Hordein variation in Brazilian barley varieties (*Hordeum vulgare* L.) and wild barley (*H. euclaston* Steud. and *H. stenostachys* Godr.)

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Abstract

SDS-PAGE was used to analyze the hordein polypeptide patterns of Brazilian barley varieties (*Hordeum vulgare* L.) and of two native species of *Hordeum* from southern Brazil (*H. euclaston* Steud. and *H. stenostachys* Godr.). Forty different hordein polypeptide bands with molecular weights ranging from 30 to 94 kDa were found in the seeds of the three species studied. Twelve of the 14 varieties examined showed intravarietal polymorphism. The number of bands ranged from 10 to 17, depending on the variety, and from 3 to 13 among individual seeds, with a total of 26 bands in *H. vulgare*. Phenograms using each seed as an operational taxonomic unit (OTU) showed that the seeds from most varieties did not form distinct clusters. Seeds from different plants of the native species varied considerably. The molecular weights of the hordein polypeptides of the two native species were quite different from those of *H. vulgare*. There was a greater similarity between the native species than with *H. vulgare*, although *H. stenostachys* was slightly closer to the cultivated species than *H. euclaston*.

INTRODUCTION

Considering the effect of variety on malting properties in the malting and brewing industries, there is a need for a quick and accurate method of varietal identification. Morphological characteristics have been used for this purpose, although these are of limited use for assessing the levels of variability because they are frequently influenced by environmental factors. Electrophoretic analysis of isoenzymes is more reliable. During the last three decades, there has been extensive characterization of barley cultivars using isoenzyme electrophoretic patterns (Almgard and Landegren, 1974; Kahler *et al.*, 1981). However, Maris (1992) showed that for Brazilian cultivars, this method is not very efficient because of the low level of allelic variation at many loci, the high degree of genetic relatedness among different cultivars, and the high degree of polymorphism within Brazilian barley cultivars.

The storage protein fraction of barley (hordein) accounts for up to half of the total nitrogen of the mature grain (Shewry *et al.*, 1978b). Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of hordein has been used to identify barley varieties from Australia (McCausland and Wrigley, 1977), the United Kingdom (Shewry *et al.*, 1978c), the United States (Heisel *et al.*, 1986) and Yugoslavia (Radovic´ and Vapa, 1996).

Variation in native species in the genus *Hordeum* has also been documented using hordein electrophoretic patterns. Doll and Brown (1979) analyzed hordein variability in natural populations of *H. spontaneum* C. Koch, the evolutionary ancestor of *H. vulgare* L., and detected more

polymorphism than with isoenzymes (Nevo *et al.*, 1979). Two native species of *Hordeum* (*H. euclaston* Steud. and *H. stenostachys* Godr.) occur in southern Brazil but have not been studied extensively. The few reports so far have dealt with the taxonomic position of these species in the genus *Hordeum* (Bothmer and Jacobsen, 1980; Bothmer *et al.*, 1995). A few genetic studies have examined the mode of reproduction and genetic variability in natural populations of *H. euclaston* (Ferreira and Cavalli-Molina, 1994), and the karyotype and meiotic behavior, agronomic parameters in relation to the biological cycle, and the morphology and fertility of *H. stenostachys* (Santos, 1992) and *H. euclaston* (Lauxen *et al.*, 1991; Hickenbick *et al.*, 1991).

In the present study, SDS-PAGE analyses of hordein polypeptide patterns were used to: 1) identify Brazilian barley varieties, 2) analyze intravarietal and intraspecific variability, and 3) assess the genetic similarity among the barley varieties and also among cultivated and native species of *Hordeum* from southern Brazil.

MATERIAL AND METHODS

Fourteen varieties (cultivars and advanced lines) of barley were analyzed. These included 12 Brazilian barley varieties, of which nine (BR-2, MN-599, MN-656, MN-607, MN-681, MN-682, MN-668, MN-685, FM-404) were selected for malting and three (IBON-216-82, CB 8501-12, CB 8501-22) for feeding purposes, along with one Japanese cultivar (Acumai) which is used for feeding, and lastly the European cultivar Hanna which was used as a control in the electrophoresis. Seeds from the Brazilian varieties were

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supplied by the breeders who developed them. Seeds from the cultivar Hanna were obtained from CENARGEN-EMBRAPA (Centro Nacional de Pesquisas de Recursos Genéticos e Biotecnologia-Empresa Nacional de Pesquisas Agropecuárias) and those from the cultivar Acumai were supplied by EMBRAPA-Trigo. Plants of two wild species, *H. euclaston* and *H. stenostachys,* were also analyzed. The seeds of *H. euclaston* were collected from three different natural populations and those of *H. stenostachys* from five populations, all from the State of Rio Grande do Sul (southern Brazil). The populations of wild barley were the same as those used in previous isoenzyme studies (Brammer, 1993; Ferreira and Cavalli-Molina, 1994).

At least 10 seeds from each variety of cultivated barley were analyzed individually. For the wild species, hordein analysis was carried out on two individual seeds from each plant and on a pool of five seeds from the same plant (*H. euclaston* = 30 plants and *H. stenostachys* = 26 plants). The analysis of individual seeds of the native species was made to determine whether variation existed between seeds from a single plant. Seeds were pooled to verify if all the bands obtained in the pool were also detected in the individual seeds. This was required due to the small size of the seeds from the wild species.

Hordein was extracted from each dry seed in buffered alcohol, and then reduced and alkylated (Doll and Andersen, 1981). SDS-PAGE was carried out in vertical gels in a discontinuous system using a Tris borate buffer (0.125 M Tris, 0.0638 M boric acid) (Shewry *et al.*, 1978a). The gels were run at a constant current of 20 mA until the tracking dye had moved 12 cm into the separating gel, after which

they were fixed in methanol and acetic acid, washed and then stained with trichloroacetic acid, methanol and Coomassie brilliant blue R-250 (Doll and Andersen, 1981). Apparent molecular weights were determined by comparison with the marker proteins phosphorylase b (MW 94,000), albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), trypsin inhibitor (20,100), and α-lactalbumin (14,400) (Amersham Pharmacia Biotech). The cultivar Hanna was run as a control in all gels.

The intravarietal, intraspecific and interspecific degrees of similarity were evaluated using the Jaccard similarity index (S_J) (Jaccard, 1908). To determine the intra- and intervarietal relationships, phenograms were constructed using each seed as an operational taxonomic unit (OTU). The Jaccard indexes and Manhattan distances were obtained from scoring (presence or absence) of the hordein polypeptide bands with and without data on band intensity. These data were used to produce clusters by the UPGMA (unweighted pair-group method using arithmetic averages) method. Phenograms were also constructed using each species as an OTU. The species were compared for the frequency of their hordein polypeptide bands using the Manhattan distance. The clusters were arranged by the UPGMA and neighbor-joining methods.

RESULTS

Forty different hordein polypeptide bands were found in the seeds from the three species. The molecular weights of the polypeptides ranged from 30 to 94 kDa. The extent of intravarietal variation was assessed using individual
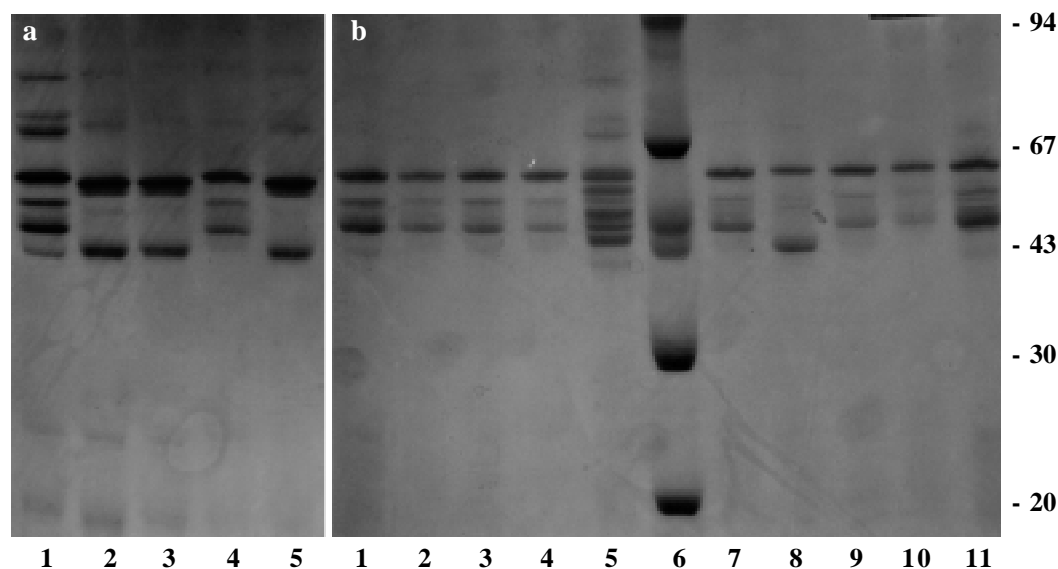

Figure 1 - **a.** Hordein polypeptide patterns of five seeds from the cultivar MN-656. **b.** Hordein polypeptide patterns from individual seeds of barley varieties: MN-599 (lanes 1 and 2), MN-682 (3 and 4), MN-681 (7), MN-668 (8), MN-656 (9), A-05 (10), BR-2 (11). Lane 5 contains the control (cultivar Hanna), and lane 6, the molecular weight markers, with the molecular weights (in kDa) indicated on the right. Cultivar A-05, also run in this gel, was not included in this study.

seeds to detect polymorphism (Figure 1a). The varieties were compared based on their hordein polypeptide profiles using the Jaccard similarity index (Table I). Twelve of the 14 varieties had intravarietal polymorphism, with the mean indices of similarity varying from 0.34 to 1.00. The ranges of intravarietal variation were very large.

Seeds of different varieties had identical or different hordein polypeptide patterns (Figure 1b). Twenty-six hordein polypeptides were detected in cultivated barley with 10-17 bands in each variety (Table II), although the number of hordein polypeptide bands ranged from 3-13 in individual seeds. No band was present in all varieties studied, although some (bands 1, 5, 11, 12, 16, 21, and 22) were present in most of them to varying degrees. Some bands occurred in only one or few varieties. Band 27 was

present only in variety FM-404 where its frequency of 100% was a distinctive trait. Band 23 was unique to cultivar MN-656 (23% frequency) and band 29 to MN-668 (27%). However, the low frequencies of these two bands meant they were not very useful as distinctive traits for these varieties.

The overall mean similarity index for *Hordeum vulgare*, calculated by the Jaccard similarity index, was 35.6%. In the phenograms constructed using each seed scored as an OTU, most of the Brazilian barley varieties did not form distinct clusters. Rather, the components from each variety were distributed among different clusters. As can be seen in the phenogram obtained using the Manhattan distances and the presence and absence of bands (Figure 2), five varieties showed greater internal homogene-

Table I - Median indices of Jaccard similarity (S_J) among seeds from different barley varieties, based on their hordein polypeptide patterns.

	Barley varieties													
	MN-656	MN-682	MN-681	MN-599	BR-2	MN-668	Acu	MN-607	Han	Ibon	MN-685	CB22	CB12	FM-404
N	13	10	11	10	15	11	12	10	14	10	10	10	10	10
$\bar{x}$	0.34	0.55	0.57	0.60	0.62	0.73	0.79	0.80	0.82	0.88	0.92	0.94	1.00	1.00
Ranges	0.00-1.00	0.30-1.00	0.27-1.00	0.33-1.00	0.27-1.00	0.40-1.00	0.50-1.00	0.62-1.00	0.62-1.00	0.55-1.00	0.85-1.00	0.83-1.00	-	-

N = Number of seeds analyzed. Acu = Acumai; Han = Hanna; Ibon = IBON-216-82; CB22 = CB 8501-22; CB12 = CB 8501-12.

Table II - Percent of hordein polypeptide bands in the barley (*Hordeum vulgare*) varieties.

Band	MN-656	MN-682	MN-681	MN-599	BR-2	MN-668	Acu	MN-607	Han	Ibon	MN-685	CB22	CB12	FM-404
1	38	20	27	20	0	27	0	70	78	90	100	100	100	100
5	8	10	0	20	0	18	91	70	71	90	100	90	100	100
6	8	0	9	0	0	0	75	70	0	0	0	0	0	100
7	31	20	0	20	13	27	0	0	78	90	100	100	100	0
8	0	0	0	0	0	0	75	50	0	0	0	0	0	100
9	8	0	27	20	0	0	0	0	64	70	100	90	100	0
10	8	0	9	0	33	0	8	0	0	0	0	0	0	0
11	54	100	100	100	100	100	100	100	100	0	0	100	100	0
12	92	70	64	100	40	0	100	100	100	100	100	100	100	100
13	46	0	0	0	0	0	0	0	0	0	100	0	0	0
14	0	30	27	30	0	0	100	100	100	0	0	0	0	100
15	0	0	0	0	60	0	0	0	0	0	100	0	0	0
16	54	100	100	100	100	100	0	30	100	100	0	100	100	0
17	23	60	73	50	60	100	91	0	0	0	100	100	100	0
18	0	0	18	0	0	0	0	100	100	0	0	0	0	100
19	54	20	36	20	66	0	100	90	100	100	100	100	100	0
20	0	0	0	0	0	9	0	0	0	0	0	0	0	100
21	46	100	100	100	100	0	42	100	100	0	100	100	100	100
22	46	100	100	100	100	0	91	100	36	100	100	100	100	0
23	23	0	0	0	0	0	0	0	0	0	0	0	0	0
24	54	0	27	0	0	0	0	100	100	90	0	0	0	0
25	0	30	45	50	0	0	0	0	0	20	0	0	0	0
26	23	40	9	40	0	100	0	0	0	0	100	100	100	0
27	0	0	0	0	0	0	0	0	0	0	0	0	0	100
28	0	0	9	0	6	64	8	100	93	100	50	0	0	0
29	0	0	0	0	0	27	0	0	0	0	0	0	0	0

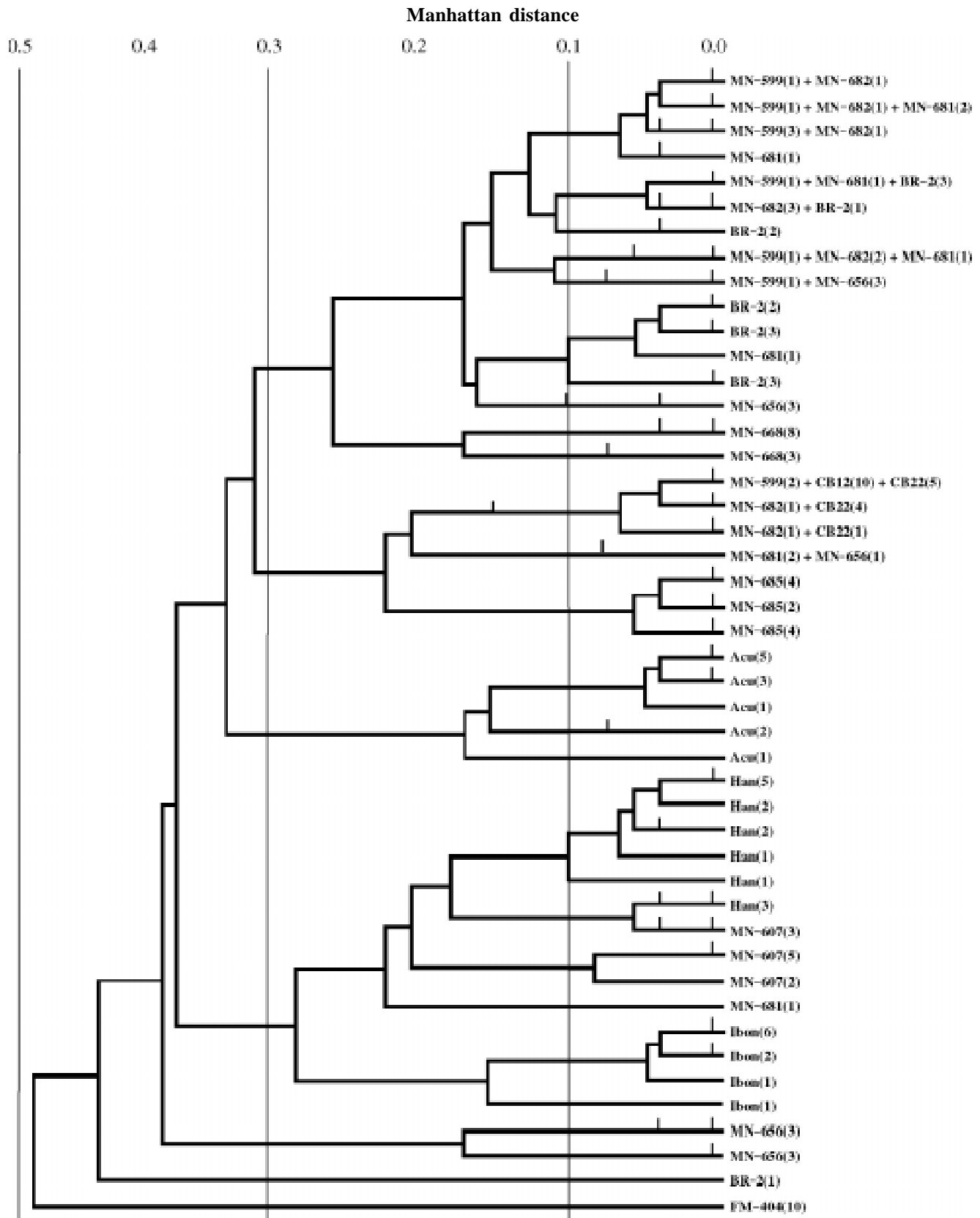For abbreviations see legend to Table I.

Manhattan distance

Figure 2 - Phenogram derived from the Manhattan distances calculated from the presence and absence of hordein polypeptide bands, and clustered by the UPGMA method. The phenogram is presented in a reduced form where OTUs which formed a well-defined cluster were joined in a single horizontal line. The number of representatives of each variety in each cluster is shown in parentheses. The vertical lines intersecting the horizontal lines indicate the highest and lowest levels of clustering for the representatives of each grouping. For abbreviations see legend to Table I.

ity and were more distinct from the other varieties, resulting in well-defined clusters: FM-404, MN-668 and MN-685 (malting varieties), Acumai (Japanese feed cultivar) and IBON-216-82 (Brazilian feed variety). MN-656 had the highest intracultivar variability as evidenced by its position in different locations throughout the phenogram. Variety FM-404 was the most distant from all the others.

The analyses of individual seeds and the pool of five seeds from a single plant of the two native species of *Hordeum* showed no banding differences between the individual seeds and the pool. This indicates that all bands present in each seed were indeed detected in spite of the small size of the kernels. Also, no genetic variation was detected between seeds from a single plant. In contrast, there was great variation in the hordein electrophoretic patterns when seeds from different plants were analyzed. The patterns varied in band number, position and relative intensity. Representative patterns of these two native species are shown in Figure 3a, b. No band had a 100% frequency in these species, although some bands did have a high frequency (Figure 4).

The S_J index among seeds from different plants of *Hordeum euclaston* ranged from 0.00 to 0.86, with an average of 0.25, while for *H. stenostachys* the indices ranged from 0.00 to 1.00, with an average of 0.26.

There were great differences in the molecular weights of hordein polypeptides among the two native species and the cultivated one (Figure 3c). Most hordein polypeptides from the cultivated species had molecular weights of 43 to 67 kDa, while in the native species they varied mainly between 30 and 43 kDa.

Analysis of the composite patterns (i.e., all of the bands of hordein polypeptides detected in each species) of *Hordeum vulgare*, *H. euclaston*, and *H. stenostachys* revealed 40 bands in the three species (Figure 4). Ten bands were detected in all species, although these differed in frequency among the species. Twenty-six were found in the cultivated species and 33 in the native ones. Seven bands were exclusive to *H. vulgare* (bands 8 to 11, 15, 20, and 23) whereas 14 were exclusive to the native species. Of the latter bands, three were unique to *H. stenostachys* (4, 31, and 35), and another three to *H. euclaston* (38, 39, and 40).

The native species, *H. euclaston* and *H. stenostachys*, had a mean S_J index of 0.21, much higher than the similarity found when these species were compared with *H. vulgare* (0.075 and 0.070, respectively). The phenogram, in which each species was an OTU and clustered by UPGMA, showed that the two native species meet at an index value of 0.10 and subsequently join the varieties belonging to *H. vulgare*, at an index value of 0.28 (Figure 5a). The phenogram obtained by the neighbor-joining method (Figure 5b) showed that the branch leading to *H. stenostachys* was shorter than that of *H. euclaston*, indicating that *H. stenostachys* was nearer to the cultivated species than *H. euclaston*.

DISCUSSION

Intraspecific polymorphism in cultivated barley

Polymorphism in seed storage proteins has been identified in several species, cultivated or wild, including pea (Thompson and Schroeder, 1978), soybean (Mori *et al.*, 1981), maize (Wilson, 1985), and wheat (Vallega and Waines, 1987). Extensive polymorphism in hordein composition has
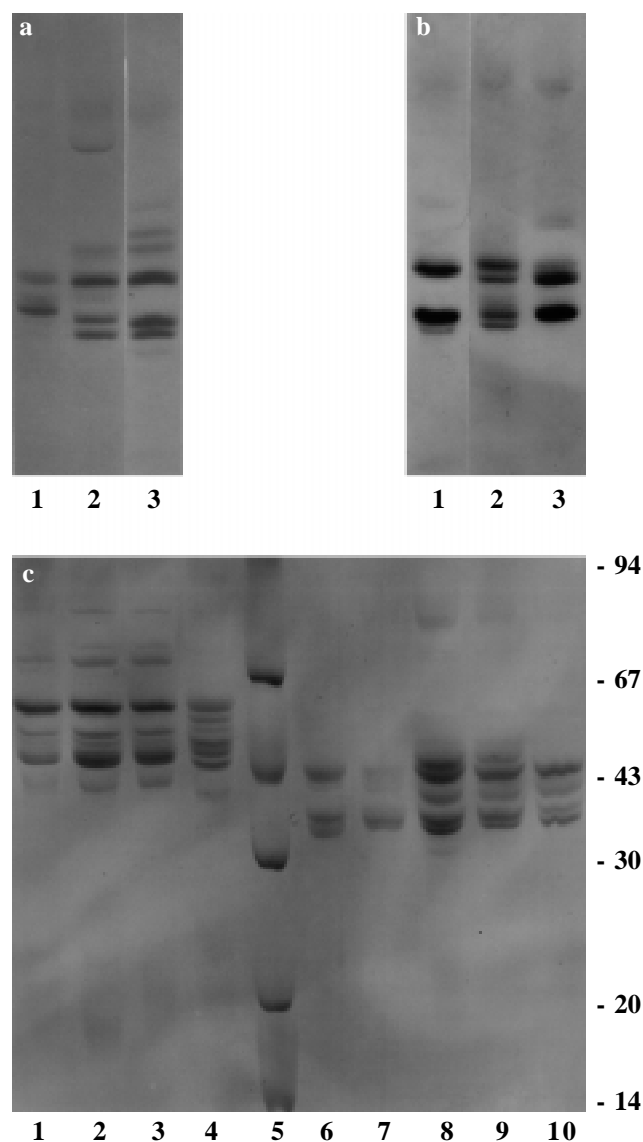

Figure 3 - **a.** Hordein polypeptide patterns of individual seeds from three plants of *Hordeum euclaston*. **b.** Hordein polypeptide patterns of individual seeds from three plants of *Hordeum stenostachys*. **c.** Hordein polypeptide patterns of seeds from the three species of *Hordeum*: *H. vulgare* (lanes 1-4), with the cultivar Hanna (control) in lane 4, *H. stenostachys* (lanes 6 and 7), *H. euclaston* (lanes 8-10), and molecular weight markers (lane 5), with the molecular weights (in kDa) indicated on the right of the gel.
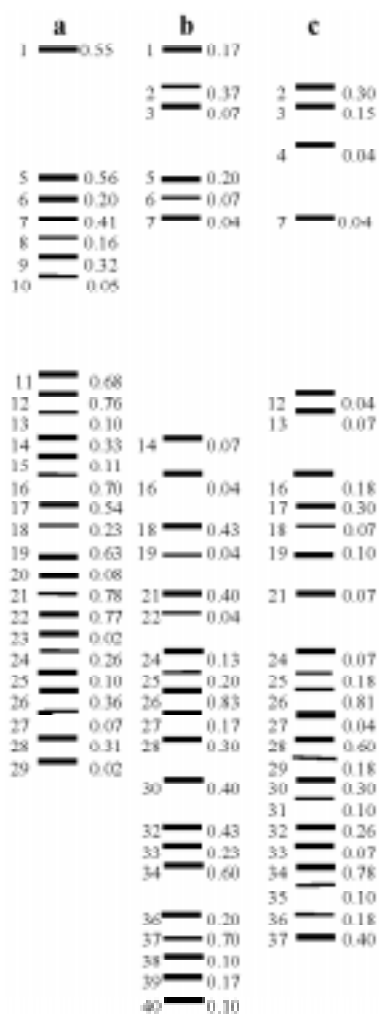
Figure 4 - Diagrammatic composite representation summarizing all the bands detected in each of the three species of *Hordeum* studied. (**a**) *H. vulgare*, (**b**) *H. euclaston*, (**c**) *H. stenostachys*. The frequency of each band is indicated.

been reported in barley (McCausland and Wrigley, 1977; Shewry *et al.*, 1978c; Marchylo, 1987), and the present study confirms these observations. The number of patterns reported here should be considered a minimum estimate since minor or doubtful differences were not recorded.

A comparison of seed protein and isoenzyme polymorphism in *Phaseolus vulgaris* L., *Triticum turgidum* L. var. *dicoccoides* and *Hordeum spontaneum* C. Koch revealed that the high levels of seed storage protein diversity contrast with the relatively lower level of isoenzyme diversity (Gepts, 1990). Maris (1992) analyzed the genetic similarity among different cultivars used for malting in Brazil and noted a great similarity among them, with most of the loci showing common alleles in all cultivars, with variation only in the allelic frequencies. This similarity reflects, at least in part, the common origin of many of these cultivars. The levels of diversity in isoenzymes

and seed storage proteins are not strictly comparable, mainly because seed storage proteins, more specifically the hordeins, are controlled by multigene families that have arisen by duplication and divergence from an ancestral gene through nucleotide substitutions, insertions and deletions. These last two mutation types have produced a variable number of repetitive sequences inside the corresponding genes (Gepts, 1990; Shewry, 1995). These multigene families provide additional opportunities for polymorphism, mainly because seed storage proteins play a less active role compared to isoenzymes. Nevo *et al.* (1983) suggested that the high diversity of hordeins could be related to the fact that hordeins are highly tolerant to mutations and are selectively neutral. In addition, although locus variation is selectively neutral, the variability in linked loci is a target for selection. The linkage of the hordein loci to the *Mla* locus, which specifies resistance to different races of powdery mildew (*Erysephe graminis* DC.; Oram *et al.*, 1975), is a possible cause of variability. Because of this linkage, the introduction of exotic sources of resistance can lead to rapid and widespread changes in the hordein polypeptide patterns found in commercial varieties (Shewry *et al.*, 1979).

Intravarietal polymorphism

The results of the present study revealed extensive intravarietal polymorphism for Brazilian varieties of barley. Analyses of individual seeds from European, Canadian, and North American cultivars, among others, indicated that some of them contain seeds with different protein electrophoretic patterns. Radović and Vapa (1996) reported intracultivar hordein polymorphism in two out of 33 Yugoslav cultivars analyzed. Heisel *et al.* (1986) reported 34 different hordein patterns for 15 barley cultivars commonly grown in the United States. Over half of the latter cultivars did not have unique patterns. These authors also found that members of certain pattern groups may be differentiated by their seed characteristics. Shewry *et al.* (1980) found only one variety with two types of seeds having different hordein patterns out of 28 varieties examined. However, Shewry *et al.* (1978c) had already reported intravarietal hordein polymorphism in five out of 88 varieties studied and that this heterogeneity existed in varieties which exhibited identical morphological characteristics for their seeds and for their plants. Marchylo (1987) found different biotypes in 12 out of 100 cultivars. Twenty-eight patterns (2-4/cultivar) were observed for these 12 cultivars.

Thus, a certain degree of intracultivar heterogeneity is common among several self-pollinated plants. But the high intravarietal polymorphism found in this study is surprising since cultivated barley is highly inbred, with very low heterozygosity, and seed breeders have devoted much effort and expense to develop homogeneous varieties with particular characteristics that include disease or drought resistance, increased yield, and improved brewing and malt-
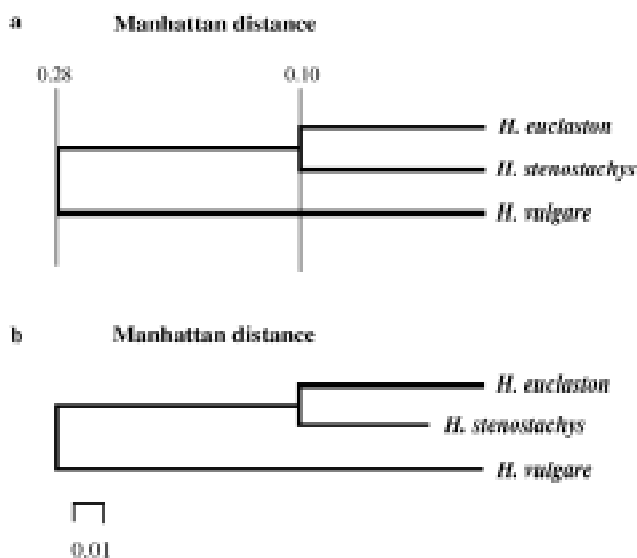
Figure 5 - Phenogram of the three species of *Hordeum*, based on Manhattan distances calculated from the band frequencies of each species. Clustering was by the UPGMA (**a**) and neighbor-joining (**b**) method.

ing properties. This variability observed in Brazilian barley varieties may be an inherent consequence of the selection process. Usually the Brazilian breeders finish the selection of new cultivars in the F_5 or F_6 generation. Thus, part of the polymorphism may be explained by residual heterozygosity. Although Maris (1992) has not found heterozygosity for isoenzyme loci in Brazilian barley varieties, it is possible to consider that homozygosity of these loci is reached in the following generations, and cultivars are a mixture of homozygotes for different alleles in many loci. Another explanation for the intravarietal variability would be the weak selection imposed by breeders on the hordein loci. Crossing or mixing after selection could be another cause of this variability. However, the frequency of crossing in barley is very low and, as already referred, the plants of Brazilian varieties are homozygous for basically all loci (Maris, 1992). Mixing in the material studied is unlikely because the seeds were obtained directly from the breeders' stocks which are routinely checked based on morphological uniformity of plants and seeds.

Intravarietal variability in Brazilian barley cultivars has already been described using isoenzyme analyses (Maris, 1992), but not to the high extent found in hordeins. Doll and Brown (1979) also detected more polymorphism in hordeins than in isoenzymes in barley. Indeed, according to these authors, the hordeins may be the most polymorphic system in plants. Although some researchers such as Almgard and Landegren (1974) criticize the lack of genotype purity of many cultivars, weak artificial selection may be beneficial in that it does not drastically reduce genetic variability.

Varietal characterization

Although considerable intraspecific variability was found in the present study, most of the varieties showed no distinct patterns which would allow their characterization. There were a few specific bands in each variety and most of them, although exclusive, appeared at such a low frequency that varietal characterization was not possible. Although researchers have found that the hordein patterns allow the identification of most cultivars (Shewry *et al.*, 1978a; Heisel *et al.*, 1986), others, including our group, have not been so successful. Marchylo and Laberge (1981) were unable to distinguish some Canadian cultivars nor were Faulks *et al.* (1981) able to distinguish European cultivars using hordein SDS-PAGE.

As noted by Wrigley *et al.* (1982), information on the polymorphic nature of a cultivar is important for identification. Authentic pure samples of cultivars are necessary to ensure representative cultivars and not inadvertent mixtures. Since pure seeds maintained by plant breeders were used in this study, the polymorphic protein patterns were considered to be representative of the varieties examined.

Variability in native species

Extensive polymorphism in hordein components has been reported for *Hordeum spontaneum* (Doll and Brown, 1979; Nevo *et al.*, 1983), the wild progenitor of cultivated barley, and a very large number of hordein patterns in *H. euclaston* and *H. stenostachys* was found in the present study. There were bands common to the three species studied but the native species had bands with similar molecular weights which were considerably different from those of cultivated barley.

The genus *Hordeum* has been subdivided by different researchers (Bothmer and Jacobsen, 1980; Bothmer *et al.*, 1995). Based on meiotic behavior, Jacobsen and Bothmer (1992) subdivided the genus into four groups, with *H. vulgare* placed in group 1 with *H. bulbosum* L. (genome I), and *H. euclaston* and *H. stenostachys* in group 4, together with all diploid species from South America, North America and Asia (genome H). A very low pairing index was observed between the species from groups 1 and 4, which agrees with the low similarity indices found in the present study between *H. vulgare* and the two native species. The great dissimilarity between the Brazilian wild barleys and *H. vulgare* makes it nearly impossible to cross *H. vulgare* with these species in order to introgress desirable genes. Crosses between *H. stenostachys* and *H. vulgare* and between *H. euclaston* and *H. vulgare* do not result in adult plants, although seed sets have been obtained when cultivated barley was used as the male parent. The endosperm was generally watery or slimy and the embryos were small, malformed or lacking. In crosses with *H. stenostachys*, germination of some embryos took place but the seedlings died at an early stage (Bothmer *et al.*, 1983). Thus, it seems unlikely that the transfer of genes

from these species into *H. vulgare* is possible through conventional crosses unless embryo culture techniques are successfully used in the rescue of interspecific plants. Alternatively, the transfer of genes may be achieved through genetic transformation using molecular biology techniques.

ACKNOWLEDGMENTS

RESUMO

No presente trabalho foi utilizada a técnica de eletroforese vertical SDS-PAGE com o objetivo de analisar os padrões de polipeptídeos de hordeína de variedades brasileiras de cevada (*Hordeum vulgare* L.) e de duas espécies nativas de *Hordeum* do Sul do Brasil (*H. euclaston* Steud. e *H. stenostachys* Godr.). A análise de representantes das três espécies permitiu a identificação de 40 bandas de polipeptídeos de hordeína com peso molecular variando de 30.000 a 94.000 Da. Das 14 variedades analisadas, 12 mostraram polimorfismo intravarietal. O número de bandas variou de 10 a 17 nas diferentes variedades e de 3 a 13 considerando sementes individuais, com um total de 26 bandas em *H. vulgare*. Fenogramas usando cada semente como OTU mostraram que as sementes da maioria das variedades não formaram blocos distintos, espalhando-se em diferentes agrupamentos, junto com componentes de diferentes cultivares. As sementes de diferentes plantas das duas espécies nativas também apresentaram grande variação. Os pesos moleculares dos polipeptídeos de hordeína das espécies nativas foram bastante diferentes daqueles de *H. vulgare*. Os resultados indicaram que há uma maior similaridade entre as duas espécies nativas e maior distanciamento destas com a cevada cultivada, embora *H. stenostachys* seja um pouco mais relacionada com *H. vulgare* que *H. euclaston*.

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