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第65号

The Research and Development on Efficient Method for Preservation and Utilization of Cold Tolerant and Freezing Hardy Plant Genetic Resources Collected by Vavilov Institute

ロシア耐寒・耐凍性植物遺伝資源の効率的保全と活用のための研究開発

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III. Main Research Results of the Project

1. Evaluation of Genetic Differentiation of Wheat Resources Using RAPD Markers

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Abstract

The DNA polymorphisms of hexaploid wheats (2n=6x=42) with genomes A, B, and D were analyzed by random amplified polymorphic DNA (RAPD). One hundred and thirty-seven polymorphic bands were obtained from 414 accessions of hexaploid wheat including *Triticum aestivum, T. compactum, T. sphaerococcum, T. petropavlovskyi, T. spelta, T. Macha, and T. vavilovii* using 28 RAPD primers. The RAPD data were analyzed by multivariate statistics to clarify the genetic relationships of the accessions.

The principal component analysis revealed nine major genetic groups that generally correspond to large ecogeographical groups of the subspecies level (*eurasiaticum, irano-turkestanicum, indicum, and sinicum*). Canonical discriminant analysis revealed that complete correspondence of the observed classification for all nine genetic groups was detected by the analysis of 32 polymorphic DNA bands. Based on the statistical analysis, 82 accessions were listed, representing all nine distinctive genetic groups that can be used as standard accessions of hexaploid wheat germplasm.

Introduction

Hexaploid species of *Triticum* genera contain three genomes (A, B, and D) and 42 chromosomes in the nuclei of somatic cells. During their distribution from the primary center of origin to different regions, these types of wheat were differentiated into a large number of forms to adapt to various ecological conditions (Flaksberger, 1935). Such differentiations appeared clearly among landraces originated from different regions.

Until now, hexaploid wheats have been divided into *T. macha* (macha wheat), *T. spelta* (spelt wheat), *T. vavilovii, T. compactum* (club wheat), *T. sphaerococcum, T. petropavlovskyi* (rice wheat), and, finally, *T. aestivum* (common or bread wheat) based on detailed investigations of morphological, physiological, cytological, and other characteristics. Morphological differences in hexaploid wheat are determined by single mutations with wide pleiotropic effects (Mac Key, 1966).

A number of botanical classification systems have been suggested for hexaploid wheats. For example, Bowden (1959) and Mac Key (1966, 1988) combined all hexaploid wheats into one species, *T. aestivum L.*, and divided it into taxons of a lower level, such as groups of cultivars or subspecies. On the other hand, Dorofeev et al. (1979) offered species status for each of these hexaploid wheats. In our report, we use this classification because it is the one adopted at the VIR. However, because the genetic investigation of hexaploid wheat has been feeble, no exhaustive genetic classifications have been established that would reflect their ecological differentiation and would allow more precise and faster selection sources for breeding with given parameters for adaptability.

At present, several methodologies for fast and detailed analyses of polymorphisms of plants on the DNA

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level have been developed. There are some publications on using RAPD and RFLP markers for the evaluation of intra- and interspecies genetic diversity of wheat (Dvorak et al., 1988; Paull et al., 1988; Sun et al., 1988; Bryan et al., 1999; Pujar et al., 1999; Kim & Ward, 2000; Luo et al., 2000; Shah et al., 2000). In this work, we attempted to include all known ecogeographical diversity in hexaploid wheat.

The main objective of this study was to investigate the hexaploid wheat polymorphism with the use of RAPD markers and to compare it with ecogeographical differentiation in the crop. The principal tasks were (i) to select primers useful for wheat genotypes that are distinct from a large number of random primers; (ii) to estimate the polymorphism of analyzed accessions by comparing the RAPD patterns; (iii) to elucidate the correspondence between the grouping of accessions determined by RAPD analysis and the ecogeographical differentiation of hexaploid wheat.

Materials and Methods

Materials

A total of 414 accessions including landraces and cultivars were studied (Table 1). Common wheat was represented by 345 accessions including 290 landraces and old cultivars of all five subspecies; 33 of 34 agroecological groups were originated from different regions of the world. In addition, 35 modern cultivars from CIS countries (former USSR), 30 old Japanese cultivars, and 20 modern cultivars from the Hokkaido region (Japan) were included in the analysis. Moreover, 69 accessions of other hexaploid wheat species, such as *T. compactum*, *T. sphaerococcum*, *T. petropavlovskyi*, *T. spelta*, *T. macha*, and *T. vavilovii*, were analyzed.

Seed material was largely selected from the VIR germplasm collection (St. Petersburg, Russia) and the MAFF Genebank (Tsukuba, Japan). Most of the analyzed landraces were selected from materials obtained during Russian expedition missions accomplished before the 1940s. Seeds of modern cultivars were obtained from the germplasm collection of HANES.

RAPD assay

Leaf tissues were harvested from 2- to 3-week-old seedlings (bulk of 15-25 seedlings per accession). DNA for the RAPD assay was extracted from freeze-dried tissue using the microscale method with CTAB (Saghai-Maroof et al., 1984). DNA concentrations were estimated and standardized on a Beckman 7400 spectrophotometer. The polymerase chain reaction (PCR) mixture (given for a 10-µl total volume) was 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2 mM $MgCl_2$, 0.2 mM each of dATP, dCTP, dGTP, and dTTP, 1 mM primer, approximately 40 ng template DNA, and 0.3 units of Gene *Taq* DNA polymerase (from Wako). PCR mixtures were prepared in 96-well microplates and subjected to 45 cycles of 94℃ (30 s), 42℃ (30 s), and 72 ℃ (1 min) on a Perkin Elmer 9600 thermal cycler. After the addition of 3 µl 6xdye solution (0.1% bromophenol blue, 0.1% xylene cyanol FF, and 15% Ficoll), 10 µl was loaded into a 2% agarose gels. Gels were 24 cm long and 12 cm wide with 26 lanes and four rows of wells. The mixture of 100 bps DNA ladders was used as a mixture of size markers. Electrophoresis was carried out in a Sunrise submarine unit (from GibcoBRL) using a 0.5xTBE buffer at 5 V/cm for approximately 1.5 h until the bromophel blue marker migrated approximately 5 cm. After staining with ethidium bromide, the gels were documented on the AL-C UV FA 1100 image system.

Statistical analyses of data scored

RAPD patterns were scored by assigning a number to each band. For subsequent numerical analyses, data were coded in a binary form, i.e., the presence or absence of a band in a line was recorded by 1 or 0, respectively. Only polymorphic and reproducible bands in multiply runs were included in the raw data matrix.

Table 1. Hexaploid wheat cultivars analyzed by RAPD markers

Classifications	Species, subspecies	Quantity of cultivars
I. Triticum aestivum L. (345 cultivars).		
a) Landraces and old cultivars from different countries selected from the VIR germplasm collection.		
Subspecies (accordingly to Vavilov, 1964)	*irano-turkestanicum*	*48*
	indicum	*36*
	Sinicum + old japanese cvs.	*50*
	eurasiaticum	*148*
	abyssinicum	*8*
b) Modern cultivars from CIS and Japan.		
	CIS	35
	Japan (from Hokkaido)	20
II. Other species (69 cultivars).		
Species (accordingly to Dorofeev at al., 1979)	*T. compactum*	35
	T. sphaerococcum	3
	T. petropavlovskyi	1
	T. macha	2
	T. spelta	26
	T. vavilovii	2
Totally analyzed - 414 cultivars.		

Statistical treatment of the results was preformed with the usage of the program packages NTSYS-pc version 2.0 (Rohlf, 1997) for cluster analysis and STATISTICA 5.0 for principal component and canonical discriminant analyses (StatSoft Inc., USA, 1995).

For cluster analysis, RAPD data matrices were used to generate a genetic distance matrix using Nei's (1972) distance calculation. A phenogram was produced using unweighted pair-group method arithmetic average (UPGMA) clustering.

Principal component analysis was used for the direct classification of accessions (Q-technique). The original data matrices were transposed, and the factor loadings were used as a direct classification score of the accession (Hays, 1988).

Canonical discriminant analysis was used for detecting primer/band combinations, which discriminate different groups, and for classifying accessions into different groups with better accuracy (Kachigan, 1986). The forward stepwise discriminant analysis, with evaluation of the results of classification at each step, was used. Classification functions were computed for each group for the direct classification of accessions. In this way, an accession can be classified into the group for which it has the highest classification score (Jennrich,

1977).

Results and Discussion

From among approximately seven hundred random decamer primers produced by Operon Technology Inc. (designated by OPX), the University of British Columbia (UBC), and WAKO Inc. Tokyo (Ew) screened with 8 accessions, the 28 primers were selected for RAPD analysis (Table 2). When PCR products were analyzed on the agarose gels, each primer generated from 1 to 12 distinctive bands ranging from 0.13 to 1 kbp. Typical RAPD patterns for 24 accessions are represented in Fig. 1. A total of 137 polymorphic DNA fragments were obtained using 28 primers, and their information was transformed into a computer data matrix. All 414 analyzed accessions differed based on the RAPD data. In order to investigate the genetic relationships of

Table 2. Nucleotide sequences of the 28 oligonucleotide primers and the number of RAPD markers they generated in the 414 genotypes.

Primer name	Sequence	Number of markers
OPA 6	GGTCCCTGAC	7
OPA 16	AGCCAGCGAA	12
OPA 19	CAAACGTCGG	4
OPA 20	GTTGCGATCC	3
OPB 13	TTCCCCCGCT	8
OPD 12	CACCGTATCC	5
OPF 19	CCTCTAGACC	10
OPE 12	TTATCGCCCC	3
OPM 9	GTCTTGCGGA	4
OPM 11	GTCCACTGTG	4
OPO 1	GGCACGTAAG	7
OPP 3	CTGAT^CGCC	1
OPP 4	GTGTCTCAGG	4
OPP 10	TCCCGCCTAC	6
OPT 17	CCAACGTCGT	5
OPU 8	GGCGAAGGTT	2
OPV 6	ACGCCCAGGT	4
OPV 9	TGTACCCGTC	10
OPZ 11	CTCAGTCGCA	1
OPAA 8	TCCGCAGTAG	6
OPAB 5	CCCGAAGCGA	6
OPAD 15	TTTGCCCCGT	3
OPAF 16	TCCCGGTGAG	1
UBC 386	TGTAAGCTCG	9
UBC 535	CCACCAACAG	6
UBC 580	GCGATAGTCC	2
Ew 5	AAGATCTTACTG	2
Ew 29	GTTATGCAAGGG	2
	total	137

accessions and to identify the genetic groups distinguishable by RAPDs, statistical analysis was carried out with increasingly more complex sets of accessions. At the beginning, the analyses of accessions belonging to each subspecies of common wheat were performed; then, accessions of all subspecies of common wheat were compared, and, finally, accessions of all hexaploid species were compared. In statistical analysis, three statistical methods of multivariate analysis were carried out to confirm the result of each analysis.

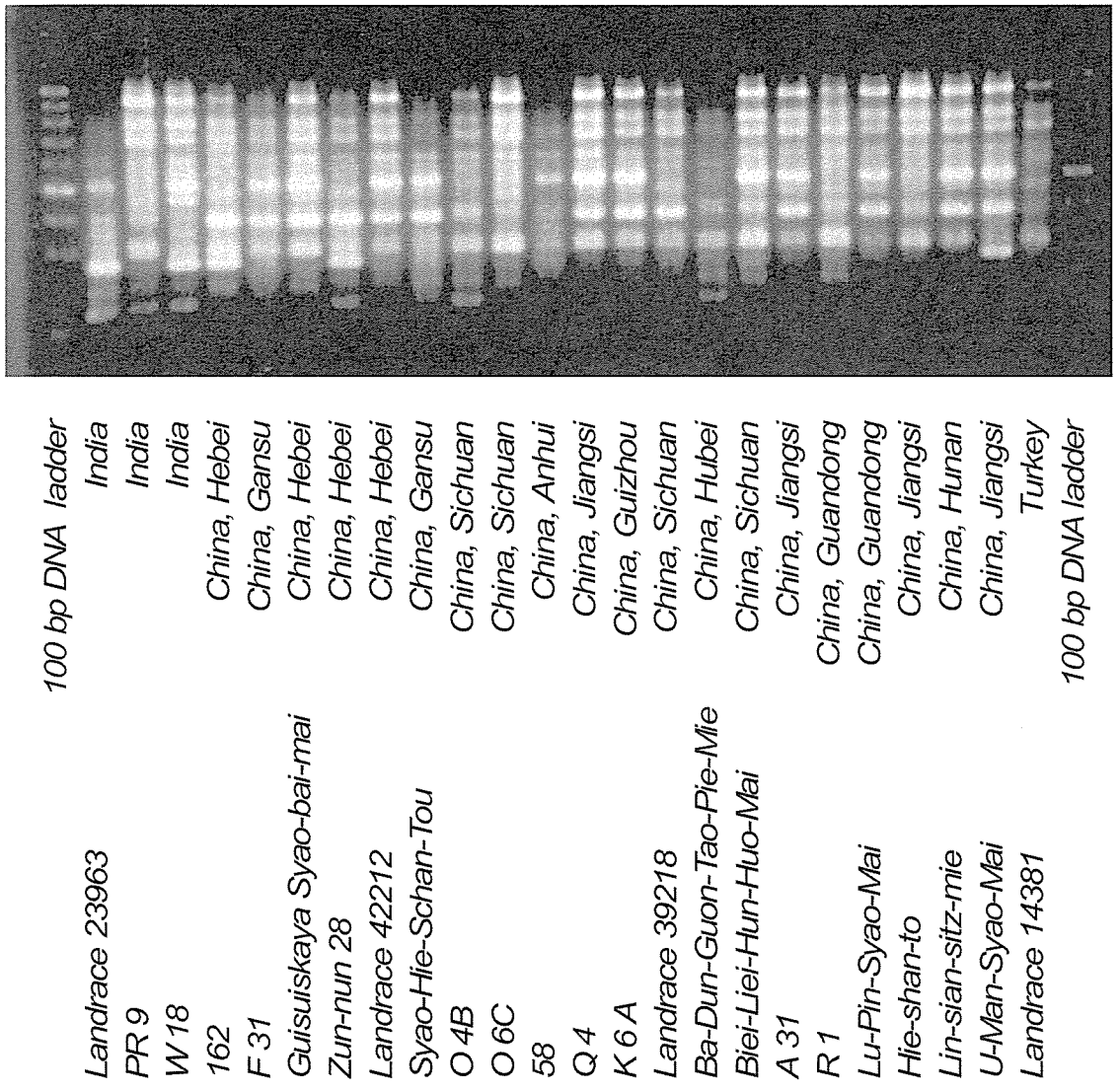

Fig. 1. RAPD analysis of 24 wheat accessions using primer OPF 19.

In the principal component analysis of RAPD data for 148 accessions of the subspecies eurasiaticum of common wheat, all accessions with factor loadings of 0.5 and more were combined into 15 groups. Three of them (groups 1, 2, and 3) were identified as major and contained 70, 30, and 18 accessions, respectively (Fig. 2). Other groups were identified as minor and usually contained 1-2 accessions. The first major group included spring and winter landraces and old cultivars from different regions. The majority of landraces of "Banatka" and "Sandomirka" types and hybrid European cultivars released before the 1940s were found in this group. The second group contained landraces and cultivars from different regions as well. In contradiction to the first group, this group mainly included spring forms such as landraces of the "Poltavka"

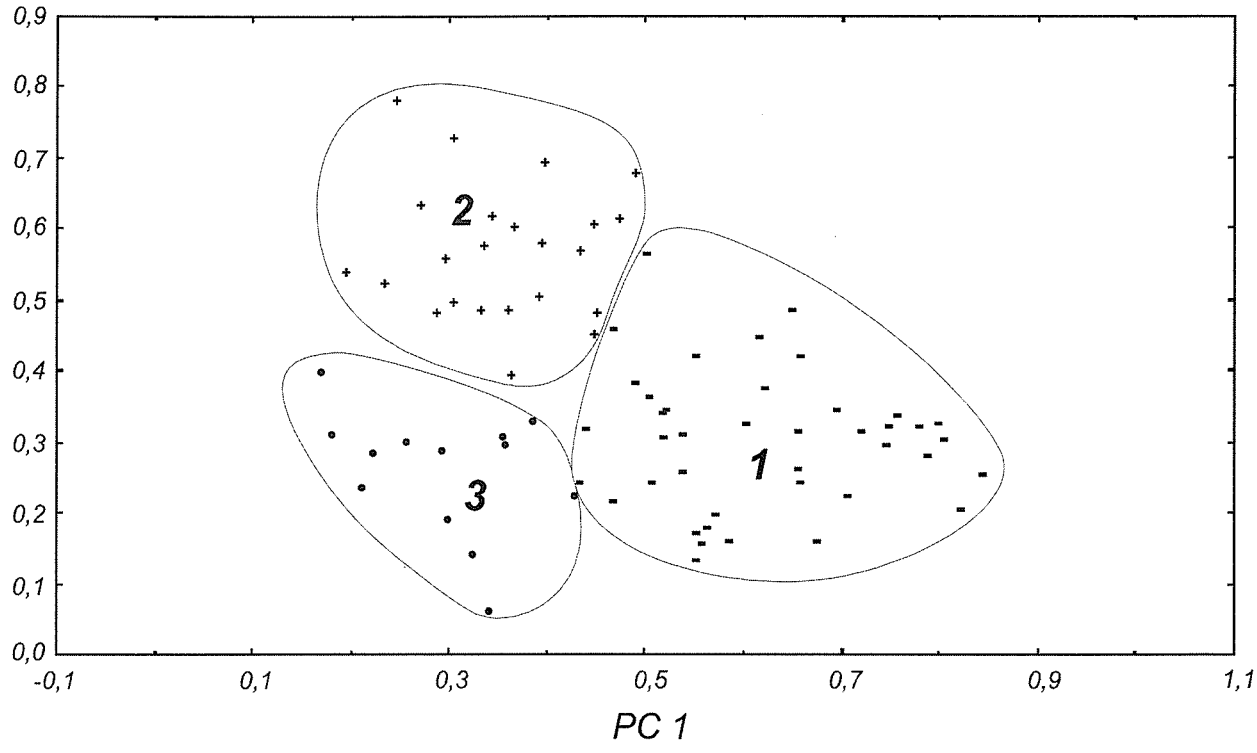

Fig. 2. Plot of the first two principal component scores for three main groups of eurasian common wheat cultivars

type and cultivars selected from landraces of this type. From the point of view of geographical origin, the accessions of the third group were the most specific. This group included landraces from the Caucasus region. On the whole, the results of cluster analysis confirmed the results of principal component analysis. In the complex structure of the phenogram (Fig. 3), a number of clusters and subclasters corresponding to the above-mentioned groups (principal components) were identified. Analysis of their composition has shown that subcluster A_1 corresponds to the third group, the part of subcluster A_2, to the first group, and clusters B and C, to the second group. It should be noted here that the part of subcluster A_2 was represented by accessions from different groups and included accessions which had essential factor loadings with two PCs simultaneously.

For the analysis of other subspecies of common wheat, the same approaches were used. Circle diagrams (Fig. 4) illustrate the genetic structure of each subspecies revealed by principal component analysis of RAPD data. Accessions of subspecies *irano-turkestanicum* were combined into nine genetic groups, *indicum*, into six, *sinicum*, into six, and *abyssinicum*, into three. The results shown in Fig. 4 were confirmed by cluster analysis (data not shown). Thus, the investigation of subspecies of common wheat has demonstrated a complex genetic structure for each subspecies.

Similar approaches were used for the analysis of the genetic differentiation of modern cultivars of *T. aestivum* from CIS countries and the Hokkaido region. Principal components analysis combined these cultivars into 5 and 4 genetic groups, respectively (Fig. 5).

All 290 landraces and old cultivars of 31 genetic groups revealed by principal component analysis were analyzed to determine the relationships of common wheat subspecies. Five major groups containing from 12 to 110 accessions were identified (Fig. 6). Other minor groups contained 1-2 accessions. Groups 1, 4, and 5 correspond to the three major groups of subspecies *eurasiaticum* mentioned above from the composition of accessions. Group 2 consisted mainly of landraces and old cultivars from China and Japan. This means that

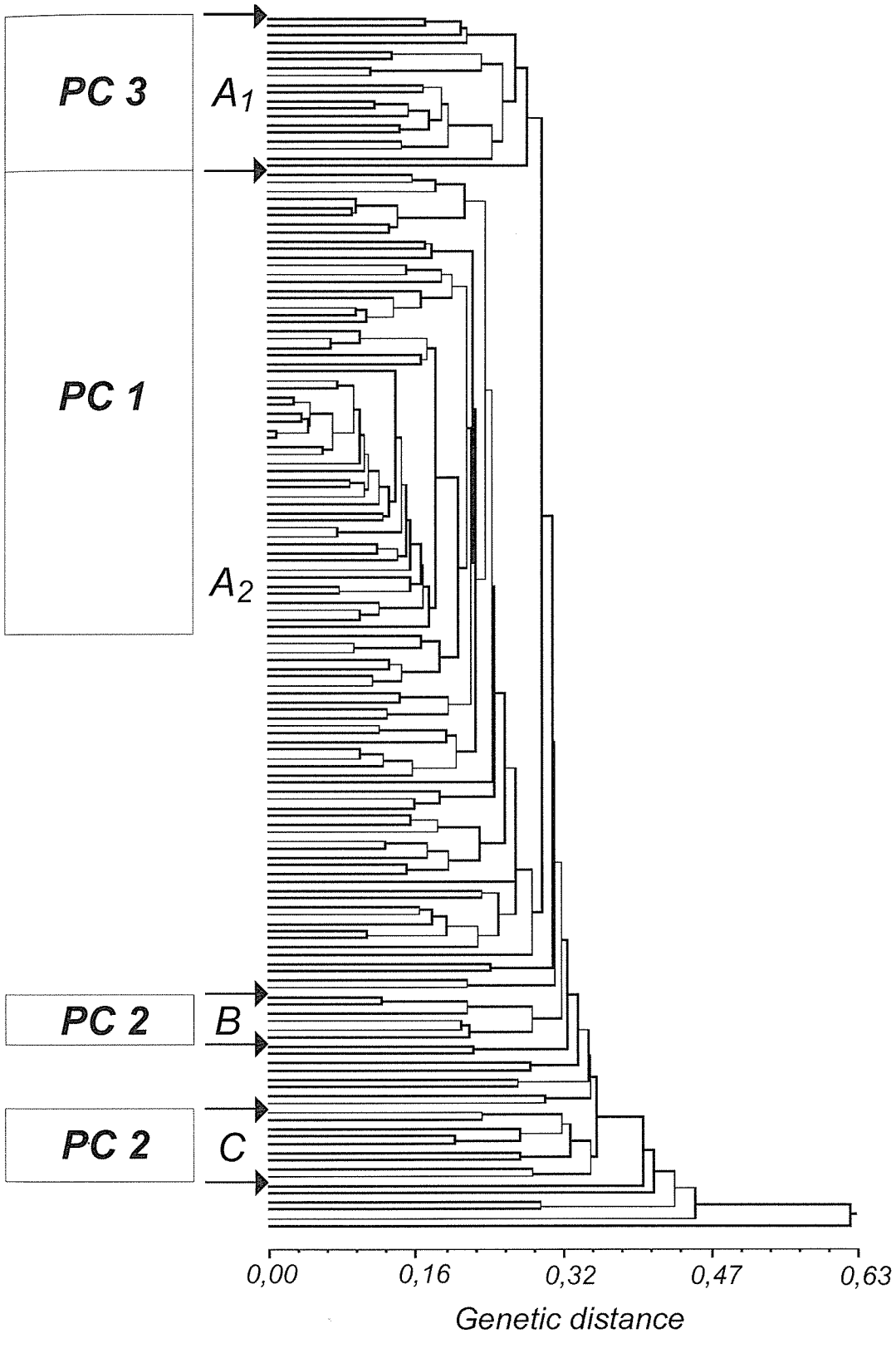

Fig. 3. Distribution on the phenogram of main clusters (A-C) of eurasian common wheat cultivars and their belonging to different PC (1-3).

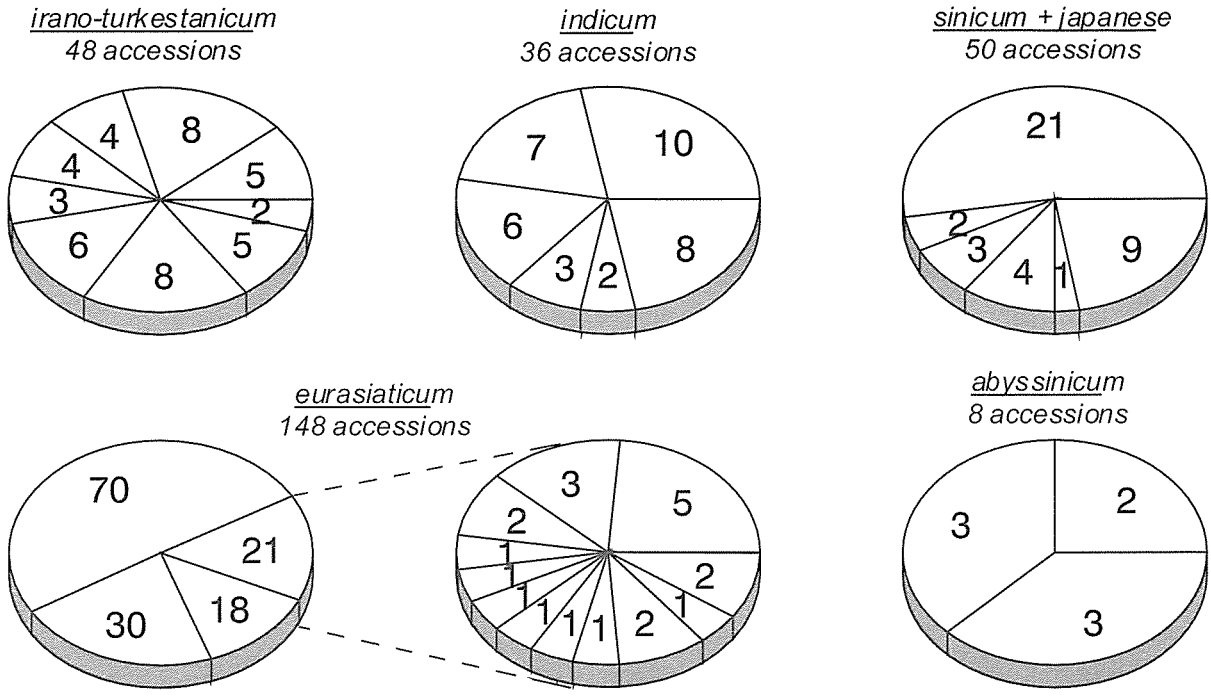

Fig. 4. Characterization of common wheat subspecies based on the principal component analysis of RAPD data. Parts of circles correspond to different PCs (genetic groups) with designation of accession quantity in each of them.

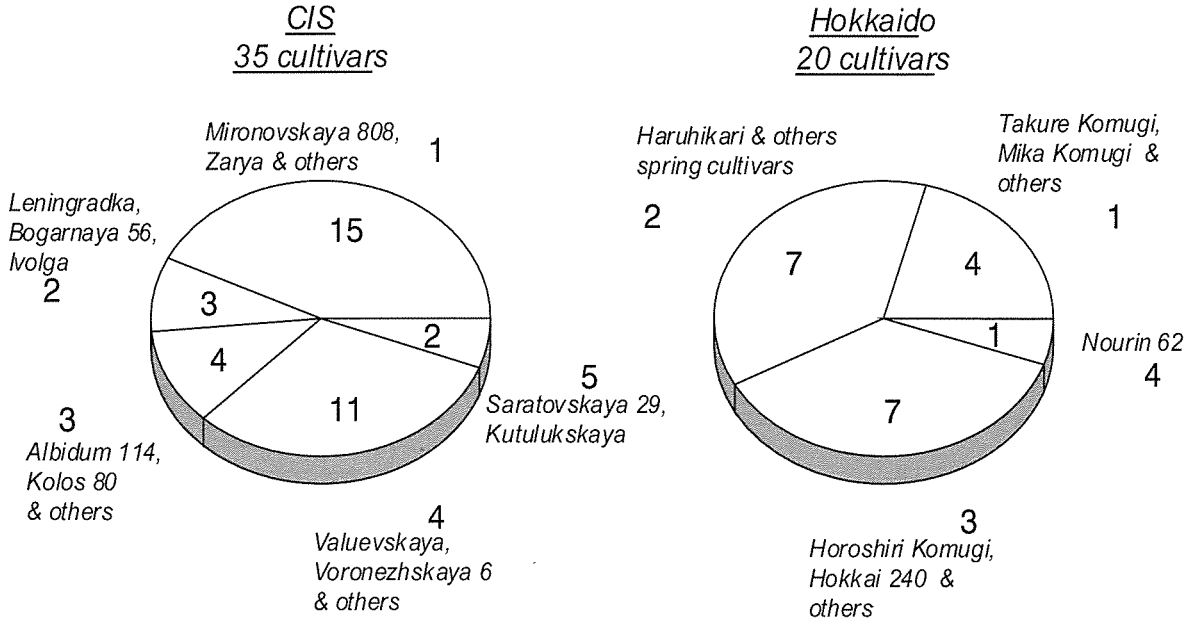

Fig. 5. Characterization of common wheat modern cultivars based on the principal component analysis of RAPD data. Parts of circles correspond to different PCs (genetic groups) with designation of accession quantity in each of them.

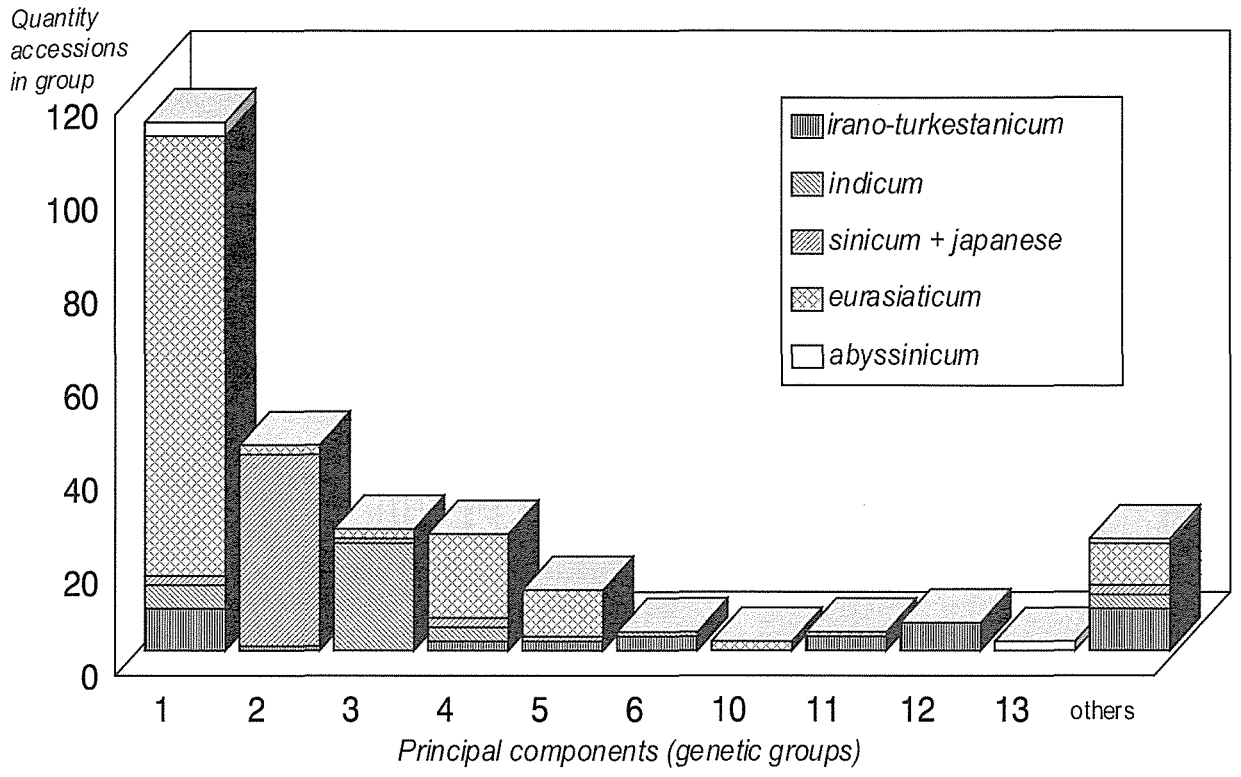

Fig. 6. Distribution of 290 landraces and old cultivars of common wheat based on the principal component analysis of RAPD data.

the accessions of subspecies *sinicum* were combined into one group in this analysis. Most accessions from India and Pakistan were combined into one group in this analysis as well (group 3). These results have shown the unique character of subspecies *sinicum* and *indicum* and the much closer genetic relation between these subspecies than between other subspecies. Accessions of subspecies *irano-turkestanicum* did not form one major group and allocated into 14 different genetic groups. Cluster analysis of 290 accessions has shown the correspondence of the clusters and subclusters to the major groups revealed by principal component analysis (Fig. 7). Similar approaches were used for the analysis of *T. spelta* and *T. compactum* (data not shown).

In Table 3, the distribution of genetic groups revealed by RAPD analysis in hexaploid wheat species is summarized (including modern cultivars from CIS and Japan). Major hexaploid wheat species in this work, *T. aestivum*, *T. compactum*, and *T. spelta*, show clear polymorphism based on the RAPD data, and their accessions were included in more than one genetic group. The accessions of common wheat are included in most genetic groups. One group usually includes accessions from different species, as can be seen in groups I, II, IV, and VI. Thus, the classification of hexaploid wheats into *T. aestivum*, *T. compactum*, *T. sphaerococcum*, and *T. vavilovii* is not in accordance with the accession grouping revealed by RAPDs in our study. Groups III, VIII, and IX included only accessions of common wheat. Group VII was represented by accessions of spelt wheat from Europe. Group X included only two accessions of macha wheat, and group XI, only one accession of rice wheat. In order to confirm the existence of groups X and XI, it is necessary to perform additional research on hexaploid wheats with more number of accessions of macha and rice wheats.

All modern Japanese cultivars were included in the first group. Half of the CIS cultivars were included in this group as well. Other cultivars from CIS (except one cultivar) formed a specific group, V.

Genetic grouping of all hexaploid wheats determined in this work was compared with all existing systems

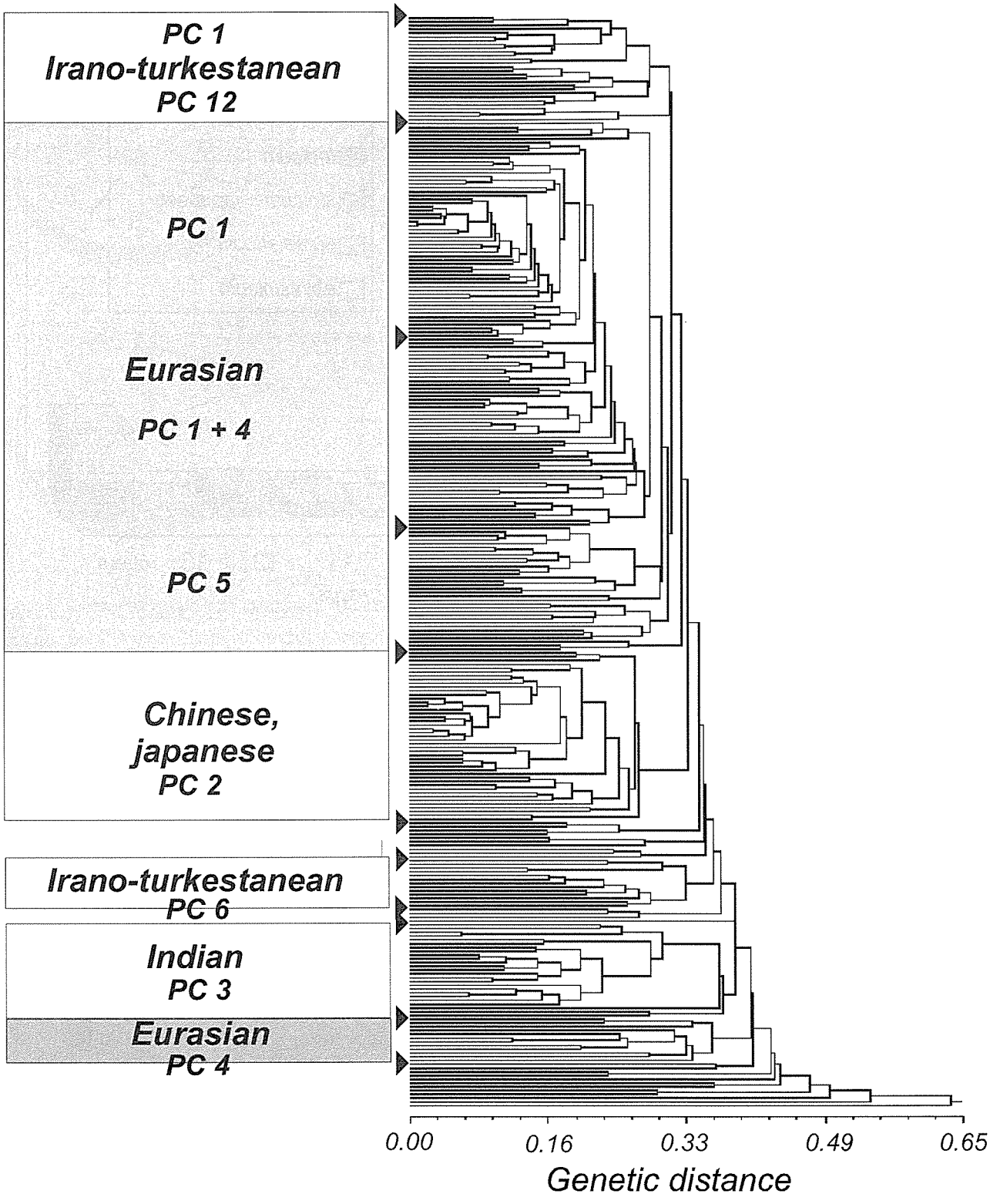

Fig. 7. Distribution of 290 landraces and old cultivars of common wheat on the phenogram and their belonging to different PC.

Table 3. Distribution of 300 cultivars of hexaploid wheats to different genetic groups revealed by principal component analysis of RAPD data.

Species, cultivars	Q-ty of cultivars	Genetic groups												Out groups
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	other	
Species:														
aestivum	189	66	17	16	18	1	15	-	6	5	-	-	21	24
compactum	35	10	13	-	2	1	2	1	-	-	-	-	1	5
sphaerococcum	3	-	-	-	3	-	-	-	-	-	-	-	-	-
petropavlovskyi	1	-	-	-	-	-	-	-	-	-	-	1	-	-
spelta	26	6	6	-	-	-	-	7	-	-	-	-	2	5
macha	2	-	-	-	-	-	-	-	-	-	2	-	-	-
vavilovii	2	1		-	-	-	-	-	-	-	-	-	-	1
Modern cultivars of common wheat from:														
CIS	26	11	-	1	-	10	-	-	-	-	-	-	3	1
Japan		11			-	-	-	-	-	-	-	-	3	2
Total		105	36	17	23	12	17	8	6	5	2	1	31	38

of hexaploid wheat classifications. It was demonstrated that this grouping mostly corresponds to the division of common wheat proposed by N. Vavilov (1964). Genetic groups I, III, VIII, and IX were represented mainly by accessions of the subspecies *eurasiaticum* of common wheat; genetic group II, by accessions of subspecies irano-turkestanicum; IV, by accessions of subspecies indicum; and VI, by accessions of subspecies sinicum. Genetic groups VIII and IX were complex and equally included accessions of subspecies eurasiaticum and *irano-turkestanicum*. According to N. Vavilov (1964), these two subspecies are ecologically much more complex than *indicum* or *sinicum*. As a result, it is conceivable that the first two were divided into several distinctive groups. Although N. Vavilov assumed this classification principle applicable to common wheat alone, our data clearly showed that this classification principle is suitable for all hexaploid wheats. Thus, the results of this work have demonstrated that all studied hexaploid wheats have the common gene pool with a complex genetic structure.

The results of canonical discriminant analysis confirmed the existence of nine major genetic groups. In the model of CDA, the analysis by 32 polymorphic bands of DNA demonstrated complete correspondence to the observed and predicted classification for all nine genetic groups. However, the analysis of 24 polymorphic bands of DNA resulted in the reduction of the level of correspondence of the observed and predicted classifications to 96.3%. Computed classification functions and a list of RAPDs included in the model of principal components (Table 4) might be recommended for the genetic grouping of wheat accessions and the building of more stable STS-markers.

Eighty-two hexaploid wheat accessions divided into nine major distinctive genetic groups are shown in Table 5. These accessions were selected from all 414 landraces and cultivars analyzed, and they are typical representatives of revealed major genetic groups. The list might be used in any research to evaluate wheat germplasm.

Table 4. Classification functions for main groups of hexaploid wheats based on canonical discriminant analysis of RAPD data.

Primer/ band of DNA, bp	Genetic groups								
	I	II	III	IV	V	VI	VII	VIII	IX
OPA6/480	-10,18	-13,56	-11,60	-8,24	-13,72	-7,92	24,44	-9,02	-13,77
OPA6/350	15,04	37,44	24,42	18,13	19,99	36,69	23,57	30,60	7,78
OPA16/1000	-3,62	-28,91	-6,94	-7,71	-22,71	-25,46	-6,77	-23,57	37,54
OPA16/650	2,97	0,31	2,89	5,17	11,62	4,17	3,45	8,46	8,82
OPA19/700	3,29	0,55	6,02	-1,13	-3,58	0,30	-5,15	-2,59	-0,86
OPA20/400	13,63	14,40	11,36	7,52	14,70	7,10	9,83	15,74	18,34
OPB13/380	4,18	-7,49	2,93	4,72	7,36	-9,97	4,26	2,93	5,74
OPD12/500	1,09	16,33	1,12	0,76	-1,59	9,01	-7,55	2,18	-1,75
OPD12/200	7,15	12,75	12,74	5,99	0,87	9,62	5,81	7,54	5,75
OPE12/370	-5,66	-1,60	-4,65	-2,84	13,56	5,57	-6,08	0,97	-4,37
OPM9/400	4,42	5,30	9,34	4,10	3,18	3,52	10,32	6,91	3,33
OPP4/500	30,26	42,04	35,82	25,54	19,99	33,59	30,17	36,11	31,27
OPP4/450	5,58	7,90	3,75	8,16	5,49	2,05	4,76	3,55	10,39
OPP4/300	7,80	5,25	5,55	5,05	17,96	9,71	2,77	11,22	11,36
OPP10/550	2,29	0,97	-1,35	-0,53	3,35	-0,25	3,55	3,74	8,35
OPU8/400	-7,58	-15,14	-13,02	-5,93	-2,61	-9,67	-10,16	-12,73	-1,70
OPU8/350	-5,43	-21,65	-10,92	-9,94	-10,06	-16,82	-12,78	-17,98	5,49
OPV9/850	4,90	2,25	1,20	0,01	8,73	5,88	4,39	10,27	8,56
OPV9/300	-5,06	-23,54	-11,11	-5,83	0,74	-18,62	-1,31	-15,26	-3,26
OPAA8/500	0,42	-2,78	6,21	2,06	-3,24	-0,47	0,30	-1,60	-6,03
OPAA8/350	12,63	8,57	9,73	7,39	13,94	9,14	6,60	15,21	26,76
UBC535/730	8,17	21,64	9,61	6,25	12,87	18,59	6,24	18,81	7,98
UBC535/200	10,12	12,94	9,22	6,51	14,02	13,64	11,66	17,01	8,16
UBC580/500	-7,15	-13,63	-9,69	-4,61	-12,22	-11,82	-1,22	-12,55	-7,13
Constant	-38,23	-64,46	-52,23	-30,87	-49,34	-45,94	-52,26	-66,15	-67,70

Table 5. Classification of analyzed hexaploid wheat accessions to different genetic groups based on the principal component and canonical discriminant analyses of RAPD data.

#	Cat #	Name	Origin	GH[1]
1	2	3	4	5
		Group I		
1	40182	Gulgeri	Russia, Daghestan	5
2	5294	Minhardi	USA	5
3	6176	Turkey Red	"	5
4	8518	Moskovskaya 2411	Russia	5
5	9204	Banatka	"	5
6	10107	"	Ukraine	5
7	10245	Gostianum 237	Russia, Saratov	5
8	10359	Zemka	Ukraine	5
9	21841	Stepnyachka	"	5
10	26550	Banatka	Poland	5
11	29924	Ferrugineum 1239	Ukraine	5
12	31919	DS 2444/2	Russia	5
13	41002	Banatka	Moldova	5
14	43072	Iohardi	USA	5
15	9701	Sandomirka	Poland	5
16	9815	"	"	5
17	22418	"	Russia	5
18	35736	"	Ukraine	5
19	36515	Landrace	Russia	5
20	25115	Wagenburger	Switzerland	1
21	5188	Sammetweizen	Sweden	5
22	31235	Heines Kolben	Germany	1
23	15594	Prelude	Canada	1
24	22132	Red Bobs	"	1
		Group II		
1	49000	Landrace	Afghanistan	5
2	44061	"	China, Xinjiang	1
3	13376	"	Russia, Siberia	1
4	26026	"	China	1
5	15616	"	Mongolia	1
6	43663	"	China, Gansu	1
7	45819	"	Iran	5

Table 5 (continued)

#	Cat #	Name	Origin	GH[1]
1	2	3	4	5
		Group III		
1	1906	Poltavka	Ukraine	1
2	23348	Landrace	Russia, Siberia	1
3	6161	Fife	Canada	1
4	6414	Pusa 4	India	1
5	44048	Syao-Hie-Schan-Tou	China, Gansu	1
		Group IV		
	23776	Punjab Type 10	India	
1				
2	23797	Punjab Tipe 19	"	1
3	23798	Punjab Tipe 20	"	1
4	23800	Punjab Tipe 18	"	1
5	23807	Punjab Tipe 21	"	1
6	23811	Punjab Tipe 15	"	1
7	23817	Punjab Tipe 16	"	1
8	24385	Pusa 12	"	1
9	30629	Gandum, 208-var.1/63	Pakistan	1
10	30636	Boojri, 210-var.6/14	"	1
11	30665	Thori, 223-var.3/33	"	1
12	30679	227-var.55/29	"	1
13	33411	Ghanum	"	1
14	23890	"	"	1
15	49916	Zarya	Russia	5
		Group V		
1	53653	Ahtyrchanka	Ukraine	5
2	53678	Kinelskaya 4	Russia	5
3	54610	Zarya 2	"	5
4	57653	Volgogradskaya 84	"	5
5	58516	Don 85	"	5
6	58801	Spartanka	"	5
7	43920	Mironovskaya 808	Ukraine	5
		Group VI		
1	44098	Lo-to-mai	China, Hubei	5
2	42212	Landrace	"	1
3	28681	O 6C	China, Sichuan	1
4	28750	58	China, Anhui	1
5	29108	K 6 A	China, Guizhou	1
6	42585	Lu-Pin-Syao-Mai	China, Guandong	1
7	42966	Lin-sian-sitz-mie	China, Hunan	3
8	44113	U-Man-Syao-Mai	China, Jiangsi	1

Table 5 (continued)

#	Cat #	Name	Origin	GH[1]
1	2	3	4	5
		Group VII (*T. spelta*)		
1	24706	Oberkulmer Rotkorn	Switzerland	5
2	24709	Leistaler Rotkorn	"	5
3	20543	Landrace	Spain	1
4	20558	Pinevas	"	1
5	20591	Landrace	"	3
6	20625	"	"	1
		Group VIII		
1	23924	Gulgeri	Russia, Daghestan	5
2	26926	Line 221	"	1
3	32467	Landrace	"	1
4	34178	Bugda	Azerbaijan	5
5	31134	Arazbugdasi	"	5
6	34695	Landrace	"	5
		Group IX		
1	23903	Gulgeri	Russia, Daghestan	5
2	41161	Tau-bugda	"	5
3	18711	Landrace	Azerbaijan	5
4	18719	"	"	5

GH[1] – grouth habitat: 1 – spring; 3 – semiwinter; 5 – winter.

Thus, based on the results of this work, the following conclusions have been reached:

1) A set of 28 primers has been formed, and the possibility of using RAPD analysis to reveal hexaploid wheat polymorphism has been demonstrated.

2) All studied hexaploid wheats form a common gene pool with a complex genetic structure: nine distinctive genetic groups of accessions were identified.

3) As shown by the investigation of the RAPD polymorphisms of complex genetic systems such as hexaploid wheats, the use of statistical analysis on various taxonomic levels with gradually increasing accession numbers is expedient. Several independent statistical analyses of specific genetic groups are recommended for the confirmation of this classification.

4) The approach to the analysis of the gene pool structure of wheat using the DNA markers described in this work might be interesting for developing a genetic classification of this crop. Eighty-two accessions, which are typical representatives of nine distinctive genetic groups, might be used in any research on the evaluation of wheat polymorphism.

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RAPDマーカーを用いたコムギ遺伝資源の遺伝的変異の評価

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要 旨

A, B, Dゲノムを持つ六倍体コムギ、*Triticum aestivum*, *T. compactam*, *T. spelt*, *T. spaerococcum*, *T. macha*, *T. vavilovi*, 及び*T. petropavlovski*の類縁関係をDNAレベルで明らかにするためRAPD法によるDNA多型解析を行った。28種類のオリゴクヌクレオチドプライマーを用いて414系統・品種のゲノムDNAを調べたところ、多型性を示す137種類のDNA断片が得られた。得られたデータをクラスター分析するとともに主成分分析した結果、9 種類の遺伝的グループに類別されることが明らかとなった。

それぞれの遺伝的グループは生態地理的な分類に基づく4 種類の亜種、*eurasiaticum*、*irano-turkestanicum*、*indicum*、及び*sinicum*に概ね対応していた。正準判別分析による解析では、32種類の多型性DNAのデータのみで主成分分析による9 種類の遺伝的グループに相当するグループを類別できることが明らかとなった。これらのRAPD法で得られる多型性DNAの多変量解析の結果に基づき、9 種類の各遺伝的グループを代表する系統・品種を抽出した。今後これらの系統はコムギ遺伝資源の遺伝的な位置付けの評価に利用できると考えられる。

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