylvestre*. Moreover, the high similarity between *S. cereale* ssp. *ancestrale* and the cultivars (60% on average) resulted in more efficient production of seeds.

In order to confirm the possible correlation of genetic polymorphism with the potential for effective crossbreeding between wild species and cultivars, it would be necessary to investigate the effectiveness of crossbreeding and development of caryopses formed as a result of crossbreeding between the species with the highest similarity. Presumably, crossbreeding between pairs of the species with high genetic similarity would reveal high effectiveness of crossbreeding and formation of seeds.

The analysis of genetic polymorphism carried out by means of RAPD molecular markers and the results of the analysis clearly revealed a high degree of similarity between the cultivars and between the cultivars and wild species. The genetic similarity ranged from 33% to 84% and indicates the presence of strong phylogenetic relationship between the wild species and cultivars. High effectiveness of crossbreeding and formation of input material for new varieties breeding with desired traits can be expected.

Conclusions

- There is a relationship between genetic polymorphism and the potential for effective crossbreeding between the wild species and cultivars of the genus *Secale*.
- The crossbreeding between *S. cereale* 'Amilo' and *S. cereale* ssp. *ancestrale* (genetic similarity ca. 70%) gives prospects for achieving high effectiveness in crossbreeding and creating new intra species variability.
- The highest genetic similarity to the cultivars can be observed in the following species: *S. strictum* ssp. *africanum*, *S. cereale* ssp. *afghanicum*, and *S. cereale* ssp. *ancestrale*. It would be necessary to confirm if they have high potential for effective crossbreeding with the cultivars.
- The considerable genetic distance between the cultivars *S. cereale* ssp. *cereale* and the wild species *S. sylvestre* and *S. cereale* ssp. *dighoricum* may cause lower effectiveness of crossbreeding or it may make crossbreeding impossible.

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Polimorfizm genetyczny dzikich gatunków i odmian uprawnych żyta *Secale cereale* L.

Streszczenie

Przedmiotem badań była analiza polimorfizmu genetycznego między dziewięcioma gatunkami dzikimi żyta i trzema odmianami uprawnymi żyta. Do oszacowania polimorfizmu genetycznego zastosowano metodę RAPD (random amplified polimorphic DNA). Na podstawie produktów amplifikacji obliczono współczynniki podobieństwa genetycznego pomiędzy badanymi obiektami i zilustrowano je za pomocą dendrogramu. Największe podobieństwo wykazano między odmianą uprawną 'Amilo' a podgatunkiem dzikim *S. cereale* ssp. *ancestrale* (70%). Najmniej podobnymi taksonami okazały się *S. sylvestre* i *S. cereale* ssp. *dighoricum* (33%). Otrzymane wyniki wskazują na dużą przydatność gatunków dzikich do krzyżowania z odmianami uprawnymi rodzaju *Secale* oraz na możliwość użycia tych taksonów jako materiałów wyjściowych do hodowli.

