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Phenotypic and genetic dissection of the contents of important metallic elements in hybrid rice grown in cadmium-contaminated paddy fields

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A B S T R A C T

Rice (*Oryza sativa* L.) is a staple food that feeds over half of the world's population, and the contents of metallic elements in rice grain play important roles in human nutrition. In this study, the contents of important metallic elements were determined by ICP-OES, and included cadmium (Cd), zinc (Zn), manganese (Mn), copper (Cu), iron (Fe), nickel (Ni), calcium (Ca), and magnesium (Mg) in brown rice, in the first node from the top (Node 1), in the second node from the top (Node 2), and in roots of 55 hybrids and their parental lines. The heritability of metallic element contents (MECs), the general combining ability (GCA) for MEC, and the correlation between MECs in different organs/tissues of hybrids were also analyzed. The results indicated that: (1) there was a positive correlation between the contents of Cd and Zn in nodes and roots, but a negative correlation between the contents of Cd and Zn in brown rice of the hybrids(2) the GCA for MECs can be used to evaluate the ability of the parental lines to improve the metal contents in brown rice of the hybrids(3) the contents of Cd, Zn, Ca, and Mg in brown rice were mainly affected by additive genetic effects(4) the restorer lines R2292 and R2265 can be used to cultivate hybrids with high Zn and low Cd contents in the brown rice.

1. Introduction

Metallic elements play important roles in plant growth and development [1]. Calcium (Ca) and magnesium (Mg) are essential macronutrients. Ca^{2+} is an important component of the plant cell wall and cell membrane and plays an important role in many

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biological processes, such as the regulation of gene transcription, programmed cell death (PCD), and chemical signal release [2,3]. Mg^{2+} can activate the enzymes related to respiration and photosynthesis in plants, and it is also an essential component of chlorophyll [4]. Zinc (Zn), manganese (Mn), copper (Cu), iron (Fe), and nickel (Ni) are essential trace elements in plants. These elements are involved in the synthesis of various proteins and/or enzymes related to photosynthesis, respiration, and redox homeostasis in the form of cofactors, and they function in many aspects of plant physiology [5,6]. Human beings also need to absorb a variety of essential metallic elements from their diet to aid in growth and development. More than 30% of the world's population suffers from iron and/or zinc deficiency due to an over-reliance on single foodstuffs in their diet, a condition known as 'hidden hunger' [7–9]. Increasing the Zn and Fe contents in staple food crops is of great significance to solve the 'hidden hunger' problem, especially in developing counties [10, 11].

When plants absorb essential elements, they also simultaneously accumulate non-essential and/or toxic heavy metals [12,13]. Cadmium (Cd) is a toxic heavy metal that enters the plant through the transport channel of essential divalent metals, and its toxicity mainly originates from non-functional binding to biological ligands of essential elements [12]. Cd enters the human body mainly through smoking and the food chain, and the half-life of Cd in the human body is as long as 10–30 years [14]. Long term exposure of the human body to Cd can increase the risk of many diseases, such as malignant tumors, kidney damage, emphysema, osteoporosis, and chondropathy [15–17]. Cd contamination in the diet typically results from anthropogenic activities such as mining, smelting, and the combustion of coal [18]. In recent years, with the continuous development of urbanization and industrialization across the world, Cd contamination of paddy fields and crops is increasing and having a direct impact on human health [19].

Rice is the staple food for more than half of the world's population, and it contributes large numbers of metallic elements to the human body. In recent decades, the selection and breeding of low-Cd-accumulating rice varieties has been a high priority [20–23]. However, not as much attention has been paid to other metallic elements which can accumulate along with Cd. Research indicates that the absorption of Cd is inhibited by Zn in the test meal [14]. Epidemiological studies have shown that the Cd content in urine is an effective medical indicator to measure the degree of exposure of the human body to Cd, but the Cd contents in food and urine are not significantly correlated [24]. The intake of Zn, Fe, and other nutrients may affect the metabolism of Cd in the human body [25,26]. Therefore, breeding rice varieties with high Zn and low Cd accumulation in the brown rice will be beneficial to human health.

Hybrid rice has been planted widely in many countries due to its high yield and wide adaptability, and it contributes extensively to the increase in worldwide rice yield [27]. The rice hybrids grown in China belong mainly to the *indica* subspecies [23]. The hybrid rice varieties (first filial generation) display a yield advantage of 10–20% over their inbred parental lines, that may be affected by additive, partial dominance and overdominance effects of genomic loci [28]. However, the Cd accumulation in brown rice of hybrid rice was dominated by additive effects, the Cd accumulation ability of brown rice of inbred parental lines significantly affected the Cd accumulation ability of hybrid rice [29,30]. To understand the phenotypic and genetic characteristics of metallic element accumulation in hybrid rice, we analyzed the contents of Cd, Zn, Mn, Cu, Fe, Ni, Ca, and Mg in brown rice, the nodes, and the roots of 55 hybrids and their parental lines. This study aimed to (1): analyze the relationship between the contents of metallic elements in hybrid rice (2), identify the elite parental lines and hybrids with high Zn and low Cd accumulation in the grains, and (3) explore the science-based development of rice hybrids with high concentrations of essential metallic elements and low concentrations of Cd in the brown rice.

2. Materials and methods

2.1. Soil features and plant materials

The Cd-contaminated paddy field was located at Beishan Town, Changsha City, Hunan Province, China (North latitude 28°24′21.15″, East longitude 113°01′13.68″). According to the environmental protection standards HJ 803–2016 and HJ 804–2016 of China, the China Certification & Inspection Group provided the features of the paddy soil in the field (Table S1). The metallic element in paddy soil was isolated by digestion with aqua regia (HCl and HNO_3, 3:1, v/v) and detected by ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometer, Agilent, USA). The metallic element contents in paddy soil were: Ca, 1.70 g kg^{-1}; Mg, 1.66 g kg^{-1}; Fe, 24.00 g kg^{-1}; Zn, 87.00 mg kg^{-1}; Mn, 147.00 mg kg^{-1}; Cu, 22.24 mg kg^{-1}; Ni, 14.80 mg kg^{-1}; and Cd, 0.97 mg kg^{-1}. Supplementary data about the metallic element contents in the paddy soil is given in Table S1.

The plant materials consisted of 55 rice hybrids and 18 parental lines. The 55 hybrids were from two sets of incomplete diallel crosses (NC II design). Group I was composed of 35 hybrids that were generated from seven male sterile (MS) lines (Ke115 A, Ke20 A, Ke108 A, Ke76-1BS, Ke244-1BS, Kebph18 A, and KeBX58S) which were pollinated by five restorer lines (R2292, R2265, R2257, R2683, and R43-07). Group II was composed of 20 hybrids derived from five male sterile (MS) lines ('ZhongqingA', 'TianfengA', 'RenwuA', 'Bing1A', and 'AnfengA') crossed with four restorer lines (R2277, R2257, R43-07, and R2683). All experimental varieties were of the *indica* rice subspecies. The field trial used a randomized complete block design with three replicates. Each plot contained two rows of 20 plants, with a density of 17 cm by 20 cm. All field management practices followed normal agricultural practices and used intermittent irrigation methods to maximize the genotypic differences in Cd accumulation [31,32].

2.2. Sample collection and elements determination

Each sample consisted of five normal plants chosen at random from 20 plants in each plot when the rice was maturing. The brown rice, Node 1 (the first node from the top), Node 2 (the second node from the top), and the roots were collected from each plant. Rinse the roots with tap water for 15 min to remove attached sandy soil, and then rinsed with deionized water for 30 min to remove any attached metal ions. Dry all samples to a constant weight at 70 °C and milled them to a fine powder by grinder (FW100, Taisite, China).

Dissolve 2 g samples of brown rice powder and 0.5 g of the other powdered samples in an HNO_3–$HClO_4$ acid mixture (5:1, v/v) respectively as described previously [33]. The national certified reference materials used in this experiment was Hunan rice GBW10045 (GSB-23), which was prepared and tested by Institute of Geophysical and Geochemical Exploration, Chinese Academy of Geological Sciences, and digested together with blank controls and samples. The digested products were diluted to 25 mL with 1% HNO_3 (the digested brown rice was diluted to 10 mL), and the metallic element contents were detected after filtration. The contents of metallic element were measured by ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometer, Agilent, USA), and the operating parameters were provided by the manufacturer (Table S4).

2.3. Data analysis

The ratio of metal elements concentration between different tissues of plants can be used to estimate the transport efficiency of metal elements in plants [34,35]. The translocation efficiency of the metallic element content (MEC) from the roots to Node 2 (TE3) was calculated as TE3 = MEC in Node 2/MEC in the roots; the MEC translocation efficiency from Node 2 to Node 1 (TE2) was calculated as TE2 = MEC in Node 1/MEC in Node 2; and the MEC translocation efficiency from Node 1 to the brown rice (TE1) was calculated as TE1 = MEC in brown rice/MEC in Node 1.

Two-way ANOVA, general combining ability (GCA), specific combining ability (SCA), narrow-sense heritability (h^2), principal component analysis (PCA) and regression analysis were performed using Data Processing System (DPS) version 16.65 in this study [36]. All figures were generated using SigmaPlot 14.0 software. The heat maps were drawn with TBtools v1.0695 [37].

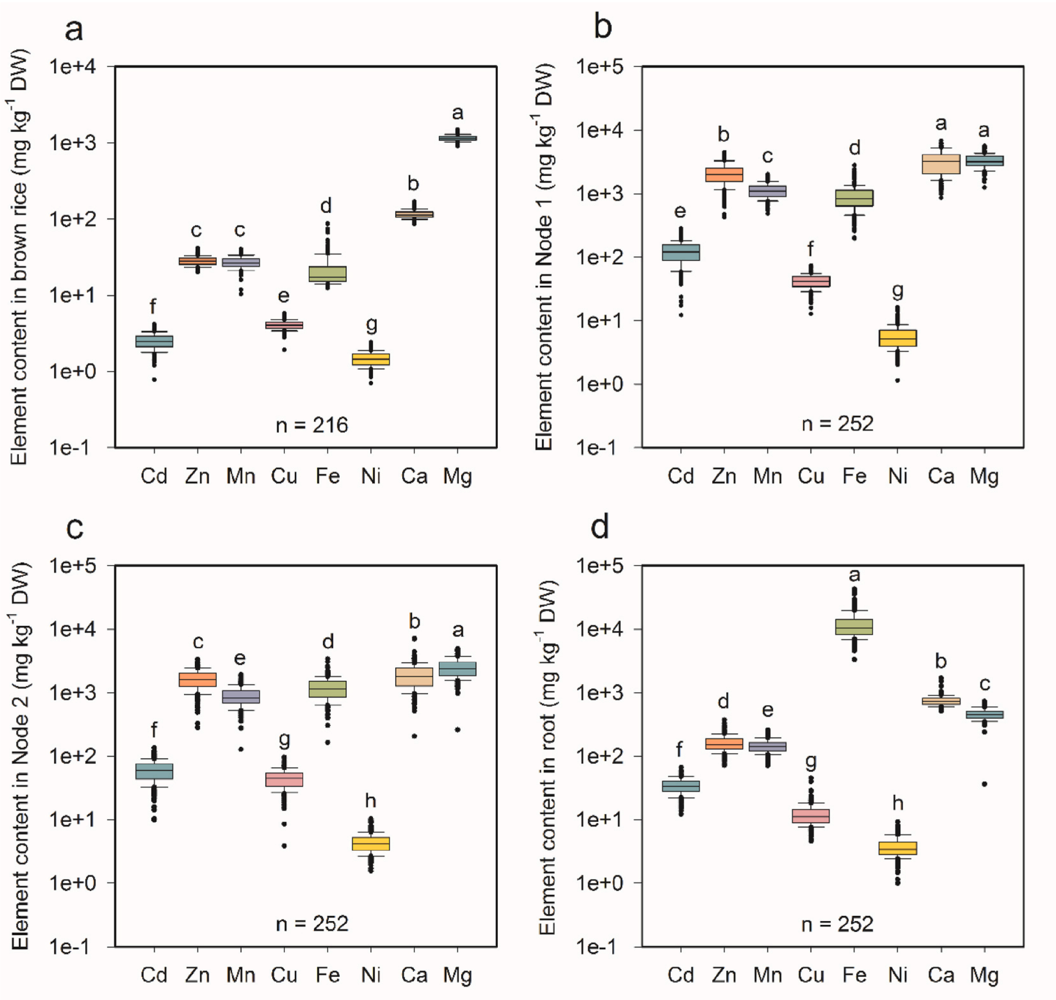

Fig. 1. Metallic element contents in four different organs/tissues of rice. (a) Metallic element contents in brown rice. (b) Metallic element contents in the first node from top (Node 1). (c) Metallic element contents in the second node from top (Node 2). (d) Metallic element contents in the root. The horizontal lines in the boxes indicate the median value, the upper and lower edges of the boxes delineate the upper and lower quartiles, and the maximum and minimum points are set at 1.5 times the interquartile range. Black circles represent outliers beyond the extreme points. Different lower-case letters indicate significant differences at the 0.05 probability level.

3. Results

3.1. Metallic elements accumulate at high levels in the nodes and low levels in the grains

The contents of metal elements in the brown rice, Node 1, Node 2, and roots are shown in Fig. 1. The contents of macro elements (Ca and Mg) in brown rice were significantly higher than the contents of the trace elements (Zn, Mn, Cu, Fe, Ni, and Cd, $p < 0.05$). The average contents of the metal elements in brown rice decreased in the order Mg > Ca > Zn and Mn > Fe > Cu > Cd > Ni ($p < 0.05$), and there was no significant difference between the Zn and Mn contents in brown rice (Fig. 1a). The average contents of the elements in Node 1 decreased in the order Mg and Ca > Zn > Mn > Fe > Cd > Cu > Ni ($p < 0.05$; Fig. 1b). The average contents of the elements in Node 2 decreased in the order Mg > Ca > Zn > Fe > Mn > Cd > Cu > Ni ($p < 0.05$; Fig. 1c). The Fe content in roots was the highest, and the average content of each element in roots was in the decreasing order Fe > Ca > Mg > Zn > Mn > Cd > Cu > Ni ($p < 0.05$; Fig. 1d).

By comparing the contents of metallic elements in different parts of the plant, we found that the lowest contents of all the elements was in brown rice (Fig. 2), and the highest contents of Cd, Zn, Mn, Ni, Ca, and Mg were in Node 1 (Fig. 2a, b, c, f, g). In addition, the highest content of Cu was in Node 2 and the highest content of Fe was in the roots (Fig. 2d and e).

3.2. Rice hybrids accumulate more metallic elements than do their parental lines

As shown in Fig. 3a, the contents of Cd, Mn, and Ca in brown rice of the hybrids were significantly higher than in the parental lines ($p < 0.05$). The contents of Cd, Zn, and Mn in Node 1 of the hybrids were significantly higher than in the parental lines ($p < 0.05$), but the Ni content in Node 1 of the hybrids was significantly lower than that of the parental lines ($p < 0.01$, Fig. 3b). The contents of Cd, Zn, Mn, Fe and Mg in Node 2 of the hybrids were significantly higher than in the parental lines ($p < 0.05$), and the Ni content in Node 2 of the parental lines was significantly higher than in the hybrids ($p < 0.01$, Fig. 3c). The contents of Cd and Mn in the roots of the hybrids were significantly higher than in the parental lines ($p < 0.05$, Fig. 3d). In general, the Ni contents of the parental lines were higher than that of the hybrids only in Nodes 1 and 2 (accounting for 6.3%), and the contents of the other elements in different organs of the hybrids were significantly higher than in the parent lines (accounting for 40.6%), or there were no significant differences between hybrids and parents (accounting for 53.1%). These results suggest that the metallic elements accumulated in the hybrids at levels that

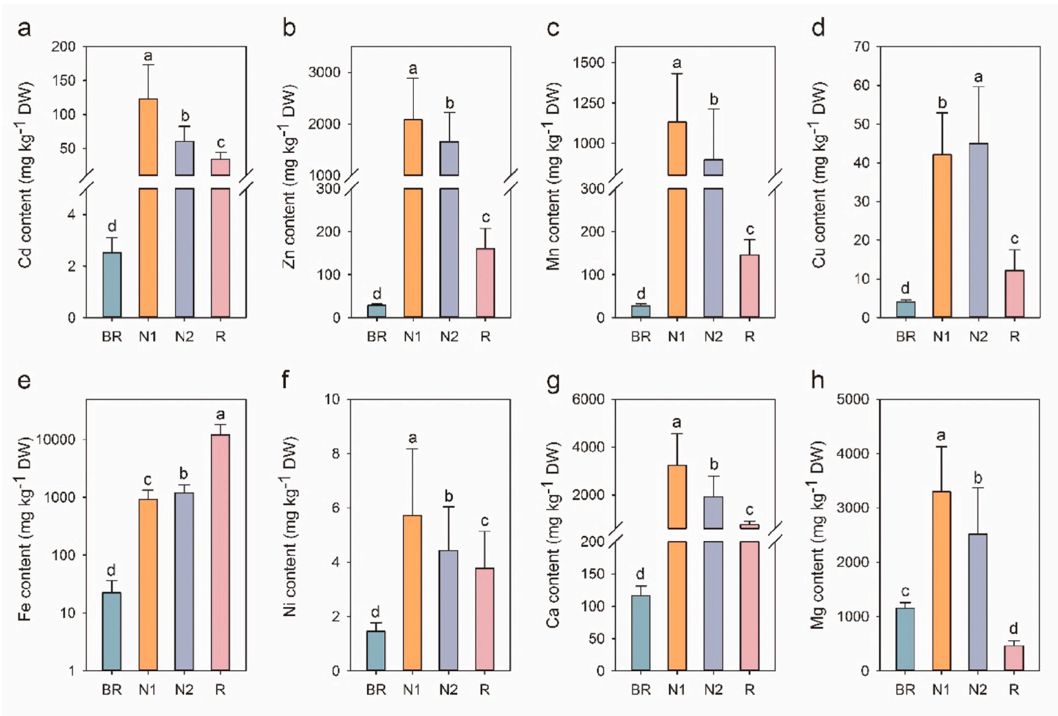

Fig. 2. Comparison of metallic element contents in the four different organs/tissues. (a) The Cd contents in the four different organs/tissues. (b) The Zn contents in the four different organs/tissues. (c) The Mn contents in the four different organs/tissues. (d) The Cu contents in the four different organs/tissues. (e) The Fe contents in the four different organs/tissues. (f) The Ni contents in the four different organs/tissues. (g) The Ca contents in the four different organs/tissues. (h) The Mg contents in the four different organs/tissues. BR, N1, N2, and R indicate brown rice, the first node from the top, the second node from the top, and the roots, respectively. Results are means ± standard deviation, averaged from three replicates for the 55 hybrids and 18 parental lines (n = 183/219/219/219). Different letters indicate significant differences at the 0.05 probability level. BR, N1, N2 and R indicate brown rice, the first node from top, the second node from top and roots, respectively.

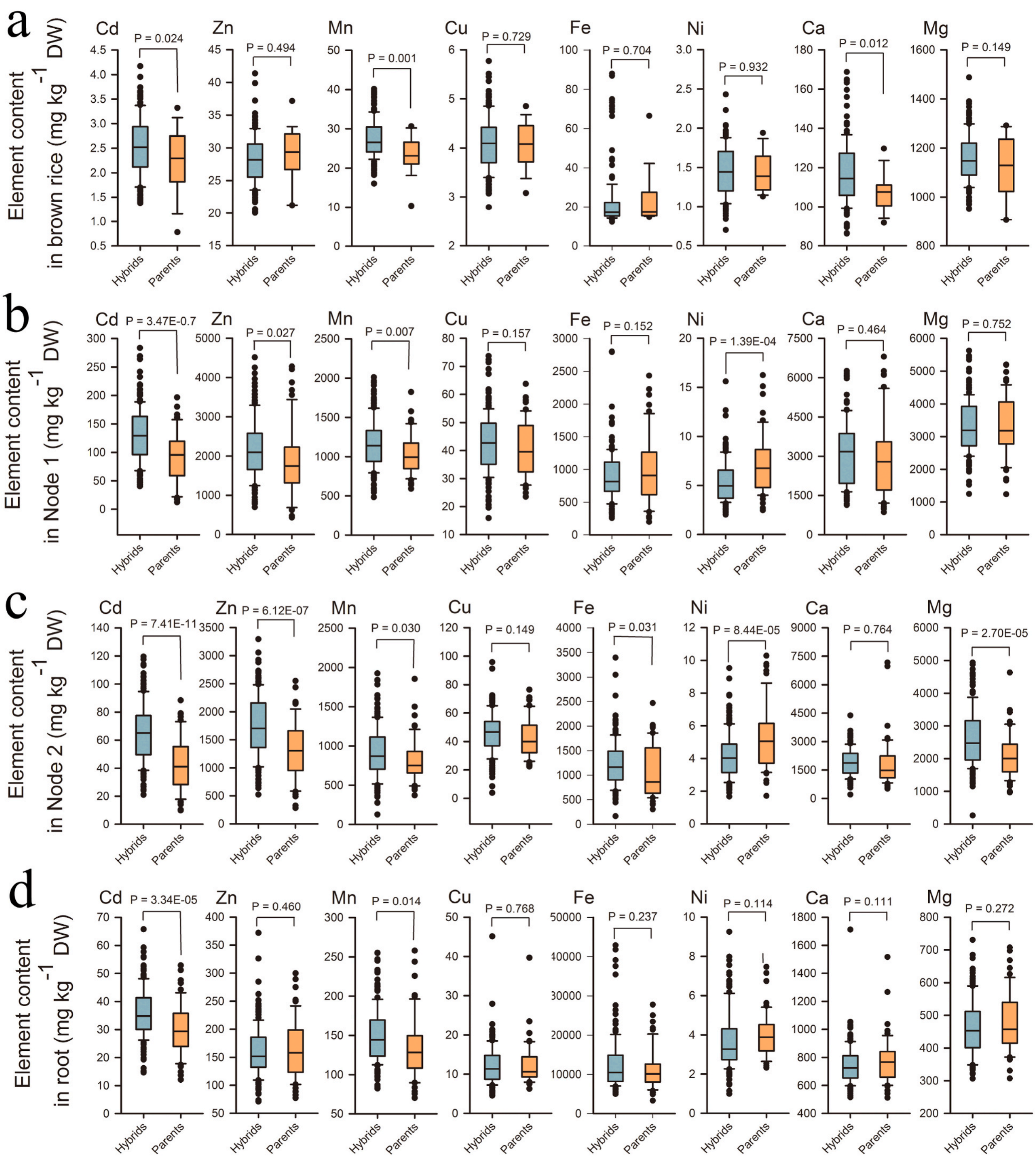

Fig. 3. Comparison of metal element contents in different organs/tissues between rice hybrids and their parental lines. (a) Comparison of metal element contents in brown rice between hybrids and parental lines. (b) Comparison of metal element contents in the first node from top (Node 1) between hybrids and parental lines. (c) Comparison of metal element contents in the second node from top (Node 2) between hybrids and parental lines. (d) Comparison of metal element contents in root between hybrids and parental lines. Horizontal lines in the boxes indicate the median value, the upper and lower edges of boxes delineate the upper and lower quartiles, and the maximum and minimum points are set at 1.5 times the interquartile range. Black circles represent outliers beyond the extreme points. Metallic element content in brown rice (n = 165/18). Metallic element content in nodes and roots (n = 165/54). *P* values were calculate using a two-tailed Student's t-test.

were generally higher than in the parental lines.

The translocation efficiency of metallic elements between different organs is shown in Table 1. Except for the translocation efficiency of the metallic element content (MEC) from the roots to Node 2 (TE3) for Zn in the hybrids, which was significantly higher than in the parental lines ($p < 0.05$), there were no significant differences in translocation efficiency from Node 1 to brown rice (TE1), from Node 2 to Node 1 (TE2), and from the roots to Node 2 (TE3) of metallic elements between the hybrids and the parental lines.

3.3. The general combining ability of the parental lines can be used to predict the metal contents in the brown rice of hybrids

The combining ability and heritability of metallic element accumulation in brown rice were analyzed by two sets of incomplete diallel crosses using the NC II design strategy. The statistical results indicated that there were significant differences in metal elements contents in brown rice between parental lines except for iron ($p < 0.05$), and there were no significant differences in the interaction between parental lines (Table 2). The results showed that the narrow sense heritabilities (h^2) of Cd, Zn, Ca, and Mg contents in brown rice were high (h^2 on average > 50%), that of Mn and Cu were medium (25% $<h^2$ on average < 50%), and that of Fe and Ni were low (h^2 on average < 25%). The general combining abilities (GCA) for Cd, Mn, Cu, and Mg contents in brown rice were higher than the special combining abilities (SCA), which indicated that the contents of these elements in brown rice are mainly affected by additive effects. The genotypic variances in Cd, Zn, Mn, Ni, and Mg contents in brown rice of the restorer lines were significantly higher than in the male-sterile (MS) lines in both of the NC II design experiments, and indicated that the restorer lines may have more important effects on the contents of the metallic elements in brown rice than do the MS lines. The genotypic variance for Ca content in brown rice of the MS lines was significantly higher than in the restorer lines, indicating that the MS lines may play more important roles in the Ca content of brown rice of the hybrids than do the restorer lines.

There was a significant linear regression between the GCA for metallic element contents (except Fe) of the parental lines and the metal element contents in brown rice of the hybrids ($p < 0.01$; R^2 >0.6; Fig. 4a), which indicated that the GCA for metallic element contents of the parental lines could be used to predict the contents of Cd, Zn, Mn, Cu, Ni, Ca, and Mg in brown rice of the hybrids. In Design I, hierarchical cluster analysis showed that the restorer line R2265 and the MS line Ke20 A have low GCA for Cd content in brown rice and high GCA for other essential element contents in brown rice, and the restorer line R2292 and MS line KeBX58S have low GCA for metallic element contents in brown rice (Fig. 4b). In Design II, the MS line 'AnfengA' was found to have low GCA for Cd content and high GCA for Zn content in brown rice (Fig. 4c). These parental lines may be useful for developing hybrids with low Cd and high essential element contents in brown rice in future breeding programs.

3.4. There is a negative correlation between the Cd and Zn contents in brown rice of the rice hybrids

As shown in by the correlation coefficients in Fig. 5, there were significant correlations in 16 character pairs of metal element contents in brown rice of the hybrids, while there were eight pairs in the parental lines ($p < 0.05$; Fig. 5a). In Node 1, there were significant correlations in 14 character pairs of metallic element contents in the hybrids, and there were 15 pairs in the parental lines (p

Table 1
Variance analysis in the translocation efficiency of metallic elements between rice hybrids and the parental lines.

Trait		Rice hybrids			Parental lines		
		Mean	MS	F-value	Mean	MS	F-value
TE1	Cd	0.02 ± 0.01^{d}	6.54E-05	1.39	0.03 ± 0.02^{cde}	4.70E-04	3.77*
	Zn	0.01 ± 0.01^{e}	6.16E-05	1.86**	0.02 ± 0.01^{de}	3.19E-04	3.21
	Mn	0.03 ± 0.01^{c}	1.21E-04	1.58*	0.03 ± 0.01^{cd}	1.14E-04	1.61
	Cu	0.10 ± 0.03^{b}	1.01E-03	1.20	0.11 ± 0.04^{b}	2.53E-03	5.42*
	Fe	0.03 ± 0.03^{c}	8.75E-04	1.28	0.03 ± 0.02^{cd}	1.88E-04	0.65
	Ni	0.31 ± 0.12^{a}	1.43E-02	0.87	0.32 ± 0.12^{a}	9.78E-03	0.88
	Ca	0.04 ± 0.02^{c}	4.34E-04	2.14**	0.05 ± 0.03^{c}	2.09E-03	5.07*
	Mg	0.37 ± 0.11^{a}	1.95E-02	2.62**	0.44 ± 0.14^{a}	2.92E-02	2.44
TE2	Cd	2.06 ± 0.57^{a}	0.32	1.00	2.25 ± 1.01^{a}	1.27	1.50
	Zn	1.34 ± 0.54^{cd}	0.39	1.57*	1.48 ± 0.62^{bc}	0.44	1.30
	Mn	1.44 ± 0.86^{bc}	0.79	1.13	1.40 ± 0.49^{bcd}	0.32	1.55
	Cu	1.09 ± 0.92^{de}	0.97	1.25	1.04 ± 0.35^{e}	0.16	1.84
	Fe	0.82 ± 0.58^{e}	0.42	1.40	1.03 ± 0.50^{e}	0.31	1.07
	Ni	1.38 ± 0.62^{bcd}	0.23	0.51	1.40 ± 0.48^{bcd}	0.35	2.03*
	Ca	1.88 ± 1.45^{ab}	2.05	0.97	1.92 ± 1.08^{ab}	2.12	3.22**
	Mg	1.41 ± 1.01^{bcd}	0.22	1.89**	1.76 ± 0.74^{ab}	1.05	3.48**
TE3	Cd	1.93 ± 0.88^{f}	1.07	1.65*	1.59 ± 0.96^{fg}	2.07	5.47**
	Zn	11.49 ± 4.73^{a}	36.08	2.30**	8.46 ± 4.15^{b}	26.94	2.02*
	Mn	6.41 ± 2.75^{bc}	12.18	2.30**	6.57 ± 3.68^{bc}	16.32	1.35
	Cu	4.15 ± 1.66^{d}	3.46	1.41	3.75 ± 1.74^{d}	5.93	3.39**
	Fe	0.12 ± 0.06^{h}	0.004	1.12	0.11 ± 0.07^{h}	0.010	2.82**
	Ni	1.27 ± 0.58^{g}	0.35	1.05	1.43 ± 0.64 g	0.75	3.24**
	Ca	2.64 ± 1.15^{c}	2.14	2.80**	2.50 ± 1.81^{ef}	4.96	1.99*
	Mg	5.75 ± 1.86^{cd}	5.66	2.64**	4.55 ± 1.64^{d}	4.24	2.08*

TE1 indicates the metal elements content (MEC) translocation efficiency from Node 1 to the brown rice. TE2 indicates the MEC translocation efficiency from Node 2 to Node 1. TE3 indicates the MEC translocation efficiency from the roots to Node 2. * and ** indicate significant differences at 0.05 and 0.01 probability levels, respectively. Multiple comparisons (Games-Hotwell method) were carried out in TE1, TE2 and TE3 separately. MS indicates mean square. Different letters indicate significant differences at the 0.05 probability level.

Table 2
Variance analysis of the effects of combining ability for Cd accumulation in rice hybrids.

Trait		Mean square			Error	Genotype variance		GCA	SCA	h^2
		Restorer lines	MS lines	R × M		Restorer lines	MS lines	variance	variance	
Group I	Cd	4.81**	0.83**	0.14	0.10	78.50%	16.24%	94.74%	5.26%	70.63%
	Zn	128.08**	33.00**	8.27	5.51	68.96%	19.93%	88.89%	11.11%	53.36%
	Mn	120.27**	39.50	19.03	19.02	77.88%	22.04%	99.92%	0.08%	24.54%
	Cu	1.82**	0.32	0.19	0.19	90.15%	9.85%	100.00%	0.00%	30.93%
	Fe	111.55	79.73	213.17	189.87	0.00%	0.00%	0.00%	100.00%	0.00%
	Ni	0.32**	0.12	0.06	0.10	73.84%	26.16%	100.00%	0.00%	14.92%
	Ca	1254.27**	2220.91**	110.72	87.84	26.86%	69.38%	96.24%	3.76%	67.15%
	Mg	112103.42**	28750.80**	5236.64	3753.39	71.16%	21.92%	93.09%	6.91%	61.04%
Group II	Cd	1.09**	0.81**	0.13	0.15	53.11%	46.89%	100.00%	0.00%	43.79%
	Zn	98.73**	49.06**	5.23	9.65	63.06%	36.94%	100.00%	0.00%	50.59%
	Mn	7.66	106.25**	3.68	16.45	3.00%	97.00%	100.00%	0.00%	34.89%
	Cu	2.06**	0.54*	0.16	0.20	79.90%	20.10%	100.00%	0.00%	43.95%
	Fe	1.99	549.82*	160.87	252.57	0.00%	100.00%	100.00%	0.00%	11.37%
	Ni	0.43**	0.03	0.03	0.10	97.81%	2.19%	100.00%	0.00%	21.60%
	Ca	458.63**	1720.62**	39.36	175.10	16.63%	83.37%	100.00%	0.00%	48.97%
	Mg	47608.29**	21422.28*	4308.10	6280.74	66.93%	33.07%	100.00%	0.00%	40.71%

* and** indicate significant differences at the 0.05 and 0.01 probability levels, respectively; Group I consisted of a 7 × 5 incomplete diallel design, and Group II consisted of a 5 × 4 incomplete diallel design. MS, male sterile; GCA, general combining ability; SCA, specific combining ability; h^2. Narrow-sense heritability.

< 0.05; Fig. 5b). In Node 2, there were significant correlations in 20 character pairs of metallic element contents in the hybrids, and there were 15 pairs in the parental lines ($p < 0.05$; Fig. 5c). In roots, there were significant correlations in 13 character pairs of metallic element contents in the hybrids, and there were 15 pairs in the parental lines ($p < 0.05$; Fig. 5d). These results indicate that there are extensive and complex correlations for the accumulation of different metallic elements in rice.

In Node 1, Node 2, and the roots of the hybrids, the Cd contents were positively correlated with the contents of Zn, Mn, and the other elements ($p < 0.05$; Fig. 5b, c, and d). Fortunately, the Cd contents were negatively correlated with the Zn, Mn, and Cu contents in brown rice of the hybrids ($p < 0.05$; Fig. 5a). The regression analysis also showed that there was a negative correlation between the Cd and Zn contents in brown rice of the hybrids (Fig. 6a; $p < 0.001$; $R^2 = 0.32$). These results mean that the essential elements in grains can be preserved while decreasing the Cd content in grains. The ratios of Zn/Cd content in brown rice of the hybrids derived from restorer lines R2292 and R2265 were significantly higher than that in hybrids derived from the other restorer lines (Fig. 6b; $p < 0.05$).

4. Discussion

Cd is a nonessential element in rice, and its absorption and transport depend on the pathways that transport Zn, Mg, and other essential elements into and within the plant [12]. Several transporters have been shown to be able to transport Cd and Zn and/or Mn simultaneously in rice [38–42]. Liu et al. (2003) found that the Cd content was positively correlated with the Zn, Mn, or Cu contents in the leaves and roots of rice, and it was presumed that Cd ions were co-transported with Zn, Mn, and Cu from the roots to the stem [43, 44]. In the present study, we found that the differences in the accumulation of metallic elements between the hybrids and their parents is because the hybrids absorb more metallic elements from the soil, rather than from differences in the translocation efficiencies of metallic elements between the hybrids and the parental lines. We also found that there was a significantly positive correlation between the Cd and Zn contents in roots and nodes of rice hybrids ($p < 0.01$; Fig. 4b, c, 4d). However, the correlation between the Cd and Zn contents in brown rice was different from that in the roots and nodes. Tan et al. (2020) investigated 11 mineral elements in 575 rice varieties and found that the Cd content in brown rice showed a significant negative correlation with the Zn, Cu, Mg, and Fe contents in brown rice. In this study, we also found that there was a significant negative correlation between Cd and Zn content in brown rice ($p < 0.01$; Fig. 6a), which was similar to previous research results. Zn and Cd in the nodes and roots of rice are mainly transported through the xylem system, while Zn and Cd in brown rice are mainly accumulated from the phloem system [45,46]. Zn, Cd and other metallic elements are difficult to transport from the xylem to phloem, which results in the Zn, Cd, and other metallic element contents in brown rice being much lower than in other organs or tissues [47,48]. The transfer of Cd, Zn and other metallic elements from xylem to phloem mainly occurs in the nodes of rice plants [49], and Huang et al. (2017) reported that the Cd and Zn contents in Node 1 had an important effect on the Cd and Zn contents in brown rice [50]. The results of the present study showed that not only was the translocation efficiency of Zn from Node 1 to brown rice (TE1) significantly higher than the TE1 for Cd in the hybrids ($p < 0.05$; Table 1), but also the Zn content in brown rice was significantly higher than the Cd content in brown rice in the hybrids ($p < 0.05$; Fig. 1a). Because the element Cd shares the same transport pathway with Zn [12], the competition between Cd and Zn translocation from Node 1 to brown rice is unavoidable and results in the negative correlation between the Cd and Zn contents in brown rice of the hybrids (Fig. 6a).

Some previous studies have reported that the narrow sense heritabilities (h^2) of the Ca, Mg, Zn, Cu, Mn, and Cd contents in brown rice were usually medium to high, while the h^2 for Fe content was low [51–53]. The accumulation of several elements in the brown rice of the hybrids was mainly affected by additive effects which were closely related to the genotypes of the parental lines [29,33,54]. In the present study, we also found that the h^2 of Zn, Cd, Ca, and Mg contents in brown rice of the hybrids was high ($h^2 > 0.5$; Table 2), and

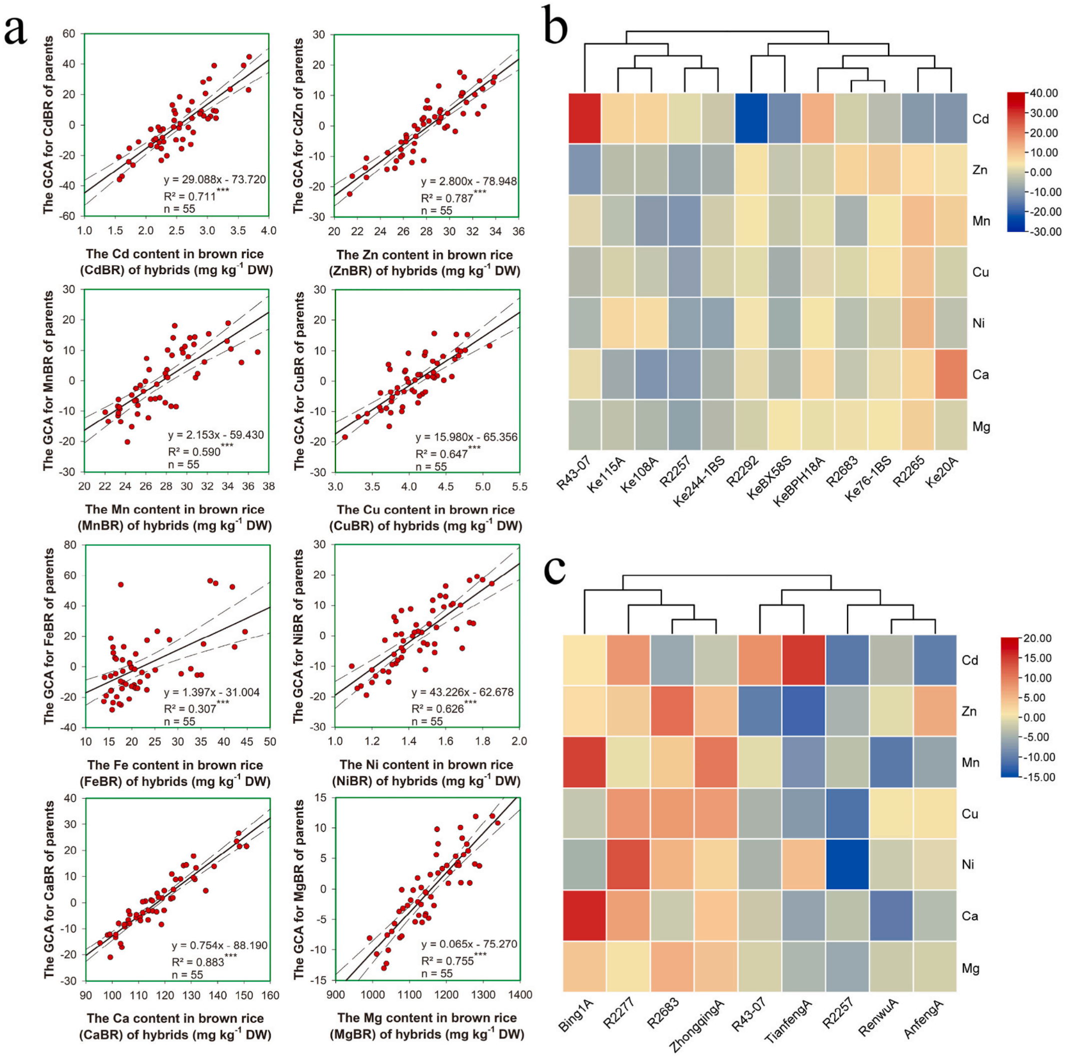

Fig. 4. Results of regression analysis and clustering analysis. (a) Correlation between metallic element contents (MEC) in brown rice of the hybrids and the relative effects of GCA for MEC of the parental lines, *** indicates significant differences at the 0.001 probability level. The hierarchical clustering analysis for group I (b) and group II (c) are based on the Euclidian distance of GCA for MEC in brown rice between the parental lines. Group I consisted of a 7 × 5 incomplete diallel design, and group II consisted of a 5 × 4 incomplete diallel design.

that the Cd, Zn, Mn, Cu, Ni, Ca, and Mg contents in brown rice of the hybrids were mainly affected by additive effects (Table 2), as well as having significant linear regression relationships between the contents of these elements in brown rice of the hybrids and the GCA for metallic element contents (MEC) of the parental lines (Fig. 4a). These studies demonstrate that the metallic element contents in brown rice can be optimized through genetic improvement. The clustering of GCA for MEC in the parental lines (Fig. 4b and c) would benefit the selection of parental lines to use in the development of hybrids with low Cd and high Zn contents in the brown rice.

5. Conclusions

Our research shows that there was a positive correlation between the contents of Cd and Zn in nodes and roots, but a negative correlation between the contents of Cd and Zn in brown rice of the hybrids. The contents of Cd, Zn, Ca, and Mg in brown rice were mainly affected by additive genetic effects, and the GCA (general combining ability) for metallic element content can be used to evaluate the ability of the parental lines to improve the metal contents in brown rice of the hybrids. These results will benefit the selection of parental lines to use in the development of hybrids with low Cd and high essential metallic elements in the brown rice.

Author contribution statement

Tengfei Liu: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed

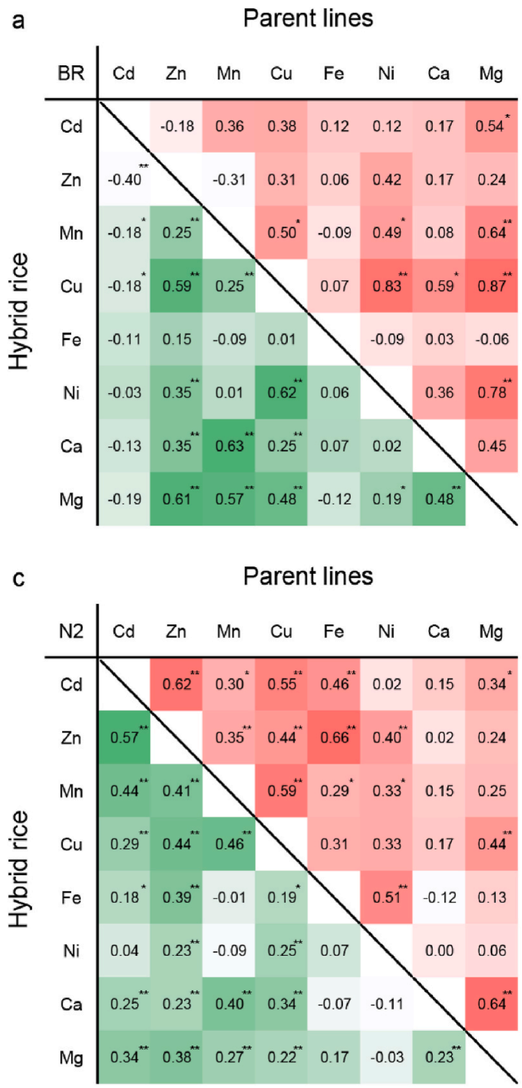

Fig. 5. Pearson correlation coefficients between the different metal element contents (MECs) in rice hybrids and the parental lines. (a) Pearson correlation coefficients between the different MECs in brown rice. (b) Pearson correlation coefficients between the different MECs in Node 1. (c) Pearson correlation coefficients between the different MECs in Node 2. (d) Pearson correlation coefficients between the different MECs in the roots. BR, N1, N2, and R indicate brown rice, the first node from the top, the second node from the top, and the roots, respectively. * and ** indicate significant differences at 0.05 and 0.01 probability levels, respectively.

reagents, materials, analysis tools or data; Wrote the paper.

Wenbin Hu: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Lvshui Weng: Lihua Deng: Jinjiang Li: Jianghui Yu: Performed the experiments.

Zheng Zhou: Ye Liu: Contributed reagents, materials, analysis tools or data.

Caiyan Chen: Teng Sheng: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Zhenghong Zhao: Guoying Xiao: Conceived and designed the experiments; Wrote the paper.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

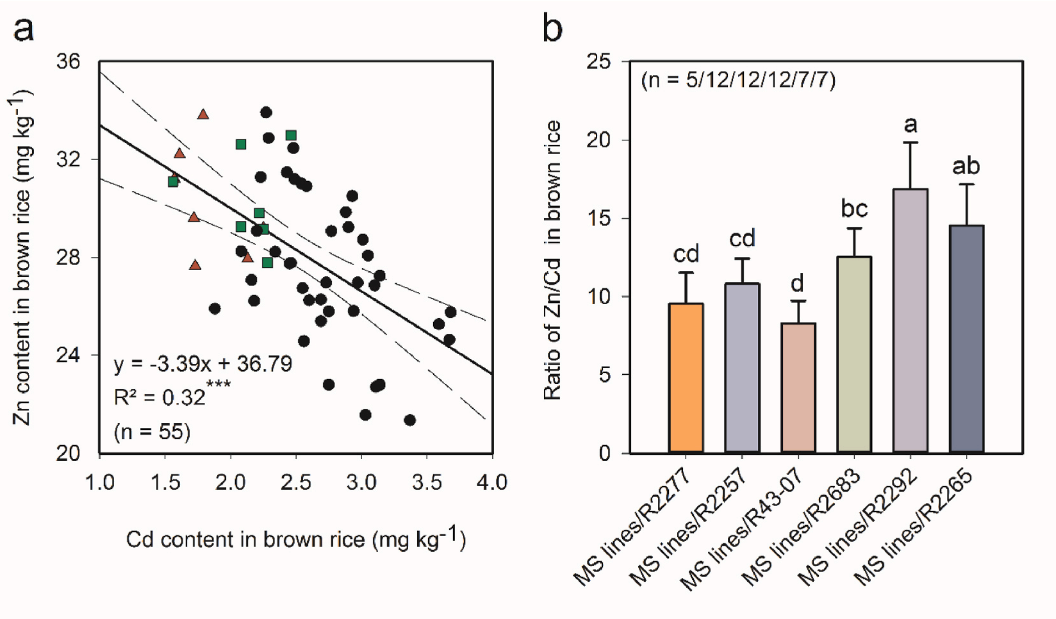

Fig. 6. (a) Regression analysis between the Cd and Zn contents in brown rice of the rice hybrids. Dashed lines indicate the 95% confidence intervals. *** indicates significant differences at the 0.001 probability level. Red triangles indicate F_1 hybrids between the MS lines and the restorer line R2292. Green squares indicate F_1 hybrids between the MS lines and the restorer line R2265. (b) The ratio of Zn/Cd contents in brown rice of the F_1 hybrids made with different restorer lines. Different letters indicate significant differences at the 0.05 probability level.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e19919.

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