Comparing the effects of calcium and magnesium ions on accumulation and translocation of cadmium in rice

Xiangying Li1,2 · Lang Teng3,4 · Tianling Fu1,2 · Tengbing He2,3 · Pan Wu1,5

Abstract
Rice (Oryza sativa L.) is one of China’s most important food crops, and it is considered the primary source of human exposure to cadmium (Cd) pollution. Adding calcium (Ca) and magnesium (Mg) to the plant nutrient solutions reduces the accumulation of Cd in the rice, but under the same condition, which one has the better effect remains unclear. Thus, hydroponic experiments were performed to compare the effects of Ca and Mg ions with concentration gradients (0.10, 0.25, and 0.50 g/L, respectively) on the absorption, distribution, and translocation of Cd in rice. The Cd contents of roots, stems, leaves, panicles, husks, and grains in different growth stages were determined. The results revealed that the supplementation of both Ca and Mg influenced the Cd accumulation and translocation in rice tissues. The Cd concentrations of different patterns were in the following order: roots > stems > leaves ≈ panicles ≈ husks > grains. Both of Ca and Mg had an apparent antagonism with Cd in different parts of the rice plant, and the antagonism was more obvious in the high Cd stress treatments. With the addition of 0.1 g/L Ca2+ and Mg2+ ions, the grain Cd contents increased, while the application of 0.25 and 0.5 g/L Ca2+ and Mg2+ ions reduced grains Cd by 19.08–38.99%, with the average value of 26.75%. Under the same concentrations, the grain Cd content of Ca treatments was lower than that of Mg treatments by 8.74%. In the Ca (Mg)-deficient and Ca (Mg)-sufficient conditions, the husks and panicles accumulated Cd to hinder Cd translocation, respectively. Altogether, the results of this study indicated that Ca had a greater effect for decreasing rice Cd accumulation and translocation than Mg, and the panicle and husk were the important parts for reducing Cd translocation to grain, and these might be a focal point for the future research. It was possible to plant and grow rice in Cd-polluted soil and that the accumulation and translocation of Cd in rice plants could be reduced by optimizing soil nutrient elements.

Keywords Accumulation · Cadmium · Calcium · Magnesium · Rice · Translocation

Introduction
Cadmium (Cd), a toxic heavy metal, has recently gained more attention due to various human activities, such as mining, smelting, sewage sludge, chemical fertilizers, and pesticides (He et al. 2013; Römkens et al. 2009). Cd is ubiquitous in the environment, as it can be accumulated in crops, and then enter the food chain (Liu et al. 2015). Rice (Oryza sativa L.) is a staple cereal crop in China, and it is the primary exposure source of Cd in non-smoking humans due to the ingestion of rice grown in Cd-contaminated paddy soils, especially in some areas of southern China (Feng et al. 2010). Long-term consumption of Cd-polluted rice may cause serious health problems, such as anemia, cancer, heart attack, proteinuria, lung disorder, emphysema, and osteoporosis (Mei et al. 2017). In recent years, the occurrence of notorious itai-itai disease in Japan was connected to the intake of Cd-contaminated rice (Järup & Åkesson 2009). In rice plant, the main pathway of Cd accumulation in grains was the absorption by roots (Fig. 1). Thus, reducing Cd accumulation and translocation in rice is a priority for food safety and human health.

Given its Karst topography, anomalously high Cd contents in carbonate rocks were observed in Guizhou Province, China (Chen et al. 2015, Zhang and Song, 2018). According to China National Environmental Monitoring Centre
China National Environmental Monitoring Center (1990), the background content of Cd in Guizhou was 1.244 mg/kg, the highest concentration in China. Cd is mainly sourced from the carbonate rocks weathering and pedogenesis, during which the soluble components leached and the insoluble components such as Cd was absorbed by clay or Fe and Mn oxides (Li et al. 2020; Xia et al. 2020). This special geological background results in a characteristic of high total heavy metals contents with low bioavailability. Pu et al. (2015) and Fang et al. (2007) found that most soil samples in Guizhou Province were rich in Ca$^{2+}$, Mg$^{2+}$, and CO$_3^{2-}$/HCO$_3^{-}$. Thus, we hypothesize that Ca$^{2+}$ and Mg$^{2+}$ ions play important roles in decreasing Cd accumulation in plants.

Calcium (Ca$^{2+}$) is an essential macromolecule and divalent cation, was enriched in carbonate rocks, and is responsible for the increase of soil pH (Hamid et al. 2020). Some studies have suggested that Cd could enter into plant cells through the Ca$^{2+}$ ions channels (Liu et al. 2020; Perfus-Barbeoch et al. 2002). Meanwhile, studies have shown considerable competition between Ca and Cd at the transport sites of plant cell membranes (Qin et al. 2020). It is believed that Ca alleviates the toxicity of Cd in plants through three processes: firstly, Ca alleviates the accumulation of Cd in plants by regulating plant growth; secondly, Ca increases the activity of antioxidant enzymes in plants to relieve oxidative stress caused by Cd; thirdly, Ca enhances photosynthetic, physiological, and metabolic activities in plants to regulate the signaling pathways that depend on calcium transmission (Fig. 1) (Guo et al. 2018; Huang et al. 2017; Khan et al. 2016). However, some studies have shown that Ca can also increase the absorption of Cd by Sesbania sesban and Brassica juncea (Franziska & Hans 2016; Suzuki 2005). Therefore, whether and how Ca$^{2+}$ ions decrease Cd accumulation requires further study.

Magnesium (Mg), a component of the chlorophyll molecule, significantly influences the synthesis of nucleic acids and proteins for photosynthetic energy (Chen et al. 2017b, Cakmak and Kirkby, 2008). Mg is the fourth essential nutrient element for plant growth and coexists with Ca, potassium (K), sodium (Na), and other elements in the soil. Mg competes with heavy metal ions during the root uptake process and affects the accumulation and transport of Cd in rice. Applying fertilizers containing Mg to Cd-polluted farmland inhibited the availability of Cd in soil. Studies found that Mg deficiency increased the absorption and accumulation of Cd in rice while adding Mg$^{2+}$ ions alleviated the Cd stress of rice seedlings. Mg deficiency reduced the physiological metabolism of rice, leading to the increased expression of Cd transporter OsiRT1, Oszip1, and Oszip3 in the plant, inducing the increment of Cd uptake and translocation by the roots (Chou et al. 2011).

Recently, several methods have been studied to control the Cd risks in soil-rice system, such as water management, fertilization, physical treatments, chemical remediation via the addition of soil amendments, bioremediation, and phytoremediation. The external environmental factors, such as soil pH, Eh, and organic matter, were concerned for influencing Cd absorption and translocation in rice (Chen et al. 2017a; Honma et al. 2016; Liu et al. 2013). Researchers showed calcium magnesium phosphate promoted the development of iron plaque on the root surface, thereby decreasing the Cd concentration and increasing the free amino acid concentration in grains, limiting the accumulation of Cd in rice root protoplasts (Cai et al. 2021; Zhao et al. 2020). Numbers of studies showed Ca and Mg reduce the Cd accumulation in rice; however, there were great differences in cultivated varieties, solution concentrations, and treatment methods. Under the same condition (e.g., cultivar, germination, sowing, concentration, and so on), Ca and Mg, which one has the greater effect to inhibit Cd uptake by rice remains unclear. Considering Ca as an essential macronutrient, participating in many physiological processes, and

![Fig. 1](image-url)
its content was more than Mg in rice plant, we hypothesized that Ca had a greater effect on releasing Cd phytotoxicity than Mg. In order to study this point, we conducted a hydroponic experiment to (i) investigate the uptake, translocation, and accumulation of Cd in rice plants; (ii) compare the effect of Ca\(^{2+}\) and Mg\(^{2+}\) ions on the absorption, distribution, and translocation of Cd in rice; and (iii) analyze the difference between Ca and Mg on Cd accumulation in rice. We assumed that the results can provide an insight comprehend to release Cd phytotoxicity by Ca and Mg and contribute in advancing strategies to mitigate Cd accumulation in rice.

**Materials and methods**

**Plant material and experimental design**

In order to avoid the interference of the complex factors such as organic matter, microorganism, and other nutrient elements in soil environment, we chose hydroponic experiment to simulate the planting environment of rice. The hydroponic experiment was conducted at the Rice Research Institute, Guizhou Academy of Agricultural Sciences in Guiyang, Guizhou Province, China (106°39′57.63″N and 26°29′57.63″E).

The rice cultivar, C Liangyouhuazhan, was obtained from the Rice Research Institute, Guizhou Academy of Agricultural Sciences. Sterilized (with 5% NaCl for 15 min) healthy seeds were cleaned with deionized water and placed on moist filter paper for germination in the dark conditions of 25 ± 1 °C. While the roots appeared, the seeds were transplanted to a floating screen and sprout in a deionized aqueous solution containing 0.5 mM CaCl\(_2\), the germination was still placed in the artificial climate with 25 ± 1 °C. After germination for 3 days, the seedlings were cultured in 1-L plastic hydroponic boxes containing modified 1/4 Hoagland’s nutrient solution. The modified 1/4 Hoagland’s nutrient solution was referred to previous studies (Wang 2015; Yoshida et al. 1976), and the nutrient element content of it was shown in supplementary material.

When the fourth leaf appeared, healthy seedlings were transplanted to 5-L hydroponic pots containing 1/4 modified Hoagland’s nutrient solution and treating solution. The pH was adjusted to pH 5.5–6.0 using 1 M HCl or NaOH. The hydroponic pots were placed into a controlled plant growth chamber. The growing conditions were 16 h of light and 8 h of darkness per day, a daytime temperature of 26 °C, and a nighttime temperature of 20 °C, while the relative humidity was 60%.

In view of the soil environmental quality risk control standard for soil contamination of agricultural land in China (GB 15618–2018), we chose the half of the risk screening value as Cd1 treatment (0.2 mg/kg), the two times of the risk screening value as Cd2 treatment (0.8 mg/kg), and the risk intervention value as Cd3 treatment (2.0 mg/kg). The treatments were designed as follows: (1) only Cd treatments, including 0.2, 0.8, and 2.0 mg/L CdCl\(_2\) solution, regarded as Cd1, Cd2, and Cd3, respectively; (2) Cd + Ca treatments, combined 0.2, 0.8, and 2.0 mg/L CdCl\(_2\) solution with 0.1, 0.25, and 0.5 g/L CaCl\(_2\) solution in pairs, for a total of nine groups, regarded as Cd1Ca1, Cd1Ca2, Cd1Ca3, Cd2Ca1, Cd2Ca2, Cd2Ca3, Cd2Ca4, Cd2Ca5, and Cd2Ca6, respectively; (3) Cd + Mg treatments, combined 0.2, 0.8, and 2.0 mg/L CdCl\(_2\) solution with 0.1, 0.25, and 0.5 g/L MgCl\(_2\) solution in pairs, for a total of 9 groups, regarded as Cd1Mg1, Cd1Mg2, Cd1Mg3, Cd2Mg1, Cd2Mg2, Cd2Mg3, Cd2Mg4, Cd2Mg5, and Cd3Mg3, respectively. The hydroponic experiment was completely randomized with three replicates. The solution was replaced every 3 days during the whole rice cultivation.

**Sample collection and preparation**

The rice samples were collected in the tillering stage (July 30), filling stage (September 14), and mature stage (October 7). The roots were soaked in EDTA (10 mM) solution for 15 min to remove the adhering Cd. The whole plants were washed with tap water first and then washed with deionized water three times. The roots, stems, leaves, panicles, husks, and grains samples were separated independently; these parts were also oven-dried to a constant weight at 75 °C. All parts were weighed, ground into a powder, and passed through a 100-mesh screen before further analysis.

**Chemical analysis**

To determine Ca, Mg, and Cd contents, over-dried (0.1 g) samples were immersed in 4 mL nitric acid and 3 mL hydrofluoric acid per sample and dissolved within a high-pressure reactor at 180 °C for 20 h. After digestion, 1 mL perchloric acid was added to it, and the digestion tank was placed at the electric hot plate to dispel the redundant acid until the white smoke stopped. The transparent liquid was filtered and diluted to 10 mL. Ca, Mg, and Cd contents were analyzed using an inductively coupled plasma optical emission spectrometer (ICP-OES, Thermo Fisher 7400). Certified reference material for rice (GBW10044) provided by National Standard Materials Center was used for quality control; the recovery rates of Cd in the plant samples were 91.3–108.8%.

**Data analysis**

**Translocation factor and bioconcentration factor**

The translocation factor (TF) was used to evaluate the Cd translocation capability of rice plant (Wang et al. 2021a, b;
Yasmin Khan et al. 2017). The TF was defined as shown below:

$$TF_{a-b} = \frac{C_a}{C_b}$$

where $C_a$ and $C_b$ represented the Cd concentrations of part “a” and part “b,” respectively. Totally, the $TF_{\text{root-stem}}$, $TF_{\text{stem-leaf}}$, $TF_{\text{leaf-panicle}}$, $TF_{\text{panicle-husk}}$ and $TF_{\text{husk-grain}}$ were calculated.

The Cd bioconcentration factor (BCF) was chosen to estimate the ability of plants to accumulate Cd and the calculation formula was as below:

$$BCF = \frac{C_{\text{grain}}}{C_{\text{solution}}}$$

where $C_{\text{grain}}$ and $C_{\text{solution}}$ are the Cd concentrations of rice grains and hydroponic solution, respectively (Jin et al. 2016; Yang et al. 2020).

**Statistical analysis**

The statistical relationship among all data were analyzed using one-way analysis of variance (ANOVA), and the least significant difference (LSD) was utilized to determine if significant differences existed between the mean values ($P < 0.05$) with SPSS 19.0 (SPSS Inc., Chicago, IL, USA). All data were expressed as the mean ± SD ($n = 3$). All of the figures were generated utilizing Origin 9.2 (OriginLab Corporation, Northampton, MA, USA), CorelDRAW X8 (Corel Corporation, Ottawa, Canada), Adobe Photoshop 2020 and Adobe Illustrator 2021 software (Adobe Systems Inc., Mountain View, California, USA).

**Results**

**Cd concentrations in rice plant**

The Cd concentrations in different tissues of rice plants was determined as roots > stems > leaves ≈ panicles ≈ husks > grains (Fig. 2). The Cd concentrations in the roots, stems, leaves, panicles, husks, and grains ranged from 0.08 to 2.43, 0.02 to 2.21, 0.04 to 1.55, 0.09 to 0.95, 0.12 to 0.53 and 0.07 to 0.41 mg/kg, respectively, and their mean values were 0.92 ± 0.63, 0.62 ± 0.54, 0.37 ± 0.37, 0.30 ± 0.20, 0.26 ± 0.11, and 0.17 ± 0.09 mg/kg, respectively. The Cd concentrations in the roots were 0.48, 1.48, 2.07, 2.53, and 4.41 times higher than those in the stems, leaves, panicles, husks, and grains, respectively, which indicated the roots had a strong ability to accumulate Cd.

**Effect of Ca and Mg on Cd absorption of rice at different stages**

At the tillering stage, the Cd concentration of all rice tissues decreased with the application of $Ca^{2+}$ and $Mg^{2+}$ ions, except for the roots of the Cd1Ca1 and Cd1Mg1 treatments (Fig. 3a). The Cd1Ca1 and Cd1Mg1 treatments slightly increased Cd concentration in roots, but the effect was not significant. Totally, the Ca treatments decreased Cd concentrations in roots, stems and leaves by 36.30%, 46.02%...
and 57.96%, respectively, and these in Mg treatments were 35.16%, 32.48%, and 47.83%, respectively. We could find that the Cd decrease in Ca treatments was more than that in Mg treatment. It is possible that the Cd ions were aggressively antagonistic to Ca and Mg ions, and the antagonism of Ca was stronger than Mg. Under excessive Cd stress, the addition of Ca and Mg ions reduced the absorption of Cd in rice during the tillering stage, and the inhibiting effects of Ca and Mg ions on Cd were enhanced with the increase of Cd concentrations in the rice plant.

During the filling stage, the Cd concentrations in the roots, stems, panicles, and brown rice ranged from 0.16 to 2.43, 0.02 to 2.21, 0.09 to 0.95, and 0.03 to 0.49 mg/kg, respectively, and their average values were 1.08 ± 0.71, 0.78 ± 0.65, 0.38 ± 0.22, and 0.20 ± 0.14 mg/kg, respectively. The absorption of Cd in the roots, stems, panicles, and brown rice was also affected by Ca and Mg application. Under the applications of Ca1, Ca2, and Ca3 treatments, the Cd concentration of rice tissues decreased significantly as compared to the Ca0 treatment in Cd2 and Cd3 levels. However, in the Cd1 level, the effects of Ca treatment were not significant. These results suggest that exogenous Ca and Mg influence Cd distribution by decreasing the absorption and translocation of Cd to the aerial parts, protecting them from toxicity. The Cd contents in roots, stems, panicles, and brown rice of Ca treatments was lower than those of Mg treatments by 5.18%, 1.94%, 26.36%, and 11.76%, respectively.

At the mature stage, the Cd1Ca1 treatments increased Cd contents in roots, leaves, and husks. Similarly, we found the Cd concentrations of all tissues increased by Cd1Mg1 treatment. This result showed that the slightly addition of Ca and Mg possibly improved rice Cd accumulation within the low Cd stress conditions. Predictively, the Ca2 and Ca3 treatments decreased Cd concentration of rice tissues in various Cd concentration gradients; the Cd contents of the roots, stems, leaves, panicles, husks, and grains were reduced by 3.07–61.70%, 7.70–85.47%, 34.41–86.19%, 23.89–62.68%, –2.32 to 56.36, and 0.80–78.60%, respectively. This phenomenon was also observed in the Mg treatment. Except for the Cd2Ca1, Cd2Ca2, and Cd3Ca1 treatments, the grain Cd concentrations in Ca treatments were lower than those in Mg treatments by 4.25–45.97%, and the average value was 25.41%. It suggests that Ca ions were
effective to reduce grains Cd accumulation. However, in Cd3, Cd3Ca1, Cd3Mg1, and Cd3Mg2 treatments, the Cd concentrations of rice grains reached 0.346, 0.411, 0.303, and 0.214 mg/kg, respectively, which exceed the Chinese maximum permissible concentration for Cd in rice (0.20 mg/kg).

Effect of Ca and Mg on Cd translocation of rice

The translocation factors among tissues in rice plants were significantly differed (Table 1). The mean TFsolution-root value was higher than those of other tissues, suggesting that roots were the main translocation channel of Cd in rice plants with hydroponic culture conditions. A similar trend was found in previous research, Khaliq et al. (2019) found that Cd translocation from soil to root was much higher (>14 times) than other migration processes among rice tissues. Additionally, the TFpanicle-husk (mean value 1.27) was higher than TFhusk-grain (mean value 0.66), indicating that the husk accumulated much Cd and played an important role in hindering Cd transport to the rice grain.

Separately, the TFsolution-root had a similar trend between Ca and Mg treatments, the TFsolution-root was reduced with the increase of Ca and Mg levels, and the TFsolution-root of Mg treatments was slightly lower than that of Ca treatments, but the difference was not significant (Fig. 4a). Both of Ca2+ and Mg2+ ions had significant negative correlations with TFsolution-root (R² = 0.69; p < 0.05 for Ca; R² = 0.73; p < 0.01 for Mg), indicating that exogenous Ca and Mg inhibited the absorption and translocation of Cd from solution to rice roots.

In the Cd1 treatment, the application of Ca and Mg initially induced a decrease in TFroot-stem, then TFroot-stem increased with their contents enhancing from 0.1 g/L to 0.5 g/L. It showed that Ca and Mg promoted Cd translocation from the roots to stems with Cd1 level. However, the change of TFroot-stem in Cd3 was conversed. The TFroot-stem in Cd2 presented a decreasing trend, indicating that Ca2+ and Mg2+ ions reduced the translocation of Cd from roots to stems while Cd concentration reached a threshold value. Still, the exact threshold at which Ca (Mg) aggravated Cd accumulation required further investigation.

There was no clear trend in TFstem-leaf, and the correlation analysis revealed that Ca and Mg had no significant impact on TFstem-leaf. TFleaf-panicle was highly and positively correlated with the Ca concentration (R² = 0.67; p < 0.01), suggesting Ca2+ ions promote Cd translocation from leaf to panicle, which was entirely different to other tissues of rice plants.

| Table 1 | Translocation factor of rice plant in different treatments |
| Cd level | Ca(Mg) Level | TFsolution-root | TFroot-stem | TFstem-leaf | TFleaf-panicle | TFpanicle-husk | TFhusk-grain | BCF |
|---------|--------------|-----------------|-------------|------------|---------------|---------------|--------------|-----|
| Cd1     | Ca0          | 1.38            | 0.83        | 0.78       | 0.75          | 1.22          | 0.83         | 0.68|
|         | Ca1          | 2               | 0.29        | 3.07       | 0.38          | 1.92          | 0.43         | 0.54|
|         | Ca2          | 1.34            | 0.49        | 0.89       | 0.83          | 1.71          | 0.69         | 0.58|
|         | Ca3          | 0.78            | 0.75        | 0.43       | 2.01          | 1.2           | 1.11         | 0.67|
| Cd2     | Ca0          | 1.73            | 0.54        | 0.75       | 0.43          | 0.99          | 0.69         | 0.2 |
|         | Ca1          | 1.4             | 0.62        | 0.44       | 1.38          | 0.84          | 0.71         | 0.31|
|         | Ca2          | 1.03            | 0.31        | 0.87       | 0.53          | 2.1           | 0.52         | 0.16|
|         | Ca3          | 0.71            | 0.19        | 1.3        | 1.19          | 0.99          | 0.51         | 0.11|
| Cd3     | Ca0          | 1.04            | 0.59        | 0.99       | 0.39          | 0.88          | 0.82         | 0.17|
|         | Ca1          | 0.88            | 1.12        | 0.67       | 0.38          | 1.03          | 0.78         | 0.21|
|         | Ca2          | 0.58            | 0.95        | 0.38       | 0.62          | 1.31          | 0.41         | 0.07|
|         | Ca3          | 0.4             | 0.82        | 0.26       | 1.06          | 1.03          | 0.4          | 0.04|
| Cd1     | Mg0          | 1.38            | 0.83        | 0.78       | 0.75          | 1.22          | 0.83         | 0.68|
|         | Mg1          | 1.86            | 0.65        | 0.76       | 1.24          | 1.22          | 0.47         | 0.64|
|         | Mg2          | 1.07            | 0.81        | 0.74       | 1.13          | 1.53          | 0.64         | 0.71|
|         | Mg3          | 0.77            | 0.95        | 0.52       | 1.36          | 1.77          | 0.76         | 0.7 |
| Cd2     | Mg0          | 1.73            | 0.54        | 0.75       | 0.43          | 0.99          | 0.69         | 0.2 |
|         | Mg1          | 1.34            | 0.26        | 0.81       | 0.81          | 1.57          | 0.76         | 0.28|
|         | Mg2          | 0.98            | 0.29        | 0.45       | 1.6           | 1.23          | 0.5          | 0.13|
|         | Mg3          | 0.65            | 0.3         | 0.57       | 1.49          | 0.92          | 1.02         | 0.15|
| Cd3     | Mg0          | 1.04            | 0.59        | 0.99       | 0.39          | 0.88          | 0.82         | 0.17|
|         | Mg1          | 0.8             | 0.73        | 0.71       | 0.83          | 0.64          | 0.69         | 0.15|
|         | Mg2          | 0.61            | 0.53        | 0.86       | 0.35          | 1.79          | 0.6          | 0.11|
|         | Mg3          | 0.38            | 0.48        | 1.04       | 0.28          | 1.79          | 0.73         | 0.07|
The increase of Ca and Mg concentrations from 0 to 0.25 g/L, the \( \text{TF}_{\text{leaf-panicle}} \) and \( \text{TF}_{\text{panicle-husk}} \) had an upward trend, and \( \text{TF}_{\text{husk-grain}} \) had a downward trend, indicating that with the addition of Ca or Mg from 0 to 0.25 g/L, Cd was translocated from leaves to panicles and then to husks; meanwhile, it reduced Cd translocation from husks to grains. Therefore, in this range, the husks play important roles for protecting grains from Cd pollution.

As shown in Fig. 5, both of the BCF in Ca and Mg treatments had the same trend under the Cd1 level, with the increase of Ca and Mg, the BCF rapidly decreased initially and then slowly increased in Ca2, Mg2, Ca3, and Mg3 treatments, respectively. Meanwhile, the range of BCF in Ca treatments was broader than that in Mg treatments, suggesting that \( \text{Ca}^{2+} \) ions had a more significant effect on BCF than \( \text{Mg}^{2+} \) ions. In Cd2 treatments, the BCF increased first and then decreased with the addition of \( \text{Ca}^{2+} \) and \( \text{Mg}^{2+} \) ions, except for the Cd2Mg3 treatment. In Cd3 treatments, the addition of \( \text{Mg}^{2+} \) ions decreased the BCF persistently, Ca treatments showed a trend of increasing first and then decreasing. Within the range of 0.25–0.5 g/L, the decrease of BCF in Ca treatments was more obvious than that in Mg treatments, and the minimum BCF in rice was found in Cd3Ca3 treatment.
The interaction between Cd and Ca (Mg) in rice plant

The Ca, Mg, and Cd concentrations among different parts of rice were significantly and positively correlated at the maturity stage (Fig. 6). Within the comparison of leaves-Cd to grains-Ca, panicles-Ca to grains-Cd, grains-Cd to grains-Ca, stems-Cd to panicles-Mg, and leaves-Cd to panicles-Mg, we could observe Cd was negatively correlated with Ca and Mg ($P < 0.05$), an apparent antagonism was observed between Cd and Ca (Mg). In addition, the Cd concentration in grains was significantly and negatively correlated with Ca content in panicles and grains, with the coefficients being $-0.586$ and $-0.631$ ($P < 0.05$), respectively. We concluded that Ca content in the rice panicle and grain have a blocking effect on Cd accumulation in grain.

Discussion

An increasing number of reports have indicated that Ca and Mg alleviated Cd accumulation in rice by reducing Cd uptake and translocation. The Cd distribution in the rice plant of our study is consistent with previous research (Khaliq et al. 2019). Cd is poorly translocated to aerial tissues and generally accumulated in its roots. The roots are directly contacting with the external environment and may
act as an effective barrier to reduce the uptake and translocation of Cd due to the unique structure of the Casparian strip of the endothelial layer in roots cells (Guo et al. 2021; Wu et al. 2018). Previous studies found that Cd concentrations in rice roots were more than ten times greater than in the aerial tissues, and Cd was mainly localized in the cell walls, indicating that the cell walls of root effectively reduced the translocation of Cd to the aerial organs (Liu et al. 2016; Yu et al. 2020).

In this study, Cd contents in all tissues decreased substantially with Ca, suggesting an interference between Ca\(^{2+}\) and Cd\(^{2+}\) ions. Cd is a nonessential and toxic element for rice plants, and it is believed to use Ca transporters to enter the cells due to the chemical similarity (Tian et al. 2016; Ye et al. 2020). Through the specific translocation channels, Ca\(^{2+}\) ions restrict the uptake and translocation of Cd\(^{2+}\) ions by root and consequently influence the plant Cd contents (Kanu et al. 2019). A previous study found that while the plant was under Cd stress, the Ca concentration in the cytoplasm rose sharply, and the signal was transmitted rapidly among cells, allowing plants to respond quickly to Cd stress (Guo et al. 2018). Ye et al. (2020) found that Cd stress increased the abundance of amino groups, hydroxyl groups, cellulose, epoxide, and reactive oxygen species in rice, inducing structural damage to the plasma membrane and cell wall, but the application of Ca reduced these adverse effects.

Previous study showed that exposure to Cd caused in structural changes in chlorophyll, mainly by replacing divalent Mg\(^{2+}\) ions, resulting in the decomposition of chlorophyll molecules (Kanu et al. 2019). Khalilq et al. (2019) found that Mg contents negatively correlated with Cd contents in upland rice, but no significant correlation was found between Cd and Mg concentration in the leaves. The difference between our studies may be the distinctions of planting patterns and rice cultivars. Reports showed the Cd contents in the shoots and roots of Mg-deficient-rice seedlings were higher than that of the normal growth rice seedlings (Chou et al. 2011). KASHEM and KAWAI (2007) suggested using Mg-containing materials as soil conditioners to improve the imbalance between exchangeable Ca and Mg in the Japanese agricultural soil. The addition of Mg in nutrient solution could promote plants’ growth in a Cd-contaminated environment, reduce the Cd concentration, and detoxify the physiological Cd toxicity in plants.

Previous reports found Ca significantly decreased the concentration of Cd in rice roots, but some researchers suggested the addition of Ca at different concentration gradients significantly increased the translocation of Cd from roots to shoots in rice plants (Ye et al. 2020; Zhang et al. 2020). The present study was different from these, we detected Ca applications that decreased Cd concentrations in both roots and stems, and the TF\(_{\text{root-stem}}\) differed in the three Cd levels. With the Ca and Mg concentration raised from 0 to 0.25 g/L, TF\(_{\text{leaf-panicle}}\) and TF\(_{\text{panicle-husk}}\) increased simultaneously; meanwhile, TF\(_{\text{husk-grain}}\) decreased, and rice grains accumulated a little Cd. These results suggest within the range of 0–0.25 g/L, the addition of Ca or Mg actually enhanced the Cd translocation from leaves to panicles and then to the husks, and simultaneously decreased Cd translocation from husks to grains. Therefore, the husks represented a barrier to prevent Cd from translocating to the grains. Another interesting finding was that when Ca (Mg) concentration reached 0.5 g/L, TF\(_{\text{leaf-panicle}}\) and TF\(_{\text{panicle-husk}}\) increased but TF\(_{\text{panicle-husk}}\) decreased concurrently, suggesting that panicles could accumulate Cd and reduce Cd translocation to husks. Thus, panicles possibly prevent Cd from translocating to edible parts in the high-Ca (Mg) environment. In sum, the husks and panicles played important roles in hindering Cd translocation from the underground parts to grains in Ca (Mg)-deficient and Ca (Mg)-rich conditions, respectively. This result may be the internal physiological regulation of the rice plant, and the mechanism remains to be studied in the future.

Comparing the bioconcentration factors, the addition of Ca could reduce BCF to a greater extent than that of Mg. The Ca1 and Mg1 treatments increased grain Cd concentration by 17.26% and 5.97%, respectively, while Ca2, Ca3, Mg2, and Mg3 reduced grain Cd concentration by 26.78%, 38.99%, 19.08%, and 22.13%, respectively. We found the treatments of low dose promoted the accumulation of grain. This result was shown in previous researches. Zhang et al. (2019) found the application of Ca–Si composite mineral resulted in Cd increased by 1.5–2.1-fold in rice roots. Li et al. (2018) suggested the synergistic accumulation was existed between Ca and Cd could be an important mechanism for Cd tolerance in A. corniculatum roots.

In addition, in both of the high and low concentrations, the impact of Ca on grain Cd content is more profound than Mg, for the greater changes of Cd. Taking all treatments into account, the BCF of Ca treatments was lower than that of Mg treatments by 8.74%. Xue et al. (2019) showed rice plants increased the internal ratio of Ca to Mg to alleviate Cd toxicity; it might establish a new ionic homeostasis in rice plants under Cd stress environment. Thus, in parallel with the same concentration of Ca and Mg, the rice plant absorbed more Ca than Mg to alleviate Cd toxicity. It might be one of the reasons for Ca had the greater effect of decreasing grain Cd accumulation than Mg. The nutritional requirement of plants for Ca was stronger than Mg, and Ca was involved in more metabolism regulation processes and competing with Mg for the transport channels. Through these ways, Ca had a more significant influence on Cd accumulation than Mg. Since the similar surface charge and ionic radius, Ca\(^{2+}\) ions may compete with Cd for transporters and channels (Wang...
et al. 2021a, b; Zhang et al. 2020). Meanwhile, Ca\textsuperscript{2+} ions protect the integrity of the plasma membrane to block the entrance of Cd into plants cells (Ye et al. 2020). Additionally, as a nutrient element, Ca enhances the activities of antioxidant enzymes and improves the rice plant biomass thus diluting the Cd contents (Kanu et al. 2019; Zhang et al. 2020).

**Conclusion**

This study identified that the addition of Ca\textsuperscript{2+} and Mg\textsuperscript{2+} ions limited the accumulation and translocation of Cd in rice plants. Cd concentrations in rice were as follows: roots > stems > leaves ≈ panicles ≈ husks > grains. In all of the growth stage, Ca and Mg decreased Cd accumulation in various tissues of rice. Both of Ca and Mg had an apparent antagonism with Cd in different parts of the rice plant, and the antagonism was more obvious in the high Cd stress treatments. With the addition of 0.1 g/L Ca\textsuperscript{2+} and Mg\textsuperscript{2+} ions, grain Cd contents increased, while the application of 0.25 and 0.5 g/L Ca\textsuperscript{2+} and Mg\textsuperscript{2+} ions reduced grains Cd by 19.08–38.99%, with the average value of 26.75%. Under the same concentrations, the grain Cd content of Ca treatments was lower than that of Mg treatments by 8.74%. In the Ca (Mg)-deficient and Ca (Mg)-sufficient conditions, the husks and panicles accumulated Cd to hinder Cd translocation, respectively.

In future studies, molecular biology, genomics, and other methods should be used further to study the internal physiological regulation of rice plants. The changes of Cd translocation in panicles and husks are also a focal point for research in the future.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s11356-021-17923-3.

**Acknowledgements** We sincerely thank the editors and reviewers for their critical comments and valuable suggestions on the manuscript.

**Author contribution** Xiangying Li: conceptualization, methodology, visualization, software, writing—original draft. Lang Teng: investigation, resources, formal analysis, data curation. Tianling Fu: validation, investigation. Tengbing He: writing, review and editing; supervision; validation. Pan Wu: writing, review and editing; project administration; funding acquisition.

**Funding** This work was supported by the Program Foundation of Institute for Scientific Research of Karst Area (NSFC-GZGOV U1612442); the Basic Condition Platform Construction Project of Guizhou Science and Technology Department [2019]5701.

**Availability of data and materials** All data generated or analyzed during this study are included in this published article and its supplementary information files.

**Declaration**

**Ethics approval and consent to participate** Not applicable.

**Consent for publication** Not applicable.

**Competing interests** The authors declare no competing interests.

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Authors and Affiliations

Xiangying Li1,2 · Lang Teng3,4 · Tianling Fu1,2 · Tengbing He2,3 · Pan Wu1,5

Xiangying Li
xyl7@gzu.edu.cn

Lang Teng
1398151435@qq.com

Tianling Fu
tlfu@gzu.edu.cn

1 College of Resource And Environmental Engineering, Guizhou University, Guiyang 550025, China

2 Institute of New Rural Development, Guizhou University, Guiyang 550025, China

3 College of Agriculture, Guizhou University, Guiyang 550025, China

4 Tongren Agriculture and Rural Affairs Bureau, Tongren 554300, China

5 Key Laboratory of Karst Georesources and Environmental, Ministry of Education, Guizhou University, Guiyang 550025, China