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Density alteration of nutrient elements in rice grains of a low phytate mutant

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Abstract

The amount and distribution of major nutritionally relevant elements was determined in the grains of a low phytate rice (*Oryza sativa* L.) mutant line (HIPi1) and its parent variety Xieqingzao (XQZ). The concentration of phytate P (PA-P) was significantly reduced in HIPi1 grains. On brown rice basis, HIPi1 had a content of PA-P about 58.4% that of XQZ, while total P levels were not significantly different between the mutant and its parent. Significant location effects were observed on the amount of the major elements, i.e., Ca, K, Mg, Fe, Zn, in various parts of grains of both HIPi1 and XQZ. In milled rice, larger amounts of these elements were found, consistently and significantly across all three locations, in HIPi1 than XQZ, at rates on average of 32.6%, 31.2%, 44.8%, 52% and 71.3%, respectively. The results showed that the low phytate mutation could not only potentially increase the bioavailability but also the amount of most important micronutrient elements, i.e., Fe, Zn and Ca, in the edible part of rice grains, and thus provide an important added-value to this mutation.

Keywords: Low phytic acid; Mineral nutrient; Mutation; *Oryza sativa* L.

1. Introduction

Humans require at least 49 nutrients including macro- and microelements to meet their metabolic needs (Welch & Graham, 2004). Malnutrition, resulting from insufficient intake of one or a number of nutrients, has become a worldwide issue (Caballero, 2002; WHO, 1999). Micronutrient malnutrition alone, predominantly resulting from Fe and Zn deficiency, is afflicting over three billion people (Welch & Graham, 2004). Recently, breeding for enriched bioavailable Fe, Zn, and provitamin A carotenoids in edible portions of several staple food crops has become a new strategy – biofortification in fighting malnutrition (Bouis, 2002; Graham, Welch, & Bouis, 2001) and is making steady progress in rice, wheat, maize, bean, and cassava (see reviews Graham et al., 2001; Welch & Graham, 2004).

One of the strategies to enhance the bioavailability of mineral element nutrients is to reduce antinutrients such as phytic acid in staple food grains (Raboy, 1997, 2002; Welch & Graham, 2004). Phytic acid (myo-inositol 1,2,3,4,5,6-hexa*kis*phosphate or Ins P_6), the major storage form of phosphorus in cereal grains and typically accounting for 65–85% of seed total P (Raboy, 1997; Strother, 1980), is an effective chelator of cations, including those of micronutrient elements Fe, Zn and Ca. Phytic acid could render micronutrient elements biologically unavailable to

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humans and non-ruminant animals (Fairweather-Tait & Hurrell, 1996) and is hence considered the uppermost anti-nutritional factor in cereal grains, though it might as well play some beneficial roles in human diets, e.g., by acting as an anticarcinogen (see review Shamsuddin & Vucenik, 1999). Reduction of phytic acid content is widely regarded as an efficient way to increase the bioavailability of mineral micronutrients in staple foods (Raboy, 2001; Welch, 2002). In light of this, low phytic acid (or phytate, a salt of the phosphorus-rich compound phytic acid) cereals and legumes are developed by using chemical or physical mutation induction, with phytate phosphorus (PA-P) reductions of about 37–95%, and beneficial effects were already demonstrated in animal studies (Raboy, 2002; Ren & Shu, 2004).

It has been well documented that large amounts of nutritionally important mineral elements, e.g., Zn, Fe, Ca and Mg, are co-enriched with PA-P in rice bran (including pericarp, seed coat, embryo, and aleurone), while fewer mineral elements are found in the starchy endosperm through mechanisms yet to be unveiled (O'Dell, de Boland, & Koirtyohann, 1972). Formation of phytate and their deposition in the globoids, mostly in bran part of rice grain (Liu, Ockenden, Truax, & Lott, 2004; Pomeranz, 1973), might be a reasonable explanation for such co-enrichment. Liu et al. (2004) recently reported that, on a brown rice basis, fewer amounts of total P, Ca, Mn and PA-P were present in a low phytic acid rice mutant (*lpa1-1*) when compared with its parent variety Kaybonnet, while the amount of K, Mg and Fe remained unchanged. Since only milled rice is usually consumed as food, it would be desirable to investigate the changes of major macro- and microelements in different parts of rice grains for a holistic evaluation of the potential value of low phytic acid rice as food or feed. In this study, we determined the changes of the amount and distribution of major nutritionally relevant elements in the grains of a low phytate mutant and its parent variety produced in three different locations during different seasons.

2. Materials and methods

2.1. Rice materials and production

The low phytate rice used in this experiment was an *indica* mutant line, *c.v.* HIPi1. It was developed through γ ray irradiation of dried seeds of Xieqingzao B (XQZ) (Wang et al., 2005). The low PA-P content of HIPi1 was controlled by a single recessive gene, which is allelic to the *lpa1-1* locus (Ren, 2005), the mutation caused phytate reduction in the mutant of Kaybonnet (Larson, Rutger, Young, & Raboy, 2000).

Both HIPi1 and XQZ were grown in different seasons/locations, side by side at all locations. The seasons and places were from March 2003 to July 2003 in Jiande of Zhejiang Province (30.2 °N, 120.1 °E), from May to September 2003 in Jiaxing of Zhejiang Province (31.3 °N, 120.6 °E) and from December 2003 to March 2004 in Linshui of Hainan Province (20.0 °N, 110.3 °E), China. The soil fertility levels of the paddy fields were low in Lingshui, medium in Jiande, and high in Jiaxing.

2.2. Rice seed partitioning

Seeds were dried by natural sunlight after harvest and stored at room temperature. Before partitioning, all samples were further dried at 60 °C for 8 h to reach comparable moisture content. Rice seeds were then divided into three components: rice hull (through dehulling using a Satake Dehuller, Satake Corporation, Japan), rice bran (the parts removed from brown rice, including pericarp, seed coat, embryo, and aleurone using a Satake Test Mill, Satake Corporation, Japan) and milled rice (including subaleurone and starchy endosperm). The degree of milling was assured similar to commercial milling by controlling the milling time. Milled rice and rice hull was ground into flour using a Cyclone Sample Mill (UDY Corporation, USA). All samples (hull, bran and milled rice flour) were passed through a 2-mm sieve and stored in desiccators till analysis.

2.3. Chemical analysis

Total P was determined colorimetrically (Chen, Toribara, & Warner, 1956). Analysis of phytate content was performed on anion-exchange HPIC (high performance ion chromatography, Dionex, USA) using phytate dodecasodium salt from rice (P-3168, Sigma) as a standard. Sample pretreatment was done according to Dorsch et al. (2003), and the chromatography assay was carried out in triplicate as described by Phillippy, Bland, and Evens (2003). Both total P and PA-P were expressed as their P (atomic weight 31) content on a dry matter basis to facilitate comparison between various components.

The concentration of major mineral elements was determined by using ICP-OES (optical emission spectrometry with inductively coupled plasma). Samples were prepared as described by Koplik, Curdova, and Suchanek (1998), subsequently measured on a PU7000 ICP spectrometer (Philips, Cambridge, UK) according to Fingerova and Koplik (1999) in triplicate. Analytical accuracy of the determinations was verified by simultaneous analysis of reference material SRM 1568a Rice Flour (NIST, USA). The element distribution was expressed by the percentage of a given element in hull, bran and milled rice. It was calculated by multiplying the weight percentage of that part (hull, bran or milled rice) in a rice grain (data not shown) by the concentration of a given element in that part.

2.4. Statistical analysis

Multiple comparison analysis was performed using the Statistical Analysis System (SAS 8.0 Institute, Inc., Cary, NC, USA). Data were expressed as the mean with standard deviation (SD) and compared by one-way analysis of variance (ANOVA), followed by the Duncan's test.

3. Results

3.1. Environmental impacts

Significant environmental effects on both concentration and distribution of elements were observed in both HIPi1 and XQZ grains. The effect seemed most profound in hull, where the concentrations of every element differed significantly among three locations (Tables 1–3). In bran, the concentrations of K, Ca, Zn and Cu also differed significantly among three locations, while other elements had similar concentrations in two of the three locations tested (Tables 1–3). In milled rice, no significant differences were observed for total P and PA-P among three locations, but the situation was similar to that in bran for other elements (Tables 2 and 3).

Another indicator of significant environmental effects on the amount of elements is the large ranges of concentration variation among the three locations. In some cases, the highest concentration attained in one location was a couple of times larger than the lowest in another location for a given element in the same part of grain, i.e., the concentration of Cu reached 11.9 μg/g in rice bran of XQZ grains produced in Jiaxing was about 5 times of that in grains produced in Lingshui (Table 3). The location differences were in many cases far larger than those between HIPi1 and XQZ in any given location for the same part and element, except for the PA-P (Tables 1–3).

Furthermore, the performances of HIPi1 and XQZ at different locations were not necessarily the same, that is, the concentrations of a given element in a given part of HIPi1 among three locations were often not in the same order as those of the same element in the same part of XQZ. For example, the Fe concentration was highest in Jiaxing, second in Jiande, and lowest in Linshui in milled rice of HIPi1, with the level of 19.9, 17.0 and 15.0 μg/g (all significantly different from each other), respectively, but the concentrations were not significantly different between the grains of XQZ produced in Jiande and Jiaxing, which were all significantly higher than that in Linshui (Table 3). The concentrations of PA-P in hull and bran, and Mg in hull of HIPi1 were in the same order as those in corresponding parts of XQZ (Tables 1 and 2).

3.2. Concentration changes

3.2.1. Phosphorus compounds

In general, the low phytate mutant line HIPi1 had slightly higher concentrations of total P than its parent XQZ either in individual parts of the grain or the rice grain as a whole, but none of the differences was statistically significant (Table 1). As expected, the amounts of PA-P were significantly lower in all parts of HIPi1 grains compared those of XZQ. Least reduction occurred in bran (36.4%), most in hull (58.3%) (Table 1), and consequently leading to reductions of 41.6% and 42.5% on the basis of brown rice and whole grain, respectively.

PA-P occupied a large proportion of total P in all parts of grains. In XQZ, it constituted as high as 68.2%, 86.7%, and 47.3% of total P in hull, bran, and milled rice, respectively. In HIPi1, although the level of total P remained almost unchanged, the proportions of PA-P to total P were all significantly lower (26.9%, 54.3%, and 22.2%, respectively) than in XQZ.

3.2.2. Macronutrient elements

Ca, K and Mg are the major macro-elements of nutritional relevance in rice grains. The concentrations of all three elements, except Ca on rice grain basis, were

Table 1
Concentration of total P and phytic acid P in rice grains of a low phytate rice mutant (HIPi1) and its parent XQZ[1]

Items	Locations		Hull	Bran	Milled rice	Brown rice	Rice grain
Total P (mg/g)	Linshui	XQZ	$0.87 \pm 0.07^{B*}$	9.05 ± 0.94^{B}	2.15 ± 0.02	3.33 ± 0.02	2.71 ± 0.06
		HIPi1	0.75 ± 0.07^{b}	9.19 ± 0.61^{c}	2.10 ± 0.17	3.32 ± 0.14	2.79 ± 0.13
	Jiande	XQZ	0.52 ± 0.04^{C}	11.38 ± 1.00^{A}	1.89 ± 0.07	3.30 ± 0.10	2.77 ± 0.08
		HIPi1	$0.79 \pm 0.07^{b*}$	11.71 ± 0.39^{a}	$2.24 \pm 0.14^{*}$	3.68 ± 0.08	3.02 ± 0.07
	Jiaxing	XQZ	1.25 ± 0.14^{A}	10.58 ± 1.07^{A}	2.06 ± 0.21	3.31 ± 0.26	2.93 ± 0.19
		HIPi1	1.42 ± 0.09^{a}	10.60 ± 1.22^{b}	$2.40 \pm 0.22^{*}$	3.46 ± 0.53	3.07 ± 0.61
	Average	**XQZ**	$\mathbf{0.88 \pm 0.37}$	$\mathbf{10.3 \pm 1.18}$	$\mathbf{2.0 \pm 0.13}$	$\mathbf{3.3 \pm 0.02}$	$\mathbf{2.8 \pm 0.11}$
		HIPi1	$\mathbf{0.93 \pm 0.38}$	$\mathbf{10.5 \pm 1.26}$	$\mathbf{2.2 \pm 0.15}$	$\mathbf{3.4 \pm 0.18}$	$\mathbf{2.9 \pm 0.15}$
Phytic acid P (mg/g)	Linshui	XQZ	$0.69 \pm 0.09^{B**}$	$7.88 \pm 0.07^{B**}$	$0.93 \pm 0.01^{**}$	$2.11 \pm 0.14^{*}$	$1.75 \pm 0.21^{*}$
		HIPi1	0.28 ± 0.06^{b}	5.06 ± 0.32^{b}	0.51 ± 0.01	1.29 ± 0.18	1.07 ± 0.04
	Jiande	XQZ	$0.27 \pm 0.04^{C**}$	$9.40 \pm 0.72^{A**}$	$0.92 \pm 0.11^{**}$	$2.17 \pm 0.32^{*}$	$1.81 \pm 0.15^{*}$
		HIPi1	0.11 ± 0.01^{c}	5.99 ± 0.39^{a}	0.51 ± 0.02	1.34 ± 0.29	1.06 ± 0.34
	Jiaxing	XQZ	$0.84 \pm 0.08^{A**}$	$9.61 \pm 0.78^{A**}$	$1.03 \pm 0.09^{**}$	$2.28 \pm 0.34^{*}$	$2.02 \pm 0.18^{*}$
		HIPi1	0.35 ± 0.07^{a}	6.04 ± 0.66^{a}	0.49 ± 0.07	1.22 ± 0.09	1.07 ± 0.34
	Average	**XQZ**	$\mathbf{0.60 \pm 0.30}^{**}$	$\mathbf{8.96 \pm 0.94}^{**}$	$\mathbf{0.96 \pm 0.06}^{**}$	$\mathbf{2.19 \pm 0.09}^{*}$	$\mathbf{1.86 \pm 0.14}^{*}$
		HIPi1	$\mathbf{0.25 \pm 0.12}$	$\mathbf{5.70 \pm 0.55}$	$\mathbf{0.50 \pm 0.01}$	$\mathbf{1.28 \pm 0.06}$	$\mathbf{1.07 \pm 1.03}$

[1] Data were shown as means ± standard error; multiple comparison results were showed in capital letters for XQZ and lower case letters for HIPi1 among three locations ($P \leqslant 0.05$), while the concentration marked by "*" and "**" differed significantly, at $P_{0.05}$ and $P_{0.01}$, between HIPi1 and XQZ at each same location, or on average across the three locations, respectively.

Table 2
Concentration of major macronutrients in grains of a low phytate rice mutant (HIPi1) and its parent XQZ[1]

Element	Locations		Hull	Bran	Milled rice	Brown rice	Rice grain
K (mg/g)	Linshui	XQZ	$6.47 \pm 0.13^{A*}$	$11.52 \pm 0.44^{C*}$	0.83 ± 0.04^{B}	2.43 ± 0.13^{A}	3.60 ± 0.26^{A}
		HIPi1	6.22 ± 0.89^{a}	10.17 ± 0.67^{a}	$1.08 \pm 0.09^{c**}$	2.45 ± 0.16^{a}	3.41 ± 0.32^{a}
	Jiande	XQZ	1.81 ± 0.22^{C}	6.47 ± 0.11^{B}	1.09 ± 0.02^{A}	1.90 ± 0.10^{B}	1.87 ± 0.11^{B}
		HIPi1	$1.97 \pm 0.33^{c*}$	$6.88 \pm 0.54^{b*}$	$1.49 \pm 0.04^{a**}$	$2.30 \pm 0.16^{b*}$	$2.23 \pm 0.19^{b*}$
	Jiaxing	XQZ	2.41 ± 0.74^{B}	5.07 ± 0.63^{A}	1.04 ± 0.01^{A}	1.64 ± 0.12^{C}	1.78 ± 0.12^{C}
		HIPi1	$3.36 \pm 0.90^{b**}$	$6.18 \pm 0.78^{c*}$	$1.30 \pm 0.01^{b**}$	$2.03 \pm 0.19^{c*}$	$2.17 \pm 0.22^{b*}$
	Average	**XQZ**	**3.56 ± 0.25**	**7.69 ± 0.34**	**0.98 ± 0.14**	**1.99 ± 0.40**	**2.25 ± 0.76**
		HIPi1	**$3.85 \pm 0.22^{*}$**	**7.74 ± 0.21**	**$1.29 \pm 0.20^{**}$**	**$2.26 \pm 0.21^{*}$**	**$2.53 \pm 0.50^{*}$**
Ca (mg/g)	Linshui	XQZ	$1.12 \pm 0.03^{C*}$	$0.52 \pm 0.01^{C*}$	0.11 ± 0.01^{C}	0.17 ± 0.01^{C}	0.34 ± 0.01^{B}
		HIPi1	1.01 ± 0.04^{c}	0.45 ± 0.01^{c}	$0.18 \pm 0.01^{b*}$	$0.22 \pm 0.02^{b*}$	$0.36 \pm 0.01^{c*}$
	Jiande	XQZ	1.25 ± 0.06^{B}	0.58 ± 0.01^{A}	0.16 ± 0.01^{A}	0.23 ± 0.02^{A}	0.41 ± 0.01^{A}
		HIPi1	$1.44 \pm 0.09^{a*}$	$0.66 \pm 0.06^{a*}$	$0.20 \pm 0.01^{a*}$	$0.27 \pm 0.02^{a*}$	$0.48 \pm 0.01^{a*}$
	Jiaxing	XQZ	$1.37 \pm 0.07^{A*}$	$0.54 \pm 0.04^{B*}$	0.14 ± 0.01^{B}	0.20 ± 0.01^{B}	0.41 ± 0.01^{A}
		HIPi1	1.27 ± 0.05^{b}	0.48 ± 0.01^{b}	$0.18 \pm 0.01^{b*}$	$0.22 \pm 0.02^{b*}$	0.41 ± 0.01^{b}
	Average	**XQZ**	**1.25 ± 0.13**	**0.55 ± 0.03**	**0.14 ± 0.03**	**0.20 ± 0.03**	**0.39 ± 0.04**
		HIPi1	**1.24 ± 0.21**	**0.53 ± 0.12**	**$0.18 \pm 0.01^{*}$**	**$0.24 \pm 0.01^{*}$**	**0.42 ± 0.01**
Mg (mg/g)	Linshui	XQZ	0.42 ± 0.01^{C}	$2.63 \pm 0.04^{A*}$	0.23 ± 0.01^{C}	0.59 ± 0.08^{C}	0.55 ± 0.02^{C}
		HIPi1	0.32 ± 0.01^{c}	2.15 ± 0.04^{b}	$0.34 \pm 0.01^{b*}$	$0.61 \pm 0.05^{b*}$	0.55 ± 0.03^{c}
	Jiande	XQZ	0.50 ± 0.01^{B}	2.19 ± 0.06^{B}	0.49 ± 0.01^{B}	0.74 ± 0.09^{B}	0.69 ± 0.07^{B}
		HIPi1	0.52 ± 0.01^{b}	2.20 ± 0.06^{b}	$0.77 \pm 0.01^{a*}$	$0.98 \pm 0.09^{a*}$	$0.90 \pm 0.10^{b*}$
	Jiaxing	XQZ	0.97 ± 0.05^{A}	2.26 ± 0.07^{B}	0.54 ± 0.01^{A}	0.80 ± 0.06^{A}	0.82 ± 0.08^{A}
		HIPi1	$1.17 \pm 0.02^{a*}$	$2.42 \pm 0.04^{a*}$	$0.71 \pm 0.01^{a*}$	$0.96 \pm 0.09^{a*}$	$1.00 \pm 0.05^{a*}$
	Average	**XQZ**	**0.63 ± 0.30**	**2.36 ± 0.23**	**0.42 ± 0.17**	**0.71 ± 0.11**	**0.69 ± 0.14**
		HIPi1	**0.67 ± 0.45**	**2.26 ± 0.15**	**$0.60 \pm 0.23^{*}$**	**$0.85 \pm 0.20^{*}$**	**$0.81 \pm 0.23^{*}$**

[1] Data were shown as means ± standard error; multiple comparison results were showed in capital letters for XQZ and lower case letters for HIPi1 among three locations ($P \leqslant 0.05$), while the concentration marked by "*" and "**" differed significantly, at $P_{0.05}$ and $P_{0.01}$, between HIPi1 and XQZ at same location, or on average across the three locations, respectively.

Table 3
Concentration of major micronutrients in grains of a low phytate rice mutant (HIPi1) and its parent XQZ[1]

Element	Locations		Hull	Bran	Milled rice	Brown rice	Rice grain
Fe (μg/g)	Linshui	XQZ	$1455.3 \pm 56.1^{B**}$	$58.5 \pm 4.1^{B*}$	9.1 ± 0.7^{B}	16.5 ± 1.0^{C}	$275.4 \pm 14.1^{B**}$
		HIPi1	862.4 ± 43.0^{a}	52.7 ± 2.3^{c}	$15.0 \pm 1.8^{c**}$	$20.7 \pm 1.6^{c*}$	123.5 ± 8.0^{a}
	Jiande	XQZ	$755.2 \pm 23.8^{C*}$	$64.8 \pm 6.8^{A*}$	10.8 ± 2.7^{A}	18.9 ± 1.4^{B}	$171.0 \pm 5.6^{C**}$
		HIPi1	625.8 ± 6.1^{c}	59.2 ± 7.8^{b}	$17.0 \pm 3.0^{b**}$	$23.4 \pm 1.9^{b*}$	97.7 ± 5.0^{b}
	Jiaxing	XQZ	$1523.7 \pm 66.7^{A**}$	68.3 ± 3.5^{A}	10.6 ± 1.6^{A}	19.3 ± 2.0^{A}	$289.9 \pm 11.1^{A**}$
		HIPi1	781.0 ± 24.9^{b}	$91.3 \pm 3.9^{a**}$	$19.9 \pm 2.2^{a**}$	$30.6 \pm 2.8^{a**}$	124.1 ± 4.4^{a}
	Average	**XQZ**	**$1244.7 \pm 425.3^{**}$**	**63.9 ± 5.0**	**10.1 ± 0.9**	**18.2 ± 1.5**	**$238.8 \pm 76.2^{**}$**
		HIPi1	**756.4 ± 120.2**	**67.7 ± 20.7**	**$17.3 \pm 2.4^{**}$**	**$24.9 \pm 5.1^{*}$**	**156.4 ± 21.7**
Zn (μg/g)	Linshui	XQZ	47.0 ± 1.8^{B}	56.4 ± 1.1^{C}	13.1 ± 0.6^{C}	19.6 ± 1.2^{B}	27.1 ± 0.8^{C}
		HIPi1	46.9 ± 2.7^{c}	54.3 ± 2.6^{c}	$17.5 \pm 0.8^{c*}$	$23.1 \pm 1.7^{c*}$	$28.8 \pm 0.6^{c*}$
	Jiande	XQZ	43.2 ± 9.3^{C}	69.7 ± 2.8^{B}	20.9 ± 2.7^{B}	28.2 ± 2.0^{A}	31.0 ± 2.1^{B}
		HIPi1	$55.1 \pm 7.9^{b*}$	$79.1 \pm 2.9^{b*}$	$28.4 \pm 1.9^{b*}$	$36.0 \pm 2.8^{b*}$	$40.1 \pm 1.7^{b*}$
	Jiaxing	XQZ	67.6 ± 4.4^{A}	77.6 ± 3.7^{A}	22.4 ± 1.1^{A}	30.7 ± 2.4^{A}	37.4 ± 0.9^{A}
		HIPi1	$83.8 \pm 1.5^{a*}$	$131.0 \pm 4.8^{a**}$	$39.7 \pm 0.6^{a*}$	$53.4 \pm 3.5^{a**}$	$57.0 \pm 1.2^{a**}$
	Average	**XQZ**	**52.6 ± 13.1**	**67.9 ± 10.7**	**18.8 ± 5.0**	**26.2 ± 5.8**	**30.8 ± 6.4**
		HIPi1	**$61.9 \pm 19.4^{*}$**	**$88.1 \pm 39.1^{**}$**	**$28.6 \pm 11.1^{*}$**	**$37.5 \pm 15.2^{*}$**	**$41.7 \pm 15.8^{*}$**
Cu (μg/g)	Linshui	XQZ	$1522.3 \pm 24.0^{A**}$	2.4 ± 0.2^{C}	2.2 ± 0.8^{C}	2.26 ± 0.34^{C}	$381.4 \pm 6.0^{A*}$
		HIPi1	725.2 ± 41.7^{b}	2.1 ± 0.3^{c}	1.9 ± 0.1^{c}	1.91 ± 0.17^{c}	157.5 ± 9.0^{b}
	Jiande	XQZ	$827.0 \pm 40.9^{C*}$	8.2 ± 0.4^{B}	6.7 ± 0.4^{A}	6.89 ± 0.59^{A}	162.4 ± 8.2^{C}
		HIPi1	675.1 ± 8.2^{c}	8.9 ± 1.1^{b}	6.2 ± 0.2^{a}	6.57 ± 0.60^{a}	158.7 ± 1.8^{b}
	Jiaxing	XQZ	$1149.7 \pm 68.4^{B**}$	11.9 ± 1.2^{A}	4.6 ± 0.4^{B}	5.70 ± 0.38^{B}	$217.7 \pm 12.6^{B*}$
		HIPi1	923.6 ± 31.3^{a}	12.6 ± 0.8^{a}	4.8 ± 0.5^{b}	5.95 ± 0.41^{b}	153.7 ± 5.1^{a}
	Average	**XQZ**	**$1166.3 \pm 347.0^{**}$**	**7.5 ± 4.8**	**4.5 ± 2.2**	**5.0 ± 2.4**	**$214.0 \pm 60.7^{*}$**
		HIPi1	**774.6 ± 131.4**	**7.9 ± 5.3**	**4.3 ± 2.2**	**4.8 ± 2.5**	**143.4 ± 24.2**

[1] Data were shown as means ± standard error; multiple comparison results were showed in capital letters for XQZ and lower case letters for HIPi1 among three locations ($P \leqslant 0.05$), while the concentration marked by "*" and "**" differed significantly, at $P_{0.05}$ and $P_{0.01}$, between HIPi1 and XQZ at same location, or on average across the three locations, respectively.

significantly higher in HIPi1 than in XQZ, both on whole rice grain and brown rice basis (Table 2). Among them, the most significant increase was observed for Mg, i.e., 19.7% or 0.14 mg/g on brown rice basis (Table 2).

Consistent and significant increase of the concentrations of Ca, K, and Mg was observed in milled rice of HIPi1, at rates on average of as high as 32.8%, 31.2%, and 44.8% over XQZ, respectively (Table 2). Except for K in hull, no significant alterations were detected in hull and bran parts.

3.2.3. Micronutrient elements

On the whole rice grain/brown rice basis, there were some increases and reductions, varying among elements, for the concentration of micronutrient elements Fe, Zn and Cu in HIPi1 when compared with those in XQZ (Table 3). Most considerably, Zn was significantly enhanced in all grain parts of HIPi1 over XQZ, with the maximum increase as high as 52.1% in milled rice (Table 3). Resulting from a big reduction in hull, Fe concentration on whole grain basis was significantly reduced in HIPi1 (Table 3). Nevertheless, the concentration of Fe in the milled rice of HIPi1 was significantly higher than that of XQZ, that is, 17.3 μg/g vs. 10.1 μg/g, or a 71.3% increase (Table 3). Except for the significant reduction in the hull, changes of Cu level in bran and milled rice were minor in HIPi1 (Table 3).

3.3. Distribution alteration

The differences of reduction degree among hull, bran and milled rice substantially altered the distribution of PA-P in rice grains of HIPi1. A significantly higher percentage of total PA-P was deposited in the bran part of HIPi1 than that of XQZ (Fig. 1).

As a result of the concentration alteration, significantly higher amounts of macronutrient elements, i.e., Ca, K, and Mg, were deposited in milled rice of HIPi1 in comparison with those of XQZ (Fig. 1), i.e., the amount of Mg in milled rice of HIPi1 grains reached 51.9% of the total while it was only 42.4% in XQZ grains (Fig. 1). Also, the distribution of micronutrient elements Fe, Zn and Cu was changed in HIPi1 (Fig. 1). More Zn seemed diverted from hull to milled rice in HIPi1, which resulted in a significant higher proportion of total Zn in milled rice of HIPi1 than in XQZ (Fig. 1), though the concentration was increased in all grain parts of HIIPi1 when compared with XQZ (Table 3). Although the hull accommodated most portions of total Cu and Fe in the grain, there were still significant increases of Cu and Fe in milled rice of HIPi1 over XQZ (Fig. 1).

4. Discussion

We reported here the amendments of the amounts and distributions of major nutritionally relevant elements in the rice grains of a low phytate mutant in comparison with its parent variety. In the study, rice grains were partitioned into milled rice, bran and hull, as in conventional milling, hence the results were more relevant to nutrient value assessment as compared with previous studies where rice grains were dissected in an anatomic manner (Liu et al., 2004; O'Dell et al., 1972). In addition, use of samples grown at three different locations enabled us to track down both genotypic and environmental effects on the alterations.

Although the low phytate mutant line (HIPi1) used in the study was an indica line, developed through gamma irradiation, the mutation leading to its PA-P reduction was allelic to the *lpa1-1* gene (Ren, 2005) in the low phytate mutant of Kaybonnet, a *japonica* line developed by Larson et al. (2000) and analyzed in Liu et al. (2004). Both the percentage of PA-P against total P and reduction of PA-P of Kaybonnet mutant (Table 2 of Liu et al., 2004), were comparable to our results on brown rice basis (Table 1). Liu et al. (2004) also found that the *lpa1-1* mutant grains were significantly higher in Zn than Kaybonnet on brown rice basis, which was consistent with our findings in this study. However, for other elements, the results seemed somehow contradictory: they found that, on a brown rice basis, the amounts of total P and Ca in *lpa1-1* mutant were lower than in Kaybonnet, while those of K, Mg and Zn were similar (Liu et al., 2004); where in our study, we found that all of these elements were higher in HIPi1 than in XQZ (Tables 1–3). Such differences might partially result from the location effects on samples, as we also observed even in this study, HIPi1 had similar contents of K and total P as XQZ in brown rice produced in Lingshui (Tables 1 and 2). Our above findings, however, were very consistent with the observation made in low phytate barley mutants by Hatzack, Johansen, and Rasmussen (2000), where they found Type A low phytate mutant lines had higher concentrations of K, Ca, Mg and Zn (Fe was not analyzed) than their parent, but other mutants (Type B) did not (Fig. 3 in Hatzack et al., 2000). The Type A mutant lines, characterized by the increase of inorganic P but not lower inositol P (Hatzack et al., 2000), was similar to *lpa1* mutants in rice. Interestingly, we also did not find similar alterations in another low phytate line, HIPj2, of which the low phytate mutation was nonallelic to *lpa1* (Ren, 2005). Both HIPj2 of rice and Type B mutants of barley might all belong to *lpa2* mutation and had no effect on element amount alteration. In a milling test of *lpa1*-like wheat mutant line, Guttieri, Bowen, Dorsch, Raboy, and Souza (2004) also found that the mutant had a higher content of Ca in all parts of wheat grains, and Fe and Mg in the inner part of grains than its parent.

The location independent consistent and significant increase of the amount of mineral nutritional elements in milled rice observed in this study, and similar observations made by other researchers in rice and other crops reviewed above, might suggest that the effect of *lpa1* mutation on mineral element accumulation and their distribution in cereal grains be a universal phenomenon. If it could be confirmed by further studies, such as analysis of progenies

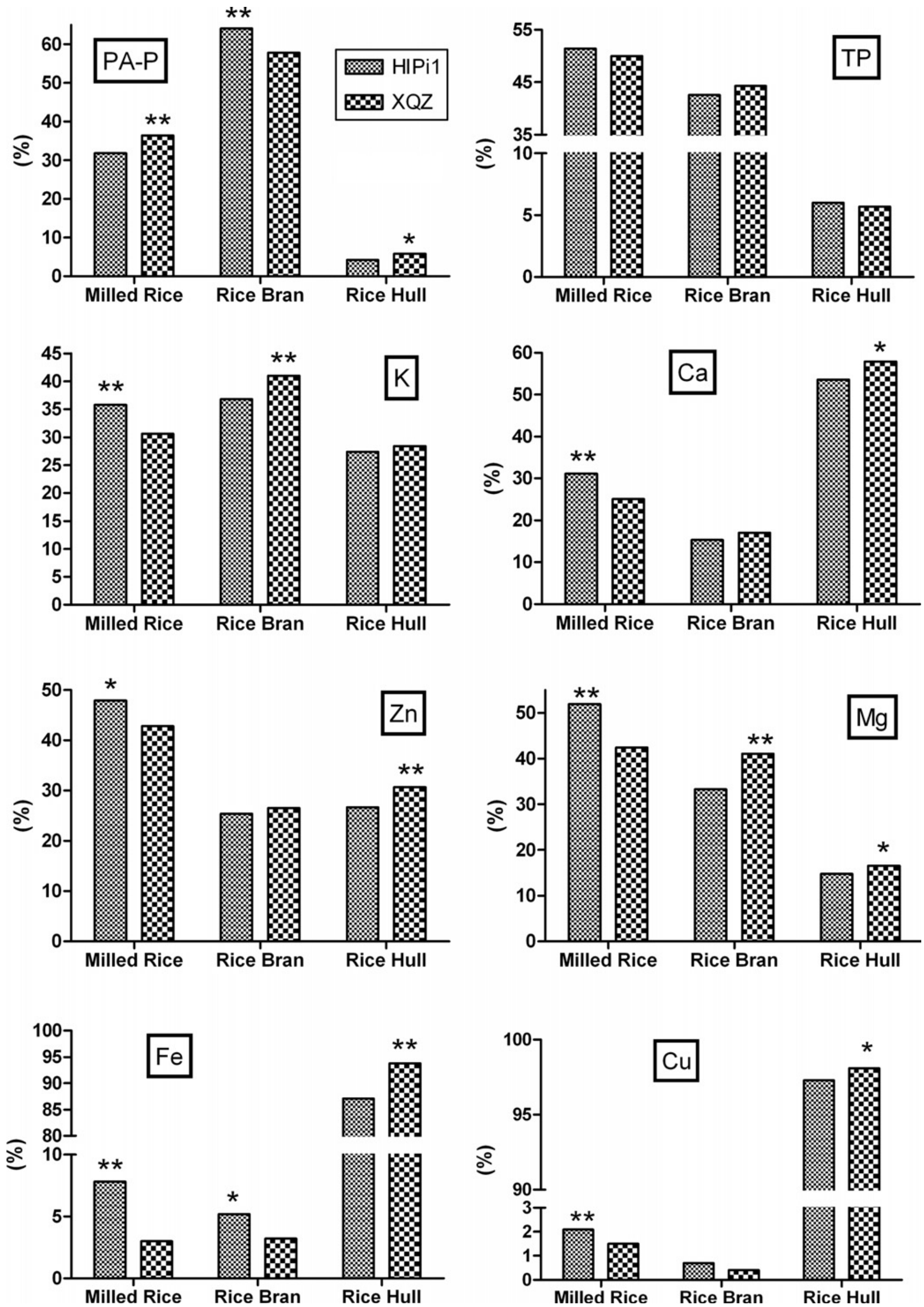

Fig. 1. Distribution of nutritionally important elements in rice grains of a low phytate mutant (HIPi1) and its parent variety (XQZ). Data were presented as % of the total amount ($^{*}p < 0.05$; $^{**}p < 0.01$).

derived from crosses between *lpa1* mutant and conventional varieties, it would constitute a significant added value of *lpa1* mutation in crop breeding for enrichment of bioavailable micronutrients.

It was already well documented that environmental conditions could greatly influence the concentration of mineral elements (Graham et al., 2001) and phytic acid (Liu, Cheng, & Zhang, 2005) in conventional rice grains, which was also confirmed in our study. However, such a study has not been yet done for low phytic acid mutant lines of any crops including rice. Therefore, our study might be the first of its kind. We found that the change of total P and PA-P concentration of HIPi1 brown rice was largely similar to that of XQZ, and in all circumstances, HIPi1 had far lower PA-P content than XQZ, which implied that the *lpa1* mutation resulted in significant reduction of PA-P which was far bigger than that might be caused by environmental effects.

Rice is inherently low in Fe and milling removes half or more of that, hence making rice the poorest of all the cereals in iron (Graham, Humphries, & Kitchen, 2000). Environmental effects surpassed the genotypic influences of *lpa1* mutation on the concentration of K, Ca, Mg, Zn, and Cu in milled rice. That is, you could easily find that the lowest content of a given element in HIPi1 was lower than the highest one in XQZ across three locations, though it was on average higher in HIPi1 than XQZ. However, this seemed not the case for Fe, of which the lowest content in milled rice of HIPi1 was much higher than the highest in XQZ across three locations tested in the study, this might be due to the relatively stability of Fe content in XZQ across locations (Table 3). Therefore, it would be a great advantage if such increase could be verified in more locations.

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