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Journal of Environmental Sciences 2010, 22(7) 1067–1072

JOURNAL OF
ENVIRONMENTAL
SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Variations between rice cultivars in iron and manganese plaque on roots and the relation with plant cadmium uptake

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Received 23 August 2009; revised 28 December 2009; accepted 15 January 2010

Abstract

To understand certain mechanisms causing variations between rice cultivars with regard to cadmium uptake and tolerance, pot soil experiments were conducted with two rice cultivars of different genotypes under different soil Cd levels. The relationships between plant Cd uptake and iron/manganese (Fe/Mn) plaque formation on roots were investigated. The results showed that rice cultivars differed markedly in Cd uptake and tolerance. Under soil Cd treatments, Cd concentrations and accumulations in the cultivar Shanyou 63 (the genotype indica) were significantly higher than those in the cultivar Wuyunjing 7 (the genotype japonica) ($P < 0.01$, or $P < 0.05$), and Shanyou 63 was more sensitive to Cd toxicity than Wuyunjing 7. The differences between the rice cultivars were the largest at relatively low soil Cd level (i.e., 10 mg/kg). Fe concentrations in dithionite-citrate-bicarbonate root extracts of Shanyou 63 were generally lower than that of Wuyunjing 7, and the difference was the most significant under the treatment of 10 mg Cd/kg soil. The results indicated that the formation of iron plaque on rice roots could act as a barrier to soil Cd toxicity, and may be a “buffer” or a “reservoir” which could reduce Cd uptake into rice roots. And the plaque may contribute, to some extent, to the genotypic differences of rice cultivars in Cd uptake and tolerance.

Key words: rice (*Oryza sativa* L.); cadmium; genotype; iron/manganese plaque

DOI: 10.1016/S1001-0742(09)60218-7

Introduction

In order to adapt to waterlogged environment, aquatic plants such as rice has a common feature which is the formation of iron plaque on the root surfaces due to the release of oxygen and oxidants in the rhizosphere and the subsequent oxidation of ferrous to ferric iron with the precipitation of iron oxide or hydroxide on root surface. In addition to Fe, the plaque contains a wide range of other metal elements. In nature, the plaque element next in importance to Fe is generally Mn (Taylor et al., 1984; Crowder and Coltman, 1993). The main forms of iron plaque are the ferric hydroxides, goethite, and lepidocrocite (Chen et al., 1980). Due to the high adsorption capacity of functional groups on iron hydroxides, iron plaque may sequester a number of metal(loid)s by adsorption or co-precipitation, and thus interferes with the availability of these elements in the rhizosphere and their uptake by and translocation in plants. Therefore, the presence of iron coating on root surface may influence metal uptake (Otte et al., 1989; Liu et al., 2007a).

Although some studies have been conducted on iron plaque and metal uptake in wetland plants, the results were not consistent with each other. Some of them indicated that the formation of Fe and Mn precipitate (plaque) on root surface of aquatic plants may provide a means of attenuation and external exclusion of metals (Greipsson and Crowder, 1992; Hansel et al., 2002). Whereas, Ye et al. (1997, 1998) reported the differences between the presence and absence of plaque in plant Cu and Zn uptake of *Typha latifolia*. The former works were mainly conducted under hydroponic conditions and/or different levels of Fe additions, few experiments were conducted under soil conditions with natural Fe.

Cd is a toxic metal to plants and animals. Although plants do not require Cd for growth and reproduction, the bioaccumulation index of Cd in plants is high and may exceed many essential elements (Kabata-Pendias and Pendias, 2001). Therefore, Cd is one of the most important heavy metals to consider in terms of food-chain contamination.

Paddy rice is one of the most important crops in the world, especially in Asia. It was reported that there were differences between rice genotypes in Fe content in iron plaque (Geng et al., 2005). Previous studies showed that rice cultivars varied significantly in Cd accumulation and tolerance (Morishita et al., 1987; Wu et al., 1999; Liu et al., 2003, 2005). However, limited information is available on the relationship between plant Cd uptake and

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Fe/Mn plaque formation on root for different rice cultivars. The present research was conducted to investigate the relationship and to understand mechanisms causing the variations between rice cultivars with regard to Cd uptake and accumulation.

1 Materials and methods

1.1 Soil preparation and analysis

The soils for pot experiments were collected from uncontaminated fields (0–20 cm). After air-drying and passing through a 2-mm sieve, the soil properties were tested and shown in Table 1. The particle size was determined using hydrometer method; pH using pH meter (soil : distilled water = 1 : 2); organic matter using sequential extraction method; cation exchange capacity (CEC) using ammonium acetate method; total N using Kjeldahl method, and total Fe, Mn and Cd using atomic absorption spectrophotometer (AAS) (Solaar S4 + Graphite Furnace System 97, Thermo Elemental, USA) following H_2O_2-HF-HNO_3-$HClO_4$ digestion (Sparks, 1996).

Table 1 shows the soil used was a silty loam with a low portion of sand and an acidic pH. The soil contained a moderate level of organic matter, CEC and nitrogen, and low level of Cd.

Four kilogram of soil was placed in each pot (18 cm in diameter and 20 cm in height). Cd in the form of $CdCl_2$ was added to the soil to obtain Cd levels of 10 and 50 mg/kg soil dw (dry weight).

$CdCl_2$ was dissolved in deionized water and poured into the soil slowly while mixing the soil at the same time. The thoroughly mixed soils were stored in pots and submerged in water (2–3 cm above the soil surface) for a month before rice seedlings were transplanted. The soils without adding Cd served as control.

1.2 Rice plant materials

Based on our previous studies (Liu et al., 2003, 2005), two rice cultivars of different genotypes with diverse Cd uptake abilities, Shanyou 63 (indica, a high Cd accumulator) and Wuyunjing 7 (japonica, a low Cd accumulator), were used in this experiment. Rice seeds were submerged in a water bath for about 48 hr at room temperature (20–25°C), germinated under moist condition (seeds were covered with two layers of moist gauze cloth) at 32°C for another 30 hr, and then the germinated seeds were grown in uncontaminated soils. After 30 days, the uniform seedlings were selected and transplanted into the pots (3 plants per pot). The pot soil was maintained under flooded conditions (with 2–3 cm of water above soil surface) during the rice growth period.

1.3 Experimental design

The experiments were carried out in a greenhouse, with a 16 hr light period and a day/night temperature of (32–35)°C/(23–25)°C. The pots were arranged in a randomized complete block design with five replicates. One gram of urea (containing 46% N) and 1 g of $K_2HPO_4 \cdot 3H_2O$ were applied to each pot on day 3 before seedling transplant, and on day 20 after the transplant.

1.4 Cold DCB extraction of iron and manganese plaque

One entire plant (including shoot and root system) was sampled from each pot at day 40 after seedling transplant. The plant samples were washed thoroughly with tap water and deionized water, and then separated into roots and shoots. Iron and manganese plaque on the roots were extracted using a modified cold dithionite-citrate-bicarbonate (DCB) method (Taylor and Crowder, 1983; Otte et al., 1989; Liu et al., 2008). The entire root systems were firstly incubated for 2 hr at 20°C in 80 mL 0.03 mol/L sodium citrate ($Na_3C_6H_5O7 \cdot 2H_2O$) and 0.125 mol/L sodium bicarbonate ($NaHCO_3$) with the addition of 1.6 g sodium dithionite ($Na_2S_2O_4$), and the flasks were shaken during the incubation. The extracts were then transferred into 100-mL glass flasks. Roots were rinsed three times with deionized water and the eluates were added to the DCB extracts. Finally, the solutions were filtered into plastic containers for subsequent analysis.

1.5 Determination of Cd concentrations in rice plants

After DCB extraction, the roots and shoots were oven-dried at 70°C to a constant weight. The oven-dried samples were ground with a stainless steel grinder to pass through a 100 mesh sieve. The Cd concentrations were determined by an AAS following HNO_3-$HClO_4$ (HNO_3:$HClO_4$, 4:1, *V/V*) digestion procedures (Allen, 1989).

Experimental data were analyzed with the statistical package SPSS10.0 and EXCEL’2000 for Windows. Two significant levels 0.05 and 0.01 were used in presenting the results.

2 Results

2.1 Plant growth

Table 2 shows the effects of soil Cd treatments on rice growth differed with rice cultivars, soil Cd levels and plant organs. In general, Shanyou 63 was more sensitive to soil Cd stress.

As compared to the control, the root and shoot weights of 10 mg/kg soil with Cd treatment increased for the both rice cultivars. However, the magnitudes of the increases differed between the cultivars. The increases were about

Table 1 Selected properties of the soil used in this experiment (n = 3)

Soil type	Soil texture	Particle size fraction (mg/kg)			pH	OM (g/kg)	CEC (cmol/kg)	Total N (g/kg)	Total Fe (mg/kg)	Total Mn (mg/kg)	Total Cd (mg/kg)
		Sand	Silt	Clay							
Paddy soil	Silty loam	184	427	389	4.71	26.2	15.9	1.25	10,024.46	738.27	ND

OM: organic matter; CEC: cation exchange capacity; ND: not detected.

Table 2 Effects of soil Cd treatments on plant biomasses of the rice cultivars

Cultivars	Soil Cd treatment	Root		Shoot		Total	
		Dry weight (g/pot)	RC (%)	Dry weight (g/pot)	RC (%)	Dry weight (g/pot)	RC (%)
Shanyou 63	Control	4.33		9.49		13.82	
	Cd10	5.25**	21.25	11.25**	18.55	16.50**	19.39
	Cd50	3.82*	–11.78	8.13**	–14.33	11.95*	–13.53
Wuyunjing 7	Control	1.49		3.84		5.33	
	Cd10	1.53	2.68	4.02	4.69	5.55	4.13
	Cd50	1.45	–2.68	3.36*	–12.50	4.81*	–9.76

Cd10, Cd50 are treatments with 10, 50 mg/kg Cd in soil (dry weight), respectively; RC: relative changes of soil Cd treatment compared to the control, e.g., RC = ((weight of Cd treatment – weight of control)/weight of control) × 100%.
**, * Significant difference between the Cd treatment and the control at $P < 0.01$ and $P < 0.05$, respectively.

20% for Shanyou 63, a significant level ($P < 0.01$); but they were less than 5% for Wuyunjing 7 at an insignificant level ($P > 0.05$).

Under soil Cd treatments of 50 mg/kg, the root and shoot weights of the rice cultivars decreased. But the magnitudes of the decreases also varied with cultivars and plant organs. With regard to root weights, the decrement was more than 10% for Shanyou 63 at a significant level ($P < 0.05$), but less than 3% for Wuyunjing 7 at an insignificant level ($P > 0.05$). With respect to shoot biomasses, the decreases were significant ($P < 0.05$, or $P < 0.01$) for the two cultivars. However, the extents of the decrease was also larger for Shanyou 63 than for Wuyunjing 7.

2.2 Cd concentrations, accumulations and distributions in rice plants

The differences between the cultivars in plant Cd concentrations, accumulations and distributions varied with soil Cd levels (Tables 3 and 4).

Cd concentrations of two rice cultivars were similar for the control, irrespective of root tissues, plaques or shoots. Under soil Cd treatments of 10 mg/kg, Cd concentrations in root tissues and shoots of Shanyou 63 were 91.9% and 106.2%, respectively, higher than that of Wuyunjing 7. But Cd concentration in the plaque of Shanyou 63 was only 9.6% higher than that of Wuyunjing 7, and the difference was insignificant ($P > 0.05$). Under soil Cd treatments of 50 mg/kg, Cd concentrations in root tissues, plaques and shoots of Shanyou 63 was 30.7%, 10.5% and 70.5%, respectively, higher than those of Wuyunjing 7, and the difference in Cd concentrations of plaques was also insignificant ($P > 0.05$).

With regard to Cd distributions, they were similar between the cultivars for the control. Under soil Cd treatments of 10 mg/kg, Cd distribution ratio in root tissue was much higher for Shanyou 63 than for Wuyunjing 7, but Cd distribution ratio in plaque was significantly lower for Shanyou 63 than for Wuyunjing 7.

Under soil Cd treatments of 50 mg/kg, Cd distribution ratio in root tissue of Shanyou 63 was also higher than that of Wuyunjing 7, but the ratio in plaque was lower for Shanyou 63 than for Wuyunjing 7. However, the magnitudes of the differences between the rice cultivars were larger under soil Cd treatments of 10 mg/kg than under 50 mg/kg treatments. Cd distribution ratios in the shoots of Shanyou 63 were obviously higher than those of

Table 3 Cd concentrations in rice plants and distribution in the roots

Cultivars	Soil Cd treatment	Cd in root tissue		Cd in plaque[a]		Total Cd concentration	
		Concentration (μg/g)	Distribution (%)	Concentration (μg/g)	Distribution (%)	In root (μg/g)	In shoot (μg/g)
Shanyou 63	Control	2.11	21.91	7.51	78.09	9.61	0.28
	Cd 10	69.29**	48.21	74.44	51.79	143.72**	19.61**
	Cd 50	196.18**	60.57	127.69	39.43	323.87*	28.20**
Wuyunjing 7	Control	1.95	20.79	7.44	79.21	9.40	0.28
	Cd 10	36.11	34.72	67.89	65.28	104.00	9.51
	Cd 50	150.11	56.51	115.51	43.49	265.62	16.54

[a] Cd concentrations in plaques are based on root dry weights. **, * Significant difference between two cultivars at $P < 0.01$ and $P < 0.05$, respectively.

Table 4 Cd accumulations and distributions in different parts of the rice plants

Cultivars	Soil Cd treatment	Cd in root tissue		Cd in plaque		Cd in shoot		Cd accumulation in rice plant (μg/pot)
		Accumulation (μg/pot)	Distribution (%)	Accumulation (μg/pot)	Distribution (%)	Accumulation (μg/pot)	Distribution (%)	
Shanyou 63	Control	9.12**	20.59	32.50**	73.41	2.66**	6.00	44.27**
	Cd 10	363.77**	37.30	390.79**	40.07	220.60**	22.62	975.15**
	Cd 50	749.41**	51.10	487.79**	33.26	229.24**	15.63	1466.45**
Wuyunjing 7	Control	2.91	19.32	11.09	73.61	1.07	7.07	15.06
	Cd 10	55.25	27.99	103.88	52.63	38.25	19.38	197.37
	Cd 50	217.65	49.38	167.49	38.00	55.58	12.61	440.73

** Significant difference between two cultivars at $P < 0.01$.

Wuyunjing 7, irrespective of 10 mg/kg or 50 mg/kg soil Cd treatments.

With respect to Cd accumulations in rice plants, Shanyou 63 was much higher than Wuyunjing 7, irrespective of in root tissues, plaques or shoots. But the magnitudes of the differences between the rice cultivars were the largest for soil Cd treatment of 10 mg/kg.

2.3 Fe and Mn concentrations and distributions in rice plants

The differences between the rice cultivars in plant Fe concentrations varied greatly with soil Cd levels (Table 5). For the control and 10 mg/kg Cd treatment, Fe concentrations in root tissues and plaques of Wuyunjing 7 were significantly higher than that of Shanyou 63 ($P < 0.01$). But under 50 mg/kg Cd treatment, the differences between the cultivars in Fe concentrations of root tissues and plaques were insignificant ($P > 0.05$).

The differences between the cultivars in shoot Fe concentrations also varied with soil Cd levels. For the control and 50 mg/kg Cd treatment, shoot Fe concentrations of Shanyou 63 were significantly ($P < 0.01$) higher than those of Wuyunjing 7. But under 10 mg/kg Cd treatment, shoot Fe concentration of Shanyou 63 was significantly ($P < 0.01$) lower than that of Wuyunjing 7.

The differences between the cultivars in plant Mn concentrations varied greatly with soil Cd levels and plant organs (Table 6). For the control and 50 mg/kg Cd treatment, Mn concentrations in root tissues, plaques and shoots of Shanyou 63 was significantly ($P < 0.01$, or < 0.05) higher than those of Wuyunjing 7, except for plaque Mn concentrations of the control. But under soil Cd treatments of 10 mg/kg, Mn concentrations of Shanyou 63 was significantly ($P < 0.01$) lower than those of Wuyunjing 7, except for shoot Mn concentrations.

3 Discussion

3.1 Variations between rice cultivars in Cd tolerance

Cadmium has been shown to affect plant metabolisms, such as chlorophyll formation, photosynthesis, nutrient absorption (Lagriffoul et al., 1998; Mendelssohn et al., 2001; Hsu and Kao, 2005). As a result, plant growth and matter accumulation were inhibited (Liu et al., 2007b).

It was reported that the deposition of iron oxide plaque or coating on rice root could ameliorate the toxic effects of Cu, Ni, and Cu + Ni on plant growth (Greipsson and Crowder, 1992; Greipsson, 1994). However, other reports showed no difference in root and shoot dry weights and leaf elongation between presence and absence of the plaque, when Cu or Ni were added to the solution (Ye et al., 1997).

In our present research, effects of Cd on rice biomasses varied with soil Cd levels and cultivars (Table 2). Under relatively lower soil Cd treatment (10 mg/kg), the biomasses of roots and shoots increased insignificantly ($P > 0.05$) for Wuyunjing 7, but the corresponding values increased by about 20% for Shanyou 63 (significant at $P < 0.01$). The stimulating effects of relatively lower level of Cd on rice growth may be another response of rice plant to Cd stress. Because Cd is not an essential element for rice growth and metabolism, the underlying mechanisms need further research.

Under relatively high level of Cd treatment (50 mg/kg), the biomasses of roots and shoots decreased significantly ($P < 0.01$, or < 0.05) for two cultivars, except for root weight of Wuyunjing 7. However, the magnitudes of the decreases were higher for Shanyou 63 than for Wuyunjing 7.

It can be seen from the results that Shanyou 63 may be more sensitive to Cd stress than Wuyunjing 7. The results also showed that DCB-Fe concentrations in the roots of

Table 5 Fe concentrations in rice plants and distributions in the roots

Cultivars	Soil Cd treatment	Fe in root tissue		Fe in plaque[a]		Total Fe concentration	
		Concentration (μg/g)	Distribution (%)	Concentration (μg/g)	Distribution (%)	In root (μg/g)	In shoot (μg/g)
Shanyou 63	Control	804.03**	1.45	54631.84**	98.55	55435.87**	245.33**
	Cd 10	746.46**	1.74	42093.79**	98.26	42840.25**	192.80**
	Cd 50	896.40	1.22	72580.18	98.78	73476.58	237.34**
Wuyunjing 7	Control	976.01	1.25	77047.67	98.75	78023.68	195.31
	Cd 10	1079.81	1.45	73372.26	98.55	74452.06	240.45
	Cd 50	897.90	1.17	76017.02	98.83	76914.92	182.69

[a] Fe concentrations in the plaque are based on root dry weights. ** Significant difference between the two cultivars at $P < 0.01$.

Table 6 Mn concentrations in rice plants and distributions in the roots

Cultivars	Soil Mn treatment	Mn in root tissue		Mn in plaque[a]		Total Mn concentration	
		Concentration (μg/g)	Distribution (%)	Concentration (μg/g)	Distribution (%)	In root (μg/g)	In shoot (μg/g)
Shanyou 63	Control	17.68**	35.75	31.78	64.25	49.46*	423.93*
	Cd 10	11.92**	26.42	33.19**	73.58	45.11**	258.29
	Cd 50	13.77**	27.28	36.70**	72.72	50.47**	221.98**
Wuyunjing 7	Control	11.59	26.23	32.58	73.77	44.17	374.08
	Cd 10	15.63	19.19	65.81	80.81	81.44	267.80
	Cd 50	9.84	27.86	25.48	72.14	35.32	150.19

[a] Mn concentrations in the plaque are based on root dry weights. **, * Significant difference between the two cultivars at $P < 0.01$ and $P < 0.05$, respectively.

Wuyunjing 7 were higher than those of Shanyou 63, and the magnitude of the difference between the cultivars was the highest for 10 mg/kg Cd treatments (Table 5). Therefore, iron plaque may provide a barrier to soil Cd stress, specifically at low soil Cd level (for example, 10 mg/kg).

3.2 Variations between rice cultivars in plant Cd uptake

Former studies indicated that the presence of iron plaque inhibited the uptake of heavy metals by plants, and Cu concentrations were low in the plants of *Lobelia dortmanna* and *Phragmites australis* in the presence of plaque (Christensen and Jensen, 1998; Batty et al., 2000). Iron plaque on rice root affected patterns of metal uptake and accumulation. Lower concentrations of Cu and Ni were also found in the leaves of rice plants with plaque than in the plants without plaque (Greipsson and Crowder, 1992; Greipsson, 1994). However, Liu et al. (2008) reported that iron plaque on rice root surface is of little significance in affecting uptake and accumulation of Cd by rice plant. The results of Ye et al. (1997) also presented that the plaque of *Typha latifolia* did not alter Cu uptake and translocation but increased Ni uptake and translocation. Their other research also indicated that Zn was absorbed fairly equally on root surfaces with and without plaque, but the roots with plaque absorbed more Pb, but less Cd, than those without. The presence of plaque did not alter Zn, Pb and Cd translocation in the seedlings of *Typha latifolia* in nutrient solutions (Ye et al., 1998).

Our present research showed that there were great differences between two rice cultivars in plant Cd concentrations and accumulations (Tables 3 and 4). The magnitudes of the differences in Cd accumulations were much larger than those in Cd concentrations, for Shanyou 63 were higher than Wuyunjing 7, not only in plant Cd concentration but also in plant biomass.

With regard to plant Cd concentrations, the magnitudes of differences between the rice cultivars varied largely with soil Cd levels (Table 3). Under soil Cd stress (10 and 50 mg/kg), Cd concentrations in root tissues and shoots of Shanyou 63 were significantly higher ($P < 0.01$, or < 0.05) than those of Wuyunjing 7, and the magnitudes of differences were much higher under 10 mg/kg Cd treatment than under 50 mg/kg treatment. But the differences between the cultivars in Cd concentrations of iron plaques were small, and did not reach significant level ($P > 0.05$).

In respect of Cd distribution in the roots and entire plants, the magnitudes of the differences between the rice cultivars varied largely with soil Cd levels and plant organs. On Cd distributions in the roots under soil Cd stress (10 mg/kg and 50 mg/kg), Cd distribution ratios in root tissues of Shanyou 63 were significantly higher than those of Wuyunjing 7 ($P < 0.01$, or < 0.05), but the distribution ratios in iron plaque of Shanyou 63 were significantly lower than those of Wuyunjing 7 ($P < 0.01$, or < 0.05) (Table 3). On Cd distributions in entire plants, Cd distribution ratios in root tissues and shoots were also significantly higher ($P < 0.01$, or < 0.05) for Shanyou 63 than for Wuyunjing 7, except for those in root tissues of 50 mg/kg Cd treatment. Cd distribution ratios in iron plaque of Shanyou 63 were also significantly lower ($P < 0.01$, or < 0.05) than those of Wuyunjing 7. The magnitudes of the differences between the rice cultivars were the largest for 10 mg/kg soil Cd treatment.

Meanwhile, DCB-Fe concentrations and the Fe concentrations in root tissues of Wuyunjing 7 were markedly higher than those of Shanyou 63, and the magnitudes of the differences between the cultivars were the largest for 10 mg/kg soil Cd treatment. Therefore, the formation of iron plaque can increase the sequestration of Cd in the rhizosphere and on the root surface, providing a means of external exclusion of soil Cd, especially in relatively low Cd-contaminated soil (i.e., 10 mg/kg).

4 Conclusions

On the basis of the experimental results, it can be concluded that the iron plaque on rice roots can act as a barrier to soil Cd toxicity, and may be a “buffer” or a “reservoir” which can reduce Cd uptake into rice roots. But the functions of plaque are limited, and only effective at relatively low soil Cd levels (for example, 10 mg/kg). Along with the increase of soil Cd levels, Cd distribution ratios in iron plaque decreased, but the ratios in root tissues increased. The results also prove that the differences in degree of Fe plaque may contribute, to some extent, to genotypic differences of the rice cultivars in Cd uptake and tolerance, especially in lower Cd-contaminated soils.

Although Mn concentrations in rice plants differed with soil Cd levels and rice cultivars, their relations with plant Cd uptake and tolerance were not obvious in the present experiments, which is needed to be studied further.

Acknowledgments

This work was supported by the Natural Science Foundation of Jiangsu Province (No. BK2008144), the Postgraduate Research and Innovation Project of the Universities in Jiangsu Province (No. CX08S-018Z) and the Research Grants Council of Hong Kong (No. HKBU2181/03M).

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