Hordein polypeptide patterns in relation to malting quality in Brazilian barley varieties[1]

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Abstract – Since there is evidence that malting quality is related to the storage protein (hordein) fraction, in the present work the hordein polypeptide patterns from 13 barley (*Hordeum vulgare* L.) varieties of different malting quality were analysed in order to explore the feasibility of using hordein electrophoresis to assist in the selection of malting barleys. The formation of clusters separating the varieties with higher malting quality from the others with lower quality suggests that there is a relationship between the general hordein polypeptide pattern and malting quality in the varieties analysed. By the Sperman's correlation test three hordein bands correlated negatively with malting quality in the germplasm studied.

Index terms: *Hordeum vulgare*, storage proteins, electrophoresis.

Padrões de polipeptídios de hordeína em relação à qualidade de malteação em variedades brasileiras de cevada

Resumo – No presente trabalho foi analisado o padrão de polipeptídios de hordeína de 13 variedades de cevada (*Hordeum vulgare* L.) que apresentam diferentes qualidades de malteação, com o objetivo de verificar a viabilidade de se usar eletroforese de hordeínas na seleção de cevadas usadas para malteação. A formação de agrupamentos separando as variedades que apresentam altos níveis de malteação daquelas com baixa qualidade sugere haver uma clara associação entre a qualidade de malteação e os padrões de hordeína, pelo menos com relação às variedades analisadas. Pelo teste de correlação de Sperman, três bandas apresentaram correlação negativa com a qualidade de malteação.

Termos para indexação: *Hordeum vulgare*, proteínas de reserva, eletroforese.

Introduction

Barley is an important crop in southern Brazil where its production is almost totally used in the brewing industry and for this purpose the malting quality must be continuously improved. Characteristics of importance for malting quality, which can differ considerably among barley cultivars, include grain size, grain protein concentration, amount of extract obtained from the malt, diastatic power and seed nitrogen (Morgan et al., 1981; Henry, 1990; Weston et al., 1993; Eagles et al., 1995).

Another factor that has been proposed as affecting the malting quality is the storage protein quality. The most abundant proteins in the cereal endosperm are the prolamins (Shewry, 1995). In barley they are known as hordeins and comprise about 40% of all the protein found in the grain (Shewry et al., 1977). Hordein was first described by Osborne (1895) as the barley seed protein soluble in aqueous alcohol. Improved methods for hordein extraction have been developed including the use of reducing agents (Shewry et al., 1978a), the modification of solvent constituents (Shewry et al., 1980b) and the addition of a buffer in the extraction medium (Doll & Andersen, 1981).

Barley is highly polymorphic regarding the hordein polypeptide composition as revealed by sodium dodecyl sulphate (SDS) electrophoretic analyses (Doll & Brown, 1979). As the migration pattern is a varietal characteristic and independent of environmental influences (Shewry et al., 1978b), electro-

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phoresis is particularly attractive as a screening test to differentiate cultivars (Shewry et al., 1980a; Marchylo, 1987) and to determine malting quality (Baxter & Wainwright, 1979; Shewry et al., 1980a). Hordein has also been investigated through different approaches including RFLP analysis (Shewry et al., 1985; Molnar & McKay, 1991), DNA sequencing (Brandt et al., 1985; Entwistle, 1988; Chernyshev et al., 1989; Vicente-Carbajosa et al., 1992; Kanazin et al., 1993), analysis of the regulatory control of hordein genes (Forde et al., 1985), and determination of the mRNA encoded by each locus (Kreis et al., 1983).

The influence of hordein polypeptides on malting quality has already been investigated. Baxter & Wainwright (1979), after studying the B fraction of hordein proteins from 16 varieties, suggested that potential malting quality is related to variations in this fraction. Shewry et al. (1980a) studied 28 barley varieties from United States which could be divided into 11 groups on the basis of hordein band patterns but the authors did not find a clear-cut relationship between B-polypeptides and malting quality. Riggs et al. (1983), in a study of 84 varieties, identified 13 groups based on the band patterns of the B-hordeins, but were unable to demonstrate that varieties known to have good potential malting quality exhibited specific or even related band patterns, although two groups contained only poor malting types. Smith & Simpson (1983) examined 35 barley varieties considering the occurrence of each one of 40 polypeptide bands detected and did not find a relationship between the different bands with malting quality.

The strategies used in these previous studies consisted in separating the varieties based on the number (and intensity in some cases) of the hordein bands (or only B-hordein bands) and to verify a possible association between presence or absence (and more or less intense staining) of these bands in varieties of good or poor malting quality. Considering the importance of identifying factors that influence malting quality and the apparent disagreement between the results obtained using these approaches, the objective of this study was to compare the total hordein pattern from barley varieties of different malting quality, in order to look for relationships between malting quality and band patterns and to explore the feasibility of using hordein electrophoresis to assist in the selection of cultivars for malting.

Material and Methods

Seeds of nine Brazilian malting varieties and four feeding varieties were analysed for the hordein electrophoretic patterns (Table 1). These varieties were selected because they represent a wide range of malting quality. Cultivar Hanna was used as an electrophoretic standard. The possibility of variability in each variety was investigated analysing at least ten seeds from each one.

The malting quality score of each variety was determined and supplied by the quality control laboratory of Companhia Cervejaria Brahma-Filial Maltaria Navegantes, Rio Grande do Sul, Brazil. The score was based on 14 malt characteristics (fine milling, extract, diastatic power, total proteins, and viscosity are the most important ones) and 10 plant characteristics (germinative power, total proteins in dry material, kernel size, among others). Seeds of the Brazilian varieties were obtained directly from breeder stocks, which are routinely subjected to accurate analysis verifying the morphological uniformity of their plants and seeds.

Hordein was extracted from single dry seeds in buffered alcohol, reduced and alkylated as described by Doll & Andersen (1981). Electrophoresis was carried out in vertical sodium dodecyl sulphate polyacrylamide gel (SDS-PAGE) in a discontinuous system with Tris borate buffer (0.125 M Tris, 0.0638 M boric acid) as described by Shewry et al. (1978b). Electrophoresis was performed at a constant current of 20 mA until the tracking dye moved 12 cm into the separating gel. Gels were fixed in methanol and acetic acid, washed and then stained with trichloroacetic acid, methanol and Coomansie brilliant blue R-250, as described by Doll & Andersen (1981). Molecular weights were estimated by comparison with the standards phosphorylase b (MW 94,000), albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), trypsin inhibitor (20,100), and α-lactalbumin (14,400), supplied by Amersham Pharmacia Biotech. Cultivar Hanna was included as a control in all gels.

Phenograms using each variety as an OTU (operational taxonomic unit) were constructed to verify the relationship between the malting quality scores and whole hordein patterns. These phenograms were based on Manhattan distance (Sneath & Sokal, 1973) calculated from frequencies of hordein bands of each variety, and clusterings by Neighbor-joining method using TREECON for Windows program (Peer & Wachter, 1994). The existence or not of an association between single hordein polypeptides and the

Table 1. Characteristics of the barley varieties used in this study.

Variety	Origin[1]	End use[2]	Malting quality scores	Pedigree
Acumaí	1	N	0	-
Ibon 216-82	2	N	0	WI 2198/EMIR-AGYPTX NACTA x CMB 77-1036
CB 8501-12	2	N	0	Ibon 186-82 (Bal 16-Manker x Choya/11012.2-5106) x CB 75137 (C2302 (Argentina x (WS 5671 x WS 5673/F7)) x C1742 (=Engledon))
CB 8501-22	2	N	0	Ibon 186-82 (Bal 16-Manker x Choya/11012.2-5106) x CB 75137 (C2302 (Argentina x (WS 5671 x WS 5673/F7)) x C1742 (=Engledon))
MN-607	3	M	1	FM-424 (((Quinn x Heda) x WS-5746) x FM-462 (Alpha x Pirolini) x Dunajsky) x Mansholtz)
MN-685	3	M	2	MN-581 (FM-434 x FM-460) x MN-578 ((Cebada capa x Volla) x (Bolívia x FM-404))
FM-404	3	M	3	Seleção da Wisa WB
BR-2	4	M	4	Nobert x FM-424 ((Quinn x Heda) x WS-5746)
MN-682	3	M	5	MN-599 x MN-610 (Duvekot x FM-434(Quinn x Heda) x Wisa-Wb)
MN-681	3	M	5	Quilmes pampa x (Ant. A-05 x FM-434)
MN-656	3	M	6	FM-434 x ((SG-4279 x FM-404) x (Ub Bacco x Union))
MN-668	3	M	6	Delisa x ((SG-4279 x FM-404) x (Ub Bacco x Union))
MN-599	3	M	7	(Ariana x Volla) x FM-462 (((Alpha x Pirolini) x Dunajsky) x Mansholtz)
Hanna	5	-	-	-

[1]1: Japan, supplied by Embrapa-Centro Nacional de Pesquisa de Trigo, RS, Brazil; 2: Brazil, developed and supplied by Estação Experimental de Capão Bonito - IAC, Capão Bonito, SP, Brazil; 3: Brazil, developed and supplied by Companhia Cervejaria Brahma-Filial Maltaria Navegantes, RS, Brazil; 4: Brazil, developed and supplied by Embrapa-Centro Nacional de Pesquisa de Trigo, RS, Brazil; 5: Europe, supplied by Embrapa-Centro Nacional de Pesquisa de Recursos Genéticos e Biotecnologia, Brasília, Brazil. [2] N: nutrition use; M: malting use.

malting quality scores was estimated by the Sperman's correlation test (Zar, 1996).

Results and Discussion

SDS-PAGE separation of hordein polypeptides from individual endosperms of the varieties analysed are shown in Figure 1. As within-variety variability was found, a compound pattern formed by joining all the bands detected in each variety was constructed (Figure 2). The frequency of each band of hordein polypeptide in each variety is shown in the Table 2.

Twenty-six different hordein polypeptide bands were found in the varieties. A range of 10 to 17 bands was detected for each variety individually. The number of bands in individual seeds ranged from 3 to 13. These numbers are similar to those obtained by Marchylo & Laberge (1981), who found a maximum of 15 bands in each Canadian-grown barley cultivar analysed individually.

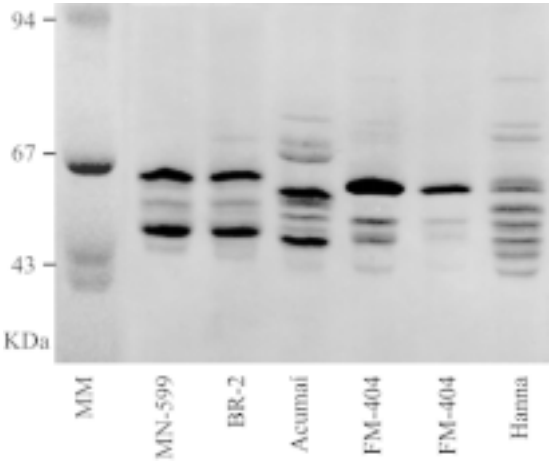

Figure 1. Hordein polypeptide patterns obtained by SDS-electrophoresis from single seeds of barley varieties. MM: molecular weight markers, with the molecular weights (kDa) indicated on the left of the gel; Hanna (standard).

Hordein polypeptides have been separated by SDS-PAGE in three fractions which were denominated A, B and C (K∅ie et al., 1976). Different analyses have indicated that C-hordein can be separated into polypeptides with molecular weight ranging between 67,000 and 86,000 Da, B-hordein between 30,000 and 60,000 Da, and A-hordein between 13,000 and 20,000 Da (Brandt, 1976; Shewry et al., 1977). In this work, the molecular weight of the polypeptides analysed ranged from approximately 43,000 to 94,000 Da, the majority of them between 43,000 and 67,000 Da. The bands observed in the present study were those of B and C fractions, the major groups of hordein that account for approximately 95% of the total hordein fraction (Entwistle, 1988). A-hordein comprises only 1-2% of the total of the three fractions and apparently is not a true hordein (Miflin & Shewry, 1977).

The phenogram which compares the different varieties shows a clear association between malting quality and the observed hordein patterns (Figure 3). The varieties with the highest malting quality scores (MN-599, MN-668, MN-656, MN-681, MN-682 and BR-2) got together in a unique block while the varieties with lower malting quality scores and the feed types were joined in two different blocks. This indicates that the total hordein pattern was effective in separating the varieties studied based on malting quality. These clusters could have also been obtained as a result of a higher genetic relationship between the grouped varieties due to common ancestors, independently of the malting quality. Nevertheless, this is not the situation of the varieties analysed as shown by their pedigrees (Table 1).

The relationship between single hordein polypeptides and malting quality was verified using the Sperman's correlation test (Table 3). Bands 5 and 12 ($P>0.05$) and band 19 ($P>0.001$) correlated negatively with malting quality scores. Thus, the occurrence of these bands, specially band 19, can be used as an indicator of low malting quality.

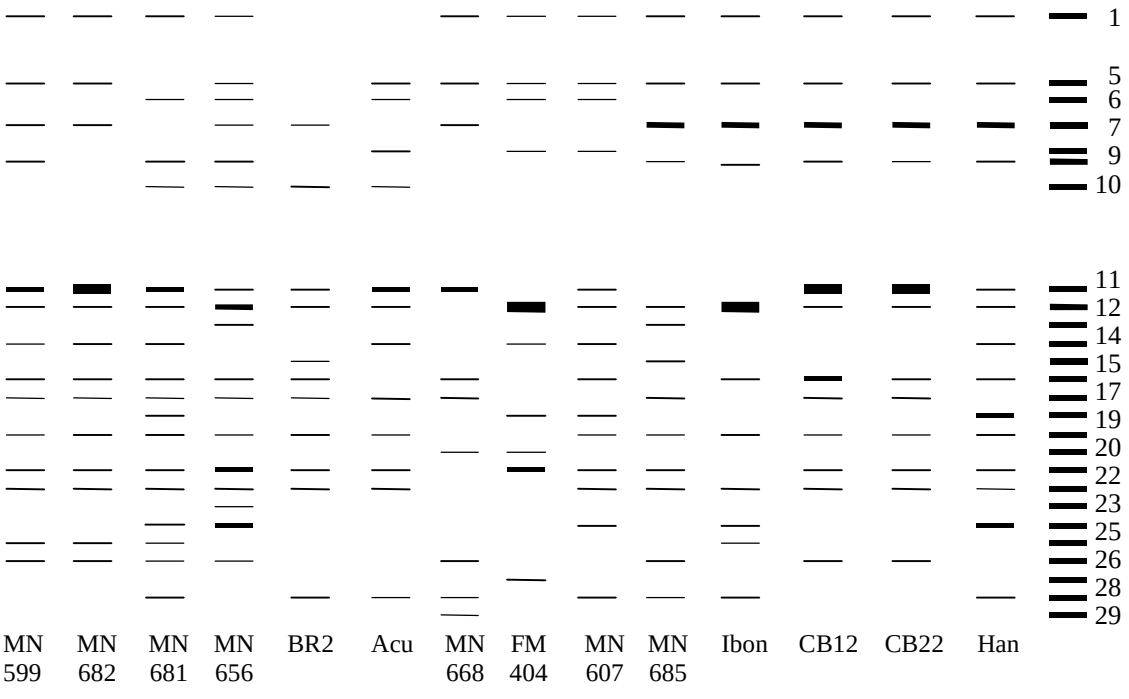

Figure 2. Compound patterns of each barley variety studied. The thickness of the line represents the most common intensity of the band for each variety. On the right, the pattern with all bands found in the species. Bands with number which do not appear in this Figure were detected in native species of *Hordeum* (*H. euclaston* and *H. stenostachys*) Acu: Acumai; Han: Hanna; Ibon: IBON 216-82; CB12: CB 8501-12; CB22: CB 8501-22.

Table 2. Frequency of the hordein polypeptide bands (in percentage) in each barley variety studied.

Hordein band	Varieties[1]													
	MN 599	MN 682	MN 668	MN 681	MN 656	BR2	Acu	Han	FM 404	MN 607	MN 685	Ibon	CB12	CB22
1	20	20	27	27	38	0	0	78	100	70	100	90	100	100
5	20	10	18	0	8	0	91	71	100	70	100	90	100	90
6	0	0	0	9	8	0	75	0	100	70	0	0	0	0
7	20	20	27	0	31	13	0	78	0	0	100	90	100	100
8	0	0	0	0	0	0	75	0	100	50	0	0	0	0
9	20	0	0	27	8	0	0	64	0	0	100	70	100	90
10	0	0	0	9	8	33	8	0	0	0	0	0	0	0
11	100	100	100	100	54	100	100	100	0	100	0	0	100	100
12	100	70	0	64	92	40	100	100	100	100	100	100	100	100
13	0	0	0	0	46	0	0	0	0	0	100	0	0	0
14	30	30	0	27	0	0	100	100	100	100	0	0	0	0
15	0	0	0	0	0	60	0	0	0	0	100	0	0	0
16	100	100	100	100	54	100	0	100	0	30	0	100	100	100
17	50	60	100	73	23	60	91	0	0	0	100	0	100	100
18	0	0	0	18	0	0	0	100	100	100	0	0	0	0
19	20	20	0	36	54	66	100	100	0	90	100	100	100	100
20	0	0	9	0	0	0	0	0	100	0	0	0	0	0
21	100	100	0	100	46	100	42	100	100	100	100	0	100	100
22	100	100	0	100	46	100	91	36	0	100	100	100	100	100
23	0	0	0	0	23	0	0	0	0	0	0	0	0	0
24	0	0	0	27	54	0	0	100	0	100	0	90	0	0
25	50	30	0	45	0	0	0	0	0	0	0	20	0	0
26	40	40	100	9	23	0	0	0	0	0	100	0	100	100
27	0	0	0	0	0	0	0	0	100	0	0	0	0	0
28	0	0	64	9	0	6	8	93	0	100	50	100	0	0
29	0	0	27	0	0	0	0	0	0	0	0	0	0	0

[1] Acu: Acumaí; Han: Hanna; Ibon: IBON 216-82; CB12: CB 8501-12; CB22: CB 8501-22.

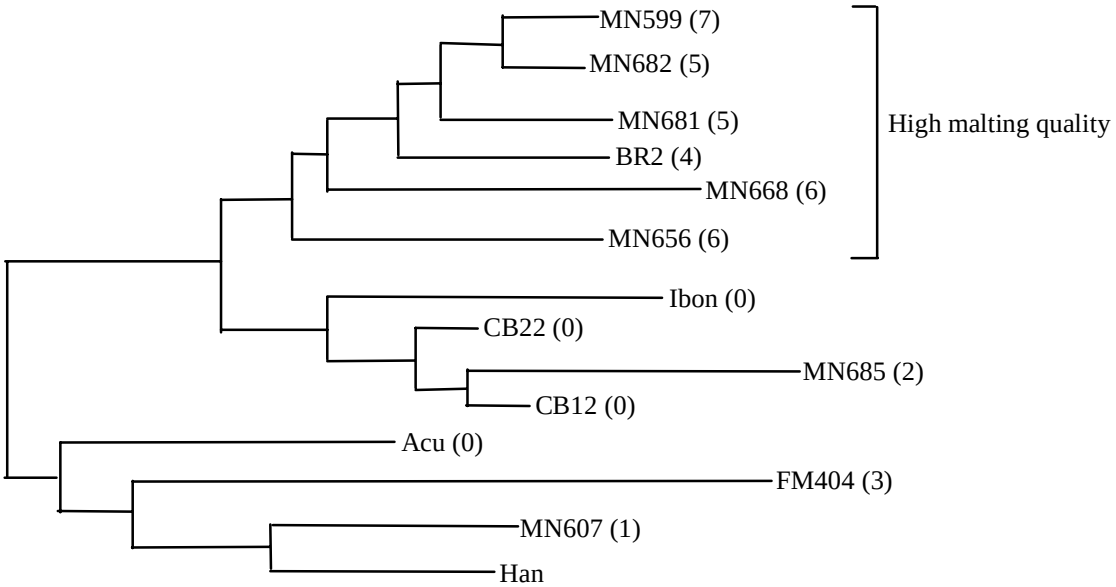

Figure 3. Phenogram of barley varieties obtained from Manhattan distance, based on hordein band frequency and clustered by Neighbor-joining method. The malting quality classification (Table 1) is shown on the right of each variety name. Acu: Acumaí; Han: Hanna; Ibon: IBON 216-82; CB12: CB 8501-12; CB22: CB8501-22.

Table 3. Sperman's correlation indexes (r_S) of each band with the values of malting quality assigned to the varieties studied.

Bands	Correlation (r_S)	Bands	Correlation (r_S)
1	-0.47	17	-0.07
5	-0.65*	18	-0.14
6	0.005	19	-0.84**
7	-0.32	20	0.27
8	-0.23	21	-0.10
9	-0.21	22	-0.12
10	0.22	23	0.35
11	0.04	24	-0.20
12	-0.64*	25	0.41
13	0.21	26	0.24
14	-0.10	27	0.04
15	0.04	28	-0.29
16	0.11	29	0.35

* and ** Significant at P>0.05 and P>0.001, respectively.

The Sperman's correlation test did not indicate any relation between band molecular weight and malting quality as was found by Baxter & Wainwright (1979), who related B-hordein with this characteristic, since the bands 5, 12, and 19, which showed significant correlation, have much different molecular weights.

Conclusions

1. The total hordein band pattern can be considered as an effective method based on malting quality scores to discriminate the varieties and can therefore be used as an early screening test to select genotypes for this characteristic in genetic improvement programs.

2. The presence of the hordein bands 5, 12 and 19 can be useful as molecular indicator of a low malting quality.

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