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Variability in monomeric prolamins in *Hordeum chilense*

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Abstract Eighty-eight lines of the species *Hordeum chilense* Roem. et Schult., representative of the variability found in the latter's distribution zone, were analysed. Monomeric prolamins (protein fraction corresponding to wheat gliadins) were studied using the SDS-PAGE technique. The analysis of the different bands was performed using the programme BIOIMAGE™ WHOLE BAND ANALYZER. Jaccard's coefficient of similarity was calculated, and the lines were grouped by cluster analysis using UPGMA. A great variability was found between the different lines studied. Forty-two different bands were identified, all of which were polymorphic. Sixty-eight different patterns of monomeric prolamins were identified within the 82 lines studied. A dendrogram was obtained from the analysis of the groups. No relationship between the distribution of the variability in the dendrogram and the geographical origin or the ecological characteristics of the species could be detected. It is concluded that *H. chilense* is an important pool of variability for storage proteins that could be used in cereal breeding.

Key words *Hordeum chilense* · Gliadins · Variability · SDS-PAGE · Molecular markers

Introduction

A serious problem agriculture currently faces is the decreasing variability in genetic resources, which has resulted from the number of economically important crops being reduced and in addition, fewer varieties of these being used in place of many traditional varieties. This homogeneity could create a problem with respect to effects on agriculture worldwide since a new agent or pathogen could destroy all of one type of crop. As well, a lack of variability reduces the possibilities of meeting the standards of agricultural products demanded at present.

Hordeum chilense Roem. et Schult. is a wild barley included in the section *Anisolepis* Nevski. This species grows exclusively in Chile and Argentina and is very variable with respect to its morphological and phenological traits. It has a perennial growth habit, being a hemicryptophyte (Tobes et al. 1995). Its economic and agricultural importance is based on its high crossability with other members of the Triticeae tribe and its interesting agronomic characteristics. Furthermore, it is believed to have the highest potential for breeding within the genus *Hordeum* (review by Martín et al. 1996). *H. chilense* seems to be salt-tolerant (Forster et al. 1990) and drought-tolerant (Gallardo and Fereres 1989), and it shows higher nitrate reductase activity than wheat (Barro et al. 1991, 1994; Maldonado et al. 1996). Furthermore, *H. chilense* has been described as showing resistance against different diseases and pests; for example, to *Schizapis graminum* (Castro et al. 1995), *Diuraphis noxia* (Clement and Lester, 1990), *Septoria tritici* (Rubiales et al. 1992), *Fusarium culmorum* and *Septoria nodorum* (Rubiales et al. 1996).

H. chilense has been successfully crossed with Triticeae species of the genera *Aegilops*, *Agropyron*, *Dasypyrum*, *Hordeum*, *Secale*, *Triticum* and ×*Triticosecale* (Martín et al. 1998). Fertile amphiploids obtained with diploid, tetraploid and hexaploid wheats have been named Tritordeums (×*Tritordeum* Ascherson et Graebner). Hexaploid tritordeum (2n = 6x = 42, $AABBH^{ch}H^{ch}$) has been the subject of a breeding programme with the goal of creating a new crop. Apart from its potential use as a new crop, tritordeums can be used as genetic bridges between wheat and the rest of *Triticeae* (Martín et al. 1998).

It is necessary to manage the variability available in both parents to convert hexaploid tritordeums into a

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crop. Hexaploid tritordeums show a low frequency of aneuploids, a wide variation in initial growth and good fertility. In addition, they show other favourable agronomic traits such as biomass, spikelets per spike, seed size and protein content (Martín et al. 1996).

In addition to affecting bread-making quality, the storage proteins, and especially the gliadins, have been used as molecular markers (Williams et al. 1993; Nieto-Taladriz et al. 1994; Metakovsky and Brandlard 1998) due to their high variability. Similarly, the gliadins have been suggested as a useful tool in identifying varieties of common bean, maize and barley (Gepts 1989). They may also be important in breeding for quality characteristics in cereals. A relationship has been found between gliadin alleles and dough strength (Metakovsky et al. 1997a, b) and the effects of alpha, beta, gamma and omega gliadins on the dough mixing properties of wheat flour (Fido et al. 1997).

The aim of this investigation reported here was to use the monomeric prolamins as molecular markers for studying the variability of these proteins in *H. chilense* and to determine if there is a relation between the genetic variability of the species and its geographical and ecological distribution.

Materials and methods

Grain samples

Eighty-eight lines of *Hordeum chilense* collected from its complete distribution area in Chile were studied (Table 1). These are being maintained at present at the IAS (Sustainable Agriculture Institute) of the CSIC (Superior Council of Scientist Research).

Table 1 Passport data of the lines used in this study

Line	Latitude	Longitude	Kingdom[a]	Line	Latitude	Longitude	Kingdom[a]
H1	Unknown			H211	32°58′	71°10′	C
H7	Unknown			H212	32°15′	71°32′	B
H8	Unknown			H213	32°25′	70°55′	C
H10	Unknown			H216	32°18′	71°31′	B
H11	Unknown			H217	34°04′	70°56′	C
H12	Unknown			H218	33°04′	70°57′	C
H13	Unknown			H220	36°45′	72°18′	C
H14	Unknown			H222	36°45′	73°09′	C
H16	Unknown			H225	32°18′	71°31′	B
H17	Unknown			H226	34°03′	71°38′	C
H33	Unknown			H228	34°04′	70°56′	C
H34	Unknown			H229	33°38′	70°18′	C
H35	Unknown			H232	32°25′	70°55′	C
H38	Unknown			H241	33°	70°57′	C
H39	Unknown			H245	34°58′	70°27′	B
H41	Unknown			H250	38°42′	73°02′	C
H46	Unknown			H251	38°26′	71°22′	B
H47	Unknown			H252	34°41′	73°24′	C
H49	Unknown			H254	34°57′	70°23′	B
H51	Unknown			H255	38°42′	73°02′	C
H52	Unknown			H261	30°23′	70°58′	B
H54	Unknown			H266	30°32′	71°42′	B
H55	Unknown			H283	30°41′	70°52′	B
H56	Unknown			H286	29°55′	71°14′	B
H57	Unknown			H290	31°53′	71°29′	B
H58	Unknown			H292	30°45′	71°32′	B
H59	Unknown			H293	30°32′	71°42′	B
H60	Unknown			H294	30°37′	71°19′	B
H61	Unknown			H295	30°41′	71°22′	B
H68	Unknown			H296	30°41′	70°52′	B
H74	Unknown			H297	30°33′	71°29′	B
H83	Unknown			H298	30°21′	71°29′	B
H91	Unknown			H299	30°15′	70°41′	B
H93	Unknown			H300	28°55′	70°45′	B
H200	34°45′	70°34′	C	H301	30°41′	71°22′	B
H202	33°01′	70°54′	C	H302	30°41′	70°51′	B
H203	32°15′	71°32′	B	H303	31°54′	70°22′	B
H204	33°	70°57′	C	H304	31°48′	71°21′	B
H205	32°58′	71°10′	C	H305	30°37′	71°14′	B
H206	33°06′	71°28′	C	H307	29°55′	71°14′	B
H207	31°54′	72°22′	B	H308	31°47′	70°35′	B
H208	32°58′	71°10′	C	H309	30°37′	71°14′	B
H209	33°06′	71°28′	C	H310	31°56′	71°31′	B
H210	33°39′	70°21′	C	H311	30°48′	71°40′	B

[a] B, Dry kingdom; C, temperate kingdom. Kingdoms have been defined by Gastó et al. (1990) based on Köppen's World Climatic Classification (1923)

Variability in *H. chilense* could has investigated using these 88 lines as they covered the area of origin of the species. A homocygotic genotype was obtained from a plant randomly chosen from each accession by means of two generations of self-fertilization. The homogeneity of the progeny of each genotype was tested using nine seeds from three different plants. Six lines in which segregation was found were eliminated from the study. When all the lines were checked for homogeneity, it was possible to take different seeds of the same line to obtain a sufficient quantity of flour, which then allowed us to perform two replicates in order to obtain consistent results.

Protein extraction

Three embryo-less seeds of each line were crushed into a fine powder and mixed in order to obtain a sufficient quantity of flour.

Albumin and globulin fractions were removed with water and saline solution (0.5 *M* NaCl), respectively. The sodium chloride that remained was eliminated by washing with pure water (1 ml per tube). Monomeric prolamins were solubilized with 1 ml of ethanol (70%) and precipitated with cold acetone. Excess cold acetone was evaporated off overnight at room temperature, and the dried pellet was solubilized in buffer containing 125 mM Tris-HCl pH 6.8+2% (w/v) ditiothreitol + 0.005% bromophenol blue in a 1:10 ratio (w/v) at 60°C 30 minutes. The tubes were kept at –20°C.

Sodium dodecyl suphate-polyacrylamide gel electrophoresis (SDS-PAGE) separation

Monomeric prolamins were fractionated in vertical SDS-PAGE slabs in a discontinuous Tris-HCl-SDS buffer system (pH: 6.8/8.8) at a polyacrylamide concentration of 12% (w/v, C = 2.67). The Tris-HCl/glycine buffer system of Laemli (1970) with the modifications proposed by Alvarez (1993) was used. Electrophoresis was performed at a constant current of 25 mA per gel at 10°C for 45 min after the tracking dye migrated off the gel.

Gels were stained overnight with a 12% (w/v) trichloroacetic acid solution containing 5% (v/v) ethanol and 0.05% (w/v) Coomassie Brilliant Blue R-250. Gels were destained with distilled water.

Data analysis

A Kodak™ camera was used to take photos of the gels. It gives digital images at a resolution of 1024 × 1024 pixels. Bands were identified using the BioImage™ Whole Band Analyzer programme and numbered consecutively. All the lines were scored for presence or absence of the different monomeric prolamins identified. The data were entered into a binary matrix as discrete variables (1 for presence and 0 for absence). Jaccard's coefficient of similarity was calculated, and the lines were grouped by cluster analysis using the Unweighted pair-group method (UPGMA). A phenogram was produced as described by (Sneath and Sokal 1973) using the programme Systat 7.0 for Windows (ICARDA, Syria).

Geographic and ecological study

Any possible relation between the geographic origin of the lines and the variability found was carried out by locating the different lines onto a map of Chile and searching for possible relation with the dendrogram groups.

Results

Out of 88 lines studied 82 had a unique pattern, and were therefore homocygotic, while the other 6 showed segregation – therefore not homocygotic – and were eliminated from the analysis.

The variability in monomeric prolamins was very high: 42 different positions of bands were identified (Fig. 1) all of them being polymorphic. The most fre-

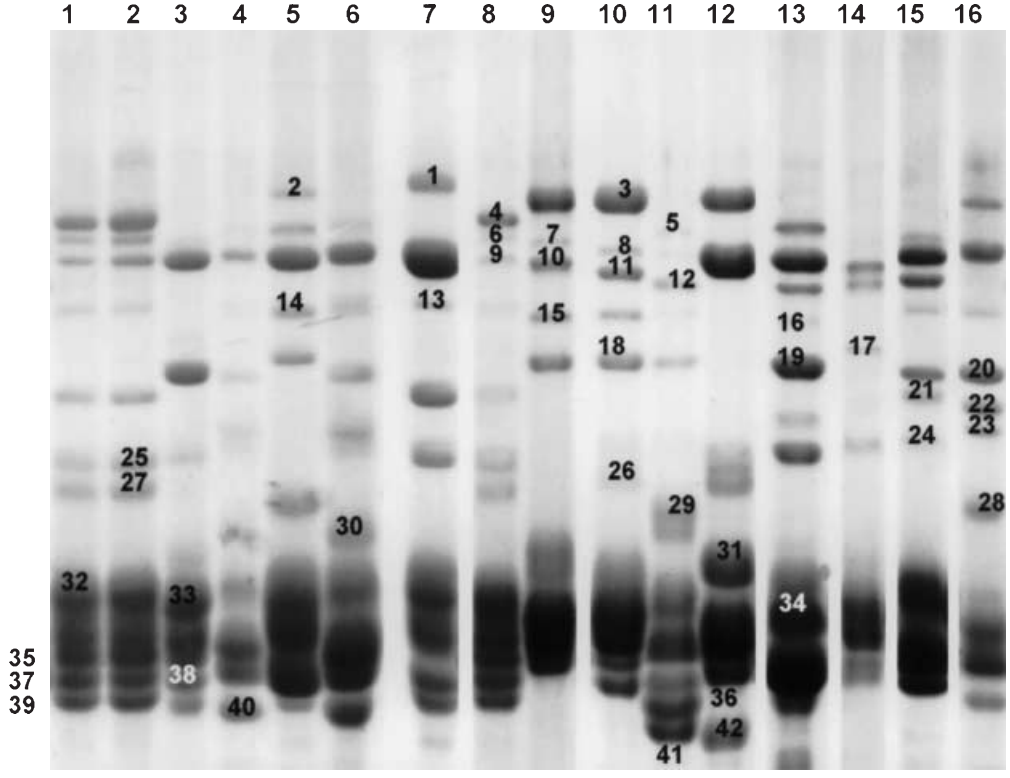

Fig. 1 Monomeric prolamins of *H. chilense* fractionated by SDS-PAGE. *Lanes: 1* H12; *2* H13; *3* H14; *4* H7; *5* H16; *6* H17; *7* H33; *8* H1; *9* H252; *10* H255; *11* H261; *12* H266; *13* H218; *14* H220; *15* H297; *16* H10

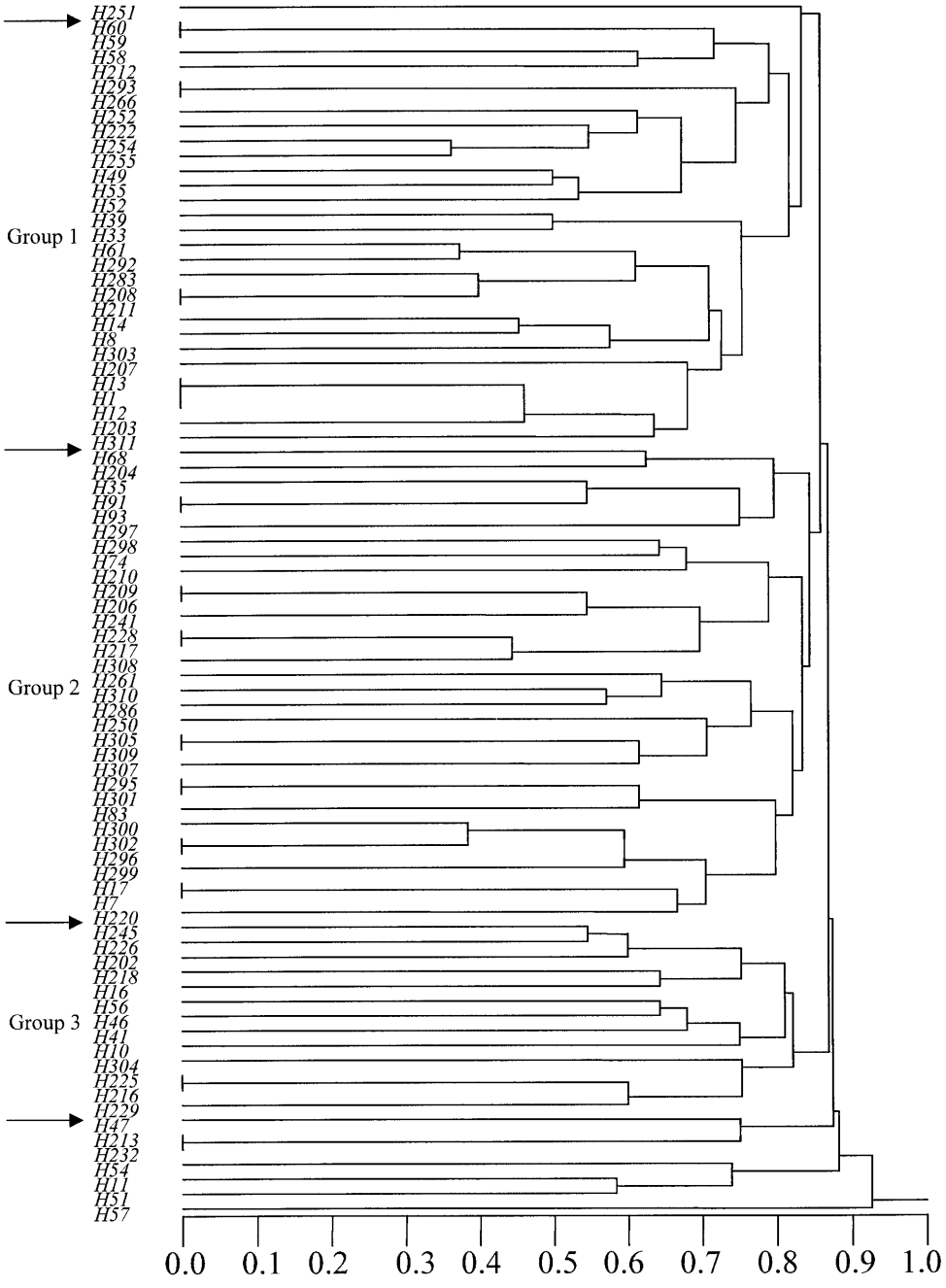

Fig. 2 Dendrogram using Jaccard's coefficient of similarity

quent band (no., 35) only appeared in 54.9% of the lines (Table 2). Likewise the number of bands per line ranged from 4 to 11, with 8 being the most frequent, occurring in 19 of 82 lines used (Table 3). Out of the 82 lines studied 68 different patterns were identified.

A dendrogram was obtained based on the analysis of groups (Fig. 2). Apart from the lines having the same pattern, there is no connection between the lines until a relative distance of 0.35. From this distance onwards, continuous connecting are produced successively. Nevertheless, it is possible to distinguish three main groups: group 1 goes from H60 to H311; group 2 from H68 to H220; group 3 from H245 to H229.

A possible relation between the genetic variation and geographic localization was studied. We plotted each of the lines on a map of Chile (Fig. 3) using the localization data available (Table 1). The three main groups of the dendrogram (Fig. 3) were identified by means of a symbol, and the lines were located on a map of Chile (Fig. 3). No relation between the groups of the dendrogram and geographic localization was observed.

The relation between ecological characteristics in the distribution area and variability for prolamins found in the dendrogram was also investigated. The lines were collected from dry and temperate kingdoms as described by Gastó et al. (1990) based on the Köppen World Climatic Classifi-

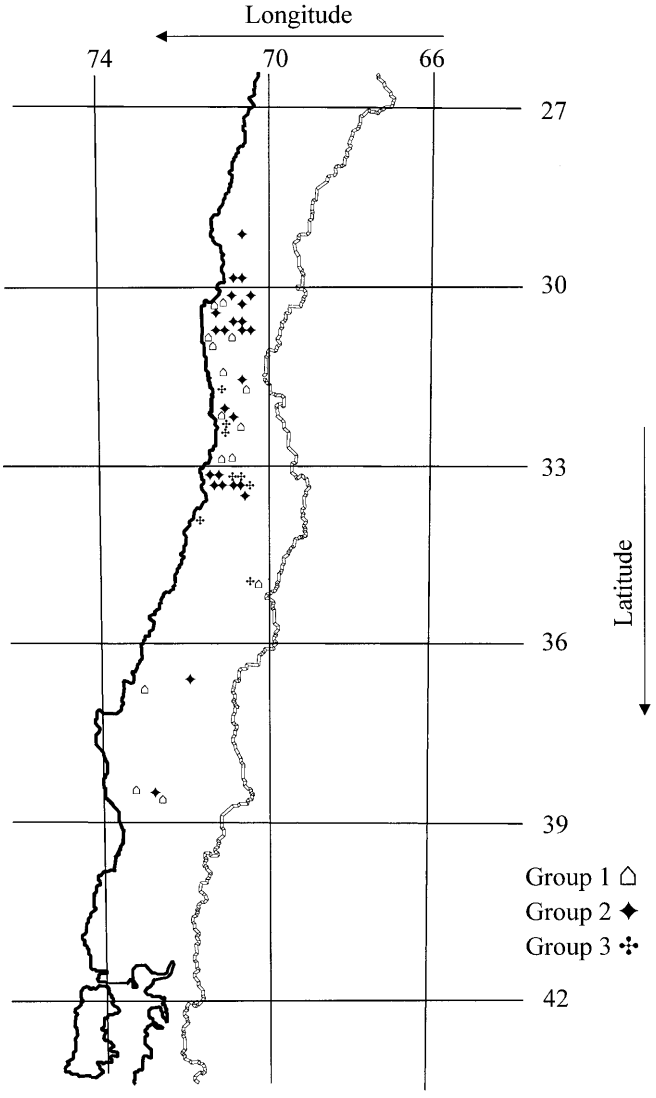

Fig. 3 Localization of the groups observed in the dendrogram on the map of Chile

Table 2 Frequency of the different bands identified within the population

Band	Lines	%	Band	Lines	%
1	7	8.54	22	14	17.1
2	3	3.66	23	7	8.54
3	14	17.1	24	8	9.76
4	14	17.1	25	31	37.8
5	24	29.3	26	6	7.32
6	16	19.5	27	16	19.5
7	8	9.76	28	6	7.32
8	12	14.6	29	6	7.32
9	30	36.6	30	6	7.32
10	28	34.1	31	16	19.5
11	20	24.4	32	20	24.4
12	7	8.54	33	25	30.5
13	10	12.2	34	31	37.8
14	15	18.3	35	45	54.9
15	34	41.5	36	22	26.8
16	6	7.32	37	35	42.7
17	4	4.88	38	16	19.5
18	14	17.1	39	24	29.3
19	14	17.1	40	21	25.6
20	16	19.5	41	2	2.44
21	15	18.3	42	10	12.2

Table 3 Number of bands for monomeric prolamins per line in *H. chilense*

Number of bands	Number of lines with this number of bands	Percentage of the population
4	1	1.22
5	1	1.22
6	12	14.63
7	12	14.63
8	19	23.17
9	16	19.51
10	14	17.07
11	7	8.54

cation (1923) and the Grassland Classification System (Gallardo and Gastó 1985). In this way 32 lines were collected from dry kingdom while 18 lines were determined to be growing in a temperate one. No association between the groups and the ecological origin of the lines was detected, which was in agreement with the lack of relationship between variability and geographical localization.

Discussion

Monomeric prolamins as molecular markers constitute a powerful tool in the identification of lines, analysis of the distribution of genetic variability and evolution processes and structure of populations as revealed by the high levels of polymorphism and the large number of patterns found. Studies are being carried out at present in our group using these markers. Monomeric prolamins have the added advantage of being cheaper than DNA markers and being directly related to cereal quality.

Therefore, the cataloguing of *H. chilense* monomeric prolamins that this investigation contributed to will be useful in tritordeum and other cereal breeding. In this way the homogeneity of wheat and triticale could be mitigated using the variability of the section Anisolepis by means of tritordeums as a genetic bridge (Martín et al. 1998).

The lack of well-defined groups revealed a continuous distribution of variability in the areas of origin of the species, although there are two main identifiable groups based on morphological characters (Martín et al. 1998) led by the H1 and H7 lines.

Likewise, lines from different environments are located in the same groups of the dendrogram (Table 4). It means there is a continuos variation of the species in the territory. Therefore, the variability of monomeric prolamins in *H. chilense* seems to be neutral to natural selection.

In conclusion, *H. chilense* presents a high variability with respect to monomeric prolamins. These could be

Table 4 Comparison between the genetic variability detected in the dendrogram and the ecological characteristics of the lines of each group

Group	Line	Kingdom[a]	Group	Line	Kingdom[a]	Group	Line	Kingdom[a]
Group 1	H203	B	Group 2	H204	C	Group 3	H202	C
	H207	B		H206	C		H216	B
	H208	C		H209	C		H218	C
	H211	C		H210	C		H225	B
	H212	B		H217	C		H226	C
	H222	C		H220	C		H229	C
	H252	C		H228	C		H245	B
	H254	B		H241	C		H304	B
	H255	C		H250	C		H10	Unknown
	H266	B		H261	B		H16	Unknown
	H283	B		H286	B		H41	Unknown
	H292	B		H295	B		H46	Unknown
	H293	B		H296	B		H56	Unknown
	H303	B		H297	B			
	H311	B		H298	B			
	H1	Unknown		H299	B			
	H8	Unknown		H300	B			
	H12	Unknown		H301	B			
	H13	Unknown		H302	B			
	H14	Unknown		H305	B			
	H33	Unknown		H307	B			
	H39	Unknown		H308	B			
	H49	Unknown		H309	B			
	H52	Unknown		H310	B			
	H55	Unknown		H7	Unknown			
	H58	Unknown		H17	Unknown			
	H59	Unknown		H35	Unknown			
	H60	Unknown		H68	Unknown			
	H61	Unknown		H74	Unknown			
				H83	Unknown			
				H91	Unknown			
				H93	Unknown			

[a] B, Dry kingdom; C, temperate kingdom

applied to breeding for cereal quality as well as molecular markers. The variability seems to be neutral to natural selection.

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