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CHARACTERISATION OF UKRAINIAN BREAD WHEAT (*TRITICUM AESTIVUM* L.) GERMPLASM BY USING MICROSATELLITE MARKERS

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Winter wheat is the most important grain crop in the Ukraine, occupying an average 19% of all arable land during the past 10-15 years. It contributes between 50% to 60% of the total quantity of annually harvested grains [5]. The high quality standards of the Ukrainian wheat have been recognized all over the world. The Ukrainian wheat germplasm has been used as important source for quality improvement of wheat varieties in many countries, including USA, Canada, France, Germany, Bulgaria and Yugoslavia. Characterization of the Ukrainian wheat germplasm using microsatellite markers permits precise germplasm identification and estimation of genetic diversity.

The International Union for the Protection of New Varieties (UPOV) is interested to involve molecular markers as additional useful tools for performing Distinctness, Uniformity and Stability (DUS) tests. Microsatellite analysis would facilitate the UPOV requirement for comparison of new registration candidates to all already released

varieties. New varieties, distinct on the basis of microsatellites could go directly to analysis of uniformity and VCU (Value for Cultivation and Use) trials [3]. Microsatellite analysis has the potential to reduce, rationalise and complement traditional morphological and physiological tests.

Microsatellites represent a highly reproducible marker system. Comparative studies in crop plants including wheat have shown that they are one of the most variable and useful marker system for hexaploid wheat. They have a codominant nature and provide a powerful methodology for discriminating genotypes of common wheat [2, 6, 7, 11, 12, 13].

The aim of this work is the characterization of germplasm of Ukrainian wheat varieties and the construction of an identification matrix for 93 Ukrainian wheat varieties and breeding lines by using wheat microsatellite markers.

Materials and methods

The following 93 wheat varieties and lines bred from the beginning of scientifically based wheat

breeding programs to nowadays were chosen: **Albatros odesskiy***, Banatka (Kherson, HTRI 142), Banatka (Kherson, HTRI 144), Belotserkovskaya 198, Belotserkovskaya semi-dwarf, Bezostaya 1, **Brigantina**, Dnepryanka, Donchanka 3, Doneckaya 46, Donskaya semi-dwarf, **ErythrospERMUM 1072**, ErythrospERMUM 127, **ErythrospERMUM 272-87**, **ErythrospERMUM 949-38**, **Fantasiya**, **Fedorovka**, Ivanovskaya ostostaya, Kharkovskaya 50, Kharkovskaya 93, Khersonskaya 86, Kievskaya 7, Kievskaya 8, Kievskaya ostistaya, Kiyanka, Kolomak 3, Kolomak 5, **Kooperatorka**, **Krassunnya odesskaya**, Krymka, Krymka mestnaya, **Lada odesskaya**, **Leleka**, **Lelya**, **Luzanovka**, **Lyubava**, Lyutescens 7, Mirich, Mirleben, Mironovskaya 27, Mironovskaya 28, Mironovskaya 33, Mironovskaya 65, Mironovskaya 808, Mironovskaya yubileynaya, Mirxard, **Nikoniya**, **Obriy**, **Odesskaya 3**, **Odesskaya 12**, **Odesskaya 16**, **Odesskaya 51**, **Odesskaya 66**, **Odesskaya 117**, **Odesskaya 132**, **Odesskaya 133**, **Odesskaya 265**, **Odesskaya 267**, **Odesskaya bezostaya**, **Odesskaya krasnokolosaya**, **Odesskaya semi-dwarf**, **Odom**, **Olesya**, **Panna**, **Polesskaya 90**, **Porada**, **Povaga**, **Priboy**, **Prima**, **Selyanka**, **Simvol odesskiy**, **Sirena**, Skifyanka, **Stepova**, **Strumok**, **Tira**, Tsyghanka 86, **Ukrainka**, **Ukrainka odesskaya**, **Ukrainka poltavskaya**, **Veselka**, **Viktoriya odesskaya**, **Vympel odesskiy**, **Yatran 60**, **Yubileynaya 75**, **Yuna**, **Yunnat odesskiy**,

Zastava, **Zbruch**, **Znahidka odesskaya**, **Zolotava**, **Zustrich**.

Seeds for the wheat varieties and lines were obtained from the Institute of Plant Breeding and Genetics (PBGi; Odessa, Ukraine), The State Commission for Testing and Protection of Plant Varieties of the Ukraine and Genebank of the Institute for Plant Genetics and Crop Plant Research (Gatersleben, Germany).

For each variety, DNA was isolated from 20 individual seeds as described by Plaschke et al. [7]. Pools of DNA from 5 seeds were made. For microsatellite analysis 14 microsatellite markers already used for analysing European, African and other wheat varieties (Röder, personal communication) were chosen (Table).

The primers for microsatellite loci are described by Röder et al. [9]. The primers for the gliadin-specific marker TagIgap was developed by Devos et al. [1]. The investigations were performed at the Institute for Plant Genetics and Crop Plant Research (IPK) in Gatersleben using ALF and ALF-express sequencer as described by Röder et al. [9]. Three reference varieties (Chinese Spring, Aztec, Soisson) having representative alleles were included in each ALF run. For data analysis Microsoft Excel-Software was used, the polymorphism information content (PIC) was calculated as described by Roder et al. [10].

Number of alleles and polymorphic information content detected for microsatellite loci

Locus	Chromosomal location	Fragment size in 'CS' (in bp)	Number of alleles PBGI excerption	Number of alleles general excerption	PIC PBGI excerption	PIC general excerption
<i>Xgwm 3</i>	3D	84	3	4	0.48	0.53
<i>Xgwm 18</i>	1B	186	4	8	0.62	0.68
<i>Xgwm 155</i>	3A	140-150*	5	6	0.71	0.71
<i>Xgwm 165</i>	4A	191	4	4	0.48	0.41
<i>Xgwm 190</i>	5D	<201	4	6	0.49	0.58
<i>Xgwm 261</i>	2D	192	4	6	0.45	0.54
<i>Xgwm 325</i>	6D	138	6	9	0.71	0.70
<i>Xgwm 357</i>	1A	123	4	5	0.64	0.65
<i>Xgwm 408</i>	5B	176	4	8	0.21	0.51
<i>Xgwm 437</i>	7D	109	9	15	0.75	0.82
<i>Xgwm 577</i>	7B	133	6	16	0.71	0.82
<i>Xgwm 631</i>	7A	197	4	6	0.53	0.65
<i>Xgwm 680</i>	6B	123	3	6	0.46	0.47
<i>TagIgap</i>	1B	255-337*	8	15	0.49	0.58

* Size of fragments was tested on wheat varieties

Results and discussion

Sixty-eight alleles were detected for varieties of PBGI and 114 alleles for all tested varieties. For the average number of alleles per locus was 4.8 and 8.1 for the gene pool of PBGI and for all varieties

analysed, respectively. The average polymorphic information content (PIC) was 0.55 and 0.61 for wheat varieties of PBGI and all tested varieties, respectively. The lowest PIC values of 0.21 and 0.41 were detected for locus *Xgwm408* between PBGI varieties and for

* Wheat varieties bred in PBGI printed with fat type.

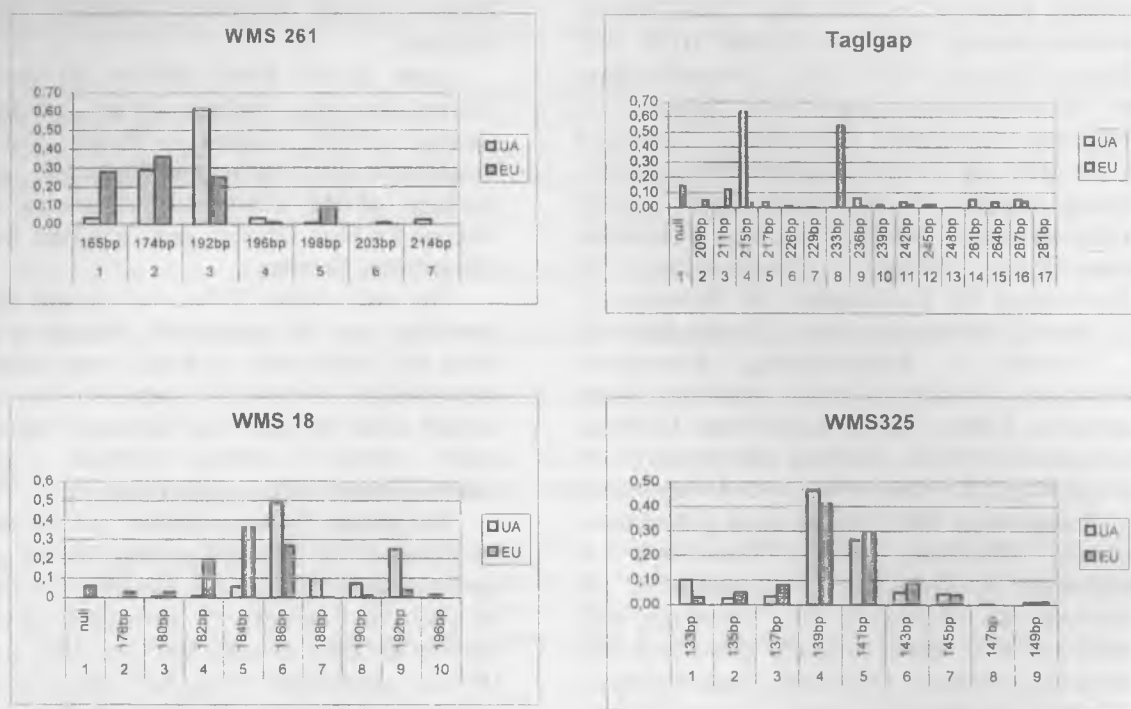


Diagram of distribution of allele frequencies for the loci *Xgwm261*, *Taglgap*, *Xgwm18* and *Xgwm325* in the Ukrainian (UA) and European (EU) wheat varieties investigated

locus *Xgwm165* in all tested varieties, respectively. Frequency of 39 revealed alleles were less than 0.02. For loci: *Xgwm3*, *Xgwm165*, *Xgwm190*, *Xgwm261*, *Xgwm408*, *Xgwm680*, *Taglgap* the prevalence of some alleles was shown. Their frequency varied from 0.61 – 0.74.

The allele frequencies of the Ukrainian varieties were compared with those detected by Roder et al. [11] for European wheats. Examples are given in Figure. In varieties from the Southern region of the Ukraine the prevalence of a 192 bp allele at the locus *Xgwm261* was detected. For wheat varieties from different geographical regions four main alleles for this locus were observed with different predominance [11, 15]. *Xgwm261* is tightly linked to the dwarfing gene *Rht8* on chromosome 2DS, a chromosomal region also carrying the gene *Ppd1* for photoperiodic insensitivity [4].

For the marker *Taglgap*, which amplifies a microsatellite in the coding region of a gamma-gliadin pseudogene on 1B chromosome [1], a major allele of 215 bp with a frequency of 0.63 was revealed in varieties from the Ukraine. At the same time a high frequency of 235 bp allele was detected for wheat varieties from different geographical regions in Europe [11]. The frequencies of the other thirteen alleles revealed at *Taglgap* locus were on average 0.02 in the investigated Ukrainian gene pool.

For microsatellite locus *Xgwm18*, located on 1B chromosome [9] we detected also significant differences in distribution of allele frequencies in Ukrainian and European gene pools.

Generally it is known that chromosome 1B is very important in wheat breeding. Centric translocations of the short arm of rye (*Secale cereale* L.) chromosome 1R were useful in wheat breeding programs and in 70-90th were developed more than 300 wheat varieties with 1BL/1RS translocations [8]. 1RS carries genes for resistance to several pests and diseases. Their major disadvantage is reduced bread making quality [14]. In Ukraine traditionally there is a big attention to bread making quality in the breeding programs, so the presence of the 1BL/1RS translocation is not frequent in Ukrainian wheat varieties. By using protein markers it was shown that in Ukrainian gene pool the allele Gli1B1 is widely distributed, whereas alleles Gli1B4 and Gli1B3 (from rye) are more common for European varieties (Poperela, personal communication).

The distribution of allele frequencies for the other microsatellite loci in the gene pool of Ukrainian wheat varieties and European wheat varieties was similar, for example in locus *Xgwm325* (Fig.).

The specific allele distribution of microsatellite loci enables to reflect the influence of selection on the genetic composition of wheat varieties, which genotypes were adapted for different geographic regions. On the base of the allele frequencies of microsatellite loci it is possible to predict the genomic regions that have been subject to selection processes.

Comparative analysis of allele distribution in the gene pools from different geographical zones could be useful for finding associations between the adaptive traits and microsatellite markers.

Although common wheat is a self-pollinating crop, we reveal a high level of intravarietal heterogeneity. Intravarietal heterogeneity is defined if more than one allele per microsatellite locus is detected in a single variety. In 85.7% of the varieties, bred at PBGI, intravarietal polymorphism of microsatellite loci were detected. The highest level of heterogeneity was revealed for varieties/landraces: Krymka mestnaya and Kooperatorka. For Odesskaya 51 and Odesskaya 16 at 10 and 9 microsatellite loci, respectively, out of 14 tested, several alleles were

detected. More than 50% of the tested loci were polymorphic for wheat varieties Odesskaya 3 and Fantasiya.

The identification matrix for 93 wheat varieties was developed on the base of 14 microsatellite loci. It could provide a rapid and effective approach for the identification of varieties and might be used for quality control in certified seed production programs and to control the maintenance of germplasm collections.

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STUDIES ON CHROMOSOMAL CONTROL OF SOME MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERS OF *TRITICUM MIGUSCHOVAE* Zhirov

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The introgressive hybridization is one of the most effective approaches of common wheat improvement. *Triticum miguschovae* Zhirov

(A^bA^bGGDD) is the member of *Timopheevii* wheat group and the artificially developed hexaploid species [8]. This species and *T. miguschovae* / *T.*