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Grain phytic acid content in japonica rice as affected by cultivar and environment and its relation to protein content

Zhenghui Liu, Fangmin Cheng, Guoping Zhang *

Department of Agronomy, Huajia Chi Campus, Zhejiang University, 310029, Hangzhou, China

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Abstract

Phytic acid and protein contents in grains of japonica rice cultivars collected from different areas of China were assayed in this study. Meanwhile, a multi-location trial was conducted to determine phytic acid content of 24 cultivars grown at 4 locations with wide differences in ecological conditions. For 72 cultivars, phytic acid content ranged from 0.685% for Xiu217 to 1.03% for Huai9746, with a mean of 0.873%, and protein content ranged from 6.45% for Xiu52 to 11.1% for K45, with a mean of 8.26%. There was no significant correlation between grain phytic acid and protein content, suggesting the possibility of breeding japonica rice cultivars with low phytic acid and high protein contents. The effects of cultivars, environments (locations) and their interactions on phytic acid content were all highly significant, with the location having the largest effect. The highly significant interaction between cultivar and environment suggests that the correct evaluation of rice germplasm by phytic acid content should be conducted in multi-environments.

Keywords: Cultivar; Environment; Phytic acid; Protein; Rice

1. Introduction

Phytic acid, *myo*-inositol 1,2,3,4,5,6-hexakisphosphate ($InsP_6$), has long been known as a form of stored phosphorus (P) in seeds. Approximately 70% of total phosphorus in seeds coexists with phytic acid and its content typically accounts for 1% or more of seed dry weight (Lott, 1984). During seed development, it is deposited as a mixed "phytate", together with some mineral cations, such as potassium, magnesium, calcium, iron, and zinc. Decomposition of phytate in germinating seeds may provide P, *myo*-inositol (MI) and mineral cations for the growth of seedlings (Raboy, 1990).

In animal, as well as human, nutrition, phytic acid in staple foods, including legumes and cereals has been of much concern (Erdman, 1981; Harland & Morris, 1995; McCance & Widdowson, 1935), as it is a strong chelator of mineral nutrients, such as Ca, Zn and Fe. The complex of phytic acid and mineral elements, in the form of phytate, results in a marked reduction in bio-availability of these nutrient elements (Erdman, 1981). $InsP_6$-induced low nutrient uptake can contribute to a major public heath problem, iron and zinc deficiency, in populations that live mainly on grains and legumes. Children and pregnant women, in particular suffer from such a risk. Moreover, more $InsP_6$ will be excreted by animals living on a high phytic acid feed, thus posing the threat of contaminating water (Raboy, 2001).

On the other hand, a beneficial effect of $InsP_6$ on human health has also been found in some recent studies (Harland & Morris, 1995; Ko & Gold, 1990; Thompon, 1993). It is reported that phytic acid has some anti-cancer and anti-oxidant functions and prevents coronary disease. Up to now it is still unclear whether phytic acid should be reduced to the lowest level in cereals and legumes intended for human food. It seems reasonable

Abbreviations: PAC = phytic acid content; $InsP_6$ = *myo*-inositol 1,2,3,4,5,6-hexakisphosphate.

* Corresponding author. Tel.: +86-571-86-971-115; fax: +86-571-86-971-115.
E-mail address: zhanggp@zju.edu.cn (G. Zhang).

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to control phytic acid contents in edible parts of crops to a level in which the medical and health functions of the food may be maintained and bio-availability of minerals is not much altered (Febles, Arias, Hardisson, Rodriguez-Alvarez, & Sierra, 2002).

Rice is one of the most important cereal crops worldwide, providing 23% of global human per capita energy and 16% of per capita protein (IRRI, 1997). It grows widely and finds many uses. Commonly, it is consumed as cooked rice or used as a raw material for food and stock-feed industries. An understanding of the relationship between grain phytic acid and protein content and the factors that affect the phytic acid accumulation in grains is essential for breeders to develop low-phytate rice cultivars with high protein content and for producers to modify agronomic practice to achieve lower phytic acid level. However, little is known about these aspects of phytic acid in japonica rice. The objectives of this study were to (a) determine the effect of cultivar and environment on phytic acid content (PAC) in rice grains, and (b) study the relationship between grain phytic acid and protein by analysis of japonica rice cultivars from different areas of China.

2. Materials and methods

Seventy-two japonica rice cultivars or breeding lines were collected from different areas of China. They were seeded in late May and transplanted in late June 2003 at the experimental farm of Zhejiang University (Huajia Chi Campus). Each genotype was grown into two replicates of an eight-row plot, 2 m long and 20 cm between rows. There were 9 hills for each row with 3 seedlings per hill. The trail was managed according to locally recommended agronomic practices. At maturity, thirty spikes were harvested from the genotypes with similar mature dates.

Meanwhile, 24 rice cultivars with substantial difference in phytic acid contents were selected and grown at four ecologically-different locations of China, i.e. Hangzhou and Jiaxing (Zhejiang province), Changzhou (Jiangsu province), and Xi'an (Shannxi province). All cultivars were grown adjacently in the same field and each cultivar consisted of three 2 m length rows with two replicates. At maturity, thirty spikes were harvested from each replicate.

Samples from each replicate were dried at 60 °C for 48 h, milled to pass through a 1.54 mm screen, and stored in a desiccator until analysis. Protein content was determined by the Kjeldahl method. For phytic acid determination, the method described by Miller, Youngs, and Oplinger (1980) was used with some modifications. In our research, phytic acid was extracted by 0.2 M HCl rather than by 3% tri-chloro acetic acid (TCA).

3. Results and discussion

Great differences could be found among rice genotypes in phytic acid and protein content (Table 1). Phytic acid content ranged from 0.69% for Xiu217, a breeding line in Zhejiang, to 1.03% for Huai9746, a commercial cultivar in Jiangsu, with a mean of 0.87% over all 72 genotypes. Protein content ranged from 6.45% to 11.1%. K45, a cultivar in Jiangsu had the highest protein content, whereas Xiu52, a cultivar in Zhejiang, ranked the lowest.

The large variation in phytic acid content among rice cultivars indicates the possibility of developing the cultivars with low phytic acid contents in grains. However, it has be shown that there is a closely positive association between the contents of phytic acid and total phosphorus (TP), protein, some mineral nutrients, including Ca, Mg, and Zn, as indicated in studies on soybean, winter wheat, and maize (Feil & Fossati, 1997; Raboy, Below, & Dickinson, 1989; Raboy & Dickinson, 1984; Raboy, Dickinson, & Below, 1984; Raboy, Noaman, Taylor, & Pickett, 1991). Hence, there is concern over selection of the cultivars with low phytic acid content, in case this causes a corresponding reduction in protein and mineral contents. Raboy (1990) suggested that breeding schemes should be designed to avoid occurrence of these negative effects accompanying the by lowering of phytic acid content in grains. In contrast, no significant correlations between phytic acid and protein content were found by using different cultivars of *faba* bean or common bean (Griffiths & Thomas, 1981; Lolas & Markakis, 1975). In this study we did not find any relationship between phytic acid and protein content among 72 rice genotypes ($r = 0.1088$, $p > 0.05$). Thus, Xiu11 and Huai6 had relatively low phytic acid contents and very high protein contents, suggesting the possibility of breeding the rice cultivars with low phytate and high protein content.

Variance analysis of PAC of twenty-four rice cultivars, grown at four locations in China, showed that the

Table 1
Frequency distribution of phytic acid and protein content in grains for 72 rice genotypes

Phytic acid (%)	Frequency	Protein content (%)	Frequency
<0.6	0	<6	0
>0.6–< 0.7	2.8	>6–< 7	14.6
>0.7–< 0.8	12.5	>7–< 8	18.8
>0.8–< 0.9	47.2	>8–< 9	20.8
>0.9–< 1.0	33.3	>9–< 10	12.5
>1.0–< 1.1	4.2	>10–< 11	4.2
>1.1	0	>11	2.1
Mean (%)	0.873		8.256
SD	0.086		0.204

Table 2
Analysis of variance of phytic acid content in grains of 24 cultivars grown at 4 locations

Source of variance	d*f*	SS	MS	*F* value
Cultivar	23	0.8883	0.0386	15.55[a]
Location	3	2.4204	0.8068	324.75[a]
Interaction	69	0.1714	0.0025	7.06[a]
Error	96	0.0334	0.00035	
Total	191	2.4130		

[a] Significant at 0.01 probability level.

effects of cultivars, locations and their interaction were all highly significant (Table 2). Comparatively, locations shared a greater contribution to the variation in PAC than cultivars or interaction, indicating that PAC is variable, depending on the environment. On average in four locations, PAC for 24 cultivars ranged from 0.675% for Bing99213 to 0.942% for Huai9726, with a mean of 0.815% (Table 3). Moreover, a great difference was noted among cultivars in the coefficient of variation (CV) when planted at different locations. For instance, Huai6 and K45 had the largest and least CV, respectively, suggesting that there is a great difference among cultivars in their response to environments in terms of PAC. Averaged over 24 cultivars, PAC of four locations ranged from 0.690% at Jiaxing to 0.966% at Changzhou (Table 3). Coefficient of variation in PAC for the 24 cultivars grown at a given location, was much smaller than that contributed by a cultivar grown in the 4 locations.

Genetic and environmental variations in the PAC of cereals and legumes have been reported in several studies (Batten, 1986; Feil & Fossati, 1997; Miller et al., 1980; Raboy et al., 1984). In the present study, significant genetic and environmental effects were also found, but the environmental effect appears to be predominant in determining phytic acid content. In a four-year (Y) experiment with four oat cultivars (C) at three locations (L), Miller et al. (1980) found that there were no significant interactions in the $C \times L$, $C \times Y$, and $C \times L \times Y$. In this study we planted 24 japonica rice cultivars at 4 locations with wide differences of ecological conditions, and found significant interaction between location and cultivar in terms of PAC, indicating the importance of using suitable cultivars for a given location, so as to control phytic acid content. In addition, it is suggested that the comprehensive evaluation of rice germplasm for phytic acid content should be conducted in multi-environments.

Table 3
Phytic acid contents (%) of 24 japonica rice cultivars grown at four locations[a]

Cultivar	Location				Mean	CV%[b]
	Changzhou	Hangzhou	Xi'an	Jiaxing		
Huai9726	1.06	1.07	0.822	0.821	0.942a[c]	14.0
K45	1.07	0.957	0.833	0.828	0.921b	11.6
9944	1.060	0.963	0.773	0.849	0.911b	13.0
Huai9140	1.05	0.971	0.756	0.751	0.881c	11.7
Wuyun11	1.051	0.940	0.768	0.747	0.876c	15.5
Chang2201	0.986	0.883	0.819	0.729	0.854c	14.3
Wuyun7	1.03	0.893	0.764	0.722	0.851d	16.5
Huai9926	0.962	0.916	0.767	0.739	0.846d	18.1
Chang164	1.023	0.937	0.697	0.706	0.840de	12.0
Huai238	0.965	0.872	0.757	0.719	0.828de	18.0
T31	0.994	0.833	0.758	0.691	0.819ef	15.7
Huai68	0.964	0.930	0.700	0.678	0.818fg	12.8
Huai9142	0.943	0.862	0.728	0.667	0.800fg	14.6
Nan06	0.945	0.862	0.657	0.721	0.796gh	15.2
Wuyun3	0.945	0.862	0.657	0.721	0.796h	17.5
Nan08	0.892	0.951	0.690	0.632	0.791h	15.1
Bing98110	0.926	0.761	0.733	0.626	0.761i	17.5
76-1	0.935	0.819	0.650	0.635	0.760i	18.8
Xiushui52	0.930	0.838	0.656	0.604	0.757i	15.2
Chang165	0.90	0.853	0.650	0.611	0.753i	17.8
Huai6	0.892	0.816	0.699	0.601	0.752i	19.2
Xiushui63	0.894	0.788	0.640	0.608	0.733j	16.9
Xiushui11	0.835	0.722	0.647	0.613	0.704k	13.1
Bing99213	0.806	0.701	0.643	0.551	0.675l	14.7
Mean	0.966a[c]	0.878b	0.725c	0.690d	0.815	
CV%	7.9	9.8	8.7	11.3		

[a] Means of duplicate analyses.
[b] CV = coefficient of variation.
[c] Means within a column followed by a different letter are significantly different ($P < 0.05$).

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