Israel Journal of Plant Sciences *Vol. 53* *2005* *pp. 135–145*

Isozymic estimation of the biodiversity of Spanish *Avena sativa* L. landraces stored in a germplasm bank

FERNANDO GONZÁLEZ-ANDRÉS,[a,*] JOSÉ M. PITA,[b] SUSANA MALMIERCA,[a] AND CELIA DE LA CUADRA[c]
[a]*Escuela Universitaria de Ingeniería Técnica Agrícola INEA, Camino Viejo de Simancas, km. 4.5, 47008 Valladolid, Spain*
[b]*Departamento de Biología Vegetal, Universidad Politécnica de Madrid, 28040 Madrid, Spain*
[c]*CRF-INIA, Autovía de Aragón, km. 36, 28800 Alcalá de Henares, Spain*

(Received 13 December 2004 and in revised form 22 July 2005)

ABSTRACT

Twenty-six accessions from the Spanish national germplasm bank of oats at the Plant Genetic Resources Centre (CRF-INIA) collection of *Avena sativa* L. were analyzed by isozyme. These accessions were a subset of the core collection and were selected on the basis of their positive agricultural characteristics. The sites where the 26 accessions to the collection were gathered were representative of the relative weightings, both in the core and in the entire collection, of the various agroclimatic regions of Spain. The objectives of the study were: (i) to ascertain the degree of isozymic variability between and within accessions; (ii) to compare the results with the variability recorded in a prior isozyme study with a larger, random, sample of the entire collection in order to validate the criteria used for the selection of the 26 accessions; and (iii) to define groupings of accessions on the basis of their genetic distances, this being a piece of information of value for the utilization of germplasm. Each of the accessions had its own distinctive array of isozyme phenotypes. High overall diversity was detected (phenotypic variability index Pj = 0.576). Almost one-third (29%) of the overall variability was intra-accession variability. There were large differences in intra-accession variability, and accessions were divided into 3 groups on the basis of their internal diversity, estimated from Pj, and the Shannon index, Hj. The isozymic variation in the 26 accessions was similar to that of the entire collection, which indicates that the method used to select the subset did not cause any loss of genetic diversity. The PCA defined 3 clusters of accessions with a few outliers. The grouping showed only a limited link to the geographical origin of the accessions, but had a noteworthy relationship with the agroclimatic conditions at the collection sites.

Keywords: isozyme, Shannon Index, phenotypic diversity, principal component analysis

INTRODUCTION

The loss of traditional landraces of cultivated plants because of their replacement by modern cultivars renders it necessary to arrange for ex situ preservation of these older types in germplasm collections (Plucknett et al., 1987). The complete characterization of a germplasm collection should comprise morphological characteristics, isozymes, and molecular-marker-based variants (Allard, 1992). As the number of accessions of a given species to a germplasm bank becomes increasingly larger, full studies need to be carried out only on a core collection (Brown, 1989) that includes the genetic diversity of the species with a minimum of redundancy. For efficient use of a germplasm collection, knowledge

*Author to whom correspondence should be addressed. E-mail: fernando.gonzalez@unileon.es

of the pattern of genetic variation for traits of economic interest is required. However, in most cases the geographic distribution of variation for a trait is unknown. In this case, sampling may be based on patterns of general genetic variation among accessions to the collection from different regions. These patterns of variations may be revealed by qualitative or quantitative morphological traits, isozymes, DNA-based markers, or other features (Beer et al., 1993).

Common cultivated oats (*Avena sativa* L.) is a hexaploid species (2n = 6x = 42) that was a major cereal crop of great importance before agriculture became mechanized. After this its importance as a crop decreased, but it is still a good option for crop rotations in areas with short vegetation growth periods (Diederichsen, 2004). For this reason the number of accessions of common oat landraces conserved in germplasm banks is very large worldwide (Phillips and Murphy, 1993; Pérez de la Vega et al., 1994; Diederichsen, 2004). The genetic variation in hexaploid oat species has been estimated from morphological (Rezai and Frey, 1988), isozyme (Gómez et al., 1991; Murphy and Phillips, 1993; Phillips and Murphy, 1993; Phillips et al., 1993; Pérez de la Vega et al., 1994; Hunter et al., 1995; Matiello et al., 1998), and DNA-based marker data (Goffreda et al., 1992; Li et al., 2000, for microsatellite, and Pal et al., 2002, for microsatellite and PCR-RFLP markers), or through pooled analysis of complementary information (Beer et al., 1993, for morphological traits, isozymes, and RFLP, and Heun et al., 1994, for isozymes and RAPD).

The collection of oats in the Spanish national germplasm bank at the Plant Genetic Resources Centre (CRF-INIA) comprises 1,157 Spanish examples of *Avena sativa* L. Ninety-nine percent are landraces. A core collection was developed using passport descriptors. The accessions were grouped by the status of the sample (landraces, improved cultivar, weedlike, and so on) and then by the agroclimatic conditions at the collection sites, according to the classification put forward by Papadakis (1966), as described for Spain by Elías and Ruíz Beltrán (1977). From each group, 20% of the accessions were randomly selected (unpublished data). They were thereafter evaluated from an agronomic viewpoint: grain yield, grain shedding, and lodging at the mature stage (de la Cuadra, C., unpublished data). The present work is an isozymic characterization of 26 agriculturally valuable *Avena sativa* L. landraces from the core collection. The criteria for selection of the 26 accessions were high grain yield with low grain shedding and lodging. The proportion of accessions from each agro-climatic region in the subset selected was consistent with the relative weighting, in terms of number of accessions, from each region in the collection as a whole.

The objectives of the work were: (i) to ascertain the distribution of overall variability between and within the 26 accessions, using isozymes; (ii) to compare the results with the variability noted in a previous isozymic study that used a larger, random, sample of the entire *A. sativa* collection (Gómez et al., 1991; Pérez de la Vega et al., 1994), in order to validate the criteria used for the selection of the 26 accessions; and (iii) to define groupings on the basis of genetic distances, this information being of value for the utilization of germplasm.

MATERIALS AND METHODS

Twenty-six *Avena sativa* L. landraces (Table 1) obtained from the national germplasm bank of oats at the CRF-INIA were analyzed by isozyme. The accessions studied were from the core collection and were chosen on the basis of their valuable agricultural characteristics (high grain yield with low grain shedding and lodging). The origins of the 26 accessions were a good match for the relative weightings of the various agricultural and climatic regions of Spain, both in the core collection and in the collection as a whole.

For every accession, 30 healthy seeds (with a translucent embryo, seeds with dark embryos being discarded) were randomly selected and the embryos were completely separated from the endosperm. Extracts were obtained by macerating one single embryo with 35 ml of distilled water. Two paper wicks (15 by 3 mm Whatman chromatography paper, no. 3) were saturated with extract and then used to load extract into gels pre-cooled for 1 hour at 4 ºC. The gels (17.5 by 12.5 by 0.8 cm) were made from 11% hydrolyzed potato starch from Sigma Chemical Company in Tris–citrate pH 6.2 buffer. Constant intensity (60 mA) was applied to the gel systems to achieve electrophoretic separation of molecules. The gels were cut horizontally to yield four assayable slices per gel. The following enzymes were stained in accordance with the procedures described by Maselli et al. (1999): alcohol dehydrogenase (ADH, EC 1.1.1.1); isocitrate dehydrogenase (IDH, EC 1.1.1.42); malate dehydrogenase (MDH, EC 1.1.1.37); phosphoglucoisomerase (PGI; EC 5.1.3.9.); phosphoglucomutase (PGM, EC 2.7.5.1); and 6-phosphogluconate dehydrogenase (6-PGD, EC 1.1.1.44). Leucine aminopeptidase (LAP, EC 3.4.11.1) and peroxidase (PER, EC 1.11.1.7) were stained by means of the procedure described by Torres et al. (2003).

Although the inheritance of isozyme polymorphisms in hexaploid oats is known for five (IDH, LAP, PER, PGM, and 6-PGD) of the eight systems studied (Salas and Murphy, 1995), the current survey revealed band patterns in addition to those published. Isozyme phe-

Table 1
Avena sativa L. landraces studied

Accession no.	CRF[1] designation	Collection site data				
		State	Province	Latitude	Longitude	Elevation (m)
#01	BG 29789	Castilla y Leon	Zamora	41º19′45″N	6º11′16″W	760
#02	BG 26964	Andalucia	Almeria	37º11′00″N	2º17′53″W	854
#03	BG 25430	Extremadura	Caceres	39º51′44″N	6º13′58″W	488
#04	BG 25425	Extremadura	Caceres	39º43′42″N	5º16′52″W	448
#05	BG 30934	Andalucia	Granada	37º11′08″N	3º09′20″W	1229
#06	BG 10413	Castilla-La Mancha	Guadalajara	40º56′14″N	2º52′55″W	841
#07	BG 18566	Castilla-La Mancha	Cuenca	40º08′47″N	2º41′25″W	840
#08	BG 10415	Castilla-La Mancha	Guadalajara	41º01′N	2º04′W	1122
#09	BG 10393	Castilla y Leon	Burgos	42º24′34″N	4º10′32″W	800
#10	BG 18541	Castilla y Leon	Salamanca	41º07′36″N	5º10′56″W	781
#11	BG 10437	Castilla y Leon	Palencia	42º08′17″N	4º50′14″W	771
#12	BG 9470	Castilla-La Mancha	Cuenca	40º09′N	2º21′W	996
#13	BG 10482	La Rioja	La Rioja	42º2705″N	3º01′36″W	724
#14	BG 9630	Castilla y Leon	Valladolid	41º5303″N	5º02′31″W	749
#15	BG 10495	La Rioja	La Rioja	42º2409″N	2º43′04″W	564
#16	BG 31124	Castilla y Leon	Segovia	41º2641″N	3º50′22″W	1082
#17	BG 9467	Castilla-La Mancha	Cuenca	40º00′59″N	2º11′44″W	924
#18	BG 18505	Extremadura	Badajoz	38º44′33″N	5º40′14″W	409
#19	BG 9466	Castilla-La Mancha	Cuenca	39º20′37″N	1º55′35″W	732
#20	BG 8164	Castilla-La Mancha	Ciudad Real	39º10′10″N	3º51′15″W	640
#21	BG 8637	Valencia	Castellon	40º37′14″N	0º05′56″W	1070
#22	BG 8119	Andalucia	Jaen	37º59′07″N	3º52′51″W	300
#23	BG 9557	Castilla y Leon	Valladolid	41º36′33″N	4º54′31″W	768
#24	BG 10397	Castilla-La Mancha	Guadalajara	41º12′03″N	2º52′10″W	1169
#25	BG 8157	Castilla-La Mancha	Albacete	39º06′54″N	1º59′48″W	688
#26	BG 9473	Castilla y Leon	Zamora	41º5816″N	5º18′58″W	707

[1]Centro de Recursos Filogenéticos (INIA, Alcalá de Henares, Madrid, Spain).

notypes were recorded and taken to be analogous to homozygous genotypes, as previously reported by Heun et al. (1994) and Hunter et al. (1995). Nine zones of band activity were defined by the eight systems stained because MDH showed two such zones.

For each individual accession an isozyme band presence or absence profile was recorded. This consisted of a series of 780 (26 accessions with 30 individuals per accession) ones for presence or zeroes for absence, describing the two possible states at each of the 57 positions where scoreable bands were observed over all eight isozymes. For each enzyme zone, bands were numbered consecutively relative to migration distance from the origin, regardless of their intensity. However, only those bands that appeared in both of the two repetitions were scored. In order to achieve a more consistent analysis of the zymograms, they were analyzed from two different viewpoints. Firstly, for each zone of band activity the possible phenotypes were described as a combination of bands (Fig. 1), and the frequency of each phenotype within each accession was calculated ($p_{ph\,i}$). Phenotypic polymorphism for each enzyme zone and accession (P_j) was estimated by using the formula from Kahler et al. (1980):

$$P_j = \sum_{i=1}^{n} p_i(1 - p_{phi}) = 1 - \sum_{i=1}^{n} p_{phi}^2$$

where *n* is the number of phenotypes observed per enzyme zone and accession.

Secondly, the frequency of each band within each type (p_{bi}) was calculated and used to estimate the Shannon index, H_j (Shannon and Weaver, 1949), as a measure of diversity:

$$H_j = -\sum_{i=1}^{m} p_{bi} \ln p_{bi}$$

where *m* is the number of bands observed.

Correlations between isozyme variation (P_j and H_j) and the geographical data of the collection site (Table 1) were assessed using the Pearson correlation coefficient.

Principal Component Analysis (PCA) based on covariance matrices of band frequencies was carried out so as to obtain an additional representation of the distance among the 26 accessions in the first three principal com-

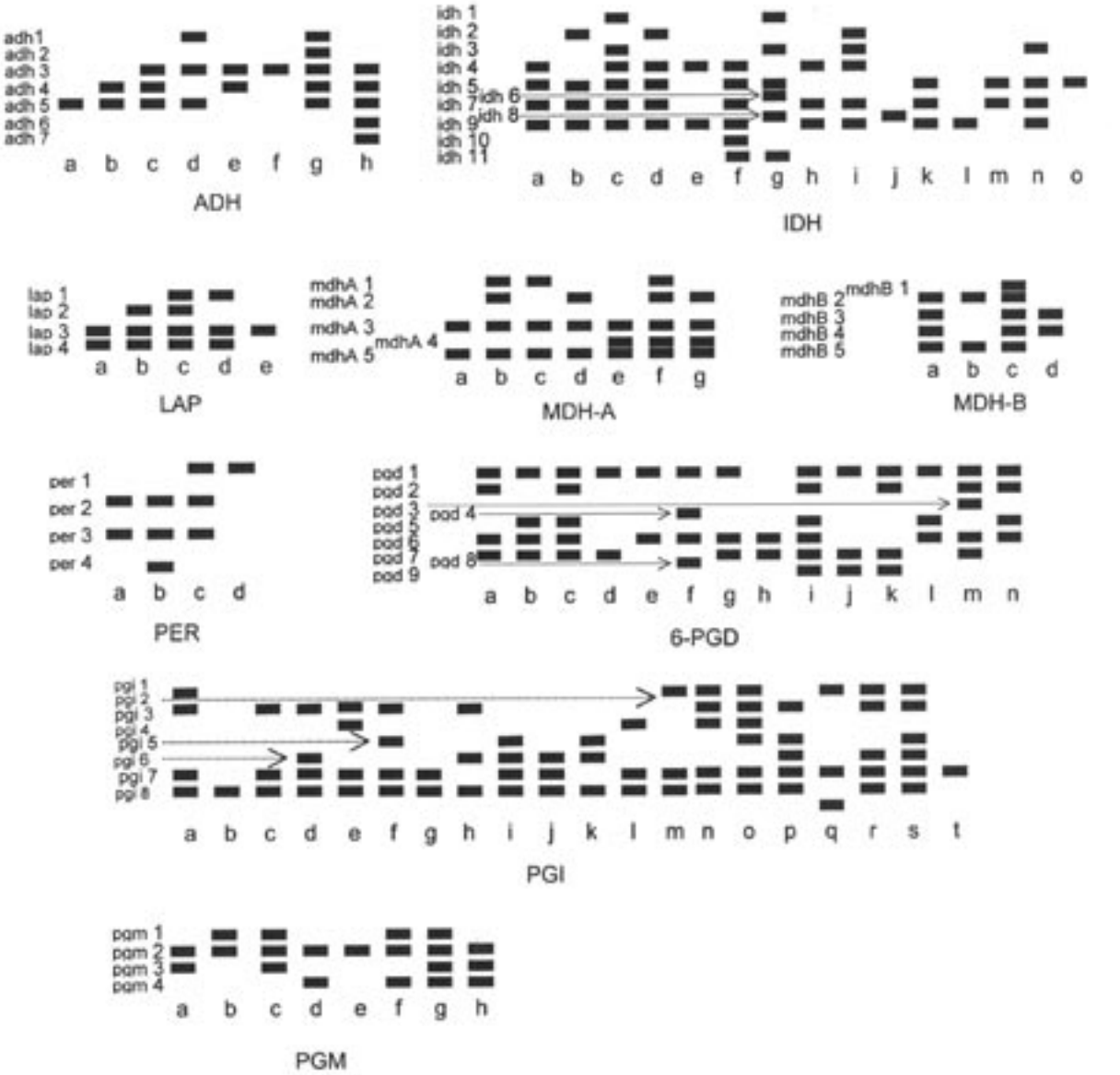

Fig. 1. Schematic diagram of isozyme band models (phenotypes) observed for 8 enzymes in 30 individuals of 26 populations studied.

ponents. The software package NTSYS (Rohlf, 2000) was used for PCA analysis.

RESULTS

Seven (ADH, IDH, LAP, PER, PGI, PGM, 6-PGD) of the eight isozymic systems displayed consistent, scoreable band activity in a single gel zone, while the MDH system had two zones of activity per gel.

Analysis based on isozymic phenotypes

For each zone of band activity a description was given of the possible alternative phenotypes defined as a combination of bands (Fig. 1). Table 2 lists the phenotypes observed in each accession, together with the percentage of polymorphic accessions for each zone (columns) and the percentage of polymorphic zones within an accession (rows). None of the zones of band activity was monomorphic throughout the collection studied. LAP and PER isozymic zones showed the lowest level of polymorphism, and ADH, PGD, PGI, and MDH-A, the highest. Accession number 16 was monomorphic for all the isozyme zones, while 18 was polymorphic for all of them.

Table 2
Isozyme phenotypes (according to Fig. 1) observed in the 26 hexaploid oat accessions studied. Phenotypes that showed a frequency lower than 10% within the corresponding accession are italicized

Accession no.	% of polymorphic zones	ADH	IDH	LAP	MDH-A	MDH-B	PER	PGD	PGI	PGM
#01	29	a,*b*	—	b	a	a	a	a	a,*b*,c	—
#02	67	b,c,*d*,*e*	a,*b*,c,*d*	a	b,*c*	a	a	*a*,b,c	c,d,*e*,*f*	a,*e*
#03	78	a,b,c,d,*f*	a,*d*,e,*f*,*g*,*h*,*i*,*j*	a	a,b,c	a	a,b	a,b,c,d,e	*a*,*c*,g,*h*,i,j,*k*	a,*b*,*c*,d,*e*,*f*,*g*
#04	33	*a*,b,c,*e*	a	a	f	c	a	*a*,b,c,*f*	c,f,*j*,*l*	a
#05	11	a	a	a	a	a	a	a,g	m	a
#06	11	b	k	b	a	a	a	b,*h*	a	a
#07	67	b,e	a,k	b	b,c,f	a,c	a	b,*c*,i	n,*o*	a
#08	50	—	k	b	d,g	a,c	a	a,c,*i*,j	g,*j*	a
#09	11	b	k	b	d	a	a	b	c,*p*	a
#10	44	b,c	k	b	b,d,f	a,c,*d*	a	a,f	n	a
#11	88	*a*,b,c	*h*,k,*l*,*m*	—	*c*,d,f,*g*	a,c	a	*a*,b,d	a,*d*,*q*,*r*	a,d
#12	22	b	k	b	d	a	a	b,c,*d*	a,*c*,*l*	a
#13	56	a,b.c	c,k	b	f,g	c	a	a,j	a,c,*r*	a
#14	56	*a*,b,*c*	k	b	a,d	a	a,*c*	*b*,c	c	a,*b*,*f*
#15	89	a,*c*	a,k	b,*d*	a,*b*,*d*	a,*b*	a,c	a,*k*	m	a,*b*
#16	0	a	d	b	a	a	a	a	o	a
#17	67	a,b,*c*	k	b	a,d,*e*,g	a,c	*a*,c	f,*k*	c,*d*	a
#18	100	b,c,*d*	a,c,e,l	*a*,b,d	*a*,*d*,f,*g*	a,c	C,d	b,c,l	*c*,*d*,*f*,*g*,*l*,o,*p*,s	a,d
#19	89	a,c	k	b	a,b,*d*,e,f	a,c	a	f,m	a,*n*,r,*t*	a,*e*,h
#20	56	*c*,g	d,*n*,*o*	a	e,*f*	a,c	a	b	c	g,h
#21	44	*b*,c	d	a	a,d	a	*a*,c	b	d,p	a
#22	56	b,c,*h*	c	c,*e*	f	c	a	c	a,r	a
#23	67	b,c,*e*	c,k	b	b,d	a	a	*b*,f	a,c,*f*	a
#24	22	b	c	b	b	b	a	f,*i*	a,r	a
#25	44	b,c	*a*,c	b	d	a	a	a,n	f	a,*d*
#26	44	a,*b*,c	c,k	b	a,b	a	a	f	c,*d*,*f*	a
% of polymorphic accessions		76	44	12	62	35	23	73	69	36

Table 3 gives the values of P_j for the various different enzyme zones and accessions. Once again, LAP and PER were the least polymorphic enzyme zones, with the lowest values for P_j. Conversely, ADH, PGI, MDH-A, and PGD were the most polymorphic, having similar P_j values. Averaged over all enzyme zones, accession numbers 3 and 18 were the most polymorphic, with the highest values for P_j. Apart from sample 16, which was monomorphic and had a P_j value of zero, the lowest values for P_j were shown by numbers 6 and 9. The overall P_j value for the whole set of accessions studied, obtained when all the individuals analyzed were included in a single artificial population, was 0.576. The average intra-accession P_j value was 0.161.

Each accession had its own distinctive array of isozyme phenotypes (Table 2). Even if those phenotypes with low frequencies (<10%) within each type (shown in italics in Table 2) were excluded, every type still showed a distinctive array of isozyme phenotypes.

Table 4 shows the overall range of frequencies for each phenotype within the set of accessions studied. Except for MDH-A, MDH-B, and PER, more than half the phenotypes showed frequencies below 10% and in most cases lower than 1%. Phenotypes marked with an asterisk in Table 4 were rare combinations of alleles in the set of accessions studied. Phenotypes were considered to be rare when they had a low frequency (under 1%) in the set of accessions overall or when their frequency was under 10% within a given accession among those studied.

Analysis based on the frequencies of each isozyme band

A total of 57 bands were scored for the 9 isozymic zones and the 26 accessions. The frequency of each single isozymic band within each population was used to

Table 3
Measures of the amount of phenotypic polymorphism (P_j) (Kahler et al., 1980) over isozyme models for estimating the phenotypic intra-accession variability for each accession, average intra-accession values of P_j and average values of P_j for each enzyme zone

Accession no.	ADH	IDH	LAP	MDH-A	MDH-B	PER	PGD	PGI	PGM	Average P_j
#01	0.064	—	0	0	0	0	0	0.464	—	0.088
#02	0.445	0.496	0	0.064	0	0	0.504	0.584	0.064	0.262
#03	0.768	0.629	0	0.624	0	0.480	0.778	0.763	0.697	0.514
#04	0.382	0	0	0	0	0	0.589	0.565	0	0.192
#05	0	0	0	0	0	0	0.490	0	0	0.061
#06	0	0	0	0	0	0	0.064	0	0	0.008
#07	0.302	0.480	0	0.494	0.198	0	0.487	0.064	0	0.225
#08	—	0	0	0.180	0.180	0	0.571	0.064	0	0.124
#09	0	0	0	0	0	0	0	0.124	0	0.014
#10	0.285	0	0	0.395	0.238	0	0.180	0	0	0.122
#11	0.426	0.296	—	0.503	0.436	0	0.253	0.199	0.231	0.293
#12	0	0	0	0	0	0	0.331	0.255	0	0.065
#13	0.616	0.420	0	0.480	0	0	0.498	0.558	0	0.286
#14	0.156	0	0	0.358	0	0.067	0.069	0	0.145	0.088
#15	0.137	0.231	0.0768	0.127	0.064	0.191	0.064	0	0.067	0.107
#16	0	0	0	0	0	0	0	0	0	0
#17	0.339	0	0	0.624	0.491	0.067	0.064	0.064	0	0.183
#18	0.464	0.711	0.4861	0.242	0.124	0.386	0.558	0.531	0.480	0.443
#19	0.198	0	0	0.704	0.420	0	0.18	0.293	0.504	0.256
#20	0.064	0.240	0	0.064	0.444	0	0	0	0.231	0.116
#21	0.328	0	0	0.311	0	0.137	0	0.358	0	0.126
#22	0.127	0	0.095	0	0	0	0	0.498	0	0.080
#23	0.287	0.480	0	0.444	0	0	0.064	0.371	0	0.183
#24	0	0	0	0	0	0	0.124	0.391	0	0.057
#25	0.500	0.124	0	0	0	0	0.180	0	0.124	0.103
#26	0.584	0.5	0	0.498	0	0	0	0.184	0	0.196
Average P_j	0.259	0.184	0.026	0.235	0.100	0.051	0.233	0.244	0.102	0.161

estimate the intra-accession Shannon index of diversity (Table 5). Accessions numbers 18 and 3 were the most variable, having the highest H_j values. Monomorphic number 16 had an H_j value of zero. Other than this, the accessions with the lowest variability were numbers 9 and 6. The overall Shannon index for the total set of accessions studied was 0.268, and the average intra-accession H_j value was 0.079 (Table 5).

Principal Component Analysis (PCA) based on covariances of isozymic band frequencies (Fig. 2), indicated that the first four components accounted for 20.7%, 15.6%, 12.7%, and 10.0% of the variability, respectively. Consequently, the first three principal components taken together accounted for 49% of the overall variability. Projection of the accessions studied along these axes defined one completely isolated accession (number 23), plus three groupings. Three accessions lay in an intermediate position between groups 1 and 2, and one accession (number 24) may be considered an outlier of group 3 (Fig. 2). Seven out of the nine isozyme zones with a total of 14 bands were heavily weighted in the eigenvectors of the first three principal components. Bands with eigenvector values >0.20 or <–0.20 (Table 6) were considered heavily weighted. An examination of the patterns of distribution of the 14 bands identified as important in the PCA (Table 6) aided interpretation of the clustering in Fig. 2. Group 1 showed either absence or predominantly low frequencies for bands ADH 3, MDH-A 1, MDH-B 1, PER 1, IDH 1, IDH 2, IDH 3, PGD 4, and PGD 8, and predominantly high frequencies for bands LAP 2 and PGD 7. Group 2 showed a high variation of frequency for most bands, but low frequencies for PGD 4 and PGD 8 and predominantly high frequencies for IDH 4, PGD 5, and PGD 7 were noticeable. Group 3 showed low frequencies for bands IDH 1, IDH 2, IDH 3, IDH 4, PGD 5, and PGD 7 and high frequencies for PGD 4 and PGD 8. Accession number 23 had high frequencies for bands that were mostly rare in the remainder of the accessions

Table 4
Isozyme phenotypes (according to Fig. 1) that showed overall frequencies for the entire collection lower than 1%, 5%, and 10%. Asterisks (*) indicate those phenotypes that showed a frequency lower than 10% within each one of the accessions studied

Phenotype	ADH	IDH	LAP	MDH-A	MDH-B	PER	6-PGD	PGI	PGM
a	>10	>10	>10	>10	>10	>10	>10	>10	>10
b	>10	<1*	>10	>10	<5*	<1	>10	<1*	<1*
c	>10	>10	<5	<5	>10	>10	>10	>10	<1
d	<5	>10	<5	>10	<1*	<5	<5	<10	<5
e	<5	<1	<1*	<5			<1	<1*	<1*
f	<1*	<1*		>10			>10	<10	<1
g	<5	<1*		<5			<5	<5	<1
h	<1*	<1*					<1*	<1*	<10*
i		<1*					<1*	<1	
j		<1*					<1	<5	
k		>10					<1*	<1*	
l		<1					<1	<1*	
m		<1*					<1	<10	
n		<1*					<1	<10	
o		<1*						<10	
p								<1	
q								<1*	
r								<5	
s								<1	
t								<1*	

(MDH-B 1, PER 1, IDH 2), but low frequencies for bands that were strongly present in most of the populations (LAP 2, PGD 7).

The Pearson correlation coefficient between the height of the collection site and P_j was –0.466 (significant at $p < 0.05$), and H_j was –0.522 (significant at $p < 0.01$). In contrast, there was no significant correlation between isozymic polymorphism and the longitude or latitude of the collection site.

DISCUSSION

ADH was the most polymorphic isozyme, this being in agreement with the results obtained by Gómez et al. (1991) and Pérez de la Vega et al. (1994). PGI was a very polymorphic isozyme as well, in contrast with the results of Gómez et al. (1991), Pérez de la Vega et al. (1994), and Kahler et al. (1980), which reported it to be the least polymorphic. However, the present study analyzed seeds, whereas the others analyzed leaves, so the differing expression of the genes encoding for PGI enzyme in these different organs might explain these differences. Maselli et al. (1999) also obtained high polymorphism for PGI when working with Brassicaceae seeds. Conversely, LAP and PER were the least polymorphic isozymes in the work reported here. Gómez et al. (1991) and Kahler et al. (1980) described high polymorphism for PER. Once again, the differences in the organ analyzed might be the reason for these differences.

The P_j value of 0.576 obtained when all the individuals analyzed were included in a single artificial population indicated that this behaved as a population in which, on average, each zone had two phenotypes with similar frequencies. This value was very similar to that obtained by Gómez et al. (1991) from a larger, random, sample of the entire Spanish *Avena sativa* collection. Consequently, the set of accessions studied had an overall level of biodiversity similar to that of the entire collection.

Both the phenotypic variability index, P_j, based on the frequency of isozyme phenotypes, and the Shannon index, H_j, based on the frequency of each single isozyme band, showed that approximately 28 or 29% of the overall variability of the collection studied was intra-accession variability and that therefore 71 to 72% of the variability was between accessions.

The accessions were ordered on the basis of their values for P_j and H_j and this defined three levels of intra-accession diversity: low (number 16, which was monomorphic, plus numbers 5, 6, 9, and 24), high (numbers 2, 3, 11, 18, and 19), and intermediate for the remainder of the accessions. There was no exact pair-by-pair corre-

Table 5
Measures of the Shannon index (H_j) (Shannon and Weaver, 1949) over isozyme bands for estimating average intra-accession variability

Accession no.	Shannon Index value (H_j)
#1	0.041
#2	0.122
#3	0.224
#4	0.078
#5	0.019
#6	0.016
#7	0.061
#8	0.050
#9	0.005
#10	0.100
#11	0.144
#12	0.046
#13	0.106
#14	0.041
#15	0.071
#16	0
#17	0.087
#18	0.241
#19	0.136
#20	0.077
#21	0.052
#22	0.049
#23	0.114
#24	0.037
#25	0.057
#26	0.077

Average (H_j) 0.079.

spondence in the ranking of accessions obtained from P_j and H_j, but the three groups mentioned were consistent. The monomorphism of number 16 might be due to the fact that this accession was not a landrace, but rather an improved cultivar. Otherwise, the great intra-accession variability is a usual characteristic of landraces.

The first three axes of the PCA accounted for 49% of the variation present, which is consistent with the 52% obtained by Heun et al. (1994) from a principal co-ordinate analysis of isozyme data from *Avena sterilis* L.

The grouping of accessions that was obtained after PCA (Fig. 2) showed only a limited relationship with their places of origin. It has been reported elsewhere for *Avena* spp. and other autogamous species that proximities obtained on the basis of isozymes were generally unrelated to the geographical distance between acccessions (Beer et al., 1993, for *Avena sterilis* L.; Phillips et al., 1993; Hunter et al., 1995). Conversely, several authors who worked with *Avena* spp. concluded that similarities in environmental features at collection sites might be the reason why oat accessions from different geographical origins clustered together (Kahler et al., 1980, for *Avena barbata* Brot; Phillips et al., 1993, and Hunter et al., 1995, for *Avena sterilis* L.; Pérez de la Vega et al., 1994, for *Avena sativa* L.). This assumes that some isozyme variability and multi-locus combinations are associated with adaptive value. The results reported here are, in a general way, consistent with these ideas. One of the groups defined after PCA analysis (Group 2) was composed exclusively of accessions from the warmer parts of Spain: the Andalusian, Estremaduran, and Valencian regions. Groups 1 and 3 included accessions from regions with a continental climate having long, cold winters (the Rioja, Castile-La Mancha, and Castile-Leon regions). They also included one accession from Granada, a province in the Andalusian Region, but its collection site was located in the mountainous area of Sierra Nevada at over 1,200 meters above sea level. Accessions from Cuenca and Zamora were distributed over groups 1 and 3. Moreover, there was a significant negative correlation between the elevation of the collection site and isozyme polymorphism, indicating a lower isozymic polymorphism for sites at a greater height. In contrast, there was no significant correlation between isozymic polymorphism and the longitude or latitude of the collection site.

An important conclusion is that the isozymic variation of the 26 accessions was similar to that of the entire collection, which indicates that the method used to obtain the subset did not cause loss of genetic diversity. This might be due to the fact (Murphy and Phillips, 1993) that isozymic variation usually has a relationship with adaptation to environmental conditions, and to the criteria used for the selection of the subset of 26 items, since the proportion of accessions from each agro-climatic region was consistent with the relative weighting, in terms of number of accessions, from each region in the collection as a whole. In consequence, the following strategy for identifying genetically distant sub-samples useful for future breeding programs is proposed. Genotypes should be chosen initially for their useful agricultural characteristics, and thereafter subsets should be selected on the basis of molecular markers from among those genotype sets that are dissimilar from one another. This strategy was previously proposed by Beer et al. (1993) based on results with *Avena sterilis*. Since the accessions studied here can all contribute good agricultural characteristics (high yield combined with lack of lodging and grain shedding), the genetically more distant accessions (Fig. 2) may serve as sources of parental material in breeding oats. This approach matches what was done by Phillips et al. (1993), who worked with the wild progenitor (*Avena sterilis* L.) of cultivated oats. They considered that accessions from

Table 6
Isozyme bands that were heavily weighted (eigenvector coefficient > 0.20 or <–0.20) in a Principal Components Analysis (PCA) of the covariance matrix of band frequencies for the 26 oat accessions, the frequencies of bands in every accessions, and the component or components in which bands were weighted heavily. The groups referred to are those defined by the scatter diagram of the PCA

	Acc. no.	ADH 3	LAP 2	MDH-A 1	MDH-A 2	MDH-B 1	PER 1	IDH 1	IDH 2	IDH 3	IDH 4	PGD 4	PGD 5	PGD 7	PGD 8
Group 1	#1	0	1	0	0	0	0	—	—	—	—	0	0	1	0
	#5	0	0	0	0	0	0	0	0	0	1	0	0	0.244	0
	#6	0	1	0	0	0	0	0	0	0	0	0	0.8174	1	0
	#8	—	1	0	1	0.051	0	0	0	0	0	0	0.124	1	0
	#12	0	1	0	1	0	0	0	0	0	0	0	0.817	1	0
	#14	0.021	1	0	0.124	0	0.017	0	0	0	0	0	1	1	0
	#15	0.038	0.800	0.017	0.034	0	0.055	0	0	0	0.635	0	0	1	0
	#16	0	1	0	0	0	0	0	1	0	1	0	0	1	0
	#7	0.097	1	1	0.184	0.057	0	0	0	0	0.225	0	1	1	0
	#9	0	1	0	1	0	0	0	0	0	0	0	1	1	0
Intermediate	#11	0.130	—	0.18	0.81	0.176	0	0	0	0	0.034	0	0.051	1	0
	#13	0.130	1	0.2254	1	1	0	0.452	0	0.452	0.452	0	0	1	0
	#25	0.304	1	0	1	0	0	0.750	0	0.746	1	0	0.050	0.689	0
Group 2	#2	0.635	0	1	0.817	0	0	0.120	0.051	0.124	0.817	0	0.742	1	0
	#3	0.350	0	0.293	0.144	0	0	0.020	0.051	0.034	0.742	0	0.293	0.517	0
	#4	0.106	0	1	1	1	0	0	0	0	1	0.017	0.684	0.817	0.017
	#18	0.452	0.423	0.635	0.817	0.742	1	0.225	0	0.225	0.635	0	1	0.225	0
	#20	1	0	0.017	0.017	0.184	0	0	0.635	0.034	0.635	0	1	1	0
	#21	0.545	0	0.562	1	0	0.728	0	1	0	1	0	1	1	0
	#22	0.817	0.776	1	1	1	0	1	0	1	1	0	1	1	0
Group 3	#10	0.585	1	0.592	0.817	0.051	0	0	0	0	0	0.684	0	0.051	0.684
	#17	0.041	1	0	0.592	0.342	0.814	0	0	0	0	0.817	0	0.017	0.817
	#19	0.667	1	0.184	0.225	0.163	0	0	0	0	0	0.684	0	0.051	0.684
	#26	0.270	1	0.293	0.293	0	0	0.293	0	0.293	0.293	1	0	0	1
	#24	0	1	1	1	0	0	1	0	1	1	0.742	0.034	0.034	0.742
	#23	0.635	0	0	0	1	1	0.633	1	0.633	0.633	0.817	0.017	0.017	0.817
Pr. Co.[1]		2	2 and 3	2 and 3	3	1	1	1	3	1	2	1	2	1	1

[1] Principal components with eigenvector coefficient > 0.20 or < –0.20.

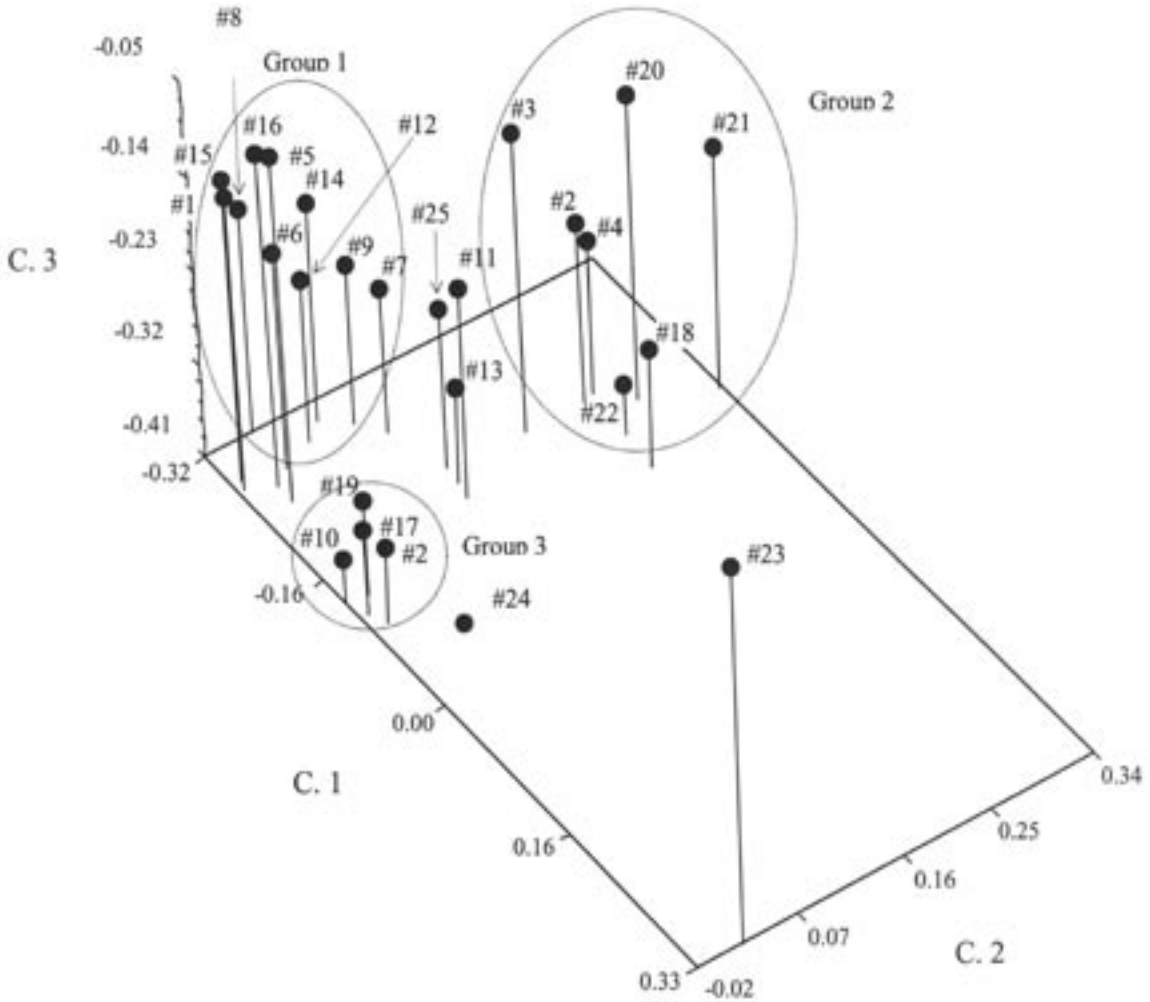

Fig. 2. Projection of the 26 oat accessions on the first three principal components based on the covariance matrix of isozyme band frequencies. The 3 first components accounted for the 49% of the variability.

some of the clusters defined after an isozyme survey could serve as sources of parental material in breeding oats, if they were identified as superior contributors of useful variations to the cultivated varieties.

Other conclusions worthy of mention are the following. The chosen subset of types has high overall diversity, because its phenotypic variability index was $P_j = 0.576$. Almost one-third (29%) of the overall variability was intra-accession variability, and the remainder was between accessions, although there were large differences in intra-accession variability among the various types.

ACKNOWLEDGMENT

This work was financially supported by INIA Project RF98-017 (Spain).

REFERENCES

Allard, R.W. 1992. Predictive methods for germplasm identification. In: Stalker, H.T., Murphy, J.P., eds. Plant breeding in the 1990s. CAB International. Wallingford, U.K., pp. 119–146.

Beer, S.C., Goffreda, J., Phillips, T.D., Murphy, J.P., Sorrells, M.E. 1993. Assessment of genetic variation in *Avena sterilis* using morphological traits, isozymes, and RFLPs. Crop Science 33 (6): 1386–1393.

Brown, A.D.H. 1989. Core collections: a practical approach to genetic resources management. Genome 31: 818–824.

Diederichsen, A. 2004. Case studies for the use of infraspecific classifications in managing germplasm collections of cultivated plants. Acta Horticulturae 634: 127–139.

Elías, F., Ruíz Beltrán, L. 1977. Agroclimatología de España. Monografías ICONA. Vol. 7. Ministerio de Agricultura,

Pesca y Alimentación, Madrid.
Goffreda, J., Burnquist, W.B., Beer, S.C., Tanksley, S.D., Sorrells, M.E. 1992. Application of molecular markers to assess genetic relationships among accessions of Wild Oat *Avena sterilis*. Theoretical and Applied Genetics 85: 146–151.
Gómez, C., Sáenz de Miera, L., Pérez de la Vega, M. 1991. Estimation of the isozymatic genetic variability of a Spanish *Avena sativa* germplasm collection. Plant Breeding 106 (4): 293–300.
Heun, M., Murphy, J.P., Phillips, T.D. 1994. A comparison of RAPD and isozyme analyses for determining the genetic relationships among *Avena sterilis* L. accessions. Theoretical and Applied Genetics 87 (6): 689–696.
Hunter, D.E., II, Murphy, J.P., Phillips, T.D. 1995. Isozyme variation in *Avena sterilis* L. collected in Turkey. Crop Science 35 (5): 1477–1482.
Kahler, A.L., Allard, R.W., Krazkowa, M., Wehrhahn, C.F., Nevo, E. 1980. Associations between isozyme phenotypes and environment in the Slender Wild Oat (*Avena barbata*) in Israel. Theoretical and Applied Genetics 56: 31–47.
Li, C.D., Rossnagel, B.G., Scoles, G.J. 2000. The development of oat microsatellite markers and their use in identifying relationships among *Avena* species and oat cultivars. Theoretical and Applied Genetics 101: 1259–1268.
Maselli, S., Pérez García, F., Aguinagalde, I. 1999. Evaluation of seed storage conditions and genetic diversity of four crucifers endemic to Spain. Annals of Botany 84 (2): 207–212.
Matiello, R.R., Neto, J.F.B., Sereno, M.J.C.M., Taderka, I., Pegoraro, D.G. 1998. Genetic variability through isozyme polymorphisms in hexaploid oats. Pesquisa Agropecuaria Brasileira 33 (6): 913–918.
Murphy, J.P., Phillips, T.D. 1993. Isozyme variation in cultivated oats and its progenitor species, *Avena sterilis* L. Crop Science 33 (6): 1366–1372.
Pal, N., Sandhu, J.S., Domier, L.L., Kolb, F.L. 2002. Development and characterization of microsatellite and RFLP-derived PCR markers in oats. Crop Science 42(3): 912–918.
Papadakis, J. 1966. Climates of the world and their agricultural potentialities. Editorial Albatros, Buenos Aires. 174 pp.
Pérez de la Vega, M., Sáenz de Miera, L.E., Allard, R.W. 1994. Ecogeographical distribution and differential adaptedness of multilocus allelic associations in spanish *Avena sativa* L. Theoretical and Applied Genetics 88: 56–64.
Phillips, T.D., Murphy, J.P. 1993. Distribution and analysis of isozyme polymorphism in North American cultivated oat germplasm. Crop Science 33 (3): 460–469.
Phillips, T.D., Murphy, J.P., Goodman, M.M. 1993. Isozyme variation in germplasm accessions of the wild oat *Avena sterilis* L. Theoretical and Applied Genetics 86 (1): 54–64.
Plucknett, D.L., Smith, N.J.H., Williams, J.T., Murthi-Anishetty, N. 1987. Gene Banks and the World's Food. Princeton University Press, Princeton, NJ.
Rezai, A., Frey, K.J. 1988. Variation in relation to geographical distribution of wild oats-seed traits. Euphytica 39: 113–118.
Rohlf, F.J. 2000. NTSYSpc. Numerical taxonomy and multivariate analysis system. Version 2.1. Exeter Software, New York.
Salas, C.A., Murphy, J.P. 1995. Inheritance and linkage of isozyme loci in hexaploid oats. Journal of Heredity 86 (5): 381–385.
Shannon, C.E., Weaver, W. 1949. The mathematical theory of communication. University of Illinois Press, Urbana, IL.
Torres, E., Iriondo, J.M., Pérez, C. 2003. Genetic structure of an endangered plant, *Antirrhinum microphyllum* (Scrophulariaceae): allozyme and RAPD analysis. American Journal of Botany 90 (1): 85–92.