Environmental Pollution 216 (2016) 86–94

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Increasing CO_2 differentially affects essential and non-essential amino acid concentration of rice grains grown in cadmium-contaminated soils☆

Huibin Wu [a, b], Zhengguo Song [b, *], Xiao Wang [b], Zhongqi Liu [b], Shirong Tang [b]

[a] Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Sciences/Key Laboratory of Agro-Environment, Ministry of Agriculture of China, Beijing 100081, PR China
[b] Centre for Research in Ecotoxicology and Environmental Remediation, Agro-Environmental Protection Institute, Tianjin 300191, PR China

ARTICLE INFO

Article history:
Received 25 March 2016
Received in revised form
13 May 2016
Accepted 19 May 2016

Keywords:
Cadmium
Rice
Amino acids
CO_2 elevation
Food safety

ABSTRACT

Environmental pollution by both ambient CO_2 and heavy metals has been steadily increasing, but we do not know how fluctuating CO_2 concentrations influence plant nutrients under high Cd pollution, especially in crops. Here, we studied the effects of elevated CO_2 and Cd accumulation on proteins and amino acids in rice under Cd stress. In this pot experiment, we analyzed the amino-acid profile of 20 rice cultivars that accumulate Cd differently; the plants were grown in Cd-containing soils under ambient conditions and elevated CO_2 levels. We found that although Cd concentrations appeared to be higher in most cultivars under elevated CO_2 than under ambient CO_2, the effect was significant only in seven cultivars. Combined exposure to Cd and elevated CO_2 strongly decreased rice protein and amino acid profiles, including essential and non-essential amino acids. Under elevated CO_2, the ratios of specific amino acids were either higher or lower than the optimal ratios provided by FAO/WHO, suggesting that CO_2 may flatten the overall amino-acid profile, leading to an excess in some amino acids and deficiencies in others when the rice is consumed. Thus, Cd-tainted rice limits the concentration of essential amino acids in rice-based diets, and the combination with elevated CO_2 further exacerbates the problem.

1. Introduction

Cadmium (Cd) is a highly toxic heavy metal that tends to contaminate agricultural soils through industrial processes, phosphatic fertilizers, and atmospheric deposition. Through its movement up the soil-plant-human chain, Cd causes considerable environmental damage and human health problems (Guo et al., 2012; Maksymiec, 2007). In China, the heavy-metal exceeding rate is 19.4% in monitored farmlands, with Cd-exceeding rate of 7%-ranked as the number-one inorganic contaminant (Ministry of Environmental Protection, 2014).

Among the major staple crops, rice has particularly high Cd uptake and accumulation (Chaney et al., 2004). Heavy metals toxicity, including Cd, altered the amino acid (AA) concentration, antioxidant activity, and nutritionally minerals of the rice cultivars (Dwivedi et al., 2010; Liu et al., 2003; Wu et al., 2004). Thus, Cd intake through rice consumption is a major health concern (Li et al., 2010), particularly after media reports of excess Cd in rice captured public attention (Bian et al., 2013). However, simply advocating the reduction of rice consumption is not desirable because rice is a highly digestible, rich source of three essential amino acids (EAAs): lysine (Lys), leucine (Leu), and isoleucine (Ile), with the other six EAAs also present at lower concentrations (Mohan et al., 1988). The negative health effects of EAA deficiency are well-documented, including low hemoglobin (Cho et al., 1984), disturbed nitrogen equilibrium, impaired growth, lowered fertility, and decreased life span (Giordano and Castellino, 1997). Rice is also rich in glycine (Gly) and glutamic acid (Glu) (Mohan et al., 1988), AAs that are among those involved in neurotransmission (Curtis and Johnston, 1974). Notably, high Cd levels are not only directly toxic to humans, but exert indirect negative health effects by decreasing AA concentrations in crops, as demonstrated in rice seedlings using pot experiments (Hsu and Kao, 2003).

Carbon dioxide is another major factor that decreases crop

☆ This paper has been recommended for acceptance by Harmon Sarah Michele.

* Corresponding author. Agro-Environmental Protection Institute, Ministry of Agriculture, No. 31, Fukang Rd, Nankai District, Tianjin 300191, PR China.
E-mail addresses: bekeyw@gmail.com (H. Wu), forestman1218@163.com (Z. Song).

http://dx.doi.org/10.1016/j.envpol.2016.05.049
0269-7491/

nutrient content, including AAs (Myers et al., 2014). Average atmospheric CO_2 concentrations have been continuously increasing in the past few decades due to global climate change (IPCC, 2013). Based on results from studies that have examined the interactive effects of elevated CO_2 and Cd-contaminated soils on plants (Guo et al., 2011; Jia et al., 2011a; Li et al., 2010; Pleijel and Högy, 2015), we hypothesized that the combination of excess Cd and high CO_2 can seriously degrade rice quality worldwide, through decreasing nutrient (protein and amino acids) concentration and increasing pollutant accumulation. Thus, high atmospheric CO_2 has the potential to worsen the existing health risk of Cd contamination in humans. Rice is a particularly critical crop to study because it is the primary grain in China, where Cd pollution is projected to increase, coexisting with heightened CO_2 concentrations. In this study, our goal was therefore to provide more data describing the consequences of elevated CO_2 on rice grown in Cd-contaminated soil and to quantify CO_2- and Cd-induced variations in the AA profiles of multiple rice cultivars. The results should allow for more accurate evaluations of AA deficiency risk under rice consumption, and contribute to the prediction of food safety.

2. Materials and methods

2.1. Experimental site and growing conditions

The tested soils were collected from the plough layer (0–20 cm) of a paddy field in Nanjing, Jiangsu Province, China. The chemical and physical properties of the soil were as follows: organic matter = 17.5 g kg^{-1}; total N = 1.55 g kg^{-1}; total P = 0.89 g kg^{-1}; total K = 10.5 g kg^{-1}; pH(H_2O) = 6.7; CEC (cation exchange capacity) = 13.4 cmol (+) kg^{-1} soil; sand = 36.9%; silt = 48.4%; clay = 14.7%; total Cd = 1.15 g kg^{-1}; extractable Cd = 0.03 g kg^{-1}.

After soil moisture content was adjusted by air-drying to 60% of the water holding capacity, soils were sieved with a 5-mm mesh. Prior to planting, all soils were fertilized with urea, KH_2PO_4, and K_2SO_4 at 0.20 g N, 0.12 g P, and 0.26 g K per kg of soil, respectively. Half of the N fertilizer and the full dose of P and K were applied as a basal dose. The remaining half of the N fertilizer was applied in two equal doses, first at the tillering stage and again during the panicle-initiation stage. Dry soil (7.5 kg) was added into individual plastic pots (22 cm diameter, 26 cm height). Pots were submerged in 3–4 cm distilled water, which was manually maintained throughout the experimental period. After planting, pots were arranged in a randomized complete block design with three replicates and rearranged monthly to minimize positional effects on rice growth.

We used 20 different rice cultivars in the experiment, including common hybrid rice, indica rice, and japonica rice (Table S1). The growing period of these cultivars was within 140 ± 10 d during 2011. Cultivar seeds were ordered from breeding institutes in south (e.g., Hunan) and north China (e.g., Shenyang). The seeds were sterilized in 1% NaClO for 10 min, rinsed with deionized water, and then germinated at 25 °C for 48 h. The germinated seeds were grown in a nursery paddy soil until three leaves were observed, then transplanted to the experiment plot.

The pot trials were conducted in six open-top chambers (OTCs; 3 m diameter, 5.2 m height). For a full description of the OTCs, please see Wu et al. (2009). The plants were split into two conditions (three OTCs each): elevated CO_2 (E [CO_2]) and ambient CO_2 (A [CO_2]). Under E [CO_2], plants were located in a 15-m-diameter ring, with pure CO_2 being sprayed from the periphery towards the center through tubes located approximately 0.5 m above the rice canopy, while A [CO_2] had no ring structures. The E [CO_2] condition was set to have a CO_2 concentration that was 300 μmol mol^{-1} higher than the A [CO_2] condition; actual season-long average CO_2 concentrations in elevated plots ranged from 200 to 300 μmol mol^{-1} higher than the average in ambient plots, which was 380 ± 30 μmol mol^{-1}. The average temperature during the growing season was 30 °C, while the average photosynthetic flux density was 540 μmol m^{-2} s^{-1}.

2.2. Sample preparation and analytical methods

At maturity, rice was harvested, separated into root and grain, oven-dried at 65 °C for 72 h, then weighed and pulverized to a homogeneous powder. Plant subsamples were then digested in a microwave oven (Mars240/50, CEM Corp., Matthews, NC, USA) with concentrated HNO_3/H_2O_2 (v/v = 7:1). The Cd concentration in the digested solution was measured with ICP-MS (inductively coupled plasma mass spectrometry; Agilent 7500a, Agilent Technologies, Santa Clara, CA, USA) equipped with a Babington nebulizer, a glass double-path spray chamber, and a standard quartz torch. All samples were analyzed in three independent replicates. As part of the quality assurance/quality control protocol, reagent blanks and standard samples (from the Center of National Standard Reference Material of China) were included in every digest of all replications to ensure analysis reliability.

2.3. Amino acid quantification

Grain powder (10 mg) was added into screw-cap tubes containing 6 M HCl (2 mL) and placed under a nitrogen atmosphere for 24 h at 110 °C. The acid was evaporated from the samples using a rotary evaporator, and the samples were washed three times in deionized water, then hydrolyzed in acetate buffer. Approximately 1 mL of the hydrolysate was put into an auto-sampler bottle and injected into an amino acid auto-analyzer (L-835-50, Hitachi). Amino acid concentrations per sample were calculated with reference to standard samples (from the Center of National Standard Reference Material of China) and expressed as percentage rice powder.

2.4. Statistical analysis

We analyzed our data using one-way ANOVA in SPSS version 21. Significant differences between individual means ($p < 0.05$) were tested using Duncan's multiple range tests if the ANOVA rejected the null hypothesis of equal means.

3. Results

3.1. Dry matter

High CO_2 concentrations changed rice grain and root biomass. Of the 20 cultivars, seven exhibited significant increases (15.4–36.8%) in biomass under elevated CO_2 compared with ambient CO_2. Additionally, several cultivars decreased significantly in grain weight when subjected to high CO_2 concentrations (Fig. 1).

3.2. Grain Cd concentration and uptake

Elevated CO_2 significantly increased rice-grain Cd concentrations by 3.6–18.1% in seven out of the 20 cultivars grown in Cd-contaminated soils (Fig. 2). Five cultivars (2, 4, 10, 13, 19) actually experienced 0.1–10.4% decreases in Cd concentrations under elevated CO_2, but of these, only the 10.4% decrease in cultivar 13 was significant.

Overall, elevated CO_2 altered Cd uptake in rice grains compared with ambient conditions. With 16 out of 20 cultivars exhibiting a 0.02–50.6% increase under elevated CO_2 levels over that ambient