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Effects of root morphology and anatomy on cadmium uptake and translocation in rice (*Oryza sativa* L.)

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ABSTRACT

A clear description of the certain mechanisms of cadmium (Cd) uptake and translocation in rice (*Oryza sativa* L.) may help to reduce Cd accumulation in rice grain. Hydroponic experiments were carried out to determine the effects of cultivation conditions (aerated and stagnant) on the uptake, translocation and subcellular distribution of Cd in relation to the morphology and anatomy of roots in two rice genotypes with different Cd accumulations in grains. Marked differences in morphology and anatomy were observed between these two genotypes under different cultivation conditions. Genotypes with low Cd accumulation in grains tended to develop fewer root tips per root surface area, larger root porosity and more mature apoplastic barriers. The stagnant cultivation condition decreased the number of root tips per root surface area but increased root porosity and accelerated apoplastic barrier formation in root tissues. Correlative Cd uptake studies revealed that rice plants with fewer number of root tips per root surface area reduced root Cd uptake ability, while mature apoplastic barriers increased root Cd retention in cell walls and the symplast. Thus, the fewer number of root tips per root surface area and the earlier formation of mature apoplastic barriers led to lower Cd uptake and translocation. The results indicated that the morphology and anatomy of roots could play important roles in Cd uptake and translocation in rice, and could be influenced by both genotype and cultivation conditions. The present results would be useful in screening and planting rice plants with low Cd accumulation.

Introduction

Agricultural soil contaminated by heavy metals has become a worldwide environmental issue in the past decades. Compared to other heavy metals, cadmium (Cd) has relatively higher mobility in soil and a higher rate of transference from soil to plants (Song et al., 2015); thus, it has attracted more public attention. It has now become clear that rice (*Oryza sativa* L.), a dominant staple food for almost half of the population of the world, especially in developing Asian countries, has become the major source of Cd intake, which is of great concern because of its toxic effects on human tissues (Li et al., 2014, 2017; Rizwan et al., 2016a). Therefore, understanding the mechanisms of Cd uptake, translocation and accumulation in rice is a key step to

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regulating Cd transfer and reducing Cd accumulation in grains. There are three major transport processes in rice plants for grain Cd accumulation: (1) Cd uptake by roots and root-to-shoot translocation by xylem flow, (2) redistribution through intervascular transfer at nodes and (3) remobilization from leaf blades to grains by the phloem stream (Uraguchi and Fujiwara, 2013). It is well known that the root is the first organ to encounter toxicants in the growth substratum, and root Cd uptake and root-to-shoot translocation are believed to be key processes for determining Cd accumulation in rice grains (Liu et al., 2007a; Rodda et al., 2011; Uraguchi and Fujiwara, 2012, 2013).

The root morphology (Huang et al., 2015; Lu et al., 2013; Wang et al., 2016) and anatomy (apoplastic barriers including Casparian bands and suberin lamellae) (Lux et al., 2004; Redjala et al., 2011; Vaculík et al., 2012) may play crucial roles in Cd uptake and translocation in plant tissues. Huang et al. (2015) reported that the cultivar "JFZ" of hot pepper, which had a larger surface area, longer root length and more root tips, had higher Cd uptake. Higher accumulation of Cd in tissues has also been found in the cultivar of peanuts with more fine roots (Lu et al., 2013). Lux et al. (2004) suggested that willow clones characterized by low Cd translocation had suberin lamellae deposited closely to the root tip. The uptake of Cd into the xylem was hindered by accelerated root apoplastic barrier development closer to the root tips of *Salix caprea* isolates (Vaculík et al., 2012). It was also confirmed that metals such as Cd in soybean shoots depended mainly on the properties of roots through grafting experiments (Sugiyama and Arao, 2007). However, the roles of root morphology and anatomy in Cd uptake and translocation in rice plants are still unclear.

The variations of root morphology and anatomy have been attributed to both genotypes (Colmer, 2003; Lu et al., 2013; Lux et al., 2004) and environmental conditions (including drought, flooding, salinity, ion deficiency, silicon application and heavy metal stress) (Akhtar et al., 2016; Enstone et al., 2002; Keller et al., 2015; Lux et al., 2011; Malamy, 2005; Rizwan et al., 2016b). Previous studies have revealed that rice plants grown in waterlogged soils or stagnant solutions possessed larger numbers of adventitious roots, larger root porosity and tighter barriers to radial oxygen loss (ROL) compared to those grown in drained soils or aerated solutions (Colmer, 2003). The exodermal apoplastic barriers of rice matured earlier in a stagnant treatment compared to those in an aerated treatment (Kotula et al., 2009). It has also been observed that different cultivation conditions (hydroponics, aeroponics and soil-grown) led to different root morphological traits and root apoplastic barrier formation in maize and thus altered the uptake of Cd in plant tissues (Redjala et al., 2011). These data imply that cultivation conditions may affect the root morphology and anatomy and thus regulate the Cd uptake and translocation in rice plants.

Available evidences indicate that different rice genotypes show significant variation in Cd uptake (Cheng et al., 2014; Wang et al., 2011), and cultivation conditions such as water regime also affect Cd accumulation in grains (Honma et al., 2016). However, previous studies have mostly focused on metal-transporting transmembrane proteins (Uraguchi and Fujiwara, 2012), ROL (Wang et al., 2011), iron plaque (Cheng et al., 2014), organic acids (Liu et al., 2007b) and variations in the chemical forms of Cd in the rhizosphere (Fulda et al., 2013; Hu et al., 2011); scant information is available on the effects of root morphology and anatomy on Cd uptake and translocation in rice, which may involve in variations in Cd accumulation in rice between genotypes under different cultivation conditions.

In this study, we hypothesized that different Cd uptake and translocation between genotypes might be strongly correlated with their root morphology and anatomy, and Cd uptake and translocation could be regulated by changing these root characteristics under different cultivation conditions. To test these hypotheses, hydroponic experiments were set up to (1) compare the root morphology and anatomy between two rice genotypes under different cultivation conditions (aerated *vs.* stagnant); (2) evaluate the Cd uptake, translocation and subcellular distribution in roots and the kinetics of Cd uptake by rice plants; and (3) assess the relationship between the uptake and translocation of Cd in rice plants and the anatomy and morphology of roots. The results of this study would provide a better understanding of the effects of root morphology and anatomy on Cd accumulation and translocation in rice plants.

1. Materials and methods

1.1. Plant material and pre-cultivation

Two genotypes of rice, Guinongzhan (GNZ) (*indica*) with high Cd accumulation in grains, and Zhonghua11 (ZH11) (*japonica*) with low Cd accumulation in grains, were selected based on our previous field-trials (Li et al., 2012). Seeds were surface-sterilized with 30% (V/V) H_2O_2 solution for 15 min and rinsed with deionized water. The seeds were then germinated in acid-washed quartz sand for 2 weeks. Uniform seedlings of each genotype were selected and transplanted to 6-L pots (eight seedlings per pot) with 25%-strength Hoagland nutrient solution (Hoagland and Arnon, 1938).

Two cultivation conditions were applied 4 weeks after the transplant: (1) Aerated condition: continuously aerated with 25%-strength Hoagland nutrient solution (to simulate an aerobic soil condition). The oxygen concentration in the aerated solution was measured randomly over the 4-day period, and the values ranged from 5.0 to 5.5 mg O_2/L. (2) Stagnant condition: deoxygenated 25%-strength Hoagland nutrient solution containing 0.1% agar (W/V) (to simulate a paddy soil condition) (Wiengweera et al., 1997). The stagnant medium was bubbled with nitrogen gas for 12 hr for deoxygenation before use (Wu et al., 2011). The oxygen concentration in the stagnant solution was measured randomly over the 4-day period, and the values ranged from 0.1 to 0.4 mg O_2/L.

Four replicates (four 6-L pots) were prepared for each genotype under each treatment. All the pots were arranged randomly in a greenhouse with a 16 hr, 28°C/8 hr, 22°C day–night regime. The pH of the solutions was adjusted to 5.8 using 0.1 mol/L KOH. The solutions were renewed every 4 days for a growth period of 4 weeks.

1.2. Growth of rice plants and characterization of root morphology and anatomy

To test the hypothesis that rice plants with different Cd accumulation abilities would differ in root morphology and

anatomy and that different cultivation conditions would lead to different root morphological and anatomical traits as well, we measured the growth characteristics, root morphology and anatomy of rice plants grown in aerated and stagnant nutrient solutions for 4 weeks.

After the 4-week cultivation, rice plants were harvested and washed with distilled water. The plants were divided into roots and shoots, and their growth characteristics, including shoot and root dry weight, shoot length, adventitious root number, porosity and ROL, were measured. The measurements of porosity and ROL were obtained according to Mei et al. (2009).

For the measurement of root morphology, fresh roots were scanned, and the images were analyzed with the WinRhizo® software (Redjala et al., 2011). Total root length, root surface area (RSA), and number of root tips (NRTs) were determined. To measure the average diameter of adventitious roots, lateral roots were removed from adventitious roots and scanned separately.

For the analysis of the development of apoplastic barriers (Casparian bands and suberin lamellae), healthy adventitious roots were cross-sectioned every 1 cm along the root axis from the apex, and proper sections were further cut into 0.5 cm intervals to accurately measure the beginning of barrier formation. To minimize the difference between the root lengths of the two genotypes under different cultivation conditions, roots were chosen for analyses when they reached an average length of 13.0, 10.0, 16.0 and 13.0 cm for ZH11 in the aerated solution, ZH11 in the stagnant solution, GNZ in the aerated solution and GNZ in the stagnant solution, respectively. To detect the development of Casparian bands, sections were stained for 1 hr with 0.1% (W/V) berberine hemisulphate and then for 30 min with 0.5% (W/V) aniline blue (Kotula et al., 2009; Lux et al., 2005). Sections were stained for 1 hr with 0.01% (W/V) Fluorol Yellow 088 to check the development of suberin lamellae (Lux et al., 2005). Sections were viewed and documented with a fluorescence microscope (Zeiss Imager, Z1, Germany) and a digital camera (Nikon, D1, Japan).

1.3. Quantification of Cd in plant tissues and the subcellular distribution of Cd in rice roots

Rice plants pre-grown under aerated or stagnant conditions were rinsed with deionized water and then grown in 25%-strength Hoagland nutrient solution (neither aerated nor stagnant) with or without 1 mg/L Cd ($CdCl_2$). The solutions were renewed every 4 days. The rice plants were harvested after a growth period of 10 days, rinsed with deionized water, divided into shoots and roots, and then oven-dried at 70°C and weighed. The concentrations of Cd in shoots and roots were then determined.

Since subcellular distribution of Cd may play a crucial role in metal tolerance and detoxification in rice, we then investigated the subcellular distribution of Cd in rice roots. Freshly harvested plants were rinsed thoroughly with deionized water. The roots were then soaked in ice-cold 5 mmol/L Na_2-EDTA solution for 10 min to remove Cd adsorbed on the surface of the roots and rinsed thoroughly with deionized water. The apoplastic fluids of the root segments were collected according to the method described by Anil et al. (2005). Approximately 1.0 g of excised roots was cut transversely into 1 cm segments and gently rocked for 1 hr in 10 mL deionized water at room temperature. The Cd content in apoplastic fluids that equilibrated with water was estimated. After collecting the apoplastic fluids, the root segments were frozen in liquid N_2. The symplast and cell wall components of root segments were obtained according to the method described by Fu et al. (2011) with some modifications (Ye et al., 2012). The frozen root segments were powdered in liquid N_2 using a mortar and pestle and then homogenized with 10 mL ice-cold extraction buffer (50 mmol/L HEPES ($C_8H_{18}N_2O_4S$), 1.0 mmol/L DTT ($C_4H_{10}O_2S_2$), 500 mmol/L sucrose, 5.0 mmol/L ascorbic acid, adjusted to pH 7.5 with NaOH). The homogenate was centrifuged at 4000 $\times g$ at 4°C for 15 min. After centrifugation, the supernatant was designated as the symplast fraction and the pellet as the cell wall fraction. The cell wall fractions were then dried at 70°C to constant weight.

1.4. Concentration-dependent kinetics of Cd

There is some evidence that the root Cd uptake rate and affinity to Cd may determine the Cd uptake by the plant. In light of this, we focused our later analyses on the concentration-dependent kinetics of Cd, according to Deng et al. (2010). Rice plants grown under aerated or stagnant conditions for 4 weeks were rinsed with deionized water. Roots were excised at the basal node and incubated in 25%-strength Hoagland nutrient solution (neither aerated nor stagnant) for 30 min at room temperature. The roots were then incubated in a test solution containing 0.5 mmol/L Ca $(NO_3)_2$, 5 mmol/L MES ($C_6H_{13}NO_4S$) (adjusted to pH 5.8 using KOH) and 0, 0.5, 1, 2, 4, 6 or 8 mg Cd/L for 20 min. After incubation, the roots were rinsed in ice-cold washing solution containing 0.5 mmol/L $Ca(NO_3)_2$, 5 mmol/L MES and 1 mmol/L K_2HPO_4 at pH 5.8 for 2 min and then incubated in ice-cold washing solution for 15 min to remove adsorbed Cd from the root free space. The roots were oven-dried at 70°C and weighed.

1.5. Determination of Cd concentrations

For Cd analysis, plant tissues and cell wall fractions of roots were wet digested with HNO_3 at 190°C for 40 min in a microwave oven (MARS-X; CEM, USA). Concentrations of Cd were determined by an Atomic Absorption Spectroscopy (AAS, HITACHI, Japan). A standard reference material (GBW-08303) (China Standard Materials Research Center, Beijing, China) and blanks were used for quality control. The Cd recovery rates ranged from 92% to 101%.

1.6. Statistical analyses

Data were presented as the mean with standard error of four replicates. Statistical analyses were performed with SPSS 19.0 statistical packages and Excel 2016 for Windows. Growth characteristics, root morphological traits, Cd concentrations and uptake kinetic parameters were analyzed by a two-way analysis of variance (ANOVA) to examine the main effects and interactions between genotypes and cultivation conditions. The Cd uptake kinetic curve fitting was done using the Origin 7.0 program, and kinetic parameters were assigned by fitting the data to the Michaelis–Menten function (Deng et al., 2010).

Table 1 – Growth and root morphological characteristics of rice plants cultivated under aerated or stagnant condition for 28 days (mean ± SE, $n = 4$).

Genotype	Condition	Shoot dry weight (mg/plant)	Shoot length (cm)	Root dry weight (mg/plant)	Maximum root length (cm)
GNZ	Aerated	217.50 ± 5.07	38.86 ± 1.33	49.48 ± 2.53	19.45 ± 1.82
	Stagnant	265.73 ± 8.52	48.49 ± 1.02	67.63 ± 4.22	15.75 ± 1.33
ZH11	Aerated	149.27 ± 4.77	33.34 ± 1.45	37.25 ± 2.03	15.97 ± 1.15
	Stagnant	198.25 ± 5.16	40.46 ± 1.29	50.98 ± 3.05	12.92 ± 0.98
Analysis of variance					
Genotype (G)		$p < 0.01$	$p < 0.05$	$p < 0.05$	$p < 0.05$
Condition (C)		$p < 0.01$	$p < 0.05$	$p < 0.05$	$p < 0.05$
G * C		NS	NS	NS	NS

Genotype	Condition	Total root surface area (cm^2)	Root average diameter (mm)	Adventitious root number	Number of root tips/surface area (cm^2)
GNZ	Aerated	51.43 ± 4.17	0.19 ± 0.01	30.20 ± 1.38	134.46 ± 11.36
	Stagnant	86.62 ± 7.35	0.29 ± 0.01	41.70 ± 1.08	95.92 ± 8.18
ZH11	Aerated	43.85 ± 3.97	0.21 ± 0.01	27.11 ± 1.11	90.13 ± 7.62
	Stagnant	70.79 ± 5.48	0.30 ± 0.01	34.71 ± 1.02	62.67 ± 6.05
Analysis of variance					
Genotype (G)		NS	NS	NS	$p < 0.01$
Condition (C)		$p < 0.01$	$p < 0.05$	$p < 0.05$	$p < 0.01$
G * C		NS	NS	NS	NS

Genotype	Condition	Porosity (%)	ROL (μmol O_2/(plant·hr))	Rate of ROL (μmol O_2/(g dry weight·hr))
GNZ	Aerated	17.42 ± 2.42	0.51 ± 0.03	10.31 ± 0.43
	Stagnant	22.37 ± 3.36	1.05 ± 0.05	15.53 ± 0.67
ZH11	Aerated	23.62 ± 3.05	0.59 ± 0.03	15.83 ± 0.76
	Stagnant	29.47 ± 3.78	0.98 ± 0.05	19.22 ± 0.81
Analysis of variance				
Genotype (G)		$p < 0.05$	NS	$p < 0.05$
Condition (C)		$p < 0.05$	$p < 0.05$	$p < 0.05$
G * C		NS	NS	NS

Results were analyzed by a two-way ANOVA. NS: not significant; ROL: radial oxygen loss; GNZ: genotype with high Cd accumulation in grains; ZH11: genotype with low Cd accumulation in grains.

2. Results

2.1. Growth of rice plants and characterization of root morphology

There were significant differences in shoot and root dry weights, shoot height and maximum root length between genotypes ($p < 0.01$), as well as between cultivation conditions ($p < 0.01$) (Table 1). ZH11 showed considerably lower shoot and root dry weights, shoot height and maximum root length than GNZ under both aerated and stagnant cultivation conditions. The aerated cultivation condition resulted in lower shoot and root dry weights and shoot height but larger maximum root lengths in the two genotypes. A significant difference between the genotypes was observed for the rate of ROL but not ROL. ZH11 had a higher rate of ROL than GNZ under both cultivation conditions. The stagnant cultivation condition resulted in significant increases in ROL and the rate of ROL in the two genotypes by an average of 85.9% and 36.0%, respectively (Table 1).

The total RSA, root average diameter and adventitious root number were significantly affected by cultivation conditions ($p < 0.05$) (Table 1). The stagnant condition increased the total RSA, root average diameter and adventitious root number in the two genotypes by an average of 64.9%, 47.7% and 33.1%, respectively, while there were no significant differences in the total RSA, root average diameter and adventitious root number between the two genotypes. The NRTs per RSA was significantly affected by both genotype and cultivation condition (Table 1). ZH11 had the least NRTs per RSA under the stagnant cultivation condition and the greatest increase (43.8%) under the aerated cultivation condition. Significant genotype and cultivation condition effects were observed for root porosity (Table 1; Fig. S1). ZH11 showed considerably larger root porosity than GNZ under both cultivation conditions, and the two genotypes produced similar increases in root porosity in response to the stagnant cultivation condition.

2.2. Characterization of root anatomy

Deposition of apoplastic barriers, including Casparian bands and suberin lamellae, was observed in both the endodermis and exodermis (Figs. 1 and S2). Regardless of genotype and cultivation

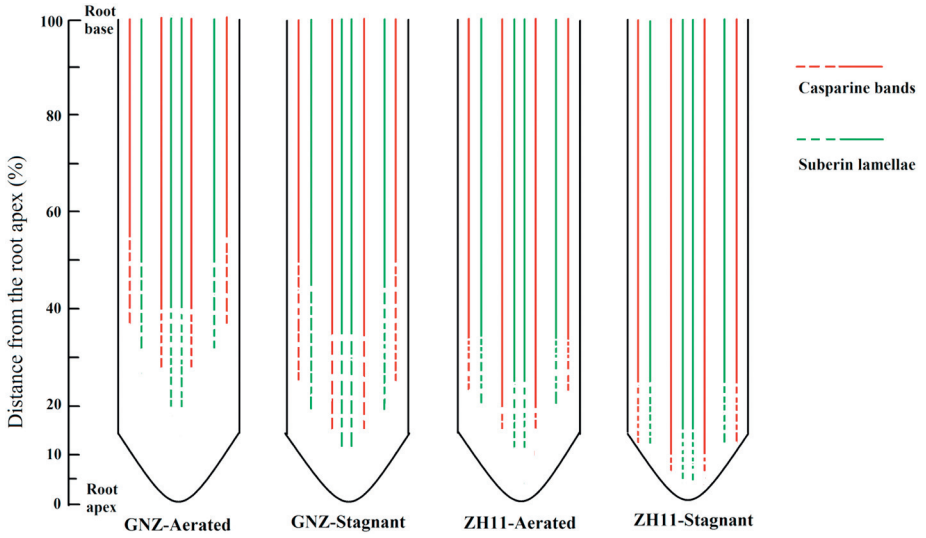

Fig. 1 – Schemes of the development of apoplastic barriers in rice roots cultivated under aerated or stagnant conditions. Casparian bands and suberin lamellae in endo- and exodermis are represented by red and green lines, respectively. Dotted lines indicate the appearance of Casparian bands or suberin lamellae and solid lines present mature Casparian bands or suberin lamellae occupying more than 50% of radial cell wall.

condition, the endodermis matured closer to the root apex than did the exodermis, and Casparian bands started to occur simultaneously with or earlier than suberin lamellae (Fig. 1).

The formations of Casparian bands and suberin lamellae varied between the two genotypes (Figs. 1 and S2). The development of the exo- and endodermis in ZH11 was earlier than that in GNZ under both growth conditions. In ZH11, the zones of the endodermal Casparian band development were between 5.0% and 11.5% from the root apex, while in GNZ, this distance was longer, between 11.5% and 20% from the apex. Similarly, the deposition of endodermal suberin lamellae in roots of ZH11 occurred at 7% and 15.4% from the root apex, while that of GNZ occurred at 15.4% and 28.1%.

Variations in the development of Casparian bands and suberin lamellae between the two cultivation conditions were observed (Figs. 1 and S2). The stagnant condition accelerated the maturity of the exo- and endodermis in both genotypes. When grown under the stagnant condition, exodermal Casparian bands were formed at 13.0% and 25.0% from the root apex of ZH11 and GNZ, respectively, but the respective values under the aerated condition were 23.1% and 37.5%. Similarly, the stagnant condition shifted the deposition of exodermal suberin lamellae more closely to the root apex, with a genotypic range of 7.0%–11.9%.

2.3. Accumulation and translocation of Cd in rice plants

Accumulation and translocation of Cd in rice plants were significantly affected by the genotype and pre-cultivation condition (Table 2). ZH11 had significantly lower tissue Cd concentrations and translocation percentage factors than GNZ. The mean values of Cd concentrations were 284 and 421 mg/kg for roots and were 41 and 75 mg/kg for shoots in ZH11 and GNZ, respectively. The stagnant pre-cultivation condition reduced the concentration of Cd in roots, the concentration of Cd in the shoots and the translocation of Cd from roots to shoots in the two genotypes by an average of 22.5%, 38.0% and 3.4%, respectively. The interaction factors between genotypes and pre-cultivation conditions were nonsignificant for all parameters, indicating that the effect of pre-cultivation conditions on Cd uptake in both genotypes was the same, and the Cd response to the pre-

Table 2 – Cd concentrations and translocation in rice plants pre-cultivated under aerated or stagnant condition for 28 days before exposed to 1.0 mg/L Cd for 10 days (mean ± SE, n = 4).

Genotype	Condition	Root (mg/kg)	Shoot (mg/kg)	Translocation factor (%)
GNZ	Aerated	463.14 ± 21.65	89.24 ± 4.97	19.27 ± 1.07
	Stagnant	379.40 ± 16.77	59.71 ± 3.18	15.59 ± 0.86
ZH11	Aerated	328.09 ± 14.85	52.07 ± 3.01	15.87 ± 0.79
	Stagnant	240.98 ± 12.11	30.58 ± 1.60	12.69 ± 0.62
Analysis of variance				
Genotype (G)		$p < 0.01$	$p < 0.01$	$p < 0.05$
Treatment (T)		$p < 0.01$	$p < 0.01$	$p < 0.05$
G * T		NS	NS	NS

Translocation factor (%) = (Cd concentration in shoot / Cd concentration in root) × 100%.
Results were analyzed by a two-way ANOVA. NS: not significant.

cultivation conditions was also comparable between the two genotypes.

2.4. Subcellular distribution of Cd in roots

The results of fractionation showed that most of the Cd was present in symplast and cell wall-containing fractions and that only a minor part of Cd was stored in the apoplastic sap (2.6%–5.1%) (Table 3). Concentrations of Cd in the different subcellular fractions were significantly affected by genotype and pre-cultivation condition (Fig. 2). GNZ had significantly higher concentrations of Cd than ZH11 in all of the different subcellular fractions. The stagnant pre-cultivation condition significantly reduced the concentrations of Cd in the apoplastic sap and symplast of the Cd-treated roots in the two genotypes. Marked differences between pre-cultivation conditions were observed for the proportions of Cd in the apoplastic sap and symplast. The stagnant pre-cultivation condition reduced the proportions of Cd in the apoplastic sap and symplast in the two genotypes by an average of 2.1% and 5.8%, respectively. However, there was no significant difference in the proportion of Cd in different subcellular fractions between the two genotypes.

2.5. Concentration-dependent kinetics of Cd

Concentration-dependent uptake kinetics for Cd influx in the roots of plants pre-cultivated in either the stagnant or aerated conditions showed similar patterns, which could be characterized by hyperbolic curves (Fig. 3). The cadmium influx in roots pre-grown under the aerated condition became saturated at high Cd concentrations, and the uptake curves could be fitted by the Michaelis–Menten function (R^2 = 0.99 for GNZ, R^2 = 0.98 for ZH11). On the other hand, the Cd influx of roots excised from plants pre-grown under the stagnant condition was not saturated even at the highest Cd concentration (8 mg/L), and the uptake curves could be resolved into Michaelis–Menten saturable and linear components (R^2 = 0.95 for GNZ, R^2 = 0.96 for ZH11).

The V_{max} values of GNZ were significantly higher than those of ZH11 under both pre-cultivation conditions, indicating that GNZ had a higher Cd uptake rate. There was no significant difference in K_m values between the two genotypes, suggesting that the affinity of the Cd carrier in roots was comparable between two genotypes (Table 4). The stagnant pre-cultivation condition significantly reduced the V_{max} values by an average of 33.1 mg/(kg dry weight·hr) but increased the K_m values by an average of 0.81 mg/kg. For the plants pre-cultivated under the stagnant condition, the slopes of the linear component of the model were (2.39 ± 0.33) and (1.49 ± 0.41) mg/(kg dry weight·hr) for GNZ and ZH11, respectively (data not shown), indicating that apoplastic Cd binding in the stagnant treatment was higher than that in the aerated treatment.

3. Discussion

3.1. Effects of genotype and cultivation condition on root morphology and anatomy

The development of root morphology and anatomy in rice plants is genotype specific and regulated by relative gene networks (Rebouillat et al., 2009). In the present study, ZH11 (the genotype with low Cd accumulation in grains) had smaller total RSA, maximum root length, and NRTs per RSA, but larger porosity than GNZ (the genotype with high Cd accumulation in grains) (Table 1). Differences in root morphological traits between genotypes that differ in Cd accumulation have also been observed in peanut (Lu et al., 2013), hot pepper (Huang et al., 2015), soybean (Wang et al., 2016) and rice (He et al., 2007). Previous studies showed that willow isolates (Lux et al., 2004; Vaculík et al., 2012) and rice genotypes (Wang et al., 2015), which differed in metal (*e.g.*, Cd and Hg) accumulation abilities, varied in the formations of Casparian bands and suberin lamellae. However, the description of the formation of Casparian bands and suberin lamellae in the exo- and endodermis between rice genotypes differing in Cd accumulation in grains has never been reported. The present data showed that ZH11 developed more mature apoplastic barriers than GNZ (Figs. 1 and S2). Thus, it could be speculated that the differences in Cd accumulation in grains between the two rice genotypes were attributed to the differences in the root morphology and anatomy.

The development of root morphology and anatomy in rice plants is also determined by external environmental factors (Rebouillat et al., 2009). Our data showed that the stagnant cultivation condition invoked significant increases in the total RSA and porosity but decreases in the maximum root length and NRTs per RSA of both genotypes (Table 1 and Fig. S1). Moreover, the stagnant cultivation condition markedly accelerated the formation of Casparian bands and suberin lamellae in the exo- and endodermis in both genotypes (Figs. 1 and S2). Similar effects of stagnant cultivation condition on root morphological traits (Deng et al., 2010) and anatomical traits (Kotula et al., 2009) have previously been observed. Under the stagnant cultivation condition, the characteristics of root morphology and anatomy were similar to those of the genotype with low Cd accumulation in grains; while under

Table 3 – Distribution ratio of Cd in different compartmentations of roots in rice plants pre-cultivated under aerated or stagnant condition for 28 days before exposed to 1.0 mg/L Cd for 10 days.

Genotype	Condition	Apoplastic sap (%)	Cell wall (%)	Apoplast (%)	Symplast (%)
GNZ	Aerated	5.12	50.45	55.57	44.43
	Stagnant	2.97	56.59	59.56	40.44
ZH11	Aerated	4.64	53.04	57.68	42.32
	Stagnant	2.58	62.69	65.27	34.73

Data in the table are presented as mean (n = 4). Apoplast (%) = Apoplastic sap (%) + Cell wall (%).

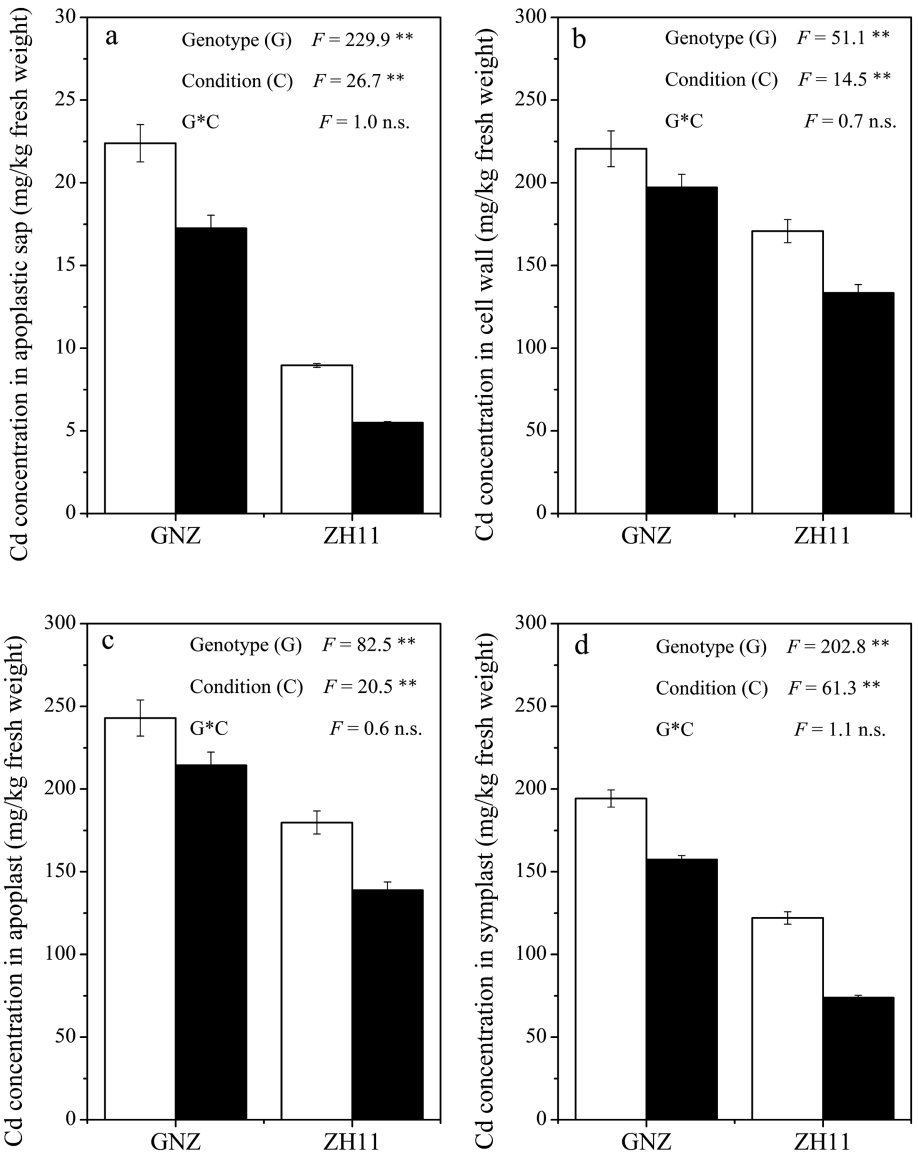

Fig. 2 – Distribution of cadmium in different compartments of roots in rice plants pre-cultivated under aerated (open column) or stagnant (close column) condition for 28 days before exposed to 1.0 mg/L Cd for 10 days. Cadmium concentration in apoplastic sap (a), cell wall (b), apoplast (c) and symplast (d), of which Cd concentration in apoplast is equal to the sum of Cd concentration in apoplastic sap and in cell wall. Data are presented as mean ± Standard Error (SE) (n = 4). n.s.: not significant, $^{**}p < 0.01$.

the aerated cultivation condition, the characteristics of root morphology and anatomy were similar to those of the genotype with high Cd accumulation in grains (Table 1 and Fig. 1). Furthermore, rice plants pre-cultivated in the stagnant condition showed lower Cd uptake and translocation than those in the aerated condition (Table 2). These results suggested that Cd uptake and translocation could be regulated by changing the root characteristics under different cultivation conditions.

3.2. Effects of root morphology and anatomy on Cd uptake and translocation in rice plants

Changes in Cd uptake and translocation in rice plants were related to their root morphology. A number of root morphological traits have been shown to be important for Cd uptake and translocation in crops, including specific root length, root diameter, RSA, NRTs, fine roots and root porosity (Huang et al., 2015; Lu et al., 2013; Wang et al., 2011, 2016). Our correlation analysis revealed that rice plants with fewer NRTs per RSA but larger root porosity had lower Cd uptake and translocation (Table 5), and suggested the NRTs per RSA played a key role in Cd uptake and translocation in rice plants. The NRT per RSA may affect the effective area for Cd uptake (Redjala et al., 2011) and determine Cd uptake ability of rice plants in this study, as previous researches showed that cation uptake is most efficient near the root apex (Boominathan and Doran, 2003; Piñeros et al., 1998). A similar observation was also found in barley that Cd uptake in wild-type barley was positively related to root hair density (Zheng et al., 2011). The present rice

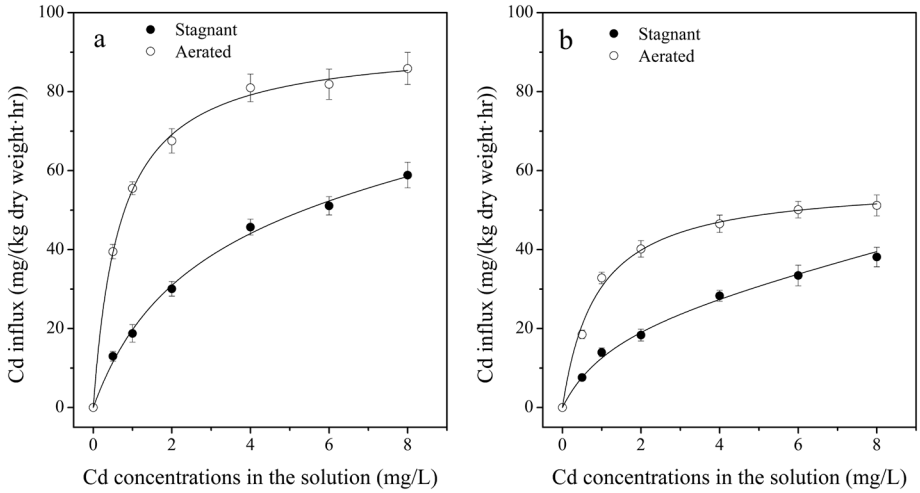

Fig. 3 – Concentration-dependent kinetics for Cd uptake by excised roots of rice plants pre-cultivated under aerated (open circle) or stagnant (close circle) condition before exposed to Cd. (a) GNZ, genotype with high Cd accumulation in grains; (b) ZH11, genotype with low Cd accumulation in grains. Bars, ±Standard Error (SE) (n = 4).

plants with higher NRT per RSA possessed a higher maximum Cd uptake rate (V_{max}) (Tables 1 and 4) and higher Cd concentrations in roots (Table 2). It has also been reported that cations can move into the xylem through the apoplastic pathway only in the apical zone of roots and where the lateral roots emerge (Moore et al., 2002; White, 2001). This explains why the concentration of Cd in shoots and the Cd translocation factor were lower in rice plants with smaller NRTs per RSA in the present study. Root porosity is an important feature for rice plants to resist metal(loid) stress and impedes toxic element accumulation (Mei et al., 2009; Wang et al., 2011). In this study, rice plants with larger porosity tended to have a higher rate of ROL and reduced Cd uptake and translocation (Tables 1 and 5). Previous studies reported that the release of oxygen from roots to the rhizosphere can prevent metal(loid)s from entering into plants by creating a protective oxidative zone around the growing roots (Mei et al., 2009; Wang et al., 2011).

RSA has been reported to affect root Cd uptake in hot pepper (Huang et al., 2015). However, RSA was not a key factor in Cd uptake in the present study, as RSA did not have any significant relationships with Cd accumulation or translocation (Table 5). The two genotypes, which differed in Cd accumulation, did not have any significant difference in their RSA ($p > 0.05$), and the rice plants pre-cultivated under the stagnant condition, which resulted in a larger RSA, had lower Cd uptake and a lower translocation factor (Tables 1 and 2). In contrast, a negative correlation was observed between RSA and root Cd concentrations in the soybean under Cd treatments (Wang et al., 2016). Although the flux of Cd to roots could occur along the whole length of the root, the uptake efficiency differs in different positions (Piñeros et al., 1998). This explains the contradictory results for the nonsignificant effect of RSA on Cd uptake and reconfirms the crucial role of NRTs per RSA in Cd uptake by rice plants.

Changes in Cd uptake and translocation in rice plants were also related to their root anatomy. Qualitative analysis indicated that the genotype ZH11 (with low Cd accumulation in grains) or rice plants pre-cultivated under the stagnant condition, which had earlier maturation of apoplastic barriers, had significantly lower Cd uptake and translocation than the genotype GNZ (with high Cd accumulation in grains) or the rice plants pre-cultivated under the aerated condition (Fig. 1 and Table 2). To the best of our knowledge, the effects of root anatomy on the Cd uptake in rice plants have never been reported. Plants with earlier maturation

Table 4 – Kinetic parameters of Michaelis–Menten function for Cd influx into rice roots pre-cultivated under aerated or stagnant condition before exposed to Cd (mean ± SE, n = 4).

Genotype	Condition	V_{max} (mg/(kg dry weight·hr))	K_m (mg/kg)	R^2
GNZ	Aerated	92.60 ± 5.92	0.68 ± 0.07	0.99
	Stagnant	59.93 ± 6.20	1.71 ± 0.61	0.95
ZH11	Aerated	57.20 ± 3.81	0.87 ± 0.09	0.98
	Stagnant	23.77 ± 3.48	1.45 ± 0.31	0.96
Analysis of variance				
Genotype (G)		$p < 0.05$	NS	
Treatment (T)		$p < 0.01$	$p < 0.05$	
G * T		NS	NS	

Results were analyzed by a two-way ANOVA. NS: not significant.
V_{max}: maximum uptake rate; K_m: Michaelis constant, represent affinity of the carrier, the higher K_m, the lower affinity to Cd.

Table 5 – Pearson correlations between root characteristics, Cd concentrations in roots and shoots, and translocation factor in rice plants (n = 4).

	Cd concentration (mg/kg)		Translocation factor (%)
	Root	Shoot	
Total root surface area (cm^2)	−0.26	−0.24	−0.25
Root average diameter (mm)	−0.57	−0.55	−0.56
Number of root tips/surface area (cm^2)	0.80**	0.81**	0.83**
Porosity (%)	−0.68**	−0.59*	−0.63**

** and * mean the correlations are significant at the 0.01 and 0.05 level (2-tailed), respectively.

of apoplastic barriers in roots could reduce Cd accumulation which was observed in maize (Redjala et al., 2011) and willow (Vaculík et al., 2012). Anatomical traits affect Cd uptake and translocation primarily *via* their effects on the hindrance of apoplasmic movement of Cd to the xylem (Lux et al., 2011). The formation of apoplastic barriers in the exo- and endodermis were reported as effective barriers to prevent the movement of heavy metals into the stele, thus influencing Cd accumulation and translocation (Baxter et al., 2009; Schreiber, 2010). Apoplastic barriers are characterized by hydrophobicity, and their formation would slow down the apoplastic flux from the epidermis to the xylem (Redjala et al., 2011). Besides, the suberization of cell walls can offer more binding sites to Cd (Nishizono et al., 1987). These explain why rice plants with earlier maturation of apoplastic barriers possessed lower concentrations of Cd in the apoplastic sap, higher retention of Cd in cell walls (Figs. 1 and 2), and lower root–shoot translocation of Cd (Table 2). It has also been reported that the mature apoplastic barriers could reduce the volume of the apoplast and the effective absorption area of plasmalemma (Waduwara, 2007), leading to lower uptake of Cd. The present kinetic results showed that the rice plants with earlier formation of the apoplastic barriers had a lower maximum Cd uptake rate (V_{max}) and a lower affinity to Cd (K_m) (Fig. 1 and Table 4) and thus reduced the Cd accumulation.

The formation of apoplastic barriers can restrict the ROL effectively (Kotula et al., 2009), and rice plants with higher rates of ROL tend to accumulate less metal(loid)s in plant tissues (Mei et al., 2009; Wang et al., 2011; Wu et al., 2012). Our data showed that rice plants with mature apoplastic barriers had higher rates of ROL, which negatively correlated with Cd accumulation and translocation (data not shown). These results suggested that the apoplastic barriers could indirectly affect the uptake and translocation of Cd in rice plants though regulating the ROL. However, the contribution and significance of root anatomy are required to be further identified.

4. Conclusions

The present results showed that genotype with low Cd accumulation in grains had lower Cd uptake and translocation due to the facts that fewer NRTs per RSA and more mature apoplastic barriers reduced the entry of Cd into the roots as well as the apoplastic flux into xylem. These results supported the initial hypothesis that different Cd uptake and translocation between genotypes were strongly correlated with their root morphology and anatomy. Our experiments also confirmed the second hypothesis that different cultivation conditions could regulate Cd uptake and translocation by changing these root characteristics. The rice plants pre-grown under stagnant condition had significantly lower Cd uptake and translocation than those pre-grown under aerated condition. And these differences were due to the facts that the stagnant cultivation condition decreased the NRTs per RSA but increased the root porosity and accelerated the apoplastic barrier formation in root tissues. The present study provided a better understanding of the significance of root features on Cd uptake, and such information would be useful in screening genotypes with low Cd accumulation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jes.2018.04.005.

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