Studying the polymorphism of cereal varieties using RAPD and ISSR markers

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Abstract. The sustainable development of the crop industry critically depends on increasing the stability of cultivated species through accelerated selection, selection of crops and varieties that complement each other, their adaptive zoning and increasing the varietal diversity of agroecosystems. Therefore, the analysis of the genetic structure of the gene pool and the assessment of the degree of genetic kinship using molecular genetic methods is necessary for achieving the most effective use of genetic resources. This study identified 28 polymorphic RAPD (random amplified polymorphic deoxyribonucleic acid) and ISSR (inter-simple sequence repeats) loci in spring triticale, wheat and oat varieties. The genetic distances of the studied varieties and breeding lines were calculated and found to vary from 0 to 1 for different markers. The data presented in this study should be utilised in future breeding research as it presents important insights into the hybridization and crossing of the source material located at a large genetic distance from each other.

1. Introduction
Adaptive intensification, which considers the relationship between high productivity and sustainability of agricultural production, low productions costs, environmental safety and profitability, is based on qualitatively new principles of rational agriculture and environmental management [1]. Factors such as the reduced genetic diversity of modern varieties, reduced immunity to diseases and insects, environmental pollution due to the use of pesticides and deterioration in the quality and degradation of land resources have caused crop yields to increase at a slower rate than population growth. However, the introduction of modern biotechnological approaches based on the use of molecular markers in breeding programs can help solve these problems [2].

Different types of markers that can detect foreign genetic material using polymerase chain reaction (PCR) can be used to target plants using marker-assisted selection (MAS) [3]. Methods based on the analysis of polymorphism of amplified DNA (deoxyribonucleic acid) fragments are widely used to determine the level of genetic variability and to identify phylogenetic relationships between plants [4]. DNA markers revolutionized the entire process of genetic and breeding research; and for the first time, it was possible to estimate the population by a large number of loci, make genetic maps and control the selection of the most valuable individuals [5]. Elucidating genetic relationships to a certain extent can be useful in planning hybridization, speeding up the selection process by selecting the right
combinations and reliably classifying genotypes [6]. Research has shown that a consistent increase in the yield of cultivated varieties is based on the improvement of their cultivation technologies and the achievements of breeding [7]. Recently, many DNA markers have been developed and proposed for the diagnosis of resistance genes and use in marker-oriented crop selection schemes. The use of molecular genetic methods in the selection process is particularly relevant at the present, which is confirmed by a long list of scientific publications on the study of genetic variability of oats [8,9], triticale [10–12] and wheat [13–16]. In this regard, the goal of the research work was to study the genetic polymorphism of varieties of cereals using RAPD (random amplified polymorphic deoxyribonucleic acid) and ISSR (inter-simple sequence repeats) markers.

2. Materials and methods
This research focused on 11 varieties and breeding lines of grain crops of the 2019 harvest: triticale – Ukro x Lana, Ukro, Lana, Ukro x Dalgau 1; wheat – Dalira, Khabarovchanka x Monakinka, Lira-98 x 334-84, Lira-98; and oats – Marshal, Cardinal, Bohun. To assess the genetic diversity of breeding material, molecular genetic analysis methods such as RAPD and ISSR were used. Three RAPD and three ISSR primers were used and tested (table 1).

Table 1. Nucleotide sequences of RAPD and ISSR primers used in the molecular genetic analysis.

| RAPD primers | Sequence          | ISSR primers | Sequence          |
|--------------|------------------|--------------|------------------|
| OPO-05       | CCCAGTCACT       | Primer8      | GTGGTGGTGGTGTTGTTG |
| OPJ-13       | CCACACTACC       | UBC856       | ACACACACACACACACACYA |
| Primer11     | CAATCGCCGT       | ISSR-24      | GAGAGAGAGAGAGAGATT |

Polymerase chain reaction (PCR) was performed in an icer amplifier (Bio-Rad, USA) using a Screen Mix PCR kit (Eurogen, Russia). To obtain the RAPD spectrum of fragments, the following program was used: 94°C – 4 min; forty cycles denaturation at 94°C – 1 min, annealing of primers at 36°C – 1 min, elongation at 72°C – 2 min; final elongation at 72°C – 10 min. To obtain the ISSR spectrum of fragments, the following program was used: 94°C – 4 min; forty cycles denaturation at 94°C – 1 min, annealing of primers at 50°C – 1 min, elongation at 72°C – 2 min; final elongation at 72°C – 10 min. At the next stage, electrophoretic separation of amplification products in 1.5% agarose gel was performed. The finished gel was stained with ethidium bromide, and the DNA fragments were analysed in a transilluminator Gel Doc XR+ (Bio-Rad) with subsequent photo documentation. Genetic distances were calculated using the method of Nei M. and Li W. H. [17] using the Treecon computer program.

3. Results and Discussion
As a result of the research, 28 polymorphic RAPD and ISSR loci were identified are represented in figure 1.

The results of the PCR of RAPD genomic DNA by primers indicated that the maximum set of amplification products was observed when using OPJ-13 (figure 1), where all the received signals were polymorphic. In the case of ISSR, the largest number of polymorphic fragments was obtained with Primer 8. It should be noted that the total number of polymorphic fragments was detected using ISSR primers, while a significant portion of the identified alleles was rare.

Among the studied cereals, the maximum polymorphism of RAPD markers was observed in triticale culture (53%), while the maximum polymorphism of ISSR markers was found in wheat varieties and breeding lines (39%). The minimum polymorphism of RAPD loci and ISSR loci was found in wheat (47%) and oats (18%), respectively (table 2).
Figure 1. Products of RAPD and ISSR amplification of DNA: spring triticale (1-4), wheat (5-8) and oats (9-11).
Table 2. Polymorphism of RAPD and ISSR markers in varieties and breeding lines of cereals.

| Culture   | Varieties and breeding lines | RAPD loci | % | mean | ISSR loci | % | mean |
|-----------|-----------------------------|-----------|----|------|-----------|----|------|
| Triticale | Ukro x Lana                 | 1         | 10 | 53   | 5         | 28 | 32   |
| Triticale | Ukro                       | 7         | 70 | 5    | 5         | 28 |      |
| Triticale | Lana                       | 7         | 70 | 8    | 44        |    |      |
| Triticale | Ukro x Dalgau 1            | 6         | 60 | 5    | 28        |    |      |
| Wheat     | Dalira                     | 4         | 40 | 35   | 5         | 28 | 39   |
| Wheat     | Khabarovchanka x Monakinka  | 2         | 20 | 5    | 28        |    |      |
| Wheat     | Lira-98 x 334-84           | 4         | 40 | 8    | 44        |    |      |
| Wheat     | Lira-98                    | 4         | 40 | 10   | 56        |    |      |
| Oat       | Marshal                    | 4         | 40 | 47   | 2         | 11 | 18   |
| Oat       | Cardinal                   | 3         | 30 |      | 2         | 11 |      |
| Oat       | Bohun                      | 7         | 70 |      | 6         | 33 |      |

The maximum polymorphism of RAPD markers was characterized by the triticale genotypes of Ukro, Lana and Bohun oats (70%), while the highest polymorphism of ISSR markers was observed in the Lira-98 wheat variety (56%). Using RAPD technology, the minimum level of polymorphism (10%) was detected during DNA amplification of the triticale Ukro x Lana breeding line. Cardinal and Bohun oats showed the smallest polymorphism of ISSR loci (11%).

Using the calculated genetic distances, dendrograms of genetic relationships between the studied breeding samples were constructed and are represented in figure 2. Samples with minimal genetic distances are included in one unifying cluster, and varieties with significant genetic distances are located in different clusters. The first cluster combines samples of triticale, the second combines samples of wheat and the third combines samples of oats. The triticale cluster had a greater genetic similarity to wheat, which corresponds to the known data on the phylogenetic origin of triticale (figure 2).

Figure 2. Dendrogram of genetic differences of the studied lines and varieties of cereals (according to the UPGMA method) in the matrix combined for each species (A) and for each individual sample (B) by RAPD markers (1), ISSR markers (2) and the sum of RAPD and ISSR markers (3). The upper scale is the genetic distance (Euclidean). Numbers indicate bootstrap values (%).

For ISSR markers, the genetic distances ranged from 1 (in the Dalira wheat variety and the triticale Ukro x Lana breeding line) to 0.12 (in the Marshall and Cardinal oat varieties). ISSR markers, similar
to RAPD, formed three unifying clusters on the dendrogram, while the triticale culture in this analysis was already clustered with oat samples. When analysing the sum of RAPD and ISSR markers, the genetic distances ranged from 1 (in the Dalira wheat variety and the Ukro x Lana triticale variety) to 0.11 (in the Marshall and Cardinal oat varieties). All genotypes analysed by the sum of RAPD and ISSR markers were clearly divided into three clusters, with triticale in this analysis clustering with oats. The minimum distance between the Marshal and Cardinal varieties is explained by the fact that these genotypes are based on the Dutch oat variety, Perona.

4. Conclusion
Thus, both marker systems used allowed identification of lines and varieties of valuable cereals. Among the studied crops, triticale had the maximum polymorphism of RAPD markers (53%), and wheat had the maximum for ISSR markers (39%). The maximum polymorphism of RAPD markers was characterized by the triticale genotypes of Ukro, Lana and Bohun oats (by 70%), and the highest level of ISSR-marker polymorphism was observed in the Dalira wheat variety (56%). Breeding material located at a significant genetic distance from each other is recommended for crossbreeding.

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