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Effects of Cd^{2+} ions on root anatomical structure of four rice genotypes

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Abstract

Root anatomical structures of four rice breeding materials (maintainer linesYixiang B and E2B, restorer lines R892 and Mianhui725), grown under different Cd^{2+} levels, were observed and the root resistance to Cd^{2+} ions was evaluated. Under low Cd stress, the new roots appeared in the cortex of four rice genotypes. The diameter of the new root in YixiangB was larger than that of E2B. The restorer line R892 generated more roots than Mianhui725. Under high Cd^{2+} stress, broken epidermis, damaged cortex and black spots appeared in both maintainer and restorerlines. In general, anatomical damages in the restorer lines (R892 and Mianhui725) were slighter than those of the maintainer lines (YixiangB and E2B). Thus, the restorer lines had more adaptive ability to Cd^{2+} stress than maintainer lines.

Key words

Accumulation type, Anatomical structure, Cadmium stress, Maintainer/restorer line rice

Introduction

Contamination of soil with cadmium (Cd) resulting from application of sewage sludge and fertilizer, emissions from mining, smelting, and other industrial activities is widespread (Yang *et al.*, 2008; Howard *et al.*, 2011). Cd is one of the most toxic heavy metal in the environment due to its high mobility and severe toxicity to the organisms (Su *et al.*, 2005).Although considered to be a non-essential element for metabolic processes, it is easily absorbed by plants and even causes toxicity symptoms in small amounts (Kuriakose *et al.*, 2008). Root is one of the vital organ of plants, which plays a very important role in the development, physiological function and substance metabolism. It can be sensitively affected by the adverse circumstance and therefore exhibits a series of corresponding reactions to environmental stresses, morphologically and physiologically (Chen *et al.*, 1995; Li *et al.*, 2009; Zheng *et al.*, 2010). Many studies have suggested that under adverse situation, the morphological structure of plants changes and the epidermal, cortex parenchymatous, exodermis, vascular cynlinder, sclerenchyma, casparian strip, and the xylem pipes are also affected in some plants (Voigt *et al.*, 2006; Probst *et al.*, 2009), such as *Thlaspicaerulescens* (Wójcik *et al.*, 2005), some wetland plants (Deng *et al.*, 2009), oregano (Panou-Filotheou *et al.*, 2004), grass (McDonald *et al.*, 2002), pea (Rodríguez-Serrano *et al.*, 2006), potato (Reid *et al.*, 2003) and so on.

Cd is more easily absorbed by plants than other heavy metals and more than 90% of the Cd is accumulated in the root of the plants (Liu *et al.*, 2003). However, so far, the investigations of plant roots under Cd stress were mainly focused on the morphological traits, such as root length, root diameter, root surface, root volume and other rhizosphere effects, such as arbuscular mycorrhizal fungus colonization (Li *et al.*, 2009; Su *et al.*, 2005). However, little information is known about the internal structural, particularly of rice roots under the Cd stress, such as changes of epidermis, cortex, vascular cylinder and vessel. The structural investigations could provide more insights into mechanism of degradation and resistance to Cd absorbed in plants.

In the present study, four rice breeding materials were selected to evaluate the root resistance capacities to three levels of Cd^{2+} ions.The results of this study firstly characterize the root anatomical changes of rice roots under Cd stress and provide some useful information for selecting rice materials grown in a Cd-contaminated circumstance.

Materials and Methods

Plant materials and Plant culture : A hydroponic culture experiment was carried out from 10th June to 20th July, 2010 in Sichuan Agricultural University, Ya'an Campus, Sichuan, China. The experiment was conducted in greenhouse with 14 hr daily photoperiod of 400 μmol $m^{-2}.s^{-1}$, day/night temperature of 25~28ºC /18~20ºC and 70% relative humidity. Four rice genotypes Yixiang B, E2B (maintainer lines), R892, Mianhui 725 (restorer lines) were sterilized with 0.1% $HgCl_2$ solution for 1 min and then thoroughly washed with distilled water. Seeds were soaked in distilled water for 36 hr (30 ºC) and then sown on a nylon net supplied with a half strength of standard rice nutrient solution. The composition of standard rice nutrient solution used for plant culture was as follows: NH_4NO_3 (1.43mM), $NaH_2PO_4 \cdot 2H_2O$ (0.323 mM), K_2SO_4 (0.513 mM), $CaCl_2$ (0.998 mM) and $MgSO_4 \cdot 7H_2O$(1.65mM) and the micronutrients $MnCl_2 \cdot 4H_2O$ (7.58mM), $(NH_4)_6Mo_7O_{24}$ (0.0598 mM), H_3BO_3 (15.1 mM), $ZnSO_4 \cdot 7H_2O$ (0.121 mM), $CuSO_4.5H_2O$(0.124 mM), $FeCl_3 6H_2O$ (0.284 mM) and citric acid (61.9 mM). At two-leaf stage, eight uniform seedlings of each rice genotype were selected and transplanted into 20 l vessels containing standard rice nutrient solution. After 7days of growth, different levels of Cd treatments were given: Control, low (0.5 mg l^{-1}) and high (3.0 mg l^{-1}). Cd was supplied as $CdCl_2.5H_2O$, and each treatment was applied for 4 replicates. The pH of nutrient solution is very important for Cd-stress-studies and was adjusted to 5.5-6.0 by adding 1M HCl or NaOH daily and the nutrient solution was renewed every 7 days. Four plants were used for observation of root anatomical structure.

Analysis methods : At booting stage, roots from four plants for each line were taken and fresh adventitious roots of 8 cm length were selected. Permanent flakes of the roots were made after the process of fixing, flushing, softening, dyeing, dehydration, transparency, waxdip, embedding, slicing, sticking, spreading, baking, dewaxing, redyeing, rehydration, and mounting(Li and Zhang, 1983). The slides were visualized with a light microscope of BX51 (Olympus, Japan), equipped with software Motic Images Advanced3.0 and the patterns were taken by CCD camera.

The whole plants were sampled from hydroponic culture. Roots were immerged in a solution containing 1.0 mM ethylene-diamine-tetra acetic acid (EDTA) for 2 hr, and then rinsed twice with de-ionized water. Plants were oven-dried at 100 ºC for 1 hr and at 60 ºC for 48 h, weighed and then grounded into fine powder and digested in concentrated $HNO_3$with a microwave digestion system (ETHOS-320, Milestome, Italy). Cd content was determined using a flame atomic absorption spectrophotometer (MK II M6, Thermo, USA) and expressed on the basis of dry weight.

Statistical analysis : A two-way analysis of variance (ANOVA) was applied to the data. Statistical analysis was performed by SPSS 13.0. Differences between means were evaluated for significance by using Duncan's multiple range test (LSD) ($P < 0.05$).

Results and Discussion

A remarkable difference in plant growth and Cd accumulation between restorer lines and maintainer lines was observed. The plant growth was not inhibited by low level of Cd (0.5mg l^{-1}) as compared with control, while high Cd concentration (3.0 mg l^{-1}) caused significant reduction in all examined growth parameters of both genotypes, such as plant height and root length. Shoot dry weights of E2B and Yixiang B were not affected in the low Cd treatment, however when treated with 3.0 Cd mg l^{-1}, it decreased by 33% and 35%, respectively. The dry weight of roots was reduced in E2B at the high Cd concentrationbut significantly promoted in YixiangB at low Cd concentration (Table1).Similar results were acquired for the restorer lines of Mianhui725 and R892. In the line of R892, the dry weight of shoots was significantly increased at the low Cd concentration but not for its roots (Table 2). The results also revealed that roots of Cd-treated plants accumulated large amount of Cd and with increased Cd concentration, the Cd content raised in roots (Tables1and 2).

As compared to control, new roots from root cortex were induced by low Cd treatment in both Yixiang B (Fig. 1b) and E2B lines (Fig. 1e).The newly-emergenced roots in YixiangB showed greater diameters than those of E2B grown under low Cd condition (Fig. 1b,e). Most of the epidermise broke off and the cortex was found empty. Also, the cankered vascular cylinder was observed. As compared with E2B, YixiangB maintained relatively intact structure of epidermis and well radial arrangement structure under low Cd treatment (Fig. 1b,e).

Under high Cd treatment, root structures of Yixiang B and E2B were observed to be less integrity than the plants grown under low Cd level (Fig. 1). Both cortex and epidermis of Yixiang B broke due to high Cd treatment. More black spots in the cortex of the pericycle of E2B were observed than Yixiang B when treated with high Cd (Fig.1c, e, f). Moreover, more xylem pipes appeared in E2B as compared with Yixiang B under high Cd treatment. New roots were observed in cortex of both R892 and Mianhui725 under low Cd treatment (Fig. 2b), but the number in R892 was greater than Mianhui 725 (Fig. 2b, e). In low Cd stressed plants, epidermis and cortex cells broke off, and incrassate cell wall of exodermis and two big pneumatophores in the cortex, faint vascular cylinder and two wrinkled xylem pipes were observed (Fig. 2). Although black spots were observed in R892, it maintained better structure in epidermis, cortex, vascular cylinder and xylem pipe than that of Mianhui 725.

When compared with control plants, both R892 and Mianhui 725 exhibited significant structure changes under high Cd treatment (Fig. 2c, f). New roots were induced in the joint

Table 1 : Biomass of plant and Cd accumulation of the maintainer lines

Breeding materials	Cd level (mg l^{-1})	Dry weight (g $plant^{-1}$)		Cd concentration (mg kg^{-1})	
		Shoot	Root	Shoot	Root
E2B	0	1.39±0.19^{a}	0.48±0.08^{ab}	0.01±0.00^{e}	0.02±0.00^{e}
	0.5	1.45±0.17^{a}	0.53±0.08^{a}	1.09±0.05^{d}	4.15±0.04^{d}
	3	0.93±0.04^{b}	0.37±0.04^{cd}	1.23±0.10^{c}	8.13±0.11^{b}
YixiangB	0	1.42±0.22^{a}	0.40±0.03^{bc}	0.02±0.00^{e}	0.02±0.00^{e}
	0.5	1.47±0.11^{a}	0.53±0.06^{a}	1.52±0.10^{b}	5.72±0.15^{c}
	3	0.92±0.06^{b}	0.41±0.05^{bc}	3.61±0.09^{a}	12.97±0.07^{a}

Data are means ± standard deviations of four replicates. Values followed by the same letters were not significantly different ($P < 0.05$) as etermined by LSD

Table 2 : Biomass of plant and Cd accumulation of the restorer lines

Breeding materials	Cd level (mg l^{-1})	Dry weight (g $plant^{-1}$)		Cd concentration (mg kg^{-1})	
		Shoot	Root	Shoot	Root
Mianhui725	0	1.70±0.04^{b}	0.52±0.07^{ab}	0.01±0.00^{d}	0.03±0.00^{e}
	0.5	1.77±0.07^{b}	0.61±0.08^{a}	1.03±0.02^{c}	2.54±0.13^{d}
	3	1.10±0.07^{c}	0.39±0.03^{b}	1.40±0.11^{b}	7.49±0.05^{b}
R892	0	1.73±0.14^{b}	0.61±0.17^{a}	0.02±0.00^{d}	0.04±0.01^{e}
	0.5	2.06±0.27^{a}	0.61±0.08^{a}	1.28±0.06^{b}	5.25±0.12^{c}
	3	1.22±0.07^{c}	0.48±0.04^{ab}	3.76±0.14^{a}	12.03±0.21^{a}

Datameans ± standard deviations of four replicates. Values followed by the same letters were not significantly different ($P < 0.05$) as determined by LSD

between exodermis and cortex of both R892 and Mianhui 725 under high Cd treatment (Fig. 2c, f). In line of Mianhui 725, most cells in epidermis and a few cells in exoderm broke off and spaces in the cortex vascular cylinder was observed. For R892, more intact structure was observed.

Under heavy metal stress, such as Cu, Zn, Pb, Ni, Cr and Cd, the structure and related physiological function of the roots occurred to change accordingly. In general, they displayed adaptive responses to the circumstance stress by changing their root external morphological traits and distributions (Li *et al*., 2009; Voigt *et al*., 2006). Under low stress, is no external change were noted but internal changes in plants. While under severe stress, it expressed significant responses in both external and internal morphological changes. Excessive stress beyond the limit of endurance of plant would result in cell death (Yu *et al*., 2009). In this study, 0.5 mg l^{-1} Cd, changed internal structures of the root were as compared with control. For example, few new roots appeared to be in cortex and the root turned to be deformed, however, the root was still growing. It is an interesting phenomenon that low Cd stress could accelerate the root growth. The appearance of new root was likely a new way to adapt to Cd toxicity, which increased the ability of nutrition uptake and transport (Cai *et al*., 2010). In fact, toxic symptoms existed in roots under low Cd stress. The result of the present study was different from previous reports in which low Cd treatment accelerated root growth in short term (Zhao *et al*., 2006). Under high Cd treatment, new roots were also observed in restorer lines. But the new roots with smaller diameter, which were close to epidermis, dyed lightly and that indicated they had began to wither. This indicated that its self-adjusting capacity was weakened and Cd toxicity in roots increased under high Cd stress. In maintainer lines under high Cd stress, no new roots and black spots appeared in cortex and pericycle, in which Cd accumulated, suggesting that the growth of root was greatly inhibited. No new roots developed or broke off, which significantly affected the absorption of water and oxygen. Accordingly, the development and growth of the plant was inhibited due to high Cd level (Zhao *et al*., 2006; Rascio *et al*., 2008). A similar conclusion was drawn by Gratão *et al*. (2009).

In previous studies, it was suggested that plants themselves possess homeostatic mechanisms to minimize damage from heavy metal toxicity. Metal compartmentation has been investigated and studies so far suggest that most of the heavy metals accumulate in epidermis, followed by cortex and pericycle (Molina *et al*., 2008). In white lupins majority of Cd accumulated in the root epidermis and cortex which had strong absorption capacity under Cd stress (Vazquez *et al*., 2007). Transfer of heavy metal from roots to shoots was observed in epidermis followed by, cortex, endoderm, vascular cylinder and xylem (Reid *et al*., 2003). The effect of external substances on roots could be estimated by the influenced part of the root. If the prominent changes were restricted to the root surface, the influence on root was relatively slight. Whereas, the prominent

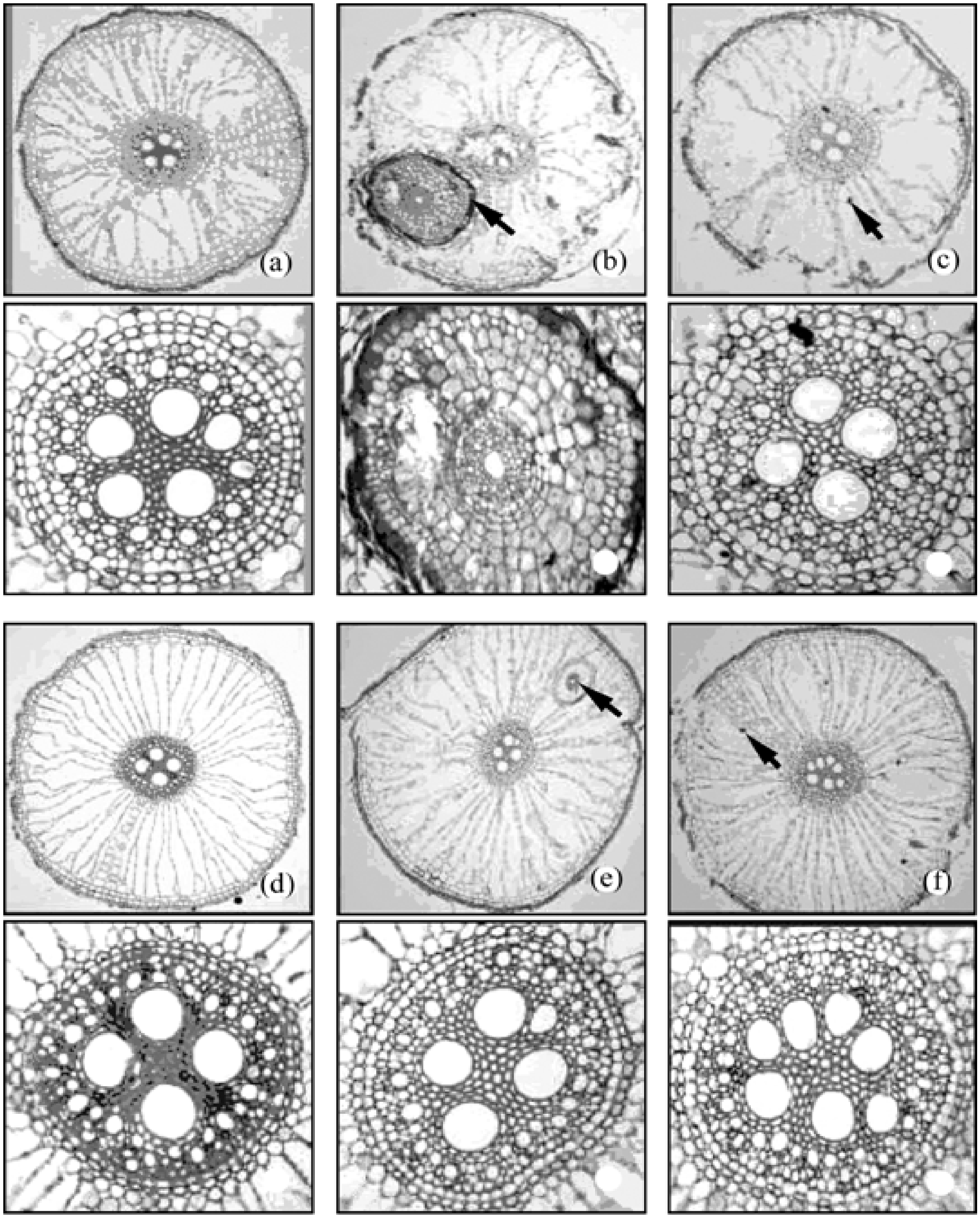

Fig. 1 : Effect of different Cd levels on root anatomical structure of maintainer lines. (a-c) root anatomical structure of Yixiang B observed under 10 × 10 times microscope and the following a row is the photographs observed under 40 × 10 times microscope (correspond to the Cd levels of 0, 0.5 and 3.0 mg L-1, respectively); (d-f) root anatomical structure of E2B observed with 10 × 10 times microscope and the following a row is the photographs observed with 40 × 10 times microscope (under the same Cd levels). Arrow indicates new roots and the black spots.

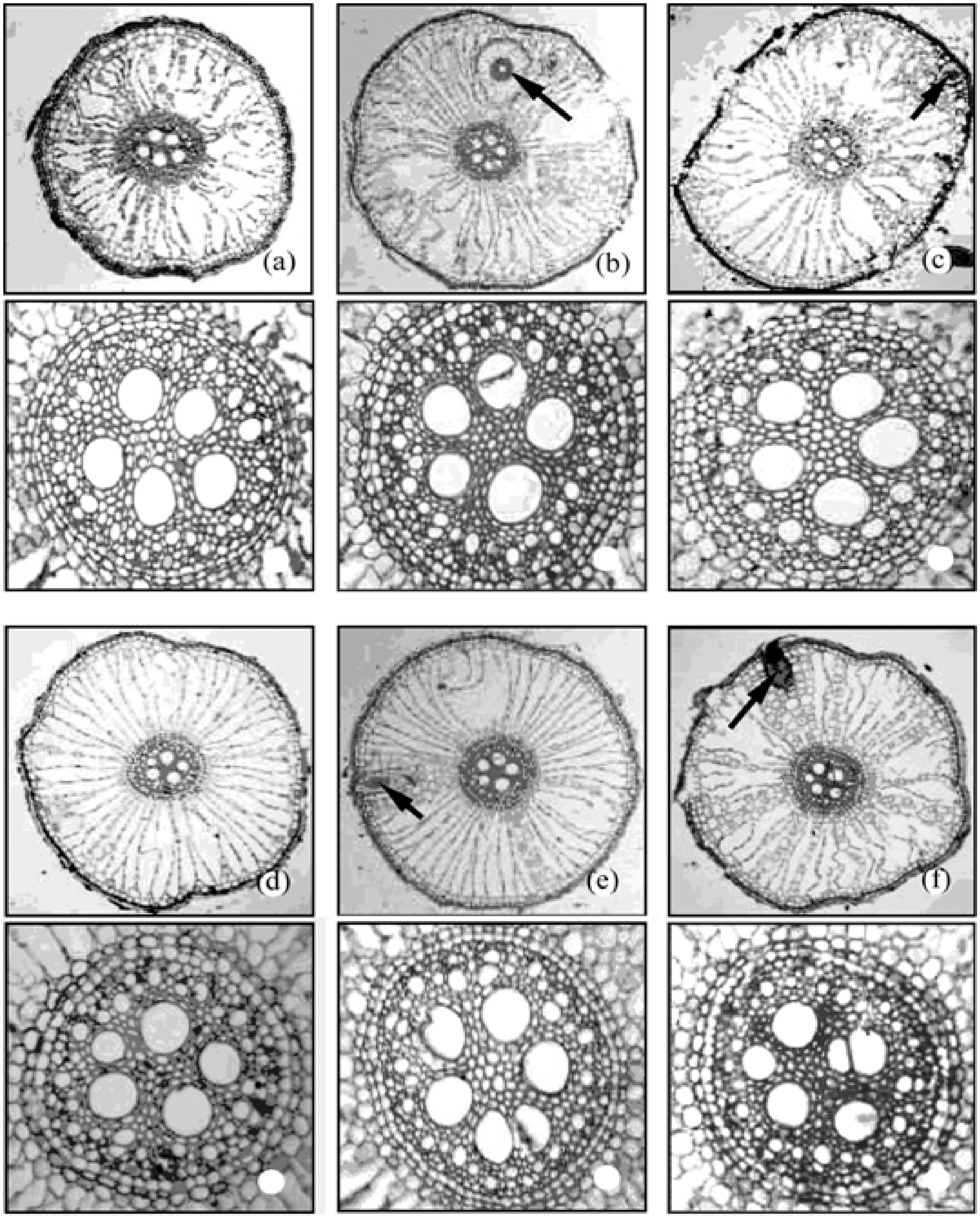

Fig. 2 : Effect of different Cd levels on root anatomical structure of restorer lines. (a-c) root anatomical structure of R892 observed under 10 × 10 times microscope and the following a row is the photographs observed under 40 × 10 times microscope (correspond tothe Cd levels of 0, 0.5 and 3.0 mg L-1, respectively).(d-f) root anatomical structure of Mianhui 725 observed with 10 × 10 times microscope andthe following a row is are the photographs observed with 40 × 10 times microscope (under the same Cd levels).Arrow indicates new roots and the black spots.

changes or remainders were close to vascular cylinder, the effect was high. In this study, the prominent changes in rice root were noted in epidermis and cortex. It displayed desquamation in epidermis and damage in exodermis. Also, the new roots appeared in cortex. The relatively small changes appeared in vascular cylinder and xylem pipe. These results were consistent with the conclusions as described by Küpper (Küpper *et al*., 1999). However, various response in cortex from different materials were distinguished under Cd stress with different concentrations. In addition, there were black spots in cortex and pericycle, exhibiting deep staining in pericycle and thickening cell wall after Cd treatment. It might be relative to the amount of Cd accumulation, which was similar to the previous reports (Li *et al*., 2009; Rascio *et al*., 2008).There were obviously differences in Cd absorption and accumulation among different rice varieties (Liu *et al*., 2003; Yu *et al.*, 2006). The current study further confirmed that there were difference in anti-Cd capacity of different rice materials based on the biomass and the pattern of root anatomical structure. For instance, low Cd level significantly promoted the biomass of YixiangB and R892 which were both high Cd accumulation type. Furthermore, different root anatomical structures were observed in maintainer Yixiang B and restorer R892 when treated with both low and high Cd. Under low Cd condition, there was only one root with big diameter and deep staining for Yixiang B, and most epidermises began to break off, the vascular cylinder turned to be cankered, and the xylem pipe were significantly destroyed. As for R892, however, only few broken epidermal cells were observed, and two new roots were observed and the wall of exodermis began to thicken. The intact structure of vascular cylinder was maintained. When treated with high Cd, most epidermises of Yixiang B began to break off and the edge of cortex was damaged. However, only few broken epidermal cells and vacuum existed in cortex were observed for R892.This toxic symptom in root was consistent with the previous studies (Panou-Filotheou *et al.*, 2004; Gratão *et al*., 2009).

For four rice breeding materials, the desquamation in epidermis and new roots in cortex were observed under low Cd stress. While under high Cd stress, root growth was inhibited in maintainer lines, new roots could not develop or broken off and a few black spots appeared in the cortex and pericycle. For restorer lines, the new roots were gradually withered and its self-adjusting capacity was weakened.

In conclusion, maintainer lines were affected more remarkably than restorer lines. The restorer lines had a stronger adaptive ability to Cd stress than the maintainer lines.

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