Theor Appl Genet (2005) 530: 530–539
DOI 10.1007/s00122-005-2044-2

ORIGINAL PAPER

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Patterns of AFLP variation in a core subset of cultivated hexaploid oat germplasm

Received: 21 January 2005 / Accepted: 18 April 2005 / Published online: 7 June 2005

Abstract Many core collections have been developed from large collections of crop germplasm, but most of these have not been characterized, particularly using molecular techniques, for germplasm management and utilization. We have attempted to characterize a structured sample representing a world collection of 11,622 cultivated hexaploid oat accessions in the hope of understanding the genetic structure of the world collection. The amplified fragment length polymorphism (AFLP) technique was applied to screen 670 accessions representing 79 countries and one group of uncertain origin. For each accession, 170 AFLP polymorphic bands detected by five AFLP primer pairs were scored. Analyses of the AFLP data showed the effectiveness of the stratified sampling applied in capturing country-wise AFLP variation. The frequencies of polymorphic bands ranged from 0.11 to 0.99, with an average of 0.72. The majority (89.9%) of the AFLP variation resided within accessions of each country, and only 6.2% of the AFLP differences existed among accessions of major geographic regions. Accessions from the Mediterranean region were the most distinct, while those from Russia and the USA were the most diverse. The two distinct groups that were observed were separated largely on the basis of common oat and red oat. Red oat was genetically more diverse than its common and hull-less counterparts, and hull-less oat was more related to common oat than red oat. Landrace and non-landrace accessions displayed similar AFLP variation patterns. These patterns are significant for understanding the domestication of cultivated oat and are useful in classifying the intraspecific diversity of oat germplasm, developing specific core subsets of the oat collection, and exploring new sources of genes for oat improvement.

Communicated by D.A. Hoisington

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Introduction

Many large collections of crop germplasm have been assembled around the world, but these have been inadequately characterized for germplasm management and utilization (FAO 1998), primarily because the size of these large germplasm collections represents a major obstacle. The establishment of an active, smaller working collection was suggested by Harlan (1972), while Frankel and Brown (1984) proposed the establishment of a core collection consisting of 10% or so of the accessions; both proposals provide good alternatives for managing large collections and exploring useful germplasm for crop improvement. Consequently, many core subsets have been developed over the last two decades to represent the range and structure of genetic variability in large germplasm collections (Brown and Spillane 1999). However, most of these core subsets are not well characterized (Liu et al. 2001; Ude et al. 2003), particularly through the application of molecular techniques (Karp 2002; Fu 2003). Many important questions have been inadequately addressed, such as how representative are the developed core subsets, what levels of genetic diversity are captured, and how the germplasm accessions are genetically structured in these core subsets. This lack of characterization partly explains why so little is known about the genetic variation and structure of many large collections and why most of these collections are still under-utilized.

Oat (*Avena* L.), as one of the major cereal crops, has received considerable attention with respect to collection and conservation. Approximately 222,000 accessions of

wild and cultivated oats are stored in seed gene banks worldwide (FAO 1998). Plant Gene Resources of Canada (PGRC; the Canadian national seed gene bank) at Saskatoon maintains a unique world collection of oat germplasm with more than 27,000 accessions of 26 oat species (Diederichsen et al. 2001). The collection includes 11,622 accessions of cultivated hexaploid oat (*A. sativa* L.), 11,461 accessions of animated oat (*A. sterilis* L.), 2,025 accessions of slender oat (*A. barbata* Pott ex Link), and 579 accessions of wild oat (*A. fatua* L.). The cultivated hexaploid oat accessions originate from 79 countries and are widely representative, although unequally, of all the continents. When these accessions were grouped following the intraspecific classification suggested by Diederichsen (2004), 77.0% of them were designated common oat (*A. sativa* L. ssp. *sativa*), 12% red oat [*A. sativa* L. ssp. *byzantina* ©. Koch) Romero Zarco], 1.6% hull-less oat [*A. sativa* L. ssp. *nudisativa* (Husn.) Rod. et Sold.], and 9.4% unclassified. To facilitate the management and utilization of the cultivated hexaploid oat germplasm, PGRC established a core subset of 646 accessions from the 11,622 accessions through a random sampling stratified with respect to country of origin and with the size equal to the natural logarithm frequency of accessions for a country. However, no molecular characterization was made on this core subset.

Exploitation of oat genetic resources for oat improvement requires a knowledge of the range and structure of genetic variability present in the oat gene pools, but to date a comprehensive characterization of the existing gene pools of cultivated hexaploid oat is largely lacking, particularly one based on molecular techniques. Efforts have been made to characterize North American germplasm of cultivated hexaploid oat using pedigree techniques (Souza and Sorrells 1989) and phenotypic traits (Souza and Sorrells 1991a, b), isozymes (Murphy and Phillips 1993; Phillips and Murphy 1993), and molecular markers (O'Donoughue et al. 1994; Fu et al. 2003, 2004). These characterizations revealed (1) genetic narrowing in the existing North American oat gene pool; (2) more genetic diversity in fall-sown, than spring-sown, oat germplasm; (3) various genetic relationships of oat germplasm of different sources and groups; (4) the existence of two cultivated gene pools. These variation patterns are useful for understanding the existing gene pools of North American oat (Jellen and Beard 2000) and would facilitate germplasm selection within the framework of North American oat improvement. However, these gene pool-specific patterns are of limited assistance for the management of oat germplasm and the exploration of useful genotypes in a large world collection. While amplified fragment length polymorphism (AFLP) markers (Vos et al. 1995) have been widely applied to characterize crop germplasm and demonstrated to be effective in detecting genetic variation (Karp 2002; Fu 2003), they have only seldom been applied to cultivated hexaploid oat (Fu et al. 2004).

The objectives of the study reported here were to (1) assess the genetic variation and structure of a stratified sample of 670 cultivated hexaploid oat accessions using AFLP markers; (2) determine the effectiveness of the stratified sampling applied in capturing genetic variability.

Materials and methods

Plant materials

The cultivated hexaploid oat germplasm used in this study consisted of 646 accessions stratified with 79 countries across all continents and 24 accessions of uncertain origin as controls (Table 1). Specifically, there were 476 common oat, 76 red oat, 33 hull-less oat, and 85 unclassified accessions. In addition, 173 accessions were recorded as landraces in this structured sample. To facilitate the inference of regional associations of oat accessions, we grouped the 670 accessions according to the regional classification of Zhukovsky (1968) [subsequently described by Zeven and Zhukovsky (1975)] for 12 crop diversity centers, not according to the eight "centers of origin" of Vavilov (1926, 1951). Zhukovsky's classification is an expansion of Vavilov's centers from 8 to 12 regions by taking into account newly accumulated evidence and is thus more informative (Zeven and Zhukovsky 1975). To reduce any bias due to accession size in this analysis, we separated the ninth region into two subregions: East Europe (9a) and West Europe (9b).

DNA extraction and AFLP analysis

About 10–15 kernels of each selected accession were obtained from the PGRC collection of cultivated hexaploid oat and grown in the greenhouse at the Saskatoon Research Centre, Agriculture and Agri-Food Canada. Young leaves were collected from 5- to 10-day-old seedlings of each accession, bulked, freeze-dried (in a Labconco Freeze Dry System for 3–5 days), and stored at −80°C. The DNA extraction and AFLP analysis were as described in detail by Fu et al. (2004).

Ten *Eco*RI:*Mse*I primer pairs were initially screened on five randomly selected samples to assess the informativeness of these primer pairs in detecting molecular variation. The five most informative pairs (Table 2) were selected for this AFLP analysis. To assess the consistency of the AFLP profiles across the seven gels, we carried out a blind test with two randomly selected DNA samples, which were placed in four different gels. Comparisons of the band patterns between gels for a primer pair revealed up to 8% mismatch, clearly reflecting the possible magnitude of a technique-related error. A test of scoring error made by two individuals revealed an additional 3–5% mismatch. Such levels of mismatch were rather high when compared with those reported by other investigators, (see, for example,

Table 1 AFLP variations for 670 cultivated hexaploid oat accessions representing 79 countries, 12 proposed regions, and one group of uncertain origin

Country and region code[a]	Number of accessions	Number of polymorphic bands[b]	Mean band frequency	Within-country variation[c]
Afghanistan (5)	3	65	0.518	43.33
Albania (7)	3	57	0.518	36.33
Algeria (7)	9	110	0.617	41.50
Azerbaijan (6)	6	95	0.586	43.20
Argentina (10)	10	119	0.605	47.96
Australia (3)	13	125	0.673	45.38
Austria (9b)	8	89	0.678	30.25
Armenia (6)	3	56	0.530	38.00
Belgium (9b)	7	92	0.613	30.24
Bolivia (10)	2	44	0.500	46.00
Bosnia (9a)	6	109	0.592	49.07
Brazil (10)	11	138	0.645	51.96
Bulgaria (9a)	14	123	0.672	35.47
Belarus (9a)	6	77	0.632	33.87
Canada (12)	14	129	0.674	41.63
Chile (10)	13	144	0.635	51.94
China (1)	12	144	0.661	48.61
Colombia (10)	3	62	0.538	41.33
Croatia (9a)	8	114	0.623	43.93
Cyprus (7)	3	57	0.509	38.00
Czech (9a)	9	104	0.629	39.33
Denmark (9b)	9	109	0.666	37.00
Ecuador (10)	8	149	0.638	62.14
Ethiopia (8)	11	125	0.628	39.65
Estonia (9a)	6	121	0.658	53.33
Finland (9b)	12	137	0.662	46.53
France (9b)	13	123	0.630	40.40
Georgia (6)	7	68	0.592	27.90
Germany (9b)	30	146	0.701	35.90
Greece (7)	11	128	0.624	48.49
Hungary (9a)	8	81	0.620	27.07
India (4)	9	133	0.641	57.72
Indonesia (2)	1	NA	NA	NA
Iran (5)	5	81	0.583	40.80
Ireland (9b)	6	90	0.641	36.47
Israel (6)	5	84	0.548	35.10
Italy (7)	7	104	0.635	46.10
Japan (1)	6	89	0.616	36.33
Kazakhstan (5)	4	61	0.557	33.17
Kenya (8)	4	106	0.576	58.50
North Korea (1)	4	57	0.618	32.50
South Korea (1)	3	26	0.551	18.00
Kyrgyzstan (5)	3	58	0.546	40.00
Latvia (9a)	8	137	0.691	50.71
Liberia (8)	2	59	0.500	60.00
Lithuania (9a)	6	93	0.692	40.87
Mexico (11)	8	85	0.658	34.71
Mongolia (1)	12	109	0.687	32.05
Moldova (9a)	3	58	0.563	40.00
Morocco (7)	9	100	0.593	34.17
Nepal (2)	1	NA	NA	NA
Netherlands (9b)	10	111	0.677	33.16
New Zealand (3)	8	118	0.626	49.68
Norway (9b)	2	46	0.500	46.00
Pakistan (4)	4	76	0.556	43.67
Peru (10)	9	133	0.627	55.39
Poland (9a)	18	137	0.669	42.31
Portugal (7)	6	104	0.591	50.80
Romania (9a)	7	110	0.638	45.33
Russia (9a)	24	151	0.680	41.40
Slovakia (9a)	1	NA	NA	NA
Slovenia (9a)	6	79	0.619	30.13
South Africa (8)	7	109	0.650	46.38
Zimbabwe (8)	6	96	0.644	42.53
Spain (7)	11	127	0.607	51.05
Sweden (9b)	18	133	0.699	34.28
Switzerland (9b)	1	NA	NA	NA
Syria (6)	2	25	0.500	26.00
Tunisia (7)	7	119	0.617	48.76
Turkey (6)	16	142	0.648	53.08
Uganda (8)	1	NA	NA	NA
Ukraine (9a)	11	122	0.648	41.67
Macedonia (9a)	9	78	0.643	25.78
Egypt (7)	6	79	0.587	32.93
UK (9b)	15	131	0.640	41.34
USA (12)	32	151	0.694	49.85
Uruguay (10)	6	119	0.593	50.00
Uzbekistan (5)	3	89	0.596	59.33
Yugoslavia (9a)	16	143	0.649	42.52
Unknown (13)	24	139	0.693	36.35

[a] Following the regional classification for crop diversity first proposed by Zhukovsky (1968) and later described by Zeven and Zhukovsky (1975), 79 countries were grouped into 12 regions (with assigned codes): East Asia (1), Indochina (2), Australia-New Zealand (3), Indian Subcontinent (4), Central Asia (5), West Asia (6), Mediterranean (7), Africa (8), East Europe (9a), West Europe (9b), South America (10), Central America (11), and North America (12). The ninth region was separated into two sub-regions to reduce the size impact in the analysis. The code for the unknown group is 13

[b] For a country with one accession, the number of polymorphic bands, the mean band frequency, and the within-country variation were not available (NA)

[c] Within-country variation calculated from the sum of squares from analysis of molecular variance

Hansen et al. 1999). Thus, extra efforts to minimize technique-related errors were made, the selected bands were scored separately by two individuals, and any band displaying more than a 5% mismatch between two scorers was discarded from the analysis.

Data analysis

The numbers of observable and monomorphic AFLP bands were counted for gels generated from each primer pair. Selective polymorphic AFLP bands that were distinct for all of the samples were manually scored as 1 (present) or 0 (absent), and these scored bands were assessed for mismatch before being selected for further analysis. The selected polymorphic bands were analyzed for the level of polymorphism with respect to primer, country of origin, and proposed region: the number of polymorphic bands was determined and summary statistics on band frequencies were generated. To visualize the variation pattern, we plotted the numbers of polymorphic bands against their frequencies of occurrence in all the assayed accessions. To assess the impact of accession size on the polymorphism observed for each country and region, we carried out a regression using

Table 2 AFLP variations revealed by five AFLP primer pairs in 670 cultivated hexaploid oat accessions

Primer pair	Number of AFLP bands[a]			Frequency of scored bands		
	Total	Mono	Scored	Mean	Minimum	Maximum
E + AAG/M + CAC	207	8	34	0.785	0.169	0.978
E + ACG/M + CTA	131	4	34	0.636	0.162	0.963
E + ACG/M + CTG	148	4	34	0.712	0.125	0.963
E + ACT/M + CGC	121	3	34	0.745	0.244	0.992
E + AGG/M + CGC	160	5	34	0.717	0.111	0.972
All	767	24	170	0.717	0.111	0.992

[a] Total, The total number of AFLP bands observed; Mono, the number of monomorphic AFLP bands detected; Scored, the number of polymorphic AFLP bands scored

Table 3 Analysis of the molecular variance (AMOVA) sum of squares partitioning of total AFLP variation into among-group and within-group components for 670 cultivated hexaploid oat accessions

Group	*df*	Among-group component (%)[a]	Within-group component (%)
Country	79	10.07****	89.93
Region	13	6.24****	93.76
Oat type	3	8.75****	91.25
Landrace	1	0.23*	99.77

*, ****Significance levels of $P < 0.05$ and $P < 0.0001$, respectively

[a] The probability that the among-group (country, region, oat type, landrace) variance component was larger than zero, as computed from 10,100 random permutations

SAS PROC REG (SAS Institute 2004) on the number of accessions over the number of polymorphic bands, the mean band frequency, and within-country (or within-region) variation measured from the sum of squares from the analysis of molecular variance (AMOVA; Excoffier et al. 1992) given below.

AFLP variations were assessed across various groups (country, region, oat type, landrace) by means of an AMOVA using ARLEQUIN ver. 2.001 (Schneider et al. 2002). This analysis not only allows the partition of the total AFLP variation into within-group and among-group variation components but also provides a measure of inter-group genetic distances as the proportion of the total AFLP variation residing between oat accessions of any two groups (called the Phi statistic; Excoffier et al. 1992; Huff et al. 1997). Models involving different levels and types of structuring (country, region, oat type, and landrace) were applied (Table 3). The significance of the resulting variance components and inter-group genetic distances was tested with 10,100 random permutations.

To assess the genetic associations of the oat accessions of different countries and regions, we analyzed the inter-country (and inter-region) distance matrices of the Phi statistic using NTSYS-PC 2.01 (Rohlf 1997) and clustered these with the algorithm of unweighted pair-group methods using arithmetic averages. The genetic associations of individual accessions were assessed by generating a pairwise accession similarity matrix for the 670 accessions using the simple matching coefficient (Sokal and Michener 1958) and converting this matrix to the Euclidean distance matrix as the square root of one minus the element-wise similarity for a principal coordinate analysis using the NTSYS-PC program. The first three resulting principal coordinate scores were plotted to assess accession associations.

Results

Five AFLP primer pairs amplified a total of 767 AFLP bands for the 670 oat accessions assayed (Table 2). The number of observable bands per primer pair ranged from 121 to 207, and the number of monomorphic bands per primer pair ranged from three to eight. Since many of the detected bands lacked clarity, only a proportion of the polymorphic bands were scored, and only 170 of the scored bands were selected for further analyses. The frequencies of the selected bands in the assayed accessions ranged from 0.11 to 0.99, with an average of 0.72. A large proportion (39%) of the selected bands had frequencies larger than 0.90 (results not shown). For each primer pair, statistics (mean, minimum, and maximum) of the band frequencies are given in Table 2, and the mean frequencies found for these primer pairs ranged from 0.64 to 0.79.

Effectiveness of stratified sampling in capturing AFLP variations

The core subset was selected by a stratified sampling of the oat collection with respect to country of origin. The number of accessions assayed for each country ranged from 1 to 32, with five countries of only one representative accession and 74 countries of 2–32 accessions (Table 1). The country with the most accessions selected was the USA (with 32 accessions), followed by Germany (30), and Russia (24). This distribution of accession sizes for country of origin was significantly associated with the variation in the number of polymorphic bands, the mean band frequency, and the within-country variation measured from the AMOVA sum of squares (Table 4), thus demonstrating the effectiveness of the stratified sampling applied in capturing country-wise genetic diversity. Also, the number of accessions for the major geographic regions greatly varied, ranging from two representative accessions for the Indochina region and eight accessions for the Central America region to 166 accessions for the East Europe region (Tables 1, 3). Such variable accession numbers were significantly associated ($P < 0.021$)

with the variation in the number of polymorphic bands, but not with those in the mean band frequency and the within-country variation measured from the AMOVA sum of squares (Table 4). Removing the accessions from the under-representative Indochina and Central America regions generated non-significant associations with any of the three genetic parameter estimates mentioned. Moreover, the unbalanced numbers of accessions for common oat, red oat, hull-less oat and unclassified accessions in the core subset (Table 3) were not significantly associated with the variations in the three genetic parameter estimates (Table 4).

Genetic variation of the core subset

Genetic variation of the core subset was quantified in this study for the number of polymorphic bands, the mean band frequency, and the within-country variation measured from the AMOVA sum of squares with respect to country and region of origin, oat type and landrace. For a country with two or more accessions, the number of polymorphic bands ranged from 25 to 151, with an average of 102; the mean band frequency ranged from 0.500 to 0.701, with an average of 0.617; the within-country variation ranged from 18.00 to 62.14, with an average of 41.90. The countries with the most polymorphic bands were Russia (151) and USA (151), followed by Ecuador (149), Chile (144), and China (144). The country with the most within-country variation was Ecuador (62.14), followed by Liberia (60.00), Uzbekistan (59.33), and Kenya (58.50). Significant variation existed among accessions of various countries, and it accounted for 10.1% of the total AFLP variation detected (Table 3).

The number of polymorphic bands for a geographic region ranged from 63 (Indochina) to 168 (East Europe and South America). The mean band frequency for a region ranged from 0.500 (Indochina) to 0.721 (West Europe). When the Indochina region with only two accessions was excluded, the South America region displayed the most within-region variation (54.81), followed by the Indian Subcontinent region (53.85). Accessions from the Central America region had the lowest within-region variation (34.71), followed by those from West Europe (37.93) and East Asia (39.05). Based on AMOVA, the among-region component accounted for 6.2% of the total AFLP variation (Table 3), and the among-countries-within-regions component explained only 4.5%.

The number of polymorphic bands for common oat accessions was 169, for red oat, 163, and for hull-less oat, 159. The mean band frequency for common oat was 0.714, for red oat, 0.700, and for hull-less oat, 0.693, and the within-type variation for common oat was 43.68, for red oat, 47.24, and for hull-less oat, 43.05. Based on AMOVA, the among-type variation component was significant and accounted for 8.75% of the total AFLP variation (Table 3). The genetic distance measured by the Phi statistic between common and red oat accessions was 0.159, between common and hull-less oat accessions, 0.027, and between red and hull-less oat accessions, 0.162. All of these genetic distances were highly significant ($P < 0.0001$).

The number of polymorphic bands was the same (169) for landrace and non-landrace accessions. The mean band frequency was 0.710 for landrace and 0.717 for non-landrace. Only a 0.23% AFLP difference existed between landrace and non-landrace accessions (Table 3).

Genetic structure of the core subset

Accession association of the core subset was analyzed with respect to oat type, country, and region of origin. A plot of the first two principal coordinate scores for the 670 individual oat accessions representing various oat types revealed two distinct groups which could be separated largely between common oat and red oat (Fig. 1). The first large group consisted of common oat and hull-less oat, and these two types of oat were well mixed together. The second group mainly included red oat. Unclassified accessions were found randomly spread over two groups (or areas) (not specified in Fig. 1).

Assessment of the genetic associations of oat accessions of 74 countries with more than one accession revealed three major distinct clusters (Fig. 2). The first cluster—at the bottom of the figure—included accessions from 15 countries, with 9 of the 15 countries belonging to the Mediterranean region. The second cluster—at the middle of the figure—consisted of accessions selected from 32 countries and originating from 22 European countries. The third cluster was

Table 4 Results for the linear regressions of three genetic parameter estimates over the number of accessions representing various groups of 670 cultivated hexaploid oat accessions

Group	Number of polymorphic bands		Mean band frequency		Within-group variation[a]	
	Coefficient	R^2	Coefficient	R^2	Coefficient	R^2
Country	4.8951***	0.56	0.0135***	0.26	0.5780*	0.06
Region	0.4005*	0.32	0.0001ns	0.16	−0.0463ns	0.01
Oat type	0.0154ns	0.20	0.0001ns	0.75	−0.0057ns	0.01

*, ***Significant at $P < 0.05$ and $P < 0.001$, respectively; ns, not significant ($P > 0.05$)

[a] Within-group variation calculated from the sum of squares from analysis of molecular variance

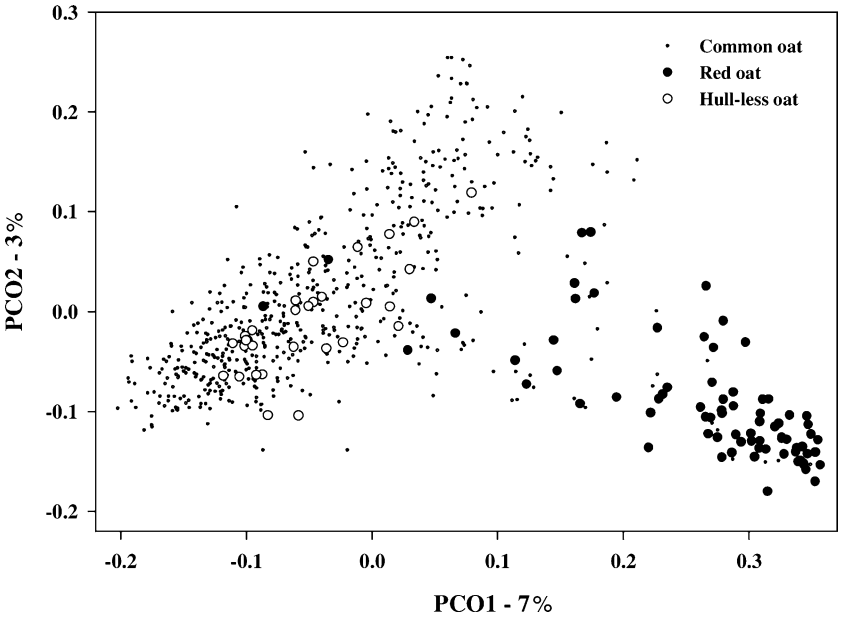

Fig. 1 Plot of the first two principal component scores based on the Euclidean distances converted from the simple matching coefficient matrix of AFLP bands for 670 cultivated hexaploid oat accessions. These two components accounted for 7% and 3% of the total variance, respectively. Germplasm of common oat (including unclassified ones) is shown by *dots*, red oat by *black circles*, and hull-less oat by *white circles*

formed by accessions of 27 countries across several regions. It is clear from Fig. 2 that accessions in the first cluster (i.e., Mediterranean cluster) are genetically more distinct than those of the other two clusters. In particular, accessions from Egypt and Ethiopia appeared to be genetically most distinct from those of the other countries.

In order to make the inference of regional associations of oat accessions more general, we excluded the oat accessions from the under-representaed Indochina and Central America regions. There were then two clear, distinct groups of 636 oat accessions representing 11 geographic regions and all three oat types (Fig. 3A). The first group consisted of accessions from the Europe, East Asia, Central Asia, and South America regions; the second group included those accessions from the Mediterranean, Africa, West Asia, Indian Subcontinent, North America, and Australia-New Zealand regions. Further assessments on the composition of these two groups revealed that the first group largely included accessions of common oat, while the second group included a higher-than-expected proportion of red oat (Table 5). Also, the accessions from the Mediterranean and Africa regions were genetically the most distinct within the first group, and accessions from the South America region were the most distinct within the second group. Assessments on Phi statistics obtained for these regions revealed that the Mediterranean accessions were located at large genetic distances from the other regions, ranging from 0.045 to 0.206 with an average of 0.114. Specifically, the largest pairwise region distances (0.201–0.206) were observed between the Mediterranean and European accessions, followed by those (0.18) between the Mediterranean and East Asia accessions. In the absence of the oat accessions from the under-represented Indochina and Central America regions, the among-region variation accounted for 7.83% of the AFLP variation. To assess the regional associations of common oat alone, we randomly selected and subsequently analyzed 88 accessions with equal representation of 11 regions: we found a pattern of regional association with two major clusters (Fig. 3B) similar to that observed for the three oat types (Fig. 3A). A major difference lied in the shift of the South America oat accessions away from the first to the second cluster dominated by the oat accessions from the West Asia, Africa, and Mediterranean regions (Fig. 3A, B).

Discussion

This AFLP analysis represents the first attempt to systematically characterize the worldwide gene pool of cultivated hexaploid oat using molecular markers and has revealed several interesting patterns. First, 89.9% of the AFLP variation resided within accessions of a country, and only 6.2% AFLP variation existed among accessions of major geographic regions. Second, accessions from the Mediterranean region were genetically the most distinct, while those from Russia and USA appeared to be the most diverse. Third, two distinct groups separated largely by common oat and red oat were observed. Red oat was genetically more diverse than common or hull-less oats, and hull-less oat was more related to common oat than red oat. Landrace and non-landrace accessions displayed similar AFLP variation patterns. These patterns are significant for understanding the domestication of cultivated oat and are also useful in classifying intraspecific diversity of cultivated oat, developing specific core subsets of the oat collection, and exploring new sources of genes for oat improvement.

This analysis also demonstrated the effectiveness of the stratified sampling applied in capturing the country-

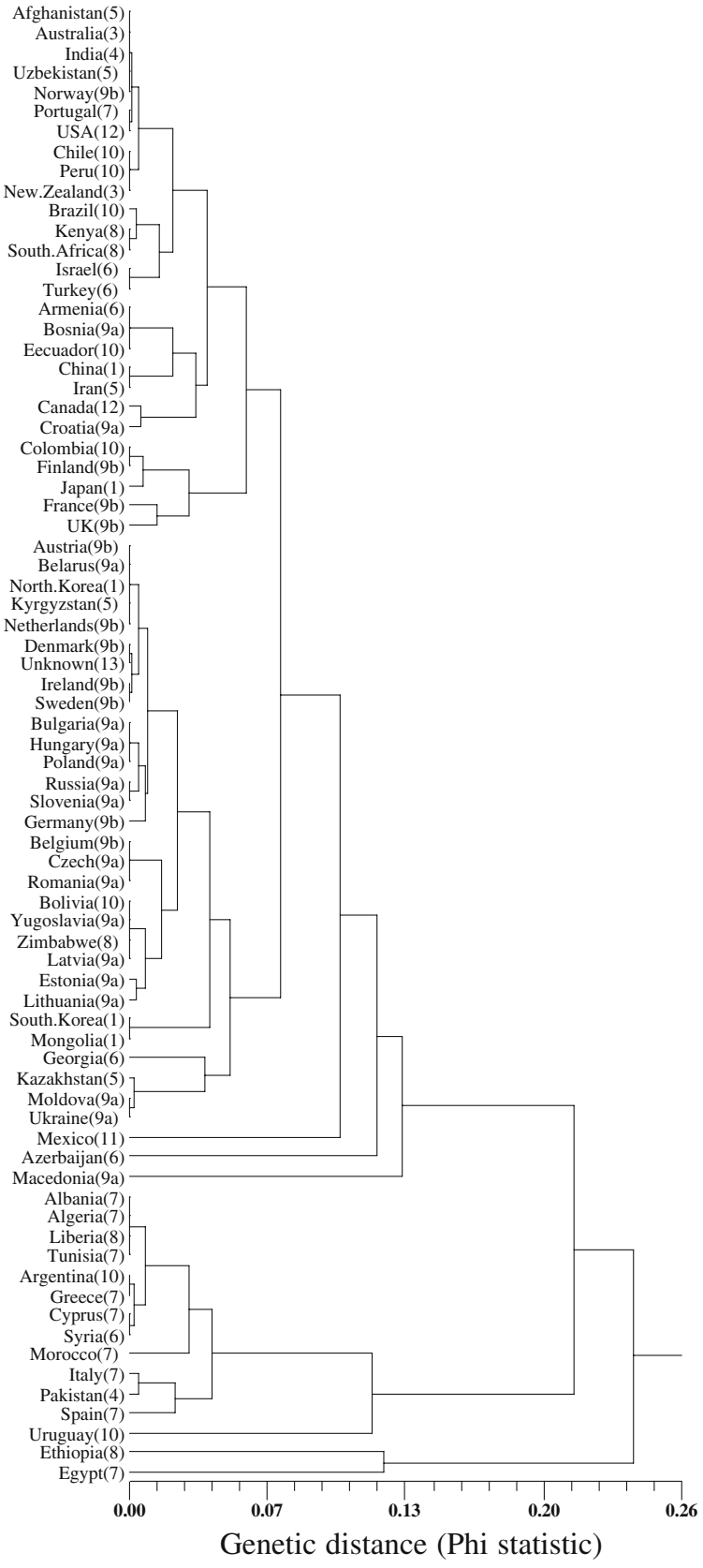

Fig. 2 Genetic associations of 670 cultivated hexaploid oat accessions representing 74 countries and one group of uncertain origin. The region code is given in parenthesis following the name of the country

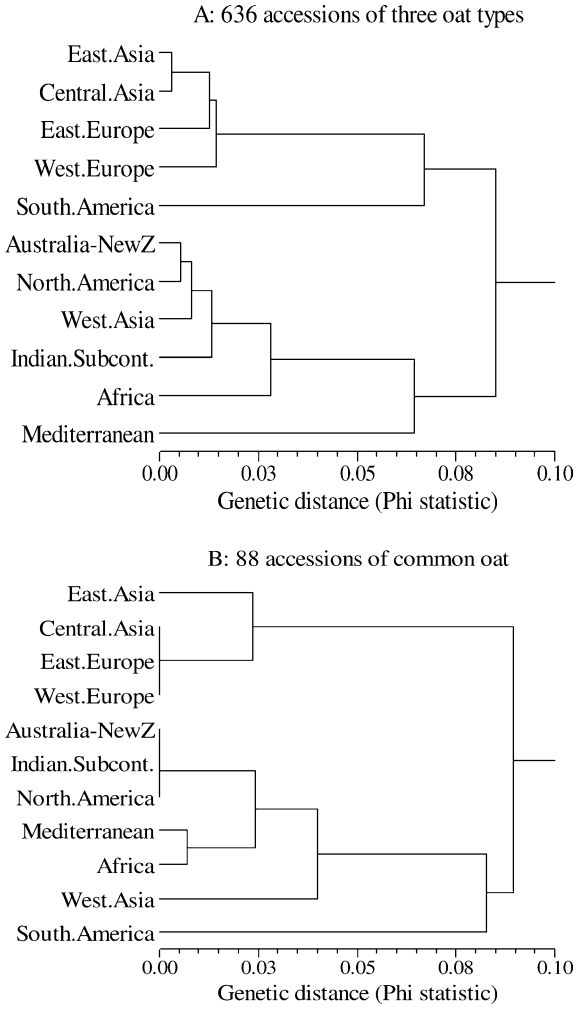

Fig. 3 Genetic associations of cultivated hexaploid oat accessions representing 11 major geographic regions. **A** 636 accessions of common oat, red oat, and hull-less oat. **B** 88 accessions of common oat for 11 geographic regions with eight randomly selected accessions each. *Australia–NewZ* Australia-New Zealand region, *Indian Subcont*. Indian Subcontinent region

wise genetic variability from the whole collection, as evidenced in the significant associations of the variable accession sizes for different countries with the variations in the number of polymorphic bands, the mean band frequency, and the within-country variation (Table 4). Thus, more country-wise variation was captured in this core subset than in those subsets developed under a completely random sampling scheme. This effectiveness was expected theoretically (Brown 1989b) but has been rarely assessed empirically (Schoen and Brown 1993). However, the sampling applied here was not well stratified with respect to region of origin and oat type (Table 4). Consequently, this core subset may not capture more regional or among-types variability than it would if a completely random sampling was made. Clearly, comparative assessments on the effectiveness of various sampling schemes in capturing molecular variability are needed in order to increase confidence in any newly developed core subset and any related inferences of variation patterns that can be made from it.

The patterns of AFLP variation reported here provide some insight into the domestication of hexaploid oat. The finding of only two distinct groups separated largely by common oat and red oat is consistent with results based on cytogenetic studies (Zhou et al. 1999; Jellen and Beard 2000) and supports the hypothesis that common cultivated oat and red oat were domesticated independently of one another (Thomas 1995; Zohary and Hopf 2000). Extensive intermingling of hull-less oat with the common oat of northern European origin offers little support for the origin of hull-less oat in central or eastern Asia, as suggested by Stanton (1923), or for China being the center of hull-less oat origin, as proposed by Vavilov (1926). In contrast, the non-separation of common oat and hull-less oat was well in line with the reasoning of Thomas (1995) that Chinese hull-less oat may trace back to hull-less variants of domesticated European hulled types that subsequently were transported to China.

The two distinct clusters of the cultivated hexaploid oat germplasm assayed represented two distinct germplasm pools as reflected in frequency differences between bands present in both pools. The same pattern was observed by Phillips and Murphy (1993) based on isozyme variation and by O'Donoughue et al. (1994) based on restriction fragment length polymorphism. Given the known history of oat cultivation (Coffman 1977; Murphy and Hoffman 1992), our finding is not surprising, but could be significant for the taxonomic treatment of *A. sativa* (Baum 1977) and/or intraspecific classification of cultivated oat germplasm (Diederichsen 2004). These two clusters accounted for about 7% of the AFLP variation detected, and their difference was highly significant. Thus, maintaining the traditional species distinction for *A. sativa* and *A. byzantina* (Rodionova et al. 1994) or forming a formal taxonomical distinction at the subspecies level (Diederichsen 2004) has an additional basis. Note that our results offer little support for the distinction of *A. sativa* subsp. *nudisativa* (Husn.) Rod. et Sold. (Rodionova et al. 1994). However, little is known as to how effective these intraspecific groupings, if carried out, would be with respect to the management and utilization of oat germplasm.

While the variation patterns obtained may be specific to this core subset, they should share some general baseline information useful for the development of specific core subsets by determining the weighting or representation for each country or region (Brown and Spillane 1999). It appears that different core subsets could be separately made for the *A. sativa* (including hull-less oat) and *A. byzantina* groups to capture more within-group variation, without any significant increases in the sizes of the core subset. The establishment of two specific subsets may require an intraspecific classification of hexaploid oat germplasm, as proposed by Diederichsen (2004). Accessions from the Mediterranean and Africa regions and accessions from the South America

Table 5 Two distinct groups of 636 cultivated hexaploid oat accessions representing 11 geographic regions with respect to AFLP variation and cultivated oat type

Group	Region[a]	Number of Accession	AFLP variation[b]			Frequency of oat types[c]			
			NPB	MBF	WRV	CO	RO	HO	UC
I	East Asia	37	157	0.716	39.05	24	0	8	5
I	Central Asia	18	136	0.687	43.07	14	2	0	2
I	East Europe	166	168	0.707	41.16	137	4	6	19
I	West Europe	131	167	0.721	37.93	112	4	9	6
I	South America	62	168	0.686	54.81	38	9	0	15
Total		414				325	19	23	47
II	Australia–NewZ.	21	142	0.677	47.07	13	2	1	5
II	North America	46	157	0.707	47.52	29	5	3	9
II	Indian Subcont.	13	138	0.667	53.85	6	5	0	2
II	West Asia	39	151	0.690	47.59	25	9	3	2
II	Africa	31	159	0.685	48.14	21	5	2	3
II	Mediterranean	72	161	0.694	48.35	31	28	0	13
Total		222				125	54	9	34

[a]Australia-NewZ, Australia-New Zealand region; Indian Subcont., Indian Subcontinent region
[b]NPB, Number of polymorphic bands; MBF, mean band frequency; WRV, within-region variation calculated from the sum of squares from analysis of molecular variance
[c]Frequency of accessions for four oat types: CO, common oat; RO, red oat; HO, hull-less oat; UC, unclassified

region may need more weight than those from other regions within the sampling of the first and second groups, respectively. Emphasis could also be given to the regions and countries of great diversity.

The core subset assayed here represents only 5.8% of the cultivated hexaploid oat accessions and theoretically could capture up to 70% of the genetic diversity present in the PGRC collection (Brown 1989a). Thus, a considerable proportion of variation may still go undetected. Also, as a bulk sample for each accession was assayed, information on within-accession variation was lacking, and bias in detecting genetic variation could exist (Fu 2003). Moreover, an effort was made to verify testing materials through passport and phenotypic data, but undetected contamination or misrepresentation (if any) could affect the comparisons of RAPD variation. However, a large amount of genetic diversity was found in the oat germplasm assayed. About 90% of the variation detected resided within the accessions of a country, implying selection from the within-country germplasm is still possible. Also, about 91% of the AFLP variation resided among common oat, red oat, and hull-less oat accessions (Fig. 3), and selection could proceed within these types of cultivated oat. These selections need not focus on the landrace materials available, as landrace accessions did not display greater variation those non-landrace accessions. The exploration of new sources of genes might focus initially on accessions originating from the Mediterranean and Africa regions, particularly from Egypt and Ethiopia, for the improvement of red oat, and on accessions from the South America region, particularly from Ecuador and Chile, for the improvement of common oat. The development of specific core subsets of oat accessions based on these variation patterns in order to facilitate the initial screening of genes of particular interest is also possible. Such core subsets would save some of the screening effort required in the exploration of new germplasm for oat improvement from large oat collections.

Acknowledgements The authors would like to thank Drs. Axel Diederichsen, James Chong, Tom Fetch, and Brian Rossnagel for their stimulating discussions on the project; Mr. Dallas Kessler and Ms. Angela Taylor for their technical assistance in sampling, planting, and screening the germplasm; and Drs. Van Ripley and Axel Diederichsen for their helpful comments on the manuscript.

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