applied sciences

Brief Report

Increased Grain Amino Acid Content in Rice with Earthworm Castings

Min Huang *, Chunrong Zhao and Yingbin Zou

Southern Regional Collaborative Innovation Center of Grain and Oil Crops (CICGO), Hunan Agricultural University, Changsha 410128, China; crzhao5@163.com (C.Z.); ybzou123@163.com (Y.Z.)

* Correspondence: mhuang@hunau.edu.cn; Tel.: +86-731-84618758

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Featured Application: Enhancing earthworm activity in the field may be a feasible way to increase grain amino acid content in rice.

Abstract: Enhancing the nutritional value of rice can improve the health of rice consumers. Grain amino acid content is an important nutritional component. This study was conducted to test the hypothesis that the application of earthworm castings could increase the grain amino acid content in rice. Results showed that total amino acid content in the grain of rice was significantly elevated by applying earthworm castings (17 kg m^{-2}), with an average increase of 8% across four tested rice cultivars. Application of earthworm castings had no significant effect on total nitrogen (N) content but significantly increased the ratio of amino acid to N (total amino acid content/total N content) in rice grains. The results of the present study suggest that application of earthworm castings can increase grain amino acid content in rice by improving the efficiency of the N to amino acid conversion, and highlight that further studies are required to assess the effects of earthworm castings on the amino acid metabolism in rice grains.

Keywords: amino acid content; earthworm castings; rice

1. Introduction

Rice feeds more than half of the global population [1], and many consumers of rice are among the world's poorest, with diets that are mainly restricted to rice as it is filling, accessible, and affordable [2]. However, rice is deficient in many nutrients [3], and thus malnutrition is common in countries where rice is the main food [4]. Therefore, it is important to enhance the nutritional value of rice to improve the health of rice consumers, especially those living in poverty.

Amino acid content is an important nutritional component on which many rice breeding programs have focused [5]. Grain amino acid content in rice is determined not only by genetic factors but also by environmental factors and management practices [6,7]. Previous studies demonstrated that there is a positive linear relationship between amino acid and nitrogen (N) contents in grains of rice [8], and that amino acid content in the rice grain can be increased by applying N fertilizer at the heading stage [6].

China is the largest producer and consumer of rice in the world, accounting for about a third of the global rice economy [9]. In the past several decades, the large input of N fertilizer across wide areas of China has caused considerable environmental costs, including soil acidification, water eutrophication, and enhanced N deposition [10–12]. Because of these effects, methods other than an artificial increase of N input are sought to increase rice grain amino acid content.

Earthworm castings, or vermicompost, are a form of fertilizer produced from earthworms as they digest soil organic matter. This excrement is enriched in mineral N [13]. Our recent study showed that application of earthworm castings could improve N uptake and utilization in rice [14]. The objective of the present study was to determine the effects of applying earthworm castings to soil on amino acid and N contents in the rice grain. We hypothesized that application of earthworm castings may increase grain amino acid content in rice.

2. Materials and Methods

A micro-plot field experiment was done in Changsha (28°11′ N, 113°04′ E), Hunan Province, China in 2017. The soil in the experimental field was a Fluvisol (FAO taxonomy) with the following chemical properties at the upper 20 cm layer: pH 5.75, 34.2 g organic matter kg^{-1}, 81.6 mg available N kg^{-1}, 34.4 mg available P kg^{-1}, and 56.7 mg available K kg^{-1}.

Thirty-two bottomless plastic boxes (length × width × height = 40 cm × 40 cm × 30 cm) were pushed into the soil of the experimental field to a depth of 20 cm to establish micro-plots. Four rice cultivars (Huanghuazhan, Liangyoupeijiu, Longliangyou 97, and Xiangliangyou 396) were grown and each micro-plot was treated with either earthworm castings (17 kg m^{-2}, EC17) or no castings (0 kg m^{-2}, EC0). The level of earthworm castings for EC17 was chosen according to the product of duration of oilseed rape-growing season (218 days) and daily production rate of earthworm castings (78 g m^{-2} day^{-1}) in a no-tillage rice-oilseed rape rotation field in Nanxian (29°21′ N, 112°25′ E), Hunan Province in 2015. The daily production rate of earthworm castings was obtained from an investigation conducted on 10 randomly selected 1-m^2 areas in the field on the first day after oilseed rape harvest. The earthworm castings (pH 7.89, 61.4 g organic matter kg^{-1}, 128 mg available N kg^{-1}, 44.2 mg available P kg^{-1}, and 254 mg available K kg^{-1}) used in the experiment were collected from several no-tillage rice-oilseed rape rotation fields in Nanxian after oilseed rape harvest in 2016. The micro-plots were arranged in a split-plot design with four replicates, using the level of earthworm castings as the main plots and cultivars as the subplots.

Rice seeds were pre-germinated and then sown in a seedbed on 10 May. Rice seedlings were transplanted on 5 June with one seedling per hill and four hills per micro-plot. Earthworm castings were applied and incorporated in the upper 20 cm of the soil on the day before transplanting. Urea was split in three applications: 6.0 g N m^{-2} at 1 day before transplanting (basal), 3.6 g N m^{-2} at 7 days after transplanting, and 2.4 g N m^{-2} at panicle initiation. Superphosphate was applied as basal at a rate of 4.8 g P_2O_5 m^{-2}. Potassium chloride (8.4 g K_2O m^{-2}) was split equally in two applications: one at basal and the other at panicle initiation. The micro-plots were flooded with a water depth of about 5 cm until 7 days before maturity. Agrochemicals were used to control diseases, insects, and weeds.

At maturity, rice grains were sampled from each micro-plot. The sampled grains were oven-dried at 70 °C to a constant weight and then hulled, ground, and sieved (100 mesh). About 1.0 g of the sieved sample was weighed, hydrolyzed, and derivatized to determine total amino acid content with high-performance liquid chromatography method [15]. Approximately 0.5 g of the sieved sample was digested by H_2SO_4-H_2O_2 method to determine the total N content. The N analysis was carried out using a segmented flow analyzer (SAN Plus, Skalar Inc., Breda, The Netherlands). The ratio of amino acid to N (total amino acid content/total N content) was calculated.

Data analysis including analysis of variance and linear regression analysis was performed using Statistix 8.0 (Analytical Software, Tallahassee, FL, USA).

3. Results and Discussion

Total amino acid content in rice grains was significantly influenced by both the level of earthworm castings and cultivar (Table 1). There was no significant interaction effect between the level of earthworm castings and cultivar on total amino acid content in the grain of rice. Averaged across the four cultivars, total amino acid content in the grain was 8% higher under EC17 than under EC0. Huanghuazhan had the highest total amino acid content in grains, more than 10% higher than that of the other three cultivars.

Table 1. Effects of earthworm casting application on total amino acid and nitrogen (N) content and on ratio of amino acid to N in grains of four rice cultivars.

Earthworm Castings	Cultivar	Total Amino Acid (mg g^{-1})	Total N (mg g^{-1})	Ratio of Amino Acid to N
EC0	Huanghuazhan	67.8 (3.52)	15.3 (0.28)	4.44 (0.232)
	Liangyoupeijiu	60.8 (2.40)	13.8 (0.05)	4.42 (0.179)
	Longliangyou 97	56.7 (3.63)	13.4 (0.22)	4.22 (0.288)
	Xiangliangyou 396	58.8 (3.40)	13.7 (0.07)	4.30 (0.250)
	Mean	61.0 (1.82)	14.1 (0.20)	4.35 (0.110)
EC17	Huanghuazhan	73.0 (3.61)	15.3 (0.07)	4.78 (0.218)
	Liangyoupeijiu	62.9 (2.49)	13.6 (0.09)	4.63 (0.198)
	Longliangyou 97	64.4 (1.67)	13.5 (0.01)	4.79 (0.125)
	Xiangliangyou 396	62.0 (2.62)	13.2 (0.11)	4.71 (0.183)
	Mean	65.6 (1.65)	13.9 (0.22)	4.73 (0.084)
Analysis of variance (*F*-value)				
Earthworm castings		4.65 *	2.20 ns	6.29 *
Cultivar		5.03 **	70.44 **	0.11 ns
Earthworm castings × Cultivar		0.34 ns	1.36 ns	0.24 ns

EC0 and EC17 represent 0 and 17 kg earthworm castings m^{-2}, respectively; Values in parentheses are SE (n = 4); *, significance at the 0.05 probability level; **, significance at 0.01 probability level; ns, non-significance at the 0.05 probability level.

N is a principal constitute element of amino acids. It is generally considered that amino acid content is positively linearly related to N content in the rice grain [8]. Some findings of this study are consistent with those of previous studies: Huanghuazhan, the cultivar with highest grain amino acid content, also had higher grain N content than the other three cultivars (Table 1). However, unexpectedly, no significant difference was recorded in the grain N content between the EC17 and EC0 treatments. Thus, increasing the grain N content was not the mechanism for the positive impact of applying earthworm castings on amino acid content in rice grains. Our study also showed that the EC17 treatment had a significantly higher ratio of amino acid to N than EC0 (Table 1). This result suggests that the increased grain amino acid content in rice induced by applying earthworm castings might be mostly attributable to the improved efficiency of converting N to amino acid. This could be further supported and informed by that the linear regression between grain amino acid and N contents was different under EC17 and EC0 (Figure 1). These results suggest that further investigations are required to determine the effects of earthworm castings on the metabolism of amino acid in rice grains.

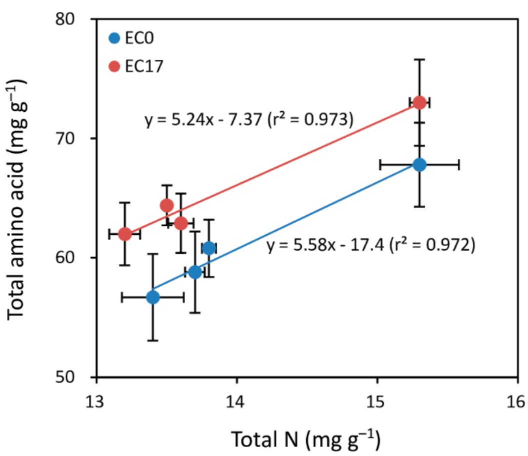

Figure 1. Linear regression between total amino acid and N contents in grains of four rice cultivars under two levels of earthworm castings. EC0 and EC17 represent 0 and 17 kg earthworm castings m^{-2}, respectively. Each data point is the mean for one cultivar under one level of earthworm castings. Error bars are SE (n = 4).

Application of earthworm castings can affect plant metabolism including protein synthetic activity [16,17]. In this regard, it is well documented that the castings and body secretions of earthworms contain a certain amount of plant hormones and hormone-like substances [18–20]. Earthworms can also enhance microbial biomass and activity due to their castings and mucus, and the byproducts of this microbial activity include plant hormones such as abscisic acids, auxins, cytokinins, ethylene, and gibberellins [21]. Although there is limited information available on the influences of plant hormones or plant hormone-like substances included in earthworm castings on amino acid metabolism in the rice crop, some relevant reports have been documented in other crops. For example, Muscolo et al. [22] found that earthworm-worked humic substances had an auxin-like effect on nitrogen metabolism in wild carrot, and Singh et al. [23] observed that gibberellic acid-like activity of vermicompost leachate resulted in an increase in amino acids in the common bean. Further study is required to determine if similar effects of plant hormones and hormone-like substances in earthworm castings are observed on amino acid metabolism in rice grains.

Application of earthworm castings has the potential for increasing amino acid content in rice grains, although the practicality of any approach must be taken into account. High labor costs are the key factor limiting the adoption of organic manures. In this study, the tested earthworm castings were not specially produced but were collected from no-tillage rice-oilseed rape rotation fields, where earthworms are known to be abundant (pers. obs.). Reduced or absent soil tillage in these fields can provide earthworms with an undisturbed biotope and hence may be favorable to increasing their populations, while oilseed rape plants can provide abundant food for earthworms by producing large volumes of residues. In addition, our previous study documented that adoption of no-tillage rice-oilseed rape cropping systems can maintain crop yields while saving labor [24]. Therefore, development of similar no-tillage systems may be an indirect but feasible way to increase grain amino acid content in rice by increasing earthworm activity.

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