Differential Uptake and Utilization of Two Forms of Nitrogen in *Japonica* Rice Cultivars From North-Eastern China

**Jun Yi, Jiping Gao, Wenzhong Zhang**, Chen Zhao, Yan Wang and Xiaoxi Zhen

Rice Research Institute of Shenyang Agricultural University, Key Laboratory of Northern Japonica Rice Genetics and Breeding, Ministry of Education and Liaoning Province, Key Laboratory of Northeast Rice Biology and Genetics and Breeding, Ministry of Agriculture, Shenyang, China

**Japonica** rice is widely planted in north-eastern China because of its superior food quality and stable grain yields. Nitrogen (N) is an essential element for rice growth, and development and its availability directly impacts on rice yields. The knowledge of N uptake and its utilization characteristics in *japonica* are thus important areas of research. Three *japonica* rice cultivars, SN265, SN1401, and SN9816, which are planted across large areas of north-eastern China, were used here to evaluate the uptake and utilization along the life cycle of both ammonium \(\text{NH}_4^+\) and nitrate \(\text{NO}_3^-\) in hydroponically grown plants. The plants were grown in one of three different solutions with varying \(\text{NH}_4^+:\text{NO}_3^-\) ratios: 1:0, 0:1, and 1:1 (The total N content was 40 mg L\(^{-1}\) for each treatment). At the tillering stage, when only \(\text{NO}_3^-\) was provided, lower rates of N uptake and enzyme activities of three rice plants resulted in reduced tiller numbers. During the reproductive stage, the \(\text{NH}_4^+\) and \(\text{NH}_4^+\) uptake rates in SN1401 were consistently maintained at high levels, whereas the rates in SN265 and SN9816 were significantly lower, across all three treatments. At the booting stage, when only \(\text{NO}_3^-\) was provided, SN1401 plants had significantly higher expression levels of *OsNRT2.1* and *OsNRT2.2*, higher activity of nitrate reductase in the roots, and higher activity levels of glutamine synthetase and glutamate synthase in the leaves, compared with the SN265 and SN9816 plants. The higher enzyme activity was beneficial to the secondary assimilation of N, which ultimately promoted panicle development in SN1401. Consequently, the grain yield per plant of SN1401 was the highest with solutions of both \(\text{NH}_4^+\) and \(\text{NO}_3^-\). These results indicate that selecting a rice cultivar with higher utilization of \(\text{NO}_3^-\) is beneficial for increasing the number of grains per panicle, grain yield, and N use efficiency.

**Keywords:** *japonica* rice, nitrogen metabolism, nitrogen use efficiency, grain yield, panicle

**INTRODUCTION**

Rice (*Oryza sativa* L.), is one of the most important global food crops and is widely cultivated in Asia, with *japonica* being a popular subspecies. In China, *japonica* rice is widely planted in the north-eastern and Jianghuai regions. In recent years, because of its food quality and increasing market demand by consumers, *japonica* rice production and cultivation have been gaining more attention.
In plant growth and development, nitrogen (N) is one of the most limiting mineral nutrients for crop production, and the forms of N that are available for plant roots to absorb from the soil are primarily nitrates, ammonium salts, and amino acids (Wang et al., 1993). In *O. sativa*, indica rice cultivars usually have greater nitrate (NO$_3^-$) uptake abilities than the *japonica* rice cultivars (Hu et al., 2015). In addition, different genotypes of *japonica* rice cultivars have been found to have different N uptake rates, grain yields, and N accumulation levels, under the same N treatments in field experiments (Zhang et al., 2007). The need of increasing the N use efficiency to increase rice production has been a long-standing problem for *japonica* rice cultivation and is complicated due to the variability in characteristics of N uptake among the different *japonica* rice cultivars (Kourouba and Ntanos, 2003).

In rice paddy fields, long-term flooding conditions inhibit the process of nitrification by soil microbes, which results from anaerobic conditions, resulting in high concentrations of ammonium (NH$_4^+$) as the main form of N (Kronzucker et al., 1998). However, under the flooded conditions, rice plants can transfer O$_2$ from their shoots to their roots and release it to the rhizosphere via special aerated tissue cells. The aerobic conditions allow soil microbial nitrification to occur and produce NO$_3^-$ in the rice plant rhizospheres for uptake and utilization by the plant (Kronzucker et al., 2000; Kirk and Kronzucker, 2005; Li et al., 2008). In plants, the assimilation pathway of NH$_4^+$ begins with the uptake of NH$_4^+$ from the soil into the plant cells by ammonium transporters, which is incorporated to the synthesis of glutamine, through the glutamate synthase (GS)/glutamate synthase (GOGAT) cycle (Lea and Miflin, 2003). Glutamate dehydrogenase (GDH) catalyzes the other NH$_4^+$-assimilation pathway by synthesizing glutamic acid from alfa-ketoglutarate (Sonoda et al., 2003). On the other side, the process of NO$_3^-$ assimilation begins after NO$_3^-$ uptake by NO$_3^-$ transporters, followed by the reduction of NO$_3^-$ to NO$_2^-$ by nitrate reductase (NR) in the cytoplasm (Lea and Miflin, 1974). Subsequently in plastids, NO$_2^-$ is reduced to NH$_2^+$ by nitrite reductase (NIR) (Xuan et al., 2017). The genes of these key N metabolism enzymes play an important role in rice plant growth and also affect the rice yield components (Tamura et al., 2010; Funayama et al., 2013). In a transgenic rice experiment, mutants lacking OsNADH-GOGAT2 had significantly lower in yields and biomasses, compared with those of their wild-type counterparts (Tamura et al., 2011). In a gene knock-out study of *OsGS1;1* (one of the glutamine synthetase genes encoding for cytoplasmic isoform in rice), the growth rate and the degree of grain filling of the transgenic rice plants was significantly reduced (Tabuchi et al., 2005).

Although the uptake and utilization of different forms of N have been investigated in rice cultivars from southern China, limited information is available on the response of NO$_3^-$ uptake and its utilization in plants grown in conditions where only NO$_3^-$ is present. Most researchers have primarily investigated the differences between NO$_3^-$ and NH$_4^+$ uptake in rice at the seedling stage, or the effects of greater NO$_3^-$ concentrations in NH$_4^+$ nutrient solutions on the processes of N uptake, utilization, and growth for rice (Yan et al., 2011). However, the uptake and utilization of NO$_3^-$ can be affected by the presence of NH$_4^+$ in the same nutrient solution (Zhang et al., 2011). For this paper, we have investigated the differences in N uptake and utilization for three common *japonica* cultivars (SN265, SN1401, and SN9816) from north-eastern China with differing N use efficiencies. We have examined the plant responses to hydroponic fertilizer solutions containing NO$_3^-$ and/or NH$_4^+$ as the N source, throughout multiple plant growth stages. The results in this study showed that the lower rates of NO$_3^-$ uptake and enzyme activities of three rice plants resulted in reduced tiller numbers at the tillering stage. During the reproductive stage, the NH$_4^+$ and NO$_3^-$ uptake rates in SN1401 were consistently maintained at high levels, whereas the rates in SN265 and SN9816 were significantly lower, across all three treatments, which also resulted in the higher grain yield in SN1401. These results provided some evidence for the N management in different growth stages of rice in the field agriculture.

### MATERIALS AND METHODS

#### Plant Materials

Three *japonica* rice cultivars, SN265, SN1401, and SN9816, were chosen based on their different responses to N applications in field trials (location: 41°48′ N, 123°25′ E) of 30 *japonica* rice cultivars carried out in 2015. Their agronomic traits are shown in **Supplementary Table 1**.

#### N Treatments and Growth Conditions

The primary nutrient solution for the hydroponically grown plants was prepared according to a formula from the International Rice Research Institute (IRRI) (Yoshida et al., 1976). The conventional nutrient solution from the IRRI was modified to create the three different conditions for our treatments, by varying the concentration ratios of NH$_4^+$ and NO$_3^-$: 1:0, 0:1, and 1:1, respectively. The total N content was 40 mg L$^{-1}$ for each treatment solution. The contents of the other nutrients in all three treatment solutions were 10 mg P L$^{-1}$, 40 mg K L$^{-1}$, 40 mg Ca L$^{-1}$, 40 mg Mg L$^{-1}$, 5.6 mg Si L$^{-1}$, 0.5 mg Mn L$^{-1}$, 0.05 mg Mo L$^{-1}$, 0.2 mg B L$^{-1}$, 0.01 mg Zn L$^{-1}$, 0.01 mg Cu L$^{-1}$, and 2 mg Fe L$^{-1}$. The nitrification inhibitor dicyandiamide was also added to each treatment solution at a concentration of 5 mg L$^{-1}$. The experiments were conducted at an experimental site at Shenyang Agricultural University (41°48′ N, 123°25′ E) from April to October in 2016, and a rain shelter was used to cover the hydroponic pots of plants.

The plants of the three rice cultivars were cultured from seed to the four-leaf stage (35 days after sowing), and then three uniform plants were equidistantly transplanted into a plastic basket with a diameter of 30.0 cm. The roots were cleaned with demineralized water, and subsequently, each plastic basket was placed in a plastic pot (30.5 cm the uppermost diameter and 20.0 cm the lowest diameter, and 25.0 cm height) with 7 L of nutrient solution in the hydroponic system. Pots without plants were also set up to detect the effect of the hydroponic environment on N content. Six replicates were used for each treatment. All pots were arranged in a randomized design and re-randomized once every 4 weeks.
10 days to minimize the position effects. The pH of the solutions was adjusted to 5.0 with either 1 M NaOH or 1 M HCl every day, while also adding an appropriate amount of demineralized water to supplement the loss by evapotranspiration. The nutrient solution was also replaced every 10 days. Three weeks before seed maturity, we replaced the culture solution with demineralized water (also at a pH of 5).

**Yield Components**
Yield components including effective panicle number, seed-setting rate, 1,000-grain weight, grain number per panicle, and yield per plant were measured for each plant that was sampled. Filled and unfilled grains of the panicle were manually separated for measurements of the seed-setting rate. All filled grains from a single plant were collected and dried at 50°C for measurement of the grain yield per plant. Randomly selected filled grains were used for the 1,000-grain weight measurements.

**Biometric Parameters and N Content**
After adding an appropriate amount of demineralized water to supplement the loss by evapotranspiration, the concentrations of $\text{NH}_4^+$ and $\text{NO}_3^-$ in the solutions were determined using a SEAL AutoAnalyzer 3 (Germany) every 10 days. Whole plants of SN265, SN1401, and SN9816 were sampled at the seed maturity stage. The plants were initially desiccated at 105°C, then oven dried at 80°C to a constant weight, and weighed. Biomasses of the leaves, stems (including sheaths), panicles, and roots were all measured prior to grinding to a powder. The N concentrations of the leaves, stems (including sheaths), panicles, and roots were all determined by semi-micro Kjeldahl digestion and distillation (Nelson and Sommers, 1980).

We calculated the following indices from data collected from the dry matter weights, and N measurements, where the indices and their parameters are defined as follows: total N accumulation per plant (g plant$^{-1}$) is the total amount of N accumulated in a plant after reaching seed maturity; N agronomic efficiency (kg kg$^{-1}$) is grain yield divided by total N application; N recovery efficiency (%) is defined as total N accumulation divided by total N application for each rice plant; and N physiological efficiency (kg kg$^{-1}$) is defined as grain yield divided by total N accumulation per rice plant.

**qRT-PCR and Enzyme Activity**
The top leaves and roots of the rice plants were sampled to examine the differences in the expression of N metabolic genes and enzyme activity at the tillering stage (30 days after transplanting) and booting stage (the day that the top second leaf completely emerged after transplanting) (Wang et al., 2017). RNA extraction was carried out using the EasyPure Super RNA extraction kit (Promega, Shanghai, China). Samples of 0.5 μg of total RNA were reverse transcribed into cDNA using the PrimeScript™ RT Master Mix kit (Takara, Dalian, China). Real-time quantitative RT-PCR (qRT-PCR) analysis was performed on cDNA using the Takara SYBR® Premix EX Taq kit and a 7500 Real-Time System (Applied Biosystems, USA). Rice ACTIN1 was used as the internal control in all analyses. The sequences of the gene-specific primers are shown in Supplementary Table 2.

The activity of NR was determined according to Gibon et al. (2004), GS according to Sun et al. (2014), NADH-GOGAT according to Singh and Srivastava (1986), and GDH was conducted according to Yamaya et al. (1984).

**Statistical Analysis**
The data were statistically analyzed with Excel 2003 (Microsoft Office 2003) and SPSS22.0 for Windows (IBM Corporation), and means were tested by least significant difference at P < 0.05 (LSD 0.05).

**RESULTS**

**Plant Architecture and Yield Components Under Different N Conditions**
In response to the different forms of N in the different treatments, the three *japonica* cultivars reached their maximum tillering stage approximately after 40 days. In the $\text{NO}_3^-$ nutrient solution, the life cycle of the *japonica* cultivars was longer than that of plants grown in the $\text{NH}_4^+$-sole solution, by about 25 days. Furthermore, plant growth and development exhibited a significant delay during the transition period from vegetative growth to that of reproductive growth (Table 1, Figures 1A, B).

With the treatment of only $\text{NO}_3^-$ in the solution, the effective panicle numbers per plant were significantly reduced. The mean effective panicle numbers of SN265, SN1401, and SN9816 were 32.3%, 27.9%, and 33.3% lower than the means of their respective cultivars grown in the $\text{NH}_4^+$-sole solution (Figure 1C). Overall, the numbers of grains per panicle in the $\text{NO}_3^-$ treatments were greater than those in the $\text{NH}_4^+$ treatments, especially in cultivar SN1401 (Figure 1D). Rice plants were significantly greater by 6.4%, 32.1%, and 7.3%, respectively, in SN265, SN1401, and SN9816. The 1,000-grain weight of the SN9816 seeds from the $\text{NO}_3^-$ treatment was significantly greater by 9.4% than that of the $\text{NH}_4^+$ treatment. However, the 1,000-grain weight of the SN265 and SN1401 seeds in the $\text{NO}_3^-$ treatment was not significantly different compared with those plants in the $\text{NH}_4^+$ treatment (Figure 1E). SN1401 showed the highest yields, regardless of the N treatment, with a better behavior under mixed nutrition.

| Stage                  | 1:0 | 0:1 | 1:1 |
|------------------------|-----|-----|-----|
| Maximum tiller         | 40 b| 55 a| 40 b|
| Start of heading       | 69 b| 86 a| 68 b|
| Physiological maturity | 120 b| 145 a| 120 b|

*Note: No differences were observed between cultivars for each growth at developmental stages, and only significant differences were observed for the N form applied. Data were the means ± SE of six biological replicates. Different letters on each row indicate significant difference at P < 0.05.*
However, the SN265 and SN9816 plants grown in the \( \text{NO}_3^- \) nutrient solution were significantly lower by 29.6% and 19.8%, respectively, compared with the plants grown in the \( \text{NH}_4^+ \) nutrient solution (Figure 1F).

**N Uptake and Utilization Under Different N Conditions**

For the entire duration of plant growth, the highest rates of N uptake in the rice were observed between 30 and 70 days after transplanting under the \( \text{NH}_4^+ \) treatments, which corresponded to the active tillering stage and to the end of the booting stage. However, the highest rates of N uptake in the \( \text{NO}_3^- \)-sole treatments were observed 40 to 60 days after transplanting, which corresponded to the maximum tillering stage and the middle of the booting stage. At the tillering stage (0–40 days after transplanting), the uptake rates of both \( \text{NH}_4^+ \) and \( \text{NO}_3^- \) increased gradually; however, the uptake rate of \( \text{NH}_4^+ \) was clearly higher and peaked earlier than that of \( \text{NO}_3^- \). Of the SN1401 plants, the uptake rate of \( \text{NH}_4^+ \) from the \( \text{NH}_4^+ \)-sole solution remained at a high level between 60 and 80 days after transplanting, the days of which were consistent with the middle of the filling stage. After
80 days, the rate clearly began to decline. However, the uptake rate of NH$_4^+$ in the SN9816 plants began to rapidly decline after plant heading, about 70 days after transplanting (Figure 2A). Under the treatment with the NO$_3^-$ nutrient solution, the uptake rate of NO$_3^-$ increased gradually in all three rice plants, reaching their maximum values at 40 days after transplanting. The uptake rates of NO$_3^-$ at 60 days after transplanting, for the three rice plants, noticeably declined. However, the uptake rate of NO$_3^-$ declined slowly in SN1401 (Figure 2B). For the treatment with both NH$_4^+$ and NO$_3^-$, the N uptake rates began to decline 70 days after transplanting, and this was mainly due to the decrease in the uptake of NO$_3^-$ by 80 days after transplanting (the middle of the filling stage), the N uptake rate of the SN1401 plants was markedly higher than those of the SN265 and SN9816 plants (Figure 2C).

The total N taken by the rice plant was higher under the NO$_3^-$-sole treatment because of not only the longer growth periods but also because the NO$_3^-$ uptake is still high after the booting stage. The total N accumulation of the SN1401 plants was significantly higher than that of the SN265 and SN9816 plants, growing in the NO$_3^-$ treatment and the treatment with both NH$_4^+$ and NO$_3^-$ . We also established various indices indicative of N utilization by the plant. Overall, SN1401 was more efficient than SN265 and SN9816 in N use because the three indices of N use efficiency in SN1401 were higher than those of S265 and SN9816, under the same N treatments. Among the different N treatments, the N agronomic efficiencies of SN265, SN1401, and SN9816 plants in the NO$_3^-$ nutrient solution were significantly lower by 41.4%, 20.1%, and 33.3%, compared with those of the NH$_4^+$ solution, respectively. The N recovery efficiencies of SN265 and SN9816 under the NO$_3^-$ treatment were also significantly lower, by 37.3% and 31.2% than that of the NH$_4^+$ treatment, respectively. However, there were no differences in the N physiological efficiency of SN265 or SN9816 between the NH$_4^+$ and NO$_3^-$ treatments (Table 2).

**Relative Expression of N-Metabolizing Genes at Tillering and Booting Stages**

The differences in grain yield per plant were mainly caused by the effective panicle number per plant and grain number per panicle, and considering that the active tillering and booting stages are the key stages for the formation of these two traits, respectively, we examined the differences in gene expression and enzyme activities involved in N uptake and those of N metabolism at the two stages (Figures 3 and 4). The results showed that at the tillering stage, the relative expression levels of OsaAMT1;1, OsaAMT1;2, and OsNADH-GOGAT1 in the plant roots were highest under the treatment with both NH$_4^+$ and NO$_3^-$ (Figures 3G–I). The expression levels of OsaGS1;2 and OsaGDH1 in the roots of all three rice plants were highest under the NH$_4^+$ treatment (Figures 3K, N). In the leaves of the three rice plants, the relative expression levels of OsaGS1;1,
OsFd-GOGAT, and OsGDH1 were higher with the NH₄⁺-sole nutrient solution than with the NO₃⁻-sole nutrient solution (Figures 3A, C, E). The relative expression level of OsNADH-GOGAT1 was higher under the mixed nutrition (Figure 3B).

In addition, because we observed differences in grain counts, we also examined the expression levels of OsDEP1 (a gene that affects the numbers of grains per panicle and N use efficiency in the three rice cultivars) and determined that in SN265, SN1401, and SN9816, the expression levels in the plant leaves were lowest under the NO₃⁻-sole treatment at the tillering stage (Figure 3F).

At the booting stage, the OsAMT1;1 and OsAMT1;2 in the plant roots were more expressed under the NH₄⁺ treatment and mixed nutrition, especially in SN1401 (Figures 4G, H).

**FIGURE 3** The transcription of genes involved in N uptake and assimilation in the leaves (A–F) and roots (G–N) at tillering stage. The proportions of NH₄⁺:NO₃⁻ were 1:0, 0:1, and 1:1. DEP1, dense and erect panicle 1, a gene that affects the numbers of grains per panicle and N use efficiency in the three rice cultivars. Actin1 was used as internal standards. Data were the means ± SE of nine biological replicates. Different letters indicated significant difference at P < 0.05.
The relative expression levels of the nitrate transporter genes, OsNRT2.1 and OsNRT2.2, in the SN1401 were also significantly higher than those for the SN265 and SN9816 roots grown in the NO$_3^-$-sole or NH$_4^+$ and NO$_3^-$ nutrient solutions (Figures 4I, J). Under the NO$_3^-$ treatment, the expression levels of the nitrate reductase gene OsNIA2 in SN9816 was significantly lower than in SN265 and SN9816 (Figure 4M). The expression levels of OsGS1;2 and OsGDH1 in the plant roots were higher under the NH$_4^+$-sole treatment (Figures 4K, N). The expression levels of OsNADH-GOGAT1 in the roots were highest in the treatment with both NH$_4^+$ and NO$_3^-$ (Figure 4L). The expression levels of OsGS1;1 and OsFd-GOGAT in the leaves of all three rice plants were also highest in the treatment with both NH$_4^+$ and NO$_3^-$ (Figures 4A, C). The expression levels of OsNADH-GOGAT2 and OsNIA2 in the leaves were significantly the highest in the NO$_3^-$ treatment (Figures 4B, D). The expression levels of OsGS1;1, and...
OsFd-GOGAT in the SN1401 leaves were significantly higher than those of the SN265 and SN9816 plants in the NO$_3^-$ solution. The expression levels of OsDEP1 were lower under the NH$_4^+$-sole treatment than with the other two treatments (Figure 4F).

**N Metabolism Enzyme Activities at Tillering and Booting Stages**

At the tillering stage, the activity levels of GS, NADH-GOGAT, and GDH in the roots and leaves of the three rice plants in the NH$_4^+$ treatment were significantly higher than those of the plants with the NO$_3^-$ treatment. Among the rice cultivars, the activities of NR and GS in roots and leaves of SN1401 were significantly higher than those of SN265 and SN9816 in the NO$_3^-$ treatment (Figure 5).

At the booting stage, the activities of NR and GS in the roots and leaves of the three rice plants showed lower enzymatic activities in the NH$_4^+$-sole treatment, and overall minimal differences were observed among the cultivars for each N treatment. On the other side, the root GDH activity was higher under the NH$_4^+$-sole nutrition. The NADH-GOGAT and GDH activities were significantly higher in the leaves of SN1401 in all N treatments (Figure 6).

**DISCUSSION**

N is an essential element for plant growth and development and is an important factor affecting rice growth and yield (Vinod and Heuer, 2012), and subsequently, improving N use efficiency is of key importance (Peng et al., 2010). In this study, we observed that after transplanting of the rice seedling, the uptake rate of NH$_4^+$ was faster than the uptake rate of NO$_3^-$ in the tillering stage (Figure 2). Besides, the NH$_4^+$ uptake is maintained until the end of the booting stage. Lower uptake rates of NO$_3^-$ in the NO$_3^-$ treatment may affect the uptake and utilization of other nutrient elements such as phosphorus (Fan et al., 2016) and iron (Zhu et al., 2016) and, thus, affect protein synthesis and metabolism in plants. In turn, plant growth and development will be affected, potentially resulting in prolonged growth periods (Figure 1, Table 2). However, the underlying mechanisms of the prolonged growth periods affected by NO$_3^-$ need further study. In addition, there were almost no change in the concentration of NH$_4^+$ or NO$_3^-$ in the pots without plants, indicating that the environment had little effect on the concentration of NH$_4^+$ and NO$_3^-$ in the experiments. Lian et al. (2012) investigated the kinetic uptake of NH$_4^+$ and NO$_3^-$ from NH$_4$NO$_3$ nutrient solutions in 23 indica rice cultivars from all over the world and found that the maximum uptake rates of NH$_4^+$ were significantly higher than those of NO$_3^-$ at both 10 mg L$^{-1}$ and 40 mg L$^{-1}$ N concentrations. In our study, we found that all three rice cultivars preferred to uptake NH$_4^+$ rather than NO$_3^-$, and the uptake rates of NO$_3^-$ were low in all three cultivars at the tillering stage, and the uptake rate of N began to decline at 60–70 days after plants transplanting, especially the significant decline in the uptake rate of NO$_3^-$ (Figure 2). In addition, under the 1:1 NH$_4^+$:NO$_3^-$ treatment, the decrease in N uptake by rice plants was mainly due to the lower uptake rate of NO$_3^-$ (Figure 2). We also found for plants at the

![Figure 5](https://www.frontiersin.org/article/https://www.frontiersin.org/article/10.3389/fpls.2019.01061)

**FIGURE 5** The activity of enzymes involved in N metabolism in the leaves (A–D) and roots (E–H) at tillering stage. The proportions of NH$_4^+$:NO$_3^-$ were 1:0, 0:1, and 1:1. Data were the means ± SE of six biological replicates. Different letters indicated significant difference at P < 0.05.
booting stage, grown in the NO$_3^-$ nutrient solution, that the expression levels of OsNRT2.1 and OsNRT2.2 in SN1401 roots and the activity of NADH-GOGAT in SN1401 were higher than those of SN265 and SN9816, and this corresponded to the higher N uptake rates of SN1401, compared with SN265 and SN9816. The higher NO$_3^-$ uptake rates of SN1401 at the reproductive growth stage may also result in higher N recovery efficiencies of SN1401 compared with those of SN265 and SN9816. Youngdahl et al. (1982) and Hu et al. (2015) also found that N uptake rates were significantly lower at the seedling and tillering stages of rice plants grown in the NO$_3^-$-sole solution, which resulted in lower dry matter and tiller numbers. The photosynthetic rates of the rice leaves were improved by topdressing with NO$_3^-$ at the later growth stage, and the grain yields of rice with high N use efficiency were also improved by partial nitrate nutrition (Luo et al., 1993; Duan et al., 2007). Therefore, according to the N uptake characteristics of the different types of rice at every stage, empirically based adjustments to N application could effectively increase the uptake of NO$_3^-$ at later stages of plant growth, which ultimately will be beneficial for increasing N utilization and the rice yields.

As excessive NH$_4^+$ in cells may cause plant poisoning, most ions of NH$_4^+$ are assimilated in the root. However, NO$_3^-$ is usually transported from root to the mesophyll cells for its reduction, and the remaining NO$_3^-$ ions are reduced in root cells and stored in the vacuoles (Tegeder and Masclaux-Daubresse, 2018). Due to the differences in N uptake of the rice grown in culture with different forms of N, the expression of related genes involved in N metabolism in leaves and roots significantly differed (Gaur et al., 2012). NH$_4^+$ assimilation mainly occurs through synergistic catalysis of GOGAT, Fd-GOGAT, and NADH-GOGAT (Yang et al., 2016). OsFd-GOGAT is mainly expressed in the plastids of mesophyll cells and is responsible for the NH$_4^+$ re-assimilation coming from NO$_3^-$ reduction and photorespiration (Chen et al., 2016). Especially, there are two types of OsNADH-GOGAT: (1) OsNADH-GOGAT1 is primarily distributed in the root epidermis and cortex cells and participates with OsGS1;2 in the assimilation of NH$_4^+$ in roots; and (2) OsNADH-GOGAT2 is primarily expressed in the vascular bundles of mature leaves (Ma et al., 2016). Moreover, the OsGS1;2 and OsNADH-GOGAT1 are important in the development of active tiller number (Tamura et al., 2010; Funayama et al., 2013), and the OsGS1;1 and OsNADH-GOGAT2 also play an important role in rice grain development (Tamura et al., 2011). In our results, the activities of GS and NADH-GOGAT of the three rice cultivars were different at different stages of plant growth (Lam et al., 1996). At the tillering stage, the expression level of OsGS1;2 and OsGDH1 and the activity of GS, NADH-GOGAT, and GDH in the roots, and the expression levels of OsGS1;1, OsFd-GOGAT, and OsGDH1, and the activity of NADH-GOGAT and GDH in the leaves were higher when NH$_4^+$ was present (either as sole N source or as mixed nutrition) than those of the plants with the NO$_3^-$-sole treatment. The higher gene expression levels and enzyme activities increased N assimilation, which also promoted the rice plants growth and development with the NH$_4^+$ treatment.

**FIGURE 6** The activity of enzymes involved in N metabolism in the leaves (A–D) and roots (E–H) at booting stage. The proportions of NH$_4^+$:NO$_3^-$ were 1:0, 0:1, and 1:1. Data were the means ± SE of six biological replicates. Different letters indicated significant difference at P < 0.05.
at the tillering stage. We also found that in the NH$_4$ treatment, the expression levels of OsGS1;1 or OsFd-GOGAT in the leaves were not significantly different among the three rice cultivars at the booting stage. However, with the NO$_3$ treatment, the expression levels of OsGS1;1 and the activity of GS, NADH-GOGAT, and GDH in the SN1401 leaves were significantly higher than those of SN265 and SN9816 at the booting stage. Those results showed that the difference in expression of genes controlling N-metabolism enzymes in relation to the genetic background of the cultivars (Gaur et al., 2012; Li et al., 2017), and SN1401 had a better capacity in N assimilation at the booting stage, which promoted the panicle development of SN1401. The higher expression levels of OsFd-GOGAT and activities of GS in the leaves may also represent that SN1401 had a better capacity in secondary assimilation of NH$_4$ at the booting stage (Yamaya and Kusano, 2014). However, this higher secondary assimilation ability of SN1401 still needs to be confirmed by further testing the expression level of GS2 and the activity of Fd-GOGAT in the leaves at booting stage.

In addition, some researchers found that plants differed in N utilization due to the differences in genotypes (Song et al., 2011). Koutroubas and Ntanos (2003) found that the N harvest index (the proportion of N content in the grain in relation to total N of the whole plant) of indica varieties was lower than those of indica varieties, which suggests that N physiological efficiencies were also lower. Qian et al. (2004) found that the 1,000-grain weight of the indica cultivar Shanyou63 was significantly greater after topdressing with NO$_3$ than with NH$_4$. Moreover, the N content of grains was also higher with the NO$_3$ topdressing. The yield of rice is mainly determined by four components: the number of effective panicles per area, the number of grains per panicle, the weight of grain, and the number of effective grains per panicle. The yield of rice plants in the reproductive growth stage, and finally, improve the conversion of NO$_3$ to NH$_4$, thereby increasing the nitrogen use efficiency of SN1401 were also significantly higher than those of SN265 and SN9816 plants. The N agronomic efficiency and N recovery efficiency of SN1401 were also significantly higher than those of SN265 and SN9816. Therefore, our results of the differences in N uptake and utilization characteristics between the three rice cultivars given varying forms of N are applicable for improving agricultural use of N fertilizer in rice production.

Moreover, according to the effects of the different forms of N on the effective panicle number and grain number per panicle, the N fertilizer application and irrigation water management could be regulated in the field (Yao et al., 2012; Sun et al., 2012), to reduce the conversion of NH$_4$ to NO$_3$ in the rhizosphere of rice plants, thereby increasing the NH$_4$ uptake of rice plants in the vegetative growth stage; and to increase the content of NO$_3$ in the rhizosphere of rice plants, thereby increasing the NO$_3$ uptake of rice plants in the reproductive growth stage, and finally, improve rice yield and nitrogen utilization.

### AUTHOR CONTRIBUTIONS

JY conducted all the experiments. JG and WZ designed the experiments and edited the manuscript. CZ and XZ measured the gene expression. YW was involved in nitrogen measurement.

### FUNDING

This research was supported by the National Key R&D Program (2018YFD0300306), Chinese Natural Sciences Foundation (31501250), Liaoning BaiQianWan Talents Program (2015-39), and Shenyang Science and Technology Plan Project (17-231-1-37).

### SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2019.01061/full#supplementary-material

### REFERENCES

Chen, H. L., Li, C. R., Liu, L. P., Zhao, J. Y., Cheng, X. Z., Jiang, G. H., et al. (2016). The Fd-GOGAT1 mutant gene lc7 confers resistance to Xanthomonas oryzae pv. Oryzae in rice. Sci. Rep. 6, 26411. doi: 10.1038/srep26411

Duan, Y. H., Zhang, Y. L., Ye, L. T., Fan, X. R., Xu, G. H., and Shen, Q. R. (2007). Responses of rice cultivars with different nitrogen use efficiency to partial nitrate nutrition. Ann. Bot. 99, 1153–1160. doi: 10.1093/abol/mcm051

Fan, X. R., Tang, Z., Tan, Y. W., Zhan, Y., Luo, B. B., Yang, M., et al. (2016). Overexpression of a pH-sensitive nitrate transporter in rice increases crop yields. Proc. Natl. Acad. Sci. U.S.A. 113, 7118–7123. doi: 10.1073/pnas.1525184113

Funayama, K., Kojima, S., Tabuchi-Kobayashi, M., Sawa, Y., Nakayama, Y., Hayakawa, T., et al. (2013). Cytosolic glutamine synthetase1:2 is responsible for the primary assimilation of amino acid in rice roots. Plant Cell Physiol. 54, 934–943. doi: 10.1093/pcp/pcr046

Gaur, V. S., Singh, U. S., Gupta, A. K., and Kumar, A. (2012). Influence of different nitrogen inputs on the members of ammonium transporter and glutamine synthetase genes in two rice genotypes having differential responsiveness to nitrogen. Mol. Biol. Rep. 39, 8035–8044. doi: 10.1007/s11033-012-1650-8

Gibon, Y., Blaising, O. E., Hannemann, J., Carillo, P., Hohne, M., Hendriks, J. H. M., et al. (2004). A robot-based platform to measure multiple enzyme activities in Arabidopsis using a set of cycling assays: comparison of changes of enzyme activities and transcript levels during diurnal cycles and in prolonged darkness. Plant Cell 16, 3304–3325. doi: 10.1105/tpc.104.025973

Hu, B., Wang, W., Ou, S. J., Tang, J. Y., Li, H., Che, R. H., et al. (2015). Variation in the expression levels of OsFd-GOGAT in indica and japonica rice under Mediterranean conditions. Field Crops Res. 83, 251–260. doi: 10.1016/S0378-4290(03)00067-4

Kirk, G. J. D., and Kronzucker, H. J. (2005). The potential for nitrification and nitrate uptake in the rhizosphere of wetland plants: a modelling study. Ann. Bot. 96, 639–646. doi: 10.1093/abol/mci216

Koutroubas, S. D., and Ntanos, D. A. (2003). Genotypic differences for grain yield and nitrogen utilization in indica and japonica rice under Mediterranean conditions. Field Crops Res. 83, 251–260. doi: 10.1016/S0378-4290(03)00067-4
Kronzucker, H. J., Kirk, G. D. I., Yeeh, S. M., and Glass, A. D. M. (1998). Effects of hypoxia on $^{15}N$ fluxes in rice roots: kinetics and compartmental analysis. *Plant Physiol.* 116, 581–587. doi: 10.1104/pp.116.2.581

Kronzucker, H. J., Glass, A. D. M., Siddiqui, M. Y., and Kirk, G. J. D. (2000). Comparative kinetic analysis of ammonium and nitrate acquisition by tropical lowland rice: implications for rice cultivation and yield potential. *New Phytol.* 145, 471–476. doi: 10.1046/j.1469-8137.2000.00606.x

Lam, H. M., Coschigano, K. T., Oliveira, I. C., Melo-Oliveira, R., and Coruzzi, G. M. (1996). The molecular-genetics of nitrogen assimilation into amino acids in higher plants. *Annu. Rev. Plant Biol.* 47, 569–593. doi: 10.1146/annurev.arplant.47.1.569

Lea, P. J., and Millen, B. J. (1974). Alternative route for nitrogen assimilation in higher plants. *Nature* 251, 614–616. doi: 10.1038/251614a0

Li, Y. L., Fan, X. R., and Shen, Q. R. (2008). The relationship between rhizosphere nitrification and nitrogen-use efficiency in rice plants. *Plant Cell Environ.* 31, 73–85. doi: 10.1111/j.1365-3040.2007.01737.x

Lian, Y., Chen, M. X., Shah., F., Wang, Q., Chen, Y. T., Cui, K. H., et al. (2012). Difference between NH$_3$- and NO$_3$- uptake kinetics of different rice (Oryza sativa L.) grown hydroponically. *J. Food Agric. Environ.* 10, 437–442.

Luo, A. C., Xu, J. M., and Yang, X. E. (1993). Effect of nitrogen (NH$_4$NO$_3$) supply on absorption of ammonium and nitrate by conventional and hybrid rice during reproductive growth. *Plant Soil* 155/156, 395–398. doi: 10.1007/BF00025066

Ma, X. L., Zhu, C. H., Yang, N., Gan, L. J., and Xia, K. (2016). g-Aminobutyric acid addition alleviates ammonium toxicity by limiting ammonia accumulation in rice (Oryza sativa) seedlings. *Physiol. Plantarum.* 158, 389–401. doi: 10.1111/pp.12473

Nelson, D. W., and Sommers, L. E. (1980). Total nitrogen analysis of soil and plant tissues. *Anal. Chem.* 52, 2210–2216.

Tabuchi, M., Sugiyama, K., Ishiyama, K., Inoue, E., Sato, T., Takahashi, H., et al. (2005). Severe reduction in growth rate and grain filling of rice mutants lacking OsGS1;1, a cytosolic glutamine synthetase1. *Plant J.* 42, 641–651. doi: 10.1111/j.1365-313X.2005.02406.x

Yamaya, T., and Kusano, M. (2014). Evidence supporting distinct functions of three ammonium transporter genes in rice. *Plant Physiol.* 164, 1809–1810. doi: 10.1104/pp.114.224063

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Yi, Gao, Zhang, Zhao, Wang and Zhen. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.