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Phylogeography of hexaploid wheat complex based on RAPD data

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Introduction

Within the hexaploid complex common wheat is the most important species from the economic point of view. The problem of broadening the wheat genepool by introducing new sources of genetic variability into the breeding programs is being extensively discussed (Feldman and Sears, 1981; Gale and Miller, 1987; Heslop-Harrison, 2002). Landraces of hexaploid wheats, which may possess potentially useful genetic variability for stress resistance and tolerance (Moghaddam *et al.*, 1998) as well as for high grain quality characters (Dotlaèil *et al.*, 2000), will become increasingly important as sources of new variations for broadening the elite germplasm. The process of landraces evolution began already in prehistoric times. For thousands of years the landraces were evolving under influence of natural and artificial selection performed by many generations of farmers. The differences in soil and climatic conditions between the areas of wheat cultivation and heterogeneity of environments within the areas have led to the development of the large number of landraces adapted to different local environments and characterized by yield stability in conditions of traditionally low input agricultural systems (Jakubtziner, 1957; Zeven, 1998). A relatively small portion of the landraces was involved into wheat breeding programs, mainly in the last Century.

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Yet there are problems for effective transferring of gene complexes and the controlled traits from landraces into improved cultivars (Heslop-Harrison, 2002). In the future the molecular biotechnologies will allow to characterize the genetic nature of valuable agronomic traits in more details, and to develop precise systems of DNA markers for controlling the purposeful transfer of gene complexes controlling these characters (Paull *et al.*, 1998; Marshall *et al.*, 2001; Koebner and Summers, 2003). This will allow much a more effective exploitation of landraces into the wheat breeding programs.

Assessment of the genetic diversity of wheat landraces is the main prerequisite for germplasm conservation and management of large-scale collections (Langridge *et al.*, 2001). In the VIR germplasm collection more than ten thousand landraces of hexaploid wheats originated in different countries of the world are maintained. Attempts of their classification with the use of morphological and agronomical traits have been undertaken (Flaksberger, 1935; Palmova, 1935; Vavilov, 1935, 1964; Jakubtziner, 1957; Dorofeev *et al.*, 1979). However, until now little information is available on phylogeography (Schaal *et al.*, 2003) of wheat providing insight into distribution of its variability in historical and geographical contexts.

Recently new approaches for fast and effective analysis of DNA polymorphism have been developed. Among them a simple and rather sensitive method of RAPD analysis is widely used for estimating the genetic diversity within the complex of hexaploid wheats (Dweikat *et al.*, 1993; Joshi and Ngyuen, 1993; Cao *et al.*, 1998, 2000; Sun *et al.*, 1998; Guadagnuolo *et al.*, 2001; Sun *et al.*, 2003). Moreover, the computer programs suitable for the classification of large sets of accessions by means of different methods of multivariate statistics are now available. The objectives of this work were to evaluate using RAPD markers the genetic variation in a set of 414 hexaploid wheats accessions originated in different regions of the world and to relate genetic diversity patterns to their geographical origin.

To accomplish these aims the following tasks were undertaken: (i) to generate, on the basis of the VIR germplasm collection, a representative set of landraces and old cultivars most completely reflecting ecogeographical diversity of hexaploid wheats; (ii) to select primers, out of available commercial sets, that most successfully reveal distinctions between the accessions; (iii) to classify the genetic variability among landraces and old cultivars of hexaploid wheats by the degree of RAPD pattern similarity, using different methods of multivariate statistics, and to define the relationships between the landraces and some of the Russian and Japanese modern cultivars; (iv) to compare the classification of accessions obtained by RAPDs with their distribution over regions of origin and taxonomy.

Material and methods

Material

In this work 329 landraces and old cultivars belonging to different hexaploid wheats with AABBDD genome composition were chosen from the VIR germplasm collection (Table 1). Selection of the accessions was carried out following the

Table 1. Accessions, their origin and classification according to N. Vavilov (1964)

Species, subspecies	Code[a]	Country (code) of origin
T. macha	MA 1–2	Georgia (GEO)
T. spelta[b]		
ssp. *spelta*	SP 1–10	Germany (DEU), Spain (ESP), Switzerland (CHE)
ssp. *kuckuckianum*	SK 1–16	Azerbaijan (AZE), Iran (IRN), Tajikistan (TJK), Turkmenistan (TKM), Uzbekistan (UZB)
T. Vavilovianum=T. vavilovii	VA 1–2	Armenia (ARM)
T. compactum		
ssp. *armeno-turkestanicum*	CA 1–19	Afghanistan (AFG), Armenia (ARM), Iraq (IRQ), Palestine (PAL), Tajikistan (TJK), Turkey (TUR), Uzbekistan (UZB)
ssp. *eurasiaticum*	CE 1–13	China (CHN), Germany (DEU), Great Britain (GBR), Italy (ITA), Mongolia (MNG), Russia (RUS), Sweden (SWE), USA
ssp. *sinicum*	CS 1–3	China (CHN)
T. vulgare =T. aestivum		
ssp. *irano-turkestanicum*	IT 1–47	Afghanistan (AFG), Azerbaijan (AZE), China (CHN), Iran (IRN), Palestine (PAL), Syria (SYR), Tajikistan (TJK), Turkmenistan (TKM), Uzbekistan (UZB)
ssp. *indicum*	IN 1–36	India (IND), Pakistan (PAK)
ssp. *sinicum*	SI 1–50	China (CHN), Japan (JPN)
ssp. *eurasiaticum*	EU 1–149	Albania (ALB), Algeria (DZA), Argentina (ARG), Armenia (ARM), Australia (AUS), Azerbaijan (AZE), Belarus (BLR), Canada (CAN), France (FRA), Georgia (GEO), Germany (DEU), Great Britain (GBR), Greece (GRC), Hungary (HUN), Italy (ITA), Moldova (MDA), Mongolia (MNG), Morocco (MAR), Netherlands (NLD), Palestine (PAL), Poland (POL), Portugal (PRT), Russia (RUS), Spain (ESP), Sweden (SWE), Switzerland (CHE), Tunisia (TUN), Turkey (TUR), Ukraine (UKR), USA, Yugoslavia (YUG)
ssp. *abyssinicum*	AB 1–8	Ethiopia (ETH)
modern cultivars	MC 1–55	Japan (JPN), Kazakhstan (KAZ), Russia (RUS), Ukraine (UKR), Yugoslavia (YUG)
T. sphaerococcum	SH 1–3	India (IND), Pakistan (PAK)
T. petropavlovskyi[b]	PT 1	China (CHN)

[a] VIR catalogue numbers and names of landraces and cultivars are shown. For old Japanese cultivars (SI 1–30) MAFF Genebank catalogue numbers are represented. MA: 1 – 28171 and 2 – 31689; SP: 1 – 1731, 2 – 20543, 3 – Pinevas (20558), 4 – 20591, 5 – 20625, 6 – 20626, 7 – Rufenach Weisskorn (24700), 8 – Oberkulmer Rotkorn (24706), 9 – Oberkulmer Rotkorn (24707) and 10 – Leistaler Rotkorn (24709); SK: 1 – 45364, 2 – 45366, 3 – 45367, 4 – 45368, 5 – 45546, 6 – 45750, 7 – 45814, 8 – 45817, 9 – 45818, 10 – 45819, 11 – 52434, 12 – 52436, 13 – 52439, 14 – 52461, 15 – 52462 and 16 – 53660;

VA: 1 – 29533 and 2 – 30085; CA: 1 – 12581, 2 – 12629, 3 – 13477, 4 – 13524, 5 – 13695, 6 – 14426, 7 – Yailya-bugdai (16067), 8 – 17369, 9 – 17385, 10 – 30070, 11 – Kila mestnaya (42937), 12 – 48997, 13 – 49000, 14 – 52634, 15 – 52640, 16 – 56557, 17 – 62952, 18 – 62953 and 19 – 62954; CE: 1 – Canuto (5167), 2 – 7985, 3 – Teremok (10442), 4 – 13376, 5 – 15616, 6 – Midea (20009), 7 – Kubbweisen (20324), 8 – 26026, 9 – Rymar (33090), 10 – 39751, 11 – Bolshaya golova (43386), 12 – Lyan-shan (43388) and 13 – 44061; CS: 1 – 43663, 2 – Lo-to-mai (44098) and 3 – 44107; IT: 1 – Krasnokoloska (9351), 2 – 12709, 3 – 12823, 4 – 13549, 5 – Buhara-bugdai (13616), 6 – Buhara-bugdai (13617), 7 – Bugdai (14632), 8 – Palestinskaya (15818), 9 – Sary-bugda (16767), 10 – 17112, 11 – 17167, 12 – 17203, 13 – 17298, 14 – 17301, 15 – 17305, 16 – 18641, 17 – 18711, 18 – 18719, 19 – 22526, 20 – 22533, 21 – 25755, 22 – 26011, 23 – Yazga (26019), 24 – 26055, 25 – 26071, 26 – Arazbugdasi (31134), 27 – 31361, 28 – 31362, 29 – 31773, 30 – 31786, 31 – 31858, 32 – 31872, 33 – 34695, 34 – 35347, 35 – 35493, 36 – 35541, 37 – Bobile (35760), 38 – 35798, 39 – 38626, 40 – 39091, 41 – 39139, 42 – Kuldzhinskaya (43245), 43 – Yarkendskaya (43251), 44 – 52499, 45 – 52504, 46 – 52506 and 47 – 52512; IN: 1 – Pusa 4 (6414), 2 – 21258, 3 – Punjab Type 8 (23773), 4 – Punjab Type 10 (23776), 5 – Punjab Type 19 (23797), 6 – Punjab Type 20 (23798), 7 – Punjab Type 24 (23799), 8 – Punjab Type 18 (23800), 9 – Punjab Type 25 (23802), 10 – Punjab Type 21 (23807), 11 – Punjab Type 15 (23811), 12 – Punjab Type 16 (23817), 13 – Punjab Type 11 (23818), 14 – PK M3 (23939), 15 – BW 1 (23940), 16 – PR 2 (23947), 17 – PR 5 (23954), 18 – PR 4 (23962), 19 – 23963, 20 – PR 9 (23974), 21 – W 18 (23982), 22 – Pusa 12 (24385), 23 – 117 var.12/564 (30614), 24 – Sur, 120-var.55/24 (30618), 25 – Spin, 121-var.12/536 (30620), 26 – Gandum, 208–var.1/63 (30629), 27 – Boojri, 210–var.6/14 (30636), 28 – Thori, 212-var.8/1 (30640), 29 – Thori, 213-var.8/5 (30645), 30 – Thori, 223-var.3/33 (30665), 31 – 227-var.55/29 (30679), 32 – Pashmak (33357), 33 – Soor Ghanum (33409), 34 – Ghanum (33411), 35 – Soor Ghanum (33476) and 36 – Soor Ghanum (33537); SI: 1 – Nourin 10 (22479), 2 – Nourin 27 (22483), 3 – Kitakami Komugi (22496), 4 – Akabouzu (22862), 5 – Akadaruma (22865), 6 – Akakomugi (22872), 7 – Igachikugo Oregon (22890), 8 – Saitama 29 (22968), 9 – Shiro Daruma (22981), 10 – Nourin 7 (23007), 11 – Nourin 42 (23012), 12 – Mikuni Komugi (23024), 13 – Kounosu 25 (23107), 14 – Shin Chuunaga (23274), 15 – Shiro Chabo (23276), 16 – Nourin 26 (23283), 17 – Junrei Komugi (23355), 18 – Hiroshima Shipuree (23368), 19 – Shirasagi Komugi (23389), 20 – Eshima Shinriki (23526), 21 – Fukuoka Komugi 18 (23527), 22 – Haya Komugi (23531), 23 – Sada Bouzu (23557), 24 – Soujuku Akage (23566), 25 – Nichirin Komugi (23570), 26 – Hiyoku Komugi (23571), 27 – Nourin 61 (23579), 28 – Gokuwase 2 (23592), 29 – Yuushouki 247 (23593), 30 – Nishikaze Komugi (23714), 31 – 162 (28348), 32 – F 31 (28654), 33 – O 4B (28675), 34 – O 6C (28681), 35 – 58 (28750), 36 – A 31 (28775), 37 – Q 4 (29045), 38 – R 1 (29093), 39 – K 6 A (29108), 40 – 39218, 41 – Guisuiskaya Syao-bai-mai (41211), 42 – Zun-Nun 28 (41562), 43 – 42212, 44 – Lu-pin-syao-mai (42585), 45 – He-shan-to (42905), 46 – Lin-sian-sitc-me (42966), 47 – Ba-dun-guon-tao-pe-me (42999), 48 – Bei-lei-hun-huo-mai (44002), 49 – Syao-he-shan-tou (44048) and 50 – U-man-syao-mai (44113); EU: 1 – Banatka pozdnyaya (457), 2 – Sandomirka (622), 3 – Poltavka (1906), 4 – Banatka (2167), 5 – Krymka (2606), 6 – Sandomirka (2718), 7 – Comeback (3686), 8 – Florence (3704), 9 – Krasnaya (3902), 10 – Marquis (5026), 11 – Sammetweizen (5188), 12 – Reliable (5226), 13 – Minhardi (5294), 14 – Harvest Queen (5813), 15 – Hussar (5907), 16 – Japhet (6025), 17 – Fife (6161), 18 – Turkey Red (6176), 19 – Lyutescens 329 (6234), 20 – Blausamliger Kolben (6260), 21 – Manitoba (7108), 22 – 7954, 23 – Kitchener (8092), 24 – Moskovskaya 2411 (8518), 25 – Ukrainka (8547), 26 – Banatka (9204), 27 – Sary-bugda (9296), 28 – Sandomirka (9463), 29 – Sandomirka (9701), 30 – Sandomirka (9815), 31 – Poltavka (9828), 32 – Banatka (9834), 33 – Banatka (10107), 34 – Dyurabl (10174), 35 – Gostianum 237 (10245), 36 – Albidum 604 (10255), 37 – Zemka (10359), 38 – Banatka (10912), 39 – Poltavka (10968), 40 – Mahmudi Belokoloska (11009), 41 – Kooperatorka (11389), 42 – 11856, 43 – 13694, 44 – 13703, 45 – Sammetweizen (14179), 46 – 14381, 47 – 14388, 48 – 14507, 49 – 14524, 50 – 14562,

51 – 14590, 52 – 14597, 53 – Ceres (14920), 54 – Sandomirka (14977), 55 – Lutescens 62 (15183), 56 – Lyutescens 479 (15188), 57 – Sary-bugda (15306), 58 – Sary-bugda (15330), 59 – Prelude (15594), 60 – 15705, 61 – 16018, 62 – Mahon (16167), 63 – 16430, 64 – Medeheba 226 (16558), 65 – Huguenot (17293), 66 – Candeal (18340), 67 – Rieti (Razza 11) (18513), 68 – Belem (18941), 69 – Amarelo de Barba Branca (20691), 70 – 21216, 71 – Vilmorin 27 (21479), 72 – Kokkinostaro (21693), 73 – Mocho de Espiga Branca (21817), 74 – Stepnyachka (21841), 75 – 21923, 76 – 21932, 77 – Red Bobs (22132), 78 – Milturum 321 (22236), 79 – Sandomirka (22418), 80 – 23348, 81 – Sary-bugda (23900), 82 – Gyulgeri (23903), 83 – Sary-bugda (23922), 84 – Gyulgeri (23924), 85 – Plantahof (24681), 86 – Diamant (25019), 87 – Rouge de Vaumarcus (25064), 88 – Rouge de la Venoge (25071), 89 – Croisement 268 (25085), 90 – Bourgeois (25091), 91 – Wagenburger (25115), 92 – Mansholt Witte Dickkopf III (25151), 93 – Wilhelmina (25162), 94 – Banatka (26550), 95 – Banatka Daleszowska (26582), 96 – Liniya 5407 (26897), 97 – Liniya 221 (26926), 98 – Dika (27878), 99 – Sarrubra (28310), 100 – Red Fife (29394), 101 – Reward (29609), 102 – San Martin (29769), 103 – Ferrugineum 1239 (29924), 104 – Tulunka (30932), 105 – Heines Kolben (31235), 106 – Thule 2 (31723), 107 – DS 2444/2 (31919), 108 – 32467, 109 – 32499, 110 – 32524, 111 – 32559, 112 – Chiddam d'Automne Aepi Blane (33344), 113 – Bugda (34178), 114 – 34576, 115 – Bencubbin (35653), 116 – Sandomirka (35736), 117 – Galgalos (35803), 118 – Dolis Puri (35921), 119 – Dolis Puri (35922), 120 – 36515, 121 – Gyulgeri (37379), 122 – Sandomirka (37481), 123 – 38162, 124 – 38312, 125 – Dolis Puri 35-4 (38398), 126 – Virtus (38481), 127 – Gabo (38574), 128 – Banatka (39008), 129 – 39511, 130 – Mulah (39794), 131 – 40001, 132 – 40003, 133 – Squarehead's Master B/4 (40072), 134 – Vilmorin 29 (40074), 135 – Gyulgeri (40182), 136 – San Giorgio (40297), 137 – Banatka (41002), 138 – Tau-bugda (41161), 139 – Cappelle-Desprez (41571), 140 – Vilmorin 53 (41574), 141 – Virgilio (41722), 142 – Barleta Benvenuto (41778), 143 – 42517, 144 – Novaro (42747), 145 – Diana (43060), 146 – Iohardi (43072), 147 – Bol-bugda (43827), 148 – 46283 and 149 – 46317; AB: 1 – Sindi (19014), 2 – 19020, 3 – 19021, 4 – 19032, 5 – 19038, 6 – 19042, 7 – 19608 and 8 – 19625; MC: 1 – Saratovskaya 29 (40599), 2 – Bezostaya 1 (42790), 3 – Valuevskaya (43738), 4 – Mironovskaya 808 (43920), 5 – Krasnodarskaya 39 (45879), 6 – Odesskaya 51 (46620), 7 – Albidum 114 (46731), 8 – Leningradka (47882), 9 – Bogarnaya 56 (48761), 10 – Moskovskaya 35 (48762), 11 – Odesskaya 66 (49255), 12 – Mironovskaya yarovaya (49882), 13 – Zarya (49916), 14 – Partizanka (51558), 15 – Krasnovodopadskaya 210 (51729), 16 – Tambovitca (51737), 17 – Ahtyrchanka (53653), 18 – Kinelskaya 4 (53678), 19 – Tarasovskaya 29 (53808), 20 – Dalnevostochnaya 10 (53986), 21 – Kutulukskaya (54213), 22 – Zarya 2 (54610), 23 – Albdum 12 (54701), 24 – Kolos 80 (55182), 25 – Olimpya (55185), 26 – Istok (56421), 27 – Simbirka (56928), 28 – Voronezhskaya 6 (57114), 29 – Volgogradskaya 84 (57653), 30 – Don 85 (58516), 31 – Spartanka (58801), 32 – Nemchinovskaya 52 (59269), 33 – Irgina (60074), 34 – Ivolga (60975), 35 – Omskaya 20 (61189), 36 – Aka kawaka, 37 – Akasabi shirazu, 38 – Chihoku Komugi, 39 – Haruhikari, 40 – Haruminori, 41 – Harunoakabono, 42 – Haruyutaka, 43 – Hokkai 240, 44 – Hokuei, 45 – Hokushin, 46 – Horoshiri Komugi, 47 – Mika Komugi, 48 – Nourin 29, 49 – Nourin 3, 50 – Nourin 35, 51 – Nourin 62, 52 – Nourin 8, 53 – Poson 1, 54 – Taisetsu Komugi and 55 – Takure Komugi; SH: 1 – 13177, 2 – 23790 and 3 – 23890; PT: 1 – Maik (43376).

[b] T. spelta is divided into two subspecies and T. petropavlovskyi designated as separate species according to classification of Dorofeev et al. (1979).

classification developed by N. Vavilov (1964). According to this classification, all the vast diversity of hexaploid wheats was pooled into a limited number of agroecological groups, subspecies and species. The accessions of each agroecological group have certain geographical areas of cultivation and are characterized by a specific combination of morphological traits and physiological properties depending on plant response to temperature, humidity, photoperiod and pathogens. The representative set of accessions included landraces and old cultivars belonging to 45 agroecological groups of hexaploid wheats: *T. macha* Dek. et Men., *T. spelta* L., *T. Vavilovianum* Jakubz. = *T. vavilovii* (Thum.) Jakubz, *T. compactum* Host, *T. sphaerococcum* Perc. and *T. vulgare* Host. Among them the common wheat (*T. vulgare* = *T. aestivum* L.) was represented by five subspecies (*irano-turkestanicum* Vav., *indicum* Vav., *sinicum* Vav., *eurasiaticum* Vav. and *abyssinicum* Vav.), and the club wheat (*T. compactum*) by three subspecies (*armeno-turkestanicum* Vav., *eurasiaticum* Vav. and *sinicum* Vav.). Accessions under analysis originated from 44 countries. Each agroecological group contained from 2 to 25 accessions.

It should be noted that the outlined classification of hexaploid wheats was based on the study of accessions collected mainly by Vavilov and his collaborators. The significant number of these accessions has been maintained in the VIR germplasm collection until the present time. Therefore the accessions mentioned by Vavilov in his classification were included in the representative set. Additionally, other accessions were included in the analysis if they were part of the VIR germplasm collection before 1940 and the regions of origin were within the wheat area of cultivation from the agroecological group described by Vavilov. Thus, origin countries of the landraces and old cultivars covered the major areas of wheat cultivation. Moreover, almost the whole described ecogeographical diversity within those areas was included.

The landraces of Asian spelt wheat *T. spelta* ssp. *kuckuckianum* Gokg. and *T. petropavlovskyi* Udacz. et Migusch were also studied. These wheat forms have been found more recently (Dorofeev *et al.*, 1979). Moreover, 30 old Japanese cultivars selected from the MAFF Genebank (Tsukuba, Japan) were studied. For the evaluation of genetic relationships between the modern cultivars and landraces, 55 common wheat modern cultivars mainly from Russia and Japan, were chosen. Out of them 20 Japanese cultivars from Hokkaido region were supplied by Dr. T. Kuwabara (Upland Agriculture Research Center, Hokkaido, Japan), and the other cultivars were selected from the VIR germplasm collection. Thus, the total number of accessions analyzed was 414.

RAPD assay

Leaf tissues were harvested from 2- to 3-week-old seedlings (bulk of 15–25 seedlings per accession). DNA for RAPD assay was extracted from freeze-dried tissue using a microscale method with cetyltrimethylammonium bromide (CTAB) (Saghai-Maroof *et al.*, 1984). DNA concentrations were estimated and standardized on a Beckman 7400 spectrophotometer. The mixtures for polymerase chain reaction (PCR)

were prepared using kits for amplification manufactured by Wako, Japan. Given for a 10 μl of total volume they contained: 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2 mM MgCl₂, 0.2 mM each of dATP, dCTP, dGTP and dTTP, 1 mM primer, approximately 40 ng of template DNA, and 0.3 units of *Taq* DNA polymerase. PCR mixtures were prepared in 96-well microplates and subjected on the Perkin Elmer 9600 thermal cycler to 45 cycles of 94°C (30 s), 42°C (30 s) and 72°C (1 min). After the addition of 3 μl 6×dye solution (0.1% bromophenol blue, 0.1% xylene cyanol FF and 15% Ficoll), 10 μl of each mixture were loaded into 2% agarose gels. Gels were 24 cm long and 12 cm broad with 26 lanes, and four rows of wells. A mixture of 100 bp DNA ladders was used as size marker. After loading of PCR products and DNA size markers the gels were run in a Sunrise submarine unit (from GibcoBRL) in 0.5×TBE buffer at 5 V/cm for approx. 1.5 h, until the bromophenol blue marker migrated approx. 5 cm. After staining with ethidium bromide the gels were documented on the UV FA 1100 image system (from AL-C, Japan).

Statistical analyses of data

RAPD patterns were scored on gels images assigning a number to each band. For subsequent statistical analyses the data were coded in a binary form as presence or absence (1 and 0) of a band in the line. Only polymorphic and reproducible bands in repetitive runs were included in the raw data matrix. For statistical treatment of the data three different methods of multivariate statistics were used: the principal component (PCA), the cluster (CA) and the canonical discriminant (CDA) analyses. Calculations were performed using the software package STATISTICA 6.0 (StatSoft, Inc., 2001). The PCA (Q-technique) was used for the identification of possible groups in analyzed set of accessions based on the RAPD data (Mohammadi and Prasanna, 2003). The original data matrix was transposed, and the factor loadings were calculated as classification scores of the accessions. All studied accessions were divided into groups according to maximal value of their factor loadings on the certain principal component obtained in the analysis (at the given threshold of *euginevalue* = 1.0). For the CA the genetic similarity matrices using Simple Matching (SMC) (Sokal and Michener, 1958) and Nei's (Nei and Li, 1979) coefficients were calculated. These matrices were submitted to CA by the hierarchical cluster analysis methods of unweighted pair group method using arithmetic average (UPGMA) (Sneath and Sokal, 1973) and Ward's minimum variance method (Ward, 1963). In UPGMA, the distance between two clusters is the average distance between pairs of observations. In Ward's method, the distance between two clusters is the analysis of variance (ANOVA) sum of squares between the two clusters summed over cluster members. The CDA was used for the construction of *a posteriori* classification of accessions into the groups obtained in the CA with a better than chance accuracy (Kachigan, 1986). The quality of classification received in the CA of RAPD data was estimated by the percentage of correct decisions about the belonging of accessions to the certain group on the basis of computed classification functions in the constructed model of CDA. Also the RAPD bands better discriminating different groups of accessions were detected in the constructed CDA model.

The forward stepwise discriminant analysis with evaluation of the classification results at each step of the analysis was used.

Results

Characterization of RAPD markers

For selection of suitable primers the screening of 708 random 10- and 12-mers oligonucleotide primers from commercial sets of Operon Technology Inc. (OP-X), University of British Columbia (UBC) and Wako (EW) was carried out using four landraces of hexaploid wheats. The majority of the analyzed primers (77.1% of the total number) initiated amplification of DNA fragments giving at electrophoretic separation excessively multicomponent and merging RAPD patterns. With the use of 73 primers (10.3%) no amplification products were registered. Electrophoretic patterns with a reduced number of polymorphic components were obtained with the use of 89 primers (12.6%). Among them, only 28 primers (4.0% of the total number) gave reproducible RAPD patterns for wheat accessions tested in repetitive experiments. They were applied to all accessions in further analysis (Table 2). The analysis of PCR products of 414 wheat accessions has shown that each of the primers initiated synthesis from 1 to 12 polymorphic DNA fragments ranging in size from 130 to 1000 bp. These primers probably amplified low copy and fast diverging genomic DNA sequences. Out of 217 recorded amplified DNA fragments 48 were not polymorphic and 32 polymorphic products were excluded from further analysis due to scoring problems. Finally, 137 polymorphic RAPD products obtained from 414 wheat accessions using 28 primers were included in the data matrix. On average 4.9 polymorphic components were revealed with each primer. Based on RAPD patterns all the analyzed wheat accessions could be distinguished.

Principal component analysis

The PCA of RAPD data indicated a complex pattern of genetic relationships between the accessions. By the degree of their RAPD pattern similarity they were pooled into 38 groups corresponding to 38 significant principal components revealed in the analysis. Nine of these, each containing from 5 to 180 accessions, were considered as major groups. The other groups, represented mainly by one or two accessions, were designated as minor groups. On the basis of the PCA, the classification of accessions represented by 38 groups covered 67.6% of the total variability of accessions detected by the RAPD patterns.

Information concerning the composition of major groups is given in Table 3. The most numerous group 1 comprised 180 accessions from different regions of the world. The landraces and old cultivars belonging to common wheat subsp. *eurasiaticum* and modern cultivars of this species were prevalent in this group. More than half of the total number of accessions (55%) represented winter wheat forms. Among them the landraces belonging to agroecological groups (proles)

Table 2. Primers selected for the analysis of 414 wheat accessions, number of polymorphic bands, and DNA fragments included in the model of canonical discriminant analysis and represented in Table 4.

No.	Name	Sequence	Number of bands	DNA fragments included in the model of CDA, bp
1.	OP-A6	GGTCCCTGAC	7	1000, 350
2.	OP-A16	AGCCAGCGAA	12	950, 400, 250
3.	OP-A19	CAAACGTCGG	4	700
4.	OP-A20	GTTGCGATCC	3	
5.	OP-B13	TTCCCCCGCT	8	
6.	OP-D12	CACCGTATCC	5	320, 200
7.	OP-F19	CCTCTAGACC	10	500, 270
8.	OP-E12	TTATCGCCCC	3	
9.	OP-M9	GTCTTGCGGA	4	
10.	OP-M11	GTCCACTGTG	4	360
11.	OP-O1	GGCACGTAAG	7	300
12.	OP-P3	CTGATACGCC	1	
13.	OP-P4	GTGTCTCAGG	4	500
14.	OP-P10	TCCCGCCTAC	6	530
15.	OP-T17	CCAACGTCGT	5	450
16.	OP-U8	GGCGAAGGTT	2	
17.	OP-V6	ACGCCCAGGT	4	380
18.	OP-V9	TGTACCCGTC	10	900, 700
19.	OP-Z11	CTCAGTCGCA	1	
20.	OP-AA8	TCCGCAGTAG	6	500, 400
21.	OP-AB5	CCCGAAGCGA	6	200
22.	OP-AD15	TTTGCCCCGT	3	
23.	OP-AF16	TCCCGGTGAG	1	350
24.	UBC386	TGTAAGCTCG	9	
25.	UBC535	CCACCAACAG	6	200
26.	UBC580	GCGATAGTCC	2	550
27.	EW-5	AAGATCTTACTG	2	
28.	EW-29	GTTATGCAAGGG	2	
	Total		*137*	

hiemale-stepposum (such as 'Banatka' type) and *hiemale-boreale* (such as 'Sandomirka' type) and also some modern cultivars had the maximum values of factor loadings. Factor loadings for all accessions studied are shown on Figure 1. Group 2 was not as numerous as group 1. It contained 52 accessions and almost completely consisted of landraces and old cultivars from Japan and China. Among them 50 accessions represented common wheat including 44 accessions of subsp. *sinicum*. Two accessions were landraces of club wheat. In group 3 landraces from India and Pakistan, which belonged mainly to common wheat subsp. *indicum*, prevaled. All three studied landraces of *T. sphaerococcum* and two landraces of club wheat were also combined there. In group 4 like in group 1 the accessions of common wheat subsp. *eurasiaticum* were mainly grouped, however the majority of accessions (69%) represented spring wheat. The landraces of proles *aestivo-stepposum* (such as 'Poltavka' type) and some landraces from Siberia had maximal values of factor loadings. Accessions of group 5 originated mostly from Central Asia, among them there were accessions of common, spelt and club wheats. The accessions of the
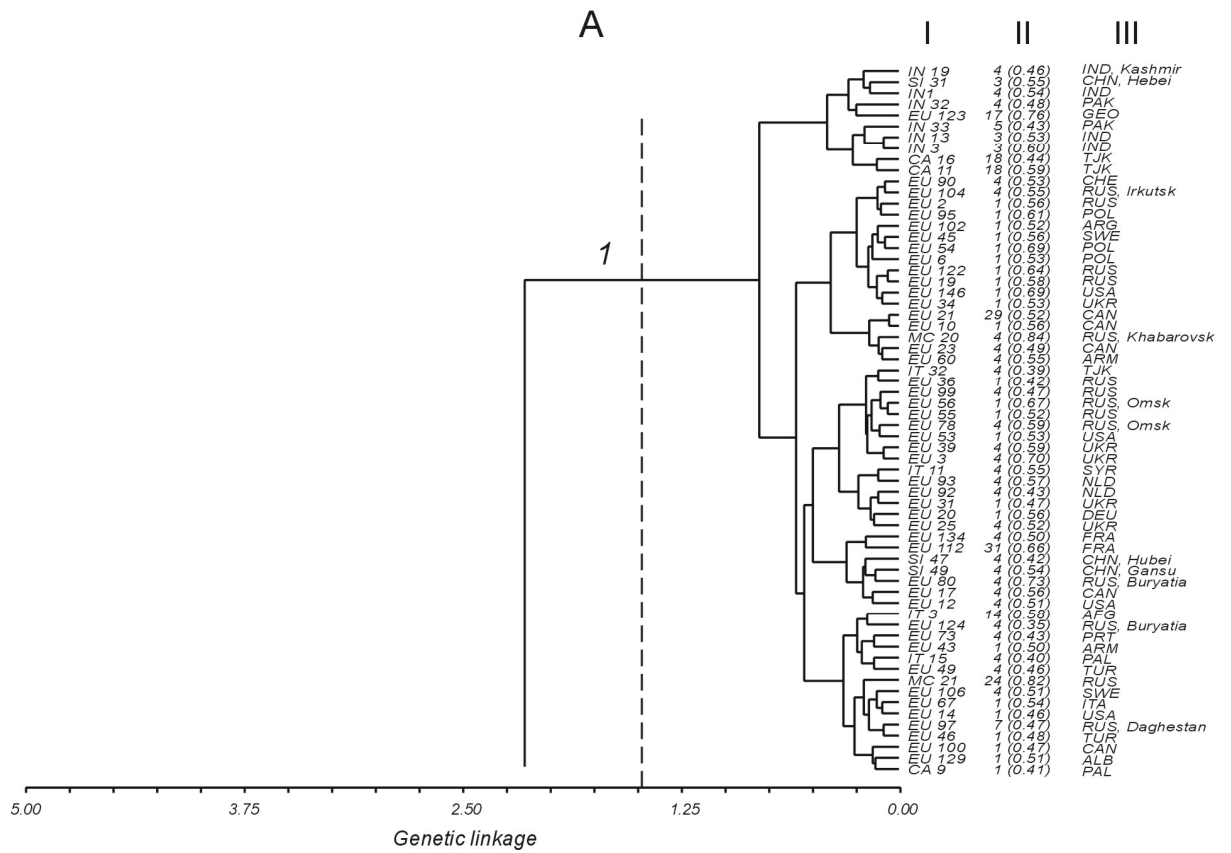
Fig. 1A.

Fig. 1. Distribution of 414 accessions of hexaploid wheats in the phenogram based on RAPD data (Simple Matching coefficient and Ward's algorithm): **A** and **B** showing clusters 1 and 2, respectively, and **C** showing clusters 3–6. (I) code and No. of accession (see Table 1); (II) group (factor loading) based on the PCA of RAPD data; (III) country code and region of origin.
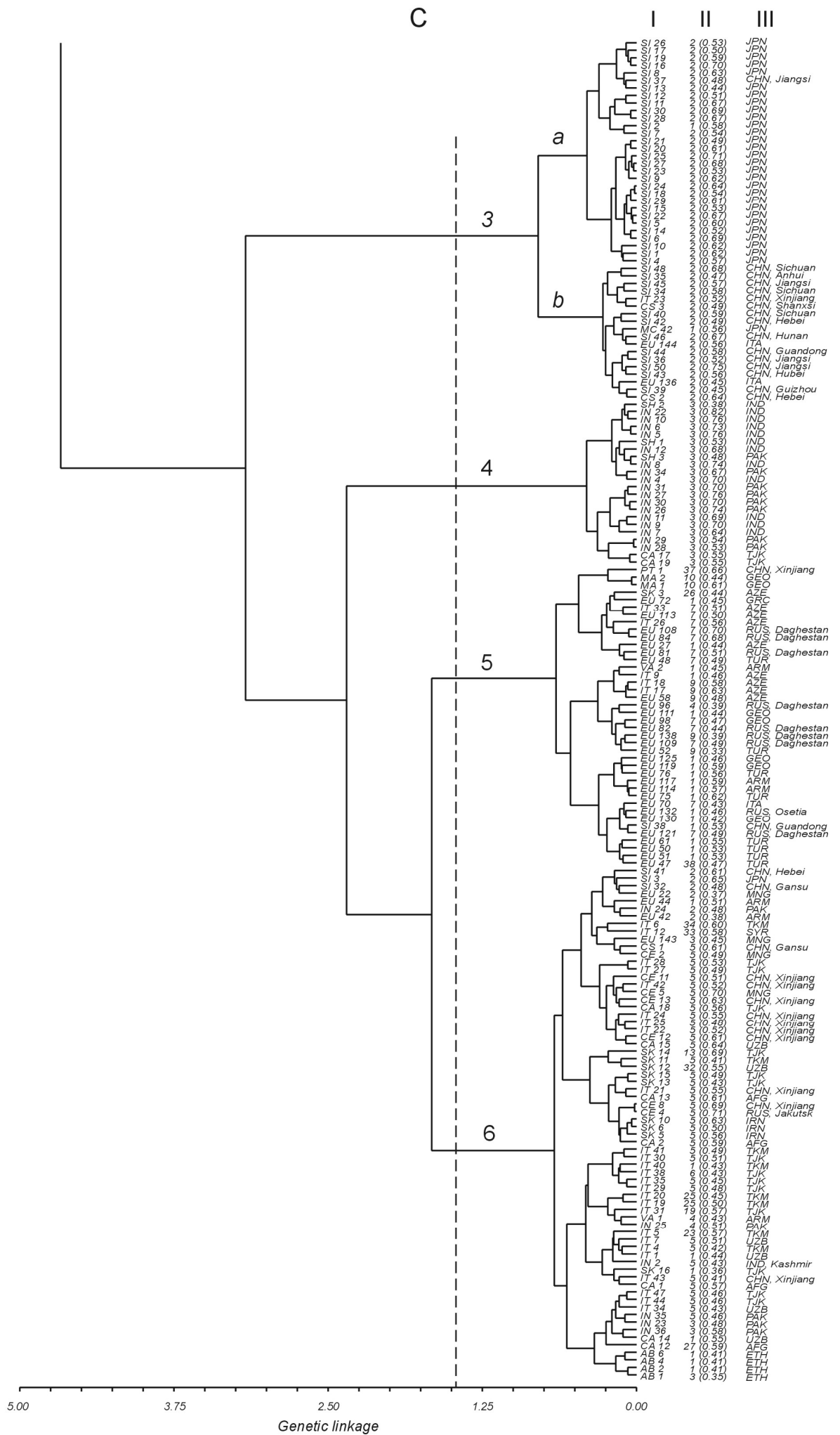
Fig. 1B.

B
          I   II   III

	I	II	III
SP 6	1	(0.58)	ESP
SP 4	21	(0.46)	ESP
SP 5	8	(0.53)	ESP
SP 3	8	(0.49)	ESP
SP 2	8	(0.49)	ESP
SP 9	1	(0.63)	CHE
SP 8	8	(0.66)	CHE
SP 10	8	(0.67)	CHE
SP 7	8	(0.55)	CHE
SK 2	6	(0.37)	AZE
SK 4	6	(0.41)	AZE
SK 1	1	(0.50)	AZE
MC 8	30	(0.61)	RUS
MC 34	30	(0.77)	RUS
IT 8	28	(0.58)	PAL
EU 63	35	(0.59)	MAR
IT 36	5	(0.42)	TKM
CE 1	1	(0.57)	PAL
CE 3	1	(0.49)	GBR
CE 6	1	(0.57)	RUS, Jakutsk
CA 4	7	(0.47)	ITA
CA 6	1	(0.59)	PAL
SP 1	1	(0.48)	TUR
EU 133	1	(0.52)	DEU
IT 46	16	(0.55)	GBR
IT 45	1	(0.55)	TJK
IT 37	1	(0.55)	TJK
EU 115	1	(0.43)	TJK
EU 7	1	(0.51)	AUS
EU 8	1	(0.59)	AUS
EU 86	1	(0.55)	AUS
SK 9	4	(0.48)	SWE
EU 127	1	(0.46)	IRN
EU 38	1	(0.53)	AUS
IN 15	1	(0.64)	HUN
IN 14	1	(0.46)	IND, Kashmir
CA 5	3	(0.50)	IND, Kashmir
CA 3	5	(0.42)	ARM
IT 10	1	(0.47)	ARM
IN 18	1	(0.56)	SYR
IN 21	1	(0.58)	IND, Kashmir
IN 16	1	(0.55)	IND, Kashmir
SI 33	1	(0.45)	IND, Kashmir
IN 20	1	(0.69)	CHN, Sichuan
EU 65	1	(0.51)	PAL
EU 64	1	(0.48)	IND, Kashmir
IT 14	3	(0.55)	TUN
EU 40	12	(0.67)	PAL
IT 2	12	(0.58)	DZA
EU 66	36	(0.50)	AFG
EU 139	1	(0.59)	ESP
EU 140	1	(0.35)	FRA
EU 71	1	(0.51)	FRA
EU 68	1	(0.45)	FRA
AB 7	35	(0.30)	PRT
MC 29	1	(0.50)	ETH
MC 2	6	(0.53)	RUS
MC 16	1	(0.80)	RUS
EU 9	6	(0.58)	CAN
MC 6	1	(0.56)	UKR
MC 19	1	(0.72)	RUS
MC 14	1	(0.84)	YUG
EU 1	1	(0.68)	BLR
MC 11	1	(0.83)	UKR
MC 1	1	(0.68)	RUS
MC 28	1	(0.57)	RUS
MC 27	1	(0.72)	RUS
EU 131	1	(0.53)	RUS, Osetia
EU 149	1	(0.56)	ARM
MC 7	1	(0.57)	RUS
MC 23	1	(0.72)	RUS
MC 15	1	(0.83)	KAZ
MC 5	1	(0.40)	RUS
MC 10	1	(0.79)	RUS
MC 9	1	(0.64)	KAZ
IT 39	1	(0.63)	IRN
AB 8	1	(0.60)	ETH
MC 24	1	(0.69)	RUS
MC 18	6	(0.61)	RUS
MC 22	6	(0.88)	RUS
MC 30	6	(0.70)	RUS
MC 4	6	(0.54)	UKR
MC 17	6	(0.77)	UKR
MC 13	6	(0.87)	RUS
MC 12	6	(0.80)	UKR
EU 57	6	(0.59)	AZE
IT 16	1	(0.53)	AZE
EU 15	1	(0.64)	USA
EU 94	1	(0.69)	POL
MC 31	6	(0.79)	RUS
EU 32	1	(0.62)	UKR
MC 25	6	(0.67)	RUS
EU 62	20	(0.51)	DZA
CA 10	1	(0.60)	IRQ
EU 145	1	(0.54)	SWE
EU 89	1	(0.61)	CHE
EU 88	1	(0.50)	CHE
CE 10	1	(0.45)	DEU
SK 8	4	(0.39)	IRN
EU 69	4	(0.40)	PRT
EU 147	4	(0.39)	AZE
CA 7	15	(0.54)	TUR
AB 5	1	(0.43)	ETH
MC 37	1	(0.56)	JPN
MC 47	11	(0.57)	JPN
MC 55	1	(0.53)	JPN
MC 51	22	(0.67)	JPN
MC 40	1	(0.63)	JPN
MC 49	1	(0.59)	JPN
MC 48	1	(0.58)	JPN
MC 50	1	(0.61)	JPN
MC 39	1	(0.66)	JPN
MC 41	1	(0.50)	JPN
MC 43	1	(0.55)	JPN
MC 44	1	(0.42)	JPN
MC 38	1	(0.66)	JPN
MC 54	1	(0.60)	JPN
MC 3	1	(0.83)	RUS
MC 46	1	(0.50)	JPN
MC 45	1	(0.64)	JPN
MC 52	1	(0.56)	JPN
EU 5	1	(0.64)	UKR
IN 17	1	(0.47)	IND, Kashmir
EU 101	1	(0.63)	CAN
EU 141	1	(0.49)	ITA
EU 142	1	(0.62)	ARG
EU 105	1	(0.73)	DEU
EU 16	1	(0.62)	FRA
EU 148	1	(0.55)	GEO
EU 41	1	(0.66)	UKR
EU 26	1	(0.73)	RUS
MC 32	1	(0.76)	RUS
MC 26	1	(0.63)	RUS
EU 118	1	(0.61)	GEO
MC 53	1	(0.67)	JPN
CE 9	1	(0.61)	USA
MC 36	1	(0.65)	JPN
EU 85	1	(0.62)	CHE
IT 13	1	(0.59)	PAL
EU 110	1	(0.51)	GEO
EU 59	1	(0.70)	CAN
EU 87	1	(0.67)	CHE
EU 128	1	(0.66)	YUG
SK 7	1	(0.62)	IRN
EU 83	1	(0.68)	RUS, Daghestan
EU 126	1	(0.65)	SWE
CE 7	1	(0.65)	SWE
MC 35	1	(0.67)	RUS, Omsk
MC 33	1	(0.69)	RUS
EU 77	1	(0.72)	CAN
EU 91	1	(0.80)	CHE
EU 120	1	(0.82)	RUS
EU 116	1	(0.81)	UKR
EU 28	1	(0.68)	POL
EU 30	1	(0.81)	POL
EU 29	1	(0.84)	POL
EU 79	1	(0.85)	RUS
EU 103	1	(0.82)	UKR
EU 37	1	(0.75)	UKR
EU 35	1	(0.79)	RUS
EU 18	1	(0.76)	USA
EU 11	1	(0.78)	SWE
EU 137	1	(0.77)	MDA
EU 33	1	(0.78)	UKR
EU 24	1	(0.72)	RUS
EU 135	1	(0.68)	RUS, Daghestan
EU 13	1	(0.49)	USA
EU 107	1	(0.80)	RUS
EU 4	1	(0.74)	RUS
EU 74	1	(0.72)	UKR
AB 3	1	(0.50)	ETH

Cluster labels: a, b, c, d, 2

Axis: 5.00 3.75 2.50 1.25 0.00

Genetic linkage

Fig. 1C.

C

I   II   III

a

b

3

4

5

6

SI 26   2 (0.53)   JPN
SI 17   2 (0.59)   JPN
SI 19   2 (0.59)   JPN
SI 16   2 (0.70)   JPN
SI 8   2 (0.63)   JPN
SI 37   2 (0.48)   CHN, Jiangsi
SI 13   2 (0.44)   JPN
SI 12   2 (0.51)   JPN
SI 11   2 (0.67)   JPN
SI 30   2 (0.69)   JPN
SI 28   1 (0.67)   JPN
SI 7   2 (0.54)   JPN
SI 21   2 (0.49)   JPN
SI 20   2 (0.71)   JPN
SI 25   2 (0.68)   JPN
SI 27   2 (0.61)   JPN
SI 23   2 (0.53)   JPN
SI 9   2 (0.62)   JPN
SI 24   2 (0.64)   JPN
SI 18   2 (0.54)   JPN
SI 29   2 (0.61)   JPN
SI 15   2 (0.53)   JPN
SI 22   2 (0.67)   JPN
SI 52   2 (0.60)   JPN
SI 14   2 (0.52)   JPN
SI 6   2 (0.69)   JPN
SI 10   2 (0.62)   JPN
SI 1   2 (0.62)   JPN
SI 4   2 (0.57)   JPN
SI 48   2 (0.68)   CHN, Sichuan
SI 35   2 (0.41)   CHN, Anhui
SI 45   2 (0.57)   CHN, Jiangsi
SI 34   2 (0.58)   CHN, Sichuan
IT 23   2 (0.52)   CHN, Xinjiang
CS 3   2 (0.49)   CHN, Shanxsi
SI 40   2 (0.59)   CHN, Sichuan
SI 42   2 (0.49)   CHN, Hebei
MC 42   1 (0.56)   JPN
SI 46   2 (0.67)   CHN, Hunan
EU 1144 2 (0.56)   ITA
SI 44   2 (0.58)   CHN, Guandong
SI 36   2 (0.59)   CHN, Jiangsi
SI 50   2 (0.75)   CHN, Jiangsi
SI 43   2 (0.55)   CHN, Hubei
EU 136   2 (0.45)   ITA
CS 2   2 (0.64)   CHN, Guizhou
SH 2   3 (0.38)   CHN, Hebei
IN 22   3 (0.82)   IND
IN 10   3 (0.76)   IND
IN 5   3 (0.76)   IND
IN 6   3 (0.73)   IND
SH 1   3 (0.53)   IND
IN 12   3 (0.68)   IND
SH 3   3 (0.48)   PAK
IN 8   3 (0.74)   IND
IN 34   3 (0.67)   PAK
IN 4   3 (0.70)   IND
IN 31   3 (0.70)   PAK
IN 27   3 (0.76)   PAK
IN 30   3 (0.70)   PAK
IN 26   3 (0.74)   PAK
IN 11   3 (0.69)   IND
IN 9   3 (0.70)   IND
IN 7   3 (0.64)   PAK
IN 29   3 (0.54)   PAK
CA 17   3 (0.55)   TJK
CA 19   3 (0.55)   TJK
PT 1   37 (0.66) CHN, Xinjiang
MA 2   10 (0.44) GEO
MA 1   10 (0.61) GEO
SK 3   26 (0.44) AZE
EU 72   1 (0.45)   GRC
IT 33   7 (0.51)   AZE
EU 113   7 (0.50)   AZE
IT 26   7 (0.56)   RUS, Daghestan
EU 108   7 (0.70)   RUS, Daghestan
EU 84   7 (0.68)   AZE
EU 27   1 (0.44)   RUS, Daghestan
EU 81   7 (0.51)   TUR
EU 48   7 (0.49)   ARM
VA 2   1 (0.45)   AZE
IT 9   1 (0.46)   AZE
IT 17   9 (0.58)   AZE
EU 58   9 (0.63)   AZE
EU 96   4 (0.48)   RUS, Daghestan
EU 111   1 (0.39)   GEO
EU 98   7 (0.44)   GEO
EU 82   7 (0.47)   RUS, Daghestan
EU 138   9 (0.39)   RUS, Daghestan
EU 109   7 (0.49)   RUS, Daghestan
EU 52   9 (0.33)   TUR
EU 125   1 (0.46)   GEO
EU 119   1 (0.59)   GEO
EU 76   1 (0.56)   TUR
EU 117   1 (0.59)   ARM
EU 114   1 (0.57)   ARM
EU 75   1 (0.62)   TUR
EU 70   7 (0.43)   ITA
EU 132   1 (0.46)   RUS, Osetia
EU 130   1 (0.42)   GEO
SI 38   1 (0.53)   CHN, Guandong
EU 121   7 (0.49)   RUS, Daghestan
EU 61   1 (0.55)   TUR
EU 50   1 (0.53)   TUR
EU 51   1 (0.53)   TUR
EU 47   38 (0.47) TUR
SI 41   2 (0.61)   CHN, Hebei
SI 3   2 (0.65)   JPN
EU 32   2 (0.48)   CHN, Gansu
EU 22   2 (0.37)   MNG
EU 44   1 (0.51)   ARM
IN 24   2 (0.48)   PAK
EU 42   2 (0.38)   ARM
IT 6   34 (0.60) TKM
IT 12   33 (0.58) SYR
EU 143   3 (0.45)   MNG
CS 1   5 (0.61)   CHN, Gansu
CE 2   5 (0.49)   MNG
IT 28   5 (0.53)   TJK
IT 27   5 (0.49)   TJK
CE 11   5 (0.51)   CHN, Xinjiang
IT 42   5 (0.52)   CHN, Xinjiang
CE 5   5 (0.70)   MNG
CE 13   5 (0.63)   CHN, Xinjiang
CA 18   5 (0.56)   TJK
IT 24   5 (0.55)   CHN, Xinjiang
IT 25   5 (0.40)   CHN, Xinjiang
IT 22   5 (0.52)   CHN, Xinjiang
CE 12   5 (0.61)   CHN, Xinjiang
CA 15   5 (0.64)   UZB
SK 14   13 (0.69) TJK
SK 11   5 (0.41)   TKM
SK 12   32 (0.55) UZB
SK 15   5 (0.49)   TJK
SK 13   5 (0.43)   TJK
IT 21   5 (0.55)   CHN, Xinjiang
CA 13   5 (0.61)   AFG
CE 8   5 (0.69)   CHN, Xinjiang
CE 4   7 (1)   RUS, Jakutsk
SK 10   5 (0.63)   IRN
SK 6   5 (0.50)   IRN
SK 5   5 (0.56)   IRN
CA 2   5 (0.59)   AFG
IT 41   5 (0.49)   TKM
IT 30   5 (0.51)   TJK
IT 40   5 (0.43)   TKM
IT 38   6 (0.43)   TJK
IT 35   5 (0.45)   TJK
IT 29   5 (0.48)   TJK
IT 20   25 (0.45) TKM
IT 19   25 (0.50) TKM
IT 31   19 (0.57) TJK
VA 1   4 (0.43)   ARM
IT 25   4 (0.51)   PAK
IT 5   23 (0.51) TKM
IT 7   5 (0.51)   UZB
IT 4   5 (0.42)   TKM
IT 1   1 (0.44)   UZB
IN 2   5 (0.43)   IND, Kashmir
SK 16   1 (0.36)   TJK
IT 43   5 (0.57)   CHN, Xinjiang
CA 1   5 (0.41)   AFG
IT 47   5 (0.46)   TJK
IT 44   5 (0.48)   TJK
IT 34   5 (0.43)   UZB
IN 35   5 (0.46)   PAK
IN 23   5 (0.48)   PAK
IN 36   3 (0.58)   PAK
CA 14   5 (0.55)   UZB
CA 12   27 (0.59) AFG
AB 6   1 (0.41)   ETH
AB 4   1 (0.41)   ETH
AB 2   1 (0.41)   ETH
AB 1   3 (0.35)   ETH

5.00   3.75   2.50   1.25   0.00

Genetic linkage

Table 3. Characterization of major groups revealed by the principal component analysis of RAPD data for 414 accessions of hexaploid wheats

Group	Code number of accessions	Country code of origin	Total accessions
1	EU (96), MC (38), IT (10), CA (7), AB (7), IN (6), CE (5), SK (4), SP (3), SI (3), VA (1)	ALB, ARG, ARM, AUS, AZE, BLR, CAN, CHE, CHN, DEU, ESP, ETH, FRA, GEO, HUN, IND, IRN, IRQ, ITA, JPN, KAZ, MDA, PAL, POL, RUS, SWE, SYR, TJK, TUR, UKR, USA, UZB, YUG	180
2	SI (44), EU (4), CS (2), IT (1), IN (1)	ARM, CHN, ITA, JPN, MNG, PAK	52
3	IN (22), SH (3), CA (2), EU (2), SI (1), AB (1)	CHN, ETH, IND, MNG, PAK, TJK	31
4	EU (23), IN (4), IT (3), SI (2), SK (1), VA (1), MC (1)	ARM, AZE, CAN, CHE, CHN, FRA, IND, IRN, NLD, PAK, PAL, PRT, RUS, SWE, SYR, TJK, TUR, UKR, USA	35
5	IT (18), CE (7), CA (6), SK (6), IN (3), CS (1)	AFG, ARM, CHN, IND, IRN, MNG, PAK, RUS, TJK, TKM, UZB	41
6	MC (11), SK (2), IT (1)	AZE, RUS, UKR	15
7	EU (11), IT (2), CE (1)	AZE, GEO, ITA, RUS, TUR	14
8	SP (6)	CHE, ESP	6
9	EU (3), IT (2)	AZE, RUS, TUR	5
	Total		379

latter species had maximal values of factor loadings. Modern cultivars from Russia prevailed in group 6. The landraces of common wheat from Northern Caucasus region, Transcaucasia and Turkey were mainly pooled in groups 7 and 9. Group 8 consisted only of the spelt wheat landraces from Europe. Among 29 minor groups it is necessary to note that group 10 contains only two investigated landraces of *T. macha* and group 37 includes the only studied landrace of *T. petropavlovskyi*. Thus, the PCA has revealed nine major groups of genetically similar accessions in the investigated set. The accessions included in each of these groups belonged as a rule to different species of hexaploid wheats, but originated mainly from the same region of Asia or Europe.

Cluster analysis

The comparison of results of the CA performed using Nei's and SMC similarity coefficients has shown, that SMCs provided a more consistent grouping with the PCA results and distribution of accessions by the origin. Therefore in the paper the results of the CA with usage of SMCs are shown. Taking into account a significant share of rare RAPD bands revealed in the analysis, it was necessary to give the same evaluation a similarity of RAPD patterns both for presence and absence of bands, to balance the importance of genome changes resulting in certain amplified DNA fragments emergence or vanishing, SMC gives such a possibility.

In the matrix of similarity coefficients, all possible 85491 pairs of comparisons for the 414 accessions were taken into account. During matrix analysis it has been

shown that the range of SMC variation was in the limits from 0.59 (between the landraces k-38162 from Georgia and k-39139 from Turkmenistan) to 0.99 (between modern Japanese cultivars Nourin 29 and Nourin 3, and also between landraces 'Sandomirka' k-36515 and k-35736 from Russia and Ukraine, accordingly) with the mean value of 0.78.

The phenogram constructed for all 414 accessions by UPGMA method was of difficult interpretation and the low level of distinctions between separate clusters indicated that identification of most of them would not be correct. At the same time the analysis of the phenogram constructed by Ward's method demonstrated that all the investigated accessions were combined into six clusters with linkage distance of separate clusters being at least 1.5 times higher than that of accessions within each of the clusters. Moreover, the major groups revealed in the PCA corresponded more precisely to separate clusters on the phenogram constructed with the usage of Ward's algorithm. It is probable that this algorithm using of SMC is more suitable for studying genetic variation in less differentiated crops (Li and Nelson, 2001; Mohammadi and Prasanna, 2003).

In the CA as well as in the PCA a complex pattern of genetic relationships between the accessions was found out. Analysis of cluster amalgamation graph (data not shown) demonstrated that the cutting point for the separation of clusters was located at a linkage distance of approximately 1.5. Six clusters determined in such a way formed two families, the linkage distance between them was about 5.0. The first family (the European hexaploid wheats) consisted of clusters 1 and 2 and grouped 236 accessions, mainly from the countries of Europe and New World. The second family (the Asian hexaploid wheats) was represented by clusters 3–6 and contained 178 accessions, largely from different regions of Asia. The analysis of matrices constructed separately for European and Asian wheats showed similar means of SMCs (0.79 and 0.79, respectively), *i.e.* the wheats from both origins possess approximately the same level of genetic variability.

Cluster 1 consisted of 61 common wheat accessions and three landraces of club wheat. The accessions originated from Russia and other countries of CIS, and also in countries of Europe, Southwestern Asia, Southern Asia and America there was a prevalence of spring wheat (69%), and they belonged mainly to common wheat subsp. *eurasiaticum*. Among them a significant number of accessions were represented by landraces such as 'Poltavka' type (EU: 36, 99, 56, 55, 78, 53, 39, 3 and 31). Only two modern cultivars, Kutulukskaya and Dalnevostochnaya 10, from Russia were included in this cluster.

Cluster 2 was the largest one and included 143, 13 and 16 accessions of common, club and spelt wheats, respectively. The accessions of winter wheat constituted more than half (58%) of the total number of accessions in this cluster. All the accessions were grouped into four subclusters, *a* to *d*. The subcluster *a* consisted of nine spelt wheat landraces from Spain and Switzerland. The landraces from each of these countries were more closely related and clustered together. In the subcluster *b* the four Asian spelt wheat landraces from Azerbaijan and Iran, eight club wheat landraces from Palestine, Turkey, Armenia, Russia, Italy and Great Britain and common wheat accessions from different countries were grouped. In the subcluster

c the modern cultivars from Russia, Ukraine and Kazakhstan were prevalent, the cultivars having in their pedigree 'Bezostaya 1' (MC 2) and 'Mironovskaya 808' (MC 4) as the closest ancestors were clustered together and grouped independently. The subcluster *d* included modern cultivars (19 from Japan and five from Russia), 21 landraces of such types as 'Banatka' (EU: 5, 41, 26, 128, 103, 37, 35, 18, 137, 33, 24, 13, 107, 4 and 74) and 'Sandomirka' (EU: 120, 116, 28, 30, 29 and 79) and nine old hybrid cultivars from American and European countries. The cultivar Valuevskaya from Russia has been combined with Japanese cultivars, in fact it was widely used in breeding of common wheat in Hokkaido region (T. Kuwabara, personal communication). It is necessary to note that 52 modern cultivars out of 55 investigated in the work were pooled into cluster 2.

Contrary to the clusters considered above, in the other ones most accessions originating from different regions of Asia have been grouped. Cluster 3 included one modern cultivar, 45 landraces and old cultivars of common wheat and two club wheat landraces. With the exception of two cultivars, all these accessions originated from Japan and China, the cultivars from Japan have formed here the separate subcluster *a*. On the contrary, in the subcluster *b* the accessions from Eastern China were mainly grouped. Old cultivars Novaro and San Giorgio from Italy included in this subcluster had in their pedigrees the old Japanese cultivar Akakomugi as the closest ancestor (Martynov *et al.*, 1992). Probably its genetic material was incorporated into Italian cultivars, explaining their relatedness to the Japanese cultivars grouped in this cluster. Cluster 4 comprised 22 spring wheat accessions, among them 17 common wheat accessions from India and Pakistan, all three studied landraces of *T. sphaerococcum* from the same countries and two club wheat landraces from Tajikistan. Cluster 5 included 35 common wheat accessions, both studied landraces of *T. macha*, a single investigated landrace of *T. petropavlovskyi*, one spelt wheat landrace from Azerbaijan and one of the two studied landraces of *T. vavilovii* (=*T. vavilovianum*). The accessions of common wheat mainly originated from regions of Northern Caucasus, Transcaucasia and Turkey. Cluster 6 grouped 68 accessions mostly of spring wheat, most of them (81%) originated in Central Asia and neighboring regions. The presence of the large number of landraces of club wheat (15), Asian spelt wheat (9) and common wheat from Tajikistan, Uzbekistan, Turkmenistan, Afghanistan, Pakistan, Mongolia, Western China (provinces Xinjiang and Gansu) was characteristic of this cluster. One of the *T. vavilovii* landraces was also included. Landraces of Asian spelt wheat and common wheat from Kashgar region (Western China) had a tendency to cluster together.

The comparison of the classifications obtained in the PCA and CA showed that the accessions of clusters 3 and 4 generally corresponded to groups 2 and 3, respectively (Figure 1). Thirty eight accessions of group 5 comprised the basis of cluster 6, and also the accessions of groups 1–3 were joined to them. Cluster 2 was mainly represented by the accessions of the group 1 (Figure 1), 129 of 180 accessions in this group were pooled in this cluster. Additionally, the accessions of groups 6 and 8 were also almost completely included in cluster 2. Similarly, cluster 5 included 17 accessions from group 1 and almost the whole groups 7 and 9. In cluster 1 the accessions from groups 4 (28 accessions) and 1 (24) were predominant (Figure 1).

Levels of genetic differentiation of hexaploid wheats complex:

Species ・ *Subspecies* ・ *Geographical races*

Genetic linkage based on RAPD data
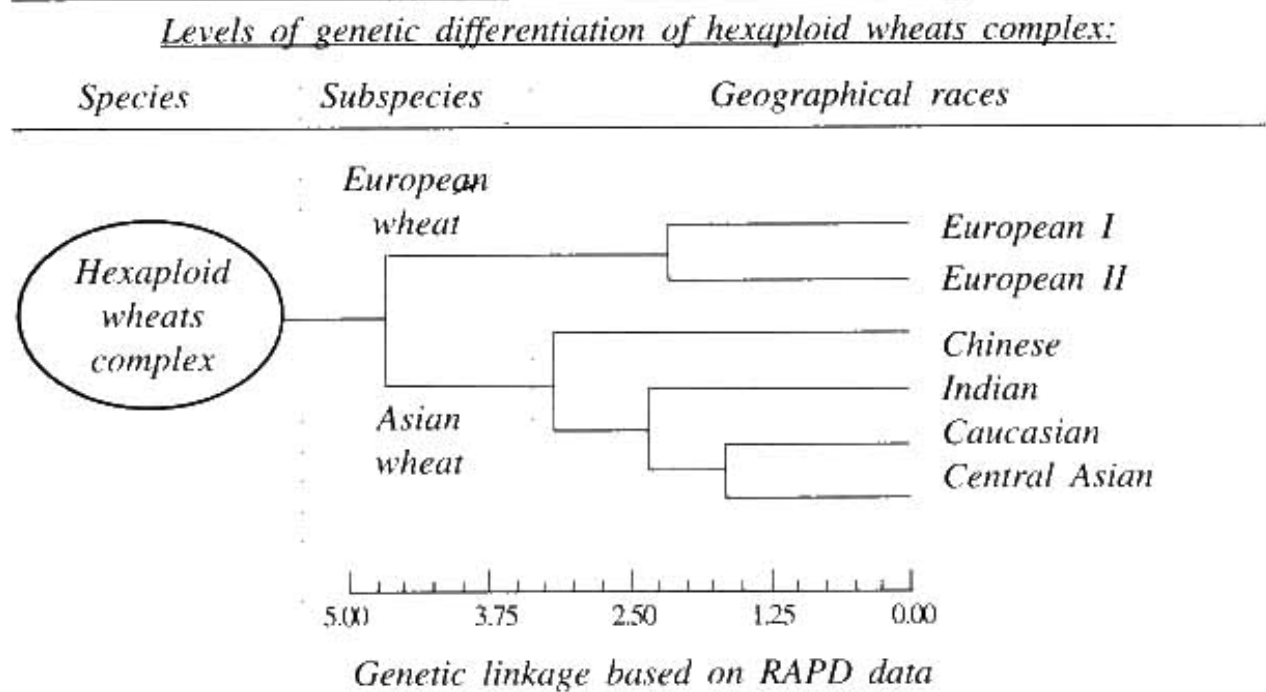
Figure 1. Division of the hexaploid wheat complex into groups and families of groups with different level of genetic differentiation

Thus, in general the CA using SMCs and Ward's algorithm provided a clear separation of the accessions and supported the conclusions from the PCA. Owing to the fact that the CA has revealed the groups of accessions of similar geographical origin more precisely and has shown a hierarchy of relationships both between accessions and groups of accessions, the classification of accessions based on the CA was accepted as a basis for further consideration and discussion. The final composition of clusters 1–6 (we will further consider these clusters as genetic groups 1–6) and hierarchy of their relationships.

Discriminant analysis and characterization of distinctions between genetic groups 1–6 based on the frequency of RAPD bands

For the estimation of the quality obtained in the CA classification and for the definition of RAPD bands (fragments of amplified DNA), which were the most important for differentiating the investigated set of accessions into six genetic groups we applied the CDA. A high average percentage of correct decisions (87%) in the model of the CDA constructed for groups 1–6 with including of 24 RAPD bands (Table 4) have confirmed the high quality of the classification obtained in the CA. The lowest percentage of the correct decisions (70%) was obtained for group 1, and the highest (100%) for group 4. Only 24 DNA fragments out of 137 studied were selected as the most important for classification of the investigated accessions. To define a pattern of distinctions between the groups the estimation of DNA fragments average frequency in groups and in all the investigated set of accessions was performed. The data obtained show that the distinctions between the groups are caused by the frequency variation of the large number of DNA fragments. In Table 4 only the data for 24 bands included in the described CDA model are shown.

Table 4. Mean of frequencies (%) of RAPD bands included in the model of canonical discriminant analysis constructed for accessions of genetic groups revealed by cluster analysis of RAPD data

Step of the CDA	RAPD band (DNA fragment, bp)	For genetic groups						For total sample
		1 (n=64)	2 (n=172)	3 (n=48)	4 (n=22)	5 (n=40)	6 (n=68)	(n=414)
1	OP-P10$_{530}$	0	2	75	0	0	2	10
2	OP-A16$_{250}$	5	4	8	0	13	66	15
3	OP-A6$_{350}$	83	94	94	5	98	93	87
4	OP-A19$_{700}$	20	23	96	96	78	87	51
5	OP-V6$_{380}$	16	3	71	91	28	4	20
6	OP-AA8$_{400}$	59	100	85	96	100	88	90
7	OP-D12$_{200}$	69	78	92	9	23	12	58
8	OP-AF16$_{350}$	22	65	8	0	85	37	45
9	OP-A6$_{1000}$	17	67	2	0	28	4	34
10	OP-A16$_{400}$	0	3	2	0	38	6	6
11	OP-AA8$_{500}$	17	12	0	59	53	10	17
12	UBC535$_{200}$	36	45	6	100	3	35	37
13	UBC580$_{550}$	28	15	0	73	10	53	24
14	OP-P4$_{500}$	72	99	98	100	100	99	95
15	OP-V9$_{900}$	14	29	2	68	3	31	23
16	OP-F19$_{500}$	5	12	54	14	15	57	24
17	OP-T17$_{450}$	6	2	50	9	25	50	19
18	OP-M11$_{360}$	73	80	85	0	80	49	70
19	OP-AB5$_{200}$	19	17	83	86	35	43	35
20	OP-D12$_{320}$	27	8	0	0	13	2	9
21	OP-O1$_{300}$	77	89	98	82	90	96	89
22	OP-V9$_{700}$	39	42	2	46	40	29	35
23	OP-A16$_{950}$	5	44	10	50	8	15	26
24	OP-F19$_{270}$	11	27	10	55	13	9	20

For the estimating the significance of distinctions and the similarity degree of genetic groups by the frequency of RAPD bands the table of the average frequencies for six genetic groups of all the analyzed RAPD bands was used as initial data matrix for the PCA. The results of the analysis confirmed the significance of distinctions between the detected groups. Only groups 1 and 2 were combined together (Table 5) by the first principal component (PC). The other genetic groups had maximal factor loadings on different PCs (PC 2–PC 5). It should be noted that genetic groups 5 and 6 also had considerable values of factor loadings by PC 1. It is evident that an essential portion of RAPD bands has approximately the same average frequency in all four groups (1, 2, 5 and 6). Groups 3 and 4 were not related genetically either with each other or with any other genetic group.

Thus, the results of CDA and analysis of average frequencies of RAPD bands have confirmed the existence of natural genetic groups in the analyzed set of accessions, which at different extents are related to each other. The distinctions between

Table 5. Factor loadings for six genetic groups of hexaploid wheats computed by the principal component analysis on the 137 RAPD bands average frequencies

Genetic group	Principal components				
	1	*2*	*3*	*4*	*5*
1	0.81	0.32	0.34	0.21	0.24
2	0.74	0.32	0.34	0.38	0.22
3	0.39	0.34	0.79	0.24	0.21
4	0.31	0.86	0.29	0.21	0.19
5	0.53	0.35	0.36	0.62	0.26
6	0.51	0.41	0.36	0.31	0.59
% of the total variation	33.4	22.6	20.0	12.8	10.1

the genetic groups are based on frequency variation of the large number of amplified DNA fragments.

Discussion

Recently the suitability of different types of DNA markers for characterization of genetic diversity and classification of accessions representing various hexaploid species (Siedler *et al.*, 1994; Cao *et al.*, 1998, 2000; Dvorak *et al.*, 1998; Sun *et al.*, 1998; Bertin *et al.*, 2001) as well as common wheat landraces and modern cultivars from different countries (Chen *et al.*, 1994; Donini *et al.*, 2000; Plaschke *et al.*, 1995; Law *et al.*, 1998; Ward *et al.*, 1998; Bohn *et al.*, 1999; Prasad *et al.*, 2000; Shah *et al.*, 2000; Stachel *et al.*, 2000; Ben Amer *et al.*, 2001; Ahmad, 2002; Parker *et al.*, 2002; Almanza-Pinzón *et al.*, 2003) have been demonstrated. Few attempts for comparative assessment of common wheat genetic diversity from different geographical regions were undertaken (Kim and Ward, 2000; Christiansen *et al.*, 2002; Hazen *et al.*, 2002; Huang *et al.*, 2002). However, in these works clear phylogeographical relationships among hexaploid wheat landraces and cultivars on a global scale have not been shown.

The results of RAPD analysis on a set of 414 accessions, which was formed applying an ecogeographical principle, have demonstrated the genetic variability in this set. The direct classification of accessions by different methods of multivariate statistics made it possible to reveal six significantly differing natural genetic groups. In the CA all the revealed genetic groups combined into two families representing the European and Asian hexaploid wheats. The existence of these families probably reflects two principal directions in evolution and geographical distribution of hexaploid wheat. The first family included two groups and was represented by accessions mainly from Europe and the New World, whereas the second family contained four genetic groups. The accessions of these groups originated from different areas of Asia and Caucasus mountain regions. Using AFLP markers we have also demonstrated similar genetic differentiation of hexaploids into the European and Asian wheats (Strelchenko *et al.*, 2003).

The number of accessions in the detected groups varied from 22 to 172. All genetic groups predominantly contained accessions of common wheat, though other

hexaploid wheats species were also represented. The accessions of different species, which were combined together with accessions of common wheat in one group as a rule had the same region of origin. Taking into consideration the origin of accessions included in each group we have designated these genetic groups as the European I, European II, Chinese, Indian, Caucasian and Central Asian. The groups correspond to the composition of clusters 1–6 in the phenogram (Figure 1).

Taking into account the character and level of genetic distinctions between the revealed groups of accessions as well as the particularities of their geographical origin and biological features (Palmova, 1935; Vavilov, 1964), we suppose that the formation of each of them could be related to the action of several factors. Firstly, the most probable could be associated with the existence in Eurasia of at least five or six different and relatively distant regions of prolonged hexaploid wheat cultivation. In fact, the areas of origin of accessions belonging to the genetic groups coincided with the geographical location of some agricultural centers, which have arisen in prehistoric times. Vavilov was the first who recognized some of these centers (Vavilov, 1926). Trying to trace the history of agriculture development on a global scale, he has come to the conclusion that the first agricultural civilizations, such as South Western Asian (there he included Transcaucasia, Asia Minor, Central Asia and North Western India), Indian, Eastern and Central mountainous Chinese and other civilizations, have arisen "independently, simultaneously or in different times... Very different ethnic and linguistic groups of the peoples ... and ... different types of cultivation techniques and domestic animals were peculiar for these civilizations" (Vavilov, 1931). According to archaeological evidences, the hexaploid wheats were cultivated in Europe since 5400–4900 BC and in Asia since 7500–6000 BC (Nesbitt, 2001). It was South Western Asia and North-Eastern Africa that Vavilov (1926, 1931) pointed out as a primary vast regions of common and club wheat cultivation, from which these wheats were spread all over the world. There are different points of view on the origin of other hexaploid wheats (Mac Key, 1966; Dorofeev *et al.*, 1979; Nessbitt, 2001). According to one of them (Flaksberger, 1935), the other hexaploid wheats were secondary derivatives from common and club wheats and could be appeared in different regions. For a long period of cultivation in different geographical regions the process of adaptation of hexaploid wheats to local soil and climatic environments occurred and has resulted in their genetic differentiation. Likely the hexaploid wheats arose during cultivation because no wild forms have been discovered (Miller, 1987). Secondly, random genetic drift could also have a large importance for the genetic differentiation of hexaploid wheats. It is possible that founders for each genetic group were not numerous and originated from genetically different wheat populations. For example, accessions from Turkey, Palestine, Syria, Transcaucasia and Northern Caucasus region have been included in the European I and European II groups. The genetic similarity of European wheat with landraces from the listed countries and regions may give evidence that ancestors of European groups could origin from them. Similarly, the inclusion in the Indian group of two accessions of club wheat from Tajikistan can indicate that wheat from Central Asia was the progenitor of this group. To prove the assumption additional data based on the study of a greater number of accessions would be

probably necessary. The Chinese group included two subgroups, the first subgroup pooled old cultivars from Japan and the second one united landraces from East China. Probably, common wheat has spread from East China to Japan and for a long time of cultivation the Japanese cultivars have genetically diverged from Chinese ancestors and that is why they have formed an independent genetic subgroup. Genetic mechanisms (including the founder effect) leading to differentiation of isolated populations were considered in details by Mayr (1963). It may be assumed that the revealed genetic distinctions between the groups have partly been caused by differences between the founders of these groups. Thirdly, in a later period of time the transfer of already formed landraces from one center to another was possible. For example, we are inclined to explain genetic similarity of a number of landraces from India, Pakistan, China and Tajikistan with European landraces by a transfer of already formed landraces from Europe to Asia.

The classification of accessions depicted in this work differs essentially from the most traditional classifications of hexaploid wheats with AABBDD genome composition (Bowden, 1959; Mac Key, 1966; Dorofeev *et al.*, 1979; Miller, 1987). These classifications were based on distinctions of hexaploid wheats by morphological traits controlled by one to several major genes (McIntosh *et al.*, 1998). On the contrary, the proposed classification is based on distinctions of geographically separated hexaploid wheats groups by variation of the frequencies of a large number of genome DNA sequences, which are probably distributed randomly in the wheat genome. A pattern and degree of distinctions of genetic groups as well as their geographical separation allowed us to consider them as original geographical races developed by prehistoric agricultural civilizations with the involvement of natural selection. Landraces within a geographical race were more similar among themselves than landraces from different races. A tendency for combining the accessions representing particular agroecological groups described by N. Vavilov (1964) was observed within certain geographical races. Since the extent of this paper is limited, these data are not shown and are reported in a previous paper (Strelchenko *et al.*, 2002).

The race classification of hexaploid wheats reflects the genetic differentiation within their genepool. None of the studied hexaploid wheats, considered by some of the researchers as species or subspecies on basis of variation for morphological traits, has formed a separate genetic group. At the same time the accessions of different species (or subspecies) were more related to common wheat than among themselves. For example, the landraces of club wheat were represented in five genetic groups, except the Caucasian one. Perhaps not only co-distribution of club and common wheats in the different regions, but also their co-evolution occurred. Accessions of spelt wheat were included mainly in the European II and Central Asian groups. Within the European II group the landraces of only European spelt wheat formed a separate subgroup. The other spelt wheat landraces in this group, including those from Iran and Azerbaijan, combined with common wheat accessions into different subgroups. Within the Central-Asian group the spelt wheat landraces also showed a tendency to joint clusterization. It should be noted that the spelt wheat accessions from Iran collected by H. Kuckuck between 1952 and 1954, and included

into the VIR germplasm collection in 1967 according to the request from the German Genebank, have turned out genetically diverse; some of them were similar to European common wheat accessions and others to Asian ones.

Landraces of *T. macha* were genetically similar to common wheat landraces from Azerbaijan. Although in the analysis of RAPD patterns by PCA the landraces of *T. macha* and *T. petropavlovskyi* have formed separate minor groups, in the CA they were included in the Caucasian group and pooled together. We cannot explain such grouping of the accessions, but it may not be occasional. The grouping of *T. petropavlovskyi* landraces with landraces of common wheat from Turkey, rather than with landraces from China was recently shown by R.W. Ward and co-workers (1998) using RFLP analysis. The understanding of the genetic relationships both among these wheats and between them and the other hexaploids will need further studies using a higher number of accessions.

The classification of the analyzed set of accessions into genetic groups of European and Asian wheats has clear analogies with former classifications suggested by some Russian botanists. Firstly, the classification based on RAPD data corresponds to the division of common wheat into European and Asian wheat in the rank of "race groups" (Vavilov, 1922–1923) or subspecies (Flaksberger, 1935; Tzvelev, 1976; Dorofeev *et al.*, 1979). Unlike European wheat, coarser ear and more difficult grain threshing characterize the Asian one. Secondly, it was demonstrated that genetic groups revealed in RAPD analysis agree with the division of common wheat into subspecies proposed by Vavilov in one of his latest work (1964). As it has been noted earlier in the paper, the ecogeographical approach was used as a basis of this division. Apparently, such an approach allowed Vavilov to reveal the genetic differentiation of common wheat, which has been confirmed with the use of RAPDs. For instance, the accessions of common wheat subsp. *irano-turkestanicum* were a basis of the Central Asian genetic group, and accessions of subsp. *sinicum* and subsp. *indicum* underlay the Chinese and Indian groups, respectively.

Unlike the classification developed by Vavilov, a quite different pooling of the accessions of common wheat subsp. *eurasiaticum* and subsp. *abyssinicum* has been shown in the classification based on RAPDs. In the first case a more complex structure of the subspecies was revealed. Accessions of subsp. *eurasiaticum* were combined into three distinctive genetic groups, the European I, European II and Caucasian ones. The first two groups formed the family of European wheat and the third group was included in the family of Asian wheat. As it has already been, noted the European II group was represented by landraces of agroecological groups (proles) *hiemale-stepposum* (such as 'Banatka' type) and *hiemale-boreale* (such as 'Sandomirka' type) and European I group included landraces of proles *aestivo-stepposum* (such as 'Poltavka' type). According to N. Vavilov (1935) and E. Palmova (1935), the accessions of the first two agroecological groups occupied southwestern steppe areas of the European part of former USSR. Compared with them, the landraces of such a type as 'Poltavka', named also "Russian wheats", had a high plasticity. Geographically they spread out of the boundaries of actually steppe area, advanced far to north, northeast and east and gained prevalent positions in Siberia. In Western

Europe the wheat of such a type was found in Central Spain, Portugal and Romania. It was also widely distributed in the north of the USA, especially in Northern and Southern Dakota. The features of this wheat are most clearly demonstrated by accessions from Armenia, Georgia and Asia Minor. Their most valuable properties are the ability to resist to droughts, slight frosts and sharp changes of daily temperatures during spring period. As to accessions of subsp. *abyssinicum* they did not form a separate genetic group and were included into different subgroups of the European II group and into Central Asian group. In order to define relationships of this wheat with other wheats the study of a larger set of accessions from Ethiopia will be necessary.

The results of the present research have demonstrated that the hexaploid wheat genepool is divided into genetic groups belonging to two families, European and Asian hexaploid wheats. The existence of these families probably reflects two principal directions in evolution and geographical distribution of hexaploid wheat. The European family consists of European I and European II groups. The Asian hexaploid wheat includes the Caucasian, Central Asian, Chinese and Indian groups. The basis of each of these groups, as well as of European ones, was formed by landraces of common wheat. However, in all groups there were also landraces of other hexaploid wheats. Accessions of different species combining in the same group originated mainly from the same geographical region. The distinctions between genetic groups revealed in this work were mainly based on the variation of frequencies of the large number of RAPD bands. It is likely that the formation of genetic groups took place in different prehistoric centers of agriculture. If the revealed geographically separated genetic groups may be designated as geographical races, the European and Asian wheat families conditionally correspond to a subspecies level of differentiation and the whole complex of hexaploid wheats should be regarded as one species. Based on the results obtained, a genetic classification of hexaploid wheat complex is suggested (Figure 1).

The application of modern technologies, that allows the study of DNA polymorphisms of large sets of wheat landraces and cultivars for estimating of their similarity, makes it possible to create a comprehensive genetic classification reflecting the history of development and adaptation of the crop. The identification of the genetic structure within wheat germplasm collections is useful for the establishment of strategies of germplasm management and will increase the efficiency for selecting the initial material for wheat breeding.

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