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Genotypic and environmental variation in cadmium, chromium, lead and copper in rice and approaches for reducing the accumulation

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HIGHLIGHTS

- Field trials evaluated situation of grain HM in main rice growing areas of Zhejiang.
- Forecasting index system to predict rice grain HM concentration was achieved.
- Hybrid rice holds higher grain Cd concentration than conventional cultivars.
- Low grain HM accumulation rice cultivars were successfully identified.
- Developed alleviating regulator which effectively reduced grain toxic HM

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ABSTRACT

The field scale trials revealed significant genotypic and environmental differences in grain heavy metal (HM) concentrations of 158 newly developed rice varieties grown in twelve locations of Zhejiang province of China. Grain Pb and Cd contents in 5.3% and 0.4% samples, respectively, were above the maximum permissible concentration (MPC); none of samples had Cr/Cu exceeding MPC. Stepwise multiple linear regression analysis estimated soil HM critical levels for safe rice production. Low grain HM accumulation cultivars such as Xiushui817, Jiayou08-1 and Chunyou689 were recommended as suitable cultivars for planting in slight/medium HM contaminated soils. The alleviating regulator (AR) of $(NH_4)_2SO_4$ as N fertilizer coupled with foliar spray of a mixture containing glutathione (GSH), Si, Zn and Se significantly decreased grain Cd, Cr, Cu and Pb concentrations grown in HM contaminated fields with no effect on yield, indicating a promising measurement for further reducing grain HM content to guarantee safe food production.

1. Introduction

Heavy metal (HM) pollution is a world-wide problem affecting the quality of terrestrial and aquatic environments, and poses a potential threate to human health via the food chain (Wei and Yang, 2010). Excessive metal concentrations in contaminated soils can result in yield reduction and poor quality of crop products. Rice, as a main food cereal in the world, is one of the major sources of HM intakes for human. Therefore, it is crucially necessary to reduce toxic HM accumulation in rice grains for safe food production.

Great effort has been implemented to remediate metal polluted soils for producing safe crop products with HM contents in edible parts below the maximum permissible concentration (MPC). Considering large-scale medium/slightly contaminated farmlands, the best approaches are to develop and plant crop cultivars with low HM accumulation in edible parts. Meanwhile, favorable agronomic practice and chemical regulators which can reduce plant HM uptake were also employed. The combination will offer a cost-effective and practically acceptable strategy for full utilization of natural resource and minimizing soil-plant transfer of HMs and guarantee safe food production. It is imperative to identify rice cultivars with low HM uptake and accumulation, thus providing reasonable rice cultivars being directly adopted in HM contaminated soils or providing genetic materials for developing rice cultivars with low grain HM content.

Many researchers studied the effects of soil factors and agronomic practices on HM accumulation in cabbage (Liu et al., 2005), wheat and barley (Adams et al., 2004), and rice (Wang et al., 2007; Cai et al., 2011; Jalloh et al., 2009; Lin et al., 2012) in hydroponic or pot experiments. However, HM contamination in natural environment is often

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much more complex than that in artificial environment (Cheng et al., 2006; Norton et al., 2009; Zeng et al., 2011). Thus, evaluating the effect of combined application of these chemicals on HM uptake and accumulation of plants in a contaminated field will provide valuable information.

In addition, genotypic differences in grain HM accumulation may result in significantly different health risks, e.g. grain Cd content is likely to exceed MPC due to planting high accumulation rice varieties even in slightly or non-contaminated soils (Chen et al., 2007a). Therefore, it is imperative to evaluate grain HM accumulation of newly developed or widely applied varieties to ensure that low-heavy-metal accumulation rice varieties were adopted especially in medium/slightly contaminated farmlands to guarantee safe food production. We therefore undertook the field scale trial (Fig. 1) to: (1) surmise the present situation and the major toxic HMs of rice grain HM contamination in different rice growing areas of Zhejiang province, and construct the forecasting index system to predict HM concentration in grain; (2) elucidate the effects and relationship of environment and cultivar on grain HM concentrations and identify low HM accumulation rice cultivars; and (3) determine the role of comprehensive alleviating regulator, containing GSH, Si, Se, Zn and $(NH_4)_2SO_4$ as N fertilizer, in decreasing grain HM accumulation.

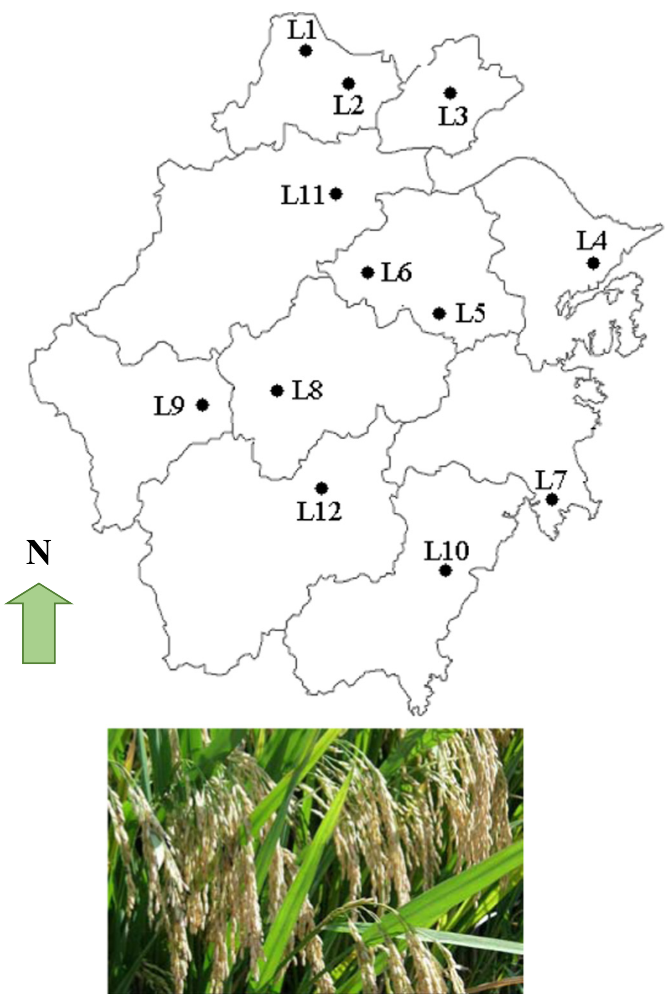

Fig. 1. Location of rice field scale trials in Zhejiang province of China.

2. Materials and methods

2.1. Experiment design

2.1.1. HM contents in paddy soils and cultivar differences in HM concentrations of rice grain in Zhejiang province

The 158 newly developed rice varieties (*Oryza sativa* L.) in 2010 regional rice variety test of Zhejiang province were grown in twelve locations (named as L1 to L12, Fig. 1) in Zhejiang, China. All the cultivars, newly developed and being planted in Zhejiang province, were categorized into six groups, according to different types of rice (Table S1). A completely randomized block design with 3 replications was constructed on each site. The plot area was 13.3 m^2 consisting of 40 rows, 2 m long and 15 cm between rows, and 25 seedlings per row. All field managements were the same as those used in local production. Rice grains were harvested at maturity. The soil species, pH and available HM concentration at each of twelve experimental sites in the different area of Zhejiang, China were listed in Table S2. Soil properties were measured according to Zeng et al. (2011).

2.1.2. Field trials for reducing HM accumulation in rice grains via integrated practices of N fertilizer and chemical regulation application (namely alleviating regulator, AR)

Cd is widely recognized as a serious environmental pollutant and easily taken up by roots and translocated into grains. Hence, we selected rice cultivars with different Cd accumulation. According to above mentioned survey, seven rice cultivars were selected including three high- (Zheda821, Yongjing21 and R8097) and three low-grain Cd accumulation (Xiushui817, Jiayou08-1 and Chunyou689), along with *cv.* Xiushui110 of low HM accumulation cultivar (Cheng, 2004). Two successive year experiments were conducted at HM polluted fields, about 200 m from the wastes of an electroplating factory that was run for more than 20 years in Jiaxing (located in North Zhejiang). Seven cultivars mentioned above and four cultivars (c.f. Zheda821 and R8097; Xiushui817 and Jiayou08-1) were used in 2011 and 2012, respectively, with two treatments of control and integrated practices of N fertilizer and chemical regulation (namely alleviating regulator, AR). The application rate of N fertilizer was 225 kg ha^{-1}, as the forms of NH_4NO_3 (643 kg ha^{-1}) and $(NH_4)_2SO_4$ (1060 kg ha^{-1}) in control and AR treatment, respectively. Concerning AR treatment, plants were foliar sprayed 300 l ha^{-1} solution containing 50 mg l^{-1} GSH, 10 mM Si ($Na_2SiO_3 \cdot 9H_2O$) and 0.2% Zn ($ZnSO_4 \cdot 7H_2O$) at tillering and full heading stages (Wang et al., 2007; Mahgoub et al., 2006). And additional 15 mg l^{-1} Se (Na_2SeO_3) was also contained in the spray solution at full heading stage. As to control plants, 300 l ha^{-1} water was simultaneously foliar sprayed. The soil had a pH of 6.68 and 6.59, with EDTA-extractable Cr, Cd, Pb and Cu of 0.323, 0.172, 17.2 and 453, and 0.352, 0.242, 16.8 and 610 mg kg^{-1} in 2011 and 2012, respectively. The experiment was laid in a split-plot design with treatment as the main plot with 6 m^2 plot size, and there were 3 replicates for each treatment. All other field managements were the same as those used in local production.

Plants were sampled at elongation, full heading and harvest stages, in both 2011 and 2012 experiments. After thoroughly washed with tap and followed by distilled water, samples were separated into leaves and stems. At harvest stage, grains were manually threshed and oven-dried at 80 °C to constant weight, and then were milled with a rice miller, and separated from chaff with chaff-removing machine. Grain samples were ground to pass through a 60-mesh sieve for HM analysis. In 2012 experiment, glume was also separated for metal analysis.

2.2. Analysis of HM concentration in rice grains

Grain HMs of 2010 samples was extracted according to Zeng et al. (2008). HMs in grains, leaves, stems and glumes in 2011 and 2012 experiments were analyzed as described by Chen et al. (2007a). HMs, including Cd, Cr, Cu and Pb, were measured with an inductively couple