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RESEARCH ARTICLE

Characterization of grain cadmium concentration in *indica* hybrid rice

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Abstract As a consequence of contamination of soil with heavy metals, cadmium accumulation in grain is of great concern worldwide, but especially in southern China. It is important to evaluate the Cd accumulation potential of grain before or when examining and approving new cultivars. An evaluation method and criteria for verifying Cd accumulation potential in rice are proposed, and the Cd accumulation potential of 56 mid-season *indica* hybrids collected from the provincial cultivar trials in 2016 were investigated. Genotype, environment and their interactions strongly affected the variation in grain Cd accumulation. Two hybrids were identified as slightly Cd accumulating. Hybrids with slight Cd accumulation potential would be suitable for safe grain production on polluted land (total Cd under 2.0 $mg \cdot kg^{-1}$) in Hunan Province (China) and should be considered for new cultivar evaluation and approval. This evaluation method and criterion could be applied for certifying Cd accumulation potential of rice cultivars.

Keywords accumulation, cadmium, hybrid, methodology, rice

1 Introduction

Cadmium is a toxic trace element belonging to group II B of the periodic table of elements. The long-term exposure to high levels of Cd poses serious health problems to humans, such as anemia, hypertension, cancer, cardiac failure, cerebrovascular infarction, emphysema, proteinuria, serious lung damage, renal dysfunction, cataract formation in eyes and osteoporosis[1]. Cd in soil and water can be taken up by certain crops and accumulated in the human body via the food chain[2]. Due to the widespread contamination of Cd in soil, mostly from anthropogenic sources, human exposure occurs mainly from consumption of Cd contaminated food. Food accounts for about 90% of Cd exposure in the general non-smoking population[3,4]. A recent nationwide soil survey in China showed that 7% of the soil samples were contaminated with Cd[5]. Regional, national and global actions are needed to decrease global environmental Cd releases and reduce occupational and environmental exposure. A land retirement program in a heavy metal contaminated area has been conducted by the government of China to minimize dietary heavy metal contamination. The Changsha-Zhuzhou-Xiangtan area of Hunan Province, a heavy metal pollution disaster area, was the first regional pilot of the land retirement program and a total of about 6.7 kha of land were fallowed in 2016[6].

Rice (*Oryza sativa*) is a major staple food and can accumulate high concentrations of Cd in its grain if grown on Cd polluted soil[7]. Hence, it is important to minimize Cd content in rice. Breeding of low Cd accumulating rice cultivars could be one of the effective ways to decrease the flux of toxic pollutants into the human food chain without any additional cost. Fortunately, there is considerable natural variation in Cd accumulation in rice that could be exploited for breeding low Cd accumulating cultivars[8–10]. The identification of Cd accumulation potential of rice can prevent cultivars with high Cd accumulation potential from being released in Cd contaminated areas.

Cropping low Cd accumulating cultivars would be a reasonable option for farmers to cope with the Cd risk and to reduce the influx of pollutants to the human food chain, especially in cases where the soil contamination conditions are unknown. Here, we propose an evaluation method and criteria for assessing Cd accumulation potential of rice and

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investigated the Cd accumulation potential of 56 mid-season *O. sativa indica* hybrids.

2 Materials and methods

2.1 Plant materials

A total of 56 mid-season *indica* hybrids were collected in 2016 from provincial cultivar trials in Hunan Province, China.

2.2 Soil environment experiment

The experiments were in four soil-filled concrete tanks (T1, T2, T3 and T4 indicating the soil treatment, see Table 1) at the experimental base of Longping High-Tech (Ningxiang City, Hunan Province, China) in 2016. The soil for each concrete tank was collected from rice paddies polluted with Cd ranging from slightly polluted (0.25 $mg \cdot kg^{-1}$) to severely polluted (2.18 $mg \cdot kg^{-1}$). All hybrids were grown in a randomized complete block design with three replicates in each concrete tank. Seedlings (25 d old) were transplanted in one row of six plants per line at spacing of 17 cm × 27 cm. The experimental tanks were flooded with water to a depth of 2–3 cm during the vegetative growth phase and about 5 cm during booting. After full heading, no surface water was maintained, but moisture management was applied through intermittent irrigation such that the soil moisture content was maintained above 70%. Cultivation was conducted in the normal season and according to standard practice. Sowing and transplanting were performed in late May and late June, respectively.

2.3 Trait evaluation

Days to heading (DTH) of each plant was recorded and DTH of each line calculated as the mean value for the six plants of that line. Plant samples and fresh soil samples from the top 15 cm of the soil profile from each tank were collected at harvest. Cd concentrations of the brown rice grains and hull were determined by atomic absorption spectrophotometry (PerkinElmer 2100, Rodgau, Germany) following HNO_3-$HClO_4$ (4:1) digestion. The standardized analytical methods of China were used (GB/T5009, 15-1996).

2.4 Statistical analyses

Analyses of variances were performed among hybrids for each and across all environments with the PROC GLM procedure (SAS Institute 2012) following the model:

$$Y = \mu + E + R + G + GEI + e$$

where Y = observed value of Cd from each test unit, μ = population mean, E = environmental effect, R = replication effect within each environment, G = genotype (hybrid) effect, GEI = interaction effect between each genotype and environment, and e = residual effect. The environment and genotype were treated as fixed factors and the replication-within-environment was considered to be a random factor. The significance of environmental variance was tested against the replication-within-environment entity.

2.5 Brown rice Cd accumulation potential

Cd accumulation potential of brown rice grain was rated by the standards detailed in Table 2.

3 Results

3.1 Statistical analysis

As shown in Table 3, the Cd accumulation in the brown rice grain differed significantly ($P < 0.001$) for environment, genotype and *GEI* with an average Cd of 0.372 $mg \cdot kg^{-1}$ that ranged from 0.061 to 1.211 $mg \cdot kg^{-1}$ across the four environments. The effects of environment (treatment), genotype and *GEI* contributed 37.6%, 34.4% and 20.9%, respectively, of the total sums of squares to the variation in grain Cd accumulation. The effects of environment, genotype and *GEI* all contributed significantly ($P < 0.0001$) to the variation in hull Cd accumulation and accounted for 21.3%, 34.5% and 33.2% of the

Table 1 Effect of different treatments on mid-season *indica* hybrid rice Cd accumulation

Treatment	Total soil Cd concentration /($mg \cdot kg^{-1}$)	Bio-available Cd concentration /($mg \cdot kg^{-1}$)	pH	Total soil organic matter content/%	Cd accumulation/($mg \cdot kg^{-1}$)		Percentage of brown rice Cd under the limit	
					Brown rice	Hull	A	B
T1	0.25	0.11	5.87	2.61	0.092 (0.010–0.374)	0.049 (0.010–0.124)	94.6	100.0
T2	0.59	0.25	6.11	2.93	0.269 (0.048–0.619)	0.086 (0.024–0.245)	37.5	83.9
T3	0.97	0.49	5.79	3.39	0.446 (0.047–1.557)	0.194 (0.031–0.855)	26.8	58.9
T4	2.18	0.95	5.95	3.17	0.779 (0.139–2.753)	0.257 (0.062–1.258)	3.6	21.4

Note: A, the percentage of brown rice samples with Cd lower than the standard of 0.2 $mg \cdot kg^{-1}$ required by National Food Safety Standard of China (GB2762-2012, NFSSC); B, the percentage of brown rice samples with Cd lower than the standard of 0.4 $mg \cdot kg^{-1}$ required by FAO/WHO.

Table 2 Rating standards for Cd accumulation potential in grain of rice genotypes

Rating	Total soil Cd concentration/(mg·kg^{-1}), pH 5.5–6.5				Cd accumulation potential
	0.25 ± 0.05	0.6 ± 0.05	1.0 ± 0.1	2.0 ± 0.2	
1	< 0.2	< 0.2	< 0.2	< 0.2	Slight
2	< 0.2	< 0.2	< 0.2	⩾ 0.2	Low
3	< 0.2	< 0.2	⩾ 0.2	⩾ 0.2	Lower
4	< 0.2	⩾ 0.2	⩾ 0.2	⩾ 0.2	Moderate
5	⩾ 0.2	⩾ 0.2	⩾ 0.2	⩾ 0.2	High
Other	Unable to rate				Undetermined

Note: The rating is determined by the lowest soil Cd concentration that resulted in grain Cd concentration of ⩾ 0.2 mg·kg^{-1} (as indicated in the body of the table).

Table 3 Analysis of variance, including degrees of freedom (df), mean squares (MS), and percent contribution to total sums of squares (SS%) across five environments for Cd content of grain and hull

Source	df	Brown rice		Hull	
		MS	SS%	MS	SS%
Environment	3	12.71***	37.62	1.42***	21.33
Genotype	55	0.63***	34.38	0.13***	34.52
GEI	165	0.13***	20.87	0.04***	33.24
Rep (environment)	8	0.02	0.17	0.00	0.07
Error	423	0.02	6.96	0.01	10.84
Total	654				

Note: *** Significant at $P < 0.0001$.

total sums of squares, respectively. As expected, the proportions of environment and *GEI* in the total variation in grain and hull Cd accumulation were relatively large due to the great differences in Cd content of the soil environments. This suggests that the hybrids responded differently to the environment in grain and hull Cd accumulation. Studies of the effect of genotype showed similar effects (34.4%) as the environment for variation in grain Cd accumulation and had major effects (37.6%) on variation in hull Cd accumulation. This result indicates that it is possible to develop rice cultivars with low Cd accumulation potential through breeding. However, for significantly lower Cd accumulation potential, more research is needed to better understand the physiological or biochemical mechanisms responsible for Cd absorption and transportation. In addition, low Cd accumulating genotypes should be used in breeding programs to develop agronomically suitable cultivars that accumulate low grain Cd concentration suited to different rice-producing regions.

3.2 Grain Cd concentration

The variation in grain Cd concentration between hybrids was highly significant ($P < 0.01$) at all levels of Cd exposure (treatments, T1–T4, Table 1). Under low Cd exposure (T1), Cd concentration in the grain ranged from 0.010 to 0.374 mg·kg^{-1} and averaged 0.092 mg·kg^{-1}. Under medium Cd exposure, Cd concentration in the grain ranged from 0.048 to 0.619 mg·kg^{-1} with a mean of 0.269 mg·kg^{-1} for T2, and from 0.047 to 1.557 mg·kg^{-1} with a mean of 0.446 mg·kg^{-1} for T3. Under high Cd exposure (T4), Cd concentration in the grain ranged from 0.139 to 2.753 mg·kg^{-1} with a mean 0.779 mg·kg^{-1}. This mean was 8.4 times higher than that with low Cd exposure (Table 1; Table 4). Fifty-three (94.6%), 21 (37.5%), 15 (26.8%) and 2 (3.6%) of the 56 hybrids of the T1, T2, T3 and T4 soil environments, respectively, produced brown rice with Cd concentration under 0.2 mg·kg^{-1} (safe Cd limit required by NFSSC, Table 1).

Based on the rating standard of the Cd accumulation potential of grain of the rice hybrids (Table 2), the tested hybrids were grouped as (1) two slight Cd accumulating hybrids, (2) 13 low Cd accumulating hybrids, (3) six lower Cd accumulating hybrids, (4) 32 moderate Cd accumulating hybrids, and (5) three high Cd accumulating hybrids (Table S1). HR46 and HR48 were identified as slight Cd accumulating hybrids that could be grown for production of Cd safe grain (Cd⩽0.2 mg·kg^{-1}) in Cd contaminated soils with total Cd content about 2.0 mg·kg^{-1}.

3.3 Stability of grain Cd concentrations in different soil environments

As shown in Table 4, the correlation coefficients among grain Cd concentration in T1, T2, T3 and T4 with different

Table 4 Correlation of Cd concentration in brown rice between four soil treatments

Treatment	T1	T2	T3	T4
T1	1.000			
T2	0.750***	1.000		
T3	0.805***	0.923***	1.000	
T4	0.752***	0.744***	0.768***	1.000

Note: *** Significant at $P < 0.0001$, see Table 1 for details of treatments T1–T4.

levels of Cd exposure were all significantly positive (r = 0.744–0.923, $P < 0.0001$). The highest correlation (r = 0.923, $P < 0.0001$) was observed between the grain Cd concentrations of two medium Cd exposure levels (T2 and T3). This result suggests a certain stability and consistency of the genotype response to medium Cd exposure (especially at about 0.6–1.0 mg·kg^{-1}) in terms of grain Cd accumulation. The two lowest correlations were observed between the grain Cd concentrations in T1 and T2 (r = 0.750), and between T2 and T4 (r = 0.744). These results suggest that the Cd accumulation potential of a rice genotype is not invariable under different Cd exposures. That is, a low Cd accumulating line in low Cd exposure does not mean it is also low Cd accumulating when exposed to high levels of Cd, and vice versa. For example, HR31 showed low Cd accumulation potential (grain Cd of 0.034 mg·kg^{-1}; ranked eighth lowest Cd concentration) in T2, but high Cd accumulation potential (grain Cd of 1.219 mg·kg^{-1}, and ranked fifth highest Cd concentration) in T4. Differences in the active uptake and passive uptake of Cd accumulation in different rice lines might be the main reason for the variability of Cd accumulation potential at different levels of Cd exposure. It is necessary that multiple trials with different levels of Cd exposure should be performed to evaluate the Cd accumulation potential of rice genotypes.

3.4 Relationship between grain Cd concentration and DTH, hull Cd concentration

The Relationship between grain Cd concentration and DTH was showed in Fig. 1. There was a significant positive correlation ($P < 0.0001$) between Cd concentration and DTH. The correlation coefficients were 0.705, 0.828, 0.845 and 0.747 in T1, T2, T3 and T4, respectively (Fig. 1). The highest correlations were found under

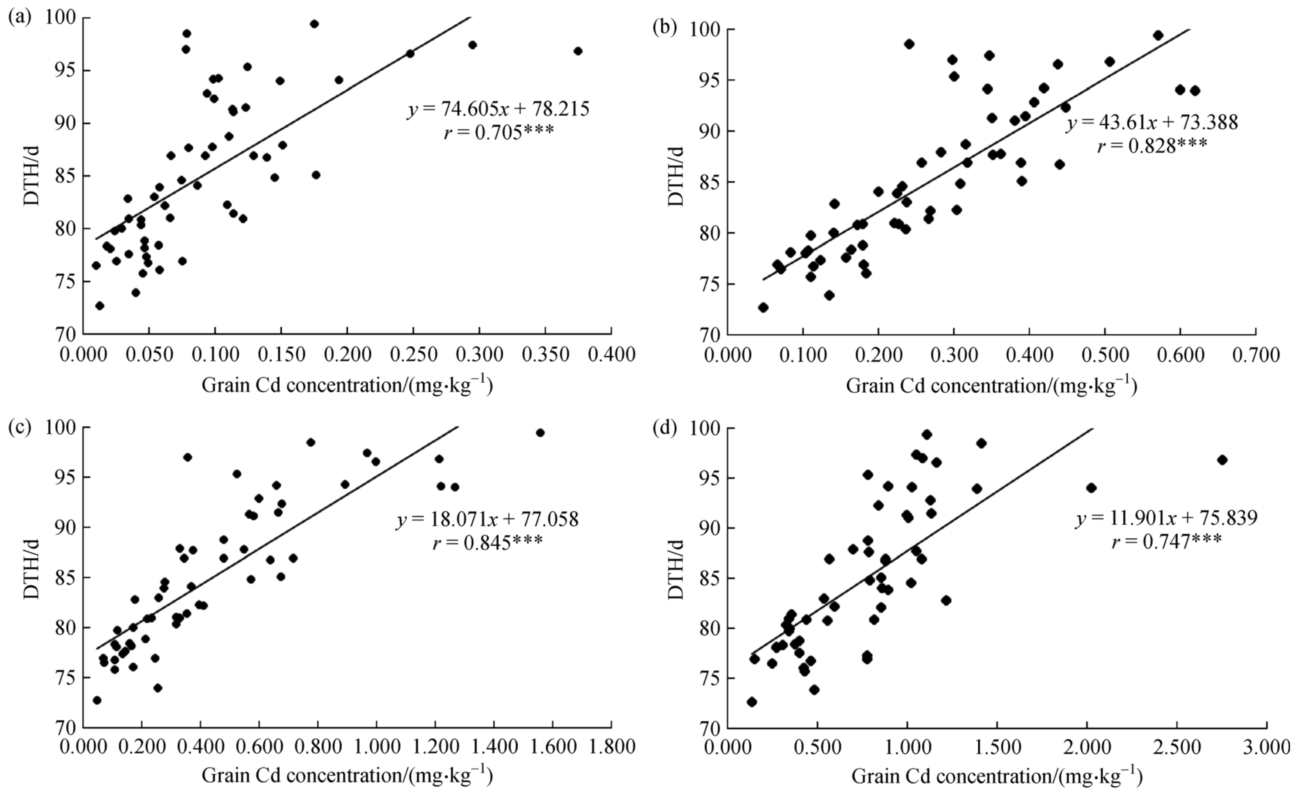

Fig. 1 Correlation between grain Cd concentration and days to heading (DTH) in T1 (a), T2 (b), T3 (c) and T4 (d) (see Table 1 for details of treatments T1–T4). *** Significant at $P < 0.0001$.

medium Cd exposure (T2 with 0.59 mg·kg^{-1} and T3 with 0.97 mg·kg^{-1}). These results indicate that the line with the longer growth period accumulates more Cd in grains, especially when was cropped in the polluted soil with Cd ranging from about 0.60 to 1.00 mg·kg^{-1}. Only two hybrids, those with the shortest DTH, produced Cd pollution-safe grains (Cd < 0.2 mg·kg^{-1}) under high Cd exposure (T4). The Relationship between grain Cd concentration and hull Cd concentration was showed in Fig. 2. A significant positive correlation ($P < 0.01$ or $P < 0.0001$) was observed between grain and hull Cd concentrations. The correlation coefficients were 0.369, 0.658, 0.711 and 0.798 in the T1, T2, T3 and T4, respectively (Fig. 2). It is noteworthy that the correlation between grain Cd concentration and hull Cd concentration increased with soil Cd exposure.

4 Discussion

Cd is a toxic heavy metal, which is known as one of the major environmental pollutants that harms human health[1]. Cd minimization must be an important requirement in rice cropping, especially in areas subject to industrial pollution. It is necessary to evaluate the Cd accumulation potential of new cultivars using reliable assessment methods before cultivar approval and promotion. There have been many studies on the characterization of grain Cd concentration in rice[9,11,12]. However, most of these studies were implemented in limited environments or in random Cd contaminated field experiments, and the evaluation methods were mostly not suitable to reliably characterize Cd accumulation potential of rice at different levels of Cd contamination of soil. An evaluation method and criteria for verifying rice Cd accumulation potential are proposed, and these were applied to 56 tested hybrids collected from the regional trial of new cultivars in Hunan Province. The effects of genotype and *GEI* contributed 34.4% and 20.9% of the grain Cd accumulation variation in four environments from slightly polluted (0.25 mg·kg^{-1}) to severely polluted (2.18 mg·kg^{-1}). This indicates that it is possible to develop rice cultivars with low Cd accumulation potential through breeding.

Based on our evaluation method and criteria two hybrids (HR46 and HR48) were identified as slight Cd accumulating hybrids. In theory, HR46 and HR48 can be grown in the most of the Cd polluted soil in Hunan Province (China) to give grain Cd concentrations under 2.0 mg·kg^{-1}. However, 62.5% of the tested hybrids were found to be moderate to high Cd accumulating cultivar. Given the risk of grain Cd accumulation, HR21, HR26 and HR36, identified as having high Cd accumulation potential,

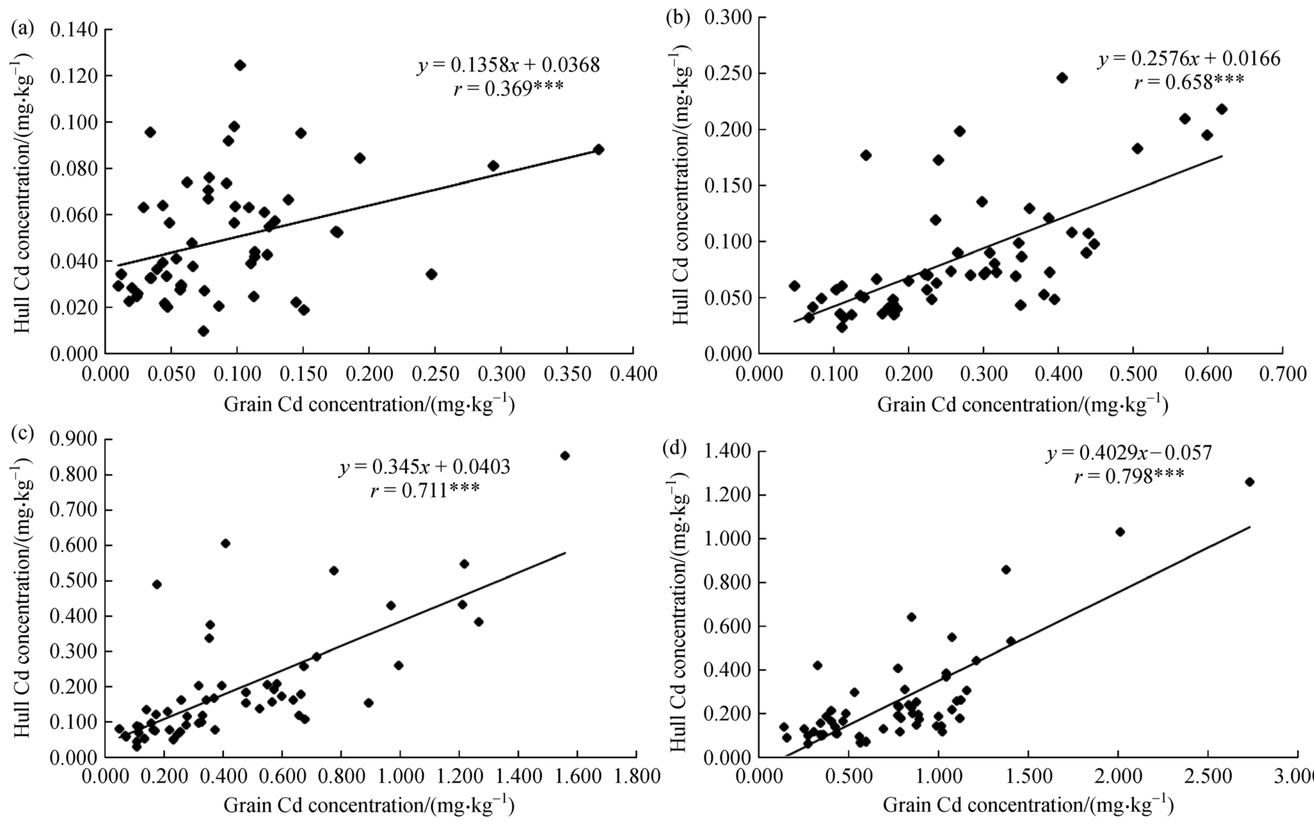

Fig. 2 Correlation between grain and hull Cd concentration in T1 (a), T2 (b), T3 (c) and T4 (d) (see Table 1 for details of treatments T1–T4). *** Significant at $P < 0.0001$.

should not be released to seed markets. These results also indicate that it is possible to develop a cultivar that can produce Cd safe grains even in Cd polluted soil, as long as the soil has a Cd contamination of under 2.0 $mg\cdot kg^{-1}$ and with normal soil pH (about pH 6). This is the first study to investigate the grain Cd accumulation potential of rice using this kind of experimental design and rating standard.

Correlation analysis of grain Cd concentration of hybrids across Cd contamination levels was used to assess the stability of our evaluation method and criteria for verifying rice Cd accumulation potential. The correlations were high, with $r = 0.774$–0.923 ($P < 0.0001$) across four Cd contamination levels with Cd exposure from slightly polluted (0.25 $mg\cdot kg^{-1}$) to severely polluted (2.18 $mg\cdot kg^{-1}$). This is higher than that in the study of Duan et al.[11] using five experiments at three field sites across two years with Cd exposure of 0.5–1.4 $mg\cdot kg^{-1}$. This evaluation method for verifying rice Cd accumulation potential is more stable than with experiments in field sites.

Significant positive correlations ($P < 0.0001$) between Cd concentration and DTH were found in this study. The two slight Cd accumulating hybrids had the shortest DTH. Similarly, Duan et al.[11] found that later flowering hybrids accumulated significantly higher Cd in grain than earlier flowering hybrids. Several other studies also detected a correlation between grain Cd concentrations and DTH, but there is no consistent agreement. Ishikawa et al.[13] found the grain Cd concentrations were negatively correlated with DTH. Sun et al.[10] did not detect a strong correlation between grain Cd concentration and heading date, and suggested that the breeding of rice with low grain Cd level should not be subjected to the limitation of heading date. These divergent results might be attributable to genetic linkage inheritance of grain Cd content and flowering time. Abe et al.[14] reported a grain Cd content QTL *qlGCd3* that mapped within the 3.5-Mb region and is collocated with flowering time gene *Hd6*. The water management difference of the paddy, especially after heading, could be a contributing factor. It is well known that intermittent flooding irrigation management of paddy soil reduces Cd bioavailability and greatly decreased the Cd concentrations in grain[8,15,16]. To further reduce the water management impact on Cd accumulation potential evaluation, our evaluation method was further improved in 2017 by (1) designing and fitting rain shelters to reduce the impact of rain, especially rain during the grain filling period, and (2) conducting experiments separately for early, middle and late heading genotypes to allow paddy water management to be tailored according to heading time.

5 Conclusions

This study proposes an evaluation method and criteria for verifying rice Cd accumulation potential. Measuring the Cd accumulation potential of hybrids revealed that genotype and environment explained a total of 72% of the variation in grain Cd accumulation. Fifty-six rice hybrids were evaluated and rated for Cd accumulation potential in grain according to our proposed evaluation method and criteria. Two slight Cd accumulating hybrids and three high Cd accumulating hybrids were identified. There was a significant positive correlation between grain Cd concentration and DTH, especially under medium levels of Cd exposure.

Supplementary materials The online version of this article at https://doi.org/10.15302/J-FASE-2019281 contains supplementary material (Table S1).

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Compliance with ethics guidelines Kai Wang, Qunfeng Zhou, Tianze Yan, Shilong Xu, Longyi Zhao, Weicheng Wang, Zhigang Jin, Peng Qin, Chenjian Fu, Liangbi Chen, and Yuanzhu Yang declare that they have no conflicts of interest or financial conflicts to disclose.

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