Journal of Zhejiang University SCIENCE
ISSN 1009-3095
http://www.zju.edu.cn/jzus
E-mail: jzus@zju.edu.cn

Protein and hordein fraction content in barley seeds as affected by sowing date and their relations to malting quality*

QI Jun-cong (齐军仓)[1,2], CHEN Jin-xin (陈锦新)[1], WANG Jun-mei (汪军妹)[3], WU Fei-bo (邬飞波)[1], CAO Lian-pu (曹连莆)[1], ZHANG Guo-ping (张国平)[†‡1]

([1]*Department of Agronomy, Zhejiang University, Hangzhou 310029, China*)
([2]*Key Laboratory of Oasis Eco-agriculture of Xinjiang Bingtuan, Shihezi 832003, China*)
([3]*Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China*)
[†]E-mail: zhanggp@zju.edu.cn

Received July 29, 2005; revision accepted Sept. 9, 2005

Abstract: The effect of sowing date on grain protein, hordein fraction content and malting quality of two-rowed spring barley was investigated by using ten commercial cultivars with different grain protein content and the relationships among these traits were examined. The results showed that grain protein content and B hordein content increased as the sowing date postponed and were significantly affected by sowing date, while C and D hordein contents were less influenced by sowing date. There were significant differences in grain protein and hordein fraction content among the ten cultivars. The coefficient of variation of D hordein content was much larger than that of B and C hordein contents, suggesting its greater variation caused by different sowing dates. Beta-amylase activity and diastatic power were also significantly affected by sowing date, with malt extract being less affected. Significant differences in measured malt quality were found among the ten cultivars. Grain protein was significantly correlated with B hordein and malt extract positively and negatively, respectively. There was no significant correlation between beta-amylase activity or diastatic power and grain protein content. B hordein was negatively and significantly correlated with malt extract, but no significant correlations between C hordein, D hordein and malting quality traits.

Key words: Barley (*Hordeum vulgare* L.), Sowing date, Protein, Hordein, Malting quality
doi:10.1631/jzus.2005.B1069 **Document code:** A **CLC number:** S511

INTRODUCTION

Barley (*Hordeum vulgare* L.) is widely used as food and feed, but its most economically important use is for malting and brewing. Many barley characters are involved in malt quality, of which protein content is the most important, as it has been observed to be related to malt qualities, such as extract and diastatic power (Weston *et al*., 1993; Eagles *et al*., 1995).

Barley suitable for malting should have low grain protein content, as high protein content will not only reduce malt extract, but also deteriorate final beer quality. On the other hand, protein content has been observed to be correlated with diastatic power (Eagles *et al*., 1995; Molina-Cano *et al*., 1997). This means that protein content has dual effects on malt quality, negative effect by deceasing malt extract and Kolbach index, and positive effect by increasing diastatic power. Diastatic power is closely correlated to beta-amylase activity. For a given cultivar, protein content is positively correlated with beta-amylase activity (Yin *et al*., 2002). However, no significant correlation was detected between beta-amylase activity and protein content among different cultivars planted in the same environment (Zhang *et al*., 2005).

‡ Corresponding author
* Project supported by the National Natural Science Foundation of China (Nos. 30270779 and 30471022) and Specialized Research Fund for the Doctoral Program of Higher Education (SRFDP) (No. 20020335028), China

Therefore, it is imperative to study the genotypic and environmental variations of protein components and their relationships to malt quality.

Hordein, as the main storage protein fraction in barley seeds, accounts for up to half of the total protein in the mature grains, and may be classified into four groups named B, C, D and γ hordeins based on their electrophoretic mobilities (Shewry *et al*., 1985). The B and C fractions account for 70%~80% and 10%~12%, respectively, of the total hordein, while the D and γ fractions are minor components. Molina-Cano *et al*.(2001) examined the genetic (G), environmental (E) and G×E effects on hordein factions in a mutant (TL43) and its parent cultivar, the malting barley cultivar Triumph, in various seasons at Dundee (E Scotland) and Lleida (NE Spain) and found that there was a G×E interaction for B hordein and no such interaction for C and D hordein contents. When studying the effect of grain protein on the malting quality, Howard *et al*.(1996) found that variation in growth conditions resulted in a wide range of grain protein contents and malt extract values, as well as obvious difference in the proportions of the individual B, C and D hordeins in grains.

Great effort has been made to clarify the relationship between hordein fractions and malting quality. Baxter and Wainwright (1979) found that B hordein influenced malting quality, while Shewry *et al*.(1980) and Riggs *et al*.(1983) reported contrasting results. Peltonen *et al*.(1994) studied the effect of B, C and D hordeins on malting quality of northern European barleys and found that the B fraction had some effect on malting quality through changing adjusting diastatic power. Howard *et al*.(1996) evaluated the relationship between D hordein and malting quality of barley under a Mediterranean-type climate in Australia and discovered that the D hordein fraction showed the strongest negative correlation with malt extract across seasons, treatments and cultivars, indicating the existence of a putative causal relationship between D hordein and malting quality. However, Brennan *et al*.(1998) utilized pairs of near-isogenic barley lines, with and without D hordeins, and did not find any relationship between D hordeins and malting quality.

It is often difficult to keep protein content below the upper limit (11.5%), because protein synthesis is variable over the environmental conditions (Smith, 1990). In general, higher available nitrogen in soil (Varvel and Severson, 1987; Weston *et al*., 1993; Eagles *et al*., 1995), and abiotic stresses including drought (Morgan and Riggs, 1981; Coles *et al*., 1991; Grant *et al*., 1991; Birch and Long, 1990) or heat, in particular combination with water stress (Macnicol *et al*., 1993; Savin and Nicolas, 1996) may increase barley protein content. Hence, an understanding of agronomic factors affecting hordein fractions and the relationship between hordein fractions and malting quality is necessary for a producer to take optimal measures to improve grain quality and for breeders to utilize the relationship to boost breeding efficiency. Thus, the objectives of the current investigation were (a) to determine the effect of sowing date on the content of hordein fractions and malting quality; (b) to evaluate the relationship between hordein fractions and malting quality by changing expression of both traits due to changed environments caused by different sowing dates, so as to sort out some of the conflictive results available.

MATERIALS AND METHODS

Cultivars

Ten commercial two-rowed spring malting barley cultivars planted currently in China were used, i.e. cv. Stark, cv. Logan, cv. Favorit, cv. B1202, cv. Xinpi 1, cv. Klages, cv. CA_2-1, cv. CDC Thompson, cv. Celink and cv. ND 14636. These cultivars showed similar growth duration, and different grain protein content.

Field experiment

The field experiment was conducted at the Agriculture Experimental Station of Shihezi University in 2004 and the previous crop was upland cotton. The soil type is grey desert soil with total N of 0.15%, available N, P_2O_5 and K_2O is 116.2 mg/g, 34.8 mg/kg and 234.2 mg/kg, respectively. A split-plot experimental design was used with three replicates, sowing date of main plot recorded and cultivar planted in the sub-plot. Sowing dates were 26 March (D1), 10 April (D2) and 25 April (D3). Each plot was 3.5 m×1.6 m, had 8 rows and 0.2 m between rows. The seeding rate was 350 seeds/m^2. Before sowing, N as urea and P as super-phosphate were applied at the rate of 75 kg/ha and 30 kg/ha, respectively. Another 75 kg/ha of N

was top-dressed in equal proportions during tillering and booting stages, respectively.

Grain sampling and chemical analysis

At maturity, six rows in the middle of each plot were harvested and threshed. The obtained grains were passed over a series of sieves and only the size of fraction between 2.5 and 2.8 mm (about 80% of total sample) was used for subsequent analyses. This eliminated the possibility of influence of grain size on protein content, as observed by Swanston and Molina-Cano (2001). Grain samples were dried at 80 °C in an oven for 2 d, ground in a Tecator Cyclotec sample mill (Tecator AB, Hoganas, Sweden), and then passed through a 0.5 mm sieve. Protein fractions were sequentially extracted according to Shewry *et al*.(1983). Isolation of hordeins was based on the method of Howard *et al*.(1996). Protein content was assayed by Kjeldahl method according to AACC (2000) and multiplied by 6.25. The B, C and D hordeins can bind Coomassie blue to almost the same extent, suggesting that their contents may be estimated accurately by scanning the relevant stained bands (Rahman *et al*., 1982). The measurement of the hordein band (fraction) was carried out using BandScan 2.0, and their contents were calculated using the following formula.

$$\text{Content of hordein fraction (mg/g)} = \frac{V_1 \times 1000/V_2 \times S}{W \times 1000}$$

V_1, the total volume of solution for extraction, ml; V_2, the volume of sample for SDS-PAGE (sodium dodecyl sulphate polyacrylaminde gel electrophoresis), μl; S, the total value of D, C or B hordeins determined by BandScan 2.0, μg; W, the sample weight for extraction.

Beta-amylase activity was measured using the commercial kits (Megazyme Ltd. Ireland) according to McCleary and Codd (1989).

Malt analysis

Grain samples (200 g) were micro-malted in Joe White Micromalting System using the following schedule; steeping: 6:14:8:14:4 h at 16 °C (wet:dry:wet:dry:wet), germination: 96 h at 15 °C, kilning: 24 h at 65 °C. Malt extract (EX) and diastatic power (DP) were measured according to EBC (1975).

Statistical analysis

Differences among means of both sowing dates and cultivars were evaluated using least significant difference (LSD) and the data analysis was performed with SPSS Version 7.5 (SPSS, Michigan Avenue, Chicago, IL, USA). Coefficients of correlation between grain protein and hordein fraction contents, beta-amylase activity, malt extract and diastatic power were calculated.

RESULTS

Effect of sowing dates on grain protein and hordein fraction contents

Significant differences were found in grain protein and B hordein content among sowing dates, although there was no the difference in C hordein and D hordein contents (Table 1). Averaged over ten cultivars, the D3 and D1 treatments had the highest and lowest grain protein and B hordein contents, respectively (Table 2), indicating that the postponed sowing dates led to increased grain protein and B hordein contents. Moreover, the effect of cultivar on grain protein and B hordein contents was much larger than that of sowing date, as reflected by the mean square value. Of the ten cultivars, Klages and Logan had the highest (14.57%) and lowest (12.56%) grain protein content, a highly significant difference. The coefficient of variation (CV) of grain protein content over 3 sowing dates among the 10 cultivars ranged from 7.52% for ND14636 to 2.01% for Klages. Similarly, a dramatic difference in B hordein contents was also found among the ten cultivars (Table 2), with Favorit and CA_2-1 being the highest (127.44 mg/g) and the lowest (34.47 mg/g), respectively. Correspondingly CV

Table 1 Analysis of variance of grain protein and hordein fraction contents (B, C and D) of ten cultivars at three sowing dates

	F-value			
	GP	*BH*	*CH*	*DH*
Sowing date (*D*)	6.98*	8.50*	0.44	0.62
Cultivar (*V*)	19.67**	18.34**	28.18**	13.56**
D×*V*	1.39	1.49	3.69**	4.47**

GP: Grain protein; BH: B hordein; CH: C hordein; DH: D hordein; *Significant at 95% probability level; **Significant at 99% probability level

Table 2 Grain protein content and hordein fraction content (B, C and D) in the 10 cultivars at three sowing dates

Factor	*GP*		*BH*		*CH*		*DH*	
	Mean (%)	*CV* (%)	Mean (mg/g)	*CV* (%)	Mean (mg/g)	*CV* (%)	Mean (mg/g)	*CV* (%)
Sowing date (*D*)								
*D*1	13.19b*	6.37	62.84b	13.34	9.60a	7.87	0.87a	15.46
*D*2	13.82a	6.11	63.85b	11.68	9.91a	6.84	0.80a	16.97
*D*3	13.83a	6.89	75.17a	12.92	9.44a	8.45	0.91a	17.99
Cultivar (*V*)								
Stark	13.16d	3.69	80.78c	10.18	6.44f	6.51	0.83bcd	16.21
Logan	12.56e	5.15	75.79c	10.15	8.36de	5.38	1.03b	11.73
Favorit	14.71a	3.75	127.44a	7.53	10.00c	6.31	1.02bc	3.05
B1202	13.96b	4.46	90.49b	6.06	8.45de	3.58	0.79bcde	14.88
Xinpi 1	13.65bc	4.96	65.74d	2.47	9.61cd	3.79	1.82a	7.13
Klages	14.57a	2.01	42.19f	0.93	8.88cde	7.98	0.96bc	11.11
CA_2-1	13.77bc	4.76	34.47g	4.34	15.13a	2.88	0.70cde	10.88
CDC thompson	12.58e	2.50	35.19g	6.73	7.97e	4.74	0.51ef	22.78
Celink	13.36cd	5.84	51.29e	9.14	12.58b	3.46	0.29f	14.60
ND14636	13.81bc	7.52	69.49d	2.11	9.08cde	3.84	0.63de	25.85
Interaction of *D* by *V*	ns		ns		s		s	

GP: Grain protein; BH: B hordein; CH: C hordein; DH: D hordein; *The same letter within the same column represents no significant difference at 95% probability level; s: Significant difference at 95% probability level; ns: No significant difference at 95% probability level

variation ranged from 10.18% for Stark to 0.94% for Klages, indicating a distinct difference among the cultivars in their environmental response.

The variation of C and D hordein contents was mainly attributed to cultivars. In terms of C hordein content, CA_2-1 ranked the highest (15.13 mg/g) and Stark the lowest (6.44 mg/g), with the difference between them being significant. The CV of C hordein content over sowing dates ranged from 7.98% for Klages to 2.88% for CA_2-1. For D hordein content, Xinpi 1 and Celink were the highest (1.82 mg/g) and the lowest (0.29 mg/g), respectively. In addition, D hordein content had much larger CV of both cultivars and sowing dates than the other three parameters, suggesting its relative instability over environments and genotypes.

Effect of sowing date on beta-amylase activity, malt extract and diastatic power

There was a significant effect of sowing date on beta-amylase activity and diastatic power, but such effect was not found for malt extract (Table 3). Beta-amylase activity and diastatic power increased as sowing date was postponed (Table 4). Thus, D1 and D3 treatments had the lowest and highest beta-amylase activity and diastatic power, respectively. The mean square value of the cultivar was much greater than that of the sowing date for malt extract, indicating that cultivar was the major factor affecting malt extract. The interactions between sowing date and cultivar were significant for all three malt quality traits.

There were significant differences in three malt quality parameters among ten cultivars (Table 4). Favorit had the highest beta-amylase activity (1280.1 U/g) and CA_2-1 the lowest (1022.0 U/g). The CV of beta-amylase activity over sowing dates ranged from 22.86% for Celink to 10.55% for Logan among the ten cultivars. In terms of diastatic power, ND14636 (399.2 WK) ranked the highest and CA_2-1 (329.8 WK) the lowest. The CV of diastatic power over sowing dates ranged from 12.02% for B1202 to 2.90% for Favorit. Concerning malt extract, Favorit and Klages were the highest (78.2%) and lowest (73.3%), with CV ranging from 1.29% (CA_2-1) to 5.71% (Favorit).

Relationships between protein and hordein fraction contents, and malt quality

The coefficients of correlation between grain protein and hordein fraction contents, beta-amylase, malt extract and diastatic power are given in Table 5. Grain protein content was positively correlated with B hordein, but not correlated with C hordein and D hordein contents, suggesting that B hordein is the main component affecting grain protein content. There was a negative correlation between grain pro-

tein content and malt extract. No correlation was found between beta-amylase activity, diastatic power and grain protein content. However, beta-amylase activity was positively correlated with diastatic power. B hordein was negatively correlated with malt extract, whereas C and D hordein were not correlated with any malt quality parameter.

DISCUSSION

It is well documented that protein content in barley grains is genetically controlled, but easily affected by the environmental conditions (Smith, 1990; Kaczmarek *et al*., 1999). The current results showed that barley protein content varied with sowing date

Table 3 Analysis of variance of beta-amylase activity, diastatic power and malt extract of ten cultivars at three sowing dates

	Mean squares			*F*-value		
	AM	*DP*	*EX*	*AM*	*DP*	*EX*
Sowing date (*D*)	1032477	3038.40	0.58	163.24**	46.86**	1.22
Cultivar (*V*)	623825	3576.72	122.43	6.48**	18.23**	58.98**
D×*V*	786207.6	3751.34	236.93	4.08**	9.56**	57.07**

AM: Beta-amylase; DP: Diastatic power; EX: Malt extract; **Significant at 99% probability level

Table 4 Beta-amylase activity, diastatic power and malt extract of 10 cultivars at three sowing dates

Factor	*AM*		*DP*		*EX*	
	Mean (U/g)	*CV* (%)	Mean (WK)	*CV* (%)	Mean (%)	*CV* (%)
Sowing date (*D*)						
*D*1	999.9c*	18.03	340.3c	11.28	76.2a	2.15
*D*2	1158.3b	12.73	365.2b	5.96	76.1a	4.05
*D*3	1260.2a	13.72	381.6a	11.27	76.0a	2.06
Cultivar (*V*)						
Stark	1047.9e	22.69	333.4g	6.43	76.5b	1.36
Logan	1197.6abc	10.55	376.9c	3.13	76.4b	1.85
Favorit	1280.1a	18.34	368.7d	2.90	78.2a	5.71
B1202	1157.9bcd	14.15	323.2h	12.02	76.6b	2.07
Xinpi 1	1112.5cde	11.59	390.3b	4.86	75.6cd	1.43
Klages	1188.6abc	12.81	392.2b	8.65	73.3e	1.41
CA_2-1	1022.0e	15.62	329.8g	16.79	73.4d	1.29
CDC Thompson	1076.0de	12.36	361.6e	4.87	76.5b	1.97
Celink	1066.8de	22.86	348.7f	12.14	76.5b	2.02
ND14636	1245.3ab	19.53	399.2a	4.61	76.0c	2.77
Interaction of *D* by *V*	s		s		s	

AM: Beta-amylase; DP: Diastatic power; EX: Malt extract; *The same letter within the same column represents no significant difference at 95% probability level; s: Significant difference at 95% probability level

Table 5 Analysis of correlation between grain protein content, hordein fraction content (B, C and D), beta-amylase activity, diastatic power and malt extract

	GP	*BH*	*CH*	*DH*	*AM*	*DP*	*EX*
GP	1						
BH	0.3827*	1					
CH	0.1479	0.1126	1				
DH	0.0267	−0.0645	−0.5573**	1			
AM	0.2589	0.2655	0.1095	−0.0182	1		
DP	0.1004	−0.1337	−0.3201	0.3560	0.4859*	1	
EX	−0.4458*	−0.4440*	0.0970	0.1710	−0.2776	0.0648	1

GP: Grain protein; BH: B hordein; CH: C hordein; DH: D hordein; AM: Beta-amylase; DP: Diastatic power; EX: Malt extract; *Significant at 95% probability level; **Significant at 99% probability level

and cultivar, which accords with the findings of Yin *et al*.(2002) and Wang *et al*.(2003). Moreover, a distinct interaction between sowing date and cultivar was not found. Yin *et al*.(2002) and Wang *et al*.(2003) reported no interactions between cultivar and seeding rate, and between cultivar and location, respectively. In contrast, Molina-Cano *et al*.(1997; 2001) found existence of interactions between genotype and location, between cultivar and environment (including location and year). A significant difference was observed among the cultivars in B, C and D hordein contents, which confirms the results reported by Molina-Cano *et al*.(2001). However, we did not find significant relation between cultivar and sowing date (environment) in B hordein content, as reported by Molina-Cano *et al*.(2001).

There was considerable difference among cultivars and environments in beta-amylase activity (Swanston, 1980; Ahokas and Naskali, 1990). Arends *et al*.(1995) reported that the variation of beta-amylase activity among 11 cultivars ranged from 501 U/g to 1100 U/g and among 4 sites from 389 U/g to 1290 U/g, with environmental effect being greater than genotypic effect. Georg-Kraemer *et al*.(2001) examined beta-amylase activity of 10 Brazilian barley cultivars and found that there was a significant difference among cultivars, ranging from 716.72 U/g to 1470.55 U/g. Macnicol *et al*.(1993) studied the changes of beta-amylase activity under the controlled environments and found that water stress in the middle of the grain filling stage enhanced accumulation of beta-amylase, while heat stress had little influence. Similarly the present study showed great variation of beta-amylase activity among sowing dates and cultivars, and the existence of interaction between sowing date and cultivar. In addition, our finding of significant differences among cultivars in the aspects of malt extract and diastatic power confirmed the previous reports (Arends *et al*., 1995; Bishop and Day, 1993; Eagles *et al*., 1995; Gibson *et al*., 1995; Molina-Cano *et al*., 1997) of similar findings. It may be concluded that the enhancement of beta-amylase activity and diastatic power due to delaying of sowing is closely associated with increased grain protein content, resulting in decreased malt extract. Thus, the complex interaction of grain protein content and malt quality traits should be fully taken into account during making the decision of sowing date for a given cultivar.

The relationships among grain protein, hordein fractions and malt quality need to be defined for efficient malting barley breeding and production. The negative and significant correlation between grain protein content and malt extract found in the present study accords with similar findings in other investigations (Howard *et al*., 1996). The positive correlation between beta-amylase and diastatic power observed in this study accorded with several similar findings (Arends *et al*., 1995; Delcour and Verschaeve, 1987; Gibson *et al*., 1995). Swanston (1980) reported a positive correlation between beta-amylase activity and grain protein content. However we did not detect significantly positive correlation between these two factors, as previously reported by Wang *et al*.(2003), indicating the possibility of developing cultivars with the desirable combination of high beta-amylase activity but low grain protein content. The negative correlation between B hordein and malt extract can be explained by the fact that B hordein is the main factor affecting grain protein content. Our finding that the correlation between C or D hordein content and malt extract was not significant further supports the conclusions of Brennan *et al*.(1998) and is contrast to that of Howard *et al*.(1996).

ACKNOWLEDGEMENT

The authors wish to express their gratitude to P. Wang and Z.Z. Jin for skilled technical assistance and the two anonymous reviewers for their valuable suggestions and comments.

References

AACC (American Association of Cereal Chemists), 2000. Approved Methods of American Association of Cereal Chemists, 10th Ed. The Association, St Paul, MN.

Ahokas, H., Naskali, L., 1990. Geographic variation of α-amylase, β-amylase, β-glucanase, pullulanase and chitinase activity in germinating *Hordeum spontaneum* barley from Israel and Jordan. *Genetica*, **82**:73-78.

Arends, A.M., Fox, G.P., Henry, R.J., Marschke, R.J., Symons, M.H., 1995. Genetic and environmental variation in the diastatic power of Australian barley. *J. Cereal Sci.*, **21**:63-70.

Baxter, E.D., Wainwright, T., 1979. Hordein and malting quality. *J. Amer. Soc. Brew. Chemist*, **37**:8-12.

Birch, C.J., Long, K.E., 1990. Effect of nitrogen on the growth, yield and grain protein content of barley (*Hordeum vul-*

gare). *Aust. J. Exp. Agric.*, **30**:237-242.

Bishop, L.R., Day, F.E., 1993. The effect of variety on the relation between nitrogen content and extract. *J. Inst. Brew.*, **39**:545-551.

Brennan, C.S., Smith, D.B., Harris, N., Shewry, P.R., 1998. The production and characterization of Hor3 null lines of barley provides new information on the relationship of D hordein to malting performance. *J. Cereal Sci.*, **28**:291-299.

Coles, G.D., Jamieson, P.D., Haslemore, R.M., 1991. Effects of moisture stress on malting quality in Triumph barley. *J. Cereal Sci.*, **14**:161-177.

Delcour, J.A., Verschaeve, S.G., 1987. Malt diastatic power. Part I. A modified EBC diastatic power assay for the selective estimation of β-amylase activity. Time and temperature dependence of the release of reducing sugars. *J. Inst. Brew.*, **93**:296-301.

Eagles, H.A., Bedggood, A.G.J., Panozzo, F., Martin, P.J., 1995. Cultivar and environmental effects on malting quality in barley. *Aust. J. Agric. Res.*, **46**:831-844.

EBC (European Brewery Convention), 1975. Analytica EBC, 3rd Ed. Verlag Hans Carl, Nurnberg, Germany.

Georg-Kraemer, J.E., Mundstock, E.C., Cavalli-Molina, L., 2001. Developmental expression of amylase during barley malting. *J. Cereal Sci.*, **33**:279-288.

Gibson, T.S., Solah, V., Glennie-Homes, M.R., Taylor, H.R., 1995. Diastatic power in malted barley: contributions of malt parameters to its development and the potential of barley grain beta-amylase to predict malt diastatic power. *J. Inst. Brew.*, **101**:277-280.

Grant, C.A., Gauer, L.E., Gehl, D.T., Bailey, L.D., 1991. Protein production and nitrogen utilization by barley cultivars in response to nitrogen fertilization under varying moisture conditions. *Can. J. Plant Sci.*, **71**:997-1009.

Howard, K.A., Gayler, K.R., Eagles, H.A., Halloran, G.M., 1996. The relationship between D hordein and malting quality in barley. *J. Cereal Sci.*, **24**:47-53.

Kaczmarek, K., Adamski, T., Surma, M., Jezowski, S., Lseniewska-Erstczak, M., 1999. Genotype-environment interaction of barley doubled haploids with regard to malting quality. *Plant Breeding*, **118**:243-247.

Macnicol, P.K., Jacobsen, J.V., Keys, M.M., Stuart, I.M., 1993. Effects of heat and water stress on malt quality and grain parameters of Schooner barley grown in cabinets. *J. Cereal Sci.*, **18**:61-68.

McCleary, B.V., Codd, R., 1989. Measurement of β-amylase in cereal flours and commercial enzyme preparations. *J. Cereal Sci.*, **9**:17-33.

Molina-Cano, J.L., Francesch, M., Perez-Vendrell, A.M., Ramo, T., Voltas, J., Brufau, J., 1997. Genetic and environmental variation in malting and feed quality of quality. *J. Cereal Sci.*, **25**:37-47.

Molina-Cano, J.L., Polo, J.P., Romera, E., Araus, J.L., Zarco, J., Swanston, J.S., 2001. Relationships between barley hordeins and malting quality in a mutant of cv. Triumph I. Genotype by environment interaction of hordein content. *J. Cereal Sci.*, **34**:285-294.

Morgan, A.G., Riggs, T.J., 1981. Effects of drought on yield and grain and malt characters in spring barley. *J. Sci. Food Agric.*, **32**:339-346.

Peltonen, J., Rita, H., Aikasalo, R., Home, S., 1994. Hordein and malting quality in northern barleys. *Hereditas*, **120**:231-239.

Rahman, S., Shewry, P.R., Miflin, B.J., 1982. Differential protein accumulation during barley grain development. *J. Exper. Bot.*, **33**:717-728.

Riggs, T.J., Sanada, M., Morgan, A.G., Smith, D.B., 1983. Use of acid gel electrophoresis in the characterization of "B" hordein protein in relation to mating quality and mildew resistance of barley. *J. Sci. Food Agric.*, **34**:576-586.

Savin, R.S., Nicolas, M.E., 1996. Effects of short periods of drought and high temperature on grain growth and starch accumulation of two malting barley cultivars. *Aust. J. Plant Physiol.*, **23**:201-210.

Shewry, P.R., Faulks, A.J., Parmar, S., Miflin, B.J., 1980. Hordein polypeptide pattern in relation to malting quality and the varietal identification of malted barley grain. *J. Inst. Brew.*, **86**:138-141.

Shewry, P.R., Franklin, J., Parmar, S., Smith, S.J., Miflin, B.J., 1983. The effects of sulphur starvation on the amino acid and protein compositions of barley grain. *J. Cereal Sci.*, **1**:21-31.

Shewry, P.R., Kreis, M., Parmar, S., Lew, E.J.L., Kasarda, D.D., 1985. Identification of *γ*-type hordeins in barley. *FEBS Lett.*, **190**:61-64.

Smith, D.B., 1990. Barley seed protein and its effects on malting and brewing quality. *Plant Varieties Seeds*, **3**:63-80.

Swanston, J.S., 1980. Use of electrophoresis in testing for high diastatic power in barley. *J. Inst. Brew.*, **86**:81-83.

Swanston, J.S., Molina-Cano, J.L., 2001. Beta-amylase activity and thermostability in two mutants derived from the malting barley cv. Triumph. *J. Cereal Sci.*, **33**:155-162.

Varvel, G.E., Severson, R.K., 1987. Evaluation of cultivar and nitrogen management's options for malting barley. *Agron. J.*, **79**:459-463.

Wang, J.M., Zhang, G.P., Chen, J.X., Shen, Q.Q., Wu, F.B., 2003. Genotypic and environmental variation in barley beta-amylase activity and its relation to protein content. *Food Chem.*, **83**:163-165.

Weston, D.T., Horsley, R., Schwarz, P.B., Goos, R.J., 1993. Nitrogen and planting date effects on low-protein spring barley. *Agron. J.*, **85**:1170-1174.

Yin, C., Zhang, G.P., Wang, J.M., Chen, J.X., 2002. Variation of beta-amylase activity in barley as affected by cultivar and environment and its relation to protein content and grain weight. *J. Cereal Sci.*, **36**:307-312.

Zhang, G.P., Wang, J.M., Chen, J.X., Wu, F.B., Ding, S.R., 2005. The effect of cultivar and environment on beta-amylase activity is associated with the change of protein content in barley grains. *J. Agron. Crop Sci.*, in Press.