Genetic Resources and Crop Evolution **46:** 193–205, 1999.

Genetic differentiation and geographical distribution of barley germplasm based on RAPD markers

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Received 2 July 1998; accepted in revised form 21 September 1998

Key words: Hordeum vulgare, diversity, germplasm, RAPD analysis, geographical distribution

Abstract

Random amplified polymorphic DNA (RAPD) analysis was used to characterize barley germplasm genetic diversity. For the analysis 303 morphologically distinctive accessions were selected from the VIR germplasm collection, St. Petersburg, Russia and the MAFF Genebank, Tsukuba, Japan to represent the principal regions of barley cultivation. A total of 93 polymorphic bands scored from RAPD patterns were used to generate a genetic distance matrix, which was used in both cluster and principal coordinate analysis. Both analysis clearly separated barley cultivars and local populations into three distinctive groups, which evidently reflect different directions in evolution and geographical distribution of barley. The hierarchy of accessions clustering in the first group indicates the westward distribution of barley from West Asia to Europe and New World across Ethiopia and then Mediterranean region. The principal breeding trends based on spike morphology are also observed in this group. The second group is associated with eastward distribution of the crop and represents a unified genetic group, which consists of East Asian and Central Asian accessions. The third distinctive group identified is connected with the evolution and dissemination of hulless forms in Central Asia and the Caucasus region. The conformity of identified genetic groups and clusters with the global centers of crops diversity (gene centers) determined by Vavilov (1926) and modern ecogeographical classification of barley is discussed.

Introduction

Genetic improvement of crops by man can be regarded as directed evolution acting upon exist intraspecific genetic variability. To optimise and accelerate breeding, it is essential to screen, evaluate and classify the genetic variability.

For barley one of the hypotheses for the classification of cultivated forms is based on a reticulate mode of differentiation resulting from mutations, domestication and hybridizations between different forms (Bothmer et al., 1995). This approach assumes that there is no simple pattern to barley phylogeny, and therefore the system of barley classification is artificial and should be seen as a means of classifying morphologically similar forms.

Another approach is based on the ecogeographical considerations of barley world genetic resources. Vavilov (1926) on the basis of numerous explorations and experimental studies of crops from different regions of the world determined the principal global centers of crop genetic diversity. He hypothesized that these centers (gene centers) were historically connected with the origin of crops. Among these centers Vavilov determined two primary (West-Asian and East-Asian) and some of the secondary ancient centers of barley cultivation, which were named as global centers of barley diversity (Vavilov, 1957). On the basis of Vavilov's ideas and further investigations of barley genetic resources, the ecogeographical classification of cultivated barley was proposed (Lukyanova et al., 1990; Trofimovskaya, 1972). Recently this classification concept for cultivated barley was recommended for developing a barley core collection (Knupffer &

Table 1. Global centers of barley diversity, countries and codes of countries from which the accessions were analyzed

Center	Countries	Code
European-Siberian	Holland	HOL
	France	FRA
	Germany	GER
	Austria	AUT
	Lithuania	LIT
	Russia	RUS
	Byelorussia	BUE
	Ukraine	UKR
New World	Canada	CAN
	United States of America	USA
	Mexico	MEX
	Peru	PER
	Bolivia	BOL
Mediterranean	Spain	SPA
	Italy	ITA
	Greece	GRE
	Cyprus	CYP
	Israel	ISR
	Egypt	EGY
	Lybia	LYB
	Algeria	ALG
	Morocco	MOR
Abyssinian	Ethiopia	ETH
West Asian	Georgia	GEO
	Armenia	ARM
	Azerbaijan	AZE
	Turkey	TUR
	Iran	IRA
Central Asian	Kazakhstan	KAZ
	Turkmenistan	TUK
	Uzbekistan	UZB
	Tadzhikistan	TAD
	Afghanistan	AFG
East Asian	China	CHI
	Japan	JAP

van Hintum, 1995). In this study we used this approach to analyze genetic diversity of barley crop (Tables 1, 3 and Figure 7).

At the present time new molecular genetic techniques such as restriction fragment length polymorphism (RFLP) and polymerase chain reaction (PCR) based approaches are powerful tools for studies of genetic differentiation in crops (see Ayad et al., 1997, for review). Recently we have found using RFLP markers clear genetic differentiation among the Russian barley germplasm (Strelchenko et al., 1996).

In this work we used RAPD analysis to study genetic variability and relationships between cultivars and landraces from different global centers of barley diversity.

Table 2. The sequence of primers used and the number of RAPD markers

#	Sequence	Number of markers
1	GTCTGACGGT	7
2	GGCGGACTGT	7
3	GCTGTGCAGC	6
4	CGTAGCGCGA	7
5	TGGCCACTGA	9
6	TGGTCACAGA	5
7	CAAACGTCGG	9
8	GTAGACCCGT	5
9	CCTTGACGCA	6
10	CTCTCCGCCA	7
11	AGACGTCCAC	1
12	GGTGACGCAG	2
13	CTATGCCGAC	3
14	CCTGCTCATC	1
15	CTGCGCTGGA	4
16	GAGGTCCACA	4
17	CAGGGGACGA	6

Materials and methods

Materials

The material analyzed included 303 cultivated and wild forms of *Hordeum vulgare* L. From 299 accessions of cultivated barley (subsp. *vulgare*) 289 accessions were selected on the basis of variation in morphological characters (two- and six-rowed, covered, naked, etc.) from the VIR germplasm collection (St. Petersburg, Russia) to represent all global centers of barley diversity (Table 1 and Fig. 7) and 10 Japanese modern cultivars were supplied by the MAFF Genebank (NIAR, Japan). The accessions of cultivated barley comprised 116 cultivars and 183 landraces (local populations) collected during this cen-

Table 3. Distribution of analyzed accessions originating from different global centers of barley diversity to different genetic groups as revealed by cluster analysis of RAPD data

Global centers & wild forms	Group A		Group B		Group C		Total
	No. Acc.	%	No. Acc.	%	No. Acc.	%	
European-Siberian	54	94.7	0	0	3	5.3	57
New World	29	93.5	0	0	2	6.5	31
Mediterranean	25	83.3	2	6.7	3	10.0	30
Abyssinian	28	96.6	1	3.4	0	0	29
West Asian	40	85.1	0	0	7	14.9	47
Central Asian	21	40.4	20	38.4	11	21.2	52
East Asian	16	30.2	31	58.5	6	11.3	53
H.v. f. *agriocriton*	1	50.0	1	50.0	0	0	2
H.v. ssp. *spontaneum*	2	100.0	0	0	0	0	2
Total	216		55		32		303

tury. In addition, accessions W-700 and W-702 of convar. *vulgare* f. *agriocriton* (Åberg) Bowd. and accessions W-34 and W-54 of subsp. *spontaneum* (C. Koch.) Thell. from the VIR germplasm collection were studied. Seeds from all analyzed accessions can be made available upon request from the authors.

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 following the method of Saghai-Maroof et al. (1984). DNA concentrations were estimated and standardised against known concentrations of lambda DNA on 1% agarose gels.

The polymerase chain reaction (PCR) mixture (given for a 10μl total volume) was 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2 mM $MgCl_2$ (all included with the 10×buffer of Perkin Elmer), 0.2 mM each of dATP, dCTP, dGTP and dTTP (from Pharmacia Biotech), 1 mM primer (manufactured by Sci-Media Ltd., Tokyo), approximately 40 ng template DNA, and 0.3 units of Ampli*Taq* DNA polymerase (from Perkin Elmer). PCR mixtures were prepared in 96-well microplates, covered with mineral oil and subjected on the PTC-100 thermal cycler (from MJ Research, Inc.) to 45 cycles of 93 °C (1 min), 36 °C (2 min), 72 °C (3 min), followed by 72 °C for 7 min to complete DNA synthesis prior to storage at 4 °C. After the addition of 3 μl 6×dye solution (0.1% bromophenol blue, 0.1% xylene cyanol FF and 15% Ficoll), 10 μl of reaction mixture was loaded into a 1.4% agarose gels. Gels were 16.5 cm long and 8 cm broad with 18 lanes, and three rows of wells. Lambda DNA digested by *Pst* I was used as a mixture of size markers. After loading the gels were run in TAE buffer at 2.5 V/cm for approx. 3 h, until the bromophenol blue marker migrated approx. 5 cm. After staining with ethidium bromide the gels were photographed on the Polaroid POLAPAN 3200 B films (print area 7.3×9.5 cm).

Statistical analysis of data scored

RAPD patterns on gels were scored by assigning a number to each band. For subsequent numerical analysis, data were coded in a binary form, i.e., presence or absence of a band in a line was recorded 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 calculation:

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

where d_{ij} is the genetic distance between accession i and accession j, x_{ki} is the i allele frequence at locus k, x_{kj} is the j allele frequence at locus k and n is the total number of loci. Phenogram was produced using unweighted pair-group method arithmetic average (UPGMA) clustering and scatter diagram resulted from principal coordinate analysis (PCA) on the genetic distance matrix. The program NTSYS-pc version 1.8 (Rohlf, 1993) was used for generation of the distance matrix, UPGMA clustering and the PCA.

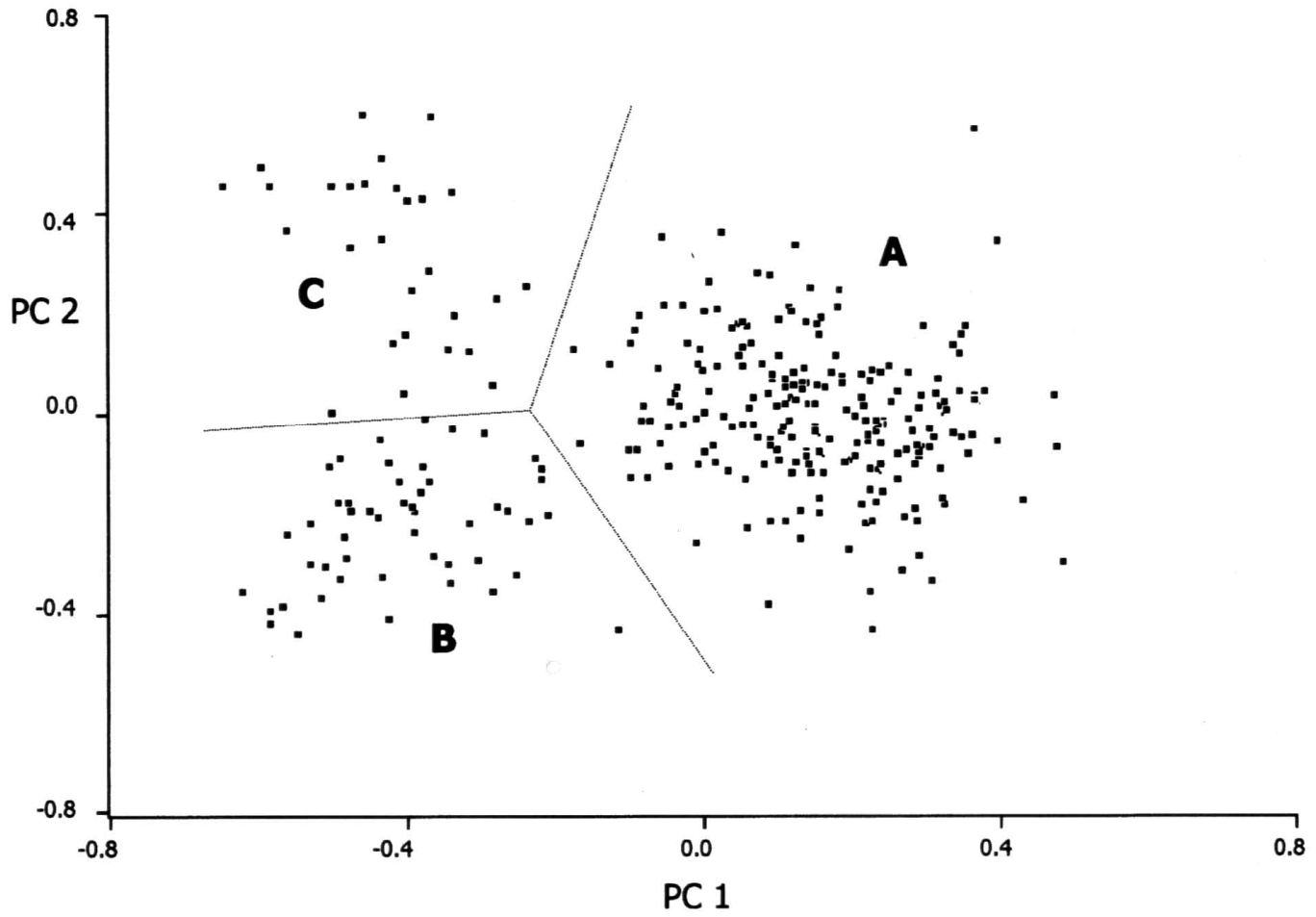

Figure 1. Plot of the first two principal coordinate scores for 303 barley accessions based on the PCR product banding patterns.

Results

Among 149 random decamer primers screened with 16 accessions, the 17 primers were selected for RAPD analysis (Table 2). When PCR products were analyzed on the agarose gels, each primer generated from 3 to 15 distinctive bands ranging from 0.3 to 3 kbp. Only polymorphic and reproducible in multiple runs bands were scored in the preparing of database. A total of 171 products were amplified against the 303 accessions. From 118 bands were scored over all 17 primers 25 bands were omitted in order to circumvent false-positive scoring of co-migrating and ambiguous RAPD fragments and only 93 bands with an average of 5.5 bands per primer were included in the final data matrix. From 303 accessions estimated on the RAPD patterns only 3 pairs of morphologically similar landraces (18844 and 18853 from Ethiopia, 14915 and 16637 from Tadzhikistan, 6154 and 16610 from Georgia) were indistinguishable from each other. They may represent duplicate accessions gathered from the same landrace population.

The means of Nei's distance ranged from 0.016 (between landraces 21544 and 21554 from Bolivia) to 1.589 (between landraces 3397 and 6557 from Turkey and Armenia, respectively). The relationships among 303 barley accessions based on RAPD genetic distance measurements were analyzed by PCA and UPGMA clustering. Both analysis divided accessions analyzed into three main genetic groups. In PCA 67.9% of the variation was accounted for by the first two principal coordinate (PC) axes. The first PC explained 45.2% of variation and clearly divided the accessions into two main groups. The first group (A) was more abundant. The second group was more heterogeneous and divided by the second PC, which explained 22.7% of variation, into two groups B and C (Figure 1). Accessions of group A originate from all global centers of barley diversity. Accessions of group B originate predominantly from East Asia and Central Asia, while the group C contains mainly Central-Asian and West-Asian accessions.

As revealed by UPGMA clustering all accessions were classified into three major groups A, B, and C

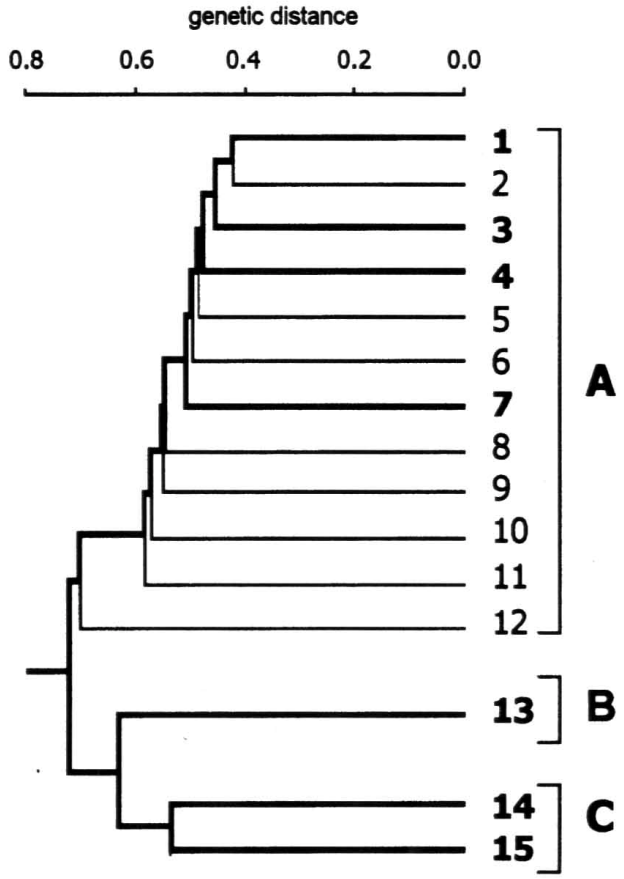

Figure 2. Distribution of clusters on the phenogram of 303 barley accessions based on the PCR product banding patterns. The major clusters are drawn by thick lines.

(Figure 2) consisting of 216, 55 and 32 members, respectively. The groups A, B and C on the phenogram correspond to the groups A, B and C on the PCA plot. Group A comprises the majority of European-Siberian, New World, Mediterranean, Ethiopian and West Asian accessions, though it also includes accessions of all other centers of barley diversity (Table 3). On the other hand 58.5% of East Asian accessions are positioned in group B, which consists largerly of East Asian (56.4%) and Central Asian (36.4%) accessions. Group C consists largerly of Central Asian (34.4%) and West Asian (21.9%) accessions. The Central Asian gene center is the most diverse based on the results obtained. The accessions originating from this gene center are distributed among groups A, B and C in proportions 40.4%, 38.4% and 21.2%, respectively. A lower diversity of barley germplasm is observed for East Asian, West Asian and Mediterranean centers.

A total of 15 clusters, consisting of 12 in group A, 1 in group B and 2 in group C were detected on the phenogram (Figure 2). From 12 clusters which were defined in group A, four clusters (1, 3, 4 and 7) are major. Taking into account the characters of accessions clustered together, these clusters may be considered as distinctive genetic groups of barley crop. The clusters 2, 5, 6, 8, 9, 10, 11 and 12 consist of a small number of accessions. These probably represent intermediate forms or genetic groups which are insufficiently represented in this study.

The most spacious is cluster 1 which is divided into two sub-clusters, *a* and *b*. Sub-cluster *a* comprises 58 accessions of two-rowed barley and 7 six-rowed forms. Within this sub-cluster two groups are defined (Figure 3). The first group includes accessions from West Europe (cvs. Igri and Malta), Central Asia (cvs. Medicum 8955, Unumli-Arpa and 4 landraces), West Asia (4 landraces) and New World (cv. Hector). Among 52 accessions of the other group, the European-Siberian center is presented by 24 accessions (46.2%) including spring West European cvs. Alexis, Arena, Aramir, Ursel, Isaria and 19 cultivars and landraces from Russia, Ukraine, Byelorussia and Lithuania. Nine accessions from Ethiopia, eight from New World and Japanese modern malting cv., Miharu Gold are also included in this group. Unlike sub-cluster *a* the sub-cluster *b* consists largerly of six-rowed accessions and was also divided into two groups. Out of 36 accessions presented in the first group (Figure 4), 14 accessions (38.9%) originate from the European-Siberian center including West European winter cvs. Vogelsanger Gold, Mammut and 12 winter and spring cultivars from Russia and Ukraine. Moreover, this group contains 7 accessions from the West Asian center and 5 accessions from the New World one. Another group of sub-cluster *b* consists of 21 accessions including 11 Russian cultivars and landraces originating largerly from the nothern regions of Russia (North Europe and Siberia).

Cluster 3 combines 25 accessions (Figure 5). The majority of those are six-rowed forms originating predominantly from the Mediterranean and the New World centers (13 and 8 accessions, respectively). The European-Siberian and Ethiopian accessions are not involved in this cluster. Thirteen out of 11 two-rowed and 19 six-rowed forms grouped in cluster 4 originate from Ethiopia. The Ethiopian accessions are divided into two groups: one includes 9 accessions having mainly dark coloured kernels and the other group includes 4 hulless accessions. Moreover, cluster 4 comprises 5 landraces from West Asian countries and 4 accessions from Caucasus region including cvs. Prikumskii 22 and Krasnodarskii 2929.

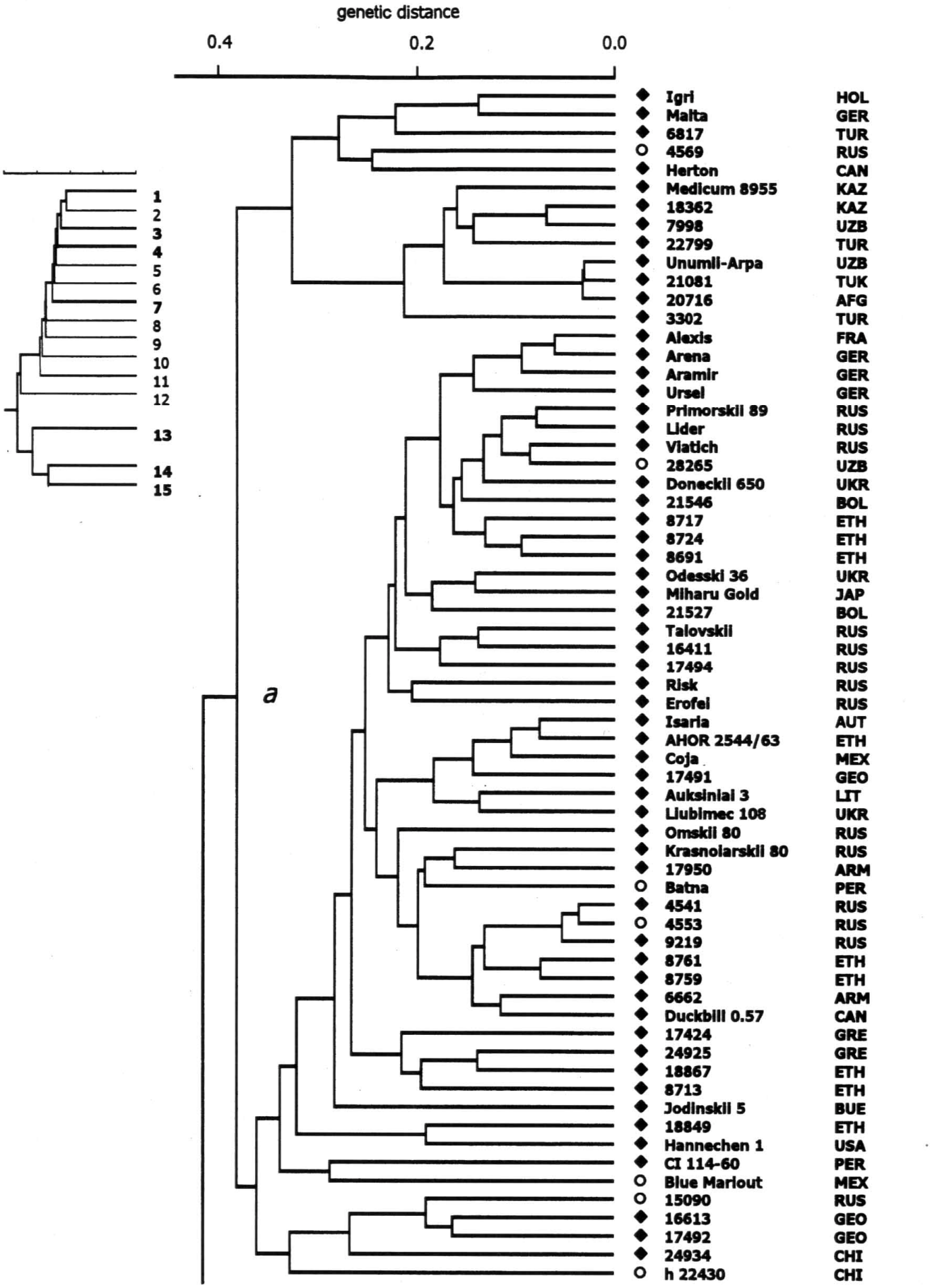

Figure 3. Phenogram of sub-cluster *a* of cluster 1 with designations of two- (♦) or six-rowed (○) accessions, name of cultivars or the VIR cat. # of landraces and country code of origin. Hulless forms are designated by «h».

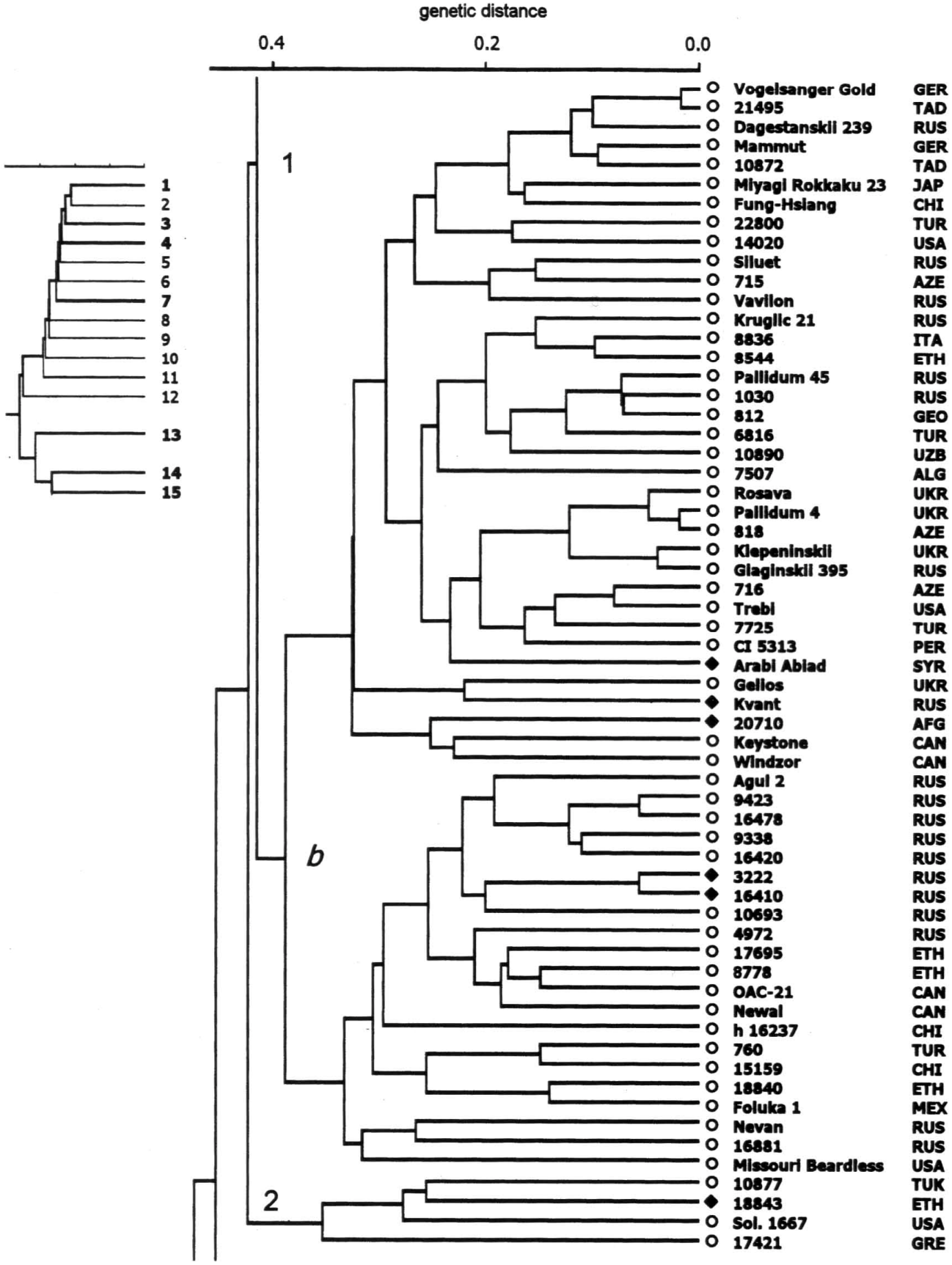

Figure 4. Phenogram of sub-cluster *b* of cluster 1.

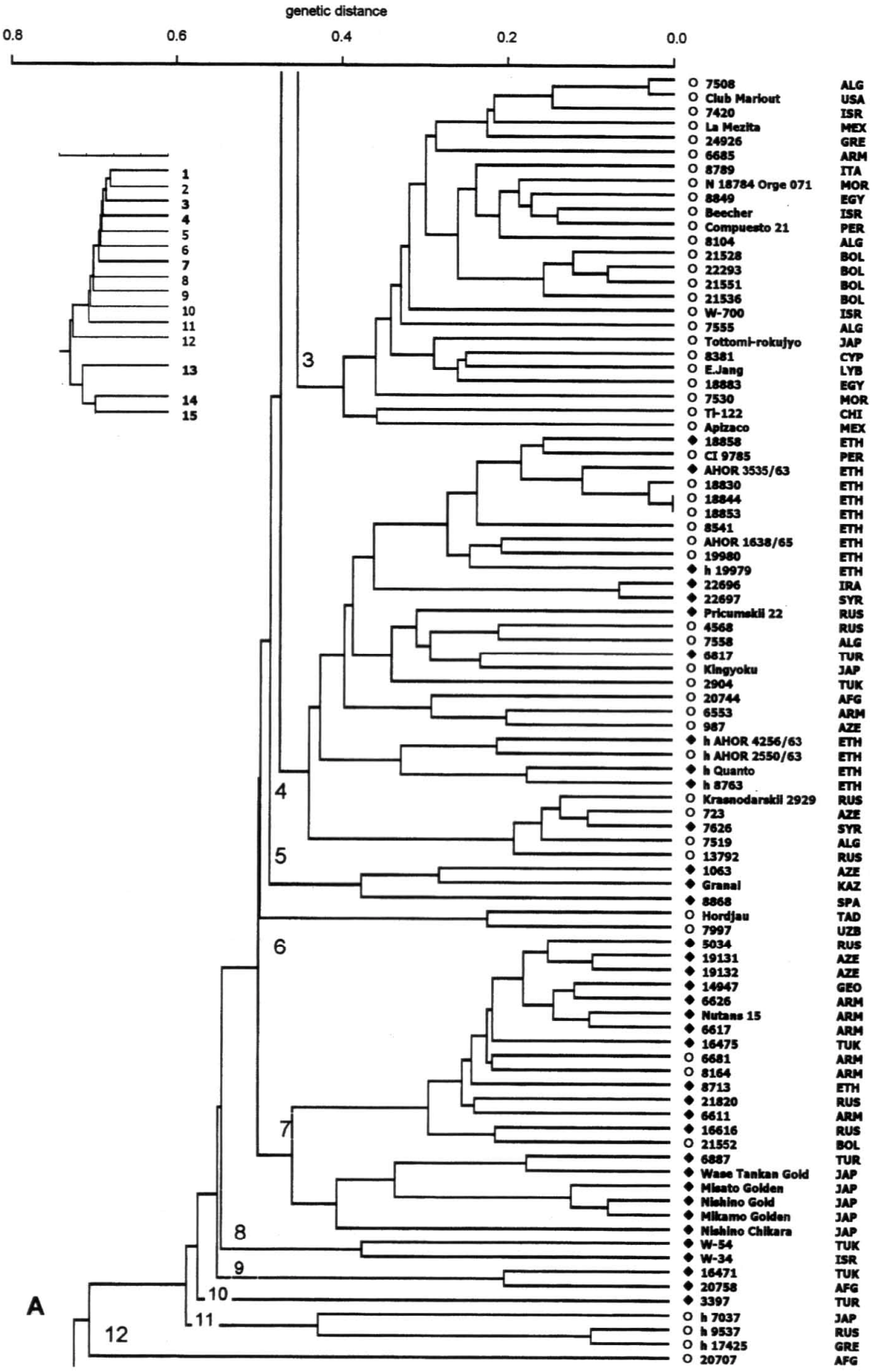

Figure 5. Phenogram of clusters 2–12.

The cluster 7 consists of 21 largely two-rowed accessions including 12 landraces from West Asian countries and Caucasus regions. It also contains 5 Japanese cultivars: old Wase Tankan Gold and modern malting Misato Golden, Nishino Gold, Mikamo Golden, Nishino Chikara.

In minor clusters there are both analyzed accessions of subsp. *spontaneum* (cluster 8) and the group of three six-rowed hulless landraces from Japan, Russia and Greece (cluster 11). Most distant cluster 12 is presented by only one six-rowed landrace from Afghanistan.

Group B is represented by only cluster 13 (Figure 6). It comprises 55 accessions, the majority of those are six-rowed forms originating predominantly from East Asia and Central Asia (31 and 21 accessions, respectively). Apart from these there are only 3 landraces from Israel, Egypt and Ethiopia. This cluster includes 7 hulless accessions and 5 Japanese modern food cvs. Ichiban Boshi, Kashima Mugi, Natori Omugi, Shan Rai and Masakado Mugi. The combining of accessions originating from East Asia and Central Asia in the one cluster probably indicates a genetic linkage between the East Asian and Central Asian centers of barley diversity.

Group C is divided into two clusters 14 and 15, which include largely hulless accessions. Cluster 14 comprises 3 groups of six-rowed accessions: the first group combines 5 hulless accessions from Japan and China, the second one consists of 9 hulless landraces of var. *coeleste* L. originating mainly from Central Asia and the third group includes 6 hulled landraces from West Asia, Cyprus and Morocco. Cluster 15 contains 12 hulless landraces which are genetically closely related and originate largerly from Central and West Asia. There are 11 two-rowed landraces of var. *nudum* (L.) Alef. and a six-rowed landrace of var. *coeleste* from Russia (Sakhalin) among them.

Discussion and conclusions

RAPD analysis of 303 morphologically distinctive accessions originating from different regions of the world revealed three major genetic groups in cultivated barley germplasm. The first group (A) comprises the majority of European-Siberian, New World, Mediterranean, Ethiopian and West Asian accessions. The second group (B) includes accessions originating predominantly from East and Central Asia. Thus, the accessions of groups A and B are not only genetically distinguishable, but also originate from different regions of the world. Vavilov (1926) was the first to point out an exotic character of barley from East Asia. Afterwards, the hypothesis of independent domestication of barley in 'oriental' and 'occidental' regions was suggested in an attempt to explain origin, dispersion and genetic differentiation of six-rowed cultivars (Takahashi, 1955; Freisleben, 1940). However, at present it is generally accepted that cultivated barley was first originated from a wild two-rowed barley, *H. vulgare* subsp. *spontaneum*, probably somewhere in the Fertile Crescent of the Near East (Harlan, 1976). Though there is much evidence for oriental-occidental differentiation of barley (Ordon et al., 1996; Takeda, 1996; Konishi, 1995; Cross, 1994; Zhang et al., 1994; Takahashi, 1987; Tolbert et al., 1979) the real cause of this differentiation is still unknown. In our study the division of barley on the occidental and oriental forms can be seen as separation of analyzed accessions into groups A and B, respectively. Moreover using RAPDs we recently found among H. *vulgare* subsp. *spontaneum* the groups of accessions related both to occidental or oriental races of cultivated barley (Strelchenko & Okuno, unpublished). Thus, the broad clustering into oriental and occidental accessions is likely to reflect different sources of wild barley germplasm contributing to the two genetic groups of cultivated barley.

While genetic differentiation of barley based on RAPDs is evident on the PCA plot and UPGMA phenogram, a pattern of geographical distribution of accessions among groups of clusters is more complex. In the group A four principal clusters linked in hierarchic order may be determined (see Figures 3, 4 and 5). In the direction from the root of phenogram and *H. vulgare* subsp. *spontaneum* (cluster 8) these are: two-rowed West Asian (cluster 7)→two- and six-rowed Ethiopian with West Asian (cluster 4)→six-rowed Mediterranean with New World (cluster 3)→separately clustered six- and two-rowed European-Siberian with some of the New World and Ethiopian accessions (cluster 1). In our opinion these clusters may be steps in the evolution and geographical distribution of the occidental type of barley after its domestication and the above presented scheme probably reflects the order of arising of global centers of barley diversity. It should be noted here that though there are distinctive genetic groups historically linked with the origin of the West Asian, Ethiopian and Mediterranean centers, many modern accessions from these centers are combined with European-Siberian

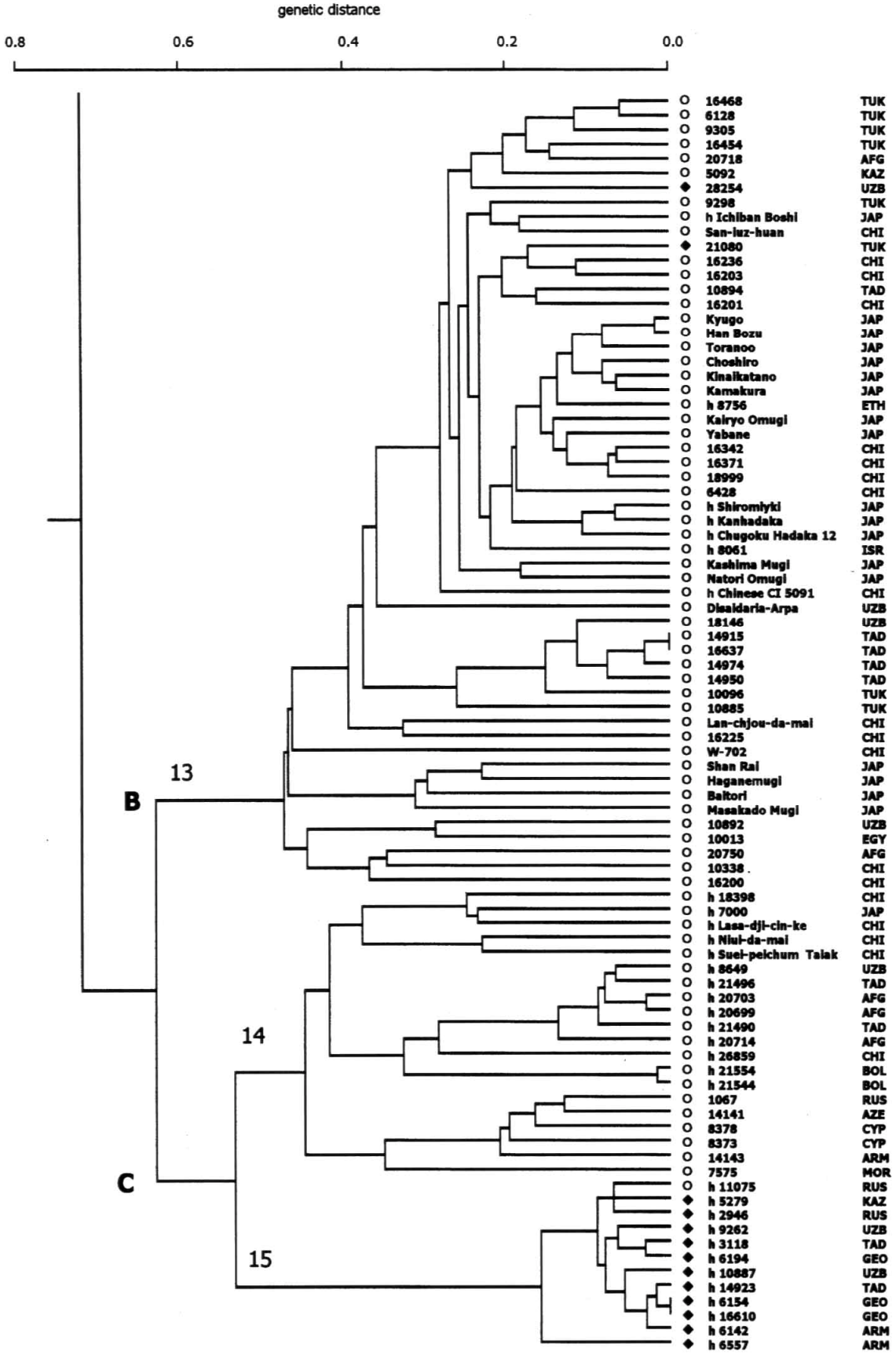

Figure 6. Phenogram of clusters 13–15.

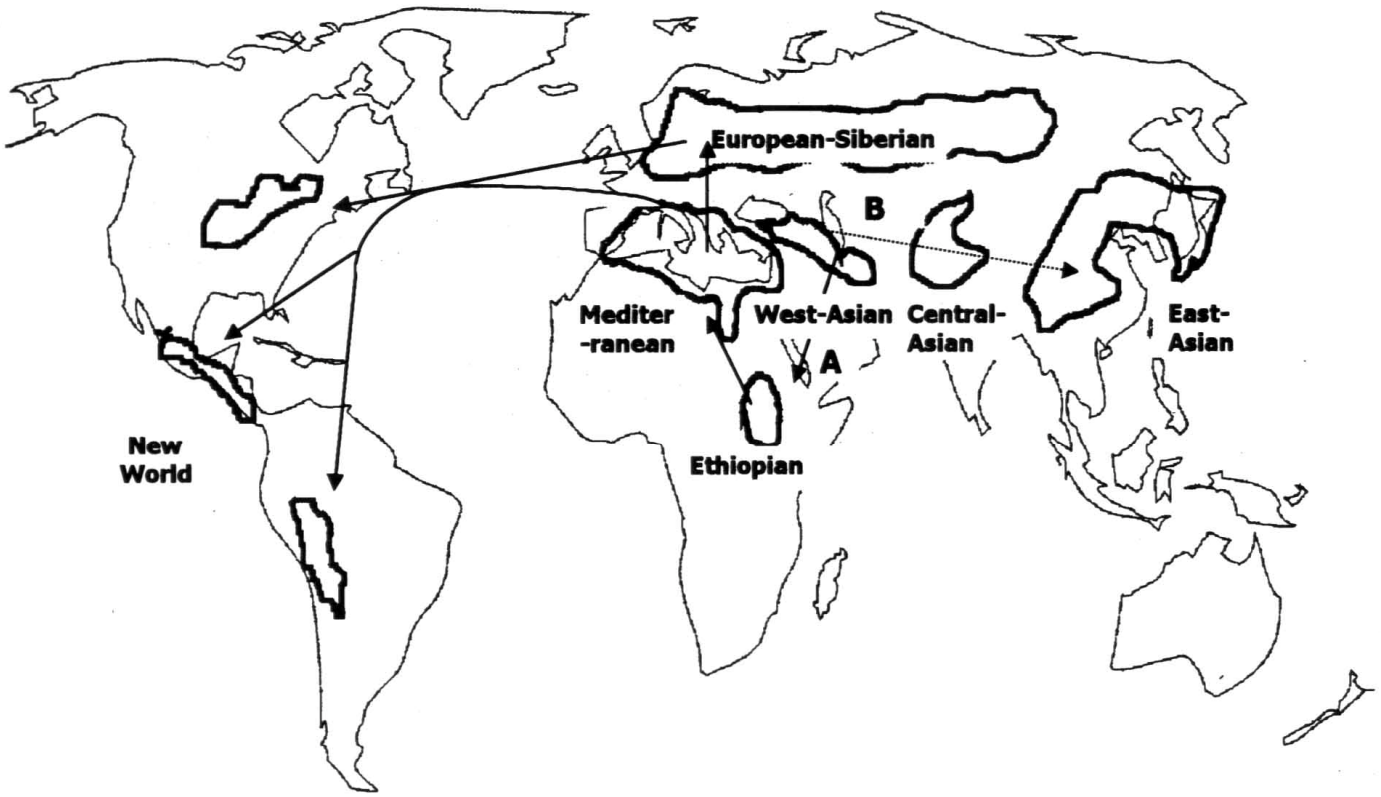

Figure 7. Global centers of barley diversity and main routes of cultivated barley distribution (⟵ - westward and ⟵ - - - eastward) as revealed by RAPDs.

accessions in a spacious cluster 1. It may be explained by intensive exchange of seed material last decades which have caused a mis-alignment of accessions to a particular origin and sometimes it is difficult to identify the real origin of cultivars or advanced landraces. We can see a similar situation among East and Central Asian accessions clustered in group A. From 16 East Asian accessions there are only 5 landraces and from 17 Central Asian landraces grouped only 6 are old entries in the germplasm collection.

New World accessions do not form a distinctive group and are genetically related to European-Siberian or Mediterranean accessions. The position of the New World accessions in the phenogram (clusters 1 and 3) might allow the supposition that barley was introduced to the New World in former times in two different ways: Spaniards might have introduced mainly Mediterranean material into Meso- and S. America, whereas most N. American barleys descended from European-Siberian material, introduced during Middle European immigration to the northern part of America.

The European-Siberian center (cluster 1) is the most spacious in this study. The accessions of this cluster are separated on the basis of spike morphology into two sub-clusters of predominantly two- or six-rowed forms *a* and *b*, respectively. Such division probably reveals two principal trends in breeding of European-Siberian, some of the New World and Ethiopian barleys. Using RAPDs similar results were recently obtained by Tinker et al. (1993) for the North American barleys, and Ordon et al. (1996) and Noli et al. (1997) for the West-European barleys. Using RFLP analysis we have demonstrated a clear difference between two- and six-rowed forms of Russian barleys (Strelchenko et al., 1996). Among sub-clusters *a* and *b* there are groups of accessions clustered together which we have also recognized in RFLP analysis of barley diversity. These groups probably represent the principal directions in breeding of both two- and six-rowed European-Siberian barleys.

The accessions of cluster 1 combine on the lowest hierarhic level on the phenogram and genetically are more similar than accessions of hierarchically higher arranged clusters. The majority of analyzed improved European and American cvs. are positioned in this cluster. Based on these considerations, we suppose that in addition to old European-Siberian cvs. cluster 1 contains most of the improved cvs. (elite genepool) of occidental barleys. The exotic genepool of occidental barleys separates into a number of genetic groups

(clusters 2–12). Some of them are probably connected with ancient agriculture centers which arose consecutively in a western direction. Furthermore, since on the base of some exotic forms improved cvs. have been received, they can combine together with initial forms in cluster 1. It should be noted that though the accessions of elite and exotic genepools are morphologically very similar and frequently belong to the same botanical variety, they differ considerably in their RAPD patterns. These data show the subjection of barley morphology to breeding effect and difficulty of using morphological characters for defining crop intraspecific genetic differentiation.

The majority of analyzed East Asian accessions (58.5%) are in group B (cluster 13) and this group evidently represents the group of oriental barleys as was claimed by Takahashi (1955). He reported that the oriental barleys were distributed in Nepal (Highland), China (proper), Korea and Japan. In this study we identified a considerable number of Central Asian accessions related to Chinese and Japanese oriental barleys. It indicates a relationship between the East Asian and Central Asian centers of barley diversity. Evidently these centers form a unified genetic group of barley which is historically connected with the common center of ancient agriculture. If so, both centers may be considered as the eastward distribution of oriental barley from its original birth place.

Analyzed accessions of *H. vulgare* f. *agriocriton* in cluster 13 (W-702 from Tibet) and in cluster 3 (W-700 from Israel) confirms the secondary origin of these forms from related cultivated barleys.

In this study a third distinctive group of barley germplasm, C, was identified. It is closer to the oriental (group B) rather than occidental barleys (group A) based on the PCA and UPGMA analysis. This group combines mainly hulless accessions from Central and West Asia and is clearly divided into six-rowed (cluster 14) and two-rowed (cluster 15) forms. The accessions of the latter cluster are closely related and hierarhically positioned higher on the phenogram than six-rowed accessions of cluster 14.

In conclusion, the results of this study show RAPDs are useful for defining intraspecific relationships of cultivated barleys. Using PCA and UPGMA analysis of RAPDs data, at least three distinctive groups among the world barley germplasm have been determined. These groups reflect different directions of evolution and dissemination of cultivated barley. The first group represents the occidental barleys. In geographical distribution these are probably historically connected with ancient agriculture centers which had been consecutively arising westwards from West Asia to Europe across Ethiopia and then the Mediterranean region (Figure 7). In barley germplasm these centers are represented by distinctive genetic groups, which coincide with centers of diversity in its ecogeographical classification. The New World has only a short history of barley cultivation, it does not form its own genetic group and accessions of this region developed largely from European-Siberian and Mediterranean barleys. The second group connected with the presumed center of origin and eastward distribution of oriental barleys in Central and East Asia. It is a unified genetic group historically connected with the common ancient center of agriculture. A high level of genetic distinction of these barleys from the occidental forms is explained not only by their geographical and ecological isolation, but also by their evolution with different forms of wild barley. The third distinctive group is connected with the origin and dissemination of hulless barleys in Central Asia and Caucasus region.

Acknowledgements

The senior author acknowledges the receipt of the Japan International Research Center for Agricultural Sciences visiting research fellowship which enables this work to be undertaken, the staff of the Plant Genetic Diversity Laboratory of NIAR for collegiality, technical assistance, materials and help during the performing of this work. The authors thank Dr. D. A. Vaughan for constructive reviewing of the manuscript.

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