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Full Length Article

Foliar Fertilization of Dual-Labeled Organic and Inorganic N in Rice; Mechanisms of Transport and Assimilation

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Abstract

The absorption, transport, and assimilation mechanism of organic and inorganic nitrogen(N) in rice organs were analyzed by applying ^{13}C-^{15}N dual-labeled organic and inorganic N directly to the leaves of rice plants (variety: C Liangyou 266) at tillering stage based on isotope tracing. The findings suggest that the dry weight and N accumulation of various rice organs under glycine N and ammonium N treatments were significantly higher than other treatments; the dry weight and N accumulation in rice organs followed the pattern of "leaf > root > stem", and there were no significant differences between control and nitrate N treatment. The ^{15}N increments were detected in the roots, stems and leaves of all treatments, showing a certain pattern of "leaf > stem > root"; there were significant differences between the ^{15}N increments of various organs ($P < 0.01$). The ^{13}C increment/^{15}N increment ratios of rice root, stem, leaf, and whole plant were 0.108, 0.158, 0.178, and 0.161 respectively. For rice plants treated by glycine N and ammonium N, the activity of GOT, GPT and GDH peaked in leaves, followed by stems and then roots; while the pattern of activity for GOT, GPT and GDH in the control (Ck) and nitrate N group was in following order "leaf > root > stem". The results showed that rice leaves directly absorb and utilize molecular glycine and the absorption rate of glycine is significantly higher than ammonium N or nitrate N. Molecular organic N absorbed into rice leaves would be transported to roots; the transportability of N in rice plants ranked in descending order is as follows: amino acid N > ammonium N > nitrate N.

Keywords: Rice; Foliar spray of N; ^{13}C-^{15}N double labeling; Absorption and transport

Introduction

Rice, one of the most widely planted crops in the world, is grown in 113 countries (Zimmernann and Hurrell 2002). The planting area of rice in China have reached 29,666,700 hm^2, making China the largest rice producer around the world. Rice has a huge demand for N during its growth, and N is of critical importance in the development and yield of rice. Putting fertilizers in the soil is the main way to satisfy the demand of rice plants for N, which is fulfilled by applying fertilizers directly to rice leaves. In recent years, excessive fertilization has caused some environmental and soil-related problems, such as soil hardening, excessive heavy metal contents, and reduced soil quality. Many countries have introduced relevant policies and measures to reduce the application amount of chemical fertilizers, and grower are encouraged to adopt precision fertilization based on the nutrient demands of crops to alleviate agricultural non-point source pollution (Eugenia *et al.* 1996; Reboredo *et al.* 2018).

Leaves are the most important nutritive organ in addition to roots as being capable of absorbing gases, nutritive elements, and pesticides. The plant leaves can absorb nutrient substances, and the utilization of absorbed nutrients by leaves resemble to roots (Peuke *et al.* 1998). Foliar fertilizers are among plant growers for being absorbable, highly nutritive, precise, highly practical, environmental-friendly, and cost-effective (Alkier *et al.* 1972; Kaya *et al.* 1999; Li *et al.* 2009). Foliar fertilization (or foliar feeding), a direct and highly-efficient supplementary measure to supply rice with nutrients, has become an important component of modern agriculture. Foliar fertilization technology has become the main focus of recent research in the world. Currently, most people pay special attention to the absorption of nutrients by leaf epidermis (Jenks *et al.* 1994; Cruickshank 1995; Flaishman *et al.* 1995), the way of absorbing nutrients by leaves (Reed and Tukey 1982; Wójcik 2004), the effects of leaf type and age on foliar nutrient absorption (Shu *et al.* 1994; Peng *et al.* 2001; Schönherr 2001), the influence of environmental factors on nutrient uptake (Rains 1968; Flore and Bukovac

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1982; Schonherr and Luber 2001), the relation between the components and the absorption of foliar nutrients (Haq and Mallarino 2000; Peryea 2000). Studies on the foliar fertilization technology of rice put emphasis on the effects of foliar fertilizers on the agronomic traits of rice (Bhuyan *et al.* 2014; Geetha and Velayutham 2016), its yield (Gangaiah and Prasad 1999; He *et al.* 2013), and economic benefits (Badole and Narkhede 1999; Slaton *et al.* 2005), etc. Very little about the direct absorption of organic N by rice leaves and the transport characteristics of the absorbed organic N in rice plants are available. In present study, isotope tracers (*i.e.*, 2-^{13}C-^{15}N-glycine, ^{15}N-ammonium sulfate and ^{15}N-potassium nitrate) were used to investigate the direct absorption of organic N into rice plants under different (organic and inorganic) N treatments as well as the transport characteristics of the absorbed N (both organic and inorganic) in plants. The findings could enrich the theory of plant nutrition and provide theoretical support for further research into the absorption and utilization efficiency of organic N in higher plants.

Materials and Methods

Seed of cv. *C Liangyou266*, provided by the Nuclear Agricultural Science and Aerospace Breeding Institute (Hunan Academy of Agricultural Sciences), was used in pot experiment; filled with the reddish paddy soil collected from the experimental fields in Hunan Academy of Agricultural Sciences. The soil physic-chemical analysis showed that it contained organic matter 24.3 g·kg^{-1}, total N 1.42 g·kg^{-1}, alkali-hydrolyzable N 178.5 mg·kg^{-1}, available phosphorus 25.4 mg·kg^{-1}, rapidly available potassium 237.4 mg·kg^{-1}, and pH 5.3. Isotope tracers 2-^{13}C-^{15}N-glycie (^{13}C abundance: 99%, ^{15}N abundance: 98%), ^{15}N-amimonium sulfate (abundance: 10.65%), and ^{15}N-potassium nitrate ($^{15}NO_3^-$-N) (abundance: 10.3%) were purchased from China Isotope Corporation.

Experimental details

The pot experiments were carried out in the net-house of the Nuclear Agricultural Science and Aerospace Breeding Institute based on isotope tracing method (Näsholm *et al.* 2001; Wei *et al.* 2013). The sun-dried soils was smashed, seived, and again air-dried out. The dimensions of pot were 18 cm (bottom diameter) x 25 cm (upper diameter) x 30 cm (depth); each contained 7.5 kg of air-dried soils. The amounts of fertilizers applied to each pot were: urea ($CO(NH_2)$) 1.63 g, potassium sulfate (K_2SO_4) 0.65 g, potassium dihydrogen phosphate (KH_2SPO_4) 1.9 g, organic fertilizer 12.5 g; the N content of organic fertilizer was 3.75%; soils were fully mixed with fertilizers before filling. The experiments were divided into four treatments: 2-^{13}C-^{15}N-glycine, ^{15}N-ammonium sulfate, ^{15}N- potassium nitrate, and distilled water; each treatment contained six replications with random arrangement. The sowing was started on 8th May; seedlings of the same growth status were chosen and transplanted to plastic pots on 21st June; three clumps in each pot and 2 plants in each clump; the seedlings were sprayed with fertilizers during 23rd to 25th July (tillering stage); N fertilizers were sprayed at the rate of 2 kg/hm^2; root, stem, and leaf samples were collected on 2nd August. The samples were treated as follows: flushed with 0.5 mmol/L $CaCl_2$ solution for four times to remove the isotope tracers adhering to the surface of the samples; clean washed with distilled water; the fresh weight of roots, stems and leaves and the activity of GOT, GPT and GDH were determined in half of the samples. De-enzyme the second half for 30 min at 110°C; dry to constant weight in an oven at 80°C; measure dry weight of each organ; smash the root, stem, and leaf samples respectively with a plant over speed pulverizer (type: RHP-400); pass the smashed samples through a 100-mesh sieve. An isotope mass spectrometer (DELTA V Advantage, America) and an elemental analyzer (Flash 2000 HT, Thermo Fisher Scientific, America) were used to determine the total C, total N, ^{13}C abundance and ^{15}N abundance of different rice organs.

The ^{15}N and ^{13}C increment originating from isotope tracers were calculated by (Taylor *et al.* 2004; Wei *et al.* 2013):

$$X_c=[C_T[\%]/12\times(^{13}C_T\text{atom}\%-^{13}C_c\text{atom}\%)\times f]\times 10^6$$

$$X_N=[N_T[\%]/14\times(^{15}N_T\text{atom}\%-^{15}C_c\text{atom}\%)\times f]\times 10^6$$

Where, X_c and X_N denote the ^{13}C and ^{15}N increment (μmoL/g, DW) in one gram of dried sample, respectively; C_T and N_T are the contents of total C and N of the samples respectively; $^{13}C_T$atom% and $^{15}N_T$atom% represent the ^{13}C and ^{15}N abundance of the samples treated by isotope labeling N; $^{13}C_c$atom% and $^{15}N_c$atom% stand for the ^{13}C and ^{15}N abundance of the samples in control treatment; f is the enrichment coefficient (or enrichment factor) of isotope tracers.

Statistical analysis

The experimental data were processed and plotted on charts with Excel 2007; Statistical analyses were performed using one-way ANOVA with by SPSS18.0; Differences were considered significant at $P < 0.05$.

Results

Distribution of dry matter in different rice organs

The dry weight of different rice organs under Gly-N and NH_4^+-N treatment followed the order of "leaf > root > stem"; The dry weight of rice leaves accounted for 40.9% and 39.2% of the total dry weight of a plant under Gly-N and NH_4^+-N treatment respectively, higher than NO_3^--N and control treatment (Ck). There were significant differences in the dry weights of various rice organs between Gly-N and NH_4^+-N treatment ($P < 0.01$); the dry weight of a whole

plant under Gly-N was 16.4% higher than under NH_4^+-N treatment. There were no significant differences in the dry weight of each organ between NO_3^--N and control treatment (Ck); the dry weights of different organs ranked in descending order were root > leaf > stem (Table 1). Therefore, spraying glycine N and ammonium N can promote the growth and development of rice plants and the absorption of nutrients by rice roots. The nutritional effect of spraying glycine is better, and the absorption of nitrate N on the leaf surface is less. Nitrate N may be more suitable for root fertilization.

Nitrogen accumulation in different rice organs

The amount of N accumulated in each organ varied greatly with the type of N treatment on rice leaves at tillering stage (Table 2). N accumulations in the root, stem, leaf, and shoot were much higher under Gly-N and NH_4^+-N treatment than NO_3^--N and control treatment (Ck) ($P < 0.01$). The N accumulations in the root, stem, leaf, and shoot were significantly higher under Gly-N treatment than NH_4^+-N treatment ($P < 0.01$); *i.e.*, was 9.3, 39.5 and 40.3% higher than NH_4^+-N, NO_3^--N, and control treatment (Ck), respectively. There were no significant differences in the N accumulation of each organ or the shoot between NO_3^--N and control treatment (Ck).

^{15}N increment in various rice organs

^{15}N increments were detected in rice root, stem, and leaf after applying different N fertilizers to rice leaves; the value of ^{15}N increment peaked in leaves, followed by stems and then roots; there were extremely significant differences in the ^{15}N increment of rice leaf, stem, and root ($P < 0.01$). The ^{15}N increment of whole plant also varied greatly among different N treatments ($P < 0.01$). The ^{15}N increment in rice leaves under Gly-N treatment was 1.35 and 4.06 times than in stems and roots, respectively. When rice leaves were sprayed with ammonium N, the ^{15}N increment in leaves was 1.31 and 4.98 times than in stems and roots, respectively; when rice leaves were sprayed with nitrate N, the ^{15}N increment in leaves was 1.36 and 3.40 times in stems and roots, respectively. When the plants were treated by glycine N, the ^{15}N increment in rice leaves was 1.19 and 4.96 times than ammonium N and nitrate N treatments, respectively; The ^{15}N increment in rice stems was 1.15 and 4.99 times than ammonium and nitrate N treatments, respectively. Likely, the ^{15}N increment in rice roots was 1.46 and 4.16 times than ammonium and nitrate N treatments, respectively. The ratios of ^{15}N increment in shoot than root under glycine, ammonium, and nitrate N sources were 7.05, 8.79 and 5.90 times, respectively (Fig. 1).

^{13}C and ^{15}N increment in rice

As shown in Table 3, ^{13}C increment were detected in rice root, stem, and leaf after spraying 2-^{13}C-^{15}N-glycine to rice leaves at tillering stage; the ^{13}C increment in leaves was 6.63 and 1.52 times of those in roots and stems respectively, and there were extremely significant differences in the ^{13}C increment of rice leaf, stem, and root ($P < 0.01$). The ^{13}C increment/^{15}N increment ratios also varied greatly from organ to organ ($P < 0.01$); the ratio peaked in leaves and hit the bottom in roots. The ^{13}C increment/^{15}N increment ratio of whole plant was 0.161, which was far from the theoretical value (1:1).

Effects on the activity of assimilation-related enzymes

The activity of GOT, GPT and GDH in different rice organs ranked in descending order was "leaf > stem > root" under Gly-N and NH_4^+-N treatment, and "leaf > root > stem" under NO_3^--N and control treatment (Ck). There were no significant differences in the GOT, GPT and GDH activity of whole plant and specific organs under NO_3^--N and control treatment. In contrast, the GOT, GPT and GDH activity of each organ and whole plant under Gly-N treatment were much higher than those under other treatments ($P < 0.01$); the GOT, GPT and GDH activity of whole plant under Gly-N treatment were 15.5, 26.5 and 21.8% higher than those under NH_4^+-N treatment, and 35.8, 43.8 and 38.4% higher than those under control treatment ($P < 0.01$). When the leaves were sprayed with Gly-N, the GOT activity of rice leaf was 112.1 and 346.7% higher than those of rice stem and root respectively; the GPT activity of rice leaf was 142.0 and 322.2% higher than those of rice stem and root respectively; the GDH activity of rice leaf was 476.9 and 761.5% higher than those of rice stem and root respectively (Table 4). This means the glycine absorbed into the plants by way of foliar spray mainly accumulated in leaves and was assimilated by transamination and deamination; only a small proportion was transported to stems and roots for assimilation, thus providing nutrients and energy for the growth and development of rice.

Discussion

N fertilizers are very important to rice production. The absorption of N elements into rice plants takes place mainly at tillering stage and during the development of young panicles (Ding *et al.* 2004). In this research, different types of N fertilizers were applied directly to rice leaves at tillering stage. The results showed that the accumulation of dry matter and N varied greatly within organs; the accumulation of dry matter and N under Gly-N treatment was significantly higher than under other N treatments or Ck ($P < 0.01$). Other studies have shown that the absorption mechanism of nutrients in rice leaves resembled to roots (Peuke *et al.* 1998), and leaves were selective about which type of N rice plant absorb (Wójcik 2004); and was connected with stomata to leaf surface (Leece 1978), hydrophilic pores in leaf cuticle (Li *et al.* 2009) and, ectodesma of leaf cells (Wu and Tao 1996). This study

Table 1: Dry weights of rice organs under different nitrogen treatments (X ± SD, g.pot^{-1})

Treatment	Leaf	Stem	Root	Plant	Shoot (g.pot^{-1})	Shoot-root ratio
Gly-N	26.4 ± 3.21a	17.6 ± 2.31a	20.5 ± 3.56a	64.5 ± 2.84a	44.0 ± 2.85a	2.15 ± 0.11a
NH_4^+-N	21.7 ± 5.42b	15.2 ± 1.56b	18.5 ± 2.69a	55.4 ± 3.21b	36.9 ± 2.56b	1.99 ± 0.08ab
NO_3^--N	15.6 ± 2.43c	13.8 ± 2.35c	17.6 ± 2.45b	47.0 ± 2.34c	29.4 ± 2.23c	1.67 ± 0.12b
Ck	15.2 ± 2.11c	13.5 ± 2.14c	17.4 ± 1.98b	46.1 ± 2.09c	28.7 ± 2.05c	1.65 ± 0.09b

Note: In each column, values followed by different small letters are significantly different at $P < 0.05$ (the same as below)

Table 2: Mass fractions of nitrogen in rice organs under different nitrogen treatments (mg.g^{-1})

Treatment	Leaf	Stem	Root	Shoot (overground part)	Total
Gly-N	854.7 ± 12.4a	376.4 ± 10.2a	635.3 ± 11.6a	1231.1 ± 10.5a	1866.4 ± 13.6a
NH_4^+-N	782.4 ± 9.8b	325.2 ± 5.3b	585.4 ± 10.4a	1107.6 ± 7.8b	1693.0 ± 10.1b
NO_3^--N	405.7 ± 6.5c	257.2 ± 4.8c	465.8 ± 14.5b	662.9 ± 8.1c	1128.7 ± 9.8c
Ck	399.7 ± 7.6c	253.8 ± 5.2c	461.1 ± 6.9b	653.5 ± 6.8c	1114.6 ± 12.4c

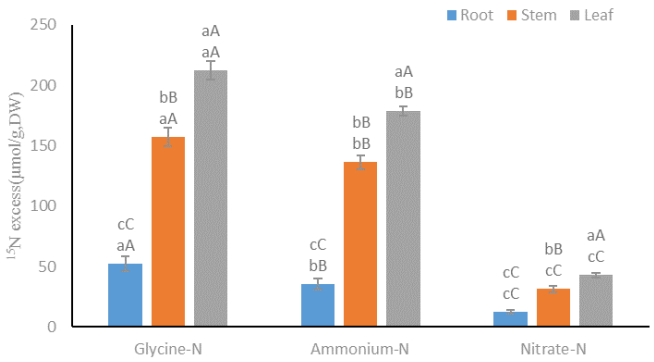

Fig. 1: ^{15}N increments in rice root, stem and leaf under various isotope N treatments
Note: Different small and capital letters at the top indicate significant differences in ^{15}N increment of root, stem, leaf, and whole plant under the same isotope treatment at the 0.05 and 0.01 levels, while those at the bottom indicate significant differences in ^{15}N increment of root, stem, leaf, and whole plant under various isotope treatments at the 0.05 and 0.01 levels

further proved that rice leaves have selectivity in the absorption of organic and inorganic N, and their absorption capacity is glycine N > ammonium N > nitrate N.

The foliar fertilizers mainly in N exist in amino acids forms. It is known that the roots and leaves are incapable of absorbing external carbon. This enables us to verify whether a plant can directly take in molecular amino acids by applying 2-^{13}C-^{15}N-glycine to the plant, if both ^{13}C and ^{15}N are detected in the plant in certain proportion, then the plant is capable of directly absorb molecular amino acids (Jones *et al.* 2005). In this study, ^{13}C increment and ^{15}N increment were detected in different rice organs when the leaves were sprayed with 2-^{13}C-^{15}N-glycine, which means rice leaves can directly take in glycine molecules. However, the increment of ^{13}C in roots, stems and leaves is significantly less than that of ^{15}N, which is significantly different from the theoretical value (1:1). This is because 2-^{13}C-^{15}N-glycine used in this study is labeled with non-carboxylic carbon, compared with labeled carboxylic carbon, it can effectively reduce the loss of ^{13}C caused by decarboxylation reaction of amino acids in plants, but non carboxylic carbon will also lose ^{13}C through deamination and respiration of tricarboxylic acid cycle. Therefore, ^{13}C absorbed by rice will decompose and decline after a certain period, resulting in the measured value of ^{13}C in glycine absorbed by rice is lower than the actual absorption value.

Both organic and inorganic can be absorbed and assimilated in plants through deamination, transamination, and other reactions. It was reported that the N elements, instead of evenly distributed in the plant, tended to accumulate in roots at the early absorption stage (Hiroaki *et al.* 2005). In this study, the ^{15}N increment in rice leaf was higher than in stem and root under various foliar N treatments, which means most organic and inorganic N absorbed into the plant was accumulated in rice leaves, and only a small proportion was transported to stems and roots. The differences between the ^{15}N increments of various rice organs could reflect their transport capacities of different N, namely glycine N > ammonium N > nitrate N. The activity of GOT, GPT and GDH in different organs also varied with the type of N source. The activity of GOT, GPT and GDH in rice leaves was significantly higher than those in stems and leaves ($P < 0.01$). Therefore, rice leaf is the main organ for the assimilation of N.

Conclusion

The findings have shown that rice leaves could absorb and assimilate organic glycine N, ammonium N, and nitrate N at tillering stage under various N treatments, and the absorption capacity of glycine N is significantly higher than that of ammonium N and nitrate N. The

Table 3: ^{13}C increment and the ^{13}C increment/^{15}N increment ratio after applying 2-^{13}C-^{15}N-glycine to rice leaves

Organs	^{13}Cincrement (μmol/g, DW)	^{15}Nincrement (μmol/g, DW)	^{13}C increment/^{15}Nincrement
Root	5.7 ± 1.1d	52.4 ± 5.3d	0.108 ± 0.05c
Stem	24.8 ± 3.2c	157.1 ± 9.5c	0.158 ± 0.06bc
Leaf	37.8 ± 5.1b	212.5 ± 11.3b	0.178 ± 0.04a
Whole seedling	68.0 ± 4.3a	422.0 ± 12.4a	0.161 ± 0.07b

Table 4: Activity of GOT, GPT and GDH in rice organs 7 days after isotope nitrogen treatments

Position	N-sources	Enzyme activity		
		GOT(μmolg^{-1}h^{-1})	GPT(μmolg^{-1}h^{-1})	GDH(U g^{-1}min^{-1})
Leaf	Gly-N	54.3 ± 6.5a	137.2 ± 10.7a	454.7 ± 15.9a
	NH_4^+-N	46.6 ± 5.2b	125.2 ± 10.5b	418.4 ± 13.9b
	NO_3^--N	34.1 ± 4.3c	102.3 ± 6.8c	392.5 ± 16.9c
	Ck	32.4 ± 4.6c	98.7 ± 9.5c	384.1 ± 15.7c
Stem	Gly-N	25.6.5 ± 3.5a	56.7 ± 5.2a	78.7 ± 9.9a
	NH_4^+-N	21.3 ± 2.9b	51.8 ± 4.3b	65.1 ± 6.8b
	NO_3^--N	10.0 ± 1.5c	25.9 ± 2.5c	33.9 ± 5.5c
	Ck	9.6 ± 1.1c	25.7 ± 5.8c	32.5 ± 6.2c
Root	Gly-N	12.7 ± 1.8a	32.5 ± 2.6a	52.7 ± 4.8a
	NH_4^+-N	11.2 ± 2.1b	29.1 ± 3.0b	45.4 ± 4.6b
	NO_3^--N	18.5 ± 2.3c	47.5 ± 2.8c	55.2 ± 3.2c
	Ck	17.9 ± 2.1c	45.6 ± 3.8c	54.3 ± 8.5c
Whole seedling	Gly-N	21.6 ± 4.1a	72.2 ± 6.3a	141.4 ± 14.6a
	NH_4^+-N	18.7 ± 3.7b	65.9 ± 6.1b	128.5 ± 12.2b
	NO_3^--N	16.8 ± 3.6c	52.1 ± 4.8c	105.1 ± 10.7c
	Ck	15.9 ± 2.3c	50.2 ± 5.2c	102.2 ± 14.8c

assimilation and transport capacity of rice leaves vary with the type of N and follow the pattern of "glycine N > ammonium N > nitrate N", This, indicate that foliar spray of glycine N offers the best nutritive effects, followed by ammonium and nitrate N. N elements would be assimilated and transformed into amino acids, proteins, sugar and energy in rice roots, stems, and leaves by way of transamination, deamination, and other reactions, among which the leaf is the main assimilation site.

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Author Contributions

Zhaohui Zou: planning and supervision of the work, methodology and editing. Xian Li: methodology and data analysis. Gangqiao Deng: fund, planning. Hongke Xie: methodology and data analysis. Yi Yang: review and editing. Jun Liu: methodology and data analysis. Yong Zhang: pot experiment. Yiji Zhou: experimental data record, sample examination. Aiguo He: data processing.

Conflicts of Interest

The authors declare no conflict of interest.

Data Availability

All data included in this study are available upon request by contact with the corresponding author.

Ethics Approval

This study does not involve animal or human experiments.

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