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GENOTYPING *TRITICUM AESTIVUM L.* CULTIVARS OF UKRAINE WITH MICROSATELLITE MARKERS

Common wheat is one of the major crops grown in Ukraine. It is an allohexaploid (2n=6x=42, AABBDD) and contains three subgenomes (ABD) derived from *Triticum urartu* (genome A), *Aegilops speltoides* (genome B) and *Aegilops tauschii* (genome D) [1–3]. Nowadays, increased yield and resistance to stress factors of new wheat cultivars require solution of many important issues, one of which is the selection of productive donors with valuable quality. Also the issue of intellectual property protection is important for breeders who create elite cultivars, seek to get the most out of their cultivars, and be competitive on the world market. Study of grain protein content and analysis of phenotypic traits are usually used for this purpose. However, these methods have some drawbacks (long time, need for grain) and do not provide accurate enough characterization of a cultivar. The study of wheat genetic pool is effective to simplify and intensify the process of plant breeding.

Molecular markers are a valuable tool to study plant genetic material. SSR-markers (microsatellites) are tandem repeats of 1–5 base pairs (bp) in eukaryotic genomes [4]. Their peculiarity is that they are codominant markers and, therefore, a comparative analysis of a DNA locus allows to check similar cultivars for varietal purity and compliance, and establishment of phylogenetic relationships [5–6].

SSR-analysis data are perspective to compose molecular genetic passports of cultivars. Writing such molecular genetic formulas allow scientists to display the structure of a cultivar, its compliance with homogeneity and stability [5–7]. Unfortunately, such a representation of a cultivar genome is not a prerequisite for cultivar registration in Ukraine today.

The objective of the work was to develop a system of DNA markers to evaluate genetic polymorphism of Ukrainian wheat cultivars by analyzing the SSR loci.

Materials and methods

The subject of the study was a set of 15 wheat cultivars of Kyiv and Myroniv origin, which are of great value for Ukrainian agriculture: Pereiaslavka, Podolianka, Yatran 60, Natalka, Kryzhynka, Vesnianka, Bohdana, Slavna, Spasivka, Sotnytsia, Hileia, Shchedrivka Kyivska, Zolotokolosa, Favorytka, Smuhlianka.

Total DNA was isolated with the modified CTAB method [8].

Polymerase chain reaction (PCR) of 20 µl included 0.5 мМ of forward and reverse primers (Metabion), 1×Reaction Buffer BD (Solis BioDyne), 2 mM $MgCl_2$, 0.2 µM of each deoxyribonucleotide-3-phosphate (Thermo Fisher Scientific), 1 unit of FIREPol® DNA Polymerase (Solis BioDyne), 30 ng of total plant DNA.

Primer sequences for loci *Xgwm18, Xgwm111, Xgwm193*, *Xgwm219*, *Xgwm261*, *Xgwm383*, *Xgwm469*, *Xgwm508, Xgwm626*, *and Xgwm642* were used in the study [9].

PCR products were separated by electrophoresis in 2 % agarose gels in lithium borate buffer, 0.1 µg/ml ethidium bromide [10–12].

Gels were visualized in UV-light with a photosystem Canon EOS 600D. Cultivars phylogenetic relationship was calculated by the unweighed pair-group method using arithmetic averages cluster analysis (UPGMA) with the software DARwin 6.0.010 [13].

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Results and discussion

The polymorphism of 10 microsatellite loci (table) was the subject of the study for establishment the genetic diversity of 15 Kyiv and Myroniv wheat cultivars, which are very important for Ukrainian agriculture. The selected SSR markers showed varying degree of polymorphism (fig. 1) among 15 cultivar of wheat.

Table

Characteristics of the SSR loci used in the study

Locus	Chromosome location [20]	Polymorphic amplified fragments	Fragment size, b.p.
Xgwm18	1B	1	190
Xgwm111	7D	2 2	125–135 175–190
Xgwm193	6B	2	134–152
Xgwm219	6B	3	150–184
Xgwm261	2D	3	148–169
Xgwm383	3D	2	187–193
Xgwm469	6D	2	155–160
Xgwm508	6B	3 1	133–139 157
Xgwm626	6B	2	106–125
Xgwm642	1B	2	169–179

Analyzing the locus *Xgwm508* (fig. 1, A), three polymorphic fragments of size 133–139 bp were amplified. Some samples that had the low bands (136–139 bp) also had a polymorphic band (157 bp) the origin of which is not known yet. However, it is clear that inheritance of the appropriate amplified locus had dominant character in contrast to codominant SSR markers.

The locus *Xgwm18* (fig. 1, B) is located on the S arm of 1B chromosome. The polymorphism of the locus was not identified among the cultivars samples; however, the absence of the amplified fragment testified that two cultivars Kryzhynka and Favorytka had wheat-rye translocation.

The results of SSR analysis were used in the research of genetic relationship as well as for construction the phylogenetic tree for 15 wheat cultivars. The denrogram (fig. 2) was assembled by means of the method UPGMA using DARwin software.

According to the dendrogram the genotypes were distributed into two main clusters. The first cluster group includes cultivars Podolianka, Bohdana, Yatran 60, Natalka, Kryzhynka,

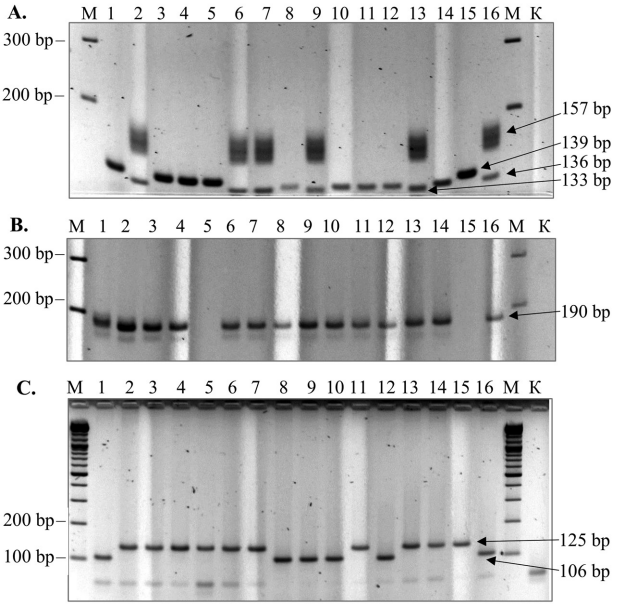

Fig. 1. Agarose gels showing the allelic segregation of the SSR markers: A – *Xgwm508*, B – *Xgwm18*, C – *Xgwm626*. Lane 1–16, samples (Pereiaslavka, Podolianka, Yatran 60, Natalka, Kryzhynka, Vesnianka, Bohdana, Slavna, Spasivka, Sotnytsia, Hileia, Shchedrivka Kyivska, Malynivka, Zolotokolosa, Favorytka, Smuhlianka, respectively); K, negative control without DNA; M, molecular weight marker GeneRuler™ DNA Ladder Mix

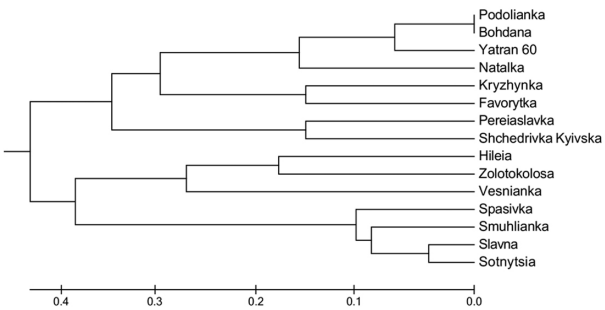

Fig. 2. The dendrogram showing similarity and clustering of 15 wheat genotypes

Favorytka, Pereiaslavka, Shchedrivka Kyivska; the second: Hileia, Zolotokolosa, Vesnianka, Spasivka, Smuhlianka, Slavna, Sotnytsia.

According to [14], all cultivars from the second cluster have 1AL.1RS wheat-rye translocation. Cultivars that do not contain it, or have 1BL.1RS translocation constitute the first cluster. Cultivars Favorytka and Kryzhynka (1BL.1RS) form a separate subcluster group. Cultivars from the second

cluster must have a common origin, considering the small number of donors with 1AL.1RS translocation. This fact confirms the efficiency of SSR markers use for the study of the genetic relationship between cultivars.

The detection of genetic diversity between a pair of Ukrainian cultivars Podolianka and Bohdana failed using ten SSR loci. Some other SSR loci as well as some valuable agricultural trait loci should be evaluated for better rating these cultivars.

Conclusions

The genetic diversity evaluation system for wheat cultivars with the SSR markers was developed. With the results of SSR analysis the phylogenetic relationship for wheat genotypes was determined. It was defined that two cultivar Bohdana and Podolianka had very similar genotypes within analysis of 10 microsatellites loci. To confirm or refute this fact further research with some other SSR markers or valuable agricultural trait loci should be carry out.

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GENOTYPING *TRITICUM AESTIVUM* L. CULTIVARS OF UKRAINE WITH MICROSATELLITE MARKERS

Aim. To develop an evaluation system of genetic polymorphism for wheat cultivars of Ukrainian origin based on analysis of SSR loci. ***Methods.*** PCRs with the following separation of amplification products by electrophoresis in agarose (2 %) were performed to find out genetic polymorphism. Phylogenetic relationship was identified with UPGMA method. ***Results.*** The dendrogram of phylogenetic relationships for 15 wheat cultivars was constructed. ***Conclusions.*** It is turned out that two wheat cultivar Podolianka and Bohdana had very similar genotype within the analysis. It is necessary to carry out the further research to establish similarity or diversity between them.

Keywords: Triticum aestivum L., SSR markers, dendrogram, phylogenetic relationship.