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Full Length Research Paper

Genetic diversity of the monomeric prolamins and hordein in hulless barley genotypes and their relation with agronomical traits

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In order to compare polymorphism resulting from monomeric prolamine and hordein, 63 genotypes of hulless barley from ICARDA were investigated. Also, to compare the patterns observed in the analyses of storage proteins and their relation with morphologic and phenologic traits, 20 agromorphological traits were recorded. In the analysis of the hordeins, no polymorphism was observed in the area D hordein. However, 10 patterns in the area C hordein, and 13 patterns in the area B hordein were observed; and in total 32 bands and 32 patterns were observed. The average genetic diversity index for these proteins was calculated as H = 0.866. In the analysis of the monomeric prolamins, which was performed with the Acid-PAGE method, 15, 9, 24, and 20 patterns were observed for the ω, γ, β, and α areas, respectively. The average of the genetic diversity index for these proteins was H = 0.889, and in total 33 bands as well as 57 patterns were observed. In the analysis of the monomeric prolamins, 51 genotype having unique patterns were identifiable while in the analysis of the hordeins, only 18 samples were identified. With regard to the fact that monomeric prolamins enjoy a greater diversity than hordeins and are more powerful in identifying samples and regarding the simple, in addition to the low cost of conducting the analysis, it can be used in a variety of genetic studies such as genetic diversity assessment, identifying genotypes and determining the phylogenic relations in barley. In the analysis of hordeins, one pattern was found among the patterns of area B hordein that was related to the traits of days to heading and days to maturity.

Key words: Genetic diversity, monomeric prolamins, hordein, hulless barley.

INTRODUCTION

Although the main application of hulless barley (*Hordeum vulgare* L.) is in the nourishment of monogastric animals (poultry) since it is rich in protein, (especially lysine amino acid and β glucan), it is appropriate for human nourishment as well (Bell et al., 1983; Bhatty, 1986; Anderson and Berglung, 1990). Traditionally, this plant has been considered a source of food among inhabitants of mountainous areas and in addition, it would be less costly than hulled barley so far as it does not need de-hulling (Newman, 1992; Bhatty, 1993). However, genetic studies and breeding programmes have been focused on hulled barley and the cultivation of hulless barley has developed slowly. Therefore, despite its high potential, this plant has not yet reached its proper position among cultivated plants and there is little information about its genetic diversity.

Hordein is the main storage protein in barley, which has four parts: A, B, C, and D (Shewry and Milfin, 1985). D hordein is a prolamin with high molecular weight and constitutes less than 5% of the total seed protein. It has a molecular weight of 105 kD, is polymeric, rich in glycine, proline and glutamine (Shewry and Tatham, 1990) and is coded by the Hor3 locus which is located at the long arm of chromosome 1H(5) (Kreis et al., 1984). C hordein is a weak sulphur prolamin and since the amount of sulfurous amino acids in its structure is negligible, it cannot be a polymer. Its molecular weight is 55 - 70 kD, is rich in glutamine and phenylalanine and is coded by the Hor3 multigenic locus located at the short arm of chromosome

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1H(5) (Shewry and Milfin, 1985; Kreis et al., 1984). B hordein is rich in sulphur and is coded by the multigenic locus located at the short arm of chromosome 1H(5), and its molecular weight is 36 - 45 kD; it is a polymer and rich in glutamine (Shewry and Milfin, 1985). But many do not consider A hordein with a molecular weight less than 20 kD a real storage protein (Shewry et al., 1978; Shewry and Milfin, 1985).

Hordein has a great inter-genotypic variation and has been used as a marker in cultivar identification, genetic diversity studies and in determining phylogenic origins (Doll and Brown, 1979; Shewry et al., 1983; Heisel et al., 1986; Moralejo et al., 1994; Radovic and Vapa, 1996; Atanassov et al., 2001; Yin et al., 2003; Listrumaite and Paplauskiene, 2007). Monomeric prolamines (according to the wheat gliadins) enjoy a great diversity both among cultivated species of barley and wild barley. Pan et al. (2007) by studying monomeric prolamines in 86 native genotypes of cultivated hulless barley from China, observed considerable diversity among this protein.

Referring to the genetic diversity observed among monomeric prolamines, they introduced Tibetan Plateau as the diversity centre of hulless barley. Alvarez et al. (2006b) examined monomeric prolamines in *Hordeum chilense*, which belongs to the wild barley family. They studied this protein diversity among samples of 9 natural populations of this plant. In another study published in the same year, Alvarez et al. (2006a) examined the diversity of monomeric prolamines among 270 seeds on 32 spikes from natural populations of this wild plant, using the A-PAGE method. These proteins can be classified as α, β, γ, and ω areas, according to the same four groups in the wheat gliadins.

There is not enough information as to the gene loci coding of these proteins. Alvarez et al. (2004), however, introduced Gli-H^{ch}, which is located on the $1H^{ch}$, as the coding gene of area ω in *H. chilense*. According to the findings of this experiment, it was suggested that areas α and β are coded at least by two different genetic loci, but no linkage was shown between these two genes; therefore, it was suggested that, according to the studies done by Payne et al. (1987) and Tercero et al. (1991), these two genes are probably located on chromosomes $5H^{ch}$ and $7H^{ch}$. However, most of the studies done on the storage proteins in the barley seed, whether among the cultivated genotypes or among the wild ancestors, have focused on hordein, and little attention has been paid to the monomeric prolamines.

MATERIALS AND METHODS

In this study, 63 genotypes of hulless barley, all of which had been provided by ICARDA (International Centre for Agricultural Research in the Dry Area) were investigated, focusing on polymorphism in hordein and monomeric prolamines. Furthermore, in order to compare the patterns observed in the analysis of the storage proteins and their relation with phonological and morphological traits, the accessions were cultivated in 10 m^2 plots under a randomized complete block design (RCBD) with three repetitions in the Moghan region in 2007 and 20 agromorphological traits were recorded. For extraction, electrophoresis and identification of hordein areas, Poperelya and Mujarinko's (2001) method was used. Hordeins were extracted from mature grains with 0.25 ml solution containing 6.9% acetic acid and 5% 2-mercaptoetanol and 16% urea and 0.01% pyronine. Extraction and electrophoresis of the monomeric prolamines were separated by the Acid-PAGE method as described by Poperelya method (1989). Seeds were individually ground and extracted overnight at room temperature with 0.2 ml solution containing 0.9% acetic acid and 18% urea and 0.01% pyronine. Identification of α, β, and ω areas was done using Bashuk and Zillman's (1977) method. But here instead of Markois cultivar, Anza cultivar which enjoys the reference band with a relative mobility of 43.5 was used. After identification of the bands, different patterns were identified among genotypes and then, using Nei's (1973) method, each pattern's frequency and genetic diversity index was calculated for each area.

$$H = 1 - \Sigma p_i^2$$

where H is genetic diversity index and P_i is each pattern's frequency. 0 and 1 coefficients were calculated for all the genotypes, depending on the presence (1) or absence (0) of the bands. It was also used in obtaining other results as well as similarity coefficients matrix of Jaccard (Jaccard, 1908). Furthermore, in order to classify the accessions, cluster analysis was done using UPGMA (Unweighted Pair Group Method with Arithmetic Means). Calculating similarity coefficients matrix and dendrogram was done by NTSYS 2.01 program. Also, in order to find the relation between agronomical traits and protein patterns, the t-test was run. That is, for all the traits, the average of those genotypes having a certain pattern was compared with the average of those genotypes lacking that certain pattern. These calculations were done by SPSS12 programme.

RESULTS

While examining the electrophoresis for genotypes, no polymorphism was observed in the area D hordein. However, in the area C, 10 patterns (Figure 1) and 14 bands were observed, among them, pattern C_1 was observed in 31.7% of the genotypes and was the most common pattern (Table 1). Among the bands in this area, all the bands had polymorphism, except one band that was a monomorph and was common in all genotypes. The genetic diversity index for all the genotypes in this area was calculated as H = 0.828. In the area B, 13 patterns and 18 bands were observed; the most common pattern was B_1, which was observed in 20.63% of the genotypes and after that was B_{11}, which was observed in 12.7% of the genotypes. However, pattern B_7 was observed only in genotype 9 and pattern B_{11} only in genotype 42 (Table 1). Among the observed patterns in this area, pattern B_8 was proved to be statistically significant in relation with two traits, days to heading and days to maturity. It means that all the genotypes having the pattern B_8 were considerably early ripening. A t-test between genotypes possessing this pattern and those lacking it in two traits confirmed this relation.

However in total, 32 bands and 32 different patterns were observed among the genotypes studied in areas B and C; among these genotypes, 18 genotypes that had their own unique patterns were identifiable with this analysis. Although the bands observed in area C were

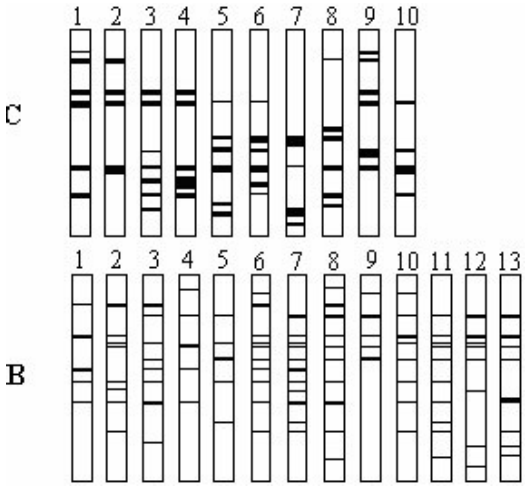

Figure 1. Ideogram of different hordein patterns in the areas of C and B observed in the genotypes studied.

clearer, the polymorphism observed in area B was much more than that. But as regards the number of bands observed in the analysis of the hordeins, genotypes 48, 60 and 63 with 9 bands had the least number of bands and genotype 9 with 18 bands had the most number of bands. The average number of bands in all the genotypes was 14.65. Figure 3 shows the dendrogram derived from Jaccard similarity coefficients for hordeins, using the PUGMA method. If the cutting is done from a distance of 0.39, the genotypes will be classified into 7 groups. The most similarity index was witnessed between genotypes 41 and 34, with similarity coefficient of 0.787 and then between genotypes 1 and 7 with similarity coefficient of 0.775.

The least similarity index was witnessed between genotypes 60 and 12, with a similarity coefficient of 0.21. Similarity mean for the total matrix for all the genotypes was calculated as 0.493.

In investigating monomeric prolamines, four distinct areas including areas α, β, γ, and ω were observed (corresponding to those four areas in the wheat gliadin). In the area ω, 15 patterns and 15 bands were observed (Figure 2). Among these patterns, pattern ω_1, which was present in 25.4% of the genotypes, was the most common pattern while patterns ω_5, ω_7, ω_8, ω_{12}, ω_{14}, and ω_{15} were found only in one genotype. The genetic diversity index among all the genotypes under study was calculated as H = 0.866. In the area γ, 9 patterns and 4 bands were found among which γ_2 which was found in 15 genotypes, was the most common, and other patterns had an average frequency, while patterns γ_4 and γ_6 were found in two genotypes only (Table 1). The genetic diversity index for this area was calculated as H = 0.854. In the area β, however, 7 different bands were observed (Figure 2). In this area, the pattern β_7 was found in 8 genotypes and had the most frequency; 10 patterns in one genotype and 5 patterns in two genotypes were ob-

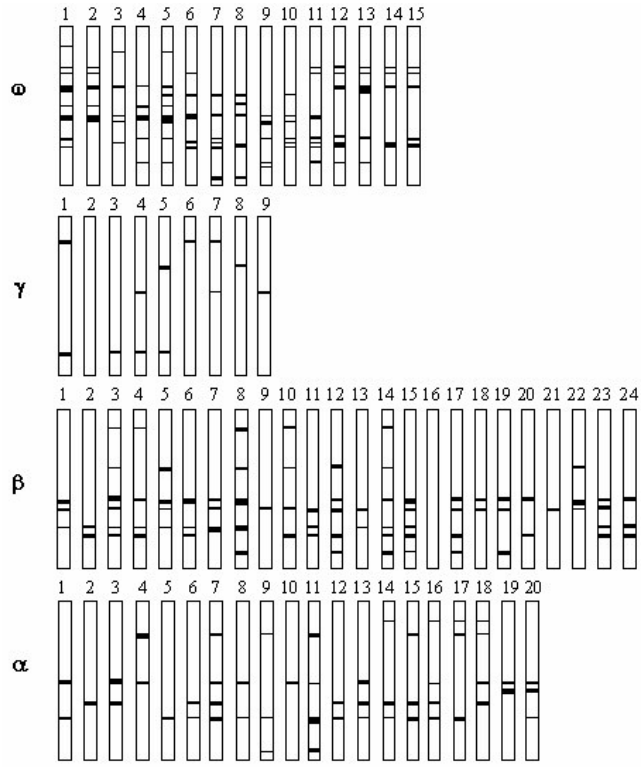

Figure 2. Ideogram of different monomeric prolamines patterns in the areas of α, β, γ and ω observed in the genotypes studied.

observed. Among the areas under study, this area with 24 different patterns and a genetic diversity index of H = 0.933 has the highest diversity. In genotypes 4 and 6, all bands were observed, and in genotypes 16, 51 and 60 no bands was observed. In the area α, 7 bands and 20 different patterns were observed, among which α_1 and α_6 were found in 10 genotypes and had the highest frequency. Nine patterns were observed only in one genotype and 15 patterns were observed only in two genotypes (Table 1). The genetic diversity index for this area was calculated as H = 0.904. Altogether, 33 bands and 57 different patterns were observed in the analysis of monomeric prolamines among which 51 patterns were found in one genotype only; A-PAGE method can be used to identify the genotypes possessing these patterns. Only two patterns were found in 6 genotypes.

As regards the number of bands observed in this area, genotypes 51 with 6 bands and genotype 21 with 17 bands had the least and the most number of bands respectively. The average number of bands in all the genotypes was 11.81. In the dendrogram obtained from the similarity coefficients of Jaccard for monomeric prolamines, if we cut from the distance of 0.35, the genotypes will be classified into 7 groups (Figure 4). As it is clear, group 7 contains 27 genotypes and group one contains one genotype. Among these 7 groups, the genotypes which are put in the same group were so much similar in terms of area ω. For example, in group 1,

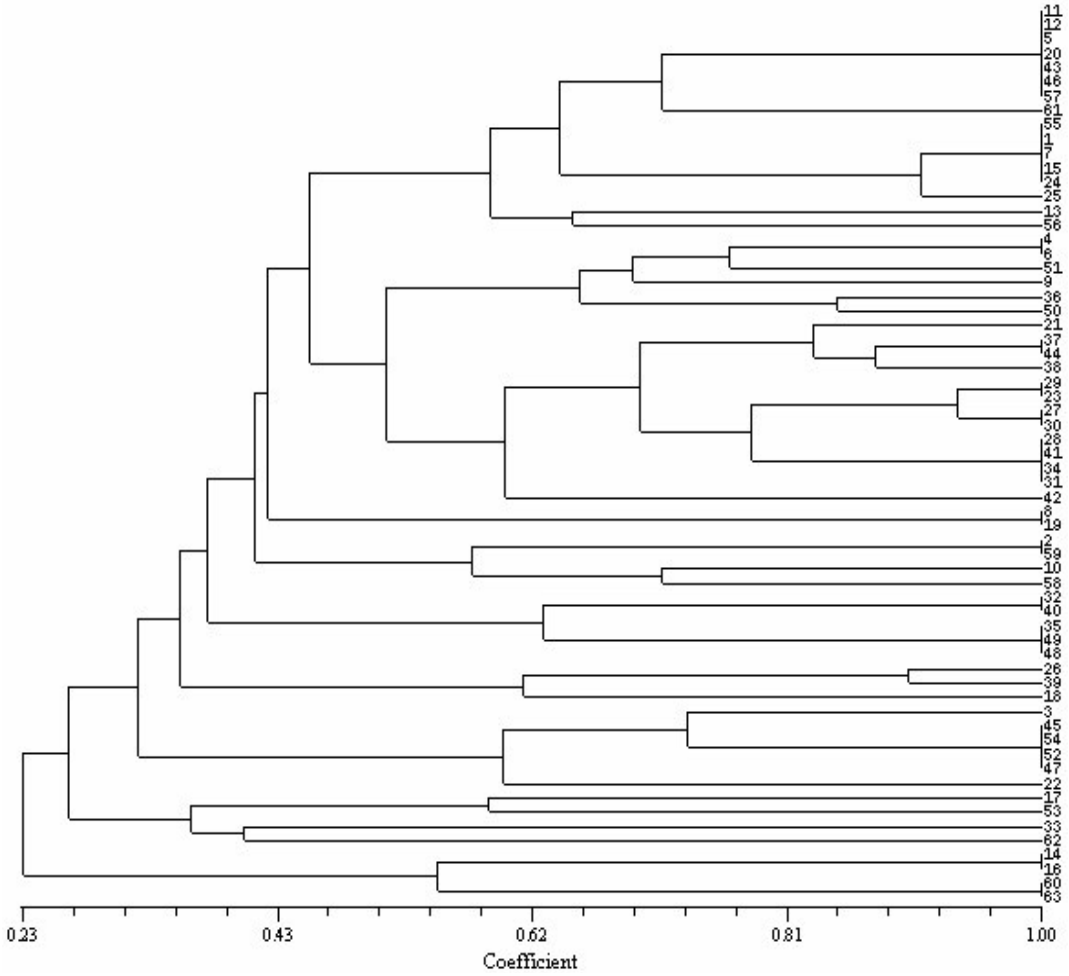

Figure 3. Dendrogram showing the relationship among 63 genotypes hulles barley based on hordein bands.

which contained genotypes 22, 33, 45, 47, 53, and 54, all these genotypes had the pattern ω_6. In other words, the patterns in the area ω had the most influence in the classification of the genotypes. The most similarity index was witnessed between genotypes 48 and 49 with a similarity coefficient of 0.792 and then between genotypes 4 and 6 with a similarity coefficient of 0.782.

The least similarity index was witnessed between genotypes 5 and 52, with a similarity coefficient of 0.217. Similarity mean for the total matrix for all the genotypes was calculated as 0.462.

DISCUSSION

In the hordein analysis for the areas B and C, 13 and 10 patterns were observed, respectively, and no polymorphism was found for the area D. Although Peltonen et al. (1994) found no polymorphism for this area, Atanassov et al. (2001) and Paplauskiene and Leistrumaite (2007) reported 4 and 3 patterns for this area, respectively. However, in other studies, more inter-genotypic variation has been reported for the C and B hordein areas. For C hordein, from 5 (Peltonen et al., 1994) to 15 (Heisel et al., 1986) patterns, and for B hordein, from 8 (Radovic and Vapa, 1996) to 24 (Shewry et al., 1978) patterns have been reported. Altogether, 32 patterns were observed in these two are as. At the same time, Radvic and Vapa (1996) reported 18 patterns among 33 Yugoslavian hulled barley cultivars, and Heisel et al. (1986) reported 34 patterns among 55 North American barley cultivars.

Also, 33 bands and 57 patterns were found among monomeric prolamines. In studying monomeric prolamines in 86 hulless barley cultivars, Pan et al. (2007) reported 43 bands and 76 patterns, while Atienza et al. (2000) studied 82 genotypes of *Hordeum chilense* and reported 42 bands and 68 patterns. Alvarez et al. (2006) studied monomeric prolamines in three areas (α, β, ω) in wild barleys and reported 41 bands and 34 patterns altogether. The genetic diversity index for these areas (α, β, ω) was calculated as 0.926, 0.795 and 0.917 respectively with the average genetic diversity index being 0.879

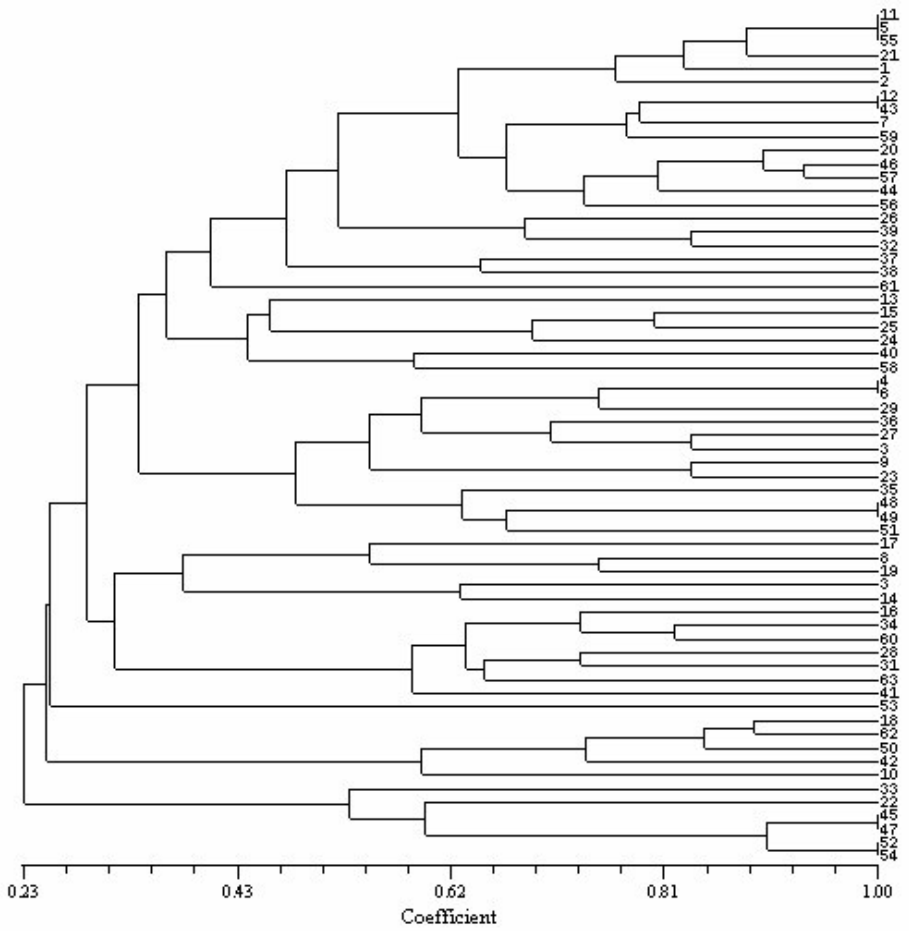

Figure 4. Dendrogram showing the relationship among 63 genotypes hulles barley based on monomeric prolamines bands.

for all these areas. In another study, Alvarez et al. (2006) reported the highest genetic diversity index in area ω and the least diversity in area β.

With regards to the fact that more patterns were found in the analysis of monomeric prolamines than that of hordeins (57 patterns versus 32 patterns), and also regarding the genetic diversity index of monomeric prolamines (H = 0.889) and hordeins (H = 0.856), it can be concluded that monomeric prolamines enjoy more diversity compared with hordeins, and this diversity is especially obvious in areas α and β. Also, in order to compare the dendrograms resulting from the two methods, cutting for each dendrogram was done from a distance of 0.35. Cutting from this distance for the dendrograms resulted from the analysis of monomeric prolamines which classified genotypes into 7 groups (Figure 4) and the dendrogram resulted from the analysis of the hordeins classifying the genotypes into 4 groups (Figure 3). As can be seen in the dendrogram of hordeins, more intense chaining can be observed. This caused 77.5% of the genotypes to be placed in one group, so that the genetic distance between them was not assessed properly. At the same time, the dendrogram obtained from monomeric prolamines was more successful in showing the genetic distance between the genotypes. In the analysis of monomeric prolamines, 51 genotypes that had unique patterns were identified among the genotypes under study, while in the analysis of hordeins, only 18 genotypes with unique patterns were observed. Therefore, compared with the analysis of hordeins, this method is more powerful in identifying genotypes.

Quick and exact recognition identifications of genotypes have long been considered major goals in studying storage proteins in seeds. In many of the studies, when hordein analysis can no longer identify numbers, more complex methods such as HPLC and two-dimensional electrophoresis, which entail more time and cost, are used. In these cases, the analysis of monomeric prolamines can be the main method for identifying genotypes, and hordein analysis can be a complementary method. Or when hordein analysis is used, monomeric prolamines

Table 1. Patterns observed in the analysis of monomeric prolamines and hordein for each genotype.

S/N	Name or pedigree	Patterns					
		α	β	γ	ω	B	C
1	BF 891 M-591	9	7	1	1	1	1
2	BF 891 M-597	10	11	1	1	5	1
3	HIGO / LINO	11	11	1	9	3	8
4	SB91488	3	3	2	4	6	3
5	BF 891M-609 (SEL.1AP)	1	1	1	1	2	1
6	SB 91925	3	3	2	4	6	3
7	AMAPA/3/ROBUR-BAR/EGYPT20 …	2	24	1	1	1	1
8	Rabano /5/CM67-B/Centeno//Cam…	19	12	9	10	1	6
9	ICNBF 8-611 (SEL.2AP)	4	4	8	4	7	3
10	PETUNIA 1	12	7	9	3	3	4
11	ICNBF 93-369	1	1	1	1	2	1
12	ICNBF-582	2	2	2	1	2	1
13	Alpha/Durra//Himalaya-26	10	14	3	12	6	9
14	ICB-102607	10	15	3	9	4	5
15	Pamir-167/Himalaya-26	13	7	5	13	1	1
16	Himalaya-13/Rhn-o3	8	16	6	11	4	5
17	BF 891 M-622 /3/ Arar // 2762 / BC-2L-2Y	3	5	3	7	8	7
18	Mala/SHAYRI//RUPO*2/JET/3/ …	5	6	2	3	9	4
19	PETUNIA2	3	15	9	10	1	6
20	ICNBF 93-328	6	2	9	1	2	1
21	MJA/BRB2/QUINA/3/CABUYA/4/ …	7	7	1	1	8	1
22	CERRAJA/3/ATACO/ACHIRA// HIGO	14	17	7	6	1	8
23	ASL-2/5/Cr.115/Pro//BC/3/Api/CM6774//…	10	24	8	4	8	3
24	Harmal	15	18	3	13	1	1
25	Rihane-03	16	19	5	13	1	1
26	ICNBF8-613	5	6	9	2	9	2
27	BF891M-614	3	11	9	4	8	3
28	BF891M-584	19	1	7	11	8	10
29	BF891M-592	3	8	2	5	8	3
30	ANCA/2469//TOJI/3/SHYRI/4/ATACO/5/…	12	11	9	4	8	3
31	Atahualpha/Iraqi Black	3	7	1	11	8	10
32	Atahualpha/IPA 7	6	20	5	2	12	2
33	INON/3/CHAMICO/TOCTE//CONGONA/4/..	2	9	3	6	10	5
34	Atahualpha/CV.Tuwaitha	3	21	7	11	8	10
35	BF891-614	11	18	5	4	12	3
36	ICNBF8-617 (SEL.5AP)	3	10	9	4	10	3
37	CI 10590/CEDRO//OLMO/3/CHINA/LINO	3	22	2	1	8	1
38	MOLA/ALELI/MORA/3/CONDOR-BAR…	10	22	3	15	8	9
39	ICNBF8-653	6	24	3	2	9	2
40	ZARA/BEREMJO/4/DS4931//GLORIA- …	6	13	3	14	12	2
41	Atahualpa	5	24	7	11	8	10
42	RABANO/4/DS4931//GLORIA-BAR/ …	20	24	2	3	11	4
43	PENCO/CHEVRON-BAR/3/ATACO/ …	2	2	2	1	2	1
44	CHENG Du 891/PENCO/CHEVRON-AR/…	6	24	2	1	8	1
45	PENCO/CHEVRON-BAR//CANTUA/3/CIRU	17	21	2	6	13	8
46	BF891M-654	6	11	9	1	2	1
47	ORA/NB1054/3/MOLA/SHYRI//ARUP*/JET	17	21	2	6	13	8
48	Alanda-01	5	7	2	4	12	3
49	LINO//ALISO/C13909.2/4/CEDRO// …	5	7	2	4	12	3

Table 1. Contd.

50	Chamico/tecte//Congona	6	2	2	3	10	4
51	REGENT-BAR/CONOR-BAR/3/MOLA/…	5	16	2	4	13	3
52	PENCO/CHEVRON-BAR//CHENG DU 105/…	17	9	8	6	13	8
53	ALELI/VIRNGA	6	13	9	8	1	7
54	RHODS//TB-B/CHZO/3/GLORIA-BAR/...	17	9	8	6	13	8
55	PINON/3/QUINN/ALOE/CARDO/4/CIRU	8	7	1	1	1	1
56	DC-B/SEN/3/AGAVE/YANALA/TUMBO…	5	23	8	1	13	1
57	BBSC Congana	6	11	4	1	2	1
58	WI 2291	6	2	3	13	3	1
59	Moroc 9-75	2	11	4	1	5	1
60	ICNBF8-654	10	16	7	11	4	10
61	LINO/HIGO/4/CEDRO//MATNAN/EH165L/…	17	9	5	2	2	2
62	TOCTE/PINON/PALTON	5	2	6	3	5	7
63	TOCTE/TOCTE//BERROS/3/PETUNIA...	18	13	7	11	4	10

analysis can be an appropriate replacement for costlier and complex methods.

Compared with hordein electrophoresis, the analysis of monomeric prolamines is easier and more economical, because the extraction method used in extracting these proteins is simpler and requires cheaper chemicals; therefore, like in other plants, the analysis of monomeric prolamines in barley also can be used in a variety of genetic studies, including genetic diversity assessment and identification and classification of genotypes.

Among the patterns in area B, one pattern was proved to be in relation with phenologic traits (days to heading and days to maturity), and this pattern can be used in identifying precocious genotypes. In addition, Jung-cong et al. (2005) reported a positive and significant correlation between B hordein percentage and phenologic traits. Although early ripening varieties yield is usually fewer than that of late ripening varieties, in many of the regions where escaping from the end season drought, diseases or pests is important, or where second cultivation is performed after harvesting barley, early ripeness is vital. Of course, in order to use this pattern as a general molecular marker, we should use QTL mapping and estimate the distance between the coding loci of phenologic traits and the genes that code these proteins, and this is the future goal of this research.

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