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Geographical and Breeding Trends within Eurasian Cultivated Barley Germplasm Revealed by Molecular Markers

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Abstract

Knowledge of genetic variability within a crop species is invaluable for its improvement. Restriction fragment length polymorphisms (RFLPs) and hordeins have been used to characterize genetic diversity of 93 barley cultivars and landraces originating from different regions of Russia and neighbouring countries. The RFLP banding patterns from 70 clone-enzyme combinations (41 map-based DNA clones, restriction enzymes *Eco* RI and *Hin*d III) yielded in total 335 polymorphic fragments. These were used to generate a genetic distance matrix, which was used in both cluster and principal coordinate analyses. Both analyses clearly separated all accessions into two major genetic groups, which are geographically linked with oriental and occidental regions of Eurasia. This confirms the existence of two principal paths in the evolution of cultivated barley. The occidental-type group consisted of more accessions and were clearly divided into two-rowed and six-rowed forms on the basis of spike morphology. Among major genetic groups, further sub-groups were apparent. These were cultivars with a similar pedigree background which clustered together. The use of RFLP and hordeins analyses for determining barley genetic variability are discussed.

Introduction

Genetic improvement of crops by man can be regarded as directed evolution acting upon the existing genetic variability in the germplasm. In order to optimize and accelerate breeding, it is essential to screen, evaluate and classify the genetic variability available in the germplasm. This is especially important for collecting, maintaining ex-situ and studying plant genetic resources in national and international germplasm programs.

Assessment of genetic variability between individuals and populations has been based on the analysis of pedigree records, morphological traits and more recently on molecular markers. However, pedigree data of lines are not always available. For example, landraces represent a large part of germplasm collections of many crops and may be a rich source of genetic variation for cultivar development. Moreover, pedigree data do not account for the effect of selection, mutation and random genetic drift. Use of morphological traits for plant diversity analysis has been criticized because genetic control is largely unknown and expression depends on environmental factors. Among biochemical markers, polymorphic proteins such as isozymes and storage seed proteins have been successfully used in different crops to characterize genetic variation in numerous taxonomic and population genetic studies (see Konarev *et al.*, 1996, for review). However, proteins often failed in the classification of crops because of the small number of available marker loci, which provided only poor genomic coverage. Recently DNA-markers such as restriction fragment length polymorphisms (RFLPs) and random amplified polymorphic DNA (RAPD) are being successfully used for assessment of genetic diversity in cultivated plant species. Such markers have the advantage of being generally independent of phenotype and, if representative of the entire genome, can provide a comprehensive survey of the genetic variation present in a sample of cultivars.

In barley (*Hordeum vulgare* L.) high-density genetic marker maps are being constructed using both RFLP and RAPD markers (Graner *et al.*, 1991; Heun *et al.*, 1991; Tragoonrung *et al.*, 1992; Graner *et al.*, 1993; Kleinhofs *et al.*, 1993). Recently, several studies have examined the genetic variation in cultivated and wild barley with RFLP (Graner *et al.*, 1990; Liao and Niks, 1991; Pecchioni *et al.*, 1993; Zhang *et al.*, 1993; Melchinger et al., 1994) and RAPDs (Dweikat et al., 1993; Tinker et al., 1993; Gonzales and Ferrer, 1993; Song and Henry, 1995). However these studies were largely restricted to the analysis of elite barley germplasm adapted to Western Europe or North America. But, cultivated barley is one of the oldest, most widely grown and polymorphic crop species and was domesticated in Asia and principal centers of its genetic diversity are situated there. N.Vavilov was the first to begin world-wide collecting and studying of genetic diversity of many crops including barley. On the basis of his work principal world centers of barley diversity (gene-centers) were determined by him (Vavilov, 1926). Afterwards, Vavilovs ideas were developed by many researchers at VIR (the Vavilov Institute of Plant Industry). Lukyanova *et al.* (1990) proposed an eco-geographical classification of barley. According to this classification the present centers of barley diversity are shown (Fig.1).

Russia occupies a considerable part of Eurasia with many different

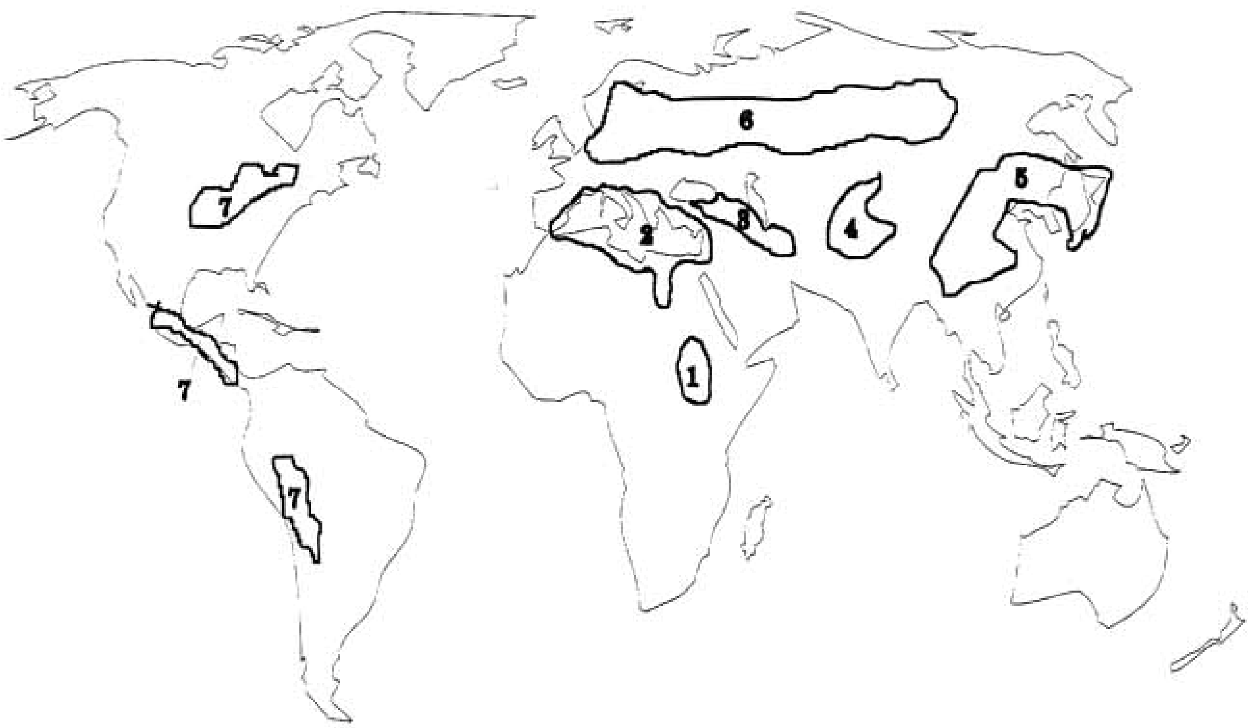

Fig.1. Global centers of barley diversity (Lukyanova *et al*., 1990): 1 -Abyssinian; 2 -Mediterranean; 3 -West Asian; 4 -Central Asian; 5 -East Asian; 6 -Europe-Siberian; 7 -New World.

agro-ecological regions. Russia borders on the primary centers of barley diversity and Russia has a long history of barley cultivation. Consequently a high level of barley genetic diversity is expected in Russia. The most representative germplasm collection of Russian barley, which includes several thousand accessions collected during this century in different regions of Russia and neighbouring countries, is being preserved at VIR.

In the present study, we assayed 93 cultivated barley cultivars and landraces originated from different regions of Eurasia. Our primary objectives were to (i) estimate the genetic relationship between barley accessions based on RFLP patterns, and (ii) compare the possibilities of RFLP and hordeins analyses for determining barley genetic variability.

Materials and Methods

Plant Material

In total 93 barley accessions including 54 cultivars (Table 1) and 39 landraces (Table 2) were used in this study. The 82 cultivars and landraces were selected from the VIR germplasm collection to represent wide geographic diversity present in Russia and other countries of the former USSR. There were 39 two-rowed and 43

Table.1. Barley cultivars used in this study.

No	Cultivar*	VIR genebank catalog number	Botanical varieties	Pedigree/Background	Region of origin
Two-rowed					
1	Viking	24700	nutans	Domen x Ingrid	Vyatka
2	Vyatich	26823	"	Brigitta x Luch	"
3	Risk	29352	"	Complex hibrid (Km1192, Temp, Hiproly, Moskovskii 121)	Moskow
4	Auksinyai 3	28117	"	Carina x Tappa 26	Lithuania
5	Zhodinskii 5	27372	"	Masurka x Km1192	Byelorussia
6	Talovskii	26261	"	Unknown	Voronezh
7	Lyubimets 108	27373	"	Unknown	Lutsk
8	Kharkovskii 82	27378	"	Union x Chernomorets	Kharkov
9	Donetskii 650	18331	medicum	Spartan x Medicum 513	Donetsk
10	Odesskii 36	19934	"	Donetskii 650 x Stepovyi	Odessa
11	Odesskii 100	26864	"	((Medicum 134 x Hiproly) x (Nutans 244 x Medicum 134)) x (Slavutich x Hml 36462/64)	"
12	Temp	22055	"	Chemomutant of Krasnodarskii 35	Krasnodar
13	Pricumskii 22	26180	medicum	Line-14094 x Line-9943	Stavropol
14	Nutans 115	19355	nutans	Selection from landrace (Armenia)	Armenia
15	Kvant	27558	"	Unknown	Ekaterinburg
16	Ilmen	26968	"	Peroga x Krasnoufimskii95	Chelyabinsk
17	Omskii 80	26179	medicum	Palisser x Omskii 13709	Omsk
18	Krasnoyarskii 80	27102	nutans	S-80 x Una	Krasnoyarsk
19	Erofei	29221	medicum	Keystone x Luch	Khabarovsk
20	Primorskii 89	27055	nutans	VIR k-19660xUssuriiskii 8	Vladivostok
21	Granal	29342	subinerme	Olimp x (VIR k-21683 x k-19991)	Kazahstan
22	Tselinnyi 213	28015	nutans	Selection from Tselinnyi 5	"
23	Medicum 8955	17386	medicum	Selection from Turkish landrace (VIR K-6857)	"
24	Alexis	(29578)	nutans	1622d5 x Trumpf	France
25	Aramir	(21875)	"	Volla x Emir	Germany
26	Ursel	(29558)	"	Aramir x Trumpf	"
27	Arena	(28947)	"	Aufhammer 39/68 x H464	"
28	Isaria	(18307)	"	Danubia x Bavaria	Austria
29	sw Unumli-Arpa	19177	"	Selection from Moroccan landrace	Uzbekistan
30	sw Nutans 27	16335	"	Selection from landrace (Uzbekistan)	"

Table.1. Barley cultivars used in this study. (Continued)

No	Cultivar*	VIR genebank catalog number	Botanical varieties	Pedigree/Background	Region of origin
31	w Igri	(24995)	erectum	Malta x ((Aurea x Carstens 2zlg.) x Ingrid)	Holland
32	w Trixi	absent	-	((Malta x Volla) x (Tria x Emir)	Germany
33	w Malta	(21827)	nutans	((Carstens 2zlg. x Aurea) x Dea) x Herfordia	"
Six-rowed					
34	Polarnyi 14	15619	pallidum	Selection from landrace (Karelia)	Murmansk
35	Belogorskii	22089	pallidum +rikotense	Chervonets x Keystone	Leningrad
36	Pallidum 45	11856	pallidum	Selection from lanrace (Saratov)	Saratov
37	Gelios	28936	rikotense	(Nutans 32 x Pallidum 125) x Athos	Odessa
38	Agul 2	27649	ricotense	Agul x Keystone	Krasnoyarsk
39	VIR-65	21833	"	Selection from Beecher (Israel)	Uzbekistan
40	sw Giaginskii 395	18122	pallidum	Selection from Chenad 395 (Rumania)	Krasnodar
41	sw Kruglic 21	13031	"	Selection from landrace (Krasnodar)	"
42	w Rosava	27404	"	Odesskii 86 x Oksamyt	Odessa
43	w Pallidum 4	13036	"	Selection from landrace (Krasnodar)	"
44	w Klepeninskii	25302	"	Vinesco x Almaz	Krimea
45	w Siluet	27704	papallelum	Rostovskii 15 x Zimran	Rostov
46	w Vavilon	29361	"	(Meteor x KNIIH84/II) x (Ager 31 x M13)	Krasnodar
47	w Skorohod	29404	"	Meteor 57 x M13 (mutant of Regia)	"
48	w Krasnodarskii 2929	16948	pallidum	Selection from landrace (Caucasus region)	"
49	w Prikumskii 43	27553	parallelum	F-2179 x F-11409	Stavropol
50	w Ararati 7	25994	pallidum	Mutant of Kaler (Armenia)	Armenia
51	w Nahichevandani	13248	"	Selection from landrace (Azerbaijan)	Azerbaijan
52	w Vogelsanger Gold	(19927)	"	(44-013 x Peragis XII) x Hauters	Germany
53	w Brunhild	absent	-	Barbo x Banteng	-
54	w Mammut	(27099)	pallidum	Vogelsanger Gold x (Madru x Wssh.382/49)	Germany

* w - winter; sw - semiwinter.

Table 2. Barley landraces used in this study.

No.	VIR genebank catalog number	Botanical varieties	Year of receiving	Region of origin	Remarks*
	Two-rowed				
1	3222	nutans	1921	Karelia	
2	16411	"	1938	Arkhangelsk	
3	4541	medicum	1923	Vologda	
4	16410	nutans	1938	"	
5	5034	medicum	1923	Smolensk	
6	21820	nutans	1972	Makhachkala	
7	2946	nudum	1914	Krasnoyarsk	h
8	18059	erectum + intermedium (six-rowed)	1951	"	
9	5279	nudum	1923	Kazahstan	h
10	18362	persicum	1954	"	
11	11749	persicum	1929	Kyrghyzstan	
12	14923	nudum	1934	Turkmenistan	h, sw
13	2904	nutans + pallidum (six-rowed)	1914	"	sw
	Six-rowed				
14	16881	pallidum	1944	Murmansk	
15	9338	"	1927	Karelia	
16	9537	coeleste	1927	Arkhangelsk	
17	9827	pallidum	1927	Vologda	
18	16420	"	1938	Vyatka	
19	9423	"	1927	Komi	
20	9511	"	1927	Kostroma	
21	11970	"	1949	Kazan	
22	4972	"	1922	Omsk	
23	16478	"	1938	Irkutsk	
24	29102	"	1986	"	
25	4825	"	1923	Chita	
26	10693	"	1927	Yakutsk	
27	11075	coeleste	1927	Sakhalin	h
28	5092	pallidum	1923	Kazahstan	
29	4847	pallidum+nutans (two-rowed)	1923	"	
30	10877	pyramidat	1926	Turkmenistan	
31	16468	nigrum (pallidum)	1938	"	
32	3038	revelatum	1917	"	h
33	17227	pallidum	1947	Uzbekistan	
34	11755	nigrum	1949	Kyrghyzstan	
35	3118	coeleste	1917	Tadzhikistan	h
36	10628	ancoberense	1928	-	h
37	21477	pallidum	1965	"	
38	8123	"	1926	Azerbaijan	w
39	6128	nigripallidum + pallidum	1924	Turkmenistan	w

* w - winter, sw - semiwinter, h - hulless.

six-rowed accessions, among which 49 were landraces or cultivars derived by selection from landraces. Also in this study 11 well known West European spring and winter cultivars from different germplasm groups were included. Seeds of the latter group were kindly provided by German breeders.

Hordeins Electrophoresis

Hordeins were extracted from crushed single seeds with 40 ml of 6M urea. After centrifugation the supernatant were used for electrophoresis. Hordein electrophoresis was carried out in slabs of 6.5% PAGE 0.013M acetic acid pH3.2 during 4-4.5 h (U = 600 V and I = 20-25 mA per slab). After electrophoresis gels were stained with 0.075% Coomassie G-250 in 10% trichloroacetic acid and photographed.

RFLP Analysis

Leaf DNA was extracted from 2-to 3-week-old seedlings (bulks of 20-25 seedlings per accessions). Isolation of genomic DNA, digestion with restriction enzymes, electrophoresis in agarose gels, Southern blotting onto nylon membranes, hybridization with ^{32}P-labelled DNA probes, autoradiography, and post-hybridization washes for stripping of probes were performed as described in detail by Graner *et al.* (1990). DNA was separately digested with restriction enzymes *Eco* RI and *Hin*d III. Electrophoresis was performed in gels 20 cm long and 15 cm broad with 20 lanes and two rows of wells. Digested DNA of all accessions was loaded on six different gels each including two check varieties ('Igri' and 'Alexis') and a lane of λ phage DNA digested by *Hin*d III. For detection of restriction fragments, we used 41 anonymous clones previously mapped, mainly, single-copy DNA clones, from *Hordeum vulgare* L.(Graner *et al.*, 1993). The clones were selected to provide a fairly uniform coverage of the barley genome with at least five clones per chromosome (Fig.2). Thirty-five were genomic DNA clones (with MWG, ABG and WG prefixes) and six were cDNA clones (with cMWG and ABC prefixes).

Data Collection and Statistical Analysis

Hordein and RFLP patterns on autoradiographs for each clone-enzyme combinations (CEC) were usually scored by assigning a number to each band. For subsequent numerical analyses, data were coded in binary form, i.e., presence or

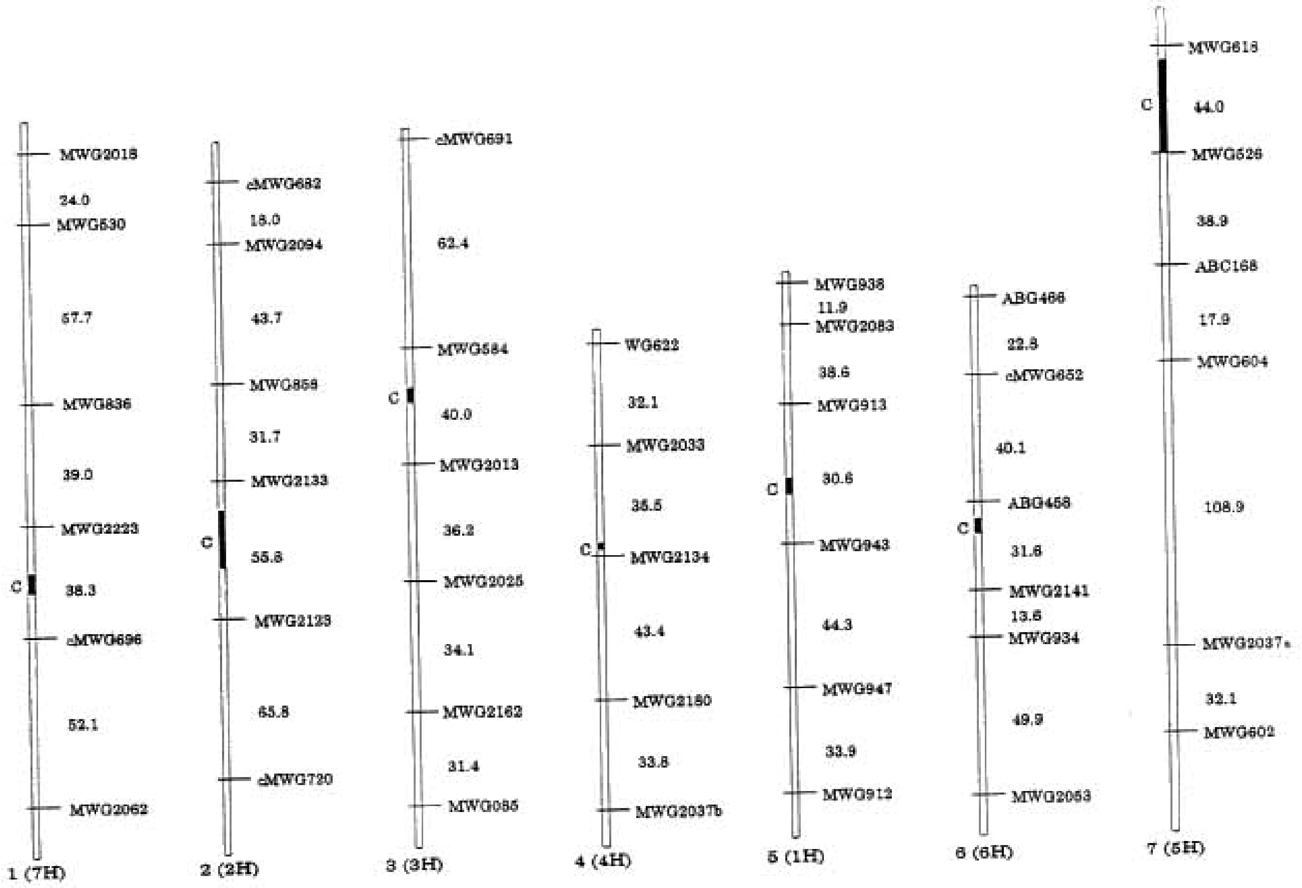

Fig.2. Chromosomal location of DNA clones assayed. Chromosomes are oriented with the short arm on top. Clone designation according to Graner *et al.* (1993). Distances in cM are presented from Igri/Franka map.

absence of a band in a line was coded by 1 or 0, respectively. Only polymorphic bands were included in the raw data matrix. This matrix was used to generate a genetic distance matrix using Nei's (1972) distance:

$$d_{ij} = -\ln \frac{\sum_{k=1}^{n} |x_{ki} x_{kj}|}{\sqrt{\sum_{k=1}^{n} x_{ki}^2 x_{kj}^2}}$$

where d_{ij} is the genetic distance between accession i and accession j , x_{ki} is the i allele frequency at locus k and n is the total number of loci. Dendrograms were produced using unweighted pair-group method, arithmetic average (UPGMA) clustering and scatter diagrams resulted from principal coordinate analyses (PCA) on the genetic distance matrix. The normalized Mantel statistic (Z) (Mantel, 1967) was used to compare the genetic distance matrixes generated from RFLP and hordeins electrophoresis data. The program NTSYS-pc version 1.8 (Rohlf, 1993) was used to generate the distance matrixes for UPGMA clustering, the PCA analysis, and the matrix comparison.

Results and Discussion

Variation for RFLPs and Hordeins

Altogether, we analyzed data from 77 CEC. Seven CEC showed completely monomorphic RFLP patterns. The DNA clones used in this study detected on average 4.9 (ranging from 2-13) polymorphic fragments per CEC for a total of 335 polymorphic fragments from 70 CEC. Restriction enzymes *Eco*RI yielded 158 polymorphic fragments from 35 CEC s and *Hin*d III yielded 177 polymorphic fragments from 35 CEC s. Typical RFLP patterns obtained are illustrated (Fig.3). All 93 accessions could be distinguished with the set of 335 polymorphic fragments.

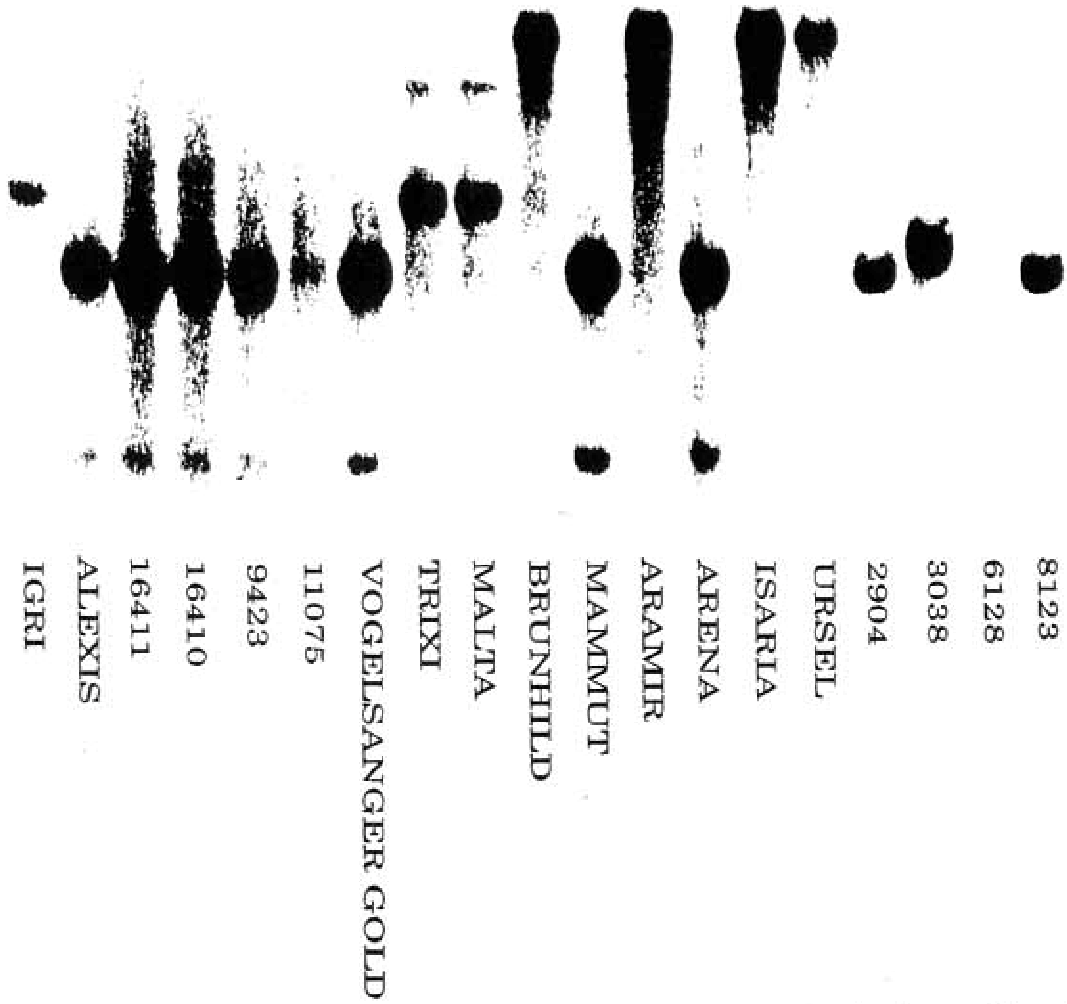

Fig.3. Restriction fragment length polymorphism banding patterns obtained on selected Eurasian cultivars and landraces with *Hin*d III and barley genome DNA clone MWG938.

From hordeins electrophoresis patterns 42 polymorphic bands were included in the raw data matrix. Twenty-seven accessions (29.0%) were polymorphic and consisted of 2-3 biotypes based on hordeins analysis of 20 seeds of each accession. For further analysis the main protein phenotypes from each accession was selected. Thus, among 93 accession 71 different protein phenotypes were determined. Thirty-four accessions formed 12 groups. Each group contains 2-4 accessions with identical pattern.

Clustering of Barley Accessions Based on RFLPs

The relationships between 93 barley accessions based on RFLP genetic distance measurement were analyzed by UPGMA clustering. All accessions, except for the hulless six-rowed landrace (acc. 10628) from Central Asia (Tadzhikistan), were separated into two major clusters (Fig.4). Cluster A comprises mainly landraces from Central Asia, Siberia and the Caucasus regions. This cluster consists of 19 landraces and 3 cultivars derived by selection from landraces. It includes both two-rowed and six-rowed accessions and all of the analyzed hulless forms. Except for the six-rowed landrace (acc. 6128), from Central Asia (Turkmenistan), there are two major sub-clusters: one is geographically linked with Central Asia and another is more widespread.

Cluster B is larger and consists of 5 sub-clusters (Fig.4). Most accessions are in sub-clusters 6 and 7 and are from a wider geographic area and distinguishable mainly on the basis of spike morphology. Sub-cluster 7 consists of two-rowed West European spring cultivars ('Alexis', 'Arena', 'Isaria', 'Aramir' and 'Ursel') and landraces and cultivars from different regions of Russia. The Russian cultivars have part of their pedigree from Western Europe, Eastern Europe and Canadian cultivars ('Trumpf', 'Ingrid', 'Isaria', 'Emir', 'Masurka', 'Chenad', 'Diamant', 'Gatway', 'Keystone' and others). Sub-cluster 6 consists mainly of six-rowed barley accessions which can be divided into three groups. One group includes spring landraces related to cultivars 'Belogorskii', 'Agul 2' and 'Erofei'. They have the Canadian cultivar 'Keystone' in their pedigree. The second group includes winter cultivars from Western Europe ('Vogelsanger Gold', 'Mammut' and 'Brunhild') and some winter Russian cultivars possibly related to them. The third includes landraces 4847 and 11970 and cultivars 'Pallidum 45' and 'Kruglic 21' possibly related to cultivar

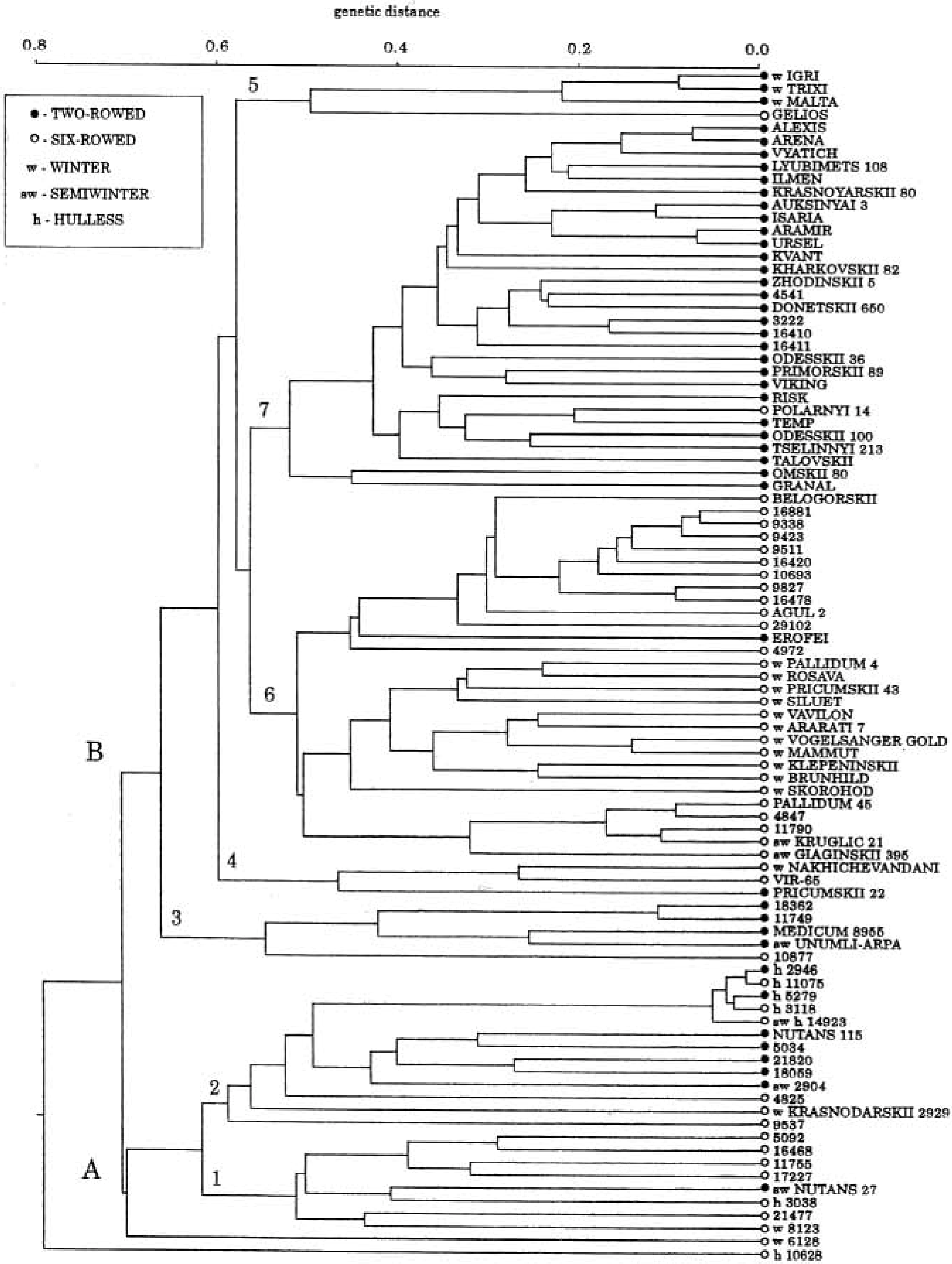

Fig.4. Dendrogram constructed from the restriction length polymorphism genetic distances matrix of 93 Eurasian barley accessions.

'Giaginskii 395', which was derived from Roumanian cultivar 'Chenad 395'.

Sub-cluster 5 comprises West European two-rowed winter cultivars 'Igri', 'Trixi' and 'Malta', which have different pedigree from the above mentioned West European six-rowed winter cultivars. This sub-cluster also includes Russian cultivar 'Gelios' related to cultivar 'Emir'.

Sub-cluster 4 includes cultivars 'Nakhichevandani' and 'Pricumskii 22', which are evidently related to cultivar 'VIR-65' selected from Israeli cultivar 'Beecher'. Finally, sub-cluster 3 includes landraces 18362 and 11749 and cultivars 'Medicum 8955' and 'Unumli-Arpa'. The latter ones were derived by selection from Turkish and Moroccan landraces, respectively.

The principal coordinate analysis (PCA) is independent from UPGMA clustering, but their results were similar (Fig.5). Some of the variation (45.5%) was accounted by the first two principal coordinate (PC) axes. Most of the variation (28.4%) was explained by the first PC, which clearly divided the analyzed accessions in two groups (A and B, see dotted line). These groups correspond exactly to clusters A and B on the UPGMA dendrogram. The second PC explained 17.1% of variation and clearly divided two-rowed and six-rowed accessions comprised in the group B into two sub-groups. This dividing of accessions according to spikelet rows is more clearly shown by a PCA plot, than by a dendrogram. On the PCA plot two-rowed accessions from sub-clusters 3 and 4 are located in sub-group of two-rowed accessions, but six-rowed cultivars 'Gelios' and 'Polarnyi' are located in sub-group of six-rowed accessions. Only two cultivars do not group according to spikelet rows of the ears: two-rowed 'Erofei' and 'Malta', they are located in the sub-group of six-rowed accessions.

The results of RFLP analysis confirm the existence of high genetic diversity present in Russian barley. This study reveals the existence of two major genetic groups in the analyzed material. Together with the West European cultivars, the majority of Russian cultivars and landraces form a large and heterogeneous group (B). The second group, which was identified in this study (A) includes a group of landraces predominantly originated from Central Asia. Vaviliov (1926) was the first to point out exotic characters of barley from Central and East Asia. The reason for this distinction is geographical isolation and evolution in the agro-ecological conditions of the region (Vavilov, 1926). The hypothesis of independent

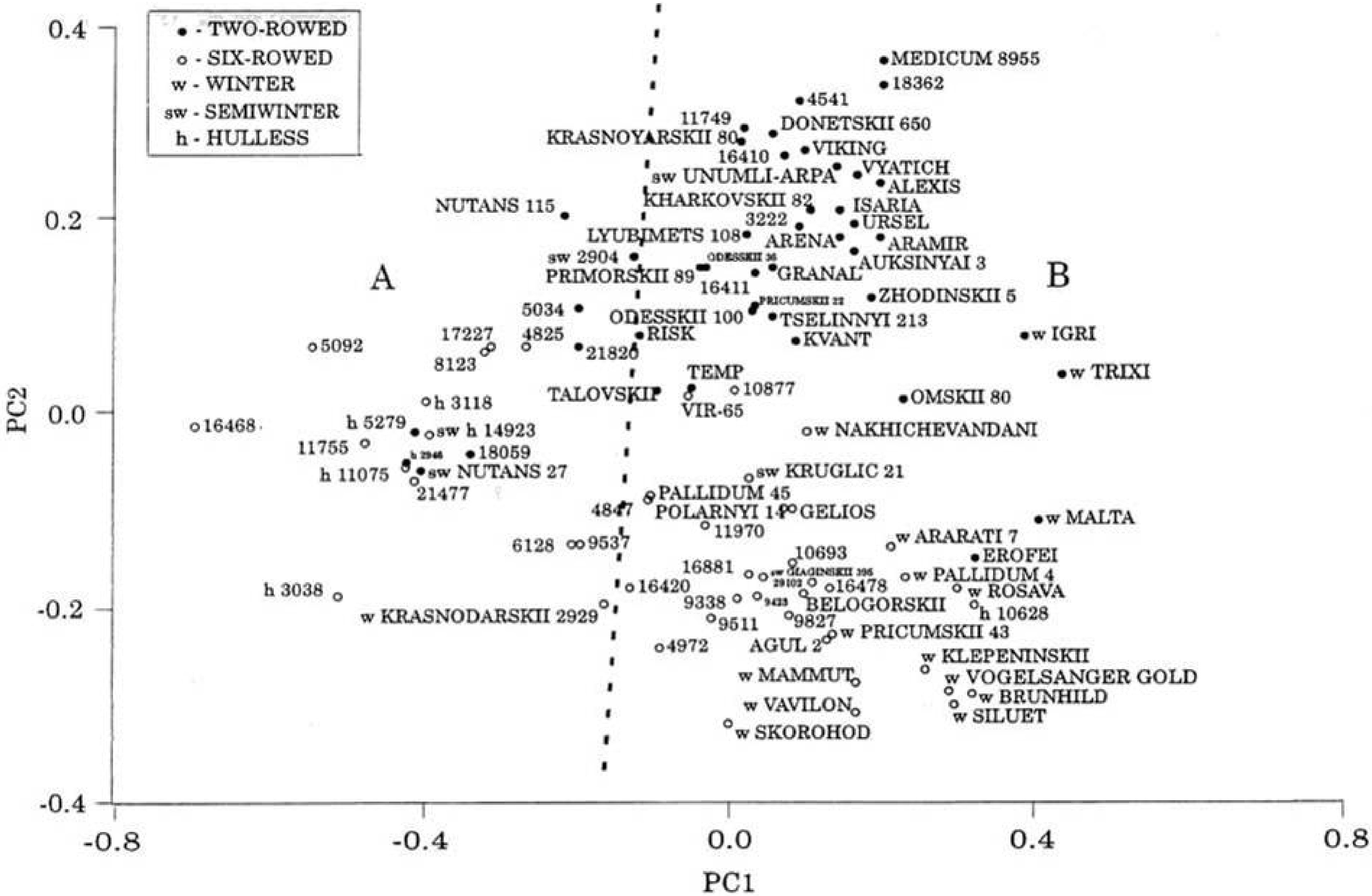

Fig.5. Plot of the principal coordinate scores from the restriction fragment length polymorphism genetic distances matrix of 93 Eurasian barley accessions. Some of the variation (45.5%) is accounted for by the two axes.

domestication of barley in oriental and occidental regions of Eurasia was suggested by a number of researchers (see Takahashi, 1955, for review). Recently Zhang *et al.* (1994) using isozyme and ribosomal DNA markers showed both broad genetic diversity of cultivated barley from Tibet and considerable oriental-occidental differentiation of barley. In our study both cluster and PCA analyses of RFLP data clearly separated all accessions into two major genetic groups, which are geographically linked with oriental and occidental regions of Eurasia. This confirms the existence of two principal trends in the evolution of cultivated barley. It is likely, that the broad clustering into oriental and occidental accessions reflects historically different sources of germplasm contributing to the two groups. According to a modern classification of global centers of barley diversity (gene-centers) adopted by VIR we may connect the above mentioned germplasm groups to Europe-Siberian (cluster B) and Central Asian (cluster A) centers (Fig.1). Central Asia might represent a valuable source of germplasm to increase the variability of barley. Group B in our study clearly divided into two sub-groups consisting predominantly of two-rowed and six-rowed accessions. Tinker *et al.* (1993) using RAPD markers differentiated 27 barley accessions into two groups, two-rowed and six-rowed forms. Similar results using RFLPs were obtained by Melchinger *et al.* (1994) in the analysis of European barley germplasm. The only exception was the position of a two-rowed winter forms, which clustered together with six-rowed winter cultivars. In our study this group ('Igri', 'Trixi' and 'Malta') formed rather distinct sub-cluster in cluster B. There are several classification systems of cultivated barley in which on the basis of spike morphology two principal sub-species (two-rowed and six-rowed) are determined (see Trofimovskaya, 1972, for review). It should be noted, that accessions of group A were both two-and six-rowed forms, but there is no order to their clustering. Moreover, genetic distinction between accessions with the same number of rows in the spike, but belonging to different groups was shown by both clustering and PC analyses. We propose the existence of two principal trends in breeding of occidental-type of cultivated barley. However, there maybe some exceptions, for example, the 'Malta' group of cultivars, which possibly have hybrid nature and derived from crossing two-and six-rowed forms. Apart from above mentioned 'Igri', 'Trixi' and 'Malta' group of cultivars related to 'Malta' there are several groups of related accessions (Fig.4). In two-rowed sub-cluster 7 the most interesting group includes both West

European ('Alexis', 'Arena', 'Isaria', 'Aramir' and 'Ursel') and Russian cultivars related to them ('Lyubimets 108', 'Ilmen', 'Krasnoyarskii 80' and 'Auksinyai 3'). In six-rowed sub-cluster 6 there are two groups of accessions. One includes both cultivars with 'Keystone' pedigree background ('Belogorskii', 'Agul 2' and 'Erofei') and 10 landraces (from 16881 to 4972). All of these accessions originated from the northern regions of barley cultivation in Russia (northern Europe and Siberia). Another group includes both West European ('Vogelsanger Gold', 'Mammut' and 'Brunhild') and 8 related Russian winter cultivars were from southern regions. In cluster A group consisting of 5 closely related hulless landraces from Central Asia and Siberia can be seen. Another one includes 5 two-rowed accessions (from Nutans 27 to 2904), which on the PCA plot are quite close to the two rowed accessions of group B (Fig.5). The third group consists of 7 six-rowed landraces and two-rowed cultivar Nutans 27. All are linked to Central Asian origin.

Comparisons between Genetic Distances Based on RFLP and Hordein Electrophoresis

In this study we attempted to compare the use of RFLP and hordeins analyses for determining barley genetic variability. For this purpose for 93 analyzed accessions the genetic distance matrixes obtained separately from RFLP and hordein electrophoresis data were compared. The normalized Mantel statistic obtained from this comparison through 500 random permutations of matrices was low ($r = Z = 0.18$) but highly significant ($p = 0.002$). UPGMA clustering based on hordeins electrophoresis data showed a picture of the accessions grouping (Fig.6) principally different from the one received from RFLP data (Fig.4). But there are several groups of related accessions (marked by grey bands), which have the same grouping on the dendrogram constructed from RFLP data. In our study among 93 accessions 71 different protein phenotypes were determined which indicates the high level of hordein polymorphism and its potential usefulness for barley cultivar identification. Taking into account the relative simplicity of isolation and electrophoresis of hordeins, this methodological approach is valuable for solving many practical problems in breeding, cultivar identification and seed control. But the possibility of using hordein electrophoresis data for studying genetic relationships of different barley cultivars are limited due to the small number of loci determining hordeins. There are only two

Fig.6. Dendrogram constructed from the hordeins polymorphism genetic distance matrix of 93 Eurasian barley accessions.

hordein-determining loci in the barley genome, which are localized on the short arm of 5th chromosome and positioned at a distance of about 15 cM from one another (Graner *et al.*, 1993). Unlike hordeins, RFLP fragments detected by a single clone represent both different alleles and different loci and the abundance of RFLP-markers permits a representative sampling of the whole genome. For these reasons, RFLP-based genetic distances provide a truer estimate of the actual genetic relationship between barley accessions.

Conclusions

In conclusion, our results of studying a diverse collection of barley from different regions of Eurasia are in accordance with recent investigations in barley (Melchinger *et al.*, 1994) that RFLPs are suitable to (i) define a germplasm group more clearly, (ii) assign lines with unknown or incomplete pedigree records to established groups, and (iii) identify diverse germplasm sources. RFLP analysis of barley cultivars and landraces from different countries of Eurasia made it possible to confirm the existence of two principal trends in the evolution of cultivated barley, which are geographically linked with oriental and occidental regions. Also in this study breeding trends were observed, such as sub-grouping of oriental forms and their further sub-grouping to groups of cultivars with similar pedigree background.

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