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RESEARCH ARTICLE

The implications of planting mode on cadmium uptake and remobilization in rice: Field experiments across growth stages

Xiao Deng, Yixuan Chen, Yang Yang, Liang Peng, Luo Si, Qingru Zeng (✉)

College of Resources and Environment, Hunan Agricultural University, Changsha 410128, China

HIGHLIGHTS

- Direct seeding (DS) method led to more distributed Cd in aerial parts of rice.
- The Cd content was significantly higher in brown rice with planting mode of DS.
- Using DS lessened the Fe plaque covering the root surface in all growth stages.
- Transplantation mode should be considered as a priority in Cd-contaminated areas.

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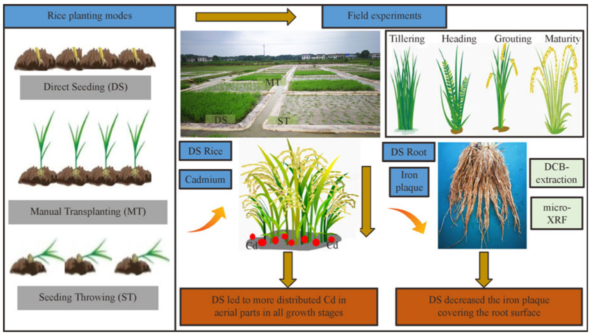
GRAPHIC ABSTRACT



ABSTRACT

Global rice production practices have gradually changed from a reliance on transplanting to direct seeding. Yet how this shift may alter cadmium (Cd) accumulation in rice is poorly known. Here we conducted field experiments with two rice genotypes cultivars that were planted using three methods: via direct seeding (DS), seedling throwing (ST), and manual transplanting (MT). Rice samples were collected during four growth stages. The formation and distribution of iron plaque were analyzed using DCB (dithionite-citrate-bicarbonate) extractions and observed under micro-XRF (micro X-ray fluorescence). The results revealed that, in each growth stage, DS rice was more apt to harbor Cd distributed in the plant's aerial parts, and the Cd concentration of brown rice from DS was 21.8%–43.3% significantly higher than those from ST and MT at maturity stage ($p < 0.05$). During the vegetative stages, the Cd uptake percentage was higher in DS than MT rice, and those plants arising from the DS method were capable of absorbing more Cd earlier in their growth and development. Conversely, using DS decreased the amount of iron plaque covering the root surface in every growth stage, especially in the critical period of Cd accumulation, such that the roots' middle areas were distinguished by a near-complete absence of iron plaque, thus weakening its role as an effective barrier to Cd uptake from soil. Collectively, this study demonstrated that implementing the DS mode of planting will increase Cd's distribution in the aboveground parts of rice, and heightening the risk of Cd contamination in grain.

1 Introduction

Cadmium (Cd), an omnipresent environmental toxin and carcinogenic metallic element to human health, is recognized as an important worldwide contaminant (Zhang et al., 2012). To prevent Cd-related diseases, the Joint FAO/WHO Expert Committee established a health-based guidance intake value for Cd of no more than 25 ug/kg bodyweight per month (Dahlin et al., 2016). Rice (*Oryza sativa* L.), a staple food for one-half of the world's population, has become a major source of Cd in human diets (Huang et al., 2021). A survey in Japan showed that about 40% of Cd intake from food came from the daily consumption of rice (Honma et al., 2016). As previously reported, rice has a strong capacity to accumulate Cd and

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✉ Corresponding author
E-mail: qrzeng@163.com

transport it into grains, especially when cultivated on acidic paddy soils (Chen et al., 2016). In China, where approximately 2.8×10^9 m^2 of its agricultural soils are polluted with Cd (Liu et al., 2015), the production of highly Cd-contaminated rice is not uncommon (Li et al., 2017). A survey of rice grain harvested from Hunan Province, a key rice-growing area in China, showed that approximately 65% of its brown rice contained Cd that exceeded the national standard of China (GB 2762-2017) (Williams et al., 2009). Similarly, Wang et al. (2016) reported that more than 70% of the grain samples exceeded the 0.2 mg/kg limit in a county in southern China, and, given the high consumption there, the daily intake of Cd by its local people posed great risks to their health. Hence, more attention should be paid to rice that contains Cd, with a view toward better understanding its health risks to humans.

Global rice production practices have gradually changed from a reliance on transplanting to direct seeding (Tao et al., 2016), mainly due to the lower labor intensity and less water consumption involved, and higher net earnings and benefit-cost ratios (Chauhan et al., 2015). For instance, in China, manually transplanting nursery seedlings was the most commonly used rice cultivation method prior to 2000, but by 2015, the regions using direct seeding of rice accounted for approximately 30% of the total 30.2 Mha under rice cultivation (Zhang et al., 2018). In some major food-producing regions in southern China, the direct seeding approach is gaining prominence, in that up to 65%–95% of the rice cultivated there now relies on this planting mode (Deng et al., 2020b). Understandably, some researchers have focused on evaluating the impact of transplanting versus direct seeding of rice upon various agronomic traits and the agroecosystem. Early work by Liu et al. (2014) showed that transplanted-flooded rice uses 15.3% more water than does the dry direct seeding of rice, while the latter increased the nitrogen-use efficiency by 11.2%–20.3%. Tao et al. (2016) also revealed that water productivity was 11.6% higher for dry direct seeding than transplanted rice, while the former's global warming potential was 60.4%–76.2% lower than the latter's. Recently, work by Devkota et al. (2020) found that, in eastern India, directly seeded rice has the lowest production cost and highest greenhouse gas reduction potential. Furthermore, it is becoming clearer that the mode of planting could also markedly impact the growth and development of rice. For example, according to Chen et al. (2009), higher transpiration rates were observed in rice plants arising from direct seeding than transplanting, whereas the stomatal conductance and net photosynthetic rates were all lower in directly seeded plants. Almost 30 years ago, Dingkuhn et al. (1991) had shown that, due to transplant shock, the growth of seedlings is delayed and the number of tiller and foliage are reduced in transplanted rice, resulting in a high LAI (leaf area index) and dry matter content observed in direct seeding rice plants. However, to our best knowledge, few empirical measurements of Cd accumulation in rice were taken from plants arising from these contrasting planting modes.

In addition, under anaerobic conditions, rice-aerated tissue will transfer 30% to 40% of the oxygen absorbed by leaves to the roots, creating an oxidizing environment in the rhizosphere that causes much Fe^{2+} and Mn^{2+} in this soil to become oxidized into Fe^{3+} and Mn^{4+} which precipitates onto the root surface, to form a reddish-brown iron-manganese oxide plaque (Cheng et al., 2014). Yang et al. (2017) revealed that this iron plaque could accumulate Cd, given the significant positive correlation between the Fe and Cd concentrations found among these plaques. According to Dong et al. (2016), increased iron plaque formation promotes the deposition of toxic metallic elements onto the root surface, thereby reducing the accumulation of Cd and As in rice. It is now widely accepted that this iron plaque on the rice root surface has great affinity for heavy metals, strongly impacting the dynamics of their uptake and translocation in plant tissues (Cao et al., 2018). As is well known, rice seedlings experience shock when they are selected and transplanted, since their roots typically get damaged in the process. Once transplanted into the soil, the roots must re-adapt themselves to the local environment belowground, while the transplanted rice roots remain unchanged. In addition, how the rice root system is spatially distributed within the cultivated soil profile can vary greatly because of the planting mode used, with directly seeded root systems found concentrated in the top 10- or 15-cm depth layer of surface soil (Naklang et al., 1996). However, whether the differences in rice as generated by planting modes will influence the formation of iron plaque on rice, as well as its Cd accumulation in plant tissues, remains understudied.

Our prior work revealed that using the direct seeding method increases the Cd and Pb concentrations in the grain of hybrid rice (Deng et al., 2020b). Yet not all rice varieties are alike, in that they can display significant differences in their ability to accumulate heavy metals, leaving it unclear whether planting modes have consistently similar effects on different genotypes of rice. In addition, Cd accumulation and translocation are regulated by a source-sink relationship across plant growth stages (Zhou et al., 2018), but it is also unknown how differing planting modes affect the dynamic variation of Cd throughout the growth period of rice. Accordingly, in this study, field experiments were conducted using two contrasting genotypes, a low-Cd-accumulating cultivar ('Xiangwanxian 13', hereon XWX) and high-Cd-accumulating one ('Tianyouhuazhan', TYHZ), which were grown under three planting modes: direct seeding (DS), seedling throwing (ST), and manual transplanting (MT). We collected and measured rice plant samples during four growth stages: tillering, heading, grouting, and maturity. This research had four objectives. 1) To compare the variation in Cd accumulation and translocation in different genotypes rice tissues induced by planting mode; 2) to investigate the dynamic variation of Cd throughout the growth period under different planting modes; 3) to evaluate the influence of MT versus DS on the formation of iron plaque, as well as 4) their influence upon Cd accumulation in plant tissues at distinct growth stages.

2 Materials and methods

2.1 Study site and soil

The moderately contaminated paddy soil was located in the Huang-gu (HG) village (27°34′27″N, 113°13′2″E), in Zhuzhou City of Hunan Province, China. This area has the subtropical monsoon humid climate, with an average annual temperature of 18°C and annual rainfall between 1300 and 1600 mm. A preliminary investigation found large areas of agricultural land polluted with heavy metals to varying degrees, largely because of past metal smelting activities (Deng et al., 2020b). The basic traits of the experimental field soils are given in Supplemental Table S1.

2.2 Field experiment: plots and design

We set up square experimental plots (4 m × 5 m), with four replications per treatment. Each plot had a separate inlet and outlet for irrigation and drainage, respectively. A three-line hybrid high-Cd-accumulating rice cultivar (TYHZ) and a conventional low-Cd-accumulating cultivar (XWX) were used both supplied by Hunan Agricultural University, Changsha, China — Whose seeds were disinfected in hydrogen peroxide and then washed. According to local planting habits, for DS, we directly sowed rice seeds into the field at a sowing rate of 22.5 kg/ha. Meanwhile, on the same day, for transplanted rice, the seedlings were raised in the uniform test field. After the seedlings had grown for approximately 27 days and reached the three-leaf stage, we selected robust seedlings and manually transplanted them into the plot (i.e., the MT rice). For ST, we sprinkled the same number of seedlings evenly across plots. The applied field water and fertilizer management practices were consistent with local practices and the same for all three treatments (DS, MT, and ST). The field fertilization and water management process was similar to our previous report (Deng et al., 2020b).

2.3 Sampling and chemical analysis

We collected rice samples during four rice growth stages (tillering, heading, grouting, and maturity stages), for which the rice and soil sampling, pretreatment, digestion, and mensuration were all similar to those described in our two recent papers (Deng et al., 2020a; 2020b). Briefly, rice samples were carefully washed, after which they were divided into root and straw at the tillering stage; root, straw, and husk at the heading stage; root, straw, and grain (husk + rice milk) at the grouting stage; and brown rice, husk, straw, and root at the maturity stage. The DCB (dithionite-citrate-bicarbonate) method was used to extract the iron plaques from fresh root surfaces. After a mixed acid (HCl-HNO_3-$HClO_4$) digestion, the total soil metal content was measured. The available Cd in soil was extracted with 0.01 mol/L of calcium chloride. Rice tissue powder was examined following its digestion with a mixed acid (HNO_3/$HClO_4$ [85:15%, v/v]). Digestion and measurement processes were accompanied by sample blanks and certified reference materials for quality assurance and quality control.

2.4 Micro X-ray fluorescence analysis

The high-Cd-accumulating rice cultivar, TYHZ, was selected for further analysis. Elemental distribution on its roots was analyzed by micro X-ray fluorescence (micro-XRF). For this, the sample preparation and parameter settings were based on Shen and Song (2017). Key details are as follows: rice root samples at maturity stage were carefully dug out, together with the soil, in order to ensure the integrity of the root system as much as possible, then put into a foam box with ice packs and moved to the laboratory, where they were carefully rinsed; from each sample, a complete root was selected and stuck to the XRF tape. The spectrometer consisted of a 50-W X-ray tube equipped with a poly-capillary lens (M4 TORNADO AMICS, Bruker Nano GmbH, Germany), for which the Rh target was the anode, and a silicon drift detector (SDD) whose resolution was 130 eV at room temperature (at 5.9 keV, full width at half maxima, [FWHM]). After calibrating the micro-XRF energy scale with a copper foil, single-point spectrum acquisition was performed on each sample to determine the interference-free characteristic line of each element. The operating parameters used were 20 keV and 0.8 mA, and a 120-s detector collection time, with a 15-μm pixel size, a stage speed of 1.5 mm/s, and an air chamber pressure of 5.1 m bar. The spectral data and Micro-XRF mapping of elements in the root surface were shown in Supplemental Fig. S2 and Fig. S3.

2.5 Field verification experiment

In the following year, the effects of the three planting modes on Cd accumulation in rice were tested and verified in another field, located in Jiaoxi (JX) Town (28°13′48″N, 113°31′22″E), in Liuyang City (Hunan Province, China). This city also has a humid subtropical monsoon climate, with an average annual temperature of 17.4°C and annual rainfall of 1680 mm. The rice cultivar of TYHZ was used for this experiment. The basic properties of this field's soil were as follows: total Cd, 0.85 mg/kg; pH, 5.7; CEC, 10.6 cmol/kg; OM, 32.3 g/kg; clay, 15.4%; available Cd, 0.23 mg/kg. This experiment's set-up and water and fertilizer management matched those of the experiment done in the previous year (as described above). At harvest, rice plants were collected to determine the Cd concentrations in their tissues.

2.6 Data and statistical analysis

The total amount of Cd in whole plants and the Cd proportion of each tissue were calculated using these two equations:

$$\text{Total Cd} = \sum C_i \times DW_i, \tag{1}$$

$$\text{Proportion } (\%) = C_i \times DW_i/\text{Total Cd} \times 100, \tag{2}$$

where, C_i represents the concentration of Cd in the ith tissue, and DW_i represents the dry weight of the ith tissue.

The translocation factor (TF) among tissues was determined by the ratio of Cd_{straw} to Cd_{root} (TFrs), Cd_{husk} to Cd_{straw} (TFsh), Cd_{grain} to Cd_{straw} (TFsg), and Cd_{BR} to Cd_{straw} content (TFsb), where Cd_{root}, Cd_{straw}, Cd_{husk}, Cd_{grain}, and Cd_{BR} are Cd concentration in rice roots, straw, husk, grain and brown rice, respectively. The uptake percentages of Cd in each growth stage were calculated this way (Zhou et al. 2018):

$$\text{Cd uptake percentage } (\%) = (C_j DW_j - C_{j-1} DW_{j-1})/C_m DW_m \times 100, \tag{3}$$

where, C_j is the Cd concentration during the jth growth stage; DW_j denotes the dry weights during the jth growth stage; C_{j-1} is the Cd concentration before the jth growth stage (j–1); DW_{j-1} denotes the dry weights before the jth growth stage (j–1); C_m is the Cd concentration during the maturity growth stage; and DW_m denotes the dry weights during the maturity stage. Negative values are the amount of Cd either released or transported that exceeded what was absorbed by rice plant tissues.

All data are expressed as the mean±SD ($n = 4$), and all statistical relationships were assessed by one-way analysis of variance (ANOVA), in SPSS 20.0 software. The relationships between the Fe concentrations in the iron plaques and Cd concentrations in rice tissues were analyzed with Pearson correlation coefficients. Duncan's test was used to check for significant differences between the mean values of the three planting mode treatments, for which a p-value < 0.05 was considered significant.

3 Results

3.1 Cd concentrations in rice tissues across growth stages

As Fig. 1 shows, across the four growth stages, the planting mode used significantly affected the Cd concentrations in rice tissues. For TYHZ rice root (without the Fe plaque), as the growing period progressed, the average Cd concentration first increased and then decreased. The highest Cd concentration in roots was obtained at the grouting stage for MT while for ST and DS it occurred at the heading stage (respective values: 6.12 mg/kg; 7.76 mg/kg and 8.89 mg/kg). Interestingly, the Cd concentration in roots of DS rice was 2.67 mg/kg and significantly lower than in MT rice plants (value: 3.76 mg/kg) at the tillering stage ($p < 0.05$), but this pattern was reversed from the heading stage onward, especially at this and the maturity stage, when the difference between DS and MT was significant ($p < 0.05$), being 49.2% and 37.1% higher, respectively. For straw tissue, the Cd concentration increased as the rice plants grew, being significantly less in DS than either MT or ST at the tillering stage ($p < 0.05$); however, in the last three growth stages, the highest mean was observed under DS, which differed significantly from the other planting mode treatments ($p < 0.05$). The Cd content of DS straw at maturity was 4.40 mg/kg, being 31.7% and 31.0% higher than that of ST and MT, respectively. For husk, at the heading stage, its Cd content was significantly higher under DS than ST and MT ($p < 0.05$), but all three treatments were similar at the maturity stage ($p > 0.05$). The most disconcerting result is the Cd concentration in rice grain. As seen in Fig. 1D, during the grouting stage the Cd concentration in the grain of DS was 0.70 mg/kg, or 1.71 and 1.87 times higher than that of ST and MT, respectively. Similarly, at the maturity stage, the Cd concentration of DS brown rice was 0.72 mg/kg, a value 36.5% and 43.3% significantly higher than ST and MT rice, respectively ($p < 0.05$). A similar trend of Cd concentrations in the tissues was also observed in the XWX rice cultivar across the growing stages. Interestingly, the Cd concentrations in roots of the low-Cd-accumulation cultivar (3.50–8.87 mg/kg) did not seem to differ significantly from those of the high-Cd-accumulation variety (2.67–11.2 mg/kg) (Fig. 1A), while the Cd concentration in the aerial part was significantly lower ($p < 0.05$), as expected. Similarly, at the maturity stage, the Cd concentration in DS straw was 22.5% and 27.3% higher than the Cd concentration in straw of MT and ST, while the Cd concentration in brown rice under DS was 0.29 mg/kg, or 33.8% and 21.8% higher than the other two treatment groups, with all differences significant ($p < 0.05$).

The corresponding results for Cd concentrations in rice plant tissues at the maturity stage in the field verification experiment are presented in Fig. 2. Using DS increased the Cd accumulation in rice, and significant differences were

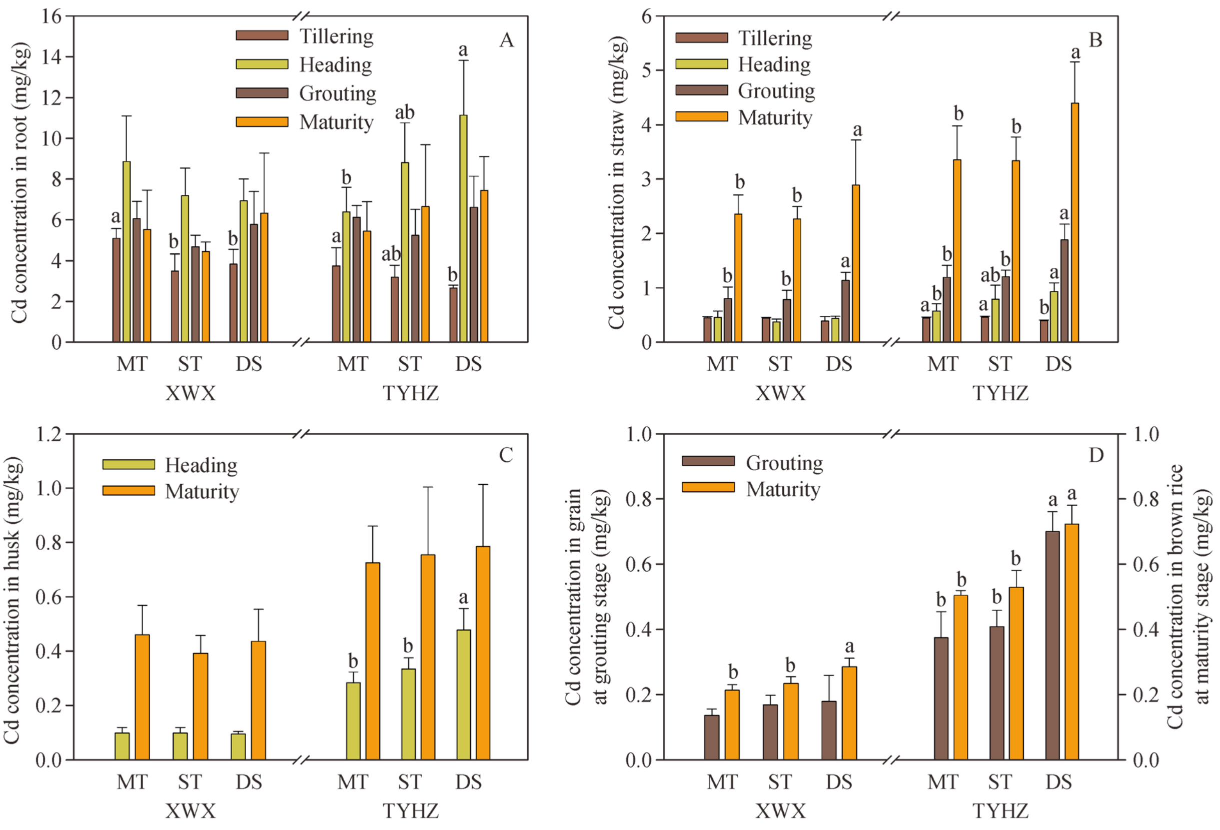

Fig. 1 Cd concentrations in tissues of two genotype rice under three planting modes at different growth stages. Cd concentrations in root (A) and straw (B); in husk at the heading and maturity stages (C); in grain at the grouting stage and in brown rice at the maturity stage (D). MT, ST, and DS mean manual transplanting, seedling throwing, and direct seeding, respectively. Different letters indicate significant differences ($p < 0.05$) among different planting modes while similar letters and parameters without letters indicate no significant difference. The format is the same in following figures and tables.

observed in roots and brown rice among the planting mode treatments ($p < 0.05$). Specifically, the Cd concentration in roots of DS rice was 6.28 mg/kg, while it was lower in ST and MT at 5.43 and 5.14 mg/kg. The disparity was more pronounced for brown rice, for which the Cd concentration under DS was 0.57 mg/kg, a value 18.4% and 29.0% higher than that of ST and MT, respectively.

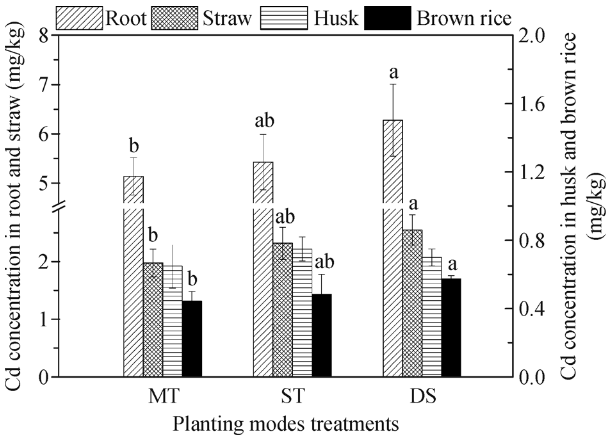

Fig. 2 Cd concentrations in rice plant tissues under three planting modes at maturity stage in field verification experiments.

3.2 Translocation factor (TF) of Cd in rice tissues across growth stages

The results for the TF of Cd in rice tissues during its different growth stages are shown in Fig. 3. The capacity of TYHZ rice roots to transfer Cd to straw was significantly affected by the growth stage. At the tillering stage, the TFrs of MT, ST, and DS was respectively 0.12, 0.15, and 0.15, with an overall average value of 0.14; while the average TFrs of the three treatments at the heading, grouting, and maturity stage were 0.10, 0.25, and 0.61, respectively. It seems that rice has a stronger capacity to transport Cd from its roots to aerial parts during the reproductive stage. By contrast, the treatments showed less disparities for the other translocation factors: the TFsh of MT, ST, and DS were respectively 0.50, 0.46, and 0.51 at heading stage; the TFsg of MT, ST, and DS were 0.31, 0.34, and 0.38 at grouting stage; the TFsb of MT, ST, and DS were 0.15, 0.16 and 0.17 at maturity stage. However, at each growth stage, the TF of Cd for various tissues was similar ($p >$

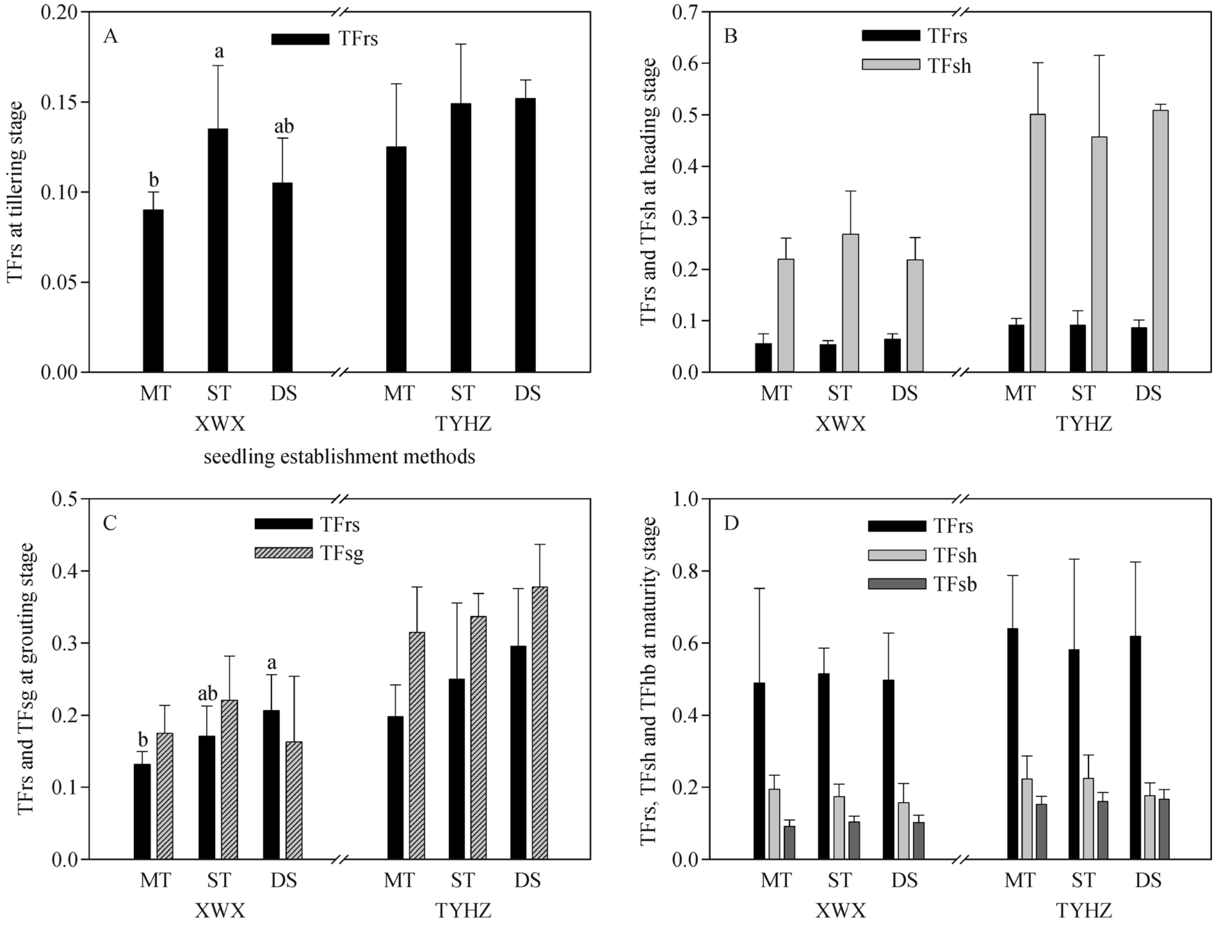

Fig. 3 Cd translocation in rice plant tissues. Translocation factor (TF), root to straw (TFrs), straw to husk (TFsh), straw to grain (TFsg), and straw to brown rice (TFsb); TFrs at tillering stage (A), TFrs and TFsh values at heading stage (B), TFrs and TFsg at grouting stage (C), and the TFrs, TFsh, and TFsb values at maturity stage (D).

0.05). For XWX, at each stage, its TF value was lower than that of the high-Cd-accumulation variety. Conversely, the TFrs of ST rice at the tillering stage significantly exceeded that of MT, and the TFsg of DS rice at the grouting stage significantly surpassed that of MT rice ($p < 0.05$). For the other two growth stages, the Cd transport capacity of each tissue was similar in that no significant differences were detected ($p > 0.05$). Generally then, the differences in Cd accumulation in rice as affected by its planting mode did not seem to have an inextricable relationship with the TF of this metal.

3.3 Distribution of Cd in rice tissues across growth stages

Figure 4 shows the effects of different planting modes on the Cd distribution in tissues of rice across its growth stages. During the tillering stage, Cd was mainly distributed in the belowground parts of rice plants. The Cd in the iron plaque of TYHZ rice from MT, ST, and DS accounted for 62.1%, 70.5%, and 55.7%, respectively, averaging 62.8%, with a mean proportion of Cd in roots of 28.2%. As rice plants' grew, the proportion of Cd allocated to their belowground parts gradually decreased, such that by the maturity stage the Cd in the iron plaque of MT, ST, and DS was reduced to 15.4%, 14.4%, and 8.04%, respectively, while its average proportion in roots was now 17.3%. For rice straw, the proportion of Cd in it increased sharply, from an average of 9.08% at the tillering stage to 63.0% at the maturity stage. By contrast, relatively little Cd was distributed to grain and brown rice tissues, for which the average proportion of grain during the grouting stage was 5.44%, and the average proportion of brown rice during the mature stage was 4.63%. More importantly, it seems that in DS rice plants there was relatively less Cd in their belowground parts and more of it in their aboveground parts, in all growth stages. During the grouting stage, the aboveground part of DS rice accounted for 64.9% of the Cd accumulation that occurred (59.6% straw + 5.38% grain), while the corresponding percentage of MT rice was 47.7% (37.9% straw + 4.8% grain). It was also observed that, during harvesting period, the aboveground portion of DS rice accounted for 76.9% of its

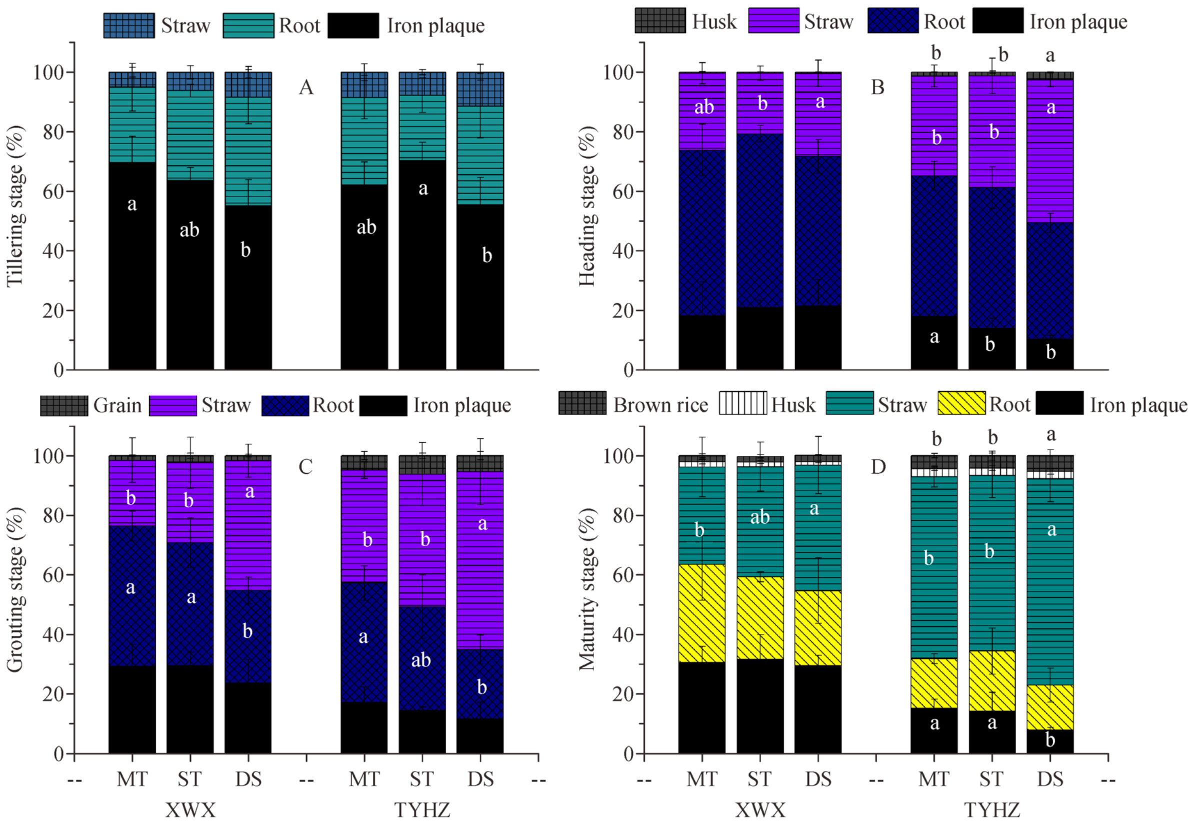

Fig. 4 Distribution of Cd in tissues of two genotype rice during different growth stages under three planting modes. Tillering stage (A), heading stage (B), grouting stage (C), and maturity stage (D).

accumulated Cd, while in the MT rice it accounted for only 68.0%, of which 5.33% was the brown rice from DS, which was 17.6% higher than that of MT. For XWX, the low-Cd-accumulation variety of rice, it had a higher proportion of Cd in its belowground part than did TYHZ from the heading stage onward. This indicated that XWX rice is able to effectively sequester Cd in the iron plaque and roots, limiting its transfer to the aerial parts. Similarly, during the grouting stage, the aboveground part of DS rice accounted for 45.2% of the Cd accumulation, this being 91.2% and 54.7% higher than the other two treatment groups. Further, during the maturity period, the aboveground portion of accumulated Cd was 23.9% higher in DS than MT rice. Hence, the DS mode of planting led to more Cd distributed into the aerial parts of cultivated rice plants.

3.4 Variation in Fe and Mn content in Fe plaque during different growth stages

Figure 5 shows the changes in the Fe and Mn content in the iron plaque across the different growth stages of rice. For TYHZ rice, the highest Fe content occurred at the tillering stage under all three planting modes; it was 34.5 g/kg in the DS iron plaque, for which the corresponding values for MT and ST were 1.67 and 1.45 times greater, respectively. During the growing period, the Fe content first showed a rapid decline but then increased slightly, for which the amount of iron plaque in DS rice was the smallest at every rice growth stage. A similar pattern was observed for Mn. At the tillering stage, the Mn content in the iron plaque of MT rice was 193 mg/kg, a value 46.5% higher than that of DS. Additionally, the Mn content of DS rice displayed a decreasing trend when compared with MT across the remaining three growth stages. For XWX rice, the trend was almost the same as those of the high-Cd-accumulation variety; hence, in this field experiment, soil conditions were probably the paramount factor driving the formation of iron plaque, and not inherent differences between the two rice varieties. Similar to TYHZ, the Fe and Mn concentrations in the iron plaque of DS rice were the smallest at every growth stage.

Pearson correlations were used to examine the linear relationships between the Fe, Mn, and Cd contents of iron plaque (Supplemental Table S2). Evidently, for both rice varieties, most of the Fe content at each growth stage was always positively correlated with its Cd content ($p < 0.05$ or < 0.01); overall, 62.5% (5/8) of the tested correlations were positive and significant, except for the grouting stage of TYHZ and the grouting and maturity stages of XWX. By contrast, Mn was weakly correlated with Cd in the iron

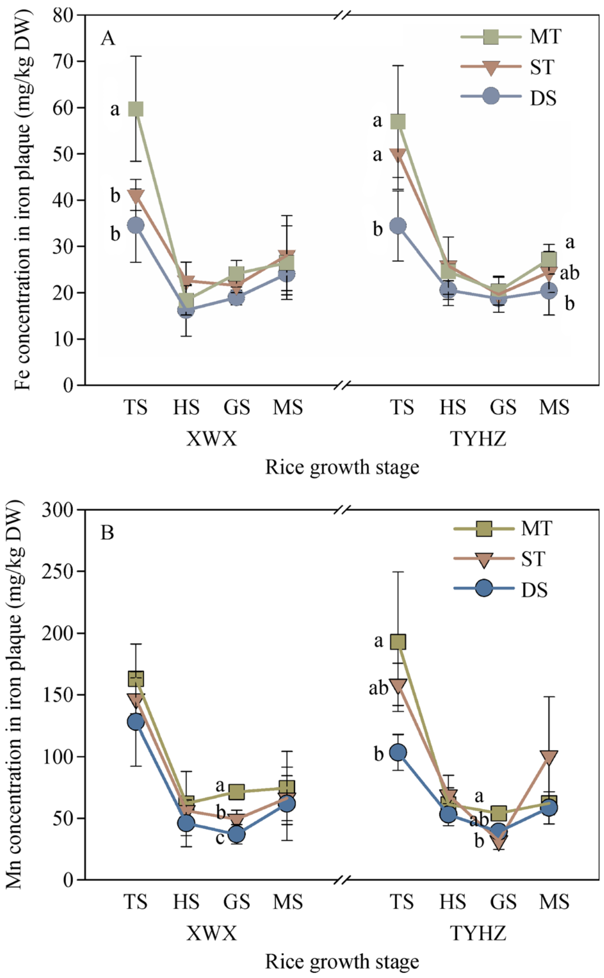

Fig. 5 Fe and Mn concentrations in iron plaque of two genotype rice under three planting modes across different growth stages. Fe (A) and Mn (B). TS, HS, GS, and MS respectively denote the tillering stage, heading stage, grouting stage, and maturity stage of rice.

plaque, and significantly so only during the maturity stage of both rice varieties and the heading stage of XWX rice ($p < 0.05$).

3.5 Location of Fe and other elements on root surfaces

The collected rice samples were brought to the laboratory where they were carefully cleaned and photographed (Supplemental Fig. S1). Evidently, there were stark physical differences in the roots among the planting modes. The DS root tissue was finer, and more heterogeneous, having a relatively whiter color with apparently less iron plaque covering the roots. A micro-XRF fluorescence scan image is shown in Fig. 6. We can clearly see that, in comparing the treatments, the iron plaque was not evenly distributed. For DS rice, there seem to be more root hairs on the whole root system. More importantly, and similarly, there were fewer iron plaque deposits visible on the surface of DS rice roots, with even a near-complete absence of iron plaque coverage in some middle areas of the root tissue. By contrast, the fluorescence signals for iron on the surface of MT and ST rice roots were much stronger, indicating more iron plaque had formed on them. Table 1 shows the mass percent of each element; for Fe, on the surface of DS rice roots, it was 0.20%, which was 27.8% and 33.3% less than the other two treatment groups. Similar results were generally observed for Mn. Taken together, this indicated that using DS in rice production is expected to decrease the formation of iron plaque.

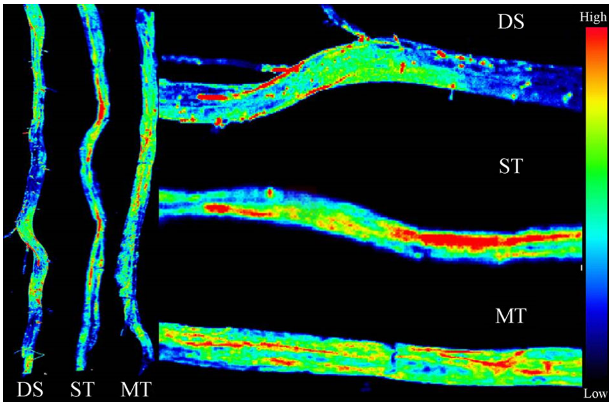

Fig. 6 Micro X-ray fluorescence images of iron (Fe) distribution in the TYHZ rice root surface of rice plants under three planting modes treatments.

4 Discussion

The direct seeding (DS) method, the process of directly sowing the seeds in the field instead of transplanting seedlings there, has been proven to reduce water requirements and require less labor for rice production, and to also work well with the mechanization of rice planting (Chen et al. 2017). However, results from this study revealed that DS rice exhibits a stronger ability to accumulate Cd, especially in the aerial parts of plants, starting from the heading stage, in that the straw and brown rice Cd concentrations in DS rice were significantly higher than those of MT and ST rice ($p < 0.05$) (Fig. 1). Additionally, the proportion of Cd that accumulated in their aerial parts was also higher, especially at the maturity stage, surpassing that of ST and MT rice, while the total amount of Cd in their roots was correspondingly lower (Fig. 4). These findings were validated in a field experiment with a different soil type in the following year (Fig. 2). This suggests the Cd accumulation in plants would be markedly enhanced by applying the DS method for rice production,

Table 1 Quantification results of mass percent (%) of some elements on TYHZ rice root using micro-XRF

Spectrum	C	O	Na	Mg	Al	Si	P	S	Cl	K	Ca	Ti	Mn	Fe	Zn
MT	30.6	22.2	0.077	0.024	0.167	0.350	0.021	0.028	0.021	0.027	0.009	0.019	0.0011	0.282	0.0004
ST	31.1	22.8	0.046	0.022	0.122	0.337	0.025	0.021	0.017	0.022	0.009	0.017	0.0012	0.305	0.0004
DS	29.5	23.1	0.068	0.029	0.114	0.252	0.014	0.018	0.026	0.015	0.010	0.020	0.0007	0.204	0.0003

especially in terms of brown rice production, thus posing greater health risks for rice consumers. The present study's results have important environmental implications for choosing an appropriate planting mode for achieving the safe production of rice grain, especially in those farmland areas characterized by substantial Cd pollution of soil.

Some previous research has investigated Cd accumulation, remobilization, and translocation at different rice growth stages, using either hydroponic or pot experiments. Zhou et al. (2018) reported that most of the Cd accumulated during the early rice growth stages. Work by Rodda et al. (2011) revealed that Cd in brown rice grain partially came from uptake by roots during the reproductive phase, while the rest was remobilized from that accumulated by the plant prior to flowering. Those findings revealed that Cd accumulation in rice grain was not only related to root uptake and root-to-aerial parts translocation, but also to the Cd remobilization and redistribution in other tissues at the plant's reproductive stage. Nonetheless, corresponding results for Cd-contaminated soils under real-world field conditions and how the planting mode could differentially affect Cd's distribution and remobilization in rice tissues are still unclear. In this study, for TYHZ rice, a higher uptake percentage of Cd in grain occurred during the grain maturation stage, on average up to 60.4% (Table 2). The ranking for the Cd uptake percentage in grain was as follows: maturing stage>grouting stage>heading stage, which suggests the grain maturation stage is key for understanding the dynamics of how Cd accumulates in rice grain. Further, a large amount of Cd was absorbed by iron plaque during the tillering stage, for which the Cd uptake percentages ranged from 257% to 295%. After this stage, however, Cd was released or transported at the heading and grouting stages, given their negative Cd uptake percentages (up to –227%), so that in the maturity stage the dynamics of absorption and transport of Cd in the iron plaque had basically attained equilibrium. For the root system of rice, the vegetative phase is when Cd is accumulated the most from soil, but more Cd is transported to the aerial part in the reproductive stage, particularly at the grouting phase; similarly, the Cd uptake percentage in straw increased initially followed by a negative value in the grouting stage. An earlier study by Kashiwagi et al. (2009) reported that substantial Cd accumulated in leaves could be remobilized within rice plants, with this remobilized Cd specifically transported to reproductive growth tissues. Yan et al. (2010) also provided evidence that the re-translocation of absorbed Cd before the heading stage in straw was also a considerable contributor to the overall accumulation of Cd pattern in brown rice. But during the heading stage, the Cd uptake percentage of both the aerial part and grain of

Table 2 Cd uptake percentages (%) at different growth stages of TYHZ rice plants in the field experiment

TYHZ	Growth stages	Iron plaque	Root	Straw	Panicle[a)]	Underground part	Aerial part
MT	Tillering	257	109	8.03	—	181	7.20
	Heading	–185	77.1	26.5	11.9	–49.5	25.0
	Grouting	12.4	–22.9	10.0	32.9	–5.82	12.4
	Maturity	15.2	–63.2	55.4	55.2	–25.3	55.4
ST	Tillering	295	58.6	7.45	—	152	6.72
	Heading	–227	103	35.2	12.5	–26.6	33.0
	Grouting	–17.0	–81.2	–5.66	34.3	–55.9	–1.74
	Maturity	48.2	19.3	63.0	53.2	30.7	62.1
DS	Tillering	285	86.5	6.53	—	157	5.91
	Heading	–173	147	51.2	28.6	33.2	49.0
	Grouting	–33.6	–145	–9.38	11.0	–105	–7.45
	Maturity	21.3	11.5	51.7	60.4	15.0	52.5

Note: a) Panicle represents the husk at the heading stage, the grain (husk and rice milk) at grouting stage, and the husk-plus-brown rice at maturity stage in the calculations. Negative values represent the amount of Cd either released or transported that exceeded what was absorbed by rice plant tissues.

DS rice exceeded that of transplanted rice. Similar results were obtained for the low-Cd-accumulation variety (Supplemental Table S3). The Cd uptake percentage for the aerial part of rice arising from DS was higher at the vegetative stages (14.4% for tillering, 36.3% for heading). This result suggests that using DS as a planting mode would foster a large accumulation of Cd in rice, by enabling rice plants to absorb more Cd sooner in their growth and development, so that they would eventually accumulate more than half of this Cd in their aerial parts during the vegetative stage.

Many research suggests that metals can be sequestered by the iron plaque on rice root surfaces, resulting in the limitation of metals' uptake and translocation in rice tissues (Wang et al. 2013). However, Liu et al. (2011) revealed that the functions of iron plaque were actually quite limited, being only effective in relatively lower or moderate Pb-contaminated soil. Recent work by Amaral et al. (2017) found that the promoted formation of this kind of Fe may still allow translocation of As to plant shoots, potentially leading to higher levels of As in both the shoots and grain of rice. According to work by Zhong et al. (2010), as the amount of iron plaque increases so too does its adsorption of Pb, resulting in a greater Pb accumulation in roots. So the current understanding is that the iron plaque can enhance or inhibit metals' uptake by rice.

In this experiment, the rice roots were well developed at the tillering stage, so they likely underwent higher radial oxygen loss (ROL) (Huang et al., 2018). Accordingly, the highest amount of iron plaque was observed at this growth stage, which was nearly three times higher than at the following stages (Fig. 5A), and this led to the highest concentration of Cd in iron plaque. Similar results were also reported by Wang et al. (2013). As seen in Figs. 7A and 7B, there was a positive correlation between the Fe content in iron plaque and the Cd concentration in root and straw tissues at tillering stage, especially for roots, for which significant relationships were evinced (r = 0.643, $p < 0.05$ for TYHZ and r = 0.584, $p < 0.05$ for XWX). During the heading stage, the amount of iron plaque began to show a negative correlation with the Cd concentration in rice tissues, but this was not significant (Figs. 7C and 7D). During grouting stage, the amount of Fe clearly declined with an increasing Cd content of all rice tissues, especially for the Cd content in the roots of TYHZ rice plants and the straw of XWX plants, for which highly significantly negative correlations were obtained (r = –0.716, $p < 0.01$ for TYHZ and r = –0.789, $p < 0.01$ for XWX) (Figs. 7E and 7F). Similar findings also characterized the maturity stage, especially for the Cd concentration in brown rice of TYHZ rice, for which a highly significantly negative correlation was found (r = –0.761, $p < 0.01$).

Previous studies by Zhang et al. (1998) have revealed that the functioning of the iron plaque, to either isolate or promote a given metal, depends on its thickness, deterioration degree, and amount of iron plaque deposited. The data of this study show that more iron plaque formed during the early stage of rice growth (up to 59.9 g/kg root dry weight), leading to excessive amounts of Cd accumulation on the roots' surface. Accordingly, root exudates such as phytosiderophores might remobilize the Cd adsorbed to the iron plaque, thereby enhancing the uptake of Cd (Zhang et al., 1998); in this way, the plaque can function as a reservoir which promotes overall Cd uptake by rice plants (Figs. 7A and 7B). Then, with the changes to ROL and flooding conditions, the plaque would become a barrier that limited Cd's uptake and transfer among rice tissues at later stages of rice growth. In previous research, we had calculated that, on a per unit area basis, less iron plaque forms on the root surface of DS rice (Deng et al., 2020b). In the present study, we observed firsthand, using micro-XRF, that indeed less iron plaque covers the root surface of rice plants (Fig. 6). Thus, the DS mode of planting inevitably leads to diminished iron plaque formation, thereby reducing Cd sequestration at the root surface of rice, especially during the key phase of development (i.e., grouting to mature stage) for Cd accumulation in the rice grain.

5 Conclusions

Here we conducted field experiments to investigate Cd's accumulation in rice tissues across its whole growing period under different modes of planting. The results show that direct seeding (DS) will increase the risk of Cd contamination to rice plants. In each growth stage, implementing DS was more inclined to distribute Cd in the plant's aerial parts. In particular, the Cd concentration in brown rice of DS rice at the mature stage will exceed that harbored by transplanted rice. At the same time, using DS reduced the iron plaque covering the root surface of rice in all growth stages, especially in the critical period of Cd accumulation, thus weakening its role as an effective barrier to Cd uptake. Therefore, the current serious situation of rice polluted with Cd in China may be linked to the promotion of DS. To rectify this, we advocate that transplantation ought to be selected as the primary mode of planting in Cd-contaminated areas for achieving the production of rice grain safe for human consumption.

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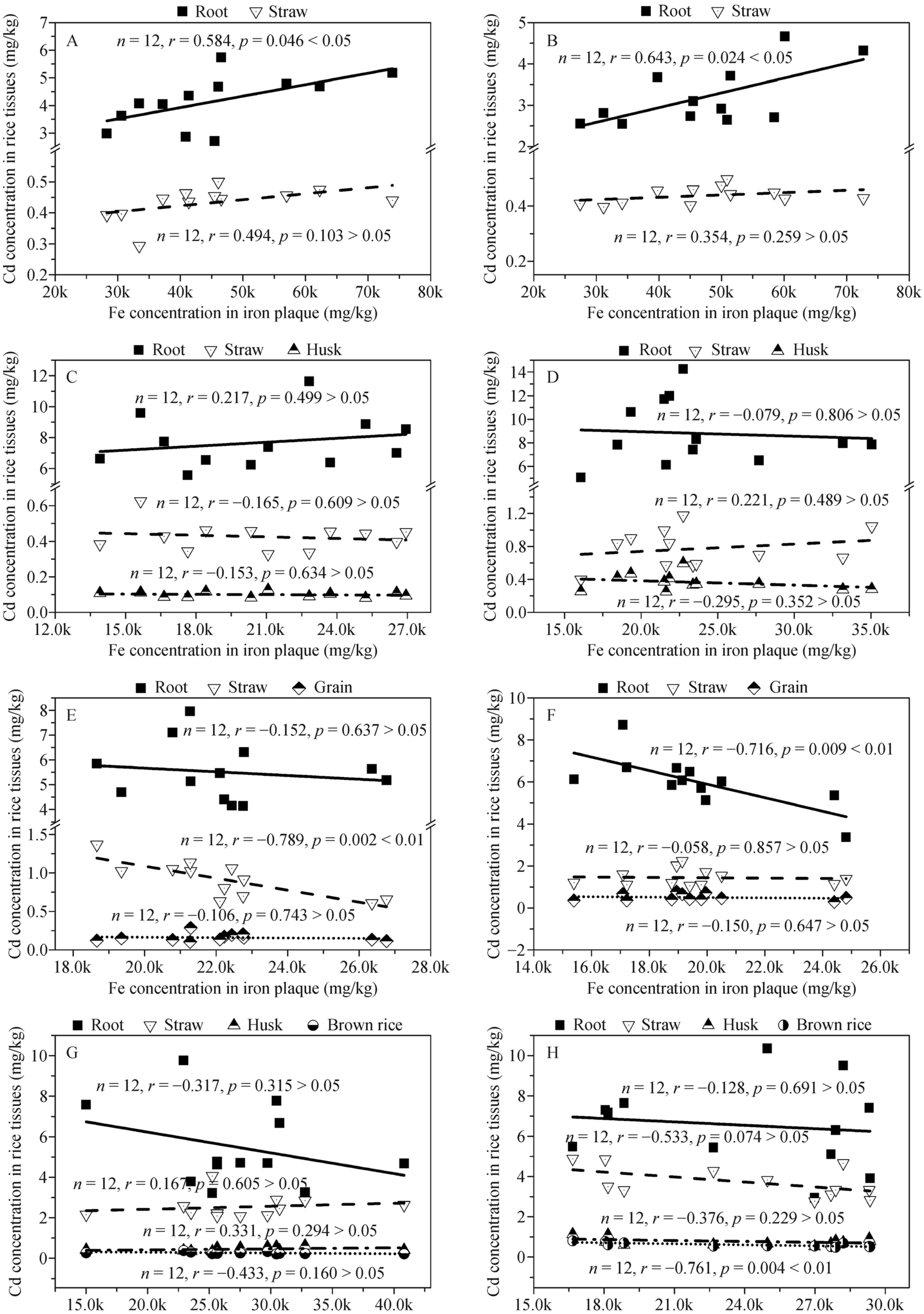

Fig. 7 Relationships between the Fe concentrations in Fe plaque and the Cd concentrations in rice tissues.

References

Amaral D C, Lopes G, Guilherme L R, Seyfferth A L (2017). A new approach to sampling intact Fe plaque reveals Si-induced changes in Fe mineral composition and shoot As in rice. Environmental Science & Technology, 51(1): 38–45

Cao Z Z, Qin M L, Lin X Y, Zhu Z W, Chen M X (2018). Sulfur supply reduces cadmium uptake and translocation in rice grains (*Oryza sativa* L.) by enhancing iron plaque formation, cadmium chelation and vacuolar sequestration. Environmental Pollution, 238: 76–84

Chauhan B S, Awan T H, Abugho S B, Evengelista G, Sudhir-Yadav (2015). Effect of crop establishment methods and weed control treatments on weed management, and rice yield. Field Crops Research, 172: 72–84

Chen D, Guo H, Li R, Li L, Pan G, Chang A, Joseph S (2016). Low uptake affinity cultivars with biochar to tackle Cd-tainted rice–A field study over four rice seasons in Hunan, China. Science of the Total Environment, 541: 1489–1498

Chen S, Cai S G, Chen X, Zhang G P (2009). Genotypic differences in growth and physiological responses to transplanting and direct seeding cultivation in rice. Rice Science, 16(2): 143–150

Chen S, Ge Q, Chu G, Xu C, Yan J, Zhang X, Wang D (2017). Seasonal differences in the rice grain yield and nitrogen use efficiency response to seedling establishment methods in the Middle and Lower reaches of the Yangtze River in China. Field Crops Research, 205: 157–169

Cheng H, Wang M, Wong M H Z, Ye (2014). Does radial oxygen loss and iron plaque formation on roots alter Cd and Pb uptake and distribution in rice plant tissues? Plant and Soil, 375(1–2): 137–148

Dahlin A S, Eriksson J, Campbell C D, Oborn I (2016). Soil amendment affects Cd uptake by wheat: Are we underestimating the risks from chloride inputs? Science of the Total Environment, 554–555: 349–357

Deng X, Chen Y, Yang Y, Lu L, Yuan X, Zeng H, Zeng Q (2020a). Cadmium accumulation in rice (*Oryza sativa* L.) alleviated by basal alkaline fertilizers followed by topdressing of manganese fertilizer. Environmental Pollution, 262: 114289

Deng X, Yang Y, Zeng H, Chen Y, Zeng Q (2020b). Variations in iron plaque, root morphology and metal bioavailability response to seedling establishment methods and their impacts on Cd and Pb accumulation and translocation in rice (*Oryza sativa* L.). Journal of Hazardous Materials, 384: 121343

Devkota K P, Sudhir-Yadav C M, Khanda S J, Beebout B K, Mohapatra G R, Singleton R, Puskur (2020). Assessing alternative crop establishment methods with a sustainability lens in rice production systems of Eastern India. Journal of Cleaner Production, 244: 118835

Dingkuhn M, Schnier H, de Datta S, Dorffling K, Javellana C (1991). Relationships between ripening-phase productivity and crop duration, canopy photosynthesis and senescence in transplanted and direct-seeded lowland rice. Field Crops Research, 26(3–4): 327–345

Dong M F, Feng R W, Wang R G, Sun Y, Ding Y Z, Xu Y M, Fan Z L, Guo J K (2016). Inoculation of Fe/Mn-oxidizing bacteria enhances Fe/Mn plaque formation and reduces Cd and As accumulation in rice plant tissues. Plant and Soil, 404(1–2): 75–83

Honma T, Ohba H, Kaneko-Kadokura A, Makino T, Nakamura K, Katou H (2016). Optimal soil Eh, pH, and water management for simultaneously minimizing arsenic and cadmium concentrations in rice grains. Environmental Science & Technology, 50(8): 4178–4185

Huang H, Chen H P, Kopittke P M, Kretzschmar R, Zhao F J, Wang P, (2021). The voltaic effect as a novel mechanism controlling the remobilization of cadmium in paddy soils during drainage. Environmental Science & Technology,55(3): 1750–1758

Huang G, Ding C, Hu Z, Cui C, Zhang T, Wang X (2018). Topdressing iron fertilizer coupled with pre-immobilization in acidic paddy fields reduced cadmium uptake by rice (*Oryza sativa* L.). Science of the Total Environment, 636: 1040–1047

Kashiwagi T, Shindoh K, Hirotsu N, Ishimaru K (2009). Evidence for separate translocation pathways in determining cadmium accumulation in grain and aerial plant parts in rice. BMC Plant Biology, 9(1): 8–17

Li H, Luo N, Li Y W, Cai Q Y, Li H Y, Mo C H, Wong M H (2017). Cadmium in rice: Transport mechanisms, influencing factors, and minimizing measures. Environmental Pollution, 224: 622–630

Liu F, Liu X, Ding C, Wu L (2015). The dynamic simulation of rice growth parameters under cadmium stress with the assimilation of multi-period spectral indices and crop model. Field Crops Research, 183: 225–234

Liu H, Hussain S, Zheng M, Peng S, Huang J, Cui K, Nie L (2015). Dry direct-seeded rice as an alternative to transplanted-flooded rice in Central China. Agronomy for Sustainable Development, 35(1): 285–294

Liu J, Leng X, Wang M, Zhu Z, Dai Q (2011). Iron plaque formation on roots of different rice cultivars and the relation with lead uptake. Ecotoxicology and Environmental Safety, 74(5): 1304–1309

Naklang K, Shu F, Nathabut K (1996). Growth of rice cultivars by direct seeding and transplanting under upland and lowland conditions. Field Crops Research, 48(2): 115–123

Rodda M S, Li G, Reid R J (2011). The timing of grain Cd accumulation in rice plants: the relative importance of remobilisation within the plant and root Cd uptake post-flowering. Plant and Soil, 347(1–2): 105–114

Shen Y T, Song Y F (2017). Effects of organic ligands on Pb absorption and speciation changes in Arabidopsis as determined by micro X-ray fluorescence and X-ray absorption near-edge structure analysis. Journal of Synchrotron Radiation, 24(2): 463–468

Tao Y, Chen Q, Peng S, Wang W, Nie L (2016). Lower global warming potential and higher yield of wet direct-seeded rice in Central China. Agronomy for Sustainable Development, 36(2): 24–32

Wang M, Chen W, Peng C (2016). Risk assessment of Cd polluted paddy soils in the industrial and township areas in Hunan, Southern China. Chemosphere, 144: 346–351

Wang X, Yao H, Wong M H, Ye Z (2013). Dynamic changes in radial oxygen loss and iron plaque formation and their effects on Cd and As accumulation in rice (*Oryza sativa* L.). Environmental Geochemistry and Health, 35(6): 779–788

Williams P N, Lei M, Sun G, Huang Q, Lu Y, Deacon C, Meharg A A, Zhu Y G (2009). Occurrence and partitioning of cadmium, arsenic and lead in mine impacted paddy rice: Hunan, China. Environmental Science & Technology, 43(3): 637–642

Yan Y F, Choi D H, Kim D S, Lee B W (2010). Absorption, translocation, and remobilization of cadmium supplied at different growth stages of rice. Journal of Crop Science and Biotechnology, 13 (2): 113–119

Yang W T, Zhou H, Gu J F, Zeng Q R, Liao B H (2017). Influence of rapeseed cake on iron plaque formation and Cd uptake by rice (*Oryza sativa* L.) seedlings exposed to excess Cd. Bulletin of Environmental Contamination and Toxicology, 99(5): 601-606

Zhang C, Ge Y, Yao H, Chen X, Hu M (2012). Iron oxidation-reduction and its impacts on cadmium bioavailability in paddy soils: A review. Frontiers of Environmental Science & Engineering, 6(4): 509–517

Zhang X, Zhang F, Mao D (1998). Effect of iron plaque outside roots on nutrient uptake by rice (*Oryza sativa* L.). Zinc uptake by Fe-deficient rice. Plant and Soil, 202(1): 33–39

Zhang Y, Liu H, Guo Z, Zhang C, Sheng J, Chen L, Luo Y, Zheng J (2018). Direct-seeded rice increases nitrogen runoff losses in southeastern China. Agriculture, Ecosystems & Environment, 251: 149–157

Zhong S, Shi J, Xu J (2010). Influence of iron plaque on accumulation of lead by yellow flag (*Iris pseudacorus* L.) grown in artificial Pb-contaminated soil. Journal of Soils and Sediments, 10(5): 964–970

Zhou H, Zhu W, Yang W T, Gu J F, Gao Z X, Chen L W, Du W Q, Zhang P, Peng P Q, Liao B H (2018). Cadmium uptake, accumulation, and remobilization in iron plaque and rice tissues at different growth stages. Ecotoxicology and Environmental Safety, 152: 91–97