The assessment of variability in Spanish populations of wild relatives of cereals

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Abstract

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Over the last few years the Plant Breeding Unit of the I.N.I.A. in Madrid has centred its attention on collecting, maintaining and studying the genetic diversity of wild species of the tribe *Triticeae* (genera *Aegilops, Agropyron, Elymus, Elytrigia, Hordelymus, Hordeum, Taeniatherum* and *Thinopyrum*) growing in Spain. The plant material collected includes samples of 609 natural populations belonging to 25 species or subspecies, both annual and perennial. These reflect the presence and distribution of the different genera and species in Spain. The present report covers aspects related to the distribution and collection of samples, and the analysis of the genetic variability of natural populations of these plant materials.

The areas explored, method of collection and material collected

Over a period of eight years (1987-1994) several expeditions were made to the greater part of Spain and its islands. Before collecting commenced a review was made of the literature concerning the regional flora of Spain and of 20 herbaria belonging to different Spanish universities and botanicgardens, to define the geographical limits of the survey. In accordance with this information, the collection area was fixed as the whole of the Spanish mainland (except for Galicia and part of the Cantabrian Mountains), the Canary Islands (the island of Tenerife) and the Balearic archipelago (the islands of Mallorca and Menorca).

In order to conserve the original structure of the natural population, specimens were collected at random and each sample was collected following the method of Hawkes (1980). About 100 ears were picked from each population. The plant material collected (Table 1) includes samples of 609 natural populations belonging to 25 species or subspecies reflecting the presence and distribution of the different genera and species in Spain.

Table 1. Spanish populations of wild species relatives of cereals collected in Spain. They include natural populations belonging to 7 genera and 25 species or subspecies and reflect their presence and distribution in Spain.

Species	2*n*=	Genome formula	System	Number of populations: Total	Spanish mainland	Balearic Islands	Canary Islands
Aegilops ovata L.	28	UM^o	annual	110	99	11	
A. biuncialis Vis.	28	UM^b	annual	17	17		
A. neglecta subsp. *recta* (Zhuk.) Hammer	42	UMU^n	annual	27	26	1	
A. triuncialis L.	28	UC	annual	67	66	1	
A. ventricosa Tausch	28	DU^n	annual	25	18	7	
A. cylindrica Host	28	CD	annual	2	2		
Hordeum bulbosum L.	14	I	perennial	27	27		
H. secalinum Schreber	28	H-?	perennial	7	7		
H. murinum subsp. *leporinum* (Link) Arcangelli	28	YY?	annual	62	53	1	8
H. murinum subsp. *murinum* L.	28	YY?	annual	17	17		
H. murinum subsp. *glaucum* (Steud.) Tzvelev	14	Y	annual	4	4		
H. marinum subsp. *marinum* Hudson	14	X	annual	41	39	2	
H. marinum subsp. *gussoneanum* (Parlat.) Thellung	14	X	annual	24	23	1	
Taeniatherum caput-medusae (L.)	14	T	annual	41	41		
Hordelymus europaeus (L.) Harz	28	T?N?	perennial	1	1		
Agropyron cristatum (L.) Gaertner	28	P	perennial	6	6		
Elymus caninus (L.) L.	28	SH	perennial	19	19		
E. hispanicus (Boiss.) Talavera	28	S?Y?	perennial	6	6		
Elytrigia pycnantha (Godr.) Löve	42	S^1S^2X	perennial	4	4		
E. repens (L.) Nevski	42	S^1S^2X	perennial	25	25		
E. pungens (Pers.) Tutin	56	$K^1K^2E^4$	perennial	18	18		
Thinopyrum junceiforme (A. & D. Löve) Löve	28	J^1J^2	perennial	6	6		

Species	$2n=$	Genome formula	System	Total	Number of populations: Spanish mainland	Balearic Islands	Canary Islands
T. junceum (L.) Löve	42	$J^1J^2E^1$	perennial	17	17		
T. elongatum (Host) Dewey	14	E	perennial	12	12		
T. ponticum (Podp.) Barkwoth & Dewey	56-70	E	perennial	4	4		
T. curvifolium (Lange) Dewey	28	E^1E^2	perennial	9	9		
T. intermedium (Host.) Barkwoth & Dewey	42	E^1E^2X	perennial	11	11		
Total				609	577	24	8

Noteworthy aspects of the collected material include the following: (1) the presenceof two populations of *Aegilops cylindrica,* one of which had not been previously cited. (2) the obtainment of four populations of *Hordeum murinum* subsp. *glaucum* which is very rare and found only locally in Spain, and (3) the obtainment of samples of the perennial endemic species *Elymus hispanicus* and *Thinopyrum curvifolium.*

Distribution and habitat of *Aegilops* species in Spain: types of population found

Spain, at the extreme western edge of its distribution, is remarkably rich in *Aegilops* and has populations of several species (Table 1). All the Spanish species are included within communities belonging to the alliance *Taeniathero-Aegilopion geniculatae* of the class *Stellarietea mediae.* They are pterophytic, subnitrophilic, of graminoid appearance and are late spring flowerers. They are found growing on uncultivated land, along the edges of paths, on abandoned cultivated land, on disturbed land and on shrubland of degraded climax vegetation. Its optimum of geographical distribution is found within regions of Mediterranean and continental climate with hot, dry summers. It gradually disappears towards the north, where the Eurosiberian biogeographical region takes over and where summers are cool and damp. The species are indifferent to soil type. Though potentially they could be distributed throughout all areas with Mediterranean influence, this has not been the case (Ruíz-Fernández & al. 1995). Fig. 1 shows the distribution of the species in Spain.

Aegilops ovata shows the widest distribution and diversity of habitats of all the Spanish species. Its range includes the whole Spanish mainland except for the North and Northwest. It is common throughout the Mediterranean biogeographical region of the Peninsula and in the Balearic archipelago. It is scarce, and exists almost certainly as a naturalized species in the Canary Isiands (Gomera, Lanzarote, La Palma and Tenerife) which fall within the Macaronesian bio-geographical region. It is found from sea level on the coast up to altitudes of 2000 m. It generally forms large or very large populations. Population density is very variable, ranging from very high in areas influenced by humans (where it usually appears associated with *A. triuncialis),* to low in areas of shrubland and in clearings arising from the degradation of woodland (where it often appears with populations of *A. triuncialis* and *A. biuncialis).*

Aegilops triuncialis also has a wide distribution though somewhat smaller than that of *A. ovata*. It occupies areas further away from the coast and is not usually found at altitudes above 1800 m in the interior. In the Balearic Archipelago it is found locally on Mallorca. It is not found in the Canary Islands. It forms populations of very variable size and density, almost always mixed with *A. ovata*. In shrubland it also appears mixed with *A. biuncialis*.

The remaining species are more localized. On the Spanish mainland *Aegilops ventricosa* is found mainly in two areas: the northern submeseta and surrounding mountainous regions, and the mountains of the southeast. In the Balearic archipelago it is found on Mallorca and, less frequently, on Menorca. It displays a preference for shady, damp habitats at the edges of woodland, close to rivers, in hollows and on grassland. Samples were taken at altitudes between 650 and 1700 m on the Spanish mainland. In the Balearic archipelago some populations are found at as low as sea level. *A. ventricosa* generally forms populations of small extension, composed of a reduced number of individuals (100 or less), Its optimum appears to be in mountainous areas where plants are more vigorous and have bigger ears. It would appear to compete poorly with surrounding herbaceous annuals which reduces its frequency.

Aegilops biuncialis grows on the Spanish mainland in an area that links the two areas of distribution of *A. ventricosa*. It is found on the southern submeseta and in the mountains adjoining the centre and southeast. In the centre, where the climate experiences greater continental influence, populations appear at lower altitude, around 600 m. In the mountains of the southeast, where Mediterranean influence is greater, it grows at higher altitudes (above 1800 m). It is also found on the island of La Gomera in the Canary Islands. Populations tend to be small, usually of some 100 to 200 individuals, and of low density. Individuals are generally well separated. In the mountains of the south very large and very dense monospecific populations can be found.

Aegilops neglecta is found mainly in an area of the West and Southwest of peninsular Spain characterized by its benign climate, large number of frost-free days and a degree of Mediterranean influence. Its distribution is limited to the north and to the east by the areas occupied by *A. ventricosa* and *A. biuncialis*. It occupies habitats in the clearings of *Quercus* sp. woods (holm oak and cork-oak woods), and in areas of degradaded communities of them. It grows over a range of altitudes, from low near the Atlantic to above 1000 m in other areas. Local populations are found in Mallorca (Balearic archipelago) and Lanzarote (Canary Islands). It forms medium to large populations (200-500 individuals) of low density. In acidophilous fields of the southwest it is usually found with *Taeniatherum caput-medusae* Nevski and occasionally with *A. ovata and A. triuncialis*.

Aegilops cylindrica is considered a species of the northern coastline of the Eastern Mediterranean and reaches as far as Central Asia. It has been described as an adventitious species in several countries of Western and Central Europe (Tutin & Humphries 1980, van Slageren 1994). Specimens were collected in two localities near each other in the southeastem part of the Spanish mainland: one corresponding to the population reported by Soriano (1983) and the other a population which had not been previously reported.

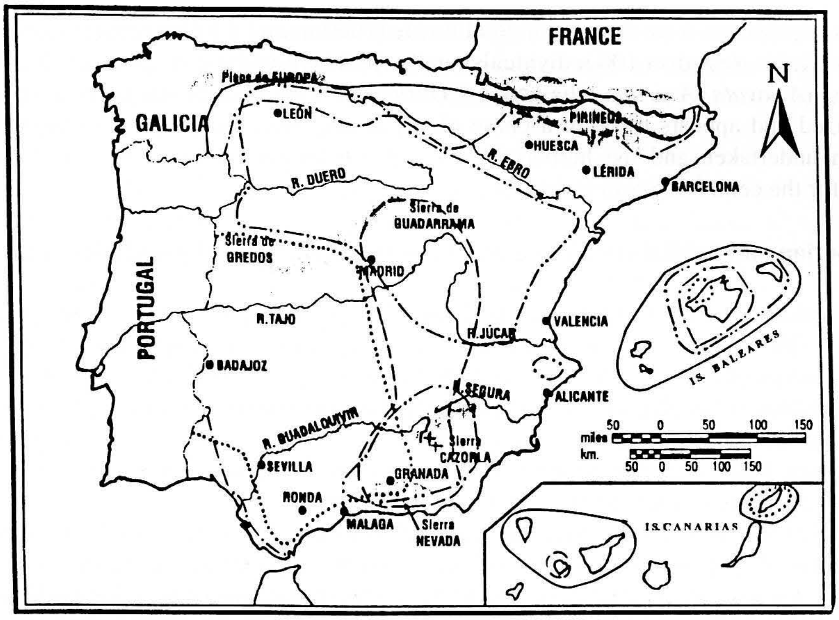

Fig. 1. Distribution of *Aegilops* in Spain: *A. ovata* (—), *A. triuncialis* (— · —), *A. ventricosa* (— ·· —), *A. biuncialis* (-------), *A. neglecta* (······), *A. cylindrica* (+).

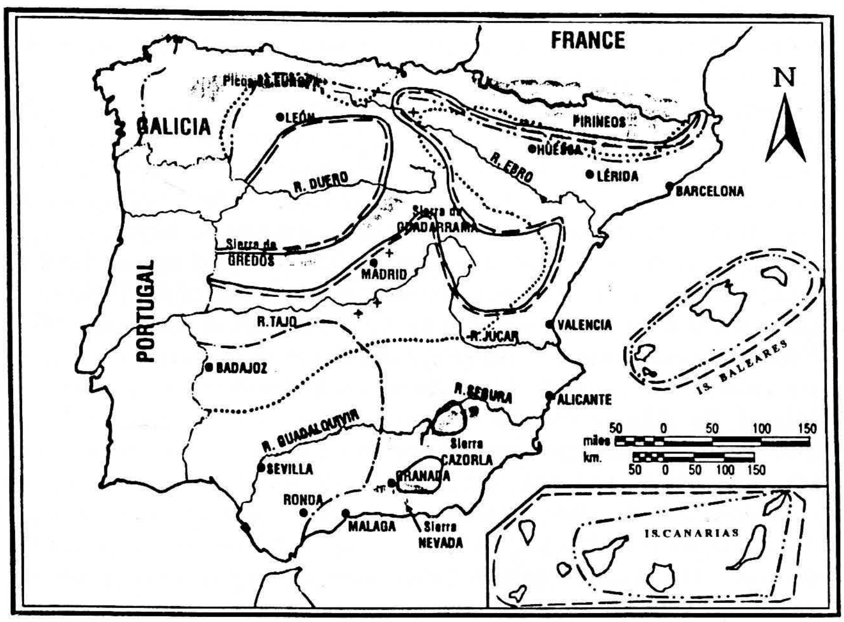

Fig. 2. Distribution of *Hordeum* in Spain: *H. bulbosum* (— · —), *H. secalinum* (······), *H. murinum* subsp. *glaucum* (+), *H. murinum* subsp. *leporinum* (-------), *H. murinum* subsp. *murinum* (—), *H. marinum* (— ·· —).

Both populations were found at high altitudes (1660 m and 1400 m respectively), were of reduced size (less than 100 individuals) and were mixed with other species of the genus *Aegilops* (*A. ovata, A. triuncialis* and *A. ventricosa*). This species should be considered naturalized and appears to be in a phase of expansion. According to the bibliographical revision undertaken and the herbaria consulted, the locality reported by van Slageren (1994) for the centre of Spain is doubtful.

Distribution and habitats of *Hordeum* species in Spain: types of population found

The species of *Hordeum* found in Spàin (Fig. 2) generally occupy very different habitats. This requires their inclusion in different classes and/or different phytosociological alliances in contrast to species of the genus *Aegilops*. The species may be nitrophilous, nitrohalophilous or halophilous. They are found in human-altered habitats, along roadsides or in hay fields.

Hordeum bulbosum is restricted to a small area of the southwest of the Spanish mainland and is more common on the plains and mountains between the Straitof Gibraltar and the Guadalquivir basin. Along the edges of paths and on disturbed land it appears in communities of the class *Rudereto-Secalinetea* where soil is sandy, acidic or neutral and which has a degree of seasonal moisture or has a fluctuating water table. Along the shores of lagoons and the banks of streams it usually appears in communities of the class *Juncetea maritimi*. Populations are small, generally of less than 50, well-separated individuals, but occupy large areas.

Hordeum secalinum is found above 400 m in the damper areas of the plains of the central Iberian peninsula. It also grows in the mountains of the centre and north of the Peninsula where it may be seen at over 1000 m. In mountainous areas it is a species characteristic of the alliance *Gaudinio fragilis-Cynosurion cristati* of the class *Molinio-Arrhenatheretea*, forming permanent meadows on deep, damp, clay soils. On the plains it is found in habitats with an almost permanently high water table, occupied by the classes *Isoeto-Nanojuncetea* and *Juncetea maritimi*. In the centre of the Peninsula populations are small (less than 100 individuals). In the Eurosiberian region they are larger.

Hordeum marinum subsp. *leporinum* is common from sea level to over 1000 m in all areas of Mediterranean climate, in the continental climatic region of the Peninsula, and in the Balearic Archipelago. It is also found in the Canary Islands where it can grow beyond 2000 m. Populations are isolated in the mountains and in the Eurosiberian region where climates are colder. In these areas it is replaced by *H. murinum* subsp. *murinum*. It is characteristic of the alliance *Hordeion leporini* (class *Rudereto-Secalietea)* which is composed of species that occupy roadsides and nitrophilous habitats altered by human action. It is indifferent to soil type and has a great capacity for colonising disturbed nitrophilous habitats. It therefore appears in communities which grow in such areas. Populations are of very variable size and density, and can form extensive monotypic or mixed populations. It can occasionally be found with *Aegilops* sp., *Taeniatherum caput-medusae* and *H. murinum* subsp. *murinum*.

Hordeum murinum subsp. *murinum* replaces *H. murinum* subsp. *leporinum* above 800-1000 m in the mountains of the interior of the Peninsula and in the entire Eurosiberian region of the North and Northwest (Galicia). It is not present in the Balearic archipelago nor in the Canary Islands. It is characteristic of the alliance *Sysimbrion officinalis.*

Indifferent to soil type it is found near buildings, rubble tips, disturbed land and along the edges of paths. It can form extensive, dense, monotypic populations which invade hay fields though it can also be found in small populations of very limited extension.

Hordeum murinum subsp. *glaucum* is found in the centre of the Peninsula and in mountainous regions of the southeast where climate is continental but moderated by Mediterranean influence. It appears locally on Ibiza (Balearic archipelago) and on Gran Canaria and Tenerife (Canary Islands). The species is characteristic of the alliance *Hordeion leporini* (class *Rudereto-Secalinietea)* along with *H. murinum* subsp. *leporinum.* It prefers soil containing nitrogen and is normally restricted to human-modified habitats, the edges of paths and the borders of cultivated land where there is a degree of humidity or environmental moisture although always where there are dry summers. It is often confused with populations of *Hordeum murinum* subsp. *leporinum* and it is therefore difficult to know its true presence in Spain which has probably been overstated. The populations found were small (less than 100 individuals), very compact and of very limited extension.

Hordeum marinum subsp. *marinum* is found mainly in regions of continental or Mediterranean climate of Spanish mainland, from the south slopes of the Cantabrian Mountains to the Mediterranean and southwest Atlantic coasts. It is also found locally on the northern coast and the coast of Galicia. It is found throughout the Balearic archipelago and on Lanzarote, Fuerteventura, Gran Canaria and Tenerife in the Canary Isiands. The species is characteristic of halophilous and nitrohalophilous communities of the alliance *Frankenion pulverulentae* (class *Frankenietea pulverulentae)* on coasts that are temporarily flooded, in the interior of the peninsula and in the Ebro Valley. Populations are found from sea level to above 1000 m in the interior. It is common to find the species in communities of *Juncetea maritimae, Arthrocnemetea, Nerio-Tamaricetea* and *Thero-Salicornietea.* Population size is very variable. In polytypic populations there are usually less than 100 individuals, but on clear land it can form very dense monotypic populations which can cover anything from a few to many hundred square metres.

On the Spanish mainland *Hordeum marinum* subsp. *gussoneanum* has a distribution similar to that of *Hordeum marinum* subsp. *marinum*, though it is a little more restricted. It is found in the interior of the Peninsula, in the mountains of the southeast (where it can grow at over 1600 m) and in pre-littoral areas of the Mediterranean. It is also found on Mallorca and Menorca (Balearic archipelago). It is not reported in the Canary Islands. It is often confused with *H. marinum* subsp. *marinum.* It appears in halophilous and nitrohalophous pterophytic communities of the alliance *Frankenion pulverulentae* (class *Frankenietea pulerulentae*). It is also found in communities of the class *Molinio-Arrhenatheretea* which reaches the Mediterranean region on damp (though not flooded), somewhat compact, sandy soils. Populations are generally small (less than 100 individuals), of variable density, sometimes mixed with populations of *H. marinum* subsp. *marinum* and with individuals intermediate between these species. It occasionally forms monotypiic populations of 500 or more individuals.

Analysis of genetic variation: methods

A first aim was to investigate the genetic variability of the populations of the different species in the order of their use in genetic improvement programmes. Knowledge of such variability is also important in the establishment of later collecting, multiplication and conservation strategies.

In order to quantify variability within and between populations, biochemical analyses were made of the endosperm proteins and leaf isozyme systems. Molecular investigations were also made of polymorphisms by random amplification of DNA (RAPDs). Endosperm proteins were analysed by vertical electrophoresis in 10% polyacrylamide gels in the presence of SDS, following the method described by Payne & al. (1981). Leaf isozymes were studied by horizontal electrophoresis in starch gels. The isozyme systems used were GOT, PGM, PGI, IPO, EST, MDH, ACPH, 6-PGD, -CPX and ACO: the first nine were used to study variability in *Aegilops* and GOT, PGI, EST and ACO used to study the same in *Hordeum*. Extraction was performed in 15 day old leaves. The buffer systems used were the Poulik (Poulik 1957), histidine (Hutchinson & al. 1983) and lithium systems (Ostergard1985). A minimum of fifteen plants per population were used in both molecular and biochemical techniques.

RAPDs were obtained using the method described by Williams & al. (1990) with modifications (González & Ferrer 1993). *Hordeum* DNA was extracted from a mixture of ten plants per population and *Aegilops* DNA from 15, according to the method Sharp & al. (1988). The products were amplified and separated on 1.5% agarose gels. Primers of 10 bases were used (OPERON Technologies). For *Hordeum* 10 primers were used and for *Aegilops* 20 were used.

To analyze interpopulational variability, the NTSYS statistical package was used (Rohlf 1989). Pearson's product-moment correlation coefficient was calculated from the band frequency matrix and a symmetric correlation matrix was obtained between populations. This showed the similarities between them. The grouping of populations in the form of dendograms was performed using the UPGMA method (Sneath & Sokal 1973).

Genetic variability in *Aegilops*

Analyses of nine isozyme systems and one endosperm protein system were made in 72 natural populations of *A. ovata, A. triuncialis, A. biuncials, A. neglecta* and *A. ventricosa* (González-Castaño 1992). The different degrees of variation observed within populations were analysed using a hypergeometric distribution test. The result of this test showed that the degree of variability detected in population samples of fifteen individuals was sufficient to be taken as representative of the population.

Endosperm proteins showed great variability within populations, differing according to species (Table 2). *A. ovata* showed the greatest number of polymorphic populations for this system at nearly 87%, followed by *A. biuncialis*. The least variable species was *A. ventricosa* at 18.20%. The mean number of different phenotypes per population for this system was also variable. *A. ovata* showed the greatest mean number (3.13) and *A. ventricosa* the least (1.72).

Of the nine isozyme systems studied only the IPO system was monomorphic and homogeneous in all materials. The remaining systems showed a range of variability both within and between populations of the different species (Table 2). *A. ovata* and *A. biuncialis* showed the highest percentages of variable populations and the greatest number of different phenotypes. As in endosperm proteins, *Aegilops triuncialis* showed intermediate variability. Six isozyme systems showed intrapopulational variability in *A. biuncialis*, 5 in *A. ovata* and *A. triuncialis*, 3 in *A. ventricosa* and 2 (-CPX and EST) in *A. neglecta*. Some populations of *A. ovata* and *A. biuncialis* were found to be variable in 4 systems.

Table 2. Intrapopulational variability in *Aegilops*.

	Endosperm proteins		Isozyme systems		Proteins and isozymes
Species	Polymorphic populations (%)	Phenotypes / population	Polymorphic populations (%)	Phenotypes / population	Multiloci Phenotypes / population
A. ovata	86.96	3.13	78.26	2.90	5.13
A. biuncialis	62.50	2.62	62.50	3.10	4.12
A. triuncialis	60.00	1.95	35.00	1.85	3.10
A. neglecta	50.00	1.80	20.00	1.20	2.20
A. ventricosa	18.20	1.72	36.36	1.54	2.81

Table 3. Intrapopulational variability in *Hordeum*.

	Endosperm proteins		Isozyme systems	
Species	Polymorphic populations (%)	Phenotypes / population	Polymorphic populations (%)	Phenotypes / population
H. murinum	100	7.08	71.41	5.28
H. marinum	100	3.25	60.00	3.70
H. secalinum	100	10.40	60.00	4.60
H. bulbosum	100	14.66	100.00	5.80

After both endosperm and leaf isozyme analyses had been performed in the same plants, complete or multiloci phenotypes were studied for all the studied systems in the 72 populations (Table 2). The mean number of phenotypes per population increases considerably for all species, reflecting intrapopulational phenotypic variability. *A. ovata* and *A. biuncialis* showed most variability. It is noteworthy that in these two species no populations shared multilocus phenotypes.

Dendrograms were produced to study interpopulational variability. As seen in the study of intrapopulational variability, the analysis of endosperm proteins provided greater resolution than did isozymes. None of the populations of any species were found to be the same by the joint study of protein and isozyme data.

The greatest differentiation between populations was found for *A. biuncialis* which showed a similarity index of approximately 0.04 (Fig. 3a). The species which showed least differentiation between some populations *A. triuncialis* and (especially) *A. neglecta* (Figs. 3b and 3c respectively).

The study of variability between populations was completed by the investigation of randomly amplified DNA fragments (RAPDs).

This was performed in 50 populations of all 5 species. As a result of amplification 325 bands were registered. 284 discriminated between at least 2 species and 116 detected variability at intraspecific level.

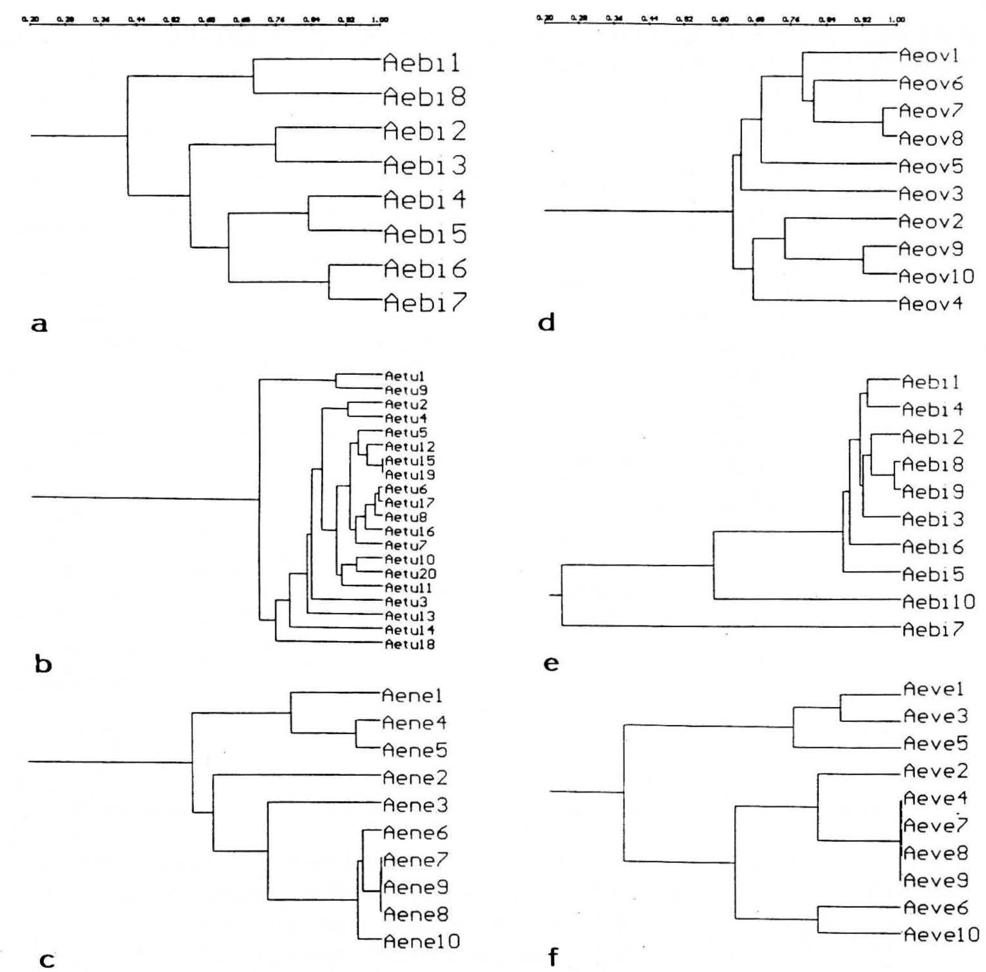

Fig. 3. Population UPGMA dendrograms based on correlation coefficient of Pearson as distance index: **a,** Data referred to endosperm proteins and isozymes in *Aegilops biuncialis.* **b,** *A.triuncialis;* **c,** A. *neglecta*; **d,** Data referred to RAPDs in *A. ovata;* **e,** *A. biuncialis;* **f,** *A. ventricosa.*

The corresponding dendograms showed that *A. biuncialis* once again showed the greatest differentiation between populations (compare Figs. 3d and 3e), followed by *A. ventricosa* (Fig. 3f) in which also four populations could not be differentiated.

Genetic variability in the genus *Hordeum*

Thirty seven populations representative of the presence and distribution of *Hordeum* species and subspecies in Spain were used. All populations were found to be polymorphic for endosperm proteins (Table 3). A noticeable degree of phenotypic variability was also observed, an outstanding feature being the high mean number of phenotypes for the two allogamous species *H. secalinum and H. bulbosum.* The populations of *Hordeum marinum* were seen to be the least variable for this system.

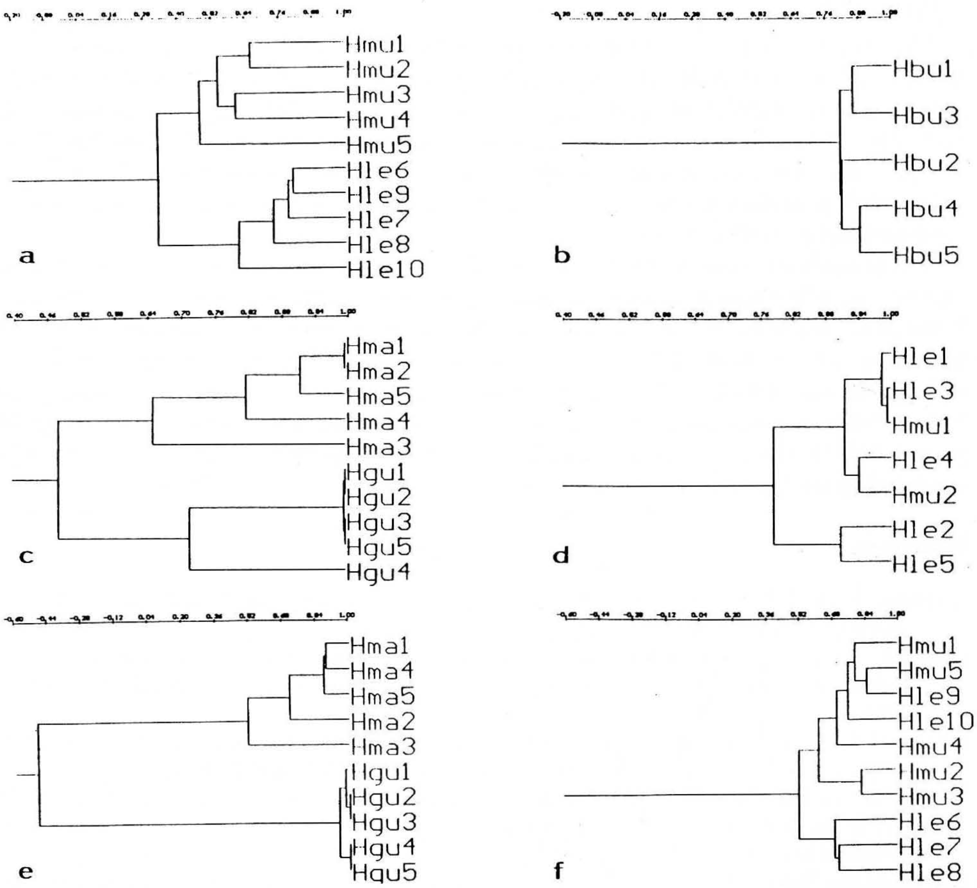

Fig. 4. Population UPGMA dendrograms based on correlation coefficient of Pearson as distance index: **a**, Data referred to endosperm proteins in *H. murinum;* **b**, *H. bulbosum;* **c**, Data referred to isozymes in *H. marinum;* **d**, *H. murinum*; **e**, Data referred to RAPDs in *H. marinum;* **f**, *H. murinum.*

With respect to leaf isozyme systems, ACO was homogeneous and the same for all materials. PGI was homogeneous in *H. marinum.* The esterase system showed no variability in *H. secalinum.* In *H. murinum* and *H. bulbosum* the tree system showed variability. Table 3 also shows the data for variability within populations obtained in the isozyme study. Again, endosperm proteins provided more information on the heterogeneity of the populations. The great variability of isozymes shown by *H. bulbosum* of the allogamous species, and *H. murinum* of the autogamous species is noteworthy.

Figure 4 shows some of the dendograms produced to study interpopulational variability. In all species and subspecies the analysis of endosperm proteins proved to be very efficient for differentiating between populations. In no cases were the results equal. The greatest differentiation between populations was seen for the autogamous species (compare Figs. 4a and 4b). It is noteworthy that on the basis of the characteristics analysed, the two subspecies of both *H. marinum and H. murinum* are clearly revealed as differentiated taxa.

The analysis of isozyme systems to determine differences between populations was insufficient to discriminate between all of those studied, since in both *H. secalinum* and *H. marinum* some populations proved to be equal in value (Fig. 4c). The considerable separation shown by the two subspecies of *H. marinum,* both in terms of endosperm proteins and isozymes, greater than that observed for the two species of *H. murinum* (where differentiation is lost in the case of isozymes and the two forms are not seen as separate taxa) is worthy of note.

RAPD analysis resolved 197 bands, of which only one was present in all the material studied, and 136 bands discriminated between populations. Here also the great separation between *H. marinum* subsp. *marinum* and *H. marinum* subsp. *gussoneanum* is evident (similarity index = -0.05) (Fig. 4e). As for isozyme analysis, *H. murinum* subsp. *murinum* and *H. murinum* subsp. *leporinum* are not differentiated into two subgroups. Despite the large number of polymorphic bands obtained in the amplification of DNA, the use of 10 primers was insufficient to distinguish between all the populations that were successfully separated by study of their endosperm proteins.

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