Bangladesh J. Bot. **45**(1): 69-74, 2016 (March)

THE EFFECT OF GLUTAMINE ON GROWTH AND CARBON-NITROGEN METABOLISM IN *ORYZA SATIVA* CV. JAPONICA

HAIXING LI, AILI BAO, ZHIJUN LIANG AND HONGMEI CAI*

Microelement Research Center, Huazhong Agricultural University, Wuhan 430070, China

Key words: Glutamine, Carbon-nitrogen metabolism, Gene expression, *Oryza sativa*

Abstract

In order to know how rice responded to environmental Gln, we analyzed the rice growth, carbon and nitrogen metabolic status under three different nitrogen sources (NH_4NO_3, Gln, NH_4NO_3 + Gln). Results showed that the rice growth and stem nitrate concentration decreased significantly under the Gln and NH_4NO_3 + Gln conditions when compared to the NH_4NO_3 condition. Various changes in the contents of soluble proteins and carbohydrates were observed in the root, stem and leaf tissues. There was a significant decrease of the intercellular CO_2 concentration under the NH_4NO_3 + Gln condition. The total carbon and nitrogen content in the root, stem and leaf tissues increased significantly under the Gln and NH_4NO_3 + Gln conditions, while the C/N ratio decreased significantly under the NH_4NO_3 + Gln condition.

Introduction

In higher plants, nitrogen (N) is an essential macroelement for their growth and development (Crawford and Forde 2002). Ammonium (NH_4^+) and nitrate (NO_3^-) are believed to be the principal nitrogen sources for plant growth in agricultural and most natural environments (Howitt and Udvardi 2000). In addition, amino acids are potential nitrogen sources for plants. Recent studies demonstrated that plants can absorb and use various amino acids and peptides from soil solution (Persson and Näsholm 2001, Näsholm *et al.* 2009).

As early as the 1970s, the root capacity of acquiring amino acids from solutions, and the identity of amino acid transporters, as well as their numbers, characteristics and specificities, were studied. Reinhold and Kaplan (1984) reported that only a single system accounted for root amino acid transport. While Kinraide *et al.* (1981) reported that there were two major transport systems accounted for root amino acid transport, one mediating uptake of neutral and acidic amino acids and another mediating that of basic amino acids. However, Li and Bush (1991) reported that there were four symport systems accounted for root amino acid transport, two mediating uptake of neutral amino acids, one mediating uptake of acidic amino acids, and one mediating that of basic amino acids.

Although amino acids uptake by higher plants has been studied for several decades and has been covered d by several reviews (Lipson and Näsholm 2001, Näsholm and Persson 2001, Rentsch *et al.* 2007), a few studies have been reported about the effect of environmental amino acids on plant growth and carbon-nitrogen metabolism, particularly in crops (Bollard 1966, Stoelken *et al.* 2010). In this study, in order to test whether Gln is a good organic N source for rice growth and development, to know how rice responded to environmental Gln, the rice growth, leaf SPAD value and photosynthesis, carbon (C) and nitrogen metabolic status were analyzed under three different N sources (NH_4NO_3, Gln, NH_4NO_3 + Gln).

---

*Author for correspondence: <caihongmei@mail.hzau.edu.cn>.

Materials and Methods

The seeds of Zhonghua 11 (*Oryza sativa* cv. japonica) were germinated and sowed in sand. The seedlings with three leaves were transplanted into the normal nutrient solution described by Yoshida *et al.* (1976). The culture solution was refreshed every three days. After a week, the seedlings were transferred into a new normal nutrient solution using 1.44 mM NH_4NO_3 as a nitrogen source (N), a nutrient solution without NH_4NO_3 but using 1.44 mM glutamine (Gln) as a nitrogen source (G) and a nutrient solution using both 0.72 mM NH_4NO_3 and 0.72 mM Gln as nitrogen sources (N+G). The root, stem and leaf were harvested at the seedling stage (2 weeks after transplanting) and the tillering stage (4 weeks after transplanting).

For the determination of leaf SPAD value and photosynthesis parameters, ten plants were randomly selected, the average SPAD value and photosynthesis parameters of the upper, middle and bottom portion of each flag leaf was analyzed. The flag leaf SPAD value was determined by a chlorophyll meter (SPAD-502), and the photosynthesis parameters were determined by a Li-6400XT portable photosynthesis system (USA, Li-COR). The carbon and nitrogen concentrations were determined by a C/N analyzer (Elementar, Vario MAX CN, German) according to the manufacturer's instructions. For the soluble protein analysis, three biological replicates of fresh roots, stems and leaves were analyzed. The fresh roots, stems and leaves were homogenized by grinding on ice with extraction buffer which described by Melo *et al.* (2003). The homogenates were then centrifuged at 12,000 g for 20 min at 4ºC. The supernatant of the extract was measured by the Bradford (1976) protein assay to determine the concentration of soluble proteins. For the soluble carbohydrate analysis, three biological replicates of pre-dried roots, stems and leaves were analyzed. The concentration of soluble carbohydrates were extracted from plant samples with boiling water and colorimetrically measured according to the anthrone procedure (Morris 1948, Maness 2010).

Results and Discussion

In order to test the effect of Gln on the rice plant growth, the root length, plant height, dry weight of the root, shoot and plant at both seedling and tillering stages grown hydroponically were analyzed under three different nitrogen forms (N, G, N+G). Results showed great effect of Gln on the rice plant growth, particularly on the root length at the seedling stage. At the seedling stage, compared to the N form, there were 59.3 and 58.3% decreases in the root length, 30.8 and 39.4% decreases in the root dry weight, 7.0 and 20.8% decreases in the shoot dry weight, 11.3 and 24.2% decreases in the plant dry weight under the N + G and G forms, respectively (Fig. 1). At the tillering stage, compared to the N form, there were 31.6 and 17.5% decreases in the root length, 7.1 and 10.6% decreases in the plant height, 27.6 and 26.0% decreases in the root dry weight, 28.9 and 21.6% decreases in the shoot dry weight, 28.8 and 22.1% decreases in the plant dry weight under the N + G and G forms, respectively (Fig. 1).

To date, a range of studies have investigated the extent to which plants can grow on amino acids as nitrogen sources. In general, it appears that plants can use most of the amino acids as a source of nitrogen, which depress plant growth relative to mineral sources of nitrogen (Wang *et al.* 2007, Näsholm *et al.* 2009). Bollard (1966) found that the plant was able to grow on just a few amino acids, such as L-Gln, L-Glu and L-Asn, while a number of amino acids (e.g, L-Arg, L-Ile and L-Val) inhibited the plant growth. Growth tests on *Lemna minor* confirm that single amino acids may inhibit, but protein (casein) hydrolysates may efficiently sustain the growth of plants (Joy 1969). Studies on obligate mycorrhizal plants also found a severely restricted capacity to utilize Gly, L-His, L-Arg and the protein BSA in the absence of their mycobionts (Stribley and Read 1980, Turnbull *et al.* 1995). Forsum *et al.* (2008) reported that the *Arabidopsis* was capable to utilize various amino acids for growth. When grown with amino acids as the sole nitrogen source, *Arabidopsis thaliana* is only capable of achieving between 1 and 50% of its potential vegetative growth on an identical concentration of nitrate (Forsum *et al.* 2008).

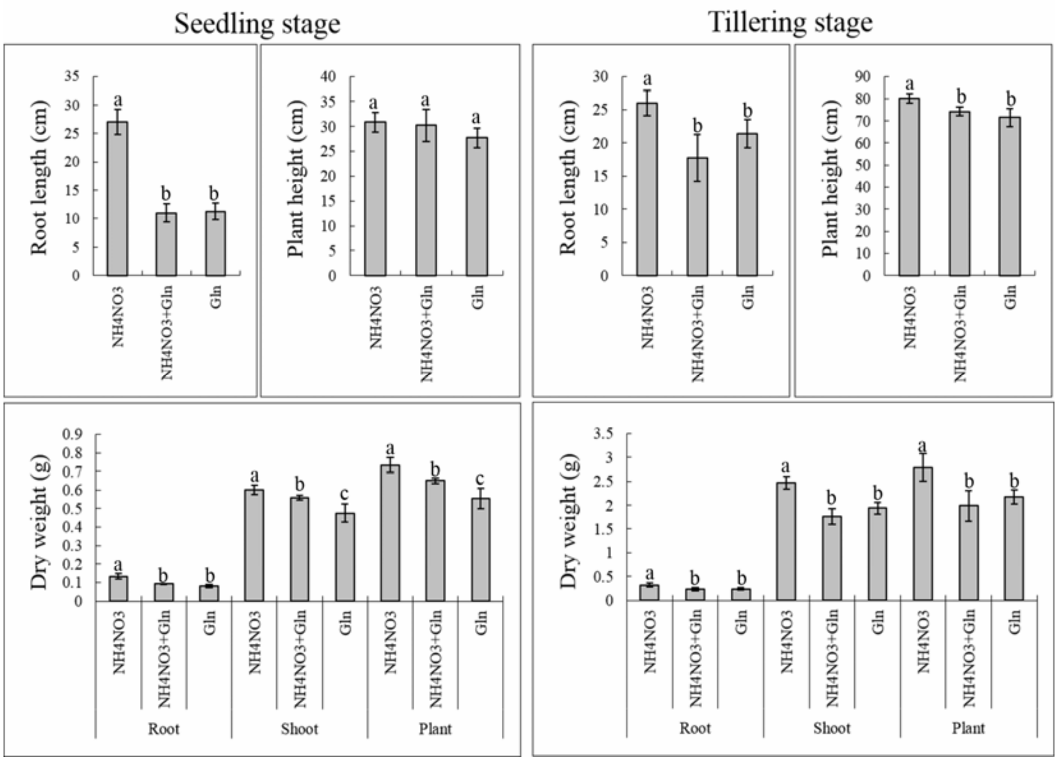

Fig. 1. The root length and plant height, the dry weight of the root, shoot and plant at the seedling stage and the tillering stage under three different nitrogen forms (NH_4NO_3, Gln, NH_4NO_3+Gln). Values are the mean ± Sd of ten randomly selected plants. a, b, c indicate the significant differences among three different nitrogen forms at the level of $p = 0.05$.

In order to study the effect of Gln on the carbon and nitrogen metabolic capacity in the rice plant, the stem nitrate concentration, the concentrations of soluble proteins and carbohydrates in the roots, stems and leaves at both the seedling stage and tillering stage, together with the leaf SPAD value and photosynthesis capacity, total carbon and nitrogen concentrations and carbon/nitrogen ratio in the roots, stems and leaves at the tillering stage were investigated under three different nitrogen forms (N, N + G and G).

For the stem nitrate concentration, compared to the N form, there were 65.0 and 57.3% decreases at the seedling stage, whereas 41.0 and 68.1% decreases at the tillering stage under the N + G and G forms, respectively (Fig. 2). For the soluble proteins at the seedling stage, compared to the N form, 23.0 and 38.4% increases in the roots, 21.6 and 11.4% increases in the leaves were displayed under the N + G and G forms, respectively (Fig. 2A). At the tillering stage, there were 34.0 and 48.9% increases of the soluble proteins in the roots under the N + G and G forms, respectively; and there was 34.3% increase in the leaves under the N + G form (Fig. 2B). For the soluble carbohydrates at the seedling stage, compared to the N form, there were 40.5% and 82.1% increases in the roots, 53.8 and 73.6% increases in the stems under the N + G and G forms, respectively (Fig. 2A). At the tillering stage, compared to the N form, there were 22.1 and 23.7% increases of the soluble carbohydrates in the roots under the N + G and G forms, respectively; there were 146.8 and 167.9% increases in the stems and leaves under the N + G form, respectively; while there were 32.0% decrease in the stems under the G form (Fig. 2B).

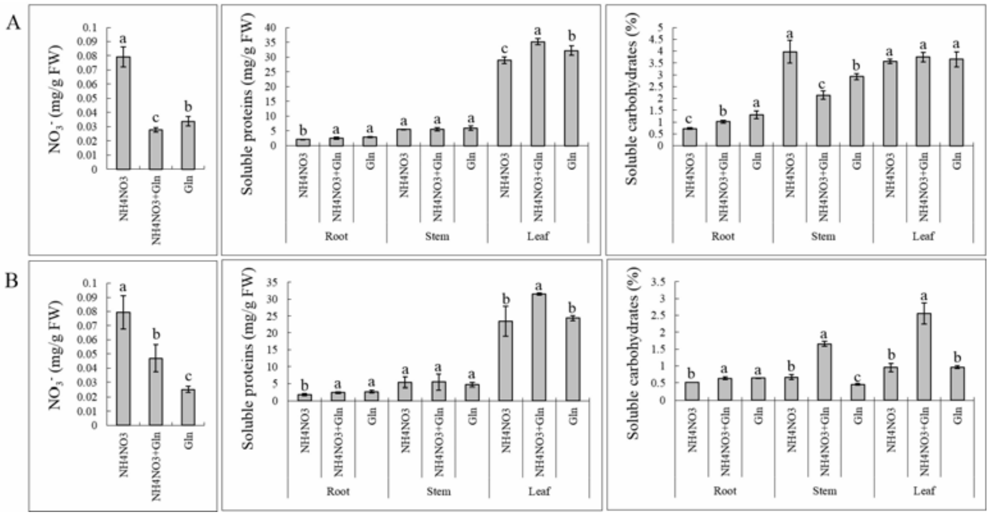

Fig. 2. The concentrations of nitrate in the stems, soluble proteins and carbohydrates in the root, stem and leaf at the seedling stage (A) and the tillering stage (B) under three different nitrogen forms (NH_4NO_3, Gln, NH_4NO_3+Gln). Values are the mean ± Sd from three biological replicates using three randomly mixed plant materials. a, b, c indicate the significant differences among three different nitrogen forms at the level of $p = 0.05$.

For the photosynthesis capacity evaluation, compared to the N form, there was 39.3% decrease was observed in the intercellular CO_2 concentration under the N + G form (Fig. 3). For the carbon and nitrogen analysis, results showed that the treatments of different nitrogen forms caused larger variations in the total nitrogen concentrations than the total carbon concentrations which resulted significant changes in the carbon/nitrogen ratio. Compared to the N form, 8.5 and 6.6% increases in the roots, 10.4 and 4.5% increases in the stems, 9.9 and 2.5% increases in the leaves of the total carbon concentrations were observed under the N + G and G forms, respectively (Fig. 3). For the analysis of total nitrogen concentrations, compared to the N form, there were 37.4 and 17.1% increases in the roots, 48.0 and 13.6% increases in the stems, 30.4 and 6.9% increase in the leaves under the N + G and G forms, respectively (Fig. 3). Oppositely, for the carbon/nitrogen ratio, compared to the N form, there were 21.0, 25.4 and 15.7% decreases in the root, stem and leaf under the N + G form, respectively (Fig. 3).

Although studies showed that plant can uptake a wide range of amino acids by root (Soldal and Nissen 1978, Shobert and Komor 1987, Persson and Näsholm 2001), the limitations in the plants' capacity to metabolize absorbed amino acids may be the primary constraints on their utilization. Therefore, several carbon-nitrogen physiological parameters in the rice plants under three different N sources (NH_4NO_3, Gln, NH_4NO_3 + Gln) were analyzed to evaluate the effect of Gln on rice carbon-nitrogen metabolism. Significant decrease in the stem nitrate concentration under the N + G and G forms when compared to the N form was observed. Stoelken *et al.* (2010) showed that the presence of Gln or Arg in a mixed solution together with NH_4^+ and NO_3^- inhibited the uptake of NH_4^+ in the tested concentration range (55 - 5500 μM total N for Gln and 75 - 7500 μM total N for Arg), while NO_3^- uptake was only affected by the presence of Gln at excess external N concentrations (5500 μM total N) and not by Arg. Similarly, NO_3^- uptake was generally decreased

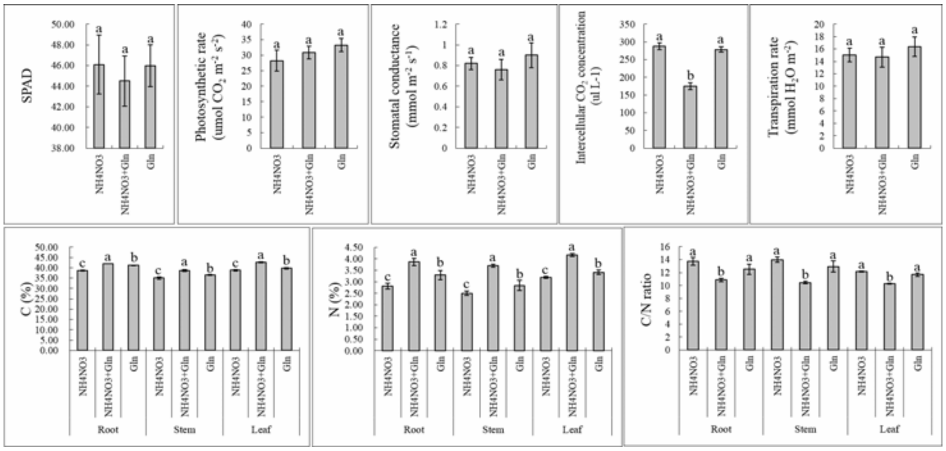

Fig. 3. The leaf SPAD value and photosynthesis parameters, the concentrations of carbon and nitrogen, the carbon/nitrogen ratio in the root, stem and leaf at the tillering stage under three different nitrogen forms (NH_4NO_3, Gln, NH_4NO_3+Gln). The values of SPAD and photosynthesis parameters are mean ± Sd of ten randomly selected plants. Other values are the mean ± Sd from three biological replicates using three randomly mixed plant materials. a, b, c indicate the significant differences among four different nitrogen levels at the level of $p = 0.05$.

in the presence of NH_4^+ in the high-concentration rage (500 μM), but not in the presence of Arg (Gruffman *et al.* 2013). It was found that the root soluble proteins and carbohydrates slightly increased under the N+G and G conditions. The total carbon and nitrogen content increased significantly under the N+G and G conditions, while the intercellular CO_2 concentration, stem carbohydrates and C/N ratio decreased significantly under the N+G condition. These results suggested that carbon-nitrogen metabolic balance was interrupted in rice when grown under the N+G and G conditions compared with N condition. This may be the main reason for the inhibited growth phenotype of rice plants under the N+G and G conditions.

Acknowledgements

This research was supported in part by grants from the National Natural Science Foundation of China (31000932) and the Fundamental Research Funds for the Central Universities (2662013PY041).

References

Bollard EG 1966. A comparative study of the ability of organic nitrogenous compounds to serve as sole nitrogen sources of nitrogen for the growth of plants. Plant and Soil **25**: 153-166.

Bradford MM 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein binding. Anal. Biochem. **72**: 248-254.

Crawford NM and Forde BG 2002. Molecular and developmental biology of inorganic nitrogen nutrition. In Meyerowitz E, Somerville C, eds, The Arabidopsis Book. American Society of Plant Biologists, Rockville, MD.

Forsum O, Svennerstam H, Ganeteg U and Nasholm T 2008. Capacities and constraints of amino acid utilization in *Arabidopsis*. New Phytol. **179**: 1058-1069.

Gruffman L, Jämtgard S and Näsholm T 2013. Plant nitrogen status and co-occurrence of organic and inorganic nitrogen sources influence root uptake by *Scots pine* seedlings. Tree physiol. **34**: 205-213.

Howitt SM and Udvardi MK 2000. Structure, function and regulation of ammonium transporters in plants. BBA-Biomembranes **1465**: 152-170.

Joy KW 1969. Nitrogen metabolism of Lemna minor. I. Growth, nitrogen sources and amino acid inhibition. Plant Physiol. **44**: 845-848.

Kinraide TB 1981. Inter amino acid inhibition of transport in higher plants. Evidence for two transport channels with ascertainable affinities for amino acids. Plant Physiol. **68**: 1327-1333.

Li ZC and Bush DR 1991. D-pH dependent amino acid transport into plasma membrane vesicles isolated from sugar beet (*Beta vulgaris* L.) leaves. Plant Physiol. **96**: 1338-1344.

Lipson D and Näsholm T 2001. The unexpected versatility of plants: Organic nitrogen use and availability in terrestrial ecosystems. Oecologia **128**: 305-316.

Maness N 2010. Extraction and analysis of soluble carbohydrates. Methods Mol. Biol. **639**: 341-370.

Melo PM, Lima LM, Santos IM, Carvalho HG and Cullimore JV 2003. Expression of the plastid-located glutamine synthetase of *Medicago truncatula*: accumulation of the precursor in root nodules reveals an *in vivo* control at the level of protein import into plastids. Plant Physiol. **132**: 390-399.

Morris DL 1948. Quantitative determination of carbohydrates with Dreywood’s anthrone reagent. Science **107**: 254-255.

Näsholm T, Kielland K and Ganeteg U 2009. Uptake of organic nitrogen by plants. New Phytol. **182**: 31-48

Näsholm T and Persson J 2001. Organic nitrogen acquisition by boreal forest plants. Physiol Plantarum **111**: 419-426.

Persson J and Näsholm T 2001. Amino acid uptake: A widespread ability among boreal forest plants. Ecology Letters **4**: 434-438.

Reinhold L and Kaplan A 1984. Membrane transport of sugars and amino acids. Ann. Rev. Plant Physiol. **35**: 45-83.

Rentsch D, Schmidt S and Tegeder M 2007. Transporters for uptake and allocation of organic nitrogen compounds in plants. FEBS Letters **581**: 2281-2289.

Shobert C and Komor E 1987. Amino-acid-uptake by *Ricinus communis* roots - characterization and physiological significance. Plant, Cell & Environment **10**: 493-500.

Soldal T and Nissen P 1978. Multiphasic uptake of amino acids by barley roots. Physiol Plantarum **43**: 181-188.

Stoelken G, Simon J, Ehlting B and Rennenberg H 2010. The presence of amino acids affects inorganic N uptake in non-mycorrhizal seedlings of European beech (*Fagus sylvatica*). Tree Physiol. **30**: 1118-1128.

Stribley DP and Read DJ 1980. The biology of mycorrhiza in the *Ericaceae*. VII. The relationship between mycorrhizal infection and the capacity to utilise simple and complex organic nitrogen sources. New Phytol. **86**: 365-371.

Turnbull MH, Goodall R and Stewart GR 1995. The impact of mycorrhizal colonisation upon nitrogen source utilization and metabolism in seedlings of *Eucalyptus grandis* Hill ex Maiden and *Eucalyptis maculata* Hook. Plant, Cell & Environ **18**: 1386-1394.

Wang HJ, Wu LH, Wang MY, Zhu YH, Tao QN and Zhang FS 2007. Effects of amino acids replacing nitrate on growth, nitrate accumulation, and macroelement concentrations in pak-choi (*Brassica chinensis* L.). Pedosphere **17**: 595-600.

Yoshida S, Forno DA, Cook JH and Gomez KA 1976. Laboratory manual for physiological studies of rice, 3rd eds. International Rice Research Institute, Manila.

(Manuscript received on 29 March, 2015; revised on 26 April, 2015)