

SELF-FERTILIZATION AND ABSENCE OF HETEROZYGOTES IN *Hordeum euclaston* (GRAMINEAE)

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ABSTRACT

Isoenzymes of *Hordeum euclaston* were analyzed by horizontal polyacrylamide gel electrophoresis. Four isoenzymatic systems were assayed in seedlings grown from seeds of 95 plants from three wild populations from Southern Brazil. The mode of reproduction was determined by analyzing progenies of several maternal plants in each population. Out of 26 loci observed, six were polymorphic, with a total of 34 alleles. No genetic variation was detected within any single plant progeny. No heterozygosity was found in the wild populations. The results show low genetic variation between plants within populations. Low interpopulational differentiation was also found, contrary to the theoretical prediction according to which different self-fertilizing populations are very different in genetic constitution.

INTRODUCTION

Hordeum euclaston is a wild grass from the same genus as the cultivated barley (*H. vulgare* L.). The species is found scattered in Southern Brazil (State of Rio Grande do Sul), Uruguay, and Argentina (Bothmer *et al.*, 1982; Bothmer and Jacobsen, 1985; Eggers and Boldrini, 1988). It is distributed in a large number of habitats, "growing in pastures, sandy steppes, in relatively open soil, near ditches, ponds and ruderal biotopes, and as a weed on cultivated ground, often together with *H. stenostachys* and *H. flexuosum*" (Bothmer *et al.*, 1982).

This species is annual and diploid ($2n=14$) and exhibits considerable morphological variability. It has a wide range of variation in habitat and size of vegetative parts, size and shape of their floral parts, lemma and indumentum (Bothmer *et al.*, 1982; Bothmer and Jacobsen, 1985; Eggers and Boldrini, 1988).

Wild species such as *H. spontaneum*, *H. bulbosum* and *H. jubatum* have been exploited as a reservoir of desirable characters in cultivated barley (Giles and Bothmer, 1985; Fedak, 1985; Bothmer, 1987; Feuerstein *et al.*, 1990). Nevertheless, characters related to the ability

to withstand local ambiental conditions, e.g. the high level of aluminium in the soil found in Southern Brazil, and those related to resistance to local diseases, must be searched for in wild species from each region.

The purpose of this investigation was to study the level of genetic variation in natural populations of *H. euclaston* by isoenzyme gel electrophoresis and to achieve a better understanding of the basic characteristics of its population structure.

MATERIAL AND METHODS

Samples were collected from three populations from the Campanha region in the State of Rio Grande do Sul, Brazil. These three populations occur in open grasslands, near roads, with intervening populations. They were identified as populations 5, 6, and 8. The three sites are 44 km (pop. 5 - pop. 6), 96 km (pop. 6 - pop. 8) and 140 km (pop. 5 - pop. 8) apart.

Seeds were collected from individual plants and were assigned with the collector's number. The number of individuals analyzed was 30, 32, and 33 for each population, respectively. Seeds were germinated in Petri dishes at 20°C under constant light in an incubator until seedlings' coleoptiles reached about 1.2 cm. Then the seedlings were frozen for later electrophoretic analyses.

The analyses were made by horizontal polyacrylamide gel electrophoresis for four enzymatic systems: glutamate oxalacetate transaminases (GOT),

malate dehydrogenases (MDH), esterases (EST), and superoxide dismutases (SOD). All the enzymes were assayed using coleoptile as well as roots, and for EST endosperm was also used. Three to six extracts of barley (cultivar MN-599) were used as control in each gel. In order to detect the largest possible number of different electromorphs, two different electrophoretic conditions were used for three out of the four enzymatic systems. For EST, 6% and 8% polyacrylamide gels and Scandalios' buffers (1969) and for MDH and SOD, 6% and 7% polyacrylamide gels and Roose and Gottlieb's buffers (1976) were used. For GOT, only one migration condition was used because the preliminary tests didn't show differences which justified the use of two distinct conditions. Seven per cent polyacrylamide gels and Brown's buffers (1983) were used. All the gels were run at about 10 V/cm until the front line reached 8 cm from the origin.

Staining procedures for EST were performed as described by Scandalios (1969), including β -naphthyl acetate. MDH and SOD were stained using the methods of Brewer (1970) with modifications for SOD which was stained with glutamate dehydrogenase staining mixture and incubated under illumination. GOT was stained according to Vallejos (1983) without using pyridoxal-5-phosphate.

The mode of reproduction was determined through the analysis of the isoenzymatic patterns of progenies (sib seedlings) from a single maternal plant from nature. For this purpose, 180 seedlings from 38 strains (strain = progeny from a single maternal plant) were analyzed; a mean of 4.7 seedlings and between 2 and 17 per progeny. The genetic control of the isoenzymes was determined by analyzing the patterns of different individuals from the three populations and of the progenies from single plants. The intrapopulation genetic variability was estimated based on the number of alleles per locus (A), proportion of polymorphic loci (P) and expected heterozygosity (H). The interpopulation genetic distance was calculated according to Nei (1972).

RESULTS

Mode of reproduction

The floral structure of *H. euclaston* is adapted to self-pollination. The plants have small anthers which do not protrude from the florets, suggesting cleistogamy.

No variations in structural loci were detected within each of the 38 progenies analyzed. This complete uniformity implies homozygosity for the maternal plants and indicates a reproductive system that is autogamous. Outcrossing, if not absent, is extremely low. Representa-

tive gels are shown in Figures 1-3. The isoenzymatic patterns of each tissue in all the individuals analyzed from the same strain were identical.

Genetic variability

Electropherograms of the four isoenzymatic systems are in Figures 4-9. The isoenzymatic patterns of all alleles are in Figure 10.

Five bands controlled by four loci were analyzed for GOT. Only the *Got-2* locus was polymorphic, with the least common allele (*Got-2²*) appearing in just one plant. *H. euclaston* GOT enzymes lose activity in storage. Because of this, the *Got-1* and *Got-2* loci were not detected in all individuals (*Got-1* was detected in 75 plants and *Got-2* in 63 individuals). All the loci showed more activity in coleoptiles than in roots.

For MDH, a total of six bands controlled by two loci were analyzed. Only the *Mdh-2* locus was polymorphic, with two alleles. The least frequent allele (*Mdh2¹*) determined a pattern of three bands, a primary one and two secondaries, while the most frequent allele (*Mdh2²*) showed just the primary band. All the loci were active in both tissues; the coleoptile showed more intense bands.

A total of 24 bands were analyzed for EST. Out of these, three showed β -esterase activity, 20 α -esterase activity and one displayed both. Eleven loci were defined, two of them polymorphic: *Est-7* with two alleles and activity in all the tissues (coleoptile, roots, and endosperm), and *Est-11*, also with two alleles, had activity restricted to the roots and endosperm. The products of *Est-11* showed a post synthesis modification that altered the mobility of bands in one of the tissues analyzed.

For SOD, a total of 16 bands were detected. Seven of these were cathodic. Nine loci were identified, two of them being polymorphic: *Sod-2* had two alleles and activity restricted to the roots, and *Sod-8* had at least four alleles and activity in both tissues (coleoptile and roots).

The 95 individuals in the samples from the three populations were homozygotes for all loci. This corroborates the data obtained in the progeny tests which confirm *H. euclaston* as an autogamous species.

Allelic frequencies in the five polymorphic loci are given in Table I. The *Sod-8* locus was also polymorphic, but its allele frequency wasn't calculated because of the difficulty in identifying the allele present in many individuals. No allelic variations were detected in the other 20 loci. Although several loci were not active in all individuals, these differences were presumed to be due to regulatory alterations (except for the GOT loci which presented storage problems) and the structural loci were considered monomorphic.

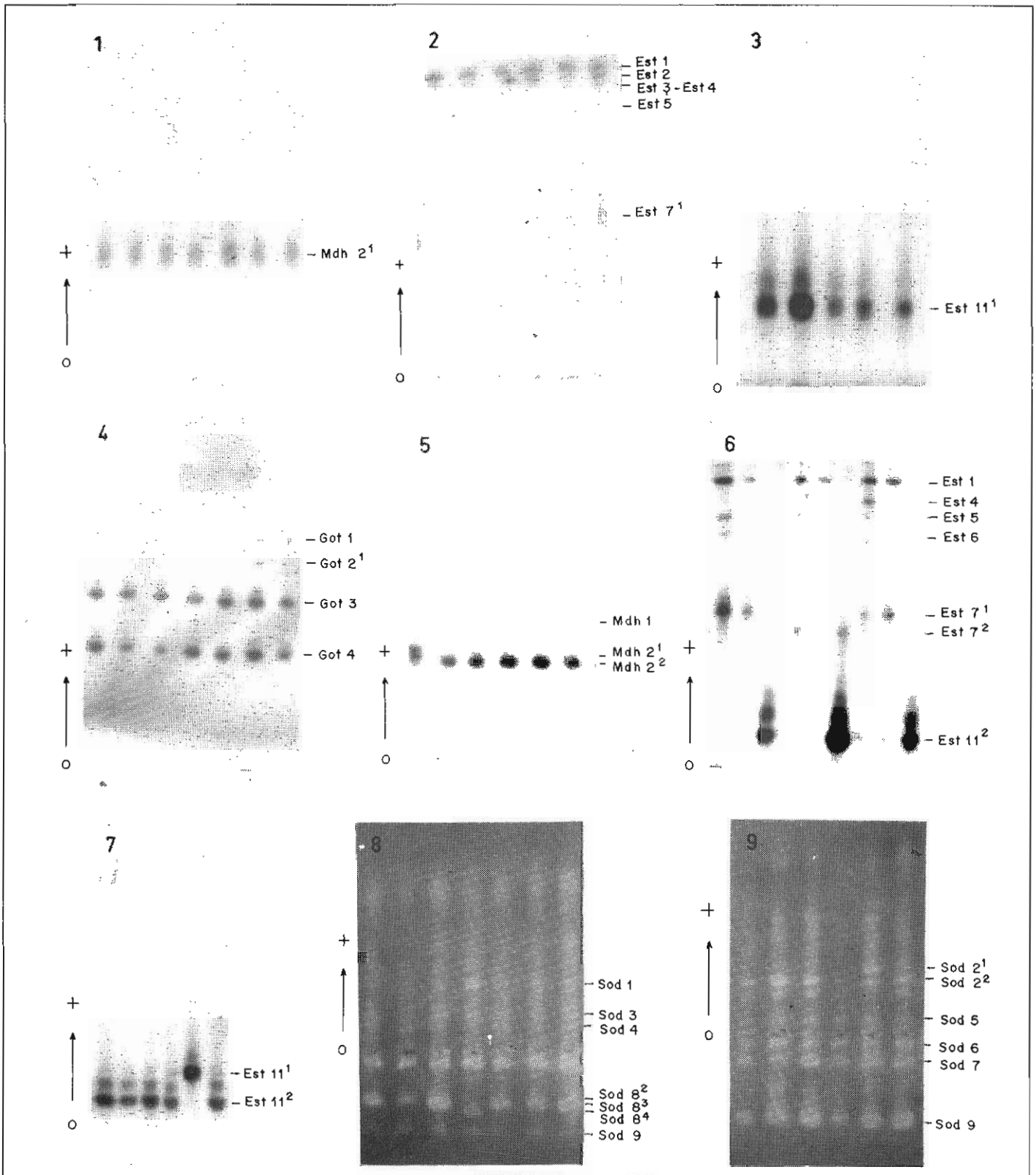


Figure 1-9 - Photographs of gels show representative isoenzymatic phenotypes of *H. euclaston*. 1. MDH: coleoptiles from seven sib seedlings from the same uniform progeny for *Mdh-2*¹. 2. EST: coleoptiles from seven sib seedlings which show uniformity for five loci, including *Est-7* which is polymorphic. 3. EST: endosperms of five seeds of the same strain, showing the least common allele of the *Est-11* locus. 4. GOT: coleoptiles from seven seedlings of different strains from the three populations showing uniformity at four loci. 5. MDH: coleoptiles from six seedlings of different strains from the three populations, the first plant displays the *Mdh-2*¹ allele and the others show the *Mdh-2*² allele. 6. EST: isoenzymatic patterns of three tissues (coleoptile, roots, and endosperm, in this order) from three seedlings of different strains showing the two alleles of the *Est-7* locus. 7. EST: endosperm from six seeds of different strains from different populations which show the two alleles of the *Est-11* locus. 8. SOD: coleoptiles from six seedlings of different strains from the three populations, showing three alleles of the *Sod-8* locus. 9. SOD: roots from six seedlings of different strains from different populations showing the two alleles of the *Sod-2* locus.

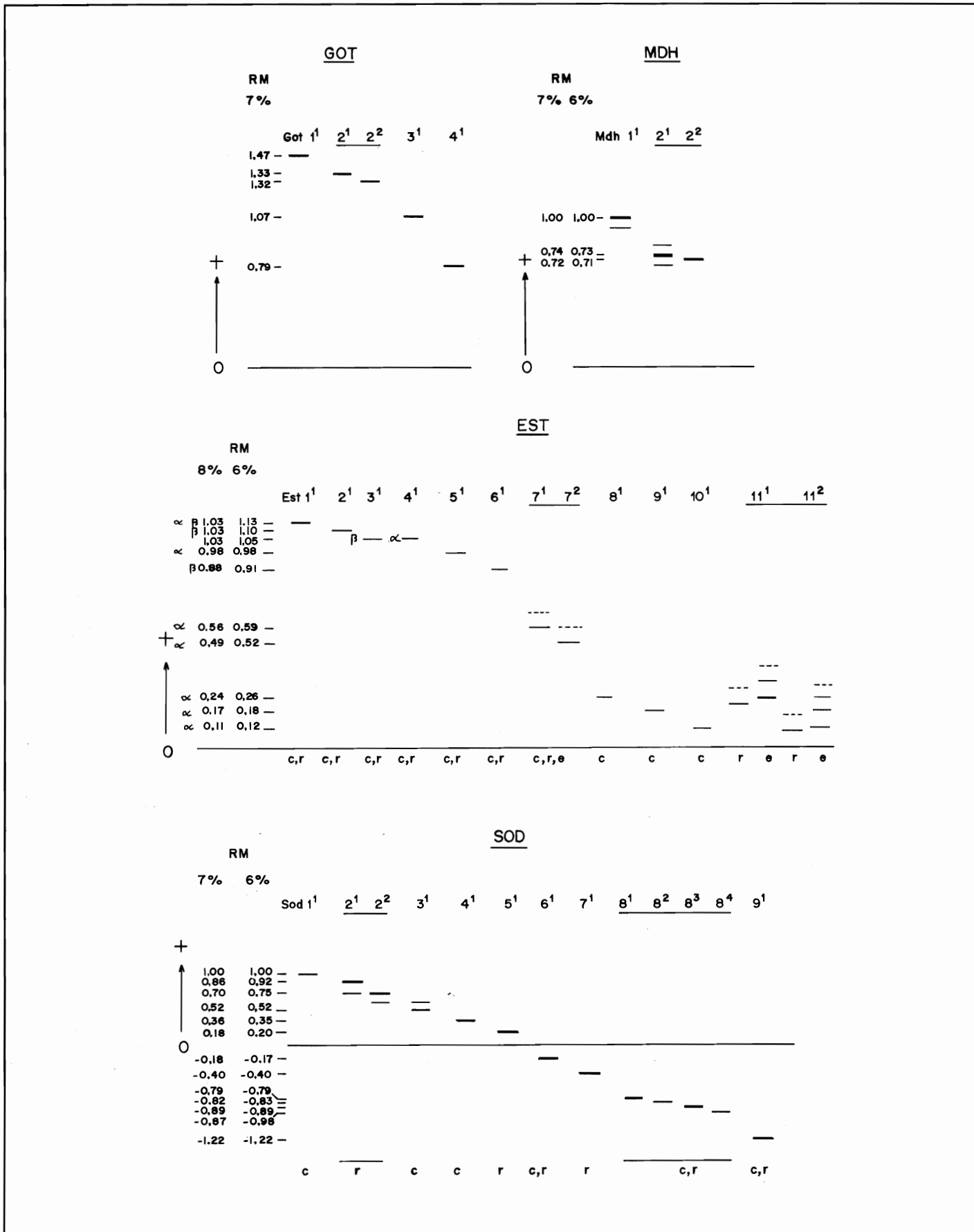


Figure 10 - Phenotypes of alleles for four enzymatic systems in *H. euclaston*. At the top of each schematic pattern the genic locus and its respective allele are indicated. On the left, the relative migration (RM) of each primary isoenzyme and the specificity of esterase activity are shown. For EST and SOD, the tissues (c = coleoptile, r = roots, and e = endosperm) where the loci were active are indicated below each loci pattern.

Table I - Allelic frequencies of five polymorphic loci of *Hordeum euclaston*. The allelic frequencies of the *Sod-8* locus aren't presented because it wasn't possible to determine accurately which allele was present in many individuals. The other 20 loci analyzed were invariant.

Alleles	Populations		
	5	6	8
<i>Got-2</i> ¹	100	95	100
<i>Got-2</i> ²	0	5	0
<i>Mdh-2</i> ¹	0	3	9
<i>Mdh-2</i> ²	100	97	91
<i>Est-7</i> ¹	48	69	100
<i>Est-7</i> ²	52	31	0
<i>Est-11</i> ¹	3	0	15
<i>Est-11</i> ²	97	100	85
<i>Sod-2</i> ¹	39	87	15
<i>Sod-2</i> ²	61	13	85

Populational differences in the allele frequencies of the polymorphic loci were few (see Table I). For the *Got-2* locus, only population 6 showed two different alleles, but the least common allele was detected in only one individual. For the *Mdh-2* locus, all the individuals in the sample from population 5 were homozygotes and were allelically identical, displaying the *Mdh-2*² allele. This allele is also the most common in the other two populations. Only one individual from population 6 and three others from population 8 had the rare *Mdh-2*¹ allele. The *Est-11*¹ allele only occurred, in low frequency, in populations 5 and 8. Just the allelic frequencies of the *Est-7* and *Sod-2* loci varied sharply among populations. All the individuals from population 8 presented the *Est-7*¹ allele, while the frequency of this allele was lower in the other two populations. For the *Sod-2* locus, the *Sod-2*² allele was more frequent in the populations 5 and 8, while in population 6, *Sod-2*¹ was the most frequent allele.

The intrapopulational genetic variability measures are given in Table II. For the percentage of polymorphic loci (P) calculations, the *Sod-8* locus, which was clearly polymorphic, was included, but for the calculations of the average number of alleles per locus (A) and the expected heterozygosity (H) it wasn't considered. The differences in the three genetic variability measures among the three populations were very low (Table II).

Table II - Intrapopulation genetic variability measures in three populations of *Hordeum euclaston*

Pop.	Average no. alleles	% of polymorphic loci	Expected heterozygosity
5	1.12	15	0.0412
6	1.16	19	0.0324
8	1.12	15	0.0272

Table III - Estimated genetic distance based on isoenzymatic loci for the three populations of *Hordeum euclaston*.

Pop.	5	6	8	Average
5	-	0.014	0.018	0.016
6		-	0.027	0.021
8			-	0.023

Genetic distances among the three populations are given in Table III. The average genetic distance among them was 0.020, which shows a great similarity.

DISCUSSION

Mode of reproduction

The analysis of progenies from single maternal plants can provide information on the amount of cross-fertilization. The result of autogamy is the establishment of pure lines. Complete or partial allogamy determines that the progeny sample shows genic segregation in a different number of loci. Although isoenzymatic patterns differed among individuals of *H. euclaston* complete genetic uniformity was encountered in all the progeny for the 26 loci. In addition, the fact that no heterozygosity was detected at any loci in none of tested plants clearly indicates self-fertilization, as did floral morphology.

In the genus *Hordeum*, there are several species in which the self-fertilization is the rule. *H. spontaneum*, the ordinarily accepted progenitor of cultivated barley, is predominantly self-pollinated and shows an overall outcrossing average of only 1.6%, with few variations which occur due to environmental differences (Brown *et al.*, 1978). *H. jubatum*, a tetraploid inbreeder, also shows low levels of outcrossing, varying from 1% to 3% (Babbal and Wain, 1977). In cultivated barley, the percentage of outcrossing is 0.57% (Weir *et al.*, 1972). *H. euclaston* then

provides another example of rare, naturally strict inbreeder.

Genic diversity

The genic diversity can be analysed in different levels: the diversity within strains, within populations, and among them.

No diversity was found within strains.

The average intrapopulational diversity estimated in *H. euclaston* was very low: the average number of alleles per locus was 1.13; the proportion of polymorphic loci 0.16; and the expected heterozygosity 0.0336. These values fall below the averages calculated for other autogamous species ($A = 1.31$, varying from 1.00 to 1.97, $P = 0.26$, from 0.00 to 0.97, and $H = 0.075$, from 0.000 to 0.290; data calculated based on Hamrick, 1979). Although only three populations of *H. euclaston* were analyzed, this result may be an overestimation of the real intrapopulational variability, since these populations occur in the center of distribution of the species, where a higher genetic variability is expected.

The analyzed loci can be divided into four groups: three highly polymorphic loci (*Est-11*, *Sod-2* and *Sod-8*); one moderately polymorphic locus (*Est-11*); two weakly polymorphic loci (*Got-2* and *Mdh-2*), and 20 invariant loci.

Though the level of intrapopulational genetic variability can differ in autogamous species, the main difference between autogamous and allogamous species resides in the frequency of heterozygotes, which is fundamentally higher in allogamous species. In *H. euclaston*, there was a total absence of heterozygotes in the three populations analyzed.

The similarity found in the three populations of *H. euclaston* two of them being separated by as much as 140 km, is unexpected. We would expect mutation, natural selection, and drift to have fixed different alleles in these isolated populations. In *H. euclaston* most of alleles occurred at similar frequencies. The average similarity among the three populations was 0.980. Even for populations which were very far from each other the similarity was the same. Only the *Est-7* and *Sod-2* loci showed greater population differentiation. This interpopulational similarity is contrary to the theoretical expectation.

Some hypotheses may be raised in an attempt to explain this high similarity among the populations of *H. euclaston*. This species may have mechanisms of gene dispersal other than through pollen-mediated gene flow, such as seed dissemination through animals, water, wind, etc. The region of the three analyzed populations doesn't seem to present topographical barriers which could prevent this interpopulational flow and these populations occur near roads, what may facilitate seed dissemination. Many highly self-pollinated species of the genera *Bromus*,

Trifolium and also *Hordeum* have evolved adaptations for animal-mediated long distance seed dispersal (Stebbins, 1974; Levin and Wilson, 1976). It is still probable that the three analyzed populations have had a common evolutionary origin and have not colonized each place for enough time since separation to incorporate different alleles. Also, the three populations occur in the same physiogeographic region, and may be under the same natural selection pressures, thus the preserved alleles would be the same.

These hypotheses are not mutually exclusive. In fact, they may have taken place together and simultaneously to promote a higher similarity among populations. Another important aspect to be considered is the possibility that autogamous species present different sources of variability mainly due to regulatory mechanisms. This situation was observed in *H. euclaston* and these results will be present elsewhere.

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RESUMO

Isoenzimas de *Hordeum euclaston* foram analisadas por eletroforese horizontal, em gel de poliacrilamida, para quatro sistemas enzimáticos. Foram analisadas plântulas, germinadas de sementes coletadas de 95 plantas, pertencentes a três diferentes populações do Sul do Brasil. O modo de reprodução foi determinado pela análise de diferentes plântulas irmãs provenientes de plantas-mãe individuais da natureza. Dos 26 locos analisados, seis são polimórficos com um total de 34 alelos. Não foi detectada variabilidade genética dentro de cada progênie. Também não foi encontrado nenhum indivíduo heterozigoto nas populações naturais analisadas. Os resultados mostram baixa variabilidade genética entre plantas de cada população. Também foi verificada baixa diferenciação interpopulacional, não confirmando a predição teórica de que diferentes populações de espécies de auto-fecundação são muito diferentes em sua constituição genética.

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