Increasing CO₂ 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, China

^b Centre for Research in Ecotoxicology and Environmental Remediation, Agro-Environmental Protection Institute, Tianjin 300191, China

*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),

Abstract

Environmental pollution by both ambient CO₂ and heavy metals has been steadily increasing, but we do not know how fluctuating CO₂ concentrations influence plant nutrients under high Cd pollution, especially in crops. Here, we studied the effects of elevated CO₂ 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 CO₂ levels. We found that although Cd concentrations appeared to be higher in most cultivars under elevated CO₂ than under ambient CO₂, the effect was significant only in seven cultivars. Combined exposure to Cd and elevated CO₂ strongly decreased rice protein and amino acid profiles, including essential and non-essential amino acids. Under elevated CO₂, the ratios of specific amino acids were either higher or lower than the optimal ratios provided by FAO/WHO, suggesting that CO₂ 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 CO₂ further exacerbates the problem.

Capsule

Combined exposure to Cd and elevated CO₂ strongly decreased both essential and non-essential amino acids in 20 rice cultivars.

Key Words: cadmium, rice, amino acids, CO₂ elevation, food safety

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 nutrient content, including AAs (Myers et al., 2014). Average atmospheric CO₂ 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 CO₂ 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 CO₂ can seriously degrade rice quality worldwide, through decreasing nutrient (protein and amino acids) concentration and increasing pollutant accumulation. Thus, high atmospheric CO₂ 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 CO₂ concentrations. In this study, our goal was therefore to provide more data describing the consequences of elevated CO₂ on rice grown in Cd-contaminated soil and to quantify CO₂- 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(H₂O) = 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, KH₂PO₄, and K₂SO₄ 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 CO₂ (E[CO₂]) and ambient CO₂ (A[CO₂]). Under E[CO₂], plants were located in a 15-m-diameter ring, with pure CO₂ being sprayed from the periphery towards the center through tubes located approximately 0.5 m above the rice canopy, while A[CO₂] had no ring structures. The E[CO₂] condition was set to have a CO₂ concentration that was 300 μmol mol⁻¹ higher than the A[CO₂] condition; actual season-long average CO₂

concentrations in elevated plots ranged from 200 to 300 $\mu\text{mol mol}^{-1}$ higher than the average in ambient plots, which was $380 \pm 30 \mu\text{mol mol}^{-1}$. The average temperature during the growing season was 30°C, while the average photosynthetic flux density was $540 \mu\text{mol m}^{-2} \text{s}^{-1}$.

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 $\text{HNO}_3/\text{H}_2\text{O}_2$ (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 CO₂ concentrations changed rice grain and root biomass. Of the 20 cultivars, seven exhibited significant increases (15.4-36.8%) in biomass under elevated CO₂ compared with ambient CO₂. Additionally, several cultivars decreased significantly in grain weight when subjected to high CO₂ concentrations (**Fig. 1**).

3.2 Grain Cd concentration and uptake

Elevated CO₂ 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 CO₂, but of these, only the 10.4% decrease in cultivar 13 was significant.

Overall, elevated CO₂ 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 CO₂ levels over that ambient CO₂. While there were seven cultivars significant differences in Fig. 2, but a significant decrease in case of one cultivar (13).

3.3 Grain protein and amino acid profile

Although 75% of the cultivars experienced an average 6.2% decrease in protein concentrations under elevated CO₂ compared to ambient CO₂, the differences were only significant in six cultivars. Furthermore, while protein concentrations appeared to increase in five cultivars under elevated CO₂, none of the increases was significant (**Fig. 3**).

Amino acid concentration in all cultivars varied naturally under ambient CO₂; Glu concentration was the highest, followed by aspartic acid (Asp) and Leu, with histidine (His) concentration being the lowest. Relative patterns in AA concentration remained unchanged at elevated CO₂, but total AA experienced a significant, 0.1–16.7% decrease in most cultivars, with the exception of cultivars 2, 8, 15, and 16.

Total EAA (threonine [Thr], valine [Val], methionine [Met], cysteine [Cys], Ile, Leu, phenylalanine [Phe], and Lys) concentration decreased significantly under elevated CO₂, by 70% (**Fig. 4**), whereas non-essential AAs (NEAAs; Asp, serine [Ser], Glu, Gly, His, arginine [Arg], and proline [Pro]) decreased significantly by 55% (**Fig. 5**). Individual reductions of EAAs ranged from 0.1% to 56.4%, and from 0.1% to 25.5% for NEAAs. Nevertheless, we also observed increases of EAAs and NEAAs

in few cultivars.

The effects of Cd accumulation differed across cultivars in terms of EAA and NEAA concentration, although all AAs decreased in every cultivar (**Tables 1 and 2**). Under ambient CO₂, both EAAs and NEAAs were negatively correlated with Cd contamination in cultivars 3, 7, 10, 16, and 19, but only Thr in cultivar 7 ($r = -1.000$, $p < 0.05$) and Pro in cultivar 3 ($r = -0.999$, $p < 0.05$) significantly decreased as Cd increased. Additionally, only AAs in cultivar 15 exhibited significantly positive correlations ($r = 0.999$, $p < 0.05$) with Cd under ambient CO₂ (**Table 1**).

Under elevated CO₂, we also observed cultivar differences in EAA and NEAA variation with the degree of Cd contamination: the relationship was more negative in cultivars 5, 6, 7, 11, 12, and 17. Cultivars 8, 16, and 20 showed positive correlations between AA and Cd levels, but they were not significant. However, other AAs (Thr, Val, Lys, Glu, and Arg) in cultivar 4 were positively and significantly correlated with Cd in grain (**Table 2**).

4. Discussion

4.1 Rice growth and Cd accumulation

We found that cultivars tended to exhibit variation in Cd accumulation under elevated CO₂, putting our results in line with previous reports of inter-cultivar differences in heavy-metal accumulation and dilution (Guo et al., 2006; Högy and Fangmeier, 2008; Li et al., 2010). Furthermore, rice biomass increased under elevated CO₂ for some cultivars, suggesting that greater biomass may be a cause of increased Cd accumulation. This explanation is supported by phytoremediation research showing that elevated CO₂ increases both plant biomass and total heavy metal (Cd) uptake. However, we note that in a few cultivars, elevated CO₂ caused a decrease in Cd concentration and uptake (Fig. 2). Previous work examining the effects of elevated CO₂ on rice cultivars (Guo et al., 2011; Li et al., 2010) and wheat grain (Pleijel, 2012) also found similar patterns to Cd concentrations. A likely explanation for this CO₂-induced decrease in Cd is the dilution effect, dilution of non-carbon compounds by increased carbohydrate concentrations from enhanced photosynthesis (Poorter et al. 1997). Another possibility is that elevated CO₂ negatively affects transpiration (Uddling et al., 2008; Wang et al., 2013), leading to improved water use efficiency. Since Cd is translocated from the root to the shoot

via the xylem (Salt et al., 1995), reduced transpiration should result in lower Cd concentrations. Although more research will be necessary to clarify the mechanisms underlying Cd reduction, our results suggest that elevated CO₂ can exert a positive effect on food security in this regard, contrasting negative effects on proteins and amino acids (see section 4.2).

Our current study only examined inter-cultivar variation in Cd accumulation under differing CO₂ levels. However, the influence of CO₂ on heavy metal concentrations can vary depending on multiple other factors, such as the type of heavy metal and plant growth conditions (e.g., water use efficiency) (Guo et al., 2011; Jia et al., 2011b; Li et al., 2010; Pleijel and Högy, 2015; Wu et al., 2009). Thus, a fruitful direction for future investigation is to examine how rice reacts to heavy metals other than Cd and to verify how heavy-metal uptake may change under various growth conditions.

4.2 Both CO₂ and Cd elevation affected rice protein and amino acid concentrations

We demonstrated that elevated CO₂ decreased AA and therefore protein concentration of rice grains in Cd-contaminated soil, suggesting that CO₂ treatment reduced rice nutrition and quality, in line with our hypothesis. These data accord with the results of previous analyses examining the effects of high CO₂ concentrations on both rice and other food crops (Taub et al., 2008; Usui et al., 2015). Our observed 5.9% mean decrease in protein concentration across 20 rice cultivars was close to other findings in rice (5.6% decrease under free-air-CO₂-enrichment experiments) (Zhou et al., 2012), and slightly less than percentage decreases from a meta-analysis including non-rice crops (9.9% decrease) (Taub et al., 2008). The significant interaction between CO₂ levels and cultivars indicates that variation in rice protein concentration has a genetic component. Specifically, cultivars may differ in N metabolism, which is closely related to AA concentration and protein synthesis.

Changes to N metabolism and uptake may be a potential mechanism behind why excess CO₂ decreases protein concentration even though CO₂ is essential for photosynthesis. Increases in CO₂ concentration alter N distribution throughout the plant via affecting stomatal closure rates-N vertical transfer increases N concentration in the roots and decreases aboveground N content. Additionally, N use by the plant increases concomitantly with a reduction in demand (Pang et al., 2006).

We also found an interactive effect between Cd contamination and CO₂ levels on AA concentration. The overall trend was a decrease in total AA across most cultivars that increased Cd uptake. This pattern was likely the result of Cd modifying and degrading proteins, subsequently leading to the inhibition of AA synthesis (Davies et al., 1987). However, the effect of Cd and CO₂ on AA concentration exhibited inter-cultivar variation, suggesting a genetic basis. Specifically, cultivars varied widely in the degree of Cd accumulation, and individual AAs within cultivars responded differently to the two pollutants. This pattern could potentially be attributed to variation in rice defense systems. For example, in some cultivars, Glu and Gly increased significantly instead of decreasing. These two AAs are components of glutathione and phytochelatins, which are involved in antioxidant and heavy-metal detoxification pathways, respectively. Although more research will be required to confirm this function, elevated CO₂ may actually allow these cultivars to increase their adaptive response against Cd, which is capable of inducing the production of reactive oxygen species (Davies et al., 1987).

Currently, we do not know the exact mechanism behind AA decline associated with Cd contamination, but one probable explanation is that many AAs are oxidized and damaged by the increased amount of reactive oxygen species. In response, the plant defense pathways are triggered, spurring an increase in the synthesis of defense-related AAs. Some examples include His and Pro. The former is involved in nickel chelation (Kramer et al., 1996), while the latter functions as a radical scavenger (Matysik et al., 2002) and may be involved in metal-ion chelation (Sharma and Dietz, 2006). Unsurprisingly, Pro is often found in plants experiencing heavy-metal stress (Matysik et al., 2002). Future studies should build on what we have shown here and further clarify the mechanisms underlying an interactive effect of CO₂ and Cd on AA concentration in rice, including which the exact roles of AAs that are selectively increased.

4.3 Implications for human health

In southern China, rice accounts for nearly 80% of total caloric intake (Du et al., 2013; Se et al., 2015). The contribution of Cd is a probable explanation for why excessive Cd accumulation in rice poses a potential health risk to populations dependent upon a rice-based diet (Bian et al., 2013; Chaney et al., 2004; Uraguchi and Fujiwara, 2013). We found that combined Cd and CO₂ stress severely lowered both

the EAA and NEAA concentration of rice, with the decline being more prominent among EAAs.

While the effects of Cd on AA concentration are unequivocally negative, the effects of elevated CO₂ in Cd-contaminated soil are more complex. Furthermore, at high levels, the products of certain AAs (e.g., ammonia, homocysteine, and asymmetric dimethylarginine) are implicated in various disorders, including neurological and cardiovascular disease. When we compared our ratios of individual EAAs to total EAAs to ratios from FAO/WHO (1973), we noted that several ratios were actually higher under our experimental conditions (**Table 3**). Additionally, elevated CO₂ lowered the ratios of select AAs, resulting in an overall more balanced EAA profile, compared with the FAO/WHO-recommended EAA ratios. These CO₂-induced deviations from the recommended AA ratio may exacerbate existing AA deficiencies in a population obtaining most of their AAs from rice. Thus, even if elevated CO₂ increases the amount of AAs, the ultimate effect is to disrupt optimal AA ratios in rice, which is unlikely to be beneficial for human health. We note, however, that the unique Cd-and-CO₂-induced changes in AA profiles suggest applications as biomarkers for the presence of multiple stressors that influenced crops during development.

5. Conclusion

We conclude that elevated CO₂ may reduce Cd uptake in rice cultivars, but this positive effect is smaller than the negative outcome of reducing protein and amino acid concentrations. Specifically, EAA synthesis was more severely inhibited under elevated CO₂ than under ambient CO₂. Thus, elevated CO₂ decreases AA and therefore protein concentration in most rice grains. The magnitude of the effects exerted by CO₂ may depend on site-specific nutrient availability in relation to the needs of rice plants. Although further research will be necessary to fully validate our findings, these data may help efforts to perform nutritional analysis on human populations to identify those most vulnerable to decreased dietary AA/protein availability from rice, allowing for targeted response efforts, including bio-fortification, supplementation, and the development of rice breeds with decreased CO₂ sensitivity. We propose that future studies examine the association between individual AA intake and Cd stress under elevated CO₂ in different populations dependent on rice-based diets.

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Figure Legends

Figure 1. Effect of elevated CO₂ in tissues of twenty rice **cultivars** grown on cadmium contamination soil. Asterisk (*) indicate significant differences between CO₂ treatments ($p < 0.05$) as determined by analysis of variance [ANOVA].

Figure 2. Effect of elevated CO₂ on cadmium concentration and uptake in grain of twenty rice **cultivars**. Asterisk (*) indicate significant differences between CO₂ treatments ($p < 0.05$) as determined by analysis of variance [ANOVA].

Figure 3. Effect of elevated CO₂ in protein of twenty rice **cultivars** grown on cadmium contamination soil. Asterisk (*) indicate significant differences between CO₂ treatments ($p < 0.05$) as determined by analysis of variance [ANOVA].

Figure 4. Effect of elevated CO₂ in profile of essential amino acids of twenty rice **cultivars** grown on cadmium contamination soil. Asterisk (*) indicate significant differences between CO₂ treatments ($p < 0.05$) as determined by analysis of variance [ANOVA].

Figure 5. Effect of elevated CO₂ in profile of non-essential amino acids of twenty rice **cultivars** grown on cadmium contamination soil. Asterisk (*) indicate significant differences between CO₂ treatments ($p < 0.05$) as determined by analysis of variance [ANOVA].

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Tables

Table 1 Correlations between essential and non-essential amino acids with grain Cd accumulation in ambient CO₂ level

amino acids	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Ile	0.655	0.915	-0.822	-0.901	-0.715	0.971	-0.982	0.500	0.143	-0.756	0.500	0.577	-0.189	0.866	0.968	-0.915	-0.945	0.992	-0.847	0.866
Leu	0.990	0.901	-0.997	-0.945	-0.655	0.961	-0.994	0.554	0.266	-0.866	0.569	0.775	0.115	0.852	0.982	-0.945	-0.945	0.990	-0.731	0.849
Val	0.655	0.901	-0.994	-0.866	-0.655		-0.982	0.596	0.500	-0.866	0.500	0.721	0.500	0.866	0.973	-0.945	-0.982	0.945	-0.648	0.918
Phe+Tyr	0.937	0.896	0.995	-0.957	-0.557	0.973	-0.979	0.490	0.097	-0.276	0.435	-0.700	0.051	0.914	1.000*	-0.931	-0.945	0.990	0.759	0.834
Lys	-0.866	0.901	-0.963	-0.655	-0.655	-0.971	-0.982	0.000	0.217	-0.500	0.444	0.277	0.189	0.839	0.971	-0.945	0.982		-0.731	0.866
Thr	-0.655	0.886	-0.997	-0.655	-0.564		-1.000**	0.397	0.327	-0.866	0.569	0.577	-0.500	0.839	0.997*	-0.961		0.945	-0.648	0.866
Met+Cys	0.945	0.934	0.997	0.990	0.655	0.971	-0.756	-0.500		-0.866	0.500	-0.721	0.000	0.866	0.982	-0.945	-0.982	-0.929	-0.292	0.866
Ala	0.982	0.892	-0.997	0.655	-0.655	-0.961		0.397	0.387	-0.866	0.596	0.775	-0.189	0.843	0.982	-0.945	0.945	0.990	-0.731	0.866
Asp	-0.945	0.901	-0.997	0.945	-0.680	-0.971	-0.968	0.500	0.277	-0.866	0.529	0.636	0.000	0.866	0.982	-0.957	0.655	0.975	-0.731	0.885
Glu	0.969	0.901	-0.981	0.959	-0.643	-0.971	-0.982	0.500	0.327	-0.846	0.500	0.839	-0.115	0.866	0.985	-0.945	0.655	0.985	-0.731	0.874
Gly	-0.990	0.907	-0.997	0.982	-0.715	-0.721	-0.929	0.500	0.327	-0.945	0.500		0.500	0.866	0.963	-0.945	0.945	0.990	-0.731	0.918
Pro	0.655	0.901	-0.999*	-0.945	-0.715		-0.994	0.470	0.327	0.803	0.466	0.721	0.000	0.846	0.977	-0.945	0.945	-0.990	-0.700	0.500
Ser	-0.976	0.897	-0.997	-0.945	-0.655	-0.971	-0.982	0.569	0.404	-0.904	0.500	0.839	0.500	0.849	0.982	-0.922		0.990	-0.731	0.896
Arg	-0.945	0.919	-0.997	-0.929	-0.655	-0.908	-0.945	0.444	0.500		0.529	0.721	0.000	0.877	0.975	-0.950	0.969	0.990	-0.766	-0.866
His	-0.901		-0.963	-0.982	-0.655	-0.971	-0.982	0.000	0.327		0.500			0.866	0.982	-0.901	0.655	0.786	-0.589	0.866

Note: *p<0.05; **p<0.01.

Table 2 Correlations between essential and non-essential amino acids with grain Cd accumulation in elevated CO₂ level

amino acids	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Ile	-0.5	-0.115	0.091	0.866	-0.933	-0.866	-0.945	0.655	0.756	0.961	-0.826	-0.924	-0.967	-0.659	0.327	0.956		0.956	-0.993	0.978
Leu	0	0	0.129	0.982	-0.933	-0.866	-0.922	0.655	0.803	0.961	-0.756	-0.924	0.965		0.356	0.956	-0.866	-0.594	-0.933	0.987
Val	0.5	-0.082	0.091	1.000**		-0.918	-0.945	0.590	0.866	0.961	-0.756	-0.924	-0.711	0.655	0.291	0.916	0.803	0.594	-0.984	0.984
Phe+Tyr	0.075	-0.247	0.098	0.999*	0.942	-0.887	-0.965	0.753	0.849	0.921	0.756	-0.892	0.970	-0.698	0.248	0.955	-0.892	0.658	0.944	0.984
Lys	0.115	-0.064	0.091	1.000**	-0.933	-0.500	-0.901	0.655	-0.866	0.961	-0.756	-0.924	-0.967	-0.655	0.434	0.975	-0.866	0.682	-0.933	0.971
Thr	0.5	-0.115	0.204	1.000**	-0.933	-0.945	-0.976	0.655	0.866	0.923	-0.826	-0.962	0.711	-0.655	0.327	0.956	-0.500	0.682	-0.933	0.975
Met+Cys	0.5	0	0.091	-0.866	-0.629	-0.866	-0.945	0.655	0.866	0.971	-0.756	-0.924	-0.967	-0.655	0.327	0.957		0.225		1.000**
Ala	0.5	0	0.027	0.993	-0.968	-0.866	-0.901	0.189	0.822	0.935	-0.756	-0.924	-0.965	-0.655	0.266	0.983	-0.756	-0.682	-0.629	0.984
Asp	-0.115	0	0.154	0.997	-0.933	-0.866	-0.935	0.189	0.803	0.961	-0.712	-0.924	-0.711	0.786	0.298	0.956	-0.500	-0.225	-0.954	0.987
Glu	-0.115	-0.044	0.060	1.000**	-0.933	-0.882	-0.939	0.688	0.866	0.961	-0.756	-0.917	0.967	0.564	0.327	0.956	-0.891	-0.710	-0.933	0.984
Gly	0.5	-0.5	0.091	0.982	-0.886	-0.866	-0.915	0.945	0.866	0.891	-0.982	-0.924	0.965	-0.655	0.404	0.956	-1.000**	0.808	-0.933	0.984
Pro	0	0	-0.204	0.993	-0.933	0.843	-0.955	0.655	-0.945	-0.693	-0.756	0.943	0.985	-0.655	-0.369		-0.822	0.808	0.933	0.984
Ser	-0.5	0	0.204	-0.866	-0.960	-0.866	-0.922	0.500	0.866	0.986	-0.756	-0.890	0.902	-0.655	0.327	0.928	-0.756	-0.532	-0.968	
Arg	0.5	0	0.129	1.000**	-0.901	-0.918	-0.929	0.564	0.756	0.996	-0.756	-0.890	0.967	0.655	0.351	0.956	-0.866	0.682	0.901	
His	0	0.5	-0.024	-0.866	-0.933	-0.945	-0.990			0.961	-0.756	-0.962	0.711	-0.189	0.327	0.656		0.682	-0.933	

Note: *p<0.05; **p<0.01.

Table 3 The ratio of essential amino acids to total amino acids in rice grain

Amino acids	RA[CO ₂]	RE[CO ₂]	FAO/WHO ^a
Thr	4.06	4.05	4
Met+Cys	4.27	4.10	3.5
Ile	4.05	3.94	4
Val	5.02	4.99	5
Lys	4.28	4.26	5.5
Leu	8.76	8.78	7
Phe+Tyr	8.71	8.81	6

Note: each value is an average of twenty rice cultivars. ^a The FAO/WHO expert panel's advice on the pattern for the requirements of nutrition.