Two Splicing Variants of OsNPF7.7 Regulate Shoot Branching and Nitrogen Utilization Efficiency in Rice

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Rice includes 93 nitrate and peptide transporters family (NPF) members that facilitate the soil uptake and internal reallocation of nitrogen for growth and development. This study demonstrated that OsNPF7.7 had two splicing variants, and altered expression of each variant could regulate shoot branching and nitrogen utilization efficiency (NUtE) in rice. The expression of both variants was down-regulated in the buds by increased nitrogen level in the Japonica rice variety ZH11. The expression level of long-variant OsNPF7.7-1 was higher in panicles at reproductive stage, however, the expression level of short-variant OsNPF7.7-2 was higher in buds and leaves at vegetative stage compared to each other in ZH11. OsNPF7.7-1 was localized in the plasma membrane, whereas OsNPF7.7-2 was localized in the vacuole membrane. Furthermore, the results indicated that the expression level of each variant for OsNPF7.7 determined axillary bud outgrowth, and then influenced the rice tiller number. Overexpression of OsNPF7.7-1 could promote nitrate influx and concentration in root, whereas overexpression of OsNPF7.7-2 could improve ammonium influx and concentration in root. RNAi and osnpf7.7 lines of OsNPF7.7 showed an increased amount of amino acids in leaf sheaths, but a decreased amount in leaf blades, which affected nitrogen allocation and plant growth. The elevated expression of each variant for OsNPF7.7 in ZH11 enhanced NUtE using certain fertilization regimes under paddy field conditions. Moreover, overexpression of each variant for OsNPF7.7 in KY131 increased significantly the filled grain number per plant. Thus, increased each variant of OsNPF7.7 has the potential to improve grain yield and NUtE in rice.

Keywords: rice, nitrogen, transporter, tiller number, grain number

INTRODUCTION

The application of nitrogen is one possible measure to obtain higher yield, and China applies more nitrogen fertilizers than any other country in the world (Narits, 2010). The loss of nitrogen is not only a serious waste of limited nutrient resources, but also negatively affects the environment through water and air emissions, eutrophication, ground water pollution, and soil...
acidiﬁcation (McAllister et al., 2012). A potential solution to this issue could be the increase of nitrogen use eﬃciency (NUE) in crop plants, including rice (Xu et al., 2012). NUE is divided into two components: N uptake eﬃciency (NUpE) and Nitrogen utilization eﬃciency (NutE) (Han et al., 2015). Therefore, NUE is a crucial strategy to increase the grain yield, which is mainly controlled by tiller number per plant, grain number per panicle, and thousand-grain weight in rice (Li et al., 2003; Liang et al., 2014).

Rice tiller experience two distinct stages in its development: the formation of an axillary bud at each leaf axil and the outgrowth of the axillary bud (Li et al., 2003). Nitrogen could enhance the metabolism of carbon and nitrogen, and the synthesis of endogenous hormones such as cytokinin, and then promote the elongation of axillary bud and the production of tiller (Sakakibara et al., 2006; Ohashi et al., 2017). Plants have evolved multiple transport systems to facilitate nitrogen uptake from the soil and internal reallocation involving in growth and development including rice tiller number (Rentsch et al., 2007; Tsay et al., 2007; Hu et al., 2014). Among Rice 93 nitrate and peptide transporters family (NPF) transporters (NPF: NRT1, low-affinity nitrate transporter; PTR, di/tripeptide transporter) (Zhao et al., 2010; Léran et al., 2014), only nine members have been functionally studied. OsNRT1 (OsNPF8.9) is a low-affinity nitrate transporter (Lin et al., 2014), only nine members have been functionally studied.

Materials and Methods

The Acquisition of Transgenic Rice With Altered Expression of OsNPF7.7

A cDNA 1,710 bp fragment of OsNPF7.7-1 or a cDNA 1,428 bp fragment of OsNPF7.7-2, containing the open reading frame (ORF) of each OsNPF7.7 variant, was inserted downstream of the 35S promoter in pCAM1301 with BglII and AflII, respectively, producing OsNPF7.7-1 or OsNPF7.7-2 overexpression plasmid. To generate OsNPF7.7 RNAi construct, two 263 bp cDNA fragments in common sequence of both variants for OsNPF7.7 were ampliﬁed by PCR and inserted downstream of the Ubi-1 promoter in rice RNAi vector pTCK303 (Wang et al., 2004) with BamHI/KpnI and SpeI/SacI, respectively. The homozygous T-DNA insertion mutant osnfp7.7 in ZH11 background was obtained from the Rice Mutant Database of Huazhong Agricultural University (Mutant ID: 04Z11EH19, T-DNA was inserted at site 1091 bp below the ﬁrst ATG in OsNPF7.7). To generate OsNPF7.7-GFP, OsNPF7.7-1 or OsNPF7.7-2 ORF (lacking the stop codon) was ampliﬁed by PCR and was cloned in front of the green ﬂuorescent protein (GFP) coding region in pCAM1302 vector3 with BglII and SpeI, respectively. Agrobacterium strain EHA105 mediated vectors were further transformed into the Japonica rice varieties ZH11 or KY131 (Hiei et al., 1997). The T2 or T3 transgenic lines were used for PCR detection. All the primers were listed in Supplementary Table S1.

Subcellular Localization of OsNPF7.7

To determine the subcellular localization of both variants of OsNPF7.7, p35S-OsNPF7.7-1-GFP, p35S-OsNPF7.7-2-GFP, and p35S-GFP were transformed into rice protoplasts as described previously (Li et al., 2015). Protoplasts were acquired from leaf sheaths of rice seedlings after sowing for 7–15 days. The co-expressed markers were plasma membrane protein OsMCA1 fusing with mCherry (Kurusu et al., 2012) and vacuole membrane protein AtTPK fusing with mkate (Voelker et al., 2006). The ﬂuorescence was observed using a confocal laser scanning microscope (Leica SP8 AOBS, Wetzlar, Germany).

Rice Growth Under Greenhouse Conditions

Germinated seeds were cultured at 28°C under white light with a 16 h light/8 h dark photoperiod for 14 days by basic rice culture solution (Yoshida et al., 1976). The composition of the basic rice solution was as follows: 1.0 mM NH4NO3, 0.32 mM NaH2PO4, 0.51 mM K2SO4, 1 mM CaCl2, 1.65 mM MgSO4, 8.9 µM MnSO4, 0.5 µM Na2MoO4, 18.4 µM

1http://rice.plantbiology.msu.edu/

2http://rmd.ncpr.cn/

3http://www.cambia.org
the 2.0 mM ammonium-treatment and ammonium-starved
concentration between the 2.0 mM nitrate-treatment and nitrate-
stage and 1/3 at heading stage. The initial nitrogen level of soil
Ltd.) was applied before seedling transplanting, 1/3 at tillering
year 2014 to 2017 at rice experimental field of Huazhong
soil under greenhouse condition continuously. Samples were
suspended with cystosepiment by the basic rice culture solution under
Harvested for RNA extraction at vegetative stage and reproductive
Conditions
32°C for sodium lamp 400 w 14 h in the daytime and 25°C for dark 10 h in the
the 2.0 mM nitrate-treatment and nitrate-

Rice Growth Under Paddy Field Conditions
The rice grew in paddy at season June to October from
year 2014 to 2017 at rice experimental field of Huazhong
Agricultural University. The 1/3 of the total amount of fertilizer
(N/P2O5/K2O = 19%/7%/14%; Hubei Batewang Chemical Co.,
Ltd.) was applied before seedling transplanting, 1/3 at tillering
stage and 1/3 at heading stage. The initial nitrogen level of soil
paddy was 30 kg/hm². Generally, the number of rice plants
was 30 for each experiment and the planting density was
19.98 cm × 19.98 cm. For the field yield trials, the number
of rice plants was 100 for each transgenic plants and ZH11.
For paddy field test of fertilization regimes, the amount of
NH4NO3 treatment was 0, 90, 180, and 270 kg/hm². For each
treatment, nitrogen was fractionated: 1/3 of the total amount at
seedling transplanting, 1/3 at tillering stage and 1/3 at heading
stage. Phosphorus and potassium provided as KH2PO4 and KCl,
respectively, were applied before transplanting at the amount of
180 kg/hm² for all treatments.

Nitrogen Influx, Concentration, and Utilization Efficiency Analysis
To analysis the nitrate and ammonium influx and concentration, 2
week seedlings of ZH11, and transgenic plants of OsNPF7.7
were placed in basic rice culture solution without nitrogen for
3 days. The nitrogen-starved seedlings were transferred to culture
solution containing 2.0 mM NaNO3 or 1.0 mM (NH4)2SO4 for
24 h. NO3⁻ influx was calculated as the difference in NO3⁻
concentration between the 2.0 mM nitrate-treatment and nitrate-
starved plants in an hour. Ammonium concentration was measured by
the colorimetric method (Kemsley et al., 2001). Total free amino
acid concentration was measured by the ninhydrin method
(Fang et al., 2013). Sole free amino acid concentration was
measured by HPLC method using amino acid analyzer L-8800
HITACHI. Total nitrogen content and total protein content were
determined using the semi-micro Kjeldahl method by using a
nitrogen analyzer (Smart Chem 200, Westco, Italy). NUtE was
determined as grain dry weight (g)/[total N in grain (g) + total
N in straw (g)].

RNA Extraction and qRT-PCR
Total RNA was extracted using TRizol reagent (Invitrogen,
Beijing, China). First-strand cDNA was synthesized using oligo
(dT) primers and MLV reverse transcriptase (TaKaRa Bio,
Beijing, China). qRT-PCR was carried out using SYBR Green
Premix (TaKaRa Bio) and monitored with the 7500 qRT-PCR
system (Applied Biosystems, Foster City, CA, United States).
To detection the expression level of two variants for OsNPF7.7,
the primers sites for longer variant OsNPF7.7-1 were situated
at the sequence of variant OsNPF7.7-1 its own, and the primers
of OsNPF7.7-2 were designed at the common sequence of both
two variants. Next, the actual expression amount of variant
OsNPF7.7-2 was that both variants amount of OsNPF7.7 deduced
the amount of OsNPF7.7-1. The primers for qRT-PCR were listed
in Supplementary Table S1.

Bud Outgrowth Analysis
To analyze bud outgrowth of different transgenic plants of
OsNPF7.7, 2 week seedlings were grown in hydroponic culture
box with size 525 mm × 360 mm × 230 mm in the rice culture
solution under greenhouse condition. The greenhouse condition
is 32°C for sodium lamp 400 w 14 h in the daytime and 25°C
for dark 10 h in the evening. The nutrient solution was renewed
every 3 days. The length of bud outgrowth was measured using a
stereoscope microscope with ImageJ software.

Statistical Analysis
For all treatments, the statistical differences were indicated by
asterisks, and the student’s t-test allowing the determination of
the significance between two sets of data was performed using the
SPSS 10 software (IBM, Inc.). Significant levels: ***p < 0.001,
**p < 0.01, *p < 0.05; NSp > 0.05.

RESULTS
Two Splicing Variants of OsNPF7.7 Have
Different Expression Patterns
We found that OsNPF7.7 in the OsNPF family has two splicing
variants (the long-splicing variant OsNPF7.7-1 and the short-
splicing variant OsNPF7.7-2) with different exons (Figure 1A).
The splicing proteins were localized at the membrane and
contained 11 transmembrane helices in the long variant and
nine in the short variant (Figure 1B). To gain insight into
the comprehensive roles of the alternative splicing mRNA
forms of OsNPF7.7 in response to various forms of nitrogen in rice, their expression patterns were investigated by qRT-PCR using inorganic nitrogen (NO$_3^-$, NH$_4^+$, or NH$_4$NO$_3$) as a sole nitrogen source. Higher levels of long splicing mRNA existed in OsNPF7.7, indicating that different mRNA splicing forms of a gene were expressed selectively in response to the form of nitrogen. In response to all nitrogen treatments, the expression of OsNPF7.7-2 was downregulated in the roots (Figure 1C) and axillary buds (Figure 1D) from low to high nitrogen concentration, whereas the expression of OsNPF7.7-1 was downregulated only in the axillary buds (Figures 1C–E). To further determine the expression level of two splicing variants for OsNPF7.7 in rice, qRT-PCR experiment of each variant for OsNPF7.7 was measured with different rice tissues (Figure 1F). The result showed that the expression of OsNPF7.7-1 is higher in panicles at reproductive stage, however, the expression of OsNPF7.7-2 is higher in buds and leaves at vegetative stage compared to each other (Figure 1F).
Subcellular Localization of OsNPF7.7

To determine the intracellular localization of OsNPF7.7, we found that the 35S: GFP (control) produced green fluorescence in the plasma membrane and the nucleus (Figures 2A–C), but the transient expression of the long variant OsNPF7.7-1-GFP in rice protoplasts produced green fluorescence in the plasma membrane (Figures 2D–F), whereas that of the short variant OsNPF7.7-2-GFP produced green fluorescence in the vacuole membrane (Figures 2G–I). Furthermore, the results showed that the OsNPF7.7-1-GFP fusion protein signal was localized at plasma membrane (Figures 2J–L) with overlap signal of the plasma membrane marker OsMCA1 fusing with mCherry, and OsNPF7.7-2-GFP fusion protein signal was localized at vacuolar membrane (Figures 2M–O) with overlap signal of vacuolar membrane marker AtTPK fusing with mkate. Overall, these data indicated that two variants of OsNPF7.7 had different localization patterns.

Altered Expression of Both Variants of OsNPF7.7 Controls Tiller Number Through Regulation of Axillary Bud Outgrowth

To determine the function of OsNPF7.7 in rice plants, we generated OsNPF7.7 overexpressing and RNAi transgenic rice plants (Figure 3). The tiller number of OsNPF7.7-1 overexpressing lines (OE1-1, OE1-2; Figures 3B,C,K,L) and OsNPF7.7-2 overexpressing lines (OE2-1, OE2-2; Figures 3D,E,K,L) was superior to that of ZH11 (Figures 3A,K,L). RNAi lines (common sequence RNAi of two variants for OsNPF7.7) showed a relatively low tiller number, dwarfism, and short panicles (Figures 3F,G,K,L). Therefore, the tiller number of mutant osnpf7.7 (common sequence knockout of two variants for OsNPF7.7) was significantly lower than that of ZH11 (Figures 3H,K,L), but the tiller number was largely recovered when either variant of OsNPF7.7 was overexpressed in osnpf7.7 (Figures 3I–L).

To further examine whether OsNPF7.7 regulated the axillary bud formation or axillary bud elongation and controlled the rice tiller number, we tested the responses of OsNPF7.7 transgenic plants raised in hydroponic culture. The results showed that the length of first (Figures 4A,B) and second (Figures 4A,C) axillary buds of OE1 and OE2 plants were longer at the seedling stage than those of ZH11, but the length of buds was shorter in RNAi lines and osnpf7.7 plants than that in ZH11 (Figures 4A–C). Furthermore, the expression of OsNPF6.5 (Figure 4D) and OsAMT1.2 (Figure 4F) increased in OE1 lines; the expression of OsNR (Figure 4E), OsAMT1.2 (Figure 4F) and OsGS1.2 (Figure 4G) increased in OE2 lines; and the expression of OsNPF6.5 (Figure 4D), OsNR (Figure 4E), OsAMT1.2 (Figure 4F), and OsGS1.2 (Figure 4G) decreased significantly in Ri and osnpf7.7 plants. Furthermore, we indicated that overexpression of OsNPF7.7-1 promoted the nitrate influx (Supplementary Figure S1A) and nitrate concentration in rice (Supplementary Figure S1B), whereas overexpression of OsNPF7.7-2 enhanced significantly the ammonium influx (Supplementary Figure S1C) and concentration in rice (Supplementary Figure S1D). The expression of the outgrowth bud elongation marker OsFC1 dramatically decreased in OE1 and OE2 lines, but increased in RNAi and osnpf7.7 plants (Figure 4H). In addition, the strigolactone signaling gene OsD3 also decreased in OE1 and OE2 lines, but increased in RNAi and osnpf7.7 plants (Figure 4I).

Effects of Two Splicing Variants of OsNPF7.7 on Nitrogen Translocation to the Leaves and Recycling to the Panicle

To investigate the reasons that OsNPF7.7 affects the rice tiller number, we measured the free amino acid and total nitrogen content in the roots, stems, and leaves of OE, Ri, and osnpf7.7 lines (Figure 5). The results showed that the free amino acid content was higher in the roots of Ri and osnpf7.7 plants than...
in those of ZH11, but lower in the leaves (Figure 5A). The amino acids that mainly accumulated in the root of Ri and osnpf7.7 plants were Asp, Thr, Ser, Ala Lys, Gln, Arg, and Pro; of these, only Ser was lower in the leaves (Supplementary Table S2). The free amino acid content in the stem of the two variant OE lines was lower (Figure 5A) than in those of ZH11, and the amino acids that showed the higher reduction were Asp, Thr, Ser, Gly, Ala, Met, Ile, Leu, Tyr, Phe, His, Arg, and Pro (Supplementary Table S2). Nevertheless, the free amino acid content of OE1 and OE2 plants was relatively higher in the root and leaves than in those of ZH11, but lower in the stem (Figure 5B). Furthermore, we found that the free amino acid content was relatively higher in the roots of Ri and osnpf7.7 plants than in those of ZH11, but lower in the leaves (Figure 5B). Nevertheless, the total nitrogen content of OE1 and OE2 lines was relatively higher in all organs than in those of ZH11, but lower in Ri and osnpf7.7 plants (Figure 5C). Therefore, both splicing variants of OsNPF7.7 participated in nitrogen allocation from the roots to the leaves and affected rice growth and development.

The effects of altered OsNPF7.7 expression on agronomic traits associated with the grain number were evaluated using paddy field-grown plants (Figure 6A). The primary branch number per panicle (Figure 6B), secondary branch number per panicle (Figure 6C), panicle length (Figure 6D), and filled grain number per panicle (Figure 6E) of OE2 lines were significantly greater than those of ZH11 and markedly higher than that in OE1 lines. Nevertheless, the same indices of Ri and osnpf7.7 plants were significantly lower than those of ZH11 (Figures 6B–E).

Therefore, we indicated that two splicing variants of OsNPF7.7 also regulate the panicle branching in rice.

**Overexpression of Both Splicing Variants for OsNPF7.7 Increases Grain Yield and Enhances Nitrogen Utilization Efficiency**

To further understand the potential practical application of OsNPF7.7, we evaluated the NUtE of transgenic plants in different concentrations of fertilizer under paddy field conditions. The results showed that NUtE of OE1 and OE2 lines was almost the same as that of ZH11, but the NUtE of Ri and osnpf7.7 plants was lower in the no-nitrogen fertilizer treatment (Figure 7A). Overexpression of either OsNPF7.7 variant led to better NUtE than that of ZH11 (Figures 7B–D). The NUtE of Ri and osnpf7.7 plants decreased in all nitrogen treatments (Figures 7B–D).

Our study showed that OsNPF7.7 played an important role in regulating tiller number and NUtE. Overexpression of both OsNPF7.7-1 and OsNPF7.7-2 in KY131, which is a japonica rice variety with the largest planting area in China, greatly increased the tiller number (Supplementary Figures S2A–E) and the effective panicle number per plant (Supplementary Figures S2F–K). The effective panicle number per plant was higher than that in OE1 and OE2 lines, whereas that of KY131 was about 23 (Supplementary Figures S2F–K). Furthermore, OE1 and OE2 lines had a significantly higher filled grain number per plant than did KY131 plants (Supplementary Figure S2L). Therefore, both variants of OsNPF7.7 enhanced rice grain yield by increasing the tiller number, effective panicle number, and filled grain number.
FIGURE 4 | Phenotype of axillary bud outgrowth and expression of related gene of OsNPF7.7 transgenic plants in ZH11. (A) Axillary buds were cultured in the basic rice culture solution containing 1 mM NH₄NO₃ under greenhouse condition at 27 days after sowing. First bud length (B) and second bud length (C) was measured at 21–33 days after sowing. Relative expression levels of OsNPF6.5 (D), OsNR (E), OsAMT1.2 (F), and OsGS1.2 (G) was measured in the root of transgenic plants at 27 days after sowing. Relative expression level of OsFC1 (H) and OsD3 (I) was measured in the axillary bud of transgenic plants at 27 days after sowing. Three replicates have been used for each experiment. Significant levels (each transgenic line VS. ZH11): ***p < 0.001, **p < 0.01, *p < 0.05. Error bars depict the SD (n = 3). Bar = 1 mm (A).
DISCUSSION

Two Variants of OsNPF7.7 Have Similar Expression Patterns Response to Nitrogen in Axillary Buds but Some Divergent Functions in Rice

Alternative splicing is one of patterns to increased functions of a gene at post transcriptional level (Kriventseva et al., 2003). In the present study, the newly found two variants of gene OsNPF7.7 in the NPF family could be assigned by nitrogen sources, and the level of each variant depended on the concentration of external nitrogen source. The expression of OsNPF7.7-1 and OsNPF7.7-2 was downregulated in axillary buds at high external nitrogen concentration (Figure 1D). qRT-PCR also indicated that the gene was highly expressed in the axillary buds and panicle (Figure 1F). Therefore, we suggest that the gene function might be related to branching growth and development.
The long-splicing variant was localized in the plasma membrane, whereas the short-splicing variant in the vacuole membrane, probably because the former had the longest sequence at the N terminus, which determines the localization. It has been shown that some NPF family members are localized in the plasma membrane (Fang et al., 2013; Hu et al., 2015; Tang et al., 2017), and some are localized in the tonoplast membrane (Li et al., 2015; Hu et al., 2016). Therefore, the different localizations of two variants of OsNPF7.7 suggest that each variant may functioned in different nitrogen transport process. In this study, our result indicated that overexpression of OsNP7.7-1 could increase nitrate influx and concentration, whereas overexpression of OsNP7.7-2 could enhance ammonium influx and concentration (Supplementary Figure S1). This demonstrated that OsNPF7.7-1 and OsNPF7.7-2 could balance the nitrate and ammonium uptake and storage at the plasma and vacuole membrane. Similarly, OsNP8.20-2 has been shown to positively influence the uptake of ammonium.
Enhanced Expression of Each Variants for OsNPF7.7 Could Increase Rice Tiller Number by Promoting the Outgrowth of Axillary Bud

In rice, nitrogen nutrition can regulate root branching (López-Bucio et al., 2003; Walch-Liu et al., 2006). However, the regulation mechanism of tiller number via nitrogen fertilization remains unclear. The high-affinity transporter OsNRT2.3 was a previously reported gene that has two variants, which differ in the regulation of rice growth and development. OsNRT2.3a is primarily expressed in root stellar cells (Tang et al., 2012), whereas OsNRT2.3b in the shoot phloem (Fan et al., 2016). Importantly, OsNRT2.3b regulates NUE (Fan et al., 2016). In addition, it was found that OsNPF8.20-2 (Fang et al., 2013) and OsNPF6.5 (Hu et al., 2015) regulate the tiller number, but the underlying mechanism was not clarified. In our study, we indicated that the two variants of OsNPF7.7 controlled the tiller number (Figure 3) by regulating the axillary bud outgrowth (Figure 4). Previously, it has been reported that OsGS1.2, a key gene in nitrogen assimilation, was implicated in the regulation of axillary bud elongation (Ohashi et al., 2015, 2017). Our results showed that the expression of OsGS1.2 was upregulated by the overexpression of OsNPF7.7-2 and down-regulated by RNAi and osnpf7.7 (Figure 4). Besides, overexpression of OsNPF7.7-1 up-regulated the expression of the nitrate transporter OsNPF6.5, which regulates nitrate transport and tiller number in rice (Hu et al., 2015).

Additionally, the bud outgrowth marker OsFC1 is required for axillary bud outgrowth and has been reported to decrease the tiller number (Minakuchi et al., 2010; Guo et al., 2013). And the strigolactone signaling gene OsD3 also participates in the repression of the tiller number in rice (Jiang et al., 2013; Zhou et al., 2013). In the present study, we showed that overexpression of each variant for OsNPF7.7 down-regulated the expression of OsFC1 and OsD3, but RNAi and osnpf7.7 up-regulated the expression of OsFC1 and OsD3. Thus, both nitrogen and hormone processes might coordinate to regulate bud outgrowth, since previous study indicated that OsGS1.2 increased the concentration of
Both Variants of OsNPF7.7 Have the Potential to Improve Grain Yield and Nitrogen Utilization Efficiency in Rice

In rice, the tiller number determines the potential effective panicle number (Yan et al., 1998; Xing and Zhang, 2010). Furthermore, it has been reported that the grain number per land area unit can be increased by increasing the panicle number or the grain number per panicle (Khush, 1995). In this study, we showed an increased effective panicle number and filled grain number accompanied by an increased tiller number in OsNPF7.7-1 and OsNPF7.7-2 overexpressing lines in KY131 (Supplementary Figure S2). Since the use of membrane transporters can improve crops and thus contribute to sustainable food production (Schroeder et al., 2013), Therefore, our results indicate that overexpression of each variant for OsNPF7.7 could enhance rice grain yield by increasing of tiller number.

Transporters for nitrogen acquisition are essential for NUE (Tegeder, 2014). Although some efforts have been made in various plants, the improvement of NUE in crops is very limited (Li et al., 2017). In the present study, we found that enhanced expression of each variant for OsNPF7.7 had better NUtE in the high-nitrogen and low-nitrogen treatments than that in ZH11, but no significant difference was found in the low-nitrogen treatment. These results suggest that overexpression of both variants for OsNPF7.7 promotes and accelerates nitrogen translocation from the roots to the leaves and from the straws to the seeds, but had no effect on NUtE when nitrogen concentration in the environment was very low. It has been reported that overexpression of OsNPF7.3 did not increase NUE in high ammonium supply (Fan et al., 2014). However, both variants of OsNPF7.7 differed from OsNPF7.3 with respect to the regulation of NUE, although both genes belong to the NPF subfamily. Overall, we showed that two variants of OsNPF7.7 regulate shoot branching and NUtE in rice.

AUTHOR CONTRIBUTIONS

ZF designed the research. ZF, WH, GB, JW, WZ, QZ, and KL performed the experiments. ZF, WH, and SS drafted the manuscript.

FUNDING

This research was supported by grants from the National Natural Science Foundation of China (31301250 and 31701990), Ministry of Agriculture transgenic major projects (2016ZX08001-003), the National Key Research and Development Program (2016YFD0100700), Hubei Natural Science Foundation (2017CFB696), Chinese Postdoctoral Science Foundation (2015MS82243), State Key Laboratory of Rice Biology (160203), and the scientific research projects of Hubei Education Department (B2017293).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2018.00300/full#supplementary-material

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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.

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