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EXPERIMENTAL ARTICLES

Genetic Differentiation of Hexaploid Wheat Inferred from Analysis of Microsatellite Loci

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Abstract—Landraces of wheat can serve as important potential sources for extending the genetic basis of selection cultivars. Analysis of microsatellites and typing of polymorphism in a representative sample of 347 genotypes, including landraces and selection cultivars, was performed using a set of 38 selected oligonucleotide primer pairs. Each genotype had a unique allele combination at 39 microsatellite loci examined. Classification of genotypes with respect to the level of their similarity was performed using cluster analysis. The data obtained pointed to genetic differentiation of hexaploid wheat. The groups of cultivars, the formation of which was thought to be associated with the main old areas of wheat cultivation in Europe and Asia, were identified. The basis of each of the groups was formed by landraces of common wheat. The differences between the groups identified were associated with multiple changes in the wheat genome and were expressed as quantitative differences in the allele frequencies of microsatellite loci. The results of the study are of interest in terms of understanding the structure of wheat genetic diversity and revealing the pathways of evolution of this culture.

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INTRODUCTION

The gene pools of wheat landraces play a special role in extending the genetic basis of modern selection cultivars. These gene pools form one of the important sources of allele combinations of monogenic and polygenic systems, controlling plant resistance to adverse environmental conditions. The process of landrace formation has started almost from the beginning of human wheat usage in prehistorical times. Various soil and climatic conditions of wheat cultivation, spontaneous mutagenesis, gene drift (associated with wheat distribution in the course of human dispersal), instinctive artificial selection, as well as natural selection (the pressure of which in the conditions of primitive agriculture was substantial), provided extreme heterogeneity and polymorphism of wheat. At present, landraces are practically absent from wheat sowings worldwide, and all their variety is mostly maintained in the cultivated plants seed collections.

The collection of Vavilov All-Russia Institute of Plant Industry (VIR), Russian Academy of Agriculture, contains more than 14000 alive landraces of wheat from different countries of the world. A titanic work on evaluation of the selection value of this material and classification of its phenotypic and agro-ecological diversity has been performed [1–6]. However, there is no clear idea on the pattern of genetic differentiation of these varieties. Elaboration of molecular biological techniques allowing rapid and effective analysis of plant DNA polymorphism, along with availability of computer programs for classification of large samples with the help of multidimensional statistics, provide new possibilities to resolve this issue.

Different types of DNA markers were used for evaluation of the genetic diversity of different species of hexaploid wheat, as well as for classification of landraces and selection cultivars of common wheat, originating from different countries [7–11]. In a number of studies, attempts to find association between wheat genetic diversity and its geographic distribution were made [12–14]. Nevertheless, general structure of this diversity, especially of that, associated with the unfavorable cultivation conditions, is far from being understood.

The main objective of this study was to investigate genetic differentiation of hexaploid wheat ($2n = 6x = 42$, genomic formula, AABBDD), based on comparative analysis of microsatellites or simple sequence repeats (SSRs).

To achieve this goal, the following tasks were set:

(1) using VIR collection, to form a sample of landraces and old breeding varieties most completely reflecting ecogeographical diversity of hexaploid wheat;

(2) to form a set of SSR markers (polymorphic microsatellite DNA sequences, or microsatellites, with known genome localization), encompassing all chromosomes of hexaploid wheat;

(3) using SSR markers and the method of cluster analysis, to characterize the structure of genetic diversity in hexaploid wheat and to compare it with other known classifications.

MATERIALS AND METHODS

The experiments were performed using a total of 319 typical accessions (genotypes), selected from landraces and old breeding cultivars derived from different species of hexaploid wheat. We believed that this genotype sample representatively reflected the range of genetic changes in wheat examined during its distribution and adaptation to local climatic conditions. This suggestion was based on the fact that the sample was formed following agro-ecological classification of wheat, elaborated by N.I. Vavilov [2, 3]. This classification is based on the subdivision of the vast territory of wheat cultivation into differing in size agro-ecological areas and regions. Furthermore, each area or region was characterized by substantial uniformity of soil and climatic conditions along with certain agro-ecological type of cultivars, defined as agro-ecological group. Agro-ecological groups are united into subspecies and species. The sizes of agro-ecological groups are different, which is determined by the history of wheat development and distribution. Substantial part of wheat cultivars, analyzed by N.I. Vavilov and served as the basis of his agro-ecological classification, has been preserved in the collection of VIR. Because of this, we had an advantage to examine the cultivars, mentioned by N.I. Vavilov in his classification. The sample examined also included other landraces and old breeding varieties, included into the collection mostly during the period from 1908 to 1940. The origin of these cultivars geographically coincided with agro-ecological zones and regions, described by N.I. Vavilov for agro-ecological groups. Taken together, the cultivars chosen for the analysis belonged to 45 agro-ecological groups of six wheat species (*Triticum macha* Dek. et Men., *T. spelta* L., *T. Vavilovianum* Jakubz. = *T. vavilovii* (Thum.) Jakubz., *T. compactum* Host, *T. sphaerococcum* Perc., and *T. vulgare* Host = *T. aestivum* L.). Among these, common wheat *T. aestivum* was represented by five subspecies (ssp. *irano-turkestanicum* Vav., ssp. *indicum* Vav., ssp. *sinicum* Vav., ssp. *eurasiaticum* Vav., and dwarf wheat, *T. compactum* was represented by three subspecies (ssp. *armeno-turkestanicum* Vav., ssp. *eurasiaticum* Vav., and ssp. *sinicum* Vav.). Each of agro-ecological groups contained from two to twenty-five cultivars. The genotypes representing Asian subspecies of spelt, *T. spelta* L. ssp. *kuckckianum* Gokg., as well as *T. petropavlovskyi* Udacz. et Migusch., were also included into the study. The latter forms of wheat were discovered rather recently [6]. In addition, to reveal the association between modern selection cultivars and landraces and old breeding varieties, 28 genotypes of selection cultivars from Russia and CIS countries were examined. Finally, the 347 genotypes tested represented the cultivars from 44 countries (see Table 2).

Analysis of microsatellites. DNA was isolated from individual 2- to 3-week-old seedlings of each wheat cultivar. DNA was extracted using the micro procedure with addition of hexadecyltrimethylammonium bromide [15]. Leaf fragments (0.2 to 0.3 g) were placed into deep 96-well plates, frozen in liquid nitrogen, and homogenized with the use of zirconia beads and a plate shaker. The samples were mixed with 0.6 ml of extraction buffer, containing 100 mM Tris–HCl, pH 8.0; 50 mM EDTA; 500 mM NaCl; and 20 mM sodium metabisulfite. The mixture was incubated for 45 min at 95°C, and then centrifuged at 4000 rpm for 10 min at +4°C. Supernatant (200 μl) was transferred to the new plates and mixed with 200 μl of isopropanol and 12 μl of 7.5 M ammonium acetate. The mixture was incubated at –20°C overnight and centrifuged as described above. DNA pellets were washed with 400 μl of 70% ethanol, centrifuged, dried, and dissolved in 50 μl of deionized water.

PCR was performed in the MJ Research PTC 225 thermal cycler according to the following scheme. First, DNA was denaturated for 3 min at 94°C, and then 35 cycles of amplification were performed (30 s at 94°C; 30 s at 45°C or 60°C, depending on primer; and 30 s at 72°C). Final elongation was performed for 5 min at 72°C [16]. For further analysis, the mixtures containing amplification products generated with the use of either two or three pairs of primers, labeled with different fluorescent dyes, were prepared. These mixtures were examined using the ABI Prism 3100 automated sequencer. The sizes of DNA fragments were determined using the GenScan v. 3.7 NT software package.

Statistical treatment of the results. Microsatellites of different sizes and belonging to one DNA fragment with known genomic localization (SSR locus) were scored as alleles of this locus. To construct binary matrix of initial data, they were coded as 1 or 0, which indicated the presence or absence of each allele at certain SSR locus.

The initial data matrix was then used to generate the genetic similarity index matrix, according to Nei and Li [17]. This matrix was used for clustering of cultivars with the help of the method of Ward [18], based on the principle of minimization of variance within clusters. All calculations and graphic constructions were performed with the help of the MVSP 3.1 and STATISTICA 6.0 software packages. The PIC (polymorphism information content) index values under the conditions of the genotypes homozygosity were calculated according to [19].

RESULTS

Characteristics of SSR Markers

In this study, microsatellite loci were examined using 37 primer pairs described by M. Réder et al. [16]. Each of these pairs enabled analysis of one locus (the

Table 1. Characteristic of microsatellite loci studied

No.	Locus*	Localization*	Total number of alleles	Number of rare (unique) alleles	Polymorphism index (PIC)
1	*Xgwm99*	1A	18	13(8)	0.801
2	*Xgwm135*	1A	25	20(4)	0.784
3	*Xgwm312*	2A	34	31(7)	0.889
4	*Xgwm372*	2A	33	28(3)	0.949
5	*Xgwm2*	3A	11	7(2)	0.786
6	*Xgwm480*	3A	14	10(0)	0.665
7	*Xcfd17h8a*	4A	12	8(4)	0.447
8	*Xgwm610*	4A	15	11(5)	0.720
9	*Xgwm186*	5A	23	16(4)	0.870
10	*Xgwm415*	5A	6	4(1)	0.531
11	*Xgwm427*	6A	22	16(3)	0.883
12	*Xgwm260*	7A	20	11(0)	0.915
13	*Xgwm11*	1B	18	13(4)	0.844
14	*Xgwm413*	1B	16	10(1)	0.853
15	*Xgwm120*	2B	17	11(3)	0.874
16	*Xgwm257*	2B	7	3(2)	0.727
17	*Xgwm285*	3B	33	28(10)	0.890
18	*Xgwm566*	3B	12	7(2)	0.807
19	*Xgwm149*	4B	12	8(3)	0.702
20	*Xgwm251*	4B	21	17(4)	0.836
21	*Xgwm234*	5B	19	10(3)	0.907
22	*Xgwm408*	5B	21	15(5)	0.880
23	*Xgwm219*	6B	21	15(2)	0.898
24	*Xgwm626*	6B	10	8(1)	0.583
25	*Xgwm46*	7B	24	18(2)	0.896
26	*Xgwm400*	7B	17	11(2)	0.868
27	*Xgwm337*	1D	21	14(3)	0.893
28	*Xgwm642*	1D	10	6(1)	0.644
29	*Xgwm261*	2D	21	18(4)	0.750
30	*Xgwm539*	2D	40	33(8)	0.944
31	*Xgwm341*	3D	25	17(8)	0.920
32	*Xgwm664*	3D	4	2(0)	0.294
33	*Xcfd17h8d*	4D	17	8(2)	0.913
34	*Xgwm190*	5D	17	11(4)	0.806
35	*Xgwm272*	5D	11	7(3)	0.695
36	*Xgwm325*	6D	13	8(2)	0.814
37	*Xgwm469*	6D	15	9(2)	0.847
38	*Xgwm44*	7D	17	9(2)	0.891
39	*Xgwm437*	7D	20	12(2)	0.921
Total			712	503(126)	0.799

* According to [16, 20].

Table 2. Distribution of 347 genotypes of hexaploid wheat among 10 groups, identified upon the analysis of 39 microsatellite loci, depending on the origin of cultivar, from which the genotype was selected

Region (genotype number)	Subregion	Country, territory	Genotype groups									
			1	2	3	4	5	6	7	8	9	10
Africa (12)		Algeria							2			
		Morocco							1			
		Tunisia				1						
		Ethiopia							8			
Asia (164)	Central (66)	Afghanistan			3				2			
		Western China		2	3			3	5			
		India, Kashmir			5							
		Iran				4			1			
		Kazakhstan								2		
		Mongolia		2	2							
		Tajikistan			8	7		3	3			
		Turkmenistan			2	2			1			
		Uzbekistan			5	1						
	Eastern (36)	Eastern China		14	2	1			6			
		Japan		5					6		2	
	Northern (8)	Russia			1				4		2	1
	Southern (28)	India	5		7	1			1			
		Pakistan	4		7	1			2			
	Western (26)	Israel			1	1			5		1	
		Iraq							1			
		Syria							3			
		Turkey			2	7	1		4			
Caucasus (46)		Azerbaijan				7			4		1	
		Armenia				1	3		2		2	1
		Georgia			2	5			3	2		
		Russia				8			2		3	
Europe (104)	Western (47)	Great Britain							2			
		Germany						4	1		1	
		Greece							1			
		Spain							6			
		Italy			1	1			3			
		the Netherlands							2			
		Portugal							3			
		France							5			
		Sweden							7			
		Switzerland						4	4		2	
	Eastern (57)	Albania							1			
		Belarus							1			
		Hungary									1	
		Moldavia									1	
		Poland							1		4	
		Russia							6	12	8	6
		Ukraine							4	5	3	3
		Yugoslavia									1	
New World (21)		Australia							4			
		Argentina									1	
		Canada							2	1	6	
		United States				1			2	2	2	

Xgwm loci, Table 1). Another pair of primers provided simultaneous analysis of two independent loci (*Xcfd17h8a* and (*Xcfd17h8d*) [20]. Thus, one locus was typed in each of chromosomes 6A, 7A, and 4D. In the remaining 18 chromosomes of the haploid set by two loci were examined. Altogether, in 347 haplotypes of hexaploid wheat 39 loci were tested.

The number of alleles identified at individual loci varied from four at *Xgwm664* to 40 at *Xgwm539*, with the mean number of alleles per loci constituting 18.3. In total, in 39 loci, 712 alleles were identified. Allele frequency in the genotype sample studied varied from 0.3% to 83.2%, with the mean frequency of 5.5%. The 503 alleles among all identified (70.6% from the total number) were found in less than 5% of genotypes. These alleles were defined as rare. Among these, 126 alleles appeared to be unique, i.e., each of alleles was present in only one genotype. The distribution of such alleles among the loci was nonrandom. They were more frequent in *Xgwm285* (chromosome 3B, 10 alleles), *Xgwm99* (chromosome 1A, eight alleles), and *Xgwm539* (chromosome 2D, eight alleles). None of these alleles were identified in *Xgwm260* (chromosome 7A). The differences in rare allele frequencies at different loci can be explained by their different mutation rates. At the prevalence of rare alleles, in each locus from one to five alleles with the frequencies of more than 10%, were identified. The total number of such alleles constituted 112.

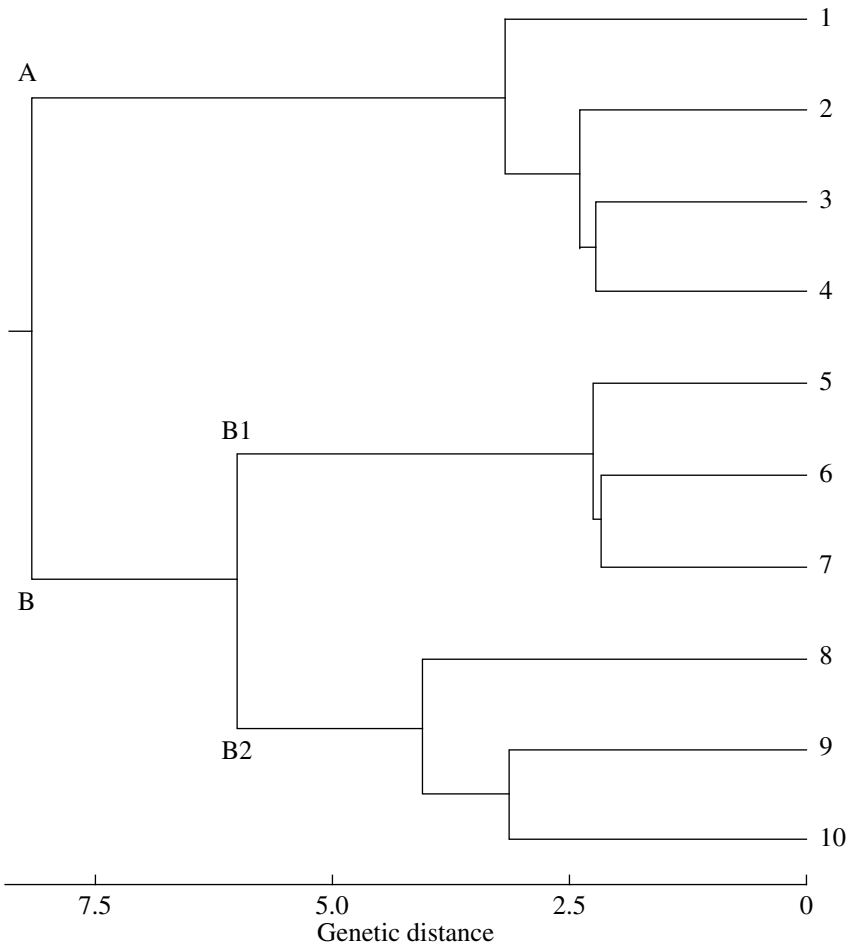
Among 347 genotypes examined, each genome of hexaploid wheat demonstrated the presence of nearly equal number of alleles, including the rare. The PIC value for genomes A, B, and D constituted 0.77, 0.82, and 0.80, respectively. Among the homeologous chromosome groups, the highest PIC value (0.90) was identified for the seventh group, while the lowest index value (0.72) was found in the fourth group. For individual microsatellite loci, the polymorphism index varied from 0.29 to 0.95, with the mean value of 0.80 (Table 1). It should be noted that unique alleles demonstrated the tendency to the appearance in certain genotypes. For instance, 44 out of 126 unique alleles identified (35.2%) were found in 20 genotypes. Furthermore, each of these genotypes contained from two to four unique alleles. Among the genotypes described, there were four out of 26 spelt genotypes identified. Although functional significance of mutations at microsatellite loci is still unknown, special attention should be focused on the preservation in the collection of the cultivars, from which the genotypes containing two or more unique mutations were selected.

In general, comparison of 347 genotypes over 39 microsatellite loci showed that each of them was characterized by a specific allele set. However, to distinguish all the genotypes studied, analysis of seven loci, *Xgwm120*, *Xgwm234*, *Xgwm260*, *Xgwm372*, *Xgwm413*, *Xgwm437*, and *Xgwm539*, was sufficient.

Cluster Analysis

The values of similarity coefficient for all possible 60031 genotype pairs varied from 0.00 to 0.95, with the mean value of 0.20. Furthermore, 27 pairs, which included 32 genotypes of different species of hexaploid wheat from Russia, China, and other countries, were absolutely dissimilar (the similarity coefficient value was equal to 0.00), and had different alleles at all microsatellite loci examined. Three genotype pairs (*T. aestivum* from India, k-23798 and k-23807; *T. compactum* from Armenia, k-13477 and k-13695; *T. vavilovii* from Turkey, k-29533 and k-30085) were fond to be most close to one another (similarity coefficient, 0.95), and had different alleles at only four loci.

Cluster analysis revealed complex relationships pattern between wheat genotypes tested. All genotypes grouped into two large clusters, A and B (figure). Cluster A included 132 genotypes, which formed groups from 1 to 4, representing landraces mostly form different Asian countries. Cluster B was comprised by 215 genotypes, manly from European countries. Within this cluster, two subclusters, B1 and B2, were identified. Each of the subclusters was represented by three groups of genotypes (5 to 7 and 8 to 10, respectively). For the convenience of dendrogram analysis, Tables 2 and 3 demonstrate combined distribution of genotypes, united in different groups, relative to different countries and geographical regions of origin, as well as relative to their belonging to different hexaploid wheat taxa in accordance with classification of N.I. Vavilov [3], recently supplemented with newly described species. As follows from the data in Tables 2 and 3, group 1 from cluster A contained seven genotypes of common wheat and two genotypes of shot wheat *T. spaerococcum*. All these genotypes originated from India and Pakistan. Among 23 genotypes, forming the second group, 19 belonged to the subspecies of Chinese common wheat (subsp. *sinicum*) and the remaining four genotypes belonged to the subspecies of Chinese clubbed wheat (subsp. *sinicum*). Genotypes of this group mostly originated from Eastern China and Japan, and four genotypes originated from Western China and Mongolia. Group 3 was presented by 51 genotypes of different species and subspecies of hexaploid wheat, including 34 of *T. aestivum*, mostly subsp. *indicum* and subsp. *irano-turkestanicum*; 14 genotypes of *T. compactum* subsp. *armeno-turkestanicum* and subsp. *eurasiaticum*; two genotypes of *T. spelta* subsp. *kuckuckianum*; and one genotype of *T. sphaerococcum*. Most of these genotypes originated from the countries of Central Asia and the neighboring regions of India and Pakistan. Among the 49 genotypes, forming the fourth cluster, 36 belonged to *T. aestivum* subsp. *eurasiaticum* and subsp. *irano-turkestanicum*; one genotype, to *T. compactum*; two genotypes, to *T. macha*; and another two, to *T. vavilovii*. The cultivars, which served as the source of the genotypes mentioned at most originated from the Caucasus and Turkey. These genotypes were joined by



Distribution of the genotype groups on the dendrogram constructed based on the results of the analysis of 39 microsatellite loci in 347 genotypes of hexaploid wheat. A and B, clusters; B1 and B2, subclusters; 1 to 10, genotype groups.

eight genotypes from the Asian subspecies of *T. spelta* from Iran, Tajikistan, and Turkmenistan.

In subcluster B1, the number of genotypes within the groups varied from four to 121. The smallest group 5 contained the genotypes of the Eurasian subspecies of common wheat from Armenia and Turkey. Group 6 was comprised of seven genotypes of European subspecies of spelt from Switzerland and Germany, three genotypes of common wheat from Tajikistan, which belonged to the group of spelt-like Irano-Turkestan common wheat, as well as all three genotypes of *T. petropavlovskyi* studied, and one genotype of clubbed wheat from Germany. Group 7 was found to be the largest and contained 121 genotypes. In this group, quantitative prevalence of common wheat genotypes from three subspecies (*eurasticum, sinicum*, and *iranoturkestanicum*) from the countries of Western Europe and Western Asia was revealed. This group also contained 13 genotypes belonging to the subspecies of Chinese and European clubbed wheat from different countries; seven genotypes of the European subspecies of spelt from Spain and Azerbaijan; and by one genotype of shot wheat from India and macha wheat from Germany. Spelt genotypes demonstrated the tendency to cluster together.

The genotypes of Eurasian common wheat (subsp. *eurasiaticum*) were mostly grouped in subcluster B2. Group 8 included the genotypes selected from winter East European landraces of common wheat, like Banatkas, and their derivates, the selections cultivars Besostaya 1, Mironovskaya 808, and their progeny. According to the data of N.I. Vavilov ([12], p. 302; [3], p. 83), before the rise of 20th century, Banatkas were the most distributed landraces of the world. They were associated with steppe and forest steppe regions, and originated from the Banat mountain region of Hungary. Detailed structure of group 8 was described earlier [21]. Group 9 comprised 41 genotypes, most of which were represented by European landraces of the Banatka and Sandomirka types and their derivatives. Landraces of the Sandomirka type are attributed to the ecological group of North European forest unbearded common wheat, which earlier occupied a wide range in nonchernozem and forest zones, and further distributed to

Table 3. Distribution of 347 genotypes belonging to different species and subspecies of hexaploid wheat among ten groups, identified upon the analysis of 39 microsatellite loci

Species, subspecies	Genotype group in cluster analysis									
	1	2	3	4	5	6	7	8	9	10
T. aestivum, including[a]:	7	19	34	36	4	3	99	24	41	11
subsp. *irano-turkestanicum*		1	11	10		3	14			
subsp. *indicum*	7		18	2			3			
subsp. *sinicum*		16	1	1			13		2	
subsp. *eurasiaticum*		2	4	23	4		53	9	37	7
subsp. *abyssinicum*							8			
Modern cultivars							8	15	1	4
T. compactum, including[a]:		4	14	1		1	13			
subsp. *armeno-turkestanicum*			9	1			8			
subsp. *eurasiaticum*		1	5			1	5			
subsp. *sinicum*		3								
T. spelta, including[b]:			2	8		7	8		1	
subsp. *spelta*						7	5			
subsp. *kuckuckianum*			2	8			3		1	
T. macha				2			1			
T. vavilovii				2						
T. sphaerococcum	2		1							
T. petropavlovskyi[b]						3				

Notes: [a] Subspecies of *T. aestivum* and *T. compactum* are given in accordance to N.I. Vavilov [3].
[b] Subspecies of *T. spelta* and the species of *T. petropavlovskyi* are given in accordance with Dorofeev et al. [6].

the steppe zone ([3], pp. 90–92). The last group 10 comprised genotypes selected from spring East European landraces of the Poltavka type and the derivative selection cultivars. Landraces of the Poltavka type represent the most flexible (universal) group of steppe wheat, distributed in many regions of steppe, forest steppe, and forest zones ([3], pp. 89–90).

Compared to landraces, genetic diversity of selection cultivars from Russia and CIS countries tested, was low. All genotypes were included into different groups of cluster B.

It should be noted that genotypes belonging to the isolated by N.I. Vavilov [3] subspecies of Abyssinian common wheat (subsp. abyssinicum), cultivated in Ethiopia and mountain Eritrea, did not form an individual cluster. On the contrary, they were the members of the largest group 7, which was mostly represented by landraces and selection cultivars from Europe.

To determine the pattern of differences between individual groups of genotypes, microsatellite allele frequencies were evaluated in the whole sample and in each of the groups. The data obtained demonstrated that the genotype groups differed from one another in terms of the frequencies of a great number of the alleles, including those characterized by high frequencies in the total sample, as well as rare alleles.

DISCUSSION

The level of microsatellite allele diversity revealed in the present study (18.3 alleles per locus, on average) agrees with the literature data. For instance, analysis of 998 common wheat accessions from the collection of the Institute of Plant Genetic and Breeding (Gatersleben, Germany) showed the presence of 18.1 alleles, on average, in each of 26 SSR loci examined [14]. In recent study of 3942 wheat accessions form the INRA collection (France) at 38 microsatellite loci, the averaged number of alleles per locus constituted 23.9 [10]. The size of the genotype sample examined in the present study was much smaller than in the studies described above. Because of this, nearly equal level of allelic diversity at the loci examined pointed to high heterogeneity of the material tested. The mean value of polymorphism index (PIC) calculated for the total genotype sample supported this proposal. It was equal to 0.80, while in the work of German researches the value of this index was 0.77 [14].

In summary, we conclude that hexaploid wheat within it range is differentiated in terms of allele composition of the SSR loci. The highest genetic differences were revealed between the groups of genotypes selected from landraces, formed on two different continents, Asia and Europe (figure). The common wheat

accessions (genotypes) representing cultivars of these large groups were different in spike roughness and thrashing difficulty. Specifically, accessions from the first group were rough with difficult thrashing, while the plants from the second group were tender with easy thrashing. These differences pointed to the belonging of the plants (genotypes) to different "race groups", according to N.I. Vavilov [22], or to different botanical subspecies, Asian subsp. *hadropyrum* (Flaks.) Tzevel., and European subsp. *aestivum*, according to classifications of Flyaksberger [4], Tsvelev [23], Dorofeev et al., [6]. At the lower difference level, each of large genotype groups split into smaller groups, characterized by different microsatellite allele frequencies. In terms of the geographic origins of the genotypes comprising these groups, they could be compared to the ancient husbandry centers, described by N.I. Vavilov [24]. Analyzing the history of the world husbandry development, N.I. Vavilov thought that the first husbandry cultures, including the cultures of Southern Asia, where he included the Transcaucasia, Asia Minor, and Central Asia, as well as the culture of India, Eastern and Central Mountain China, and the others, appeared "autonomously, at one or different times… They were characterized by substantially different ethnic and linguistic groups of populations… and … different types of farming tools and domestic animals". According to archaeological data, hexaploid wheat was already cultivated in Europe as early as four thousand years B.C. [25, 26]. In Asia, this process began even earlier, eight thousand years B.C. [27]. It was suggested that cultivars, differing in the complexes of morphological and physiological characters, and adapted to the corresponding local soil and climatic conditions, as well as the conditions of cultivation, were formed during the long period of wheat cultivation within the isolated husbandry centers. Establishment of the landrace groups identified in the present study implies the existence of the following husbandry centers: Southern Asian, for group 1; Eastern Asian, for group 2; Central Asian, for group 3; Caucasian, for group 4; Western European, for subcluster B1; and Eastern European, for subcluster B2.

The hexaploid wheat classification generated in the present study is very similar to that constructed in our previous studies based on the analysis of RAPD markers [28, 29]. This classification was roughly consistent with agro-ecological classification of common wheat suggested by N.I. Vavilov [2, 3]. On the other hand, it was radically different from known botanical classifications, based on the analysis of hexaploid wheat differences in morphological characters controlled by a small number of genes with wide pleiotropic effects [6, 30–32].

Microsatellite analysis showed that common wheat was most differentiated among all wheats, and consisted of at least ten groups of cultivars. At the same time, neither genotypes representing endemic species, *T. sphaerococcum*, *T. vavilovii*, *T. macha*, and *T. petropavlovskyi*, nor the genotypes selected from the landraces of earlier rather widely cultivated species, *T. compactum* and *T. spelta*, formed individual clusters. On the contrary, they grouped with common wheat. In other words, they were more similar to common wheat, then to each other. Genotypes of clubbed wheat and spelt were the members of different groups, as well as of both clusters A and B. This genotype distribution can be considered as the evidence in favor of coevolution of these wheats and common wheat. Close relatedness of common wheat and spelt also followed from the data on differential chromosome staining [33]. Furthermore, in this study, the legitimacy of subdivision of the subspecies of European spelt into ecogeographical groups was confirmed, which was consistent with our data. Specifically spelt genotypes belonging to Iberian group clustered together and were included into group 6, characterized by the prevalence of common wheat from Europe. In turn, genotypes belonging to Bavarian group grouped with common wheat cultivars from Southwest Asia, which relative to the spike characters were very similar to spelt. In different classifications, this speltlike common wheat is isolated into the *speltiforme* group [3, 4]. According to the data obtained, the phenotypic similarity described could be determined by genotypic relatedness of this wheat and spelt.

Thus, application of modern DNA technologies, providing analysis of polymorphism of wheat landraces at a great number of genomic regions, and enables construction of genetic classification of this culture, which reflects the history of its evolution and dispersal.

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