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Positional variation in grain mineral nutrients within a rice panicle and its relation to phytic acid concentration*

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Abstract: Six *japonica* rice genotypes, differing in panicle type, grain density, and phytic acid (PA) content, were applied to investigate the effect of grain position on the concentrations of major mineral nutrients and its relation to PA content and grain weight within a panicle. Grain position significantly affected the concentrations of the studied minerals in both the vertical and horizontal axes of a rice panicle. Heavy-weight grains, located on primary rachis and top rachis, generally had higher mineral concentrations, but were lower in PA concentration and molar ratios of PA/Zn, compared with the small-weight grains located on secondary rachis and bottom rachis, regardless of rice genotypes. However, on the basis of six rice genotypes, no significant correlations were found among mineral elements, PA, and grain weight. These results suggested that some desired minerals, like Zn and Fe, and their bioavailability, can be enhanced simultaneously by the modification of panicle patterns, and it will be helpful in the selection of rice genotypes with low PA and high mineral nutrients for further breeding strategy without sacrificing their high yields.

Key words: Grain position, Minerals, Phytic acid, Rice, *Oryza sativa* L.
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1 Introduction

Micronutrient malnutrition, often called 'Hidden Hunger', now affects more than two billion people worldwide (McLaughlin *et al*., 1999). Populations in developing countries are particularly at risk as they live largely on plant-based diets without sufficient micronutrients gained from meat, fruits, or vegetables (Frank *et al*., 2009). The case is particularly severe for women, infants, and children (Umeta *et al*., 2005). It is therefore imperative to improve the nutrient composition of plant food so as to alleviate the widespread micronutrient malnutrition (Ferguson *et al*., 1988).

Rice is one of the major cereal crops, consumed by nearly two billion people worldwide. As a primary dietary source of energy, especially for the low-income and rural population, rice plays an important role in providing plant proteins, carbohydrates, and other nutrients (Ferguson *et al*., 1988). However, rice grain is relatively low in some micronutrients such as iron (Fe), zinc (Zn), and calcium (Ca), compared with other staple crops such as wheat, maize, and legumes (Adeyeye *et al*., 2000).

In the past few decades, great efforts have been made to elucidate the effects of genotype and environment on mineral uptake and accumulation in rice plants (Schachtman and Barker, 1999). It has been demonstrated that mineral concentrations in rice grain vary greatly according to genotype, geographical location, and other environmental factors (Anandan *et al*., 2011). Although the extent of difference is cultivar-dependent, the former studies showed that grain position within a panicle had a considerable impact on grain chemical composition, like protein or micro-elements (Calderini and Ortiz-Monasterio, 2003; Liu *et al*., 2005a), suggesting that the improvement of spikelet architecture and morphological pattern is possible for obtaining the cultivars with high yield

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and balanced nutrition (Calderini and Ortiz-Monasterio, 2003).

Large variation among rice grains in a panicle is unfavorable for commercial value and quality (Jeng *et al*., 2006). Some research has been performed on the effect of grain position within a panicle on rice quality, so as to reduce the variation through improved agronomic practices, including application of plant growth regulators, and better water and fertilizer management (Jongkaewwattana and Geng, 1991). It was reported that there were large variations in grain quality (chalky occurrence, palatability, amylose, protein, and amylographic characteristics) between primary and secondary rachides (Matsue *et al*., 1995) and among the different positions within a rice panicle (Kobayasi *et al*., 2002). However, to date, little research has been done on the positional variation of micronutrient concentration in relation to rice panicle morphology.

Phytic acid (PA) in crops, including cereals and legumes, is an anti-nutritional factor for animals and human, as it can strongly chelate mineral nutrients such as Zn, Fe, Ca, and magnesium (Mg) (Raboy, 2001), leading to a marked reduction in their bioavailability (Raboy *et al*., 1984). Moreover, animals possibly excrete more phytates if they are given high PA feed, which poses a threat of contaminating water. Therefore, it was important to reduce PA concentration in rice grain for the improvement of crops' nutritional quality (Raboy, 2001).

In this experiment, six *japonica* rice genotypes, differing in panicle type, grain density, and PA content, were used with the following objectives: (1) to investigate the effect of grain position on mineral elements (potassium (K), Mg, sodium (Na), Ca, Zn, Fe, manganese (Mn), and copper (Cu)), as well as PA concentration; (2) to identify the positional distribution of mineral elements in grains within a panicle as affected by the panicle morphology of rice genotypes; and, (3) to illustrate the relationships among mineral elements, PA content, and grain weight at different positions within a panicle.

2 Materials and methods

2.1 Plant materials and field experiments

According to previous surveys on grain PA and panicle type of 56 rice cultivars and 32 breeding lines, collected from different locations of China (Liu *et al*., 2005a; Cheng *et al*., 2007), six *japonica* rice genotypes (98110, HIPj, Xiushui63, Xiushui994, Chunjiang15, and Xiushui11) were selected for the current study. All genotypes were seeded in late May and transplanted in late June at the experimental farm of the Huajiachi Campus of Zhejiang University, China. Each genotype was grown in two replicates of an eight-row plot, 2 m long and 20 cm wide between rows with nine hills in each row and three seedlings per hill. The trial was managed according to locally recommended agronomic practices.

2.2 Grain sampling and analysis

Grains from six positions within a panicle, i.e., top primary, top secondary, middle primary, middle secondary, bottom primary, and bottom secondary rachides, were sampled for analysis. The classification of these positions for each genotype is shown in Table 1. Panicle traits, including panicle length and bending degree of panicle (an angle from the tip of panicle to the extending top of stem), were determined at maturity with 20 individual measurements of main stems in each plot (Table 1).

At maturity, 30 panicles were harvested from each replication. After being dried naturally, rice grains were collected separately from six positions, then husked, milled, and passed through a 0.5-mm sieve. The flour was then stored in a desiccator until analysis.

The concentrations of K, Na, Mg, Ca, Zn, Fe, Mn, and Cu were determined by an atomic absorption spectrophotometer (Perkin-Elmer Model AA6300) after wet digestion with nitric acid. PA was determined as described by Miller *et al*. (1980) with modification (Liu *et al*., 2005a). Triplicate measurements were performed for each sample.

2.3 Data analysis

Statistical analysis was performed using statistics software version SPSS 11.0. The data were submitted for variance analysis and the means were tested by least significant difference. Correlation analysis was conducted to characterize the relationships among eight mineral elements, PA content, and grain weight for different grain positions and six genotypes, respectively.

Table 1 Classification of grain position within a panicle and panicle morphological traits for six rice cultivars

Cultivar	Total number of primary branches	Classification of rachis within a panicle			Panicle morphological character				
		Upper rachis	Middle rachis	Lower rachis	PL (cm)	GPP	GD (grain/cm)	BD (°)	Yield (kg/hm^2)
98110	14	4	5	5	16.2	151.2	9.3	9.0	8427.5
HIPj	14	4	5	5	16.0	148.7	9.3	9.2	8132.7
Xiushui63	12	3	5	4	15.8	126.5	8.0	9.7	7737.1
Xiushui994	14	4	5	5	15.4	132. 6	8.6	7.8	7945.3
Chunjiang15	12	4	4	4	21.5	103.7	4.8	76.2	6863.8
Xiushui11	12	4	4	4	19.2	110.0	5.7	86.1	7142.0

PL: panicle length; GPP: grains per panicle; GD: grain density; BD: bending degree of panicle

3 Results

3.1 Positional variation in K, Mg, Na, Ca, Zn, Fe, Mn, and Cu concentrations within a panicle

As shown in Table 2, grain position was an important source of variation for grain mineral concentrations. Of all eight minerals, there were significant differences among grains located on the different positions within a panicle. The grains on the top primary and middle primary rachides had substantially higher minerals concentrations than those on the bottom secondary and middle secondary rachides. The order of positional differences in mineral concentrations was: top primary rachides>middle primary rachides>bottom primary rachides/top secondary rachides>middle secondary rachides>bottom secondary rachides, although the extent of difference was genotype-dependent and varied largely with various mineral elements. This result clearly indicated that grain position affected the concentrations of all eight minerals in both vertical and horizontal axes of the rice panicle. In comparative terms, the effects of grain positions on Na, Fe, and Cu concentrations were more substantial than those on Ca and Zn concentrations, as reflected by their differences in coefficients of variation (CVs) and the variable range of different positions (Table 2).

The ratio of the eight mineral concentrations was relatively stable or showed only slight variations among different grain positions in a rice cultivar (Table 2). However, relatively great variability in the ratio of various minerals was found among cultivars, although the concentrations of Zn, Fe, Mn, and Cu were found to be remarkably lower than those of K, Mg, Na, and Ca in rice grains, with the lowest values for Cu and Fe, and the highest for K and Mg. For instance, 98110 had much higher Ca and Mg concentrations than Xiushui11, whereas the concentrations of Fe, Mn, and Cu for 98110 were lower than those for Xiushui11, in terms of an average of six different positions. This result suggested that grain positions had a relatively small impact on the ratio of mineral concentrations compared with the rice genotypes, despite the different sensitivities to the effect of grain positions among different mineral elements.

Considering the yield potential of rice genotypes (Table 1) and grain mineral concentrations (Table 2), little relationship was found between the eight mineral concentrations and rice genotypes, differing in grain density in a panicle and grain yield, in terms of average different positions. In contrast, the extent of positional variation in some mineral elements (K, Na, Fe, and Cu), particularly the variable range between top primary rachides and bottom secondary rachides, even tended to be more pronounced than that of the six rice genotype variations (Table 2). This implied that the concentrations of mineral elements in rice grains were not inherently related to the level of grain yield for different rice genotypes.

3.2 Positional variation in PA content, grain weight, molar ratios of PA/Zn and PA/Fe within a panicle

From Table 3, the marked differences among different positions were found in PA concentration, grain weight, and molar ratios of PA/Zn and PA/Fe. For grain weight, the grains located on the primary rachis (PR) and top rachis (TR) were significantly heavier than those on the secondary rachis (SR) and bottom rachis (BR), irrespective of rice genotype. On the other hand, the grains on PR and TR had

Table 2 Positional variations in the concentrations of K, Mg, Na, Ca, Zn, Fe, Mn, and Cu in six different rice cultivars

Cultivar	Grain position	Concentration (μg/g)							
		K	Mg	Na	Ca	Zn	Fe	Mn	Cu
98110	TPR	1627a	1518a	260.6a	263.7a	25.28a	10.27a	24.82bc	2.780a
	TSR	1568b	1453ab	219.8ab	256.5b	24.58b	9.57c	24.20c	2.471b
	MPR	1468c	1398b	203.3bc	253.9bc	24.55b	9.89b	26.08a	2.425b
	MSR	1396d	1315c	191.7bcd	250.7bc	24.07c	9.31d	26.03a	2.273b
	BPR	1440cd	1378bc	170.4cd	247.8cd	23.48d	8.96e	25.19b	1.866c
	BSR	1314e	1324c	156.3d	242.3d	22.78e	8.59f	24.63c	1.671c
	Mean	1469	1398	200.4	252.5	24.12	9.43	25.16	2.248
	CV	7.76%	5.56%	18.57%	2.93%	3.69%	6.49%	3.04%	18.28%
HIPj	TPR	1862a	1704a	265.6a	282.1a	26.76a	11.93a	31.62a	2.815a
	TSR	1732b	1585b	200.5cde	269.4b	25.97b	10.90c	28.78b	2.567bc
	MPR	1583c	1440c	241.7ab	267.7b	25.83b	11.33b	27.52c	2.688ab
	MSR	1285e	1321d	215.1bc	263.8c	25.29c	10.92c	25.55c	2.459cd
	BPR	1536d	1389c	192.6de	257.6d	24.75d	10.42d	26.81c	2.302d
	BSR	1328e	1329d	184.1e	253.1e	24.38d	9.88e	25.07d	2.022e
	Mean	1554	1461	216.6	265.6	25.50	10.90	27.56	2.476
	CV	14.43%	10.47%	14.49%	3.83%	3.41%	6.53%	8.71%	11.49%
Xiushui63	TPR	1836a	1757a	312.2a	290.1a	22.87a	9.68a	46.83a	2.505a
	TSR	1790a	1671ab	267.7b	283.2b	22.50a	9.42bc	43.27c	2.167b
	MPR	1802a	1655b	275.8b	281.8b	21.99b	9.33cd	45.03b	2.225b
	MSR	1570b	1415c	249.9bc	276.9c	21.28c	9.16d	38.87de	2.168b
	BPR	1737a	1715ab	209.7c	270.3d	20.88cd	8.70e	42.87c	2.010b
	BSR	1498b	1429c	191.9c	262.4e	20.44d	8.40f	38.73e	1.643c
	Mean	1706	1607	251.2	277.5	21.66	9.11	42.60	2.120
	CV	8.12%	9.19%	17.66%	3.57%	4.39%	5.25%	7.66%	13.40%
Xiushui994	TPR	1655a	1359a	312.2a	304.2a	20.84a	10.81a	36.88a	3.270a
	TSR	1587ab	1297ab	239.1b	296.1b	20.46ab	10.18b	32.36c	2.908b
	MPR	1492b	1305ab	218.4b	292.3c	19.89ab	10.23b	33.81b	2.868b
	MSR	1571ab	1245b	202.2bc	287.4d	19.75ab	9.76c	32.52c	2.673bc
	BPR	1514b	1291ab	167.6cd	282.7e	19.21b	9.17d	32.98c	2.442cd
	BSR	1465b	1225b	150.6d	276.1f	18.79b	8.96e	30.29d	2.198d
	Mean	1547	1287	215.0	289.8	19.82	9.85	33.14	2.727
	CV	4.54%	3.68%	26.80%	3.44%	3.84%	7.09%	6.55%	13.84%
Chunjiang15	TPR	1571a	1456a	228.9a	254.0a	24.81a	9.72a	34.02a	3.208a
	TSR	1308bc	1339b	179.9bc	247.8b	22.40b	9.26b	32.30b	2.975b
	MPR	1395b	1310bc	202.0abc	247.0b	21.55c	9.14b	31.93b	2.832b
	MSR	1336bc	1250c	218.7ab	240.2cd	22.40b	8.81c	31.22bc	2.870b
	BPR	1278c	1274bc	187.0abc	236.9d	21.26c	8.40d	30.84c	2.603c
	BSR	1285c	1375b	174.9c	231.6e	20.45d	8.18e	30.18c	2.525c
	Mean	1362	1334	198.7	242.9	22.15	8.92	31.75	2.836
	CV	8.13%	5.59%	10.99%	3.37%	6.77%	6.41%	4.24%	8.78%
Xiushui11	TPR	1527a	1458a	273.8a	229.6a	24.48a	11.71a	30.58a	2.868a
	TSR	1347bc	1343a	228.2b	223.9b	23.74a	11.42b	26.95b	2.672b
	MPR	1421ab	1333a	228.9b	218.8cd	24.04a	11.20c	25.15bc	2.568c
	MSR	1345bc	1365a	216.1bc	214.6d	23.49a	10.86d	24.30c	2.458d
	BPR	1276c	1368a	206.0bc	209.5e	22.33b	10.34e	24.06c	2.283e
	BSR	1098d	864b	191.5c	203.2f	21.92b	10.08f	22.65d	2.132f
	Mean	1336	1289	224.1	216.6	23.33	10.94	25.62	2.497
	CV	10.81%	16.50%	12.57%	4.43%	4.29%	5.78%	10.99%	10.65%

TPR: top primary rachis; TSR: top secondary rachis; MPR: middle primary rachis; MSR: middle secondary rachis; BPR: bottom primary rachis; BSR: bottom secondary rachis; CV: coefficient of variation. The values followed by the same letters within a column are not significantly different in term of the same cultivar (P>0.05)

Table 3 Positional variations in phytic acid (PA) concentration, grain weight, and the molar ratios of PA/Zn and PA/Fe in six different rice cultivars

Cultivar	Grain position	PA concentration (mg/g)		Grain weight (mg)		PA/Zn molar ratio		PA/Fe molar ratio	
		PR	SR	PR	SR	PR	SR	PR	SR
98110	TR	4.68b	4.55b	25.47a	20.12c	18.35c	18.34c	45.17e	47.09d
	MR	4.89a	4.49b	24.35a	20.63c	19.74b	18.50c	49.00c	47.81cd
	BR	5.07a	5.25a	22.16b	17.06d	21.39a	22.85a	56.02b	60.58a
	Mean 1	4.88	4.76	23.99	19.27	19.83	19.89	50.06	51.83
	Mean 2	4.82		21.63		19.86		50.95	
	CV	6.24%		14.10%		9.48%		11.80%	
HIPj	TR	4.54a	4.43ab	24.12a	19.05d	16.82a	16.90a	37.73c	40.26b
	MR	4.18c	4.32ab	23.16b	19.01d	16.03b	16.91a	36.54cd	39.17b
	BR	3.68d	4.23c	20.50c	18.09e	14.73c	17.17a	35.00d	42.39a
	Mean 1	4.13	4.32	22.59	18.72	15.86	16.99	36.42	40.61
	Mean 2	4.23		20.65		16.43		38.52	
	CV	7.10%		11.89%		5.57%		6.90%	
Xiushui63	TR	5.64bc	5.60c	26.54a	23.04c	24.66d	25.24d	58.26c	60.30c
	MR	6.13a	6.06a	25.68ab	22.40c	27.88c	28.48b	65.70b	66.16b
	BR	5.97ab	5.98ab	25.26b	22.45c	28.59b	29.26a	68.62b	71.19a
	Mean 1	5.91	5.88	25.83	22.63	27.04	27.66	64.19	65.88
	Mean 2	5.90		24.23		27.35		65.04	
	CV	3.77%		7.48%		7.01%		7.56%	
Xiushui994	TR	4.58ab	4.46b	25.71a	23.18ab	21.98c	21.80c	42.37d	43.81c
	MR	4.28c	4.36c	24.14a	18.62c	21.52c	22.07c	41.84d	44.67c
	BR	4.43bc	4.64a	20.29bc	17.57c	23.06b	24.69a	48.31b	51.78a
	Mean 1	4.43	4.49	23.28	19.79	22.19	22.85	44.17	46.75
	Mean 2	4.46		21.58		22.52		45.46	
	CV	3.01%		15.04%		5.27%		8.47%	
Chunjiang15	TR	4.87bc	4.90b	27.69a	21.41d	19.63c	21.88b	50.10c	52.92bc
	MR	4.98ab	5.09a	26.51ab	25.07bc	23.11a	22.72b	54.49b	57.78a
	BR	4.85bc	4.75c	24.68c	21.03d	22.81b	23.23a	57.74a	58.07a
	Mean 1	4.90	4.91	26.29	22.50	21.85	22.61	54.11	56.26
	Mean 2	4.91		24.40		22.23		55.18	
	CV	2.38%		11.01%		6.12%		5.90%	
Xiushui11	TR	4.23b	4.31a	29.00a	28.61ab	17.28c	18.15b	36.12c	37.74c
	MR	4.49a	4.25b	28.48ab	24.26c	18.68b	18.09b	40.09b	39.13b
	BR	4.51a	4.38a	27.49b	19.73d	20.21a	19.98a	43.62a	43.45a
	Mean 1	4.41	4.31	28.32	24.20	18.72	18.74	39.94	40.11
	Mean 2	4.36		26.26		18.73		40.03	
	CV	2.74%		13.84%		6.14%		7.57%	

PR: primary rachis; SR: secondary rachis; TR: top rachis; MR: middle rachis; BR: bottom rachis. The crossing column between TR and PR means TPR (top primary rachis), and the corresponding expressions were for TSR (top secondary rachis, TR-SR), MPR (middle primary rachis, MR-PR), MSR (middle secondary rachis, MR-SR), BPR (bottom primary rachis, BR-PR), and BSR (bottom secondary rachis, BR-SR), respectively. Mean 1 is the average of three different positions for PR or SR; Mean 2 is the average of all six different positions; CV is the coefficient of variation. The values followed by the same letters within a column are not significantly different in term of the same cultivar (P>0.05)

somewhat lower PA concentrations relative to those on SR and BR. This result suggested that the grains with heavier weights were generally lower in PA concentrations in a comparison of different positions within a panicle, which was opposite to the effect of grain positions on mineral concentrations. However, the extent of positional variation in PA concentrations was considerably smaller compared with that in grain weight and most of minerals, in terms of their small CVs and narrow-range in PA among different grain positions for all six rice genotypes presented here (Tables 2 and 3).

The molar ratio of PA/Zn was commonly considered as an important parameter for the evaluation of Zn bioavailability in cereal and legumes (Raboy *et al*., 1984). As shown in Table 3, the average molar ratio of PA/Zn ranged from 18.73 (Xiushui11) to 27.35 (Xiushui63) among the six genotypes, and the extent of positional variation in the molar ratio of PA/Zn was much larger than that in PA concentrations among different grains within a panicle, as reflected by their CVs. In general, PR and TR tended to have lower molar ratio of PA/Zn relative to SR and BR, which was almost consistent with the tendency of positional differences in PA concentration. This result implied that grain position was closely related to Zn bioavailability in rice grains, with relatively high Zn bioavailability noted for PR and TR within a panicle.

The average molar ratio of PA/Fe ranged from 38.52 to 65.04 among all six genotypes, wider variation compared with that of average molar ratio of PA/Zn (Table 3). Moreover, the extent of the variation in the molar ratio of PA/Fe among different grain positions was also somewhat greater than that in the molar ratio of PA/Zn, despite the similar trend for difference between PR and SR for both molar ratios of PA/Fe and PA/Zn.

3.3 Correlations among K, Mg, Na, Ca, Zn, Fe, Mn, Cu, PA contents and grain weight

The Pearson correlation coefficients among eight minerals, PA and grain weight were calculated for six different grain positions in a panicle (Table 4). There were highly positive correlations ($P<0.01$) between Zn and Fe, and statistically positive correlations ($P<0.05$ or $P<0.01$) among Ca, Zn, Fe, and Cu, regardless of genotypes. However, the statistical significance varied greatly with rice genotype for most of their correlations among other mineral concentrations, and also among minerals, PA, and grain weight. Generally, there were positive correlations among eight minerals, and between minerals and grain weight, but negative correlations between minerals and PA content.

Table 5 presents the correlations among the eight minerals, PA and grain weight for six genotypes. The correlation coefficients among the minerals were statistically significant only for K-Ca ($r=0.833$), but no significant correlations ($P>0.05$) were observed among PA and minerals, except for PA-Mn ($r=0.818$). Furthermore, grain weight was poorly correlated with minerals and PA. This result implied that grain PA concentration was relatively independent of grain weight and most metal minerals for different rice genotypes.

4 Discussion

The yield potential of a rice cultivar is closely related to grain number per panicle and grain weight (Cheng *et al*., 2007). However, it was practically difficult to make a balance of grain number and grain weight for a rice panicle owing to the limiting assimilation supply from leaves (Mohapatra and Sahu, 1991). In the past two decades, the enhancement of yield level for newly released *japonica* cultivars was mostly attributable to the elevated grain number per panicle (Padmajarao, 1995), and there was a narrow variation of grain weight between modern *japonica* cultivars and landraces (He *et al*., 2011). In fact, the *japonica* cultivars now being widely planted in China were mainly characterized by compact and erect or semi-erect panicles with more grains per panicle (Cheng *et al*., 2007). On the other hand, the compact-panicle cultivars were often found to show a great variation in floret development and grain filling among different rachides within a panicle (Liu *et al*., 2005b; Wang *et al*., 2006); also there were dramatic differences in protein composition, amylose content, grain weight, and chalkiness among different positional grains (Cheng *et al*., 2007). In our current study, grain position significantly affected the concentrations of minerals in both vertical and horizontal axes of the rice panicle. The grains located on top primary and middle primary rachides were substantially higher than those on bottom secondary rachides and middle secondary rachides for all measured minerals (K, Mg, Na, Ca, Mn, Zn, Fe, and Cu). This result was generally in agreement with several previous findings, which demonstrated that the levels of Zn and Fe concentrations in wheat grain decreased at grain positions far distal from rachis (Calderini and Ortiz-Monasterio, 2003), and the heavy-weight grains had higher Zn and Fe concentrations relative to the small-weight grains within a wheat spike (Liu *et al*., 2006). However, little association was found between mineral concentrations and the rice yield for the rice

Table 4 Correlation coefficients of K, Mg, Na, Ca, Zn, Fe, Mn, Cu, phytic acid (PA), and grain weight (GW) among different positions within a panicle

Cultivar	Feature	Correlation coefficient									
		K	Mg	Na	Ca	Zn	Fe	Mn	Cu	PA	GW
98110	K	1.000									
	Mg	0.960^{**}	1.000								
	Na	0.932^{**}	0.889^{*}	1.000							
	Ca	0.953^{**}	0.891^{*}	0.991^{**}	1.000						
	Zn	0.904^{*}	0.810	0.959^{**}	0.981^{**}	1.000					
	Fe	0.856^{*}	0.796	0.936^{**}	0.955^{**}	0.981^{**}	1.000				
	Mn	−0.302	−0.430	−0.151	−0.092	0.064	0.143	1.000			
	Cu	0.874^{*}	0.777	0.962^{**}	0.971^{**}	0.993^{**}	0.970^{**}	0.054	1.000		
	PA	−0.577	−0.353	−0.662	−0.677	−0.730	−0.612	−0.117	−0.768	1.000	
	GW	0.702	0.663	0.711	0.770	0.794	0.854^{*}	0.361	0.733	−0.315	1.000
HIPj	K	1.000									
	Mg	0.963^{**}	1.000								
	Na	0.601	0.650	1.000							
	Ca	0.816^{*}	0.891^{*}	0.880^{*}	1.000						
	Zn	0.826^{*}	0.886^{*}	0.859^{*}	0.990^{**}	1.000					
	Fe	0.709	0.751	0.951^{**}	0.957^{**}	0.953^{**}	1.000				
	Mn	0.961^{**}	0.982^{**}	0.744	0.923^{**}	0.907^{*}	0.825^{*}	1.000			
	Cu	0.743	0.753	0.883^{*}	0.934^{**}	0.956^{**}	0.978^{**}	0.812^{*}	1.000		
	PA	0.351	0.567	0.512	0.665	0.669	0.537	0.492	0.492	1.000	
	GW	0.684	0.624	0.902^{*}	0.744	0.729	0.839^{*}	0.741	0.795	0.161	1.000
Xiushui63	K	1.000									
	Mg	0.938^{**}	1.000								
	Na	0.746	0.541	1.000							
	Ca	0.807	0.614	0.985^{**}	1.000						
	Zn	0.821^{*}	0.651	0.953^{**}	0.972^{**}	1.000					
	Fe	0.753	0.533	0.984^{**}	0.994^{**}	0.963^{**}	1.000				
	Mn	0.956^{**}	0.925^{**}	0.763	0.782	0.800	0.718	1.000			
	Cu	0.780	0.618	0.947^{**}	0.963^{**}	0.881^{*}	0.947^{**}	0.767	1.000		
	PA	−0.473	−0.531	−0.483	−0.533	−0.661	−0.503	−0.477	−0.433	1.000	
	GW	0.789	0.836^{*}	0.552	0.547	0.503	0.465	0.896^{*}	0.634	−0.201	1.000
Xiushui994	K	1.000									
	Mg	0.669	1.000								
	Na	0.880^{*}	0.853^{*}	1.000							
	Ca	0.848^{*}	0.875^{*}	0.978^{**}	1.000						
	Zn	0.879^{*}	0.807	0.963^{**}	0.989^{**}	1.000					
	Fe	0.772	0.819^{*}	0.961^{**}	0.979^{**}	0.962^{**}	1.000				
	Mn	0.734	0.924^{**}	0.873^{*}	0.852^{*}	0.780	0.830^{*}	1.000			
	Cu	0.827^{*}	0.865^{*}	0.974^{**}	0.995^{**}	0.977^{**}	0.990^{**}	0.872^{*}	1.000		
	PA	0.142	−0.053	0.056	−0.110	−0.098	−0.179	−0.081	−0.164	1.000	
	GW	0.587	0.945^{**}	0.862^{*}	0.909^{*}	0.854^{*}	0.898^{*}	0.834^{*}	0.905^{*}	−0.162	1.000
Chunjiang15	K	1.000									
	Mg	0.690	1.000								
	Na	0.805	0.191	1.000							
	Ca	0.802	0.460	0.607	1.000						
	Zn	0.869^{*}	0.542	0.792	0.850^{*}	1.000					
	Fe	0.820^{*}	0.488	0.641	0.993^{**}	0.881^{*}	1.000				
	Mn	0.888^{*}	0.618	0.655	0.967^{**}	0.933^{**}	0.971^{**}	1.000			
	Cu	0.818^{*}	0.480	0.715	0.944^{**}	0.949^{**}	0.972^{**}	0.956^{**}	1.000		
	PA	0.111	−0.567	0.575	0.309	0.251	0.329	0.161	0.367	1.000	
	GW	0.764	0.124	0.863^{*}	0.592	0.615	0.567	0.602	0.540	0.439	1.000
Xiushui11	K	1.000									
	Mg	0.871^{*}	1.000								
	Na	0.921^{**}	0.690	1.000							
	Ca	0.916^{*}	0.751	0.938^{**}	1.000						
	Zn	0.944^{**}	0.733	0.887^{*}	0.940^{**}	1.000					
	Fe	0.909^{*}	0.714	0.911^{*}	0.990^{**}	0.970^{**}	1.000				
	Mn	0.842^{*}	0.645	0.973^{**}	0.938^{**}	0.813	0.892^{*}	1.000			
	Cu	0.928^{**}	0.744	0.950^{**}	0.998^{**}	0.955^{**}	0.992^{**}	0.938^{**}	1.000		
	PA	−0.337	−0.189	−0.507	−0.503	−0.458	−0.500	−0.548	−0.522	1.000	
	GW	0.838^{*}	0.882^{*}	0.709	0.804	0.734	0.771	0.700	0.782	0.041	1.000

$^{**, *}$ Correlation is significant at the 0.01 and 0.05 levels (2-tailed), respectively

Table 5 Correlation coefficients of K, Mg, Na, Ca, Zn, Fe, Mn, Cu, phytic acid (PA) and grain weight (GW) among six rice cultivars

Feature	Correlation coefficient									
	K	Mg	Na	Ca	Zn	Fe	Mn	Cu	PA	GW
K	1.000									
Mg	0.801	1.000								
Na	0.670	0.657	1.000							
Ca	0.833*	0.403	0.270	1.000						
Zn	−0.195	0.200	−0.210	−0.444	1.000					
Fe	−0.216	−0.269	0.076	−0.304	0.490	1.000				
Mn	0.724	0.621	0.699	0.597	−0.611	−0.562	1.000			
Cu	−0.573	−0.759	−0.590	−0.112	−0.318	0.036	−0.236	1.000		
PA	0.562	0.726	0.568	0.285	−0.339	−0.744	0.818*	−0.543	1.000	
GW	−0.420	−0.133	0.316	−0.661	−0.209	−0.057	0.153	0.030	0.285	1.000

**, * Correlation is significant at the 0.01 and 0.05 levels (2-tailed), respectively

genotypes differing in grain density and grain weight (Table 2), and between grain weight and the mineral concentrations, in term of an average of six different grain positions (Table 5). The results indicated the feasibility of enhancing the desirable mineral concentrations in rice grains without causing reduction in grain weight and yield level by appropriate breeding strategies and agronomic management for the compact rice cultivars. Moreover, in light of the fact that the high yield potentials of compact rice cultivars were mostly achieved by high grain density and floret numbers in a panicle (Wang *et al*., 2006; Cheng *et al*., 2007), it might be practically efficient to increase grain mineral concentrations of whole rice panicles, with the variation of mineral concentration among grains within a panicle being simultaneously decreased, through the modification of their rachis patterns in a panicle, i.e., reducing grain number on the secondary rachis, or increasing grain number on the secondary rachis at the top rachis instead of at the bottom rachis.

Previous studies have showed that the enhancement of yield potentials could cause a significant reduction in nitrogen (N) and phosphoros (P) concentrations of wheat (Calderini *et al*., 1995), rice (Jongkaewwattana and Geng, 1991), maize (Simmonds, 1995), and sunflower grains (López Pereira *et al*., 2000). Additionally, initial findings indicated that grain weight was negatively related to grain PA, prolamin, glutelin, and total protein contents among different positions in a wheat spike (Herzog and Stamp, 1983; Ishimaru *et al*., 2005) or a rice panicle (Matsue *et al*., 1995; Liu *et al*., 2005b). Our present research concluded that the heavy-weight grains in a rice panicle generally had lower PA relative to the light ones (Table 3), which coincided well with previous reports (Liu *et al*., 2005b). Interestingly, our experimental results revealed that Fe and Zn concentrations of heavy-weight grains were generally higher than those of light-weight grains (Table 2), but the positional variations in Fe and Zn concentrations were poorly related to those in grain PA concentration for the different rachides within a rice panicle (Table 4). Thavarajah *et al.* (2010) investigated the impact of environmental temperature on PA, Zn and Fe concentrations in lentil seeds, concluding that high PA content was independent of the increase in total Zn and Fe concentrations in lentil seeds for high temperature regimes. Furthermore, Iwai *et al.* (2012) revealed that total Fe, Zn, and K concentrations in filling grains increased constantly with rice grain development, and the well-developed mature grains had higher concentrations of Fe, Zn, and K minerals than the poorly-developed filling grains. According to our findings, the heavy-weight grains in a rice panicle generally had higher Fe and Zn concentrations relative to the small ones (Table 2). This trend was basically similar to the results reported previously by Thavarajah *et al.* (2010) and Iwai *et al.* (2012), but it was contradictory to a previous hypothesis, which

presumed that the low N and P concentrations in heavy-weight grains were partially caused by the dilution of additional starch accumulation to other chemical components (Calderini and Ortiz-Monasterio, 2003). Nevertheless, these discordant phenomena did not exclude the possibility that grain N and P concentrations were quite different from many metal minerals in their positional variations within a panicle/spike, which could be caused by their difference in the translocation and accumulation in the rice plant and grain location (Schachtman and Barker, 1999; Vasconcelos *et al*., 2003). Considering the extent of variation in grain mineral concentration among different positions within a rice panicle, the effect of grain position was more substantial for Na, Fe, and Cu than for Ca and Zn (Table 2). For rice breeding strategies, the different sensitivities to the effect of grain position on these metal minerals suggested that the improvement of panicle architecture and morphological pattern should be attended to more carefully if the deficiency of desired minerals was caused by low Fe concentration rather than low Zn and Ca concentrations in a compact rice cultivar. However, it should be stressed that the effect of grain position on the concentrations of PA and metal minerals (e.g., Fe, Zn, and Ca) was somewhat variable, depending on rice cultivars, and also the extent of positional variations in PA and metal minerals within a panicle was, in many cases, not so great as the cultivar-dependent difference in PA and metal minerals (Tables 2 and 3). For instance, Xiushui11 had relatively higher Fe, but inherently lower Ca concentration in rice grains than Chunjiang15, irrespective of grain position in the rice panicle. Thus, the selection of rice genotypes with low PA and high desirable minerals was an effective approach to directly reduce grain PA and to enhance the desirable mineral concentrations in rice grains for the improvement of its nutritional quality.

The inhibitory effect of PA on mineral absorption can be estimated by molar ratios of PA/mineral concentrations. The molar ratio of PA/Zn was most widely applied to evaluate Zn bioavailability for human and animal absorption (Raboy *et al*., 1984). The molar ratios of PA/Zn for cereals and legumes, maize, wheat, sorghum, and rice ranged approximately from 20 to 40, and were relatively high compared with millet and soybean. In our study, the molar ratio of PA/Zn in brown rice grains was very close to those reviewed by Adeyeye *et al.* (2000), but it was slightly lower than the previous report of Wei *et al.* (2012), possibly owing to differences in PA and mineral determination methods. Considering the variations of the molar ratios of PA/Zn and PA/Fe among different grain positions, PR and TR generally were lower in both the molar ratios of PA/Zn and PA/Fe compared with BR and SR (Table 3), and also there was a significantly positive association between Zn and Fe concentrations for different grain positions within a panicle (Table 4) and positive correlation for various rice cultivars (Table 5). Our result was in agreement with the previous finding that transgenic rice with the ferritin gene enhanced Zn accumulation in rice grains (Vasconcelos *et al*., 2003). Therefore, it could be concluded that grain position had a large impact not only on grain Zn and Fe concentrations, but also on their bioavailability. Meanwhile, the lower PA and higher Zn or Fe concentrations for the heavier weight grains within a panicle implied that grain Zn and Fe concentrations in rice grains and their bioavailability could be concomitantly enhanced by a selection of good panicle architecture and morphological pattern for the rice genotypes, particularly for the compact rice genotypes with high grain density and yield levels.

Compliance with ethics guidelines

Da SU, Faisal SULTAN, Ning-chun ZHAO, Bing-ting LEI, Fu-biao WANG, Gang PAN, and Fang-min CHENG declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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中文概要：

本文题目：水稻穗内不同粒位间的矿质营养变化差异及其与籽粒植酸含量的关系

Positional variation in grain mineral nutrients within a rice panicle and its relation to phytic acid concentration

研究目的：阐明水稻穗内不同粒位间的主要矿质营养元素和植酸含量差异、粒位分布特点及其与品种穗型间的联系。

创新要点：将水稻品种的穗型变化与稻米营养品质结合起来，从水稻穗粒结构角度，对同一稻穗内不同籽粒间的主要矿质营养元素与植酸含量差异、粒位分布特点及其与水稻品种穗型间的相互关系进行了较系统的探讨分析。

研究方法：以典型的直立穗型和弯穗型粳稻品种为材料，通过对两类水稻品种在相同栽培条件下籽粒矿质营养元素和植酸含量的测定分析，并依据水稻籽粒在稻穗上的着生部位，将同一稻穗内的不同籽粒划分为六个粒位，比较分析了两类品种同一稻穗内不同部位间矿质营养元素和植酸含量的差异变化及其粒位分布特点。

重要结论：水稻穗型虽然与品种间的籽粒矿质营养元素和植酸含量高低没有直接关系，但对其穗内不同籽粒间的主要矿质营养元素和植酸含量存在着较大影响；与稻穗中下部的弱势粒相比，同一稻穗内着生在稻穗上中部的强势粒通常具有相对较高的锌、铁矿质元素含量，而籽粒植酸含量和植酸/锌（铁）摩尔比则有所降低，稻米营养品质也相对较好；不同矿质营养元素相比，粒位效应对铁矿质营养的影响作用要略大于对钙和锌营养元素含量。

关键词组：水稻穗型；粒位效应；营养品质；矿质元素；植酸