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MICROSATELLITE LOCI POLYMORPHISM IN MIGUSCHOVA WHEAT AND COMMON WHEAT CULTIVARS

Fusarium head blight is one of the most widespread and dangerous wheat diseases worldwide. Resistance to Fusarium is controlled by some main genes from different Triticinae genomes; however, common wheat has few such resistance genes. Miguschova wheat (Triticum miguschovae Zhir.) with A^bA^bGGDD genome is characterized by the genotype resistant to Fusarium head blight. In order to effectively use it as a source of introgressions to common wheat genome, molecular genetic polymorphisms should be identified, which could later be used for identification of Miguschova wheat introgressions in the common wheat genome. Microsatellite PCR analysis using primers to SSR loci with a known chromosome localization for common wheat identified 14 highly informative loci with specific to Miguschova wheat amplicons, localized on chromosomes of 6 homoeological groups. Seven other SSR loci were identified to have a limited informative value, as DNA of Miguschova wheat did not form any specific PCR product with corresponding primers (null allele). The informative value of those loci was limited to differentiation of wheat cultivars.

Keywords: Miguschova wheat, common wheat, SSR loci, polymorphism, introgression.

Fusarium head blight is one of the most dangerous and widespread diseases of cereals and wheat in particular. Characteristic feature of this disease is that fungi from the Fusarium genus not only harm plants, but also a number of mycotoxins accumulate in grain. Therefore, on contrast to other phytopathogenic lesions, the harvest becomes unusable. No fully resistant to Fusarium head blight wheat cultivars have been developed up to know [1-3]. Resistance to the disease is controlled by a number of main genes with different chromosome localization; and wheat wild relatives are important sources of new resistance genes to Fusarium head blight. Miguschova wheat (*Triticum miguschovae* Zhir., A^bA^bGGDD genome) is resistant to Fusarium head blight according to G. Fedak [4]. The use of this species for development of introgressive lines with common wheat as a recipient genome (*T. aestivum* L, AABBDD genome) is promising for enrichment of common wheat with genes for resistance to Fusarium head blight. The process of introgressive lines development includes as a required element screening of hybrid progeny with the use of molecular genetic markers for identification of alien chromatin in their genomes. Use of microsatellite (SSR) loci for identification of introgressions in wheat is widespread and productive direction for screening introgressive derivatives of distant crosses [5-8]. Search for polymorphism of chromosome specific SSR loci in components of initial cross (common wheat x Miguschova wheat) is a key stage in preparation for screening introgressive progeny, as on the presence of such polymorphism depends possibility of the following selection of those hybrids that contain introgressions in their genomes. The article offers the results of a comparative microsatellite analysis of genomes of Miguschova wheat and common wheat cultivars, and these results could be the basis for selection of cross combinations perspective for the future work with their derivatives.

Materials and methods

Wheat genotypes analyzed in the study: 1. Synthetic hexaploid species *Triticum miguschovae* Zhir. (A^bA^bGGDD), 2. Winter common wheat cultivars (*T. aestivum,* AABBDD): Odeska 267, Panna, Vdala, Leleka from selection of Selection Genetic Institute – National Centre of Seed Science and Cultivar Studies NASU.

DNA was extracted from leaves using buffer containing CTAB. DNA was amplified with primers to microsatellite loci in PCR with conditions according to the originators of primers. DNA was extracted from individual plants. Chromosome and chromosome arm specificity of studied SSR loci are shown in the tables 1-7. Amplification products were electrophoretically separated in 8 % PAAG with 6M carbamide.

Results and discussion

Search for polymorphism between Miguschova wheat and common wheat cultivars were conducted through comparison of electrophoretic spectra of amplification products obtained using primers to microsatellite loci with chromosome specificity for common wheat. Polymorphism was identified as different electrophoretic mobility of spectra components (+/+), or as presence/absence of particular component in the compared spectra (–/+).

For microsatellite loci *Xpsp2999*-1A, *Xgpw2069*-1A, *Xgwm550*-1B, *Xcfa2158b*-1B, *Xgpw1143, Xcfd92, Xgwm337, Xgwm106* no polymorphism was identified for mobility of spectra components (table 1). Differences in spectra were identified only for three SSR loci (fig. 1), and in all cases polymorphism was identified as different weight (mobility) of amplification products obtained with DNA of Miguschova and common wheat.

Out of the six studied SSR loci specific to the chromosomes of homoeological group 2 (table 2), three loci were polymorphic (fig. 2): *Xbarc124*-2A and *Xgwm122*-2A having alleles of different length, and locus *Xgwm311*, for which amplification products were obtained only with DNA of Miguschova wheat, and no amplification products were obtained for studied common wheat cultivars. Locus *Xgwm261*-2D was monomorphic between studied genotypes, and primers to loci *Xgwm539*-2D and *Xgwm304*-2A produced no amplification products.

Only three microsatellite loci were studied for chromosomes of homoeological group 3, and for all these loci polymorphisms of "+/-" type were

Table 1. **Characteristics of the primers to microsatellite loci on chromosomes of the homoeological group 1**

Locus and its chromosome localization	Presence of amplification product		Polymorphism presence and type
	Miguschova wheat	Common wheat	
Xpsp2999-1A	+	+	–
Xgpw2069-1A	+	+	–
Xgwm550-1B	+	+	–
Xcfa2158b-1B	+	+	–
Xgpw1143-1B	+	+	–
Xcfd92-1B	+	+	–
Xbarc188-1B	+	+	+/+
Xgwm337-1B	+	+	–
Xgpw2118a-1D	+	+	+/+
Xbarc149-1D	+	+	+/+
Xgwm106-1D	+	+	–

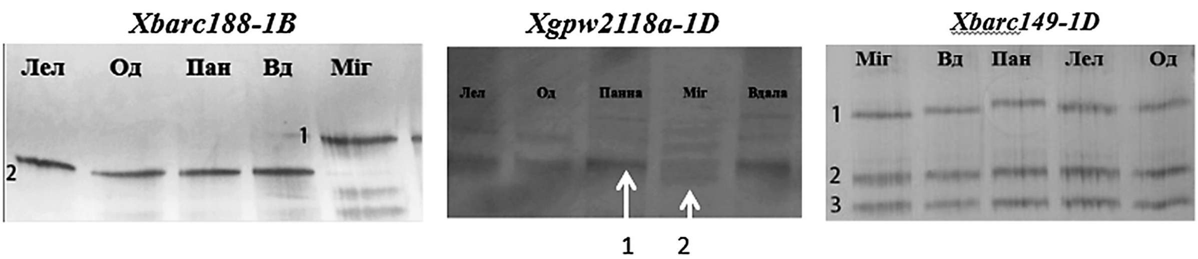

Fig. 1. Polymorphic amplicons (1, 2 and 3) obtained with primers to the indicated SSR loci specific to chromosomes of the homoeological group 1. Міг – Miguschova wheat, Вд – Vdala, Пан – Panna, Лел – Leleka, Од – Odeska 267

Table 2. **Characteristics of the primers to microsatellite loci on chromosomes of the homoeological group 2**

Locus and its chromosome localization	Presence of amplification product		Polymorphism presence and type
	Miguschova wheat	Common wheat	
Xgwm539-2D	–	–	–
Xgwm122-2A	+	+	+/+
Xgwm311-2A,2B,2D	+	–	+/–
Xgwm261-2D	+	+	–
Xbarc124-2A	+	+	+/+
Xgwm304-2A	–	–	–

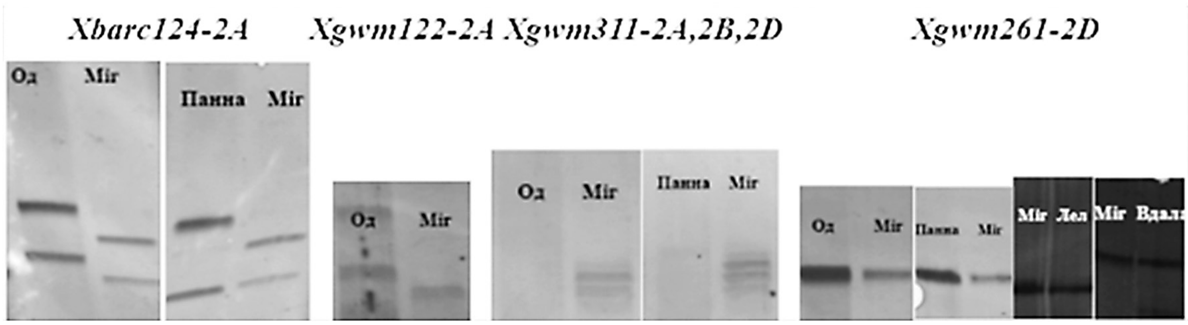

Fig. 2. Polymorphic amplicons obtained with primers to the SSR loci *Xbarc124*-2A and *Xgwm122*-2A, informative polymorphism –/+was identified for locus *Xgwm311*, monomorphic spectra obtained with primers to locus *Xgwm261*-2D, amplification of DNA of Miguschova wheat and common wheat cultivars. Міг – Miguschova wheat, Вдала – Vdala, Панна – Panna, Лел – Leleka, Од – Odeska 267

Table 3. **Characteristics of the primers to microsatellite loci on chromosomes of the homoeological group 3**

Locus and its chromosome localization	Presence of amplification product		Polymorphism presence and type
	Miguschova wheat	common wheat	
Xcfd55-3D	+	–	–/+
Xcfd152-3D	+	–	–/+
Xcfd141-3D	+	–	–/+

identified: presence of amplicons in spectra of common wheat cultivars and absence of amplification products with DNA of Miguschova wheat (table 3). Informative value of such polymorphism is unreliable and limited for analysis of introgressive plant material.

Three SSR loci specific to chromosome of homoeological group 4 of wheat were monomorphic for studied genotypes, and two loci demonstrated polymorphism (fig. 3, table 4). With primers to locus *Xwmc285* DNA of Miguschova wheat produced three amplicons whereas common wheat cultivars produced two amplicons; informative value of this locus is high. With primers to locus *Xgwm194* DNA of common wheat produced four amplicons, and DNA of Miguschova wheat produced no amplification products; informative value of this locus is limited.

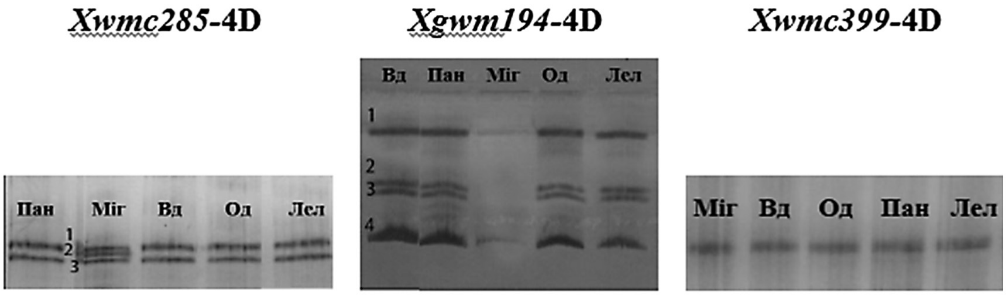

Fig. 3. Polymorphic (loci *Xwmc285*-4D and *Xgwm194*-4D) and non-polymorphic (locus *Xwmc399*-4D) amplicons for chromosomes of homoeological group 4. Міг – Miguschova wheat, Вд – Vdala, Пан – Panna, Лел – Leleka, Од – Odeska 267

Table 4. **Characteristics of the primers to microsatellite loci on chromosomes of the homoeological group 4**

Locus and its chromosome localization	Presence of amplification product		Polymorphism presence and type
	Miguschova wheat	common wheat	
Xcfd84-4D	+	+	–
Xcfd106-4D	+	+	–
Xwmc399-4D	+	+	–
Xwmc285-4D	+	+	+/+
Xgwm194-4D	–	+	–/+
Xwmc89-4B	+	+	–

For eight out of the 13 studied microsatellite loci specific to chromosomes of homoeological group 5 no amplification products were obtained with DNA of both wheat species (table 5). Alleles of SSR loci *XCfd156*-5B and *Xbarc18*-5D were identical for Miguschova wheat and common wheat. Only loci *Xcfd86-5D*, *Xgwm179-5A* and *Xbarc230-5A* were identified as having informative value, as amplification products produced with primers to these loci had different mobility in electrophoretic spectra (fig. 4).

Among microsatellite loci specific to chromosomes of homoeological group 6 only loci *Xbarc196*-6D and *Xcfd287*-6D were identified to be informative, as their amplification products with DNA of Miguschova wheat and common wheat cultivars had different mobility on electrophoretic spectra (table 6, fig. 5). SSR loci *Xcfd132-6D* and *Xcfd76*-6D had limited informative value, as DNA of Miguschova wheat did not produce any amplification products with primers to these loci. Alleles of *Xbarc96*-6D locus were monomorphic.

Comparing the components of spectra obtained after electrophoresis of samples amplified with primers to SSR loci *Xbarc53, Xwmc506, Xbarc111*, *Xgwm44*, *Xcfd69* and *Xbarc154,* it was demonstrated that all studied genotypes produced identical components, therefore, no polymorphism was identified. Polymorphism was identified for SSR loci *Xcfd66* and *Xbarc172* (table 7, fig. 6). For locus *Xbarc172* Miguschova wheat DNA formed amplicons with different electrophoretic mobility

Table 5. **Characteristics of the primers to microsatellite loci on chromosomes of the homoeological group 5**

Locus and its chromosome localization	Presence of amplification product		Polymorphism presence and type
	Miguschova wheat	common wheat	
Cfd156-5B	+	+	–
Cfd8-5D	–	–	–
Cfd86-5D	+	+	+/+
Barc18-5D	+	+	–
Gwm179-5A	+	+	+/+
Gwm234-5B	–	–	–
Cfd189-5D	–	–	–
Barc143-5D	–	–	–
Barc205-5D	–	–	–
Barc216-5B	–	–	–
Barc230-5A	+	+	+/+
Barc316-5A	–	–	–
Wmc537-5B	–	–	–

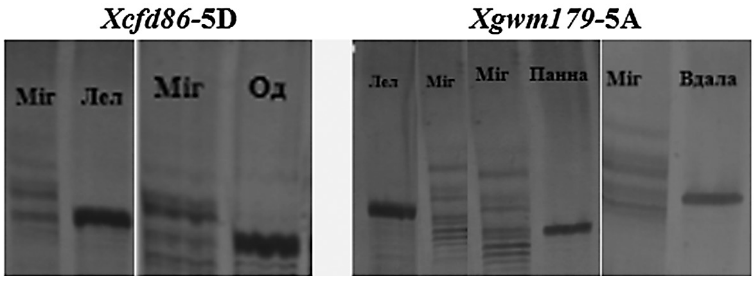

Fig. 4. Polymorphism for SSR loci *Xcfd86*-5D and *Xgwm179*-5A between Miguschova wheat and common wheat cultivars. Міг – Miguschova wheat, Вдала – Vdala, Панна – Panna, Лел – Leleka, Од – Odeska 267

Table 6. **Characteristics of the primers to microsatellite loci on chromosomes of the homoeological group 6**

Locus and its chromosome localization	Presence of amplification product		Polymorphism presence and type
	Miguschova wheat	common wheat	
Xcfd132-6D	–	+	–/+
Xcfd76-6D	–	+	–/+
Xbarc96-6D	+	+	–
Xbarc196-6D	+	+	+/+
Xcfd287-6D	+	+	+/+

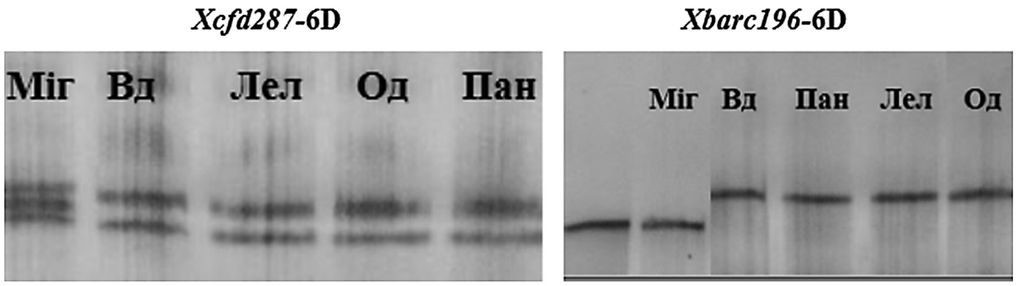

Fig. 5. Polymorphism for SSR loci *Xcfd287*-6D and *Xbarc196*-6D between Miguschova wheat and common wheat cultivars. Міг – Miguschova wheat, Вд – Vdala, Пан – Panna, Лел – Leleka, Од – Odeska 267

compared to amplicons obtained with DNA of common wheat cultivar Vdala. Spectra of Miguschova wheat obtained with primers to locus *Xcfd66* had two additional components that were absent in spectra of all the studied common wheat cultivars (fig. 6). Three other components were present in all the studied samples.

informative value: 3, 3, 0, 1, 3, 2, 2, according to chromosomes of homoeological groups from 1 to 7. Seven other SRR loci were identified to have limited informative value, because DNA of Miguschova wheat did not produce specific amplification products with primers to these loci (null allele). Thereby, according to microsatellite analysis not all

Table 7. **Characteristics of the primers to microsatellite loci on chromosomes of the homoeological group 7**

Locus and its chromosome localization	**Presence of amplification product**		**Polymorphism presence and type**
	Miguschova wheat	**common wheat**	
Xbarc172-7D	+	+	+/+
Xcfd66-7D	+	+	+/+
Xbarc53-7D	+	+	–
Xwmc506-7D	+	+	–
Xbarc111-7D	+	+	–
Cgwm44-7D	+	+	–
Xbarc154-7D	+	+	–
*Xcfd*69-7D	+	+	–

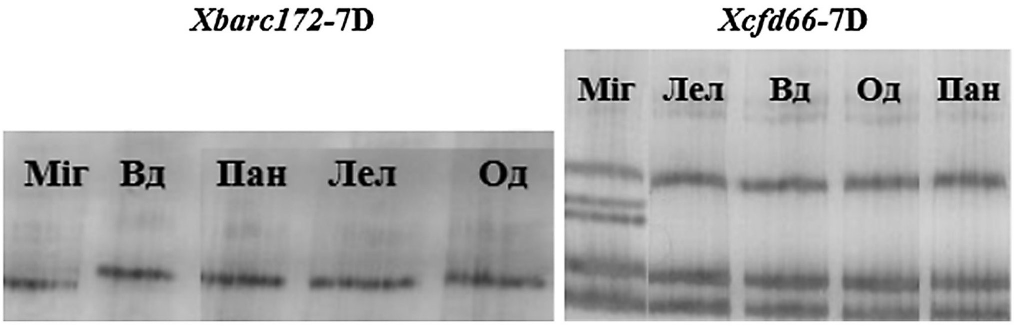

Fig. 6. Polymorphism for loci *Xbarc172*-7D and *Xcfd66*-7D between Miguschova wheat and common wheat cultivars. Міг – Miguschova wheat, Вд – Vdala, Пан – Panna, Лел – Leleka, Од – Odeska 267

Conclusions

Polymorphism between Miguschova wheat and common wheat was studied using molecular genetic markers specific to chromosomes of different homoeological groups of wheat. Among 52 studied microsatellite loci with known localization on chromosomes of seven wheat homoeological groups [9-12], 14 loci were identified to have high chromosomes of Miguschova wheat could be identified as substituted in the introgressive derivatives from its cross with common wheat cultivars. However, if in addition to microsatellite markers, we compare results of protein polymorphism study between Miguschova wheat and common wheat cultivars, chromosomes of all homoeological groups of wheat with genome A^bA^bGGDD could be identified [13].

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ПОЛІМОРФІЗМ ЗА МІКРОСАТЕЛІТНИМИ ЛОКУСАМИ У ПШЕНИЦІ МІГУШОВОЇ ТА СОРТІВ ПШЕНИЦІ М'ЯКОЇ

Фузаріоз колоса є одним із поширених по всьому світу та найнебезпечніших захворювань пшениці. За стійкість рослин до збудника відповідають кілька головних генів із різних геномів Triticinae, однак пшениця м'яка таких генів практично не має. Пшеницю Мігушової (*Triticum miguschovae* Zhir.) з геномом A^bA^bGGDD визнано генотипом, стійким до фузаріозу колоса. Для її ефективного залучення як джерела інтрогресій до геному пшениці м'якої потрібно ідентифікувати молекулярно-генетичні маркери, за якими можна детектувати наявність чужинного хроматину в інтрогресивних лініях, що будуть створені на основі геному пшениці м'якої за участю пшениці Мігушової. Як джерело поліморфізмів для створення молекулярно-генетичних маркерів використано мікросателітні локуси (SSR), хромосомна локалізація яких для пшениці м'якої є відомою. Метод ідентифікації поліморфізмів – ПЛР з праймерами SSR-локусів, хромосомна локалізація яких для пшениці м'якої встановлена. З 52 перевірених мікросателітних локусів, локалізованих на хромосомах A, B та D семи гомеологічних груп, продукти ампліфікації ДНК виявились однаковими для досліджених видів пшениці для 31 локуса. Високоінформативними були 14 локусів: 3, 3, 0, 1, 3, 2, 2, відповідно, на хромосомах гомеологічних груп від 1-ї до 7-ї. За цими локусами ДНК пшениці Мігушової утворює специфічний продукт на спектрі. Ще сім локусів визнано такими з обмеженою інформативністю, тому що ДНК пшениці Мігушової не формує з відповідними праймерами специфічного продукту (нуль-алель). За даними мікросателітного аналізу, не всі хромосоми пшениці Мігушової можуть бути ідентифіковані як заміщені у складі інтрогресивних похідних від її схрещування з сортами пшениці м'якої. Однак якщо до мікросателітних маркерів додати наявні результати вивчення поліморфізму між пшеницею Мігушової та сортами пшениці м'якої за генами запасних білків та ферментів, ідентифікуються хромосоми всіх гомеологічних груп пшениці з геномом A^bA^bGGDD.

Ключові слова: пшениця Мігушової, пшениця м'яка, SSR-локуси, поліморфізм, інтрогресія.

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