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EFFECTS OF EXOGENOUS NITROGEN FORMS ON RICE GROWTH AND NITROGEN ACCUMULATION

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Abstract

In order to optimize the concentration ratio of different forms of nitrogen and improve the effect of foliar fertilization, the effects of different concentration ratios of exogenous glycine nitrogen, nitrate nitrogen and ammonium nitrogen on biomass, nitrogen content, root morphology and leaf physicochemical indexes of rice were investigated. The results indicated that the effects of different exogenous nitrogen concentration compositions on the biomass, nitrogen content and physiological and biochemical indexes of rice were significantly different ($p<0.05$) when they were applied at the tillering stage of rice. The highest biomass and nitrogen content of rice were 68.6 mg · p^{-1} and 1888.5 mg · g^{-1} respectively, under the treatment of high glycine nitrogen (2500μmol·L-1), ammonium nitrogen (2500μmol·L-1) and low nitrate nitrogen (2500μmol·L-1). The changes of total root length, root surface area, root volume and root activity were consistent with those of biomass. The highest contents of chlorophyll, soluble protein, soluble sugar and SOD activity were 7.35mg/g, 39.25mg/g, 38.81mg/g and 342.56 U·g-1 in high glycine-nitrogen and low inorganic nitrogen treatment, respectively. A certain increase of Gly-N concentration and reduction of inorganic nitrogen concentration can contribute to the growth of rice.

Key words: Foliar spraying, Nitrogen concentration ratio, Root morphology, Biomass.

Introduction

Nitrogen is one of the most influencing factors for the growth of a plant (Mooney *et al*., 1987). Nitrogen nutrition has a direct impact on the photosynthesis and growth of crops and thus has a direct impact on the yield and quality of crops. Classical mineralization theory suggests that only inorganic nitrogen can be used as the nitrogen source of plants. However, a large number of studies have shown that both wild plants and crops can directly absorb and utilize various forms of soluble organic nitrogen (Näsholm *et al*., 2009), such as amino acids (Jones *et al*., 2005; Hill *et al*., 2011), polypeptides (Nie *et al*., 2011) and proteins (Mulholland & Lee, 2009; Warren, 2014; Louis, 2015). Different nitrogen forms affect plant carbon metabolism, which provides carbon source and energy for nitrogen metabolism, and nitrogen metabolism provides enzymes and photosynthetic pigments for carbon metabolism. The root, a major organ for plants to absorb nitrogen, is also an important place for the synthesis of some amino acids, hormones and other physiological active substances. The morphological and physiological characteristics of the root would affect the nitrogen nutrition of plants and further influence the growth and development of the shoot (Zhang *et al*., 2009; Zhang *et al*., 2012; Qi *et al*., 2013). Lots of previous studies have investigated the relationship between the morphological and physiological characteristics of root and the nitrogen efficiency (Himmelbauer *et al*., 2004; Dorlodot *et al*., 2007; Shimizu *et al*., 2008; Wei *et al*., 2008) and they found that different proportions of forms (inorganic and organic nitrogen) all presented significant effects on the carbon and nitrogen metabolism of crops (Gunes *et al*., 1996; Wu *et al*., 2020). Besides, nitrogen can also be absorbed through the foliage and enters the plant to take part in the metabolism process and synthesis of organic matters of the crop. However, most current studies focus on the effects of single inorganic nitrogen and partial replacement of inorganic nitrogen by organic nitrogen spray on crop growth and carbon and nitrogen metabolism (Zhao *et al*., 2016; Zhao *et al*., 2018; Zhao *et al*., 2022). There are few reports on the effects of mixed nitrogen source concentration ratio spray on carbohydrate accumulation and nitrogen absorption in crops.

Glycine (Gly), the smallest molecular weight and simplest structure, is an amino acid with a high content in soil (Bardgett *et al*., 2003) Compared with other amino acids, Gly is not easily decomposed by microorganisms (Lipson *et al*., 1999) and has long been an ideal nitrogen source for the research of plant amino acid nitrogen. In this study, Gly-N was selected as the test amino acid nitrogen, and monocotyledonous rice (*Oryza sativa* L.) was selected as the test material to investigate the response of rice growth, nitrogen absorption and chlorophyll content to different exogenous nitrogen concentration compositions (Gly-N, NO^{-}_{3}-N, NH^{+}_{4}-N). It aimed to provide a theoretical basis for comprehensive and efficient management of rice nutrients.

Material and Methods

Rice variety: The tested rice C *Liangyou* 266 was provided by the breeding room of the Institute of Nuclear Agronomy and Aerospace Breeding, Hunan Academy of Agricultural Sciences and the tested soil was Quaternary red and yellow clay from the experimental field of Hunan Academy of Agricultural Sciences. The organic matter of the soil was 24.3g · kg^{-1}, total nitrogen 1.42 g · kg^{-1}, alkali-hydrolyzed nitrogen 178.5 mg · kg^{-1}, available phosphorus 25.4 mg · kg^{-1}, available potassium 237.4 mg · kg^{-1}, pH value 5.3. After fully dried in the sun, smashed, screened and spread out for drying, the soil was filled into different porcelain pots with the inner diameter of 26 cm and height of 30 cm. Each pot was filled with 15 kg of soil and watered layer by layer to allow the soil to settle naturally. Then 5g of compound fertilizer was applied in each pot as base fertilizer.

Experimental design: The experiment was carried out in the net room of Nuclear Aviation Institute of Hunan Academy of Agricultural Sciences by pot method. Fertilizer application rate of rice: urea ($CO(NH_2)$)1.63g/ basin, potassium sulfate (K_2SO_4)0.65g/ basin, Monopotassium phosphate (KH_2PO_4)1.9g/ basin, organic fertilizer 12.5g/ basin, organic fertilizer nitrogen content 3.75%. Soil and fertilizer in each basin were fully mixed before loading into the basin. The nitrogen sources tested were Gly-N, NO^-_3-N and NH^+_4-N, with two levels of 250 and 2500μmol· L^{-1} for each nitrogen source and a total of 8 treatments (Table 1). Each treatment was composed of 8 randomly arranged repetitions, the total nitrogen concentrations of which were 750, 3000, 3000, 5250, 3000, 5250, 5250, and 7500 μmol·L^{-1}, respectively. The experiment was carried out on May 8, and the seedlings with the same three-leaf monotropy were transplanted into the pots on June 21, with 2 plants planted in each pot and 1 plant left after greening. The seedlings were sprayed from July 23 to July 25 (tillering stage) with the nitrogen spraying amount of 2kg/hm^2, and harvested on August 2.

Table 1. Exogenous nitrogen concentration composition in different treatments.

Treatment*	NH^+_4-N/μmol · L^{-1}	NO^-_3-N/μmol · L^{-1}	Gly-N/μmol · L^{-1}
Gna	250	250	250
GNa	250	2500	2500
GmA	2500	250	2500
gNA	2500	2500	250
gnA	2500	250	250
Gna	250	250	2500
gNa	250	2500	250
GNA	2500	2500	2500

Note: *Combined treatment codes "a", "n" and "g" represent low concentrations (250 μmol · L^{-1}) of NH^+_4- N, NO^-_3- N and Gly-N, respectively; "A", "N" and "G" represent high concentrations (2 500 μmol · L^{-1}) of NH^+_4- N, NO^-_3- N and Gly-N, respectively. The same below

Measurements: When rice seedlings were harvested, the shoots and roots were separated and weighed separately. The morphological structure of the root system was scanned and analyzed using root scanner (STD1600, Epson, Nagano, Japan). The samples were crushed after drying and disinfected with concentrated sulfuric acid-hydrogen peroxide method. The nitrogen content of the samples was determined by semi-micro Kjeldahl method; the total free amino acid was determined by water and ninhydrin colorimetric method; the total soluble protein was determined by Coomassie brilliant blue staining method with BSA (bovine serum albumin) as the standard curve; the total soluble sugar was measured by anthrone colorimetric method; the root activity was tested by TTC (triphenyl tetrazolium chloride) staining method; the leaf chlorophyll content was measured by ethanol extraction method (Ming *et al.*, 2007); the SOD (superoxide dismutase) activity was determined by NBT (nitro-blue tetrazolium) photo-reduction method (Wang *et al.*, 2007) and superoxide dismutase (SOD) activity was determined by nitrogen blue tetrazole (NBT) photoreduction method.

Data analysis: The data were analyzed with ANOVA (analysis of variance) using STATISTICA 5.5 (StaSoft Inc, USA) software, and the significance of different treatments for each index was tested using Duncan's new multiple range method ($p<0.05$).

Results

Effects of different exogenous nitrogen concentrations on biomass and nitrogen content of rice: The effects of exogenous nitrogen concentrations on shoot and root biomass of rice under different treatments were significantly different ($p<0.05$) (Fig. 1). The biomasses of the root, shoot and whole plant in the GnA treatment were the highest, reaching 21.5 g, 47.1 g and 68.6 g respectively, significantly higher than other seven treatments (except for the root biomass, $p<0.05$). At low Gly-N concentration (250 μmol · L^{-1}), the biomasses of the shoot and whole plant in the gna, gnA and gNa treatments were not significantly differen among gna, gnA and gNa treatments. Under the condition of high Gly-N concentration (2500μmol·L^{-1}), the biomass of whole plants treated with GnA increased by 12.8%, 33.5% and 29.9% compared with that treated with Gna, GnA and GNA, respectively. Although the nitrogen concentration of the GNA treatment was 10 times of that of the gna treatment, the biomass of the whole plant in the GNA treatment only increased by 5.7% compared with the gna treatment. Under equal nitrogen condition (2500 μmol · L^{-1}), the biomass of the whole plant in the Gna treatment was significantly higher than that of the gnA and gNa treatments ($p<0.05$). Under the condition of 5250 μmol · L^{-1}, the biomass of the whole plant in the GnA treatment with low NO^-_3- N was significantly higher than the gNA and GNa treatments with high NO^-_3- N ($p<0.05$).

As indicated in Fig. 2, the nitrogen contents of the root, shoot and whole plant of rice in the GnA treatment were significantly higher than those of other treatments ($p<0.05$). The nutrient solution concentration of GNA treatment was the highest, but the total nitrogen content of Gna and GnA treatment decreased by 9.3% and 22.9%, respectively. Under the condition of isonitrogen content (3000μmol·L^{-1}), the order of nitrogen content in the whole plant was Gna>gNa>gnA . Under the condition of 5250μmol·L^{-1}, the order of nitrogen content in whole plant was GnA>gNA>GNa.

The three-factor ANOVA showed that the three nitrogen sources of NO^-_3-N, NH^+_4-N and Gly-N showed significant main effects on the biomass of the whole rice plant ($p<0.05$), that is, the three nitrogen sources of NO^-_3-N, NH^+_4-N and Gly-N with different concentrations and their interaction also significantly had a significant effect on the growth of rice (Table 2). However, the main effects of Gly-N, NO^-_3-N and NH^+_4-N sources and their interaction effects on the total nitrogen content of rice were not significant.

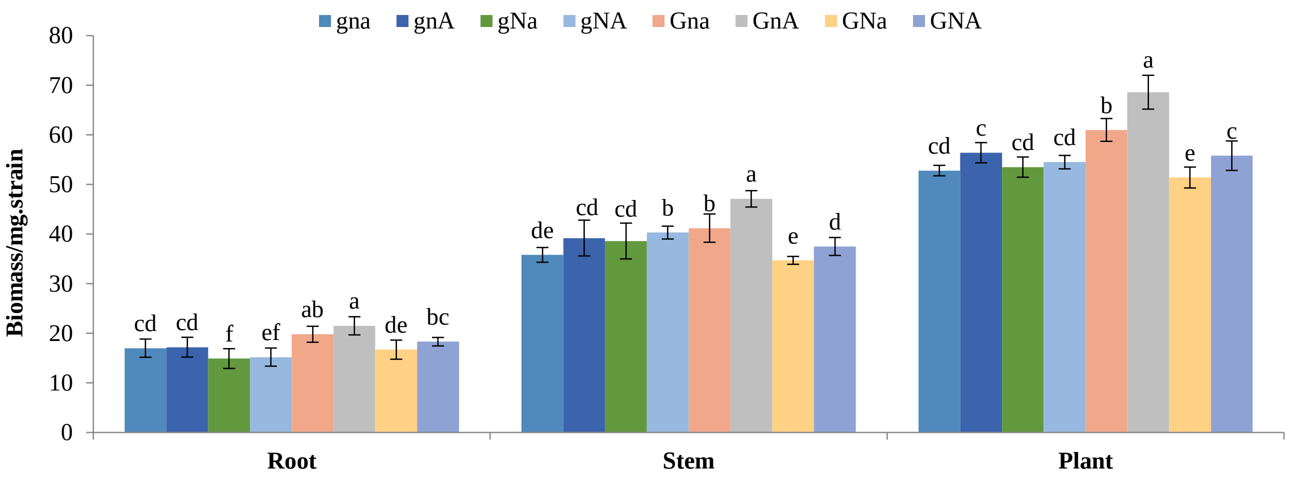

Fig. 1. Effects of different exogenous nitrogen concentration ratios on biomass of rice.
Note: a, b, c, d, e indicated significant differences between different treatments of root, shoot and whole plant of rice (p<0.05). The same below.

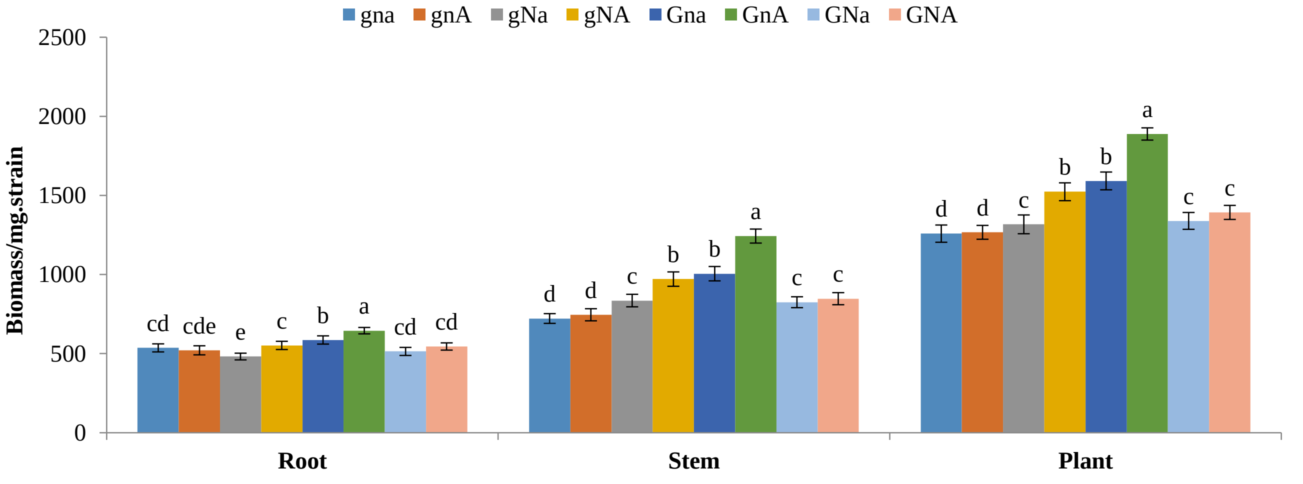

Fig. 2. Effects of different exogenous nitrogen concentration ratios on nitrogen content of rice.

Table 2. Main and interaction effects of Gly-N, NO_3^-, and NH_4^+-N on biomass and nitrogen content of rice using three-factor analysis of variance(ANOVA).

Effect	Biomass		Nitrogen content	
	F	P	F	P
Gly	25.974	<0.001	269.356	<0.001
NO_3^-	37.658	<0.001	68.548	<0.001
NH_4^+	18.632	0.001	120.503	<0.001
Gly × NO_3^-	30.388	<0.001	418.181	<0.001
Gly × NH_4^+	3.703	0.072	7.321	0.016
NO_3^- × NH_4^+	2.275	0.151	0.632	0.438
Gly × NO_3^- × NH_4^+	0.024	0.878	70.998	0.000

Table 3. Effects of exogenous nitrogen concentration ratios on root morphology and root activity of rice.

Treatments	Total root length /m	Root surface area /cm^2	Root diameter /mm	Root volume /cm^3	Adventitious roots /a	Root activity /mg · h^{-1} · g^{-1}
gna	17.68 ± 12de	721.23 ± 23de	0.36 ± 0.004b	15.11 ± 3d	121 ± 7d	0.512 ± 0.012de
gnA	19.11 ± 12d	786.47 ± 17c	0.37 ± 0.006ab	18.21 ± 2bc	137 ± 8cd	0.577 ± 0.025cd
gNa	20.65 ± 16cd	752.12 ± 16cd	0.40 ± 0.007ab	17.15 ± 3cd	149 ± 9c	0.526 ± 0.035de
gNA	22.35 ± 20bc	815.98 ± 18bc	0.39 ± 0.007a	19.35 ± 3bc	164 ± 13bc	0.624 ± 0.032bc
Gna	23.61 ± 24ab	827.28 ± 26ab	0.38 ± 0.011ab	20.11 ± 4ab	186 ± 16a	0.7451 ± 0.036ab
GnA	25.56 ± 27a	867.41 ± 34a	0.41 ± 0.011a	21.32 ± 5a	172 ± 14ab	0.798 ± 0.047a
GNa	15.39 ± 11e	621.27 ± 25f	0.40 ± 0.018a	16.24 ± 2cd	158 ± 10bc	0.501 ± 0.015de
GNA	16.67 ± 10e	681.56 ± 20ef	0.42 ± 0.009a	13.65 ± 3e	143 ± 11cd	0.425 ± 0.018e

Note: a, b, c, d, e, f indicated significant differences between different treatments of root morphology and activity of rice (p<0.05)

Root morphology and activity of rice: According to Table 3, the total root volume, root activity, root length and root surface area of rice in the GnA treatment were the highest, reaching 25.56 m, 867 cm^2, 21.32 cm^3, 0.798 mg · h^{-1} · g^{-1} respectively, significantly higher than those of other treatments (except for Gna treatment, $p<0.05$). At high Gly-N concentration, the increase of NO^-_3-N concentration (such as GNa and GNA treatments) could significantly inhibit the root morphological characteristics of rice, and the total root length, root surface area, root volume and root activity were significantly lower than those of the Gna and GnA treatments. At low Gly-N concentration, the main root morphological indexes of rice were the highest with the gNA treatment. The change trend of main root morphological indexes and root activity was similar to that of biomass, and the change trend of total root length, root surface area, root volume and root activity was GnA>Gna>gNA> gnA>gNa>gna>(GNa, GNA).

The effect of different exogenous nitrogen concentrations on rice root diameter was not significant. The root diameter of gna treatment was the lowest (0.36mm), and there was no significant difference among other treatments.

Physiological and biochemical indexes of rice leaves: The contents of chlorophyll, soluble sugar, soluble protein and SOD activity in rice leaves under different treatments were significantly different due to the different concentration ratio of exogenous nitrogen ($p<0.05$) (Fig. 3). The active contents of chlorophyll, soluble sugar, soluble protein and SOD in Gna treatment were the highest, reaching 7.35, 39.25, 38.81 and 342.56mg·g-1, respectively. Under the condition of isonitrogen content (3000μmol·L^{-1}), the contents of chlorophyll, soluble sugar, soluble protein and SOD activity of Gna treatment were increased by 40.93%, 32.65%, 74.70% and 83.85%, respectively, compared with the average value of gnA and gNa treatment. Under the condition of 5250 μmol · L^{-1}, the contents of chlorophyll, soluble sugar and soluble protein in GnA and GNa treatment were significantly increased compared with gNA treatment ($p<0.05$), but the increase trend of SOD activity was not significant. In the high Gly-N concentration treatments, the contents of soluble sugar, soluble protein and chlorophyll were significantly reduced by the high NO^-_3-N and NH^+_4-N concentrations (e.g. GNA treatment), whereas this trend was not obvious in low Gly-N concentration treatments.

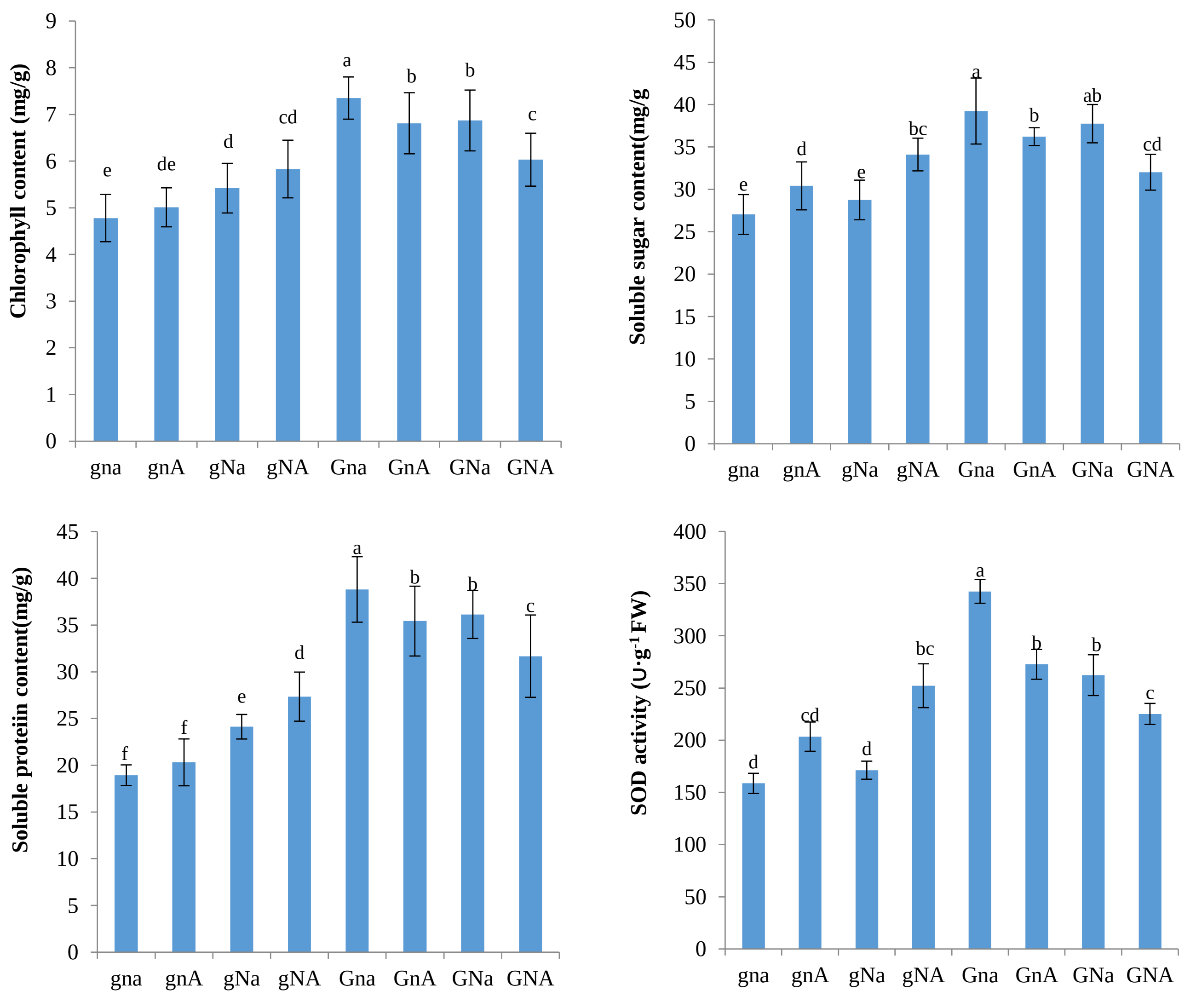

Fig. 3. Effects of exogenous nitrogen concentrations ratios on the physiological and biochemical indexes of rice leaves.

Discussion

Plant leaves are an important nutrient organ of plants. The application of spraying exogenous nitrogen on the leaves can improve the chlorophyll content of leaves, enhance the photosynthesis and increase the biomass of the plant. Furthermore, exogenous nitrogen can also be absorbed and utilized by crops without photosynthesis, increasing the nitrogen content and promoting the growth of the plant (Lipson *et al*., 1999). The results of this experiment showed that the biomass and nitrogen content of rice were significantly affected by the nitrogen concentration and its composition, and reached the highest value in high Gly-N, NH^+_4-N and low NO^-_3-N concentrations (GnA). Under the condition of low Gly-N concentration, the biomass of the whole plant of rice affected by nitrogen concentration was not significantly different among different treatments. At high Gly-N concentration, the increase of NO^-_3-N concentration (such as GNa and GNA treatments) resulted in a significant decrease in the biomass and nitrogen content of rice due to the intense competition for the absorption of Gly-N and NO^-_3-N in rice leaves as speculated. However, NH^+_4-N showed the opposite trend. Increasing the concentration of NH^+_4-N at low NO^-_3-N concentration significantly increased the biomass and nitrogen content of rice (such as GnA), indicating that appropriately increasing the concentrations of Gly-N and NH^+_4-N in mixed nitrogen sources and decreasing the concentration of NO^-_3-N was conducive to the increase of rice biomass. Cao *et al*., (2015) found that under sterile conditions, the biomass of Chinese cabbage increased with the increase of the proportion of exogenous Gly-N: NH^+_4-N (Cao *et al*., 2015), which was consistent with the results of Gunes *et al*., (1966) studied on onion and Wu *et al*., (2020) on wheat and our experiment on rice. High concentrations of exogenous Gly-N and NH^+_4-N increased the biomass and total nitrogen content of rice leaves, indicating that rice leaves were also prone to absorb reduced amino acid nitrogen and NH^+_4-N in addition to NO^-_3-N and enhanced the nitrogen metabolism process *In vivo*. This may be related to the existence of a feedback regulation mechanism of nitrogen metabolism in rice. Besides, the assimilation of NH^+_4-N in the plant body requires less energy than that of NO^-_3-N. Therefore, theoretically plants are more prone to use NH^+_4-N as the nitrogen source. However, the effects of exogenous amino acids on the absorption and transport mechanism of NH^+_4-N in plants are still unclear.

As a reduced product of NO^-_3-N, amino acid has a feedback regulation effect on nitrogen metabolism. When the plant itself has enough reduced nitrogen, the plant will not absorb or only absorb a small amount of NO^-_3-N so as to save the energy used in the absorption, reduction and final amino acid synthesis of NO^-_3-N. Therefore, under the conditions of mixed nitrogen sources, rice preferentially absorbs Gly-N, thus inhibiting the absorption of NO^-_3-N in plant leaves, and thus reducing the nitrate content in the body. Compared with Gna treatment, GnA treatment significantly increased the biomass and nitrogen content of rice, but its nitrate content remained at a lower level, indicating that under the condition of low NO^-_3-N concentration, high concentration of exogenous NH^+_4-N would inhibit the absorption of high-affinity NO^-_3-N, which was consistent with the results of Nazoa *et al.*, (2003) and Vidmar *et al*., (2000).

As an important organ for plants to absorb nutrients, the root is also a place for the assimilation, transformation or synthesis of many substances as well as an organ to realize material exchanges with the shoot. The vitality of the root has a direct effect on the growth and yield of plants (Zhu *et al*., 2020; Xu *et al*., 2017). This study showed that the effects of different nitrogen concentrations on rice root morphology showed a trend of GnA>Gna>gNA>gnA>gNa>gna>(GNa, GNA), indicating that good root morphological characteristics were conducive to the growth of shoot. High Gly-N and NH^+_4-N concentrations and low NO^-_3-N concentrations could promote total root length, root surface area, root volume and root activity of rice roots, but there was no significant difference in root diameter among all treatments. At low NO^-_3-N concentration, the increase of NH^+_4-N concentration in mixed nitrogen sources can promote the development and growth of rice. This may be due to the fact that the foliar acidification caused by the absorption of NH^+_4-N can partially offset the foliar alkalization caused by the absorption of NO^-_3-N forming a suitable environment for plant growth. However, high NH^+_4-N concentration would inhibit the crop growth by reducing the delivery of photosynthates to the root, and consequently the growth of the root was inhibited and thin root or black root and low root/shoot ratio appeared. Therefore, it is of great significance to seek suitable nitrogen concentration composition for building ideal plant growth environment.

This study showed that compared with single source of NH^+_4-N or NO^-_3-N, ammonium and nitrate mixed nitrogen source can significantly enhance the growth rate of crops, increase the total nitrogen absorption and protein content, and improve the nutritional quality of crops (Chen *et al*., 2010; Li *et al*., 2010; Liu *et al*., 2013). In this study, the method of folia spraying was adopted and adding appropriate amount of amino acid nitrogen without affecting the biomass of rice increased the content of chlorophyll, soluble sugar, soluble protein and SOD activity of rice, which contributed to the better growth and development of the rice. After partial substitution of NO^-_3-N by amino acid nitrogen and NH^+_4-N soluble sugar content in rice leaves increased, which may be related to the decrease of NO^-_3-N quantity in vacuoles and the entry of soluble sugar and amino acid into vacuoles to maintain osmotic balance.

Conclusion

Under the condition of folia spraying, increasing the concentration of Gly-N and decreasing the concentration of inorganic nitrogen (especially NO^-_3-N) in the mixed nitrogen source could increase the contents of chlorophyll, soluble sugar, soluble protein and SOD activity of rice at tillering stage, and provide sufficient nutrition for the subsequent growth and development of rice. The variation trend of the indexes of the total root volume, root activity, root length and root surface area of rice was consistent with that of the biomass, indicating that good root activity and root morphological characteristics were beneficial to the growth of shoot.

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