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Genotypic and environmental variation in phytic acid content and its relation to protein content and malt quality in barley

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Abstract

Phytic acid or phytate is a chelating agent, which is involved in binding minerals (such as K^+, Ca^{2+}, Zn^{2+}, Fe^{2+}, etc.) and making them unavailable for dietary absorption. It is also involved in forming complexes with protein, making protein less soluble, and affecting enzymatic degradation, gastric absorption, and malting processes. The phytic acid and protein contents of barley grains are influenced by genetic and environmental factors. This study investigated differences in phytic acid and protein contents in grains of 100 barley (*Hordeum vulgare* L.) genotypes or cultivars. Eight barley cultivars were selected and grown at seven locations for two years to study the effects of genotypic and environmental factors on phytic acid content (PAC) and its relation to malt quality. The phytic acid contents of 100 barley genotypes ranged from 3.85 mg g^{-1} to 9.85 mg g^{-1}, with a mean of 7.01 mg g^{-1}. The effects of cultivars, locations, time and their interactions were highly significant, but the variation was mainly attributed to the environment (location and time). The correlation between grains phytic acid and protein content was significant and positive. Whereas, the correlation between grain phytic acid content and malt extract was significant and negative. The relationship between phytic acid and protein contents of barley is important as it affects the malting process, malt yield and quality, and final beer quality. Barley grain for malting and feed uses should have low phytic acid content.

Keywords: Barley (*Hordeum vulgare* L.); Environment; Genotype; Phytic acid; Protein; Malt quality

1. Introduction

Phytic acid, *myo*-inositol 1,2,3,4,5,6-hexakisphosphate (InsP6), has long been known as a form of stored phosphorus in seeds grains, where it is reported to be 1% or more of the dry weight and 50–80% of the total phosphorus in seeds (Liu, Cheng, & Zhang, 2005b; Loewus & Murthy, 2000; Lott, Ockenden, Raboy, & Batten, 2000; Ockenden et al., 2004). It is reported that more than 80% of phytic acid in barley grain is localized in the aleurone layer, and the remainder in the germ (O'Dell, de Boland, & Koirtyohann, 1972). Phytic acid is ubiquitous in eukaryotic species and its metabolism may play a number of roles in the eukaryotic cells (Shears, 2001), including phosphorus and mineral storage and homeostasis in developing and germinating seeds (Dorsch et al., 2003). It is reported that phytic acid as an effective chelator of cations, is deposited during seed development, together with some mineral cations, such as K^+, Mg^{2+}, Ca^{2+}, Fe^{2+} and Zn^{2+} (Liu et al., 2005b; Ockenden et al., 2004).

Phytic acid is regarded as an "anti-nutrient" because of its direct or indirect ability to bind minerals, and alter their solubility, functionality, digestibility and absorption, which affect their bio-availability (Bilgiçli, Elgün, & Türker, 2006). Phytic acid is also reported to have the ability to react with some proteins to form complexes, which make the proteins less soluble, and affect enzymatic degradation and peptic digestion (Carnovale, Lugaro, & Lombardi-Boccia, 1988; Elsheikh, Fadul, & El Tinay, 2000).

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Barley grains contain appreciable amount of minerals, but their availability is low because of the formation of insoluble complexes with phytate, a salt of phytic acid (Sandberg et al., 1999; Sandström, Bügel, McGaw, Price, & Reid, 2000), which is one of the main inhibitors for iron and zinc absorption in humans (Eklund-Jonsson, Sandberg, & Alminger, 2006). Efforts have been made by scientists to reduce phytic acid content (PAC) in grains, throught fertilization (Elsheikh et al., 2000). Saastamoinen, Plaami, and Kumpulainen (1992) reported that the phytic acid content in oat grains was increased by nitrogen and phosphorus fertilizer application as well as by high mean temperature during grain filling stage, and was decreased by high precipitation. Understanding of the factors that affect phytic acid accumulation in barley grains is essential for breeders to develop low phytic acid barley cultivars.

It has been reported that barley grains used for malt should have relative low protein content, as higher protein was involved in prolonging the malting process and deteriorated malting produce and final beer quality (Bertholdsson, 1999). The relationship between phytic acid and protein content of barley is also very important as it affects the malting process, malt yield and quality, and final beer quality. The objectives of this study were (1) to determine the influence of genotype and environment on PAC in barley grains and (2) investigate the relationships between phytic acid and malting quality.

2. Materials and methods

One hundred barley (*Hordeum vulgare* L.) genotypes were grown in a field of Zhejiang University, Huajiachi campus, China in 2002–2003 growing seasons for phytic acid and protein content studies. All genotypes were planted in early November, 2002. Eight two-rowed barley cultivars with similar maturity time were selected for the multi-location experiment study that was conducted in two successive years (2000–2001 and 2001–2002 growing seasons) at seven locations. The multi-location experiment was to study the effects of genotypic and environmental factors on phytic acid content and its relation to malt quality. All cultivars were grown in adjacent plots in the same field, and each cultivar consisted of 2-m-length rows with three replications. The field management was the same as applied locally. At maturity, the plants in the mid-line were harvested and grains were mixed as the samples for evaluation.

Grain samples from each replicate were dried at 80 °C for 48 h, milled to pass through a 0.5 mm screen, and stored for analysis. Phytic acid assay was conducted according to Miller, Youngs, and Oplinger (1980) and Liu et al. (2005b) with some modification. The barley sample (0.3 g) was placed into a 50 ml centrifuge tube and 10 ml 0.2 M HCl was added, vibrated in a shaker for 2 h, then centrifuged at 10,000*g* for 10 min. To 2.5 ml of the supernatant 2 ml 0.2% $FeCl_3$ was added, boiled in a bath for 30 min, then centrifuged again at 10,000*g* for 15 min after cooling. Supernatant was discarded and the tube was washed twice with 5 ml de-ionized water. Three millilitres 1.5 M NaOH was added into the residue, vortexed for 2 min and then centrifuged at 10,000*g* for 10 min. Supernatant was discarded and 10 ml 0.5 M HCl was added to dissolve the residue. Finally, de-ionized water was added to the solution up to the volume of 50 ml. The Fe content in the solution was determined by atomic absorption spectrophotometer (Shimadzu, AA6300, Japan). Phytic acid content was calculated by multiplying by the factor of 4.2.

Total protein content in barley grains was determined by near infrared reflectance spectroscopy (NIRA, Matrix-1, Bruker Co., Germany) using a previously established calibration curve (Yin, Zhang, Wang, & Chen, 2002). Protein fraction was separated and analyzed according to Liu, Cheng, Cheng, and Zhang (2005a) with some modification. The 4 protein fractions were sequentially extracted in the order given below by stirring the flour (0.5 g sample using 25 ml solvent) for 2 h at room temperature in the following solvents: albumin, 10 mM Tris-HCl, pH 7.5; globulin, 1 M NaCl, 10 mM Tris-HCl, pH 7.5; hordein, 55% (v/v) *n*-propanol, 10 mM Tris-HCl, pH 7.5; glutelin, 0.24% $CuSO_4$, 1.68% KOH, 0.5% potassium sodium tartrate, and 50% (v/v) *iso*-propanol. After centrifugation at 4000*g* for 10 min at room temperature, the contents of the former 3 protein fractions were determined according to Bradford (1976), with bovine serum albumin (BSA) as the standard protein. Glutelin content was analyzed by the Biuret method, using a calibration curve established by the Kjeldahl method (Holme & Peck, 1998).

Grain samples (200 g) were micro-malted in a Joe White Micro-malting System Apparatus (Adelaide, Australia), with the regime: steeping (6 h, 16 °C), air-rest (14 h, 16 °C), steeping (8 h, 16 °C), air-rest (14 h, 16 °C), steeping (4 h, 16 °C); germination for 96 h at 15 °C; kilning for 24 h at 65 °C; followed by removal of rootlets and acrospires. The malts were milled with a Cyclotec 1093 sample mill (Tecator AB, Hoganas, Sweden) through a 0.5 mm screen. The malt extract was determined according to Analytica EBC Official Methods (European Brewery Convention, 1975).

Analysis of variance was performed using SAS statistical software (PROC GLM). Differences among means were evaluated using the Duncan's multiple range test.

3. Results and discussion

3.1. Phytic acid and protein contents of 100 barley genotypes

The phytic acid content (PAC) of 100 barley genotypes ranged from 3.85 mg g^{-1} to 9.85 mg g^{-1}, with a mean of 7.01 mg g^{-1} (Fig. 1 and Table 1). Most of the barley genotypes (71%) had PAC in the range of 6 mg g^{-1} to 8 mg g^{-1} and only few genotypes (3%) had PAC below 5 mg g^{-1}. The results indicated that differences were found in PAC of 100 barley genotypes, and the PAC range was quite large. The total protein contents of barley cultivars

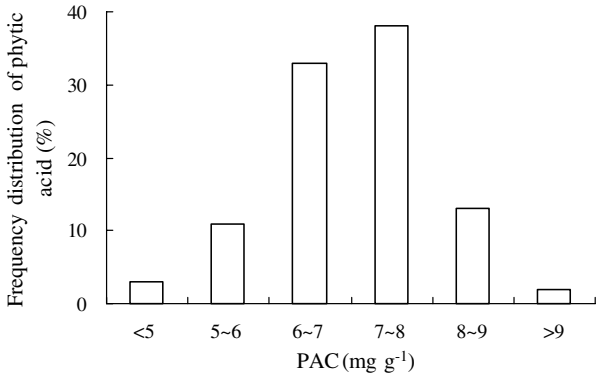

Fig. 1. The phytic acid contents of 100 barley cultivars.

Table 1
The phytic acid content (PAC), total protein content (TPC) and protein fractions of 100 barley cultivars (mg g^{-1})

	PAC	TPC	Globulin	Albumin	Glutelin	Hordein
Highest	9.85	65.2	1.14	5.39	34.7	12.3
Lowest	3.85	151	3.90	18.6	84.4	48.9
Mean	7.01	100	2.19	12.4	57.9	27.6
CV (%)	13.9	14.7	27.6	21.0	17.8	30.7

CV: Coefficient of variation.

ranged from 65.2 mg g^{-1} to 151 mg g^{-1}, with a mean of 100 mg g^{-1} (Table 1). The ranges for barley protein fractions were globulin 1.14 mg g^{-1} to 3.90 mg g^{-1}, albumin 5.39 mg g^{-1} to 18.6 mg g^{-1}, glutelin 34.7 mg g^{-1} to 84.4 mg g^{-1} and hordein 12.3 mg g^{-1} to 48.9 mg g^{-1}; with average protein fractions of 2.19 mg g^{-1} (globulin), 12.4 mg g^{-1} (albumin), 57.9 mg g^{-1} (glutelin), and 27.6 mg g^{-1} (hordein), respectively (Table 1). The correlation between phytic acid (PAC) and total protein content or protein fractions was significant and positive (Fig. 2a–c). The results indicated that phytic acid and protein contents of some barley genotypes were high, and it would be better for barley breeders to develop cultivars with low phytic acid and protein contents.

3.2. Analysis of variance (ANOVA) of phytic acid content of barley cultivars

The analysis of variance (ANOVA) of PAC in 8 barley cultivars grown at seven locations in two successive years showed that the effects of cultivars, locations, time and their interactions were highly significant (Table 2). Comparatively, locations shared the largest contribution to the variation in PAC and followed by years or time, indicating that PAC in barley grains is variable, and environment factors (location and time) had greater impact on it than genotype (cultivar). Genetic and environmental variations in the PAC of cereals and legumes have been reported in several studies (Batten, 1986; Feil & Fossati, 1997; Liu et al., 2005b; Miller et al., 1980; Raboy, Dickinson, & Below, 1984). The results obtained in the present studies showed that environmental and genotypic factors had significant influence on PAC of barley grains, but the former was a predominant factor in influencing the phytic acid content. Liu et al. (2005b) reported similar results in rice, and identified environmental factor as the main contributor of PAC in grains.

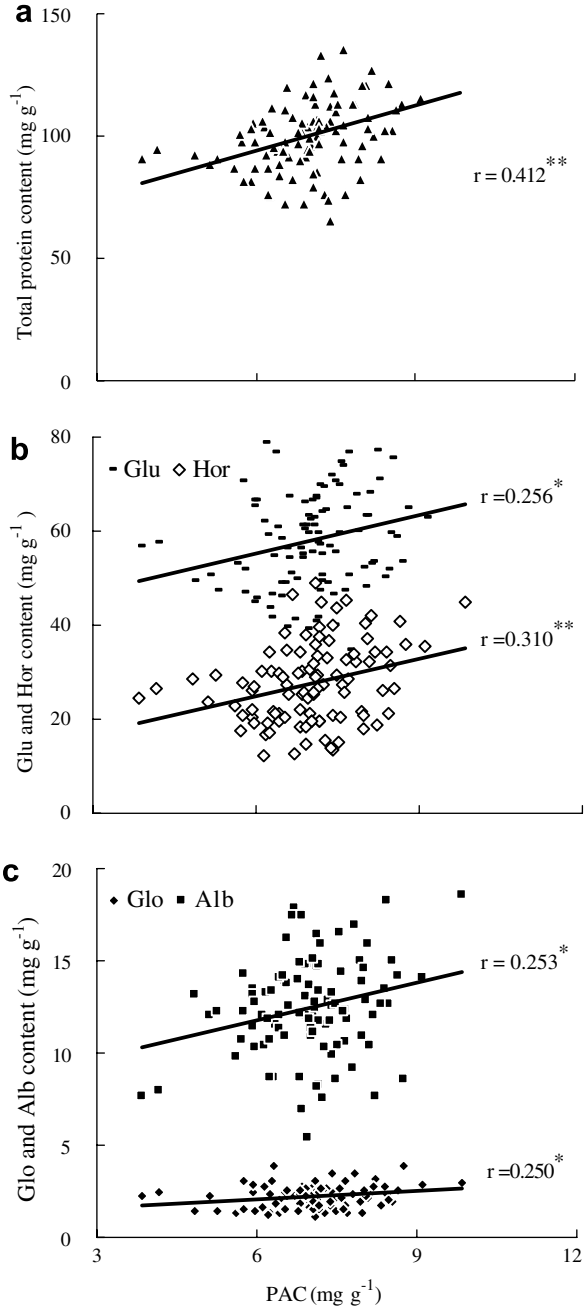

Fig. 2. The correlation between phytic acid content (PAC) and protein content of 100 barley cultivars ($N = 100$): (a) Total protein content versus phytic acid content (PAC); (b) Protein fractions (Glutelin and Hordein) versus PAC and (c) Protein fractions (Globulin and Albumin) versus PAC. Glo, globulin; Alb, albumin; Glu, glutelin; Hor, hordein; * and ** means significant at 0.05 and 0.01 probability level, respectively.

Table 2
The analysis of variance (ANOVA) of phytic acid content (PAC) of eight barley cultivars grown at seven locations in two years

Source	DF	SS	MS	F
Year	1	84.21	84.21	1503.5**
Location	6	296.04	49.34	881.0**
Year × Location	6	16.23	2.70	48.3**
Cultivar	7	16.58	2.37	42.3**
Year × Cultivar	7	9.41	1.34	24.0**
Location × Cultivar	42	39.06	0.93	16.6**
Year × Location × Cultivar	42	28.01	0.67	11.9**
Error	112	6.27	0.056	

SS, sum of square; DF, degrees of freedom; MS, mean square.
** Means significant at 0.01 probability level.

3.3. The effects of genotypic and environmental factors on phytic acid content (PAC) of barley cultivars

The effects of barley cultivars, locations and time on phytic acid contents are presented in Tables 3 and 4. The PAC in barley grains differed between two years in all the seven locations, with 2000–2001 being significantly higher than 2001–2002. The average PAC of the eight barley cultivars grown at seven locations in two years were significantly different, ranging from 2.50 mg g^{-1} for Nanchong to 6.65 mg g^{-1} for Tian'an in 2000–2001, and 2.00 mg g^{-1} for Nanchong to 5.71 mg g^{-1} for Tian'an in 2001–2002, respectively (Table 3). The average PAC was constant, with Nanchong having the lowest values, and Tian'an having the highest values in the two years. Tian'an location, however, had relatively low CV in PAC, indicating that variation in PAC between cultivars was also dependent on environmental (location) factors.

On an average over seven locations, PAC in 8 barley cultivars ranged from 4.82 mg g^{-1} for Dan'er to 6.20 mg g^{-1} for Xiumai 3 in 2000–2001 and 3.72 mg g^{-1} for Dan'er to 4.37 mg g^{-1} for Gangpi 1 in 2001–2002, respectively (Table 4). There was no significant difference between Dan'er and Zheyuan 18 in 2000–2001, and Gangpi 1 and Yanyin 1 in 2001–2002 as presented in Table 4. Dan'er barley cultivar had the lowest PAC in both years. However, the barley cultivar with the highest PAC differed in the two years. The ANOVA results indicated that there was a significant difference in the PAC of barley cultivars grown at different locations, and the variation in location may be responsible for the higher CV value. The relatively small difference among cultivars and large difference among locations in PAC indicated that the variation in PAC was mainly dependent on the environmental factors.

3.4. Relationship between phytic acid content, protein content and malt extract

Analysis of the correlation among PAC and protein content of 100 genotypes indicated that PAC was significantly and positively associated with total protein or protein fraction contents (globulin, albumin, glutelin and

Table 3
The effect of location on phytic acid content (PAC) of eight barley cultivars grown at seven locations in two years

Location	2000–2001				2001–2002			
	Lowest	Highest	Mean	CV (%)	Lowest	Highest	Mean	CV (%)
Hangzhou	5.50	6.39	5.90b	6.2	4.35	5.38	4.68b	7.5
Jingzhou	4.74	5.83	5.39c	6.1	4.17	5.68	4.72b	11.7
Nanchong	1.19	3.38	2.50d	29.5	1.00	2.89	2.00e	36.9
Putian	3.78	6.46	5.25c	15.3	3.10	3.88	3.56c	7.0
Yancheng	4.51	8.72	6.00b	22.9	3.93	4.99	4.57b	8.3
Taian	5.95	8.33	6.65a	12.1	5.01	6.19	5.71a	7.1
Zhengzhou	3.79	6.36	5.38c	17.3	2.05	4.18	3.24d	21.1
Mean (mg g^{-1})			5.30				4.07	

CV: Coefficients of variation; means with different alphabets within a column were significant at 0.05 probability level.

Table 4
The effect of barley cultivars grown at seven locations in two years on the phytic acid content (PAC)

Cultivar	2000–2001				2001–2002			
	Lowest	Highest	Mean	CV (%)	Lowest	Highest	Mean	CV (%)
Zheyuan 18	1.19	6.29	4.88e	36.3	1.51	5.44	3.81cd	31.9
Zhepi 4	2.48	6.28	5.23cd	25.0	2.00	5.79	3.78d	33.6
ZAU 3	1.79	7.25	5.34c	33.3	2.35	6.16	4.09bc	29.8
Xiumai 3	3.38	8.72	6.20a	29.5	1.09	6.19	4.04cd	38.4
Gangpi 1	2.64	6.76	5.58b	24.7	2.86	5.50	4.37a	21.0
Suyinmai 2	2.29	6.61	5.15d	27.9	2.31	6.00	4.26ab	30.2
Yanyin 1	3.08	6.74	5.17d	24.9	2.89	5.68	4.48a	24.4
Daner Damai	3.13	6.11	4.82e	22.2	1.00	5.38	3.72e	44.2
Mean (mg g^{-1})			5.30				4.07	

CV: Coefficients of variation; means with different alphabets within a column were significant at 0.05 probability level.

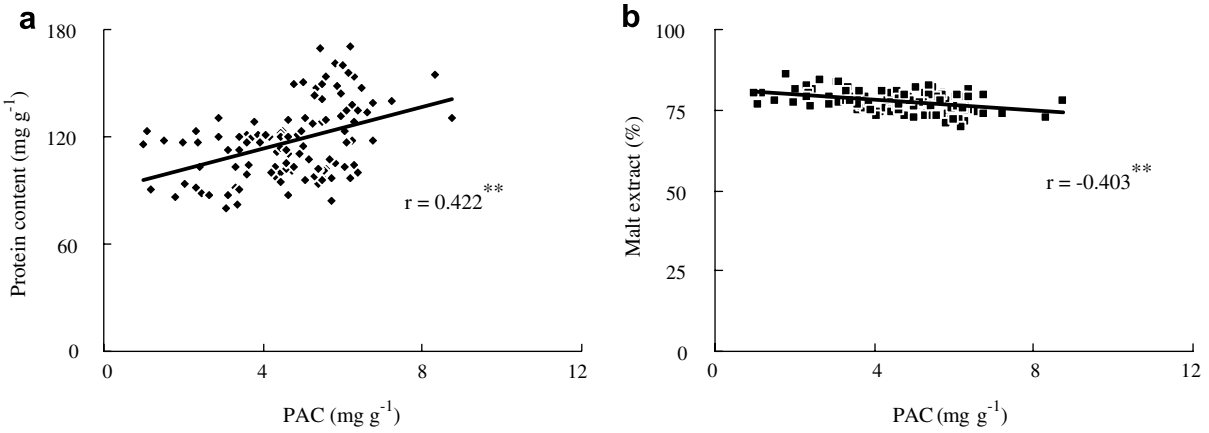

Fig. 3. The correlation between: (a) Phytic acid content (PAC) and total protein content and (b) PAC and malt extract from eight barley cultivars grown at seven locations in two years. **Means significant at 0.01 probability level.

hordein) as presented in Fig. 2a–c. The correlation between PAC and total protein content was also highly significant and positive for eight barley cultivars grown at seven locations in two years. In contrast, PAC was significantly and negatively correlated to malt extract (Fig. 3). The results indicated that high PAC in barley grains may interfere with the malting process, malt yield and quality, and beer quality.

Several studies reported that there was a correlation between phytic acid and protein contents faba bean or legumes (Chitra, Vimala, Singh, & Geervani, 1995), oat (Saastamoinen et al., 1992) and soybean (Raboy et al., 1984). In contrast, some researchers found no significant correlation between phytic acid and protein contents in *faba* bean or common bean (Griffiths & Thomas, 1981; Lolas & Markakis, 1975), and rice (Liu et al., 2005b). The results from the present study showed that the correlation between phytic acid and protein contents was significant and positive, indicating the possibility of developing the barley cultivars with low phytic acid and protein contents, simultaneously. Moreover, as an important quality parameter, malt extract was significantly and negatively correlated with PAC, indicating that high PAC in barley grains was also undesirable for malt production. In malt barley production, the grains with low phytic acid content should be selected for improving malt quality.

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