Uptake and utilization of different nitrogen forms in erect panicle *japonica* rice cultivar

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**ABSTRACT**

The response of rice to nitrogen (N) was compared between three rice cultivars and three N treatments: 1:0, 0:1, and 1:1 NH₄⁺:NO₃⁻. The three rice cultivars used were: Shennong1401 (SN1401, erect panicle rice cultivar) and Habataki and Sasanishiki (curved panicle rice cultivars). At the booting stage, higher gene expression and enzyme activity associated with N metabolism of rice in the including-NO₃⁻ solutions effected the secondary assimilation of N metabolism, and ultimately increased grain development. During the tillering stage, lower NO₃⁻ uptake rates and glutamine synthetase and NADH-dependent glutamate synthase activity in Habataki and Sasanishiki under the NO₃⁻ treatments resulted in lower effective panicle number per plant. However, no significant differences were observed in the grain yield per plant of SN1401 between NH₄⁺ and NO₃⁻ treatments. Our results indicate that the erect panicle rice cultivar can grow better under the including-NO₃⁻ treatment.

**Introduction**

Rice (*Oryza sativa* L.) is one of the most important food crops and is widely cultivated in Asia. During its breeding process, the applications of dwarf genes and heterosis have been two important advances that have led to increased rice yield (Sasaki et al. 2002; Cheng et al. 2007). For super-high yield production, breeding based on ideal plant architecture plays an important role in improving rice growth (Lu et al. 2013). The erect panicle, an important morphological adaptation, adapted to the requirement for higher yields, and promoted the development of ideal plant architecture breeding (Jiao et al. 2010). Compared with the curved panicle rice plants, those with erect panicles were not sensitive to nitrogen (N) during the vegetative growth stage, which could accumulate and assimilate more N, resulting in improving harvest index and grain yield (Sun et al. 2014). However, N utilization by rice plants remains low in northeastern China, where the erect panicle *japonica* rice is more common (Peng et al. 2010; Wang, Cai, et al. 2018).

N is an important mineral nutrient for plant growth and development. The forms of N that are available to plant roots to absorb from the soil are primarily nitrates, ammonium salts, amino acids, and other organic N compounds (Kropp 2015). In the uptake and utilization of NO₃⁻ between *indica* and *japonica* rice cultivars, *indica* usually have higher NO₃⁻ uptake ability than *japonica*. In addition, different genotypes of *japonica* were found to have different N uptake rates under the same N treatment conditions during the seedling stage (Hu et al. 2015). In rice paddy fields, long-term flooding conditions inhibit the process of nitrification by soil microbes, which results in high concentrations of NH₄⁺ (Kronzucker et al. 1998). Therefore, the majority of rice plant usable N is consumed as NH₄⁺ from flooded fields.

However, in well-aerated fields, NO₃⁻ is the main form of available N because the aerobic condition allows soil microbial nitrification to produce NO₃⁻ in the rhizosphere for rice plants to uptake and utilize (Wang et al. 2017).

The uptake and utilization of different forms of N have been widely investigated in rice cultivars (Zhang et al. 2009; Zhao et al. 2011). Most previous studies have investigated differences between NO₃⁻ and NH₄⁺ uptake in rice during the seedling stage, or the effects of greater NO₃⁻ concentrations in NH₄⁺ nutrient solutions on the processes of N uptake and utilization and rice growth (Song et al. 2011). However, the uptake and utilization of NO₃⁻ can be affected by the presence of NH₄⁺ under the same nutrient solution treatment (Zhang et al. 2011). Moreover, limited information is available regarding the response of NO₃⁻ uptake and utilization in rice plants to growth conditions where NO₃⁻ is the only source of N present, especially among the different plant architectures of rice. In the present study, we investigated the differences in N uptake and utilization between Shennong1401 (SN1401, *japonica* rice cultivar, erect panicle), Habataki (*indica* rice cultivar, curved panicle), and Sasanishiki (*japonica* rice cultivar, curved panicle) and examined plant responses to hydroponic fertilizer solutions containing NO₃⁻ and/or NH₄⁺ as N sources throughout multiple plant growth stages. These results may also provide insights into the most effective N management strategies for improved rice production.

**Materials and methods**

**Plant materials**

The rice cultivars SN1401, Habataki, and Sasanishiki were selected based on their response to N application in field trials.
of 30 rice cultivars carried out in 2015. Their agronomic traits are shown in Table 1.

Experimental design

The experiments were conducted at an experimental site at Shenyang Agricultural University (41°48′ N, 123°25′ E) in Shenyang, China, from 22 April (date of sowing) to 29 September (date of harvesting), 2017. The primary nutrient solution for hydroponically grown plants was prepared according to a formula developed by the International Rice Research Institute (IRRI) (Yoshida et al. 1976). The conventional nutrient solution of the IRRI was modified with different concentration ratios of the two forms of N to establish three treatments of 1:0, 0:1, and 1:1 NH4+ and NO3− concentrations. Thus, there were nine treatments in total: three rice cultivars for each of three N treatments. Using a randomized-block design, each treatment had six replicates.

The total N content was 40 mg L−1 for each treatment solution. Therefore, the N levels of the three treatments 1:0, 0:1, and 1:1 were 40 mg L−1 NH4+ and 40 mg L−1 NO3−, and 20 mg L−1 NH4+ and 20 mg L−1 NO3−, respectively. The content of other nutrients in the three treatment solutions were: 10 mg L−1 K, 40 mg L−1 Ca, 40 mg L−1 Mg, 5.6 mg L−1 Si, 0.5 mg L−1 Mn, 0.05 mg L−1 Mo, 0.2 mg L−1 B, 0.01 mg L−1 Zn, 0.01 mg L−1 Cu, and 2 mg L−1 Fe. As a nitrification inhibitor, 5 mg L−1 dicyandiamide was also added to each treatment solution. A rain shelters were used to cover the hydroponic pots of plants from the rain.

Plant growth

The plants of the three rice cultivars were cultured from the seed to the four-leaf stage (35 days after sowing), and then three uniform plants were equidistantly transplanted into a plastic basket containing 7 L of nutrient solution in a hydroponic system. Pots without plants were also set up to test the effect of the hydroponic environment on N content of nutrient solution. All pots were arranged in a randomized design and re-randomized once every 10 d to minimize position effects. The pH of the solutions was adjusted to 5.0 with either 1 M NaOH or 1 M HCl, while also adding an appropriate amount of demineralized water to counter the water lost through evapotranspiration. The nutrient solution was replaced every 10 d. Three weeks before seed maturity, we replaced the culture solution with demineralized water (also at a pH of 5).

| Cultivars | Effective panicle numbers per plant | NO. of grains per panicle | Seed setting rate (%) | 1000-grain weight (g) | Grain yield (t ha−1) |
|-----------|-------------------------------------|--------------------------|----------------------|---------------------|---------------------|
| Habataki  | 12.0 c                              | 204.0 a                  | 84.0 a               | 21.5 b              | 9.9 a               |
| SN1401    | 14.6 b                              | 153.8 b                  | 80.2 b               | 24.6 a              | 10.3 a              |
| Sasanishiki | 18.8 a                             | 123.0 c                  | 82.2 a               | 23.0 ab             | 10.1 a              |
| Mean      | 15.1                                | 160.3 c                  | 82.1                 | 23.1                | 10.1                |
| LSD (0.05)| 0.000                               | 0.000                    | 0.161                | 0.014               | 0.479               |

Note: Data were the means ± SE of five biological replicates. Different letters on each line indicate significant difference at P < 0.05 according to LSD.

Measurement of N content

The concentrations of NH4+ and NO3− in the solutions were determined by a SEAL AutoAnalyzer 3 (Seal Analytical GmbH, Norderstedt, Germany). Whole plants were sampled at the seed maturity stage. Plants were initially desiccated at 105°C, and then oven dried at 80°C to a constant weight, weighed, and finally ground to a powder. Biomass of the leaves, stems (including sheaths), panicles, and roots were measured prior to grinding. The N concentrations of leaves, stems (including sheaths), panicles, and roots were determined by semi-micro Kjeldahl digestion and distillation methods (Nelson and Sommers 1980).

qRT-PCR and measurement of enzyme activity

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

The activity of nitrate reductase (NR) was determined according to Gibon et al. (2004). Activity of glutamine synthetase (GS) was determined according to Sun et al. (2014). Activity of NADH-dependent glutamate synthase (NADH-GOGAT) was determined according to Singh and Srivastava (1986).

Measurement of yield components

Yield components, including effective panicle number, seed-setting rate, 1000-grain weight, grain number per panicle, and yield per plant, were measured for each sampled plant. Filled and unfilled grains of the panicle were manually separated to test the effect of the hydroponic environment on N content of nutrient solution. All filled grains from a single plant were collected and dried at 50°C to measure grain yield per plant. Randomly selected filled grains were used to measure 1000-grain weight.

Calculations and statistical analysis

We calculated the following indexes from data collected from dry matter weights and N measurements, where the indexes and parameters are defined as follows: total N accumulation per plant (g) is the total amount of N accumulated in a

Table 2. Primers sequences used for quantitative real-time PCR.

| Primer            | Forward sequence | Reverse sequence |
|-------------------|------------------|------------------|
| NIA2   | tgcagcagtctaccagtcg | cgtagcggctacacgccttg |
| NiR    | cggagagaggaacagacag | tgcagccttacacgagaga |
| GS1/3  | caccaagagagcaagtcc | acctccacgtctcgcatc |
| GS1/2  | tgccttccatccttcgc | tcaacgctttctggcc |
| NADH-GOGAT1 | tggtgctgcgtgatgcataaa | cggtgactcactgtcagcaatrc |
| NADH-GOGAT2 | ctcctgccagctgtaaggctgaaccc | tgcactgcctctactctgcacta |
| Dep1   | ggcattgcagttggcagctggtg | tgcgtagcagctacctgctctg |
| ACTIN1 | accattgtggtgtagcgtgggtt | cgacagcttcatcctgtagaag |
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 to each rice plant; and N physiological efficiency (kg kg\(^{-1}\)) is defined as grain yield divided by total N accumulation per rice plant.

Data were statistically analyzed in Excel (Microsoft Office 2003) and SPSS22.0 for Windows (IBM Corporation). Means were tested by least significant difference at a significance level of \(P < 0.05\) (LSD 0.05).

### Results

#### Yield components under different N conditions

The effective panicle number per plant and grains per panicle of rice yield components were affected by the interaction between cultivar and N treatment (Table 3). In the NO\(_3^-\)-only solution, the mean effective panicle numbers of Habataki, SN1401, and Sasanishiki were significantly lower by 35.4\%, 21.5\%, and 36.3\% than the means of their respective cultivar grown in the NH\(_4^+\)-only solution. However, the number of grains per panicle of plants under the NO\(_3^-\) treatment was 9.3\%, 27.5\%, and 10.6\% higher than that of plants in the NH\(_4^+\) treatment. The seed setting rate and 1000-grain weight of the three rice cultivars under the NO\(_3^-\) treatment were not significantly different compared with those under the NH\(_4^+\) treatment. The grain yield per plant of Habataki or Sasanishiki plants grown in the NO\(_3^-\) nutrient solution were significantly lower by 29.7\% or 25.6\% compared with those grown in the NH\(_4^+\) nutrient solution. An exception was observed in SN1401 plants, and almost no significant differences were observed between plants when comparing yield between NO\(_3^-\)-only and NH\(_4^+\)-only nutrient solutions.

Table 3. Yield and yield components of three rice cultivars.

|          | NH\(_4^+\):NO\(_3^-\) | Effective panicle numbers per plant | NO. of grains per panicle | Seed setting rate (%) | 1000-grain weight (g) | Grain yield per plant (g) |
|----------|------------------------|-------------------------------------|---------------------------|-----------------------|------------------------|--------------------------|
| Habataki | 1:0                    | 11.3 ± 0.5 b                        | 127.6 ± 2.1 d             | 82.4 ± 3.3            | 21.42 ± 0.59 b         | 25.51 ± 1.54 abc         |
|          | 0:1                    | 7.3 ± 0.5 e                         | 139.5 ± 2.1 c             | 80.9 ± 3.3            | 21.60 ± 0.18 b         | 17.94 ± 2.18 d           |
|          | 1:1                    | 10.0 ± 0.0 c                        | 135.1 ± 0.7 c             | 86.4 ± 2.9            | 21.50 ± 0.11 b         | 25.09 ± 0.87 bc          |
| SN1401   | 1:0                    | 9.3 ± 0.5 cd                        | 124.9 ± 2.4 d             | 84.7 ± 3.4            | 24.39 ± 0.39 a         | 24.11 ± 1.05 c           |
|          | 0:1                    | 7.3 ± 0.5 e                         | 159.3 ± 10.0 a            | 83.7 ± 1.0            | 24.76 ± 1.06 a         | 24.05 ± 1.00 c           |
|          | 1:1                    | 9.0 ± 0.9 d                         | 146.0 ± 10.2 b            | 85.0 ± 3.3            | 24.45 ± 0.26 a         | 27.40 ± 1.50 ab          |
| Sasanishiki | 1:0                  | 15.7 ± 1.0 a                        | 79.3 ± 1.0 f              | 83.8 ± 8.0            | 24.62 ± 0.50 a         | 25.75 ± 4.11 abc         |
|          | 0:1                    | 10.0 ± 0.9 c                        | 87.7 ± 0.5 e              | 87.1 ± 1.8            | 24.94 ± 2.28 a         | 19.17 ± 3.39 d           |
|          | 1:1                    | 15.7 ± 0.5 a                        | 89.1 ± 0.8 e              | 85.5 ± 4.2            | 23.57 ± 1.34 a         | 28.10 ± 2.01 a           |

Note: Data were the means ± SD of six biological replicates. Different letters indicated significant difference at \(P < 0.05\) according to LSD. C, cultivar; T, treatment.
*significant at the 0.05 probability level; **significant at the 0.01 probability level; ns, not significant.

![Figure 1](image-url). The N uptake rates of three rice cultivars under different N nutrient solutions. From transplanting to three weeks before seed maturity, the N uptake rate of three rice cultivars under NH\(_4^+\) (A), NO\(_3^-\) (B) or NH\(_4^+\) and NO\(_3^-\) (C) nutrient solution every ten days. (C), the three histograms at every ten days represented the N uptake rate of Habataki, SN1401 and Sasanishiki, respectively.
In the solution with both NH₄⁺ and NO₃⁻, the grain yield of SN1401 was the highest (Table 3).

N uptake and utilization under different N conditions

For the entire duration of plant growth, the highest rates of NH₄⁺ uptake in rice were evidently higher than those of NO₃⁻. At the tillering stage (0–40 d after transplanting) of the three rice cultivars, the uptake rate of NH₄⁺ or NO₃⁻ increased gradually. The uptake rate of NH₄⁺ was clearly higher and peaked earlier than that of NO₃⁻. In SN1401 plants, the uptake rate of NH₄⁺ from the solution including NH₄⁺ was evidently lower than those of the other two rice cultivars in the early growth stage. However, the N uptake rates of SN1401 plants in the filling stage were higher when grown in the sole N solution (Figure 1(A and B)).

Table 4. N use efficiency of three rice cultivars.

| NH₄⁺:NO₃⁻ | Total N accumulation (g N per plant) | N agronomic efficiency (kg·kg⁻¹) | N recovery efficiency (%) | N physiological efficiency (kg·kg⁻¹) |
|-----------|--------------------------------------|---------------------------------|--------------------------|-------------------------------------|
| Habataki  | 1:0 0.70 ± 0.04 a 30.4 ± 2.0 b 75.5 ± 4.8 a 40.4 ± 3.5 e | | | |
| 0:1       | 0.46 ± 0.04 d 15.5 ± 2.6 e 49.5 ± 4.2 cd 43.0 ± 1.9 de | | | |
| 1:1       | 0.50 ± 0.05 cd 29.9 ± 1.2 b 54.1 ± 6.5 bc 55.7 ± 5.4 ab | | | |
| SN1401    | 1:0 0.58 ± 0.09 bc 21.7 ± 0.4 cd 62.2 ± 11.0 b 47.0 ± 6.0 cd | | | |
| 0:1       | 0.63 ± 0.02 ab 17.5 ± 1.1 e 56.4 ± 1.6 bc 50.8 ± 2.4 bc | | | |
| 1:1       | 0.57 ± 0.08 bc 24.7 ± 0.3 c 61.6 ± 10.2 b 54.1 ± 9.5 abc | | | |
| Sasanishiki | 1:0 0.60 ± 0.06 abc 36.2 ± 2.5 a 64.1 ± 7.0 ab 47.6 ± 4.3 cd | | | |
| 0:1       | 0.44 ± 0.01 d 18.7 ± 3.7 de 39.2 ± 1.3 d 58.0 ± 8.6 a | | | |
| 1:1       | 0.52 ± 0.06 bcd 34.2 ± 3.2 a 56.1 ± 7.4 bc 60.0 ± 3.9 a | | | |
| C         | ns                                   | ns                             | ns                       |
| T         | **                                   | **                             | **                       |
| C×T       | ns                                   | ns                             | ns                       |
| Mean      | 0.56 ± 0.02 d    28.7 ± 0.4 cd   57.6 ± 9.1 bc 50.7 | | | |
| LSD (0.05) | 0.11 ± 0.06 cd | 3.3 ± 0.3 e   7.1 ± 0.3 bc | 6.7 | | |

Note: Data were the means ± SD of six biological replicates. Different letters indicated significant difference at P < 0.05 according to LSD. C, cultivar; T, treatment. *significant at the 0.05 probability level; **significant at the 0.01 probability level; ns, not significant.

N treatment significantly affected N utilization. Total N accumulation of SN1401 plants was significantly higher than that of Habataki and Sasanishiki plants growing in the NO₃⁻ nutrient solutions. In the NO₃⁻ nutrient solution, the N agronomic efficiency and N recovery efficiency of Habataki and Sasanishiki plants were significantly lower than that of plants grown in the NH₄⁺ solution, respectively. However, there was no significant difference in the N recovery efficiency of SN1401 between NH₄⁺ and NO₃⁻ treatments. The highest N physiological efficiency of the three rice cultivars was observed in the solution with both NH₄⁺ and NO₃⁻ (Table 4).

Relative expression of N metabolic genes

The differences in yield per plant are mainly caused by the effective panicle number per plant and grains per panicle
under the treatments with different N forms. Therefore, we examined the differences in expression of N metabolic genes at the tillering and booting stages. The results showed that, at the tillering stage, the expression levels of OsNIA2 in Habataki were significantly higher than those of SN1401 and Sasanishiki roots grown in the NO$_3^-$-only or NH$_4^+$ and NO$_3^-$ nutrient solutions (Figure 2(A)). However, the expression levels of OsNiR in SN1401 and Sasanishiki were significantly higher than that of Habataki roots under the including-NO$_3^-$ treatments (Figure 2(B)). Moreover, under the including-NH$_4^+$ treatment, the expression levels of OsGS1;2 and OsNADH-GOGAT1 in Habataki and Sasanishiki were significantly higher than those of plants roots under the NO$_3^-$-only treatment, respectively. However, no significant differences were observed in SN1401 among the N treatments (Figure 2(C and D)). In the leaves, the expression of OsNIA2 in Habataki was also significantly higher than that of SN1401 and Sasanishiki in the NO$_3^-$ nutrient solutions (Figure 3(A)). The lowest expression levels of OsGS1;1, OsNADH-GOGAT1, and OsFd-GOGAT in the three rice cultivars were observed under the NO$_3^-$ treatment (Figure 3(C–E)). Especially, there was no significant difference in the expression levels of OsFd-GOGAT in SN1401 leaves among the N treatments (Figure 3(E)).

At the booting stage, the expression levels of OsNIA2 and OsNiR in SN1401 and Sasanishiki roots were significantly higher than those of Habataki grown in the including-NO$_3^-$ nutrient solutions (Figure 4(A and B)). Under the NO$_3^-$ treatment, the expression levels of OsGS1;2 and OsNADH-GOGAT1 in SN1401 roots were also significantly higher than those of Habataki and Sasanishiki (Figure 4(C and D)). In the top second leaves of three rice plants, the expression levels of OsNIA2 and OsNiR were clearly upregulated at the booting stage and the highest expression was observed under treatment with only-NO$_3^-$ in the solution (Figure 5(A and B)). In SN1401 plants, the expression levels of OsGS1;1, OsNADH-GOGAT2 and OsFd-GOGAT were significantly higher than those of Habataki and Sasanishiki under NO$_3^-$ treatment. Furthermore, under the NO$_3^-$ treatment, the expression levels of OsGS1;1 and OsFd-GOGAT in SN1401 were significantly higher than those of plants grown in the NH$_4^+$ nutrient solution (Figure 5(C–E)). In addition, we also
examined the expression levels of OsDEP1 and determined that, in SN1401, the expression level was higher under the including-NO$_3^-$ treatment than that under the NH$_4^+$ treatment (Figure 5(F)).

Activity of N metabolizing enzymes

At the tillering stage, the activity of GS and NADH-GOGAT of Habataki and Sasanishiki roots under the NO$_3^-$ treatment was significantly lower than that in plants under the NH$_4^+$ treatment. There were no significant differences in SN1401 among the N treatments. The activity of NR of Habataki and Sasanishiki roots under the NO$_3^-$ treatment was significantly higher by 41.9% and 33.3% compared with plants under the NH$_4^+$ treatment, respectively. However, no significant differences were observed for Sasanishiki between NH$_4^+$ and NO$_3^-$ treatments. The activity of GS and NADH-GOGAT of Habataki and Sasanishiki leaves under the NO$_3^-$ treatment was significantly lower than that in plants under the NH$_4^+$ treatment. However, no significant differences were observed for Sasanishiki between NH$_4^+$ and NO$_3^-$ treatments. Moreover, the activity of GS in SN1401 leaves under the NO$_3^-$ treatment changed less than that of the other two rice plants among the N treatments (Table 5).

At the booting stage, the activity of NR of the rice plant roots under the NH$_4^+$ treatment was significantly lower than that in plants under the NO$_3^-$ treatment. The activity of NADH-GOGAT in SN1401 roots was also the lowest under the NH$_4^+$ treatment. In the leaves, the activity of NR, GS, and NADH-GOGAT of the three rice cultivars was lowest under the NH$_4^+$ treatment. There were no significant differences observed among the three rice cultivars under the other two N treatments (Table 6).

Discussion

N is an important factor affecting rice growth and yield. Rice is being increasingly grown under intermittent irrigation, or even in the aerobic upland field, in which NO$_3^-$ is the main form of N available to rice (Kant 2018). Therefore, improving N use efficiency in rice plants is of key importance to rice growth. 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 10 mg L$^{-1}$ and 40 mg L$^{-1}$ N concentrations. In this study, we also found that, after transplanting, the uptake rates of NH$_4^+$ in the three rice cultivars were higher than those of NO$_3^-$.

In conventional and hybrid rice, the chlorophyll content and photosynthetic rate of leaves were improved by topdressing with NO$_3^-$ at the late growth stage (Luo et al. 1993). The high photosynthetic rates were used for the accumulation of photosynthate. During the filling stage, the NO$_3^-$ uptake rate of SN1401 was obviously higher than that of Habataki and Sasanishiki plants, which also resulted in there being no significant change in the yield of SN1401 between NH$_4^+$ and NO$_3^-$ treatments. Therefore, rational application of N could help improve the utilization of NO$_3^-$ and yield of rice based on the N uptake.

Figure 4. The transcription of genes involved in N uptake and assimilation in the roots at the booting stage. 1:0, 0:1 and 1:1 were the proportions of NH$_4^+$:NO$_3^-$ actin1 were used as internal standards. Data were the means ± SD of nine biological replicates. Different letters indicated significant difference at $P < 0.05$ according to LSD.
characteristics of different types of rice at later stages of plant growth.

Owing to the differences in N uptake of rice plants grown in the culture with different forms of N, the expression of genes involved in N metabolism in leaves and roots were always significantly different (Qiu et al. 2009). After transport into plant cells, the assimilation of NO₃⁻ begins with the reduction of NO₃⁻ to NO₂⁻ by NR.

Table 5. Activity of enzymes involved in N metabolism in the roots and leaves at the tillering stage.

|                     | Roots                     |                      |                     |                      |
|---------------------|---------------------------|----------------------|---------------------|----------------------|
|                     | NR (umol g⁻¹ h⁻¹)         | GS (U mg⁻¹ protein h⁻¹) | NADH-GOGAT (nmol min⁻¹ g⁻¹) |                     |
|                     | Habataki 1:0               | 0.2 ± 0.0 c           | 45.2 ± 4.2 a        | 336.1 ± 12.7 a       |
|                     | 0:1                       | 0.5 ± 0.0 a           | 35.1 ± 3.2 c        | 223.8 ± 16.8 b       |
|                     | 1:1                       | 0.5 ± 0.0 a           | 45.4 ± 3.2 a        | 328.2 ± 23.6 a       |
|                     | SN1401 1:0                | 0.2 ± 0.0 c           | 49.1 ± 1.5 a        | 350.2 ± 32.3 a       |
|                     | 0:1                       | 0.5 ± 0.0 a           | 48.6 ± 1.3 a        | 346.9 ± 44.3 a       |
|                     | 1:1                       | 0.5 ± 0.0 a           | 50.2 ± 3.3 a        | 362.5 ± 31.7 a       |
|                     | Sasanishiki 1:0           | 0.2 ± 0.0 c           | 46.7 ± 4.2 a        | 348.4 ± 45.2 a       |
|                     | 0:1                       | 0.4 ± 0.1 b           | 40.1 ± 2.6 b        | 232.5 ± 5.6 a        |
|                     | 1:1                       | 0.4 ± 0.0 b           | 46.9 ± 1.6 a        | 360.4 ± 37.5 a       |
|                     | C, T                      | **                   | **                  | **                   |
|                     | C × T                     | ns                   | **                  | **                   |
|                     | Mean                      | 0.4                  | 45.3                | 321.0                |
|                     | LSD (0.05)                | 0.0                  | 3.1                 | 31.9                 |

Note: Data were the means ± SD of four biological replicates. Different letters indicated significant difference at P < 0.05 according to LSD. C, cultivar; T, treatment. *significant at the 0.05 probability level; **significant at the 0.01 probability level; ns, not significant.
in the cytoplasm (Wang, Cheng, et al. 2018), and then NO$_3$ is reduced to NH$_4^+$ by nitrite reductase (NIR) in plastids (Maeda et al. 2014). The assimilation pathway of NH$_4^+$ begins with the transportation of NH$_4^+$ from soils into plant cells for the synthesis of glutamine through the GS/GOGAT cycle (Singh and Ghosh 2013). In our study, compared with Sasanishiki, we observed higher expression of OsNIA2 and activity of NR in Habataki and SN1401 at the tillering stage, which also indicated greater reduction rates of NO$_3$ in Habataki and SN1401. The assimilations of NH$_4^+$ were divided into primary assimilation and secondary assimilation according to the source of NH$_4^+$. In rice plants, OsGS1;2 and OsNADH-GOGAT1 are involved in the primary assimilation of NH$_4^+$ in roots (Xuan et al. 2017). The secondary assimilation NH$_4^+$ mainly includes the reutilization of NH$_4^+$ produced by protein degradation during leaf senescence by the participation of OsGS1;1 and OsNADH-GOGAT2, and the reutilization of NH$_4^+$ produced by the participation of OsFd-GOGAT in leaf photorespiration (Yamaya and Kusano 2014; Yang et al. 2016). Our results suggested that at the tillering stage, the primary assimilation and secondary assimilation of NH$_4^+$ in Habataki and Sasanishiki under the NO$_3^-$only treatment were lower than those under the other two N treatments. However, there was no significant difference in SN1401 under all three N treatments. At the booting stage, the primary assimilation and secondary assimilation of NH$_4^+$ were higher under the including-NO$_3^-$ treatment. Moreover, Tamura et al. (2011) reported that both OsGS1;1 and OsNADH-GOGAT2 play important roles in rice grain development. Therefore, the higher abilities of N assimilation were benefit to panicle development in the three rice plants at the booting stage under the including-NO$_3^-$ treatment (O’Brien et al. 2016; Wang, Cheng, et al. 2018).

The erect panicle architecture of *japonica* rice cultivars was caused by a mutation at the DEP1 locus. The mutation led to shorter internodes, resulting in an increased number of grains per panicle, and a consequent increase in grain yield (Huang et al. 2009). This architecture could improve the air flow capacity in rice canopy and reduce the shadowing by the panicle and upper leaves to the lower leaves (Xu et al. 2010). In addition, Sun et al. (2014) found that DEP1-encoded G protein γ subunit would affect N use efficiency of rice by interacting with α and β subunits. The plants carrying the *dep1* allele were not insensitive to N at the vegetative growth stage, thus increased the N uptake and utilization. The tiller number per plant of erect panicle rice plants showed no difference between the high or low nitrogen fertilization levels (0, 60, 200, 300 kg ha$^{-1}$). In the present study, we also found that, under the NO$_3^-$only condition, the effective panicle number per plant of the erect panicle *japonica* rice cultivar, SN1401, was less different from the two other treatments. Moreover, under the including-NO$_3^-$ treatment, the number of grains per panicle in the three rice cultivars was significantly higher than that under the NH$_4^+$ treatment. Therefore, the grain yield per plant of SN1401 was significantly higher under the treatment with both NH$_4^+$ and NO$_3^-$ in the solution. However, owing to the differences in the effective panicle number per plant, the grain yield per plant of *indica* rice cultivar Habataki and curved panicle *japonica* rice cultivar Sasanishiki under the NO$_3^-$ treatment was significantly lower than that of under the other two treatments. Consequently, compared with Habataki and Sasanishiki, there were no significant differences in the N recovery efficiency and N physiological efficiency of SN1401 under the three N treatments.

**Conclusion**

In this hydroponic experiment of varying the availability of the two main forms of N to three rice cultivars, plants showed different growth patterns and N uptake rates and utilization among the cultivars, SN1401 (*japonica* rice cultivar, erect panicle), and Habataki (*indica* rice cultivar, curved panicle), Sasanishiki (*japonica* rice cultivar, curved panicle). During growth, the NO$_3^-$ uptake rates of rice plants were lower than those of NH$_4^+$. Furthermore, under the NO$_3^-$ conditions and when plants were at the tillering stage, the higher expression levels of genes and activity of enzymes associated with N metabolism likely improved N utilization in SN1401 compared with that of Habataki and Sasanishiki. At the booting stage, NO$_3^-$ promoted a higher grain number per panicle. Consequently, when using the solution with both NH$_4^+$ and NO$_3^-$, the grain yield per plant of SN1401 was higher than that in the other two treatments. Therefore, our results regarding the differences in N uptake and utilization characteristics between the rice cultivars given different forms of N, are applicable toward improving the use of N fertilizer, and thus yield, in rice production.
Disclosure statement
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