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Short Communication

Effects of Exogenous Amino Acids Application on Nitrogen Accumulation, Yield and Grain Quality in Rice

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Abstract

The improvement of nitrogen utilization efficiency and the increase of essential amino acid contents in rice is the main purpose of reducing fertilizer and increasing quality in rice production. An experiment was conducted in order to explore the effects of exogenous amino acids on the nitrogen accumulation and the content of essential amino acids in rice: for the 6 treatment groups, 500 mg/L of eachL-leucine-^{15}N and L-lysine-^{15}N sprayed on the rice leaves at the tillering, heading and the ripening stages, and for the control group, only water was sprayed. The results showed that exogenous L-leucine-^{15}N and L –lysine-^{15}N increased the content of nitrogen and essential amino acid in leaves, stems of rice ($P< 0.05$), and with the increase of spraying times, the content of nitrogen and essential amino acid increased. At the same time, it was found that the 50% of N^{15} absorbed by leaves is mainly transported to rice grains. In conclusion, the exogenous application of amino acids could significantly increase the accumulation of nitrogen and the content of essential amino acids in grain; improve the yield and quality of rice.

Keywords: Rice; L- leucine -^{15}N; L-lysine -^{15}N; Nitrogen content; Essential amino acid

Introduction

Essential amino acid is a key component of food protein that cannot be synthesized by the body itself, or whose synthesis cannot meet the needs of the body, and therefore it must be obtained from food. Essential amino acid has an irreplaceable role in human's growth and health care. The daily needs of it by toddlers, children and adults are about 714 mg/kg, 352 mg/kg and 84 mg/kg of the body weights (Shi, 2012; Ghosh and Uauy, 2016). People mainly absorb essential amino acids from animal and plant protein, but overly relying on meat will cause hypertension, hyperlipidemia, coronary heart disease and other associated diseases (Swain *et al*., 2008). It is an important means to solve this problem by changing the composition of plant protein and increasing the content of essential amino acids. Rice is the staple food for more than half of the world's population. Therefore, if the exogenous essential amino acids can be transferred into rice, their contents will be increased, and people's dietary pattern will be changed. It is of great significance to strengthen the nutrition and health of human body.

At present, there is a common concern all over the world, that is, the excessive application of fertilizers and pesticides has caused water eutrophication and influenced the quality and safety of agricultural products (Fang *et al*., 2013). Reducing the amount of fertilizers and pesticides, and improving the yield and quality of rice is a hot topic in the field of scientific research (Tan, 2015). In recent years, some studies have been done on the improvement of the content of essential amino acids in rice and the related factors (Wu *et al*., 2004; Rong *et al*., 2007; Sha *et al*., 2012; Zhou *et al*., 2015), the methods and factors of improving the content of essential amino acids in rice (Dai *et al*., 2006; Lu *et al*., 2007; Wang *et al*., 2011; Zhang *et al*., 2014). Strain differences, cultivation measures, environmental factors, fertilization and chemical regulation can affect the content of amino acid in rice, and the most important external factor is fertilization: different fertilizing methods, especially the fertilizers amount and time of application can significantly affect the content of amino acid in rice (Xu *et al*., 2003; Dwivedi *et al*., 2012; He *et al*., 2013). In agricultural production, spraying amino acid is an important means to improve the quality of rice, and it can reduce amount of fertilizer and pollution to the environment (Li *et al*., 2009; Lu *et al.*, 2010; Sã *et al.*, 2016). But there are few studies on the mechanism how amino acids influence rice quality improvement, and the specific effects of a certain amino acid spray on the quality of rice. In the previous studies, YS-45 endophytic *Bacillus subtilis* was separated from the citrus and used to ferment soybean residue, to make

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soybean-amino acid nutrient solution, which can be sprayed on rice leaves to increase the amino acids content in rice (Liu *et al.*, 2011; Zhang *et al.*, 2009, 2013). Are the amino acids absorbed by the rice plant directly used for protein synthesis? And what effects do amino acids exert on the growth and development of rice and the content of essential amino acids in rice? To solve these problems is the premise to clarify the mechanism of how exogenous amino acids can improve their contents in rice. This study explores the effects of exogenous amino acids on the nitrogen accumulation and their contents in rice, based on the spraying certain concentrations of exogenous L-leucine-^{15}N and L-lysine-^{15}Non the rice leaves at tillering, heading and ripening stages. This research is of great significance in the reuse of agricultural by-products processing wastes and the improvement of rice quality.

Materials and Methods

Experimental Details and Treatments

The C-liangyou166 used in the experiment was offered by the breeding lab of the Institute of Nuclear Agriculture and Space Breeding in Hunan Province; the L-leucine-^{15}N with abundance of 98.87% and the L-lysine-^{15}N with abundance of 98.54%, have been both bought from China Isotope Cooperation; the cultivation ceramic pots bought from Changsha Hongxing Flower Market.

General Condition of the Experiment

The experiment was conducted from June to September, 2014, in the natural network room of the Institute of Nuclear Agriculture and Space Breeding in Hunan Province. The cultivation soil was the reddish clayey soil developed from the quaternary red clay, the compound including organic matter 24.3 $g \cdot kg^{-1}$, total nitrogen 1.42 $g \cdot kg^{-1}$, available nitrogen of 178.5 $mg \cdot kg^{-1}$, phosphorus of 25.4 $mg \cdot kg^{-1}$, K 237.4 $mg \cdot kg^{-1}$, with pH of 5.3. Before the transplanting the rice plant, every pot was fertilized with 10 g compound fertilizer (the total primary nutrient ≥ 40.0%, N-P_2O_5-K_2O being 18-10-12), and 6g urea at the tillering stage.

Methodology and Procedures

The rice plants were pot-cultivated. This experiment was consisted of seven treatments, each treatment repeated three times, and each time two 25-days old rice seedlings were planted.

The plants were sprayed with 200 mL solution of L-leucine-^{15}N (L-Leu-^{15}N), each of 0.05%, 0.1% and 0.15% and L-Lysine-^{15}N (L-Lys-^{15}N) of 0.05% , 0.1% and 0.15% , using double distilled sprayed water as control (CK). L-Leu-^{15}N 0.05% and L-Lys-^{15}N 0.05% were prayed at tillering stage, L-Leu-^{15}N 0.1% and L-Lys-^{15}N 0.1% at tillering and heading stages, while, L-Leu-^{15}N 0.15% and L-Lys-^{15}N 0.15% at tillering, heading and ripening stages.

When L-leucine-^{15}N and L-lysine-^{15}N in every growth period, the 3 pots of rice plants in every treatment were sprayed and put into triangle. And in order to ensure the L-leucine-^{15}N and L-lysine-^{15}N are fully absorbed, sprayed at 10:00 am and 5:00 pm every day.

The leaves were cut down together with their sheathes, and washed 3 times with clean water and once with deionized water, dried with sterilized gauze, wrapped in 105°C for 12 h, and then dried to the constant weight. The stems, with their sheathes and grains stripped off, were cut off in the root neck, and dried with the same method as for the leaves. And the height of the plants was measured from the surface of the soil to the highest ear (not including the awn).Ear grain number is the average of the panicle grain number of every plant. Seed setting rate (%) was obtained by dividing the average total grain number per panicle to the average solid grain number per panicle. Thousand grain weight was calculated as the weight of 1000 dried clean grains, after mixing the samples, with the difference of two weights no greater than 3% of their average. Single rice dry weight is the weight of the dried rice from a single rice plant. The amino acid was measured by HPLC (referring to the methods for measurement of the free amino acid of fruits and vegetable juice according to GB 12292-90). The grains were dried to constant weight after being washed by the deionized water. Milled rice was made from dried grains by a NZJ15/15-F Mini Rice Sheller (Hebei Deco Mechanical Technology Co. Ltd) specially used for experiments. The dried straw stems, leaves and grains were weighed with JY5001scale (Shanghai Precision Testing Equipment CO. Ltd).

Total nitrogen content were measured by Kjeldahl method and abundances of ^{15}N were using improved ZHT mass spectrometer (Beijing analytic instrumental factory) to measure at maturity.

Statistical Analysis

Excel2007 was used for the data processing and diagrams, and SPSS18.0, for the significance with LSD test.

Results

Effects on Rice Yields

Yield components showed that timing of spray had no significant effects on 1000 grain weight of rice ($P>$ 0.05), but do have on spike grain number and ripening rate ($P<0.05$), especially on the single rice dry weight ($P<0.01$). Spraying only at the tillering or at the tillering and heading stage has no significant effects on rice plant height and ear length($P>0.05$), but spraying at the tillering, heading and the ripening stages had significant effects on plant height, ear length, spike grain number and ripening rate of rice ($P<0.05$).Therefore, in the middle and late period of rice growth, spraying L-leucine and L-lysine can supply nutrition, prevent premature senility and degenerated spikelets of rice, reduce the amount of shrunken grains, and increase the amount of spike grain number and ripening rate, to improve the yield of rice (Table 1).

Effects on Nitrogen Accumulation in Rice

Leaf nitrogen content in rice: In the present study, the effects of exogenous amino acids on nitrogen content in rice leaves were significant ($P<0.05$), the content and frequency of spraying was positively correlated. The nitrogen content in rice leaves in all treatments, were significantly higher than in the control group (CK), by 22.5%, 36.5%, 45.5%, 22.2%, 36.9%, 46.1%, respectively. The content of ^{15}N in all treatments were 5.9%, 41.6%, 42.4%, 9.6%, 42.9% and 45.3% higher than in CK. These figures showed that the foliar absorption of L-leucine-^{15}N and L-lysine-^{15}N could promote the absorption of nitrogen fertilizer in rice roots (Fig. 1).

Effects on nitrogen content of rice stem: The contents of total N and ^{15}N in rice stems of six treatment groups were higher than in CK group: the content of total nitrogen (N) of all treatments were 53.6%, 71.2% and 84.1%, 55.1%, 73.1% and 100.9% higher, and the content of ^{15}N, 6.3%, 22.6%, 35.1%, 10.8%, 25.8% and 37.2% higher respectively (Fig. 2). The highest N contents were at the ripening stage, followed by the heading stage. These figures showed that leaves blades can absorb L-leucine and L-lysine efficiently, and transport them to the stem of rice to promote the growth of the rice stem and improve the yield (Fig. 2).

Influence on the content of nitrogen in milled rice: Spraying the exogenous amino acid had a significant influence on the content of nitrogen in rice. The contents of N and ^{15}N of milled rice in the all treatments was higher than those in CK group and the content of N in the all treatments were 13.7, 24.5, 40.4, 17.4, 28.3, and 41.3% higher, and ^{15}N, 3.5, 16.3, 21.5, 5.6, 19.2 and 22.9% higher respectively (Fig. 3). It is showed that the quality of rice improves with the increase in the content of total amino acid and protein in rice (milled rice), with the best effect when spraying exogenous amino acid at the heading and the ripening stage (Fig. 3).

Effects on ^{15}N content in the leaves, stems and rice(%): The contents of ^{15}N in the leaves, stems and rice by spraying at the tillering and the heading stages was higher than by spraying only at the tillering stage, while the contents even higher by spraying at all three stages than at the first two stages (Table 2). This reveals that the rice absorbs and transfers the L-leucine-^{15}N and L-lysine-^{15}N in the way of distribution to the nearest and addictive effect. In the same group the content of ^{15}N in leaves, stems, and rice presented obvious differences ($P \leqq 0.05$) (Table 2).

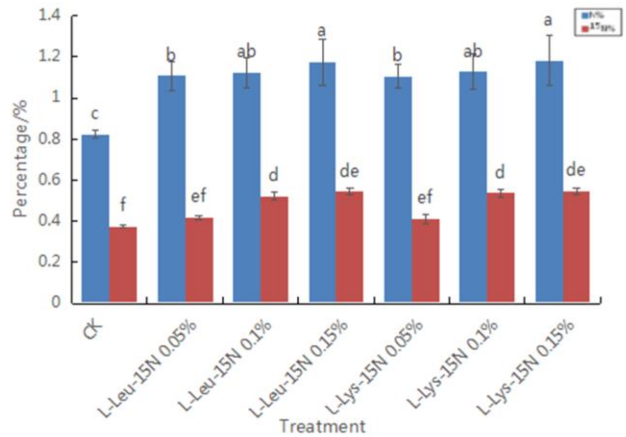

Fig. 1: Effects of spraying L- leucine-^{15}N and L- lysine-^{15}N on nitrogen content in rice leaves

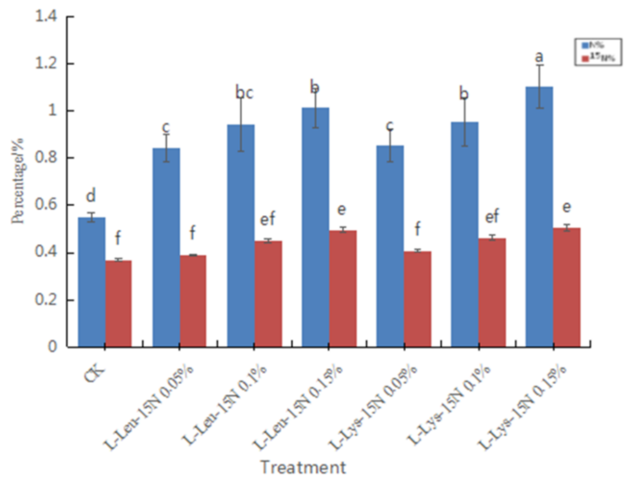

Fig. 2: Effects of spraying L-leucine-^{15}N and L-lysine-^{15}N on the nitrogen content in rice stems

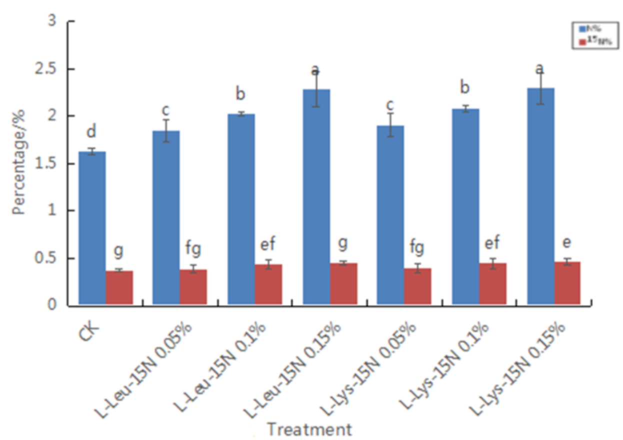

Fig. 3: Effects of spraying L-leucine-^{15}N and L-lysine-^{15}N on the nitrogen content in rice

Table 1: Effect of L-leucine-^{15}N and L-lysine-^{15}N on yield of rice

Treatment		Plant height/ cm	ear length/ cm	ear kernel number	Seed setting rate/%	The 1000 grain weight/ g	Single rice dry weight/ g
CK		113.5±3.0b	22.9±1.5ab	130.4±9.2b	81.0±2.8b	25.4±0.2a	54.0±1.9d
L- leucine -^{15}N	(0.05%)	113.6±2.5b	23.1±1.4b	137.3±9.0ab	81.5±3.3b	25.3±0.2a	63.8±4.5c
	(0.1%)	113.8±3.5b	23.1±1.2b	138.9±8.5ab	82.4±3.0ab	25.5±0.1a	70.2±1.8b
	(0.15%)	114.1±3.3a	24.2±1.8a	139.8±10.0a	84.6±2.1a	25.4±0.2a	78.5±2.8a
L- lysine -^{15}N	(0.05%)	113.4±2.6b	23.0±1.5b	138.3±9.0ab	81.4±3.2b	25.3±0.2a	64.1±4.2c
	(0.1%)	113.7±3.2b	23.3±1.1b	138.8±8.8ab	82.5±3.2ab	25.4±0.1a	70.4±1.7b
	(0.15%)	114.2±3.1a	24.1±1.9a	139.7±9.9a	84.7±2.1a	25.5±0.2a	78.8±2.7a

Note: In each column, values followed by different small letters are significantly different at $P<0.05$.The same as following

Table 2: ^{15}N content increased in leaves, stems and rice (%)

Organs	CK	L- leucine -^{15}N			L- lysine -^{15}N		
		(0.05%)	(0.1%)	(0.15%)	(0.05%)	(0.1%)	(0.15%)
Leaf	5.3±0.1d	5.9±0.4d	41.6±0.2b	42.4±0.5b	9.6±0.5c	42.9±0.7b	45.3±1.0a
Stem	5.9±0.2f	6.3±0.1f	22.6±0.3d	28.1±0.3b	10.8±0.4e	25.8±0.3c	37.2±0.8a
Rice	3.4±0.1e	3.5±0.2e	16.3±0.1c	21.1±0.6a	5.6±0.3d	19.2±0.5b	22.9±0.5a

Table 3: Percentage of nitrogen in leaves, stems and rice from ^{15}N (%)

Organs	CK	L- leucine -^{15}N			L- lysine -^{15}N		
		(0.05%)	(0.1%)	(0.15%)	(0.05%)	(0.1%)	(0.15%)
Leaf	2.45±0.21c	2.65±0.35c	7.29±0.81b	10.66±0.87a	2.74±0.24c	7.50±0.81b	11.07±0.35a
Stem	1.20±0.11c	1.24±0.12c	4.67±0.45b	7.79±0.85ab	1.29±0.08c	4.79±0.23b	7.92±0.56a
Rice	3.78±0.25c	3.87±0.54c	10.14±0.65b	14.54±0.54a	3.95±0.20c	10.40±0.62b	14.77±0.41a

Table 4: Influence of spraying L- leucine-^{15}N and L- lysine-^{15}N on the content of essential amino acid in rice

Test item (mg/100 g)	CK	L- leucine -^{15}N			L- lysine -^{15}N		
		(0.05%)	(0.1%)	(0.15%)	(0.05%)	(0.1%)	(0.15%)
His	129±10d	135±7c	148±8b	157±7a	138±8c	149±7b	155±8a
Thr	220±8d	245±8c	264±11b	285±14a	248±8c	269±12b	290±9a
Met	110±7d	130±6c	138±8c	142±7b	132±10c	141±12b	147±8a
Val	425±12f	440±8d	477±12c	482±10b	446±10e	480±12b	489±9a
Phe	390±7d	400±9c	425±10b	450±7a	403±8c	430±10b	455±6a
Ile	337±5d	365±6bc	386±6b	412±10a	355±5c	379±12bc	405±6a
Leu	580±11e	650±15cd	785±5bc	820±9a	628±12d	750±9c	793±12ab
Lys	234±7f	250±10e	263±8d	282±7bc	275±7c	308±5b	329±9a
Total	2425	2615	2886	3030	2625	2906	3063
Relative to CK±%	-	7.8%	19.0%	24.9%	8.2%	19.8%	26.3%

Distribution of amino acid nitrogen (^{15}N) absorbed in leaves, stems and rice: Among the treatments, T3 and T6 absorbed the greatest rate, amounting to 33% of the sprayed ^{15}N (Table 3). About 50% of the exogenous amino acid absorbed was transferred to the rice, 30% to the leaves, and 20% to the stems.

However, the leaves' absorption rate of exogenous amino acid was not high because of the loss in the process of spraying, the influence of temperature, humidity, light and so on, and all lead to the waste of exogenous amino acid and the low absorption (Table 3).

Influence on the Essential Amino Acids Contents in Rice

Spraying L-leucine-^{15}N and L-lysine-^{15}N increased the contents of the essential amino acid in rice (Table 4). In treatments applied with L-Leu-^{15}N L-leucine-^{15}N contents increased, and likely L-Lys-^{15}N with respective treatments increased L-lysine-^{15}N and the contents of the essential amino acids in rice was higher by spraying at the tillering and the heading stages than spraying only at the tillering stage and even higher by spraying at all three stages than at the first two stages. Compared with CK, the content of essential amino acid in the all treatment groups had significant difference ($P \leqq 0.05$), 7–27% higher than CK (Table 4).

Discussion

Most of terrestrial plants absorb nutrients by roots, but the leaves can also absorb exogenous substances, such as gas, nutrients and pesticides (Pinder III *et al*., 2006). Various forms and kinds of nutrients sprayed and absorbed by leaves can be utilized by the plants with the same effect as root fertilization (Peuke *et al*., 1998). There are many factors that affect the nutrient absorption by crop leaves, such as the type and the age of leaves, the nutritional status and growth stages of crops, the nutrient status of leaves and environmental factors (Shinde and Haldankar, 2010). The results of this experiment showed that rice leaves can effectively absorb exogenous amino acids and transport them to all parts of the plant. But the proportion and utilization rate of different organs is different. Leaves had the largest content of amino acids increased, amounting to about 45%. Rice grains had the highest utilization rate about 14% higher. Given the same concentration of the exogenous amino acids sprayed, the yield of rice and the nitrogen accumulation in various organs were higher by spraying at the tillering and the heading stages than by only at the tillering stage, and even higher by spraying at all three stages than at the first two stages. The yield of rice and the nitrogen accumulation in various organs in each of the treatment groups was significantly higher than in the control. Rice leaves can absorb exogenous amino acids and efficiently promote the absorption of nitrogen fertilizer by the roots at the same time (Li *et al*., 2009).

The composition and balance of rice protein and amino acid is an important index to evaluate the nutritional quality of rice, and the rationality determines the nutritional value of rice (Bressani *et al*., 1971). According to some researches, though the content and composition of amino acid are more balanced in the rice than in other grain crops. The content of essential amino acid are still not enough to meet people's need for the nutritional quality of rice. The content of protein and the composition of amino acid in rice are not only controlled by the DNA, but also influenced by the temperature, light and fertilization (Xie *et al.*, 2008). Therefore, to enhance the physical quality and food security, it is significant to discuss the factors influencing the content and composition and seek the ways to increase the content of amino acid, especially of the essential ones. The results of this research shows that spraying L-leucine-^{15}N and L-lysine-^{15}N on the rice leaves can increase the content of amino acid, especially increase the both amino acids in rice significantly and the amino acid absorbed by the leaves can get into the rice, maybe part of what is decomposed into raw materials for the synthesis of amino acids, and still a part, directly used for protein synthesis (Wu and Tao, 2000).

Conclusion

Spraying exogenous amino at the tillering, heading and ripening stages can significantly increase content of nitrogen in grains, stems and leave, and promote the growth of rice and the nitrogen absorption of root. At the same time, it also can increase the rice production and the content of essential amino acids in rice. The exogenous amino acids absorbed by leave translate to grains, rendering nearby allocation and the cumulative effect. Thus, the best period of spraying exogenous amino acids for rice are the heading and ripening stages.

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