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Variations in Concentration and Distribution of Health-Related Elements Affected by Environmental and Genotypic Differences in Rice Grains

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Abstract: A research work was conducted to investigate the variations in concentration and distribution of health-related elements affected by environmental and genotypic differences in rice grains. The grain of Xieqingzao B (indica rice variety) and Xiushui 110 (japonica rice variety) were divided into: hull, bran and milled rice, based on the conventional rice consumption and process. Xieqingzao B was grown at four different locations, and at one location, it was planted in the same field and season as Xiushui 110. In addition, another four indica and four japonica varieties were cultivated in the same field and time to analyze the elements in milled rice. The average concentrations of total P and phytic acid P were the highest in the bran, followed by milled rice and hull; Zn, K, Mg, and As concentrations were the highest in bran, followed by hull and milled rice, while Fe, Ca, and Cu concentrations were the highest in the hull, but similar in bran and milled rice. The result indicated that genotype and environment significantly affected the concentrations of all the tested elements, while the distribution of the above elements in grains was not in the same order as concentration. Moreover, all the elements except 97.7% of Cu and 93.2% of Fe was deposited in the hull on average, were mostly distributed either in the bran (37.3% and 57.7% for K and phytic acid P) or in milled rice (41.7%, 42.6%, 40.3%, 49.8% for Zn, Mg, As, total P, respectively).

Key words: distribution; concentration; micronutrient mineral; biofortification; breeding; rice

Malnutrition is one of the major problems in most of the developing countries, especially among women, infants and children. Biofortification is a very new strategy to enhance the bioavailability of micronutrients in staple food by using advanced breeding methods [1-2], which has recently become part of international initiatives such as the HarvestPlus Program (http://www.harvestplus.org/ index.html) and the Bill & Melinda Gates Foundation's Grand Challenges in Global Health Program (http://www. grandchallengesgh.org/). Rice is one of the staple diet of nearly two billion people worldwide, while over 50% of total rice consumption is in Asia [3]. Moreover, rice has been on the priority list of initiatives of crop biofortification in most of the Asian countries. It can play a significant role in improving the quality through breeding, although improvements have already been achieved by increasing potential head rice recovery (milling quality), reducing amylose content (eating and cooking quality) [4], and recently also in optimizing the viscosity profile (related to cooked rice texture) [5]. In addition, nutritional elements, genotypic differences against toxic heavy metals such as arsenic (As) and cadmium (Cd), were also found in recent studies in rice [5-6], implying the potential for reducing the accumulation of toxic metal in rice grains through breeding.

The coarse rice grains are eventually consumed both as food (milled rice, including part of the subaleurone and the whole starchy endosperm) and feed (hull and bran, including pericarp, seed coat, embryo and aleurone). In some cases, brown rice (milled rice plus bran) is also directly or indirectly (after processing) consumed. Therefore, the important elements essential for health largely depend on their concentration and distribution in various parts of rice grains. The primary analysis and the spatial distribution of various elements in the different anatomical parts of brown rice [7-8] or milled rice [9] indicated that the majority of the elements were enriched in the bran or the outer part of milled rice. The genotypic and environmental effects on Fe and/or Zn content in brown rice has been investigated by

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Graham's group [10-12]. To further investigate the genotypic and environmental effects on the concentration and distribution of major health-related elements in rice grains, which is of pivotal importance for evaluation of biofortification breeding potential in rice, a comprehensive investigation was conducted by analyzing both microelements and bioavailability-affecting phytic acid phosphorus (PAP) and other important elements in the various parts of rice grains.

MATERIALS AND METHODS

Rice seed production

The experiment was conducted during 2003 to study the environmental effects on rice verities i.e. indica rice variety Xieqingzao B (XQZB), at four different predominated rice growing areas, namely, Lingshui of Hainan Province (20°N, 110.3°E), Wenzhou (28.0°N, 120.5°E), Jiande (30.2°N, 120.1°E) and Jiaxing (31.3°N, 120.6°E) of Zhejiang Province, with varying soil fertility such as poor (Lingshui), medium (Jiande and Wenzhou), fertile (Jiaxing). To investigate the effect of genotypic difference on the element concentration in milled rice, four indica (Xieyou 7954, II you 3027, Taiziyubo, Taiziyuzhu) and four japonica (Sun 365, Xiushui 110, Xiushui 63 and Xuenuo) rice varieties were grown in the same field and season in Jiande of Zhejiang Province. To observe the distribution of elements, one indica rice variety, Xieqingzao B and one japonica rice variety, Xiushui 110 were harvested from the same field and in the same season in Jiaxing of Zhejiang Province.

Xieyou 7954 [13] and II you 3027 [14] are indica hybrid rice. Xieyou 7954 is famous for its high yield potential and widely grown in Zhejiang and Anhui Provinces as a single crop variety. II you 3027 has a good grain quality and is grown in double crop areas of Zhejiang and Jiangxi provinces. Taiziyubo and Taiziyuzhu are two traditional rice varieties with good quality, which are grown in some areas of Xiaogan in Hubei Province, and sold under a special brand 'Taizi' (http://www.hb.xinhuanet.com/xgtxb/xw/old/ts8.html). XQZB is the maintainer line of the widely used commercial cytoplasmic male sterile line Xieqingzao A, which was bred in 1980s and is still used as female parent of many hybrid combinations (e.g. Xieyou 7954 is a cross of Xieqingzao A and Zhehui 7954) in Zhejiang, Jiangxi, Jiangsu and Anhui provinces [15]. Among the japonica rice varieties, Xiushui 63 and Xiushui 110 are both newly developed superior grain quality and high yielding varieties, and are widely grown in areas of single crop production, i.e., Zhejiang and Jiangsu Provinces [16-17], while Xuenuo is a traditional black rice and Sun 365 is a foreign variety.

Rice seed partitioning

After harvest, rice seeds were sun-dried and stored at room temperature. Before milling, all samples were further dried at 60℃ for 8 h to get the required moisture content. The seeds were then separated into: hull (through dehulling by using a Satake Dehuller, Satake Corporation, Japan), bran (the parts removed from brown rice, including pericarp, seed coat, embryo, and aleurone, by using a Satake Test Mill, Satake Corporation, Japan), and milled rice (including sub-aleurone and starchy endosperm). The degree of milling was similar to the commercial rice by controlling the milling time. Milled rice and rice hull was ground into flour with a Cyclone Sample Mill (UDY Corporation, Rome). All samples (hull, bran and milled rice flour) were passed through a 2-mm sieve and stored in desiccators for analysis.

Chemical analysis

The total P content was determined colorimetrically [18]. Analysis of phytic acid content was performed by anion-exchange HPIC (high performance ion chromatography, Dionex, USA) using phytic acid dodecasodium salt (P-3168, Sigma) as a standard. All the samples were pretreated according to Dorsch et al [19], while the chromatography assay was carried out in triplicate as described by Philliphy et al [20]. The amount of total P and phytic acid P were expressed as their P (atomic weight =31) content on 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). The samples were prepared according to Koplik et al [21], and then measured with PU7000 ICP spectrometer (Philips, Cambridge, UK) according to Fingerova and Koplik [22] in triplicate. Analytical accuracy was verified by simultaneous analysis of the reference material SRM 1568a Rice Flour (NIST, USA). The element distribution was expressed as the weight percentage of a given element in hull, bran and milled rice and calculated by multiplying the weight percentage of that part (hull, bran or milled rice) in grain (data not shown) by the weight percentage of a given element in that part.

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) compared by one-way analysis of variance (ANOVA) and the Duncan's test. The Person-Spearman-Kendall correlation matrix from the SPSS 10.0 statistical package was used to analyze the level of correlation.

RESULTS

Concentration and distribution of non-metal elements

In all the locations, the concentrations of total P (TP), phytic acid P (PAP) and arsenic (As) among the three parts of rice grain were significantly different from each other (Table 1). All components seemed most concentrated in the bran, TP and PAP were found the least in hull and As in milled rice (Table 1). On average, the levels of TP and PAP were almost 15

Table 1. Distribution and variation of phosphorus and arsenic in rice grains.

Items and varieties	Location	Hull		Bran		Milled rice		Brown rice	Rice grain
		Concentration	% of grain	Concentration	% of grain	Concentration	% of grain	Concentration	Concentration
Total P (mg/g)									
XQZB	Lingshui	0.87±0.07^{Cb}	8.1	9.05±0.94^{Ab}	42.8	2.15±0.02^{Bab}	49.1	3.33±0.02^{a}	2.71±0.06^{a}
XQZB	Jiande	0.52±0.04^{Cd}	3.6	11.38±1.00^{Aa}	49.1	1.89±0.07^{Bb}	47.3	3.30±0.10^{a}	2.77±0.08^{a}
XQZB	Wenzhou	0.66±0.04^{Cc}	3.4	12.74±0.17^{Aa}	43.2	2.45±0.06^{Ba}	53.4	3.69±0.13^{a}	3.11±0.06^{a}
XQZB	Jiaxing	1.25±0.08^{Ba*}	7.9	10.58±0.20^{Aab}	43.1	2.06±0.04^{Bb}	45.0	3.31±0.26^{a}	2.93±0.04^{a}
Average of XQZB		0.83	5.7	10.78	44.5	2.14	49.8	3.43	2.88
CV of XQZB (%)		38.52	44.9	10.69	6.8	10.97	5.2	6.95	6.22
XS 110	Jiaxing	0.66±0.13^{C}	4.8	14.04±0.95^{A}*	43.2	1.72±0.15^{B}	52.0	2.87±0.22	2.48±0.20
PAP (mg/g)									
XQZB	Lingshui	0.69±0.09^{Cb}	9.3	7.88±0.07^{Ac}	54.5	1.02±0.01^{Bb}	36.2	2.11±0.14^{b}	1.82±0.05^{b}
XQZB	Jiande	0.27±0.06^{Cd}	2.8	9.40±0.84^{Ab}	62.1	0.92±0.07^{Bc}	35.1	2.17±0.32^{b}	1.81±0.12^{b}
XQZB	Wenzhou	0.50±0.06^{Cc}	3.8	11.60±0.41^{Aa}	57.4	1.22±0.10^{Ba}	38.8	2.78±0.20^{a}	2.13±0.14^{a}
XQZB	Jiaxing	0.84±0.05^{Ca*}	7.7	9.61±0.40^{Ab}	56.8	1.03±0.04^{Bb}	35.5	2.28±0.34^{b}*	2.02±0.06^{a}
Average of XQZB		0.58	6.0	9.62	57.7	1.05	36.3	2.34	1.95
CV of XQZB (%)		42.85	53.1	15.87	5.6	12.96	5.0	13.06	8.06
XS 110	Jiaxing	0.44±0.09^{C}	5.1	11.37±1.38^{A}*	56.7	0.79±0.09^{B}	38.2	1.78±0.19	1.54±0.13
As (μg/g)									
XQZB	Lingshui	0.13±0.02^{Bc}	16.7	0.55±0.09^{Ac}	47.1	0.07±0.00^{Cc}	36.2	0.08±0.02^{c}	0.14±0.05^{c}
XQZB	Jiande	0.65±0.14^{Bb}	26.1	0.90±0.16^{Aa}	24.1	0.32±0.08^{Ca}	49.8	0.37±0.08^{a}	0.45±0.09^{a}
XQZB	Wenzhou	0.79±0.13^{Aa}	45.6	0.74±0.08^{Ab}	26.9	0.11±0.01^{Bb}	27.6	0.21±0.05^{b}	0.30±0.11^{b}
XQZB	Jiaxing	0.67±0.13^{Bb}	25.7	0.89±0.12^{Aa}	22.7	0.34±0.09^{Ca}	51.6	0.37±0.06^{a}	0.47±0.07^{a}
Average of XQZB		0.56	28.5	0.77	30.1	0.21	41.4	0.26	0.34
CV of XQZB (%)		52.37	42.8	21.29	37.9	66.55	30.0	54.50	45.12
XS 110	Jiaxing	1.04±0.19^{A}*	35.4	1.05±0.22^{A*}	16.8	0.32±0.06^{B}	48.8	0.39±0.10	0.50±0.09

Data were shown in mean ± SD, means followed by the same uppercase letters in the same row and the same lowercase letters in the same column for a item were not significantly different (P>0.05).

* denoted the multiple comparison results between indica rice (Xieqingzao B, abbreviated as XQZB) and japonica rice (Xiushui 110, abbreviated as XS 110) in the same column, values with this asterisk were significantly different ($P \leqslant 0.01$).

and 18 times higher in bran than those in the hull, and about 5.6 and 10 times higher than those in milled rice, respectively. The difference of As level between hull and bran was significant at three out of four locations, while the concentrations of As in the hull and bran were 3-4 times higher than those in milled rice.

Milled rice contains the main part of rice seeds (mostly 65-70%, data not shown), while the distribution of these elements was not in the same order as their concentration. The results indicated that less than 6% of TP and PAP were in rice hull, but large portions remained in rice bran and milled rice. PAP was higher in rice bran than in milled rice, though TP was higher in milled rice (Table 1). On the other hand, the distribution pattern of As was quite different from that of P. On average, it was more or less evenly partitioned into rice hull and bran, and slightly more into milled rice (Table 1).

The concentration of TP, PAP and As in all the parts of rice grains (Table 1) were significantly affected by environment. For example, the highest TP in hull was noted in Jiaxing (1.25 mg/g), while lower in grain compared those in Wenzhou (Table 1). Environmental effects were also found in PAP and As (Table 1). Moreover, large distribution differences of these components were also found among samples produced at four locations (Table 1), e.g., the ratio of hull vs whole rice grain varied from 3.4% to 8.1% for TP and from 2.8% to 9.3% for PAP, 16.7% to 45.6% for As, all showed about 2-3 (fold) times differences. The differences in concentration of TP, PAP and As were only significant in hull and bran between Xieqingzao B and Xiushui 110 grown in Jiaxing (Table 1). However, the differences in concentration of these elements were significant in milled rice of the eight varieties grown in Jiande, where japonica rice varieties had higher average As levels than indica (Table 2).

PAP is widely regarded as an anti-nutritional component [23], therefore, its ratio to TP is also an important parameter. In this study, it has been noted that more than 80% of the TP in bran existed as PAP, while less than half was found in milled rice. Environmental effects on the ratio seemed most significant in hull, and the largest difference was about 50% (Jiande vs Lingshui), while only slight difference (3%) was found in milled rice (Table 1).

Concentration and distribution of metal minerals

On the whole rice grain basis, the concentration of K was higher than all the other metal mineral elements (Table 3). However, the level of metal minerals in the three parts of the rice grain was considerably different. Similar trends were found for K and Mg concentrations, the highest level was noted in bran, followed by the rice hull and milled rice, with significant differences each other(Table 3). On the other side, the concentration pattern of Ca was almost

Table 2. Concentration of total P, phytic acid P (PAP) and mineral micronutrients in milled rice of different varieties.

Variety	Total P (mg/g)	PAP (mg/g)	K (μg/g)	Fe (μg/g)	Ca (μg/g)	Zn (μg/g)	Mg (μg/g)	Cu (μg/g)	As (μg/g)
indica									
Xieyou 7954	4.03±0.34^{AB}	1.85±0.05^{AB}	766.40±6.11^{C}	10.55±1.54^{B}	167.07±20.69^{B}	14.15±0.68^{CD}	321.53±6.54^{CD}	2.15±0.04^{D}	0.24±0.07^{B}
II you 3027	4.27±0.26^{A}	1.97±0.11^{A}	734.80±20.17^{C}	10.60±1.56^{B}	159.97±6.24^{B}	17.50±2.35^{B}	369.66±5.85^{B}	2.70±0.33^{B}	0.33±0.04^{B}
Taiziyubo	3.76±0.18^{BC}	1.65±0.02^{ABC}	645.03±16.15^{D}	12.39±1.92^{AB}	201.77±34.35^{A}	19.91±3.53^{A}	315.07±6.10^{D}	2.25±0.09^{D}	0.28±0.06^{B}
Taiziyuzhu	3.58±0.22^{BCD}	1.59±0.02^{ABC}	576.77±24.14^{E}	12.73±1.23^{AB}	190.97±41.97^{AB}	17.98±1.90^{B}	269.60±8.75^{E}	2.16±0.06^{D}	0.49±0.09^{A}
Average	3.91±0.30	1.77±0.18	680.75±86.30	11.57±1.15	179.94±19.69	17.39±2.40	318.97±40.94	2.32±0.26	0.34±0.11
japonica									
Sun 365	3.94±0.35^{AB}	1.74±0.03^{ABC}	1158.33±20.55^{A}	16.74±2.00^{A}	169.57±5.69^{AB}	17.37±1.21^{B}	462.57±4.33^{A}	2.59±0.08^{B}	0.55±0.12^{A}
Xiushui 110	3.66±0.29^{BC}	1.69±0.05^{ABC}	935.00±27.99^{B}	13.38±2.18^{AB}	187.07±5.25^{AB}	17.86±1.63^{B}	392.90±5.52^{B}	3.03±0.25^{A}	0.51±0.14^{A}
Xiushui 63	3.15±0.25^{D}	1.37±0.06^{C}	734.60±13.50^{C}	12.92±2.04^{AB}	172.63±14.57^{AB}	13.54±0.65^{D}	228.13±13.47^{F}	2.42±0.19^{BC}	0.49±0.13^{A}
Xuenuo	3.33±0.19^{CD}	1.48±0.03^{BC}	616.50±8.00^{DE}	13.43±2.85^{AB}	160.00±25.21^{B}	15.56±2.34^{C}	337.70±2.72^{C}	2.39±0.14^{CD}	0.47±0.11^{A}
Average	3.52±0.35	1.57±0.17	861.11±237.79*	14.12±1.76*	172.32±11.21	16.08±1.96	355.33±99.00*	2.61±0.30	0.51±0.03*
Average of all	3.72±0.37	1.67±0.19	770.93±191.63	12.84±1.94	176.13±15.38	16.73±2.14	337.15±72.78	2.46±0.30	0.42±0.12
CV (%)	9.91	11.57	24.86	15.10	8.73	12.81	21.59	12.25	28.09

Data were shown in mean ± SD, means followed by the same uppercase letters in the same column were not significantly different (P>0.01).

*denoted the multiple comparison results between indica rice and japonica rice in the same column, values with this asterisk were significantly different ($P \leqslant 0.01$).

Table 3. Distribution and variation of metal minerals in rice grains.

Items and samples		Hull		Bran		Milled rice		Brown rice	Rice grain
		Concentration (μg/g)	% of grain	Concentration (μg/g)	% of grain	Concentration (μg/g)	% of grain	Concentration (μg/g)	Concentration (μg/g)
K	XQZB 1	6471.33±132.58^{Ba}	44.8	11523.33±442.99^{Aa}	40.9	826.63±43.96^{Cb}	14.3	2645.00±60.85^{a}	3599.39±58.13^{a}
	XQZB 2	1807.00±21.70^{Bd}	18.3	6467.00±106.79^{Ab}	41.4	1089.11±22.35^{Ca}	40.3	1883.64±31.82^{b}	1869.13±4.34^{b}
	XQZB 3	2927.67±71.02^{Bb}	25.1	5846.00±51.26^{Ac}	32.8	1127.58±18.49^{Ca}	42.1	1815.34±20.95^{b}	1878.17±13.29^{b}
	XQZB 4	2408.33±74.23^{Bc}	25.2	5073.33±63.00^{Ac}	34.1	1039.13±13.71^{Ca*}	40.7	1624.26±15.47^{c*}	1773.86±19.66^{b}
	Average	3403.58	28.4	7227.42	37.3	1020.61	34.3	2002.06	2280.14
	CV (%)	61.58	40.4	40.40	12.1	13.16	39.0	22.02	38.63
	XS 110	5145.00±95.14^{B*}	45.9	7657.00±237.27^{A*}	29.6	651.98±29.43^{C}	24.5	1306.66±46.65	1987.50±38.56*
Fe	XQZB 1	1455.33±56.15^{Ab}	95.1	58.54±4.08^{Bc}	2.6	9.11±0.74^{Cb}	2.3	16.52±0.33^{c}	275.36±14.13^{a}
	XQZB 2	755.20±23.76^{Ad}	89.9	64.78±6.76^{Bb}	5.1	10.76±2.74^{Ca}	5.0	18.86±2.38^{b}	171.02±5.57^{c}
	XQZB 3	1369.33±9.81^{Ac}	93.3	99.95±5.92^{Ba}	4.5	8.04±1.90^{Cc}	2.2	21.83±3.57^{a}	257.62±2.77^{b}
	XQZB 4	1523.67±66.67^{Aa*}	94.6	68.32±3.45^{Bb*}	2.8	10.62±1.56^{Ca*}	2.6	19.28±2.89^{b*}	289.89±11.10^{a*}
	Average	1275.88	93.2	72.90	3.8	9.63	3.0	19.12	245.15
	CV (%)	27.65	2.5	25.35	33.8	13.48	44.5	11.36	25.90
	XS 110	391.90±20.32^{A}	86.3	60.38±1.43^{Bb}	5.3	8.71±0.51^{C}	8.4	13.36±1.34	77.99±2.37
Ca	XQZB 1	1117.00±26.91^{Ac}	67.4	521.07±6.50^{Ba}	16.1	109.46±7.26^{Cc}	16.5	179.63±7.40^{c}	413.43±11.82^{b}
	XQZB 2	1251.67±61.58^{Ab}	56.5	582.77±2.00^{Ba}	16.6	163.51±3.32^{Ca}	26.9	225.45±2.55^{a}	419.73±13.18^{b}
	XQZB 3	969.70±12.49^{Ad}	47.7	588.70±42.38^{Ba}	18.9	163.64±9.85^{Ca}	33.4	217.40±12.26^{a}	328.06±14.73^{c}
	XQZB 4	1374.00±72.08^{Aa*}	60.7	542.30±41.09^{Ba}	15.4	144.14±7.91^{Cb*}	23.9	193.86±19.57^{b*}	530.33±6.97^{a*}
	Average	1178.09	58.0	558.71	16.8	145.19	25.2	209.09	422.89
	CV (%)	14.78	14.2	5.81	9.0	17.58	27.9	10.69	19.61
	XS 110	1048.00±9.54^{A}	60.3	581.70±22.81^{B}	14.5	104.09±9.05^{C}	25.2	148.73±10.28	308.24±9.39
Zn	XQZB 1	46.99±1.82^{Bc}	43.27	56.35±1.06^{Ac}	26.55	13.12±0.63^{Cc}	30.18	20.47±0.52^{c}	27.08±0.84^{b}
	XQZB 2	43.19±9.29^{Bc}	26.41	69.70±2.80^{Ab}	26.96	20.90±2.69^{Cab}	46.63	28.11±2.42^{b}	30.96±2.10^{b}
	XQZB 3	57.00±1.08^{Bb}	24.89	93.85±4.18^{Aa}	26.74	28.79±0.65^{Ca}	48.37	36.55±2.97^{a}	35.11±0.46^{a}
	XQZB 4	67.58±4.42^{Ba*}	33.51	77.55±3.66^{Ab}	24.75	22.43±1.05^{Cb*}	41.75	28.70±3.05^{b*}	37.37±0.92^{a*}
	Average	53.69	32.02	74.36	26.25	21.45	41.73	29.46	32.63
	CV (%)	20.38	26.20	21.06	3.86	36.75	19.64	25.31	13.96
	XS 110	59.52±2.88^{B}	36.80	72.37±2.80^{A}	19.39	16.86±2.91^{C}	43.81	22.04±2.86	28.69±2.34
Mg	XQZB 1	423.27±2.48^{Bd}	18.12	2626.67±39.00^{Aa}	57.52	227.91±0.78^{Cc}	24.37	635.68±6.63^{c}	582.70±4.86^{d}
	XQZB 2	503.47±7.48^{Bc}	13.74	2194.67±56.22^{Ac}	37.91	485.28±10.99^{Cb}	48.35	737.83±14.40^{b}	693.46±12.89^{c}
	XQZB 3	739.77±8.25^{Bb}	15.42	2367.00±70.41^{Ab}	32.19	575.64±12.01^{Ca}	52.39	804.34±38.46^{a}	773.70±13.09^{b}
	XQZB 4	973.40±48.98^{Ba*}	21.88	2259.00±66.81^{Ac}	32.68	538.46±4.68^{Ca*}	45.44	796.54±35.98^{ab*}	824.20±10.80^{a*}
	Average	659.98	17.29	2361.84	40.08	456.82	42.64	753.60	718.52
	CV (%)	37.64	20.55	8.06	29.73	34.38	29.33	11.93	14.66
	XS 110	582.37±17.42^{B}	19.18	2593.33±167.10^{A*}	37.01	316.45±11.86^{C}	43.81	529.24±14.17	538.67±14.08
Cu	XQZB 1	1522.33±24.01^{Aa}	99.56	2.41±0.16^{Bc}	0.08	2.23±0.04^{Bc}	0.36	2.26±0.05^{c}	381.40±5.96^{a}
	XQZB 2	827.03±40.85^{Ad}	96.42	8.15±0.37^{Bb}	0.75	6.67±0.36^{Ba}	2.84	7.18±0.74^{a}	162.39±8.24^{c}
	XQZB 3	923.57±31.28^{Ac}	96.95	12.61±0.83^{Ba}	0.86	4.78±0.54^{Cb}	2.19	5.95±0.38^{b}	153.65±5.14^{c}
	XQZB 4	1149.67±68.39^{Ab*}	97.88	11.91±1.20^{Ba}	0.65	4.60±0.37^{Cb}	1.47	5.70±0.49^{b*}	217.65±12.56^{b*}
	Average	1105.65	97.70	8.77	0.59	4.57	1.72	5.27	228.77
	CV (%)	27.94	1.41	53.25	59.39	39.81	61.96	40.02	46.17
	XS 110	455.33±29.54^{A}	95.78	9.13±0.56^{B}	0.83	3.83±0.09^{C}	3.39	4.33±0.13	84.33±5.13^{b}

Data were shown in mean±SD, means followed by different uppercase letters in the same row and by different lowercase letters in the same column for an element in XQZB 1 to 4 were significantly different (P≤0.05); means of samples (XQZB 4 or XS 110) followed by a '*' in the same column of each element were significantly different ($P \leq 0.05$).

XQZB 1 to XQZB 4 refer to indica rice samples of Xieqingzao B produced in Lingshui of Hainan Province, and Jiande, Wenzhou and Jiaxing of Zhejiang Province, respectively, and XS 110 refers to the japonica rice sample of Xiushui 110 produced in Jiaxing of Zhejiang Province.

similar to Fe concentration, which was the highest in hull and followed by the bran and milled rice, differed each other at significant levels (Table 3). In contrast to Cu, which had a far higher level in rice hull than in bran and milled rice, the differences of Zn concentration among various parts were rather small (Table 3).

Determining the ratio of mineral elements in hull, bran and milled rice compared to the whole rice grain, indicated that the most of Cu, Fe and Ca were

Table 4. Correlation coefficients among total P (TP), phytic acid P (PAP), K, Fe, Ca, Zn, Mg, Cu, As in the milled rice.

	TP	PAP	K	Fe	Ca	Zn	Mg	Cu
PAP	0.992**							
K	-0.271	-0.206						
Fe	0.601*	0.540	0.023					
Ca	0.678*	0.668*	-0.036	0.572*				
Zn	-0.288	-0.222	0.548	-0.311	0.190			
Mg	-0.240	-0.161	0.813**	-0.099	0.067	0.836**		
Cu	-0.680*	-0.629*	0.622*	-0.397	-0.192	0.653*	0.722**	
As	0.426	0.367	-0.119	0.843**	0.460	-0.272	-0.095	-0.221

Data was calculated on the basis of Table 1-3.

**, Significantly different at $P<0.01$; *, Significantly different at $P<0.05$.

deposited in the rice hull, whereas more than 40% of Zn and Mg were in milled rice, while K was accumulated almost evenly in hull, bran and milled rice (Table 3).

It has been examined that both environment and genotype had significant effects on the concentration and distribution of mineral elements (Tables 2, 3). The concentration of Mg in the rice hull and milled rice from Jiaxing was more than two times higher than that from Lingshui, but the Mg concentrations in bran were significantly higher in Lingshui compared to other locations (Table 3). Such the significant environmental effects were found for all minerals in all the grain parts except for Ca in rice bran (Table 3).

In Jiaxing, the concentrations of all the minerals of Xieqingzao B were significantly different from those of Xiushui 110 in different parts of grain, except Ca, Zn and Cu in bran and Cu in milled rice (Table 3). In most cases, Xieqingzao B showed higher mineral concentrations than Xiushui 110 except for K (Table 3). The genotypic difference was further reflected in milled rice of the eight varieties grown in Jiande, where both K and Mg had a two-time difference between the lowest and highest values, while others had only around 50% difference (Table 2).

Correlation among the concentrations of elements

The correlation of elements in milled rice showed that the concentrations of several metallic minerals were ($P<0.05$ or <0.01) positively correlated, e.g., Cu with K, Zn and Mg, Mg with K and Zn (Table 4). In addition, the correlations between metal and non-metal mineral concentrations were also significant, e.g. positively between Fe and As, and negatively between Cu and total P and phytic acid P (Table 4).

Molar ratio of phytate to minerals

The molar ratio of phytate to micronutrient minerals is an important factor influencing the bioavailability of certain micronutrients in food. It was readily calculated based on the data of Tables 1-3, e.g. the highest ratio of phytate to Zn was in the bran (>40:1), the medium in milled rice (>15:1) and the lowest in hull (≈5:1).

DISCUSSION

The results of concentration and/or distribution of elements obtained in this experiment were in agreement with previous reports that in brown or milled rice, where not all elements had been investigated, and the partitioning approaches were also greatly different [7-9]. O'Dell et al [7] partitioned rice seeds into germ, endosperm and pericarp (including aleurone), and the concentrations of total P, PAP, Zn, Fe, Mn, Cu, Ca, Mg and K were determined. Moreover, Tanaka et al [8] determined the transversal distribution of P, K, Mg, Ca, Fe, and Mn in outer layers (including aleurone and subcellular particles of the aleurone) of the mature rice grains; while Yoshizawa et al [9] divided the white rice grain into germ, back and front sides, and K and P were detected. It is clear from the above studies as well as ours that micronutrients are rich in the bran and outer layers of rice endosperm.

Thus, it indicates that brown rice would be superior to milled rice, when only mineral nutrients are considered. However, Table 1 indicates, that PAP was highly concentrated in bran, representing about 58% of its total amount in the rice grain, whereas in milled rice the percentage was only about 36%. However, milled rice contained about 70% of the total rice weight in contrast with 10% for the bran. This finding was consistent with previous reports on wheat flour [23-24]. PAP as a portion of total P (TP) was on average 89.2% in bran and 49% in milled rice (Table 1), which were generally in agreement with previous report [7] that PAP was made up of 40% of the TP in milled rice and 90% of that in rice bran.

The results of the current experiment implied that the benefit of the rice bran should be further evaluated. Though high concentrations of mineral micronutrients do exist in rice bran, but the phytic acid is regarded as an anti-nutritional component [25], and the toxic elements such as As and Cu were also denser in rice bran than that in milled rice (Table 1). Moreover, there was sufficient evidence of As for carcinogenicity to humans, therefore the World Health Organization has announced 10 µg/L (0.01 mg/L) for arsenic in drinking-water as the practical quantification limit (WHO, 1996, see http://www.who.int/). Though there is no general guideline yet, but in most cases 0.1 mg/kg is accepted as the upper limit for As in food. In our study the concentration of As was higher than 0.1 mg/kg in most of the tested samples, therefore, a balance proper for As is needed for rice bran in food consumption. Firstly, phytic acid is mainly account for the lower bioavailability of Fe, Zn and Ca for human beings in cereal grains [26]. In light of the WHO guidelines, Zn bioavailability is largely dependent on the phytate/Zn molar ratio, i.e. the smaller the ratio, the higher the bioavailability. Usually bioavailability of zinc is assigned as high (55%), intermediate (35%) and low (15%) corresponding to phytate/Zn molar ratios that range between 0-5, 5-15, and >15, respectively [27]. During current experiment, the phytate/Zn ratio was much higher than 15:1, particularly in rice bran, indicating that the bioavailability of Zn is very low, which would even become worse by consumption of such kinds of rice bran. Although there is no such a ratio for judgment of bioavailability of Fe and Ca yet, but the mechanism should be similar because phytic acid can chelate these elements in the same way as Zn [28]. Therefore, it clearly implies that eating rice together with bran, or brown rice, without proper preprocessing such as dephytinization, will provide little extra bioavailable minerals to human, and probably will reduce the bioavailablity of micronutrients in milled rice. Secondly the toxic elements such as As and Cu were found higher in concentration of bran than in milled rice (Tables 1, 3), so eating bran may impose potential risks of intaking these toxic elements. Therefore, further studies are needed before making a sound judgment.

The finding on concentration and distribution of health-related elements in rice grain has also been implicated in breeding of nutrition-denser rice. For biofortification breeding of rice, both the increase in concentration of mineral micronutrients, e.g. Zn and Fe, by double or triple of current value [12, 29], and a 50% reduction of phytate P, e.g. in low phytic acid rice grain [30], may have marked effects on the level of the bioavailability of micronutrient elements, due to the reduced molar ratio of phytate/Zn will be much lower in milled rice.

In most of the cases both environment and genotype significantly affected the concentration and distribution of elements in rice grain with various degrees for different elements. Take the most important micronutrients Fe, Zn, Ca in milled rice for an example, the concentration ratio of the highest/ lowest among the eight varieties was about 1.6, 1.5, and 1.3 respectively (Table 2); it was about 1.2, 2.2, and 1.5, respectively, for Xieqingzao B among the four locations (Table 3). Therefore, both genotypic and environmental effects were strong. The results were consistent with Gregorio et al [12], who also found that genotypes had greater effects on Fe than environment in brown rice. Similar to the results of Banziger and Long [31] who found that the environment had higher effect on Zn concentration in maize grain than the genotypes. It has been noted that, based on the CV value (Tables 1, 3), the environmental effect was tissue-dependent. The variations of K, TP and PAP concentration were in the order of hull > bran > milled rice, while for Mg, Ca and As, the smallest concentrations were noted in bran, but mechanisms yet to be explored. The significant genotypic difference of these health-related elements implied a potential to enhance/reduce the concentration of health promoting/risking elements through breeding in order to produce safer rice varieties. On the other hand, the existence of environmental effects suggested that selection of location and proper husbandry procedure is also important in production of health rice grains.

To our knowledge, no report on the concentration profiles of various elements in rice grain was documented. During experiment distinct patterns were observed, namely K and Mg type (bran> hull> milled rice, all significantly), Ca and Fe type (highest in hull, significantly lower in bran and far lower in milled rice), Cu type (far significantly higher level in hull than in bran and milled rice) and Zn type (rather evenly in hull, bran and milled rice). The different mineral profile in rice grains might be the result from different mechanisms of ion transport channels and active partitioning of element into various parts of the rice grain, but it could also be the result of interactions among chemicals, e.g. divalent cations with phytic acid, and phytic acid and mineral elements with protein [32-33].

Further investigation is needed to know more about the mechanism of significantly negative/positive correlations among minerals observed in this study, but it was well documented in previous reports that most of the metal minerals are positively correlated [34-35].

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