1 **Running title: OsNLP3 enhances NUE and grain yield in rice**

2 **Rice NIN-LIKE PROTEIN 3 plays a significant role in nitrogen use efficiency**

3 **and grain yield under nitrate-sufficient conditions**

4

5 Zi-Sheng Zhang¹, Jin-Qiu Xia¹, Alamin Alfatih¹, Ying Song¹, Yi-Jie Huang¹, Liang-Qi

6 Sun¹, Guang-Yu Wan¹, Shi-Mei Wang², Yu-Ping Wang³, Bin-Hua Hu³, Guo-Hua

7 Zhang³, Peng Qin³, Shi-Gui Li³, Lin-Hui Yu^{1,4}, Jie Wu¹, and Cheng-Bin Xiang¹

8 ¹School of Life Sciences and Division of Molecular & Cell Biophysics, Hefei

9 National Science Center for Physical Sciences at the Microscale, University of

10 Science and Technology of China, The Innovation Academy of Seed Design, Chinese

11 Academy of Sciences, Hefei, Anhui Province 230027, China

12 ²Rice Research Institute, Anhui Academy of Agricultural Sciences, Hefei 230031,

13 China

14 ³Rice Research Institute, State Key Laboratory of Hybrid Rice, Sichuan Agricultural

15 University, Chengdu, Sichuan, China

16 ⁴Present address: Biology Department, Brookhaven National Laboratory, Upton, New

17 York, NY 11973, USA

18

19 Corresponding authors:

20 Cheng-Bin Xiang (Email: xiangcb@ustc.edu.cn; Phone: 86-55163600429)

21 Jie Wu (Email: wujie199104@163.com; Phone: 86-55163607332)

22 Lin-Hui Yu (Email: yulh@ustc.edu.cn)

23 **Abstract**

24 Nitrogen (N) is an essential macronutrient for crop growth and yield, but
 25 excessive application of N fertilizer has caused serious environmental pollution and
 26 greatly increased the cost of agricultural production. One of the effective and
 27 economical solutions to this dilemma is to improve the N use efficiency (NUE) of
 28 crops. Although some components involved in regulating NUE have been identified,
 29 the underlying molecular mechanism remains largely elusive in rice. Here we report
 30 that the OsNLP3 (NIN-like protein 3) is an important regulator in NUE and grain
 31 yield under nitrate-sufficient conditions. Both NUE and grain yield were significantly
 32 improved by enhanced *OsNLP3* expression in the field, but reduced in *osnlp3* mutants.
 33 The expression of *OsNLP3* responds to both nitrate and ammonium, while OsNLP3
 34 nuclear retention is only induced by nitrate, not by ammonium. OsNLP3 regulates the
 35 expression of a set of genes involved in N transport and assimilation by directly
 36 binding to the nitrate-responsive *cis*-element in the promoters of these genes. Our
 37 study demonstrates that OsNLP3 is significant for the regulation of NUE and grain
 38 yield, particularly in nitrate-rich conditions, thus providing a candidate for improving
 39 NUE and grain yield in rice.

40

41 **Key words:** rice NIN-like protein 3 (OsNLP3), nitrogen use efficiency (NUE), grain
 42 yield, nitrogen availability, rice (*Oryza sativa*)

43

44

45 **Introduction**

46 Nitrogen (N) is an essential macronutrient for plant growth and development and
 47 lower N availability in the field limits the crop productivity (Crawford and Forde,
 48 2002; Robertson et al., 2009). N fertilization is one of the key factors improving crop
 49 yield and reducing world hunger (Good et al., 2004). To increase crop yield, farmers
 50 in many countries excessively apply N fertilizer but less than 50% of the applied
 51 fertilizer is utilized by crops, while the leftover is lost to the environment, causing the
 52 collateral environmental pollution (Garnett et al., 2009; Mueller et al., 2014;
 53 Undurraga et al., 2017). One of the promising solutions to this dilemma is to improve
 54 the N use efficiency (NUE) of crops (Hirel et al., 2011; Mandal et al., 2018). NUE,
 55 mainly consisting of NUpE (N uptake efficiency) and NUtE (N
 56 utilization/assimilation efficiency), is defined by the grain yield per unit of available
 57 N in the soil (Xu et al., 2012; Han et al., 2015).

58 Nitrate is one of the main N sources for plant utilization, and approximately 40%
 59 of the total N taken up in paddy grown rice is absorbed as nitrate, due to nitrification
 60 in the rhizosphere (Kirk and Kronzucker, 2005). Plants uptake and transport nitrate by
 61 four protein families, including low-affinity transporter NPF/NRT1 (peptide
 62 transporter family/nitrate transporter1), high-affinity transporter NRT2, CLC (chloride
 63 channel), and SLAH (slow anion channel-associated homologues) families (Krapp et
 64 al., 2014). Once taken up in the plant, nitrate is reduced to ammonium by the action of
 65 NR (nitrate reductase) and NiR (nitrite reductase), then fixed into Glu (glutamate) and
 66 Gln (glutamine) by GS (glutamine synthetase) and GOGAT (glutamate synthase)

67 respectively, and thereafter converted to N-containing molecules, such as proteins,
68 nucleotides and hormones (Crawford, 1995; Xu et al., 2012).

69 Nitrate also act as an important signaling molecule in plants (Camargo et al.,
70 2007; Ho and Tsay, 2010; Undurraga et al., 2017). AtNRT1.1/AtNPF6.3 is a
71 transceptor that functions as a receptor to perceive external nitrate signaling and as a
72 dual-affinity nitrate transporter (Liu et al., 1999; Ho et al., 2009; Hu et al., 2009).

73 After nitrate is sensed by AtNRT1.1, the signal is transduced into the cell through a
74 Ca^{2+} -dependent or Ca^{2+} -independent pathway (Mu and Luo, 2019). There are also
75 some other proteins, mostly transcription factors (TFs) that were identified
76 participating in the nitrate signaling transduction and regulating N-related metabolism,
77 growth, and development in Arabidopsis, such as AtANR1 (Arabidopsis nitrate
78 regulated 1), AtNRG2 (nitrate regulatory gene 2), AtLBD37/38/39 (lateral organ
79 boundary domain 37/38/39), AtTGA1/4 (TGACG sequence-specific binding protein
80 1/4), AtAFB3 (auxin signaling F-box 3), and AtbZIP1 (basic leucine zipper 1) (Zhang
81 and Forde, 1998; Rubin et al., 2009; Vidal et al., 2010; Alvarez et al., 2014; Para et al.,
82 2014; Xu et al., 2016). Moreover, several NIN-like proteins (NLPs) act as central
83 transcription factors in nitrogen responses and nitrate signaling (Konishi and
84 Yanagisawa, 2013; Chardin et al., 2014). AtNLP7 acts as a master regulator in nitrate
85 signaling (Konishi and Yanagisawa, 2013; Wang et al., 2018). The knockout mutant
86 *atnlp7* exhibits an impaired PNR (primary nitrate response), which is a rapid
87 nitrate-induced transcriptional response but without protein synthesis (Castaings et al.,
88 2009; Wang et al., 2018). Noteworthy, AtNLP7 orchestrates the early response to

89 nitrate via a special nuclear retention mechanism, which is regulated by
 90 nitrate-AtNRT1.1-Ca²⁺-AtCPK10/30/32 (Ca²⁺-dependent protein
 91 kinase10/30/32)-AtNLP7 signaling cascade (Marchive et al., 2013; Liu et al., 2017;
 92 Alvarez et al., 2020; Oldroyd and Leyser, 2020). In addition, AtNLP7 also plays a
 93 central role in plant NUE. The knockout mutant *atnlp7* displays several features of N
 94 starvation, whereas overexpression of *AtNLP7* significantly improves the growth and
 95 NUE (Castaings et al., 2009; Yu et al., 2016). AtNLP8 acts as a regulator for
 96 nitrate-promoted seed germination by reducing ABA content (Yan et al., 2016).
 97 Overexpression of the maize *ZmNLP6* and *ZmNLP8* can complement *atnlp7* mutant
 98 by restoring nitrate signaling and assimilation (Cao et al., 2017). Furthermore,
 99 *ZmNLP5* acts as a central hub in a molecular network associated with N metabolism
 100 and modulates the nitrogen response in maize (Ge et al., 2020).

101 Rice is an important food crop for a large part of the world population and serves
 102 as a monocot model plant for research. Over the past decade, much progress has been
 103 made to enhance NUE in rice. Overexpression of *OsNRT1.1A/OsNPF6.3* improves N
 104 utilization and confers high yield and early maturation (Wang et al., 2018). Variation
 105 in *OsNRT1.1B/OsNPF6.5* and *OsNR2* (nitrate reductase 2) contributes to nitrate use
 106 divergence between rice subspecies (Hu et al., 2015; Gao et al., 2019). Moreover,
 107 *OsNRT1.1B* also acts as a nitrate sensor and interacts with phosphate signaling
 108 repressor *OsSPX4* (SPX domain containing protein 4) to form a nitrate–*OsNRT1.1B*–
 109 *OsSPX4* regulatory module, integrating the nitrogen and phosphorus signaling
 110 networks (Hu et al., 2019). *OsNAR2.1* (*OsNRT2* associate protein) interacts with

111 nitrate transporters OsNRT2.1, OsNRT2.2 and OsNRT2.3a, enable a rapid response to
 112 external nitrate concentrations (Yan et al., 2011). OsNPF6.1^{HapB} was trans-activated
 113 by the transcription factor OsNAC42 (NAC family transcriptional factor 42),
 114 enhancing NUE and grain yield (Tang et al., 2019). Overexpression of *OsPTR9*
 115 (peptide transporter 9) improves NUE and grain yield in rice (Fang et al., 2013).
 116 Increasing the OsGRF4 (growth-regulating factor 4) abundance in the balance
 117 between OsGRF4 and growth inhibitor DELLA improves NUE and grain yield in
 118 green revolution varieties (Li et al., 2018). Increased OsNGR5 (nitrogen-mediated
 119 tiller growth response 5) activity promotes rice tillering from nitrogen regulation,
 120 boosting rice yield and enhancing NUE (Wu et al., 2020).

121 Although significant progress has been made in understanding nitrate signaling
 122 and improving NUE, the regulatory mechanisms remain largely elusive in rice. We
 123 recently reported *OsNLP1* and *OsNLP4* that rapidly respond to N availability and
 124 improves grain yield and NUE in rice (Alfatih et al., 2020; Wu et al., 2020). Here we
 125 report that *OsNLP3*, the closest homolog to Arabidopsis *AtNLP7* in rice, also plays a
 126 significant role in nitrate uptake, assimilation and signaling. Similar to *OsNLP1* and
 127 *OsNLP4*, *OsNLP3* expression level is significantly induced by N deficiency and
 128 repressed by nitrate/ammonium resupply in shoot. However, it does not respond to N
 129 starvation but is induced by nitrate/ammonium resupply in the root. *OsNLP3*
 130 nucleocytosolic shuttling is only responsive to nitrate but not to ammonium.
 131 Overexpression of *OsNLP3* significantly improved grain yields and NUE, particularly
 132 in high N conditions, whereas *osnlp3* mutants resulted in severe growth reduction,

133 lower grain yield and NUE in field. Our results indicate that *OsNLP3* is a promising

134 candidate to improve NUE and grain yield in rice for sustainable agriculture.

135

136

137 **Results**

138

139 ***OsNLP3* expression responds to N availability**

140 To investigate the response of *OsNLP3* to N availability, we quantified the
 141 *OsNLP3* transcript level with different nitrogen treatments in the wild type Zhonghua
 142 11 (ZH11). *OsNLP3* transcript levels in the shoot were gradually induced after shifted
 143 to N deficiency (0 N) conditions from normal N supply conditions (NN) and
 144 plateaued at 24 hours after the shift. Upon N resupply (either nitrate or ammonium),
 145 the transcript levels declined to its pre-induction level in 1 hour while the KCl control
 146 treatment did not alter the transcript level (Figure 1A). Interestingly, *OsNLP3*
 147 transcript level in the root exhibited a different pattern. In response to N starvation, it
 148 slightly increased 1 hour after the shift but gradually declined to its pre-induction
 149 level and remained at this low level. Upon N resupply, *OsNLP3* transcript level was
 150 induced and peaked 2 hours after the shift, then declined to its pre-induction level
 151 (Figure 1B). Our data show that *OsNLP3* is responsive to both nitrate and ammonium
 152 while the response patterns are different between shoot and root.

153 RT-qPCR analyses of *OsNLP3* transcript levels in different tissues revealed that
 154 it was expressed in all the tissues analyzed but with higher levels in leaves and leaf
 155 sheaths (Figure 1C).

156

157 ***OsNLP3* localization is regulated by nitrate-dependent nuclear retention**

158 To test if the *OsNLP3* also have nitrate induced nucleocytoplasmic shuttling, we

Figure 1. *OsNLP3* expression responds to nitrogen availability.
A. Response of *OsNLP3* expression to N availability in shoots of wild type (ZH11). Seedlings were hydroponically grown and treated as described in Materials and Methods (NN, normal nitrogen; 0N, nitrogen starvation; re-N, N resupply). KCl was used as a negative control. Shoots were sampled at indicated time points for RNA analyses with RT-qPCR. Values are the mean $\pm$ SD (n = 3).
B. Response of *OsNLP3* expression to N availability in root of wild type (ZH11). Roots of the same seedlings as in A were sampled at indicated time points for RNA analyses with RT-qPCR. KCl used as a negative control. Values are the mean $\pm$ SD (n = 3).
C. Expression of *OsNLP3* in different tissues at flowering stage of wild type (ZH11). RNA was extracted from tissues as indicated and subjected to RT-qPCR analyses. Values are the mean $\pm$ SD (n = 3).

generated transgenic rice expressing *pActin1::OsNLP3-GFP* fusion construct in ZH11 background. We found that in seedlings grown in normal N conditions (NN) the subcellular localization of OsNLP3-GFP fusion protein was both in the nucleus and cytosol (Figure 2A), whereas the GFP signals mostly exist in the cytosol in the root of plants grown in N deficiency (0 N) condition (Figure 2B).

We also examined the nucleocytoplasmic shuttle of OsNLP3-GFP in response to nitrate and ammonium. In response to resupplied nitrate after N-starvation, OsNLP3-GFP rapidly accumulated in the nucleus within minutes (Figure 2C). This nuclear accumulation was fully reversible when nitrate was withdrawn (Figure 2C). However, when ammonium or KCl was resupplied, the nuclear accumulation of OsNLP3-GFP was not observed (Figure 2D and E), indicating that OsNLP3 nuclear accumulation was specifically responsive to NO₃⁻ but not to NH₄⁺ or K⁺.

OsNLP3 significantly affects seedling growth in nitrate-sufficient conditions

To further investigate the function of OsNLP3 in rice, we generated three loss-of-function mutants (*osnlp3-1*, *osnlp3-2*, *osnlp3-3*) with CRISPR/Cas9 system which targeted two different sequences in the first exon of *OsNLP3* (Figure S1A) and

Figure 2. OsNLP3 nuclear retention is only induced by nitrate.
A and B. Subcellular localization of OsNLP3-GFP in *pActin1::OsNLP3-GFP* transgenic rice seedlings grown on normal nitrogen medium (A) and N-free medium (B) for 18 days. Bars = 20 μ m.
C-E. Localization of OsNLP3-GFP in response to nitrate and ammonium. 18-day-old *pActin1::OsNLP3-GFP* seedlings grown on N-free medium (0 N) were transferred to medium with 50 mM KNO_3 (C) or NH_4Cl (D) or KCl (E, as a negative control) for 5 min and 15 min, and then transferred to N-free medium for 50 min and 120 min. The images of root cells were captured with a confocal microscope at indicated time points. Bars = 20 μ m.

two *OsNLP3* overexpression lines (OE-1, OE-10) (Figure S1B). These lines were hydroponically grown in the modified Kimura B solution contained different concentrations of either nitrate or ammonium as the sole N source for 25 days after germination.

Under nitrogen starvation (0 N), seedling growth was obviously retarded for all genotypes without any visible difference (Figure 3A, E-J). Under low N (0.2 mM nitrate as the sole N source) conditions, *osnlp3* mutants only displayed a slight decrease in shoot and root weight, but no obvious difference in shoot length, shoot/root ratio or chlorophyll content of leaves (Figure 3B, E-J), while *OsNLP3* OE plants did not display obvious difference from the wild type except slightly shorter shoot (Figure 3B and I) and higher chlorophyll content (Figure 3J).

By contrast, under normal nitrate (2 mM) and high nitrate (5 mM) conditions the *osnlp3* mutants exhibited constitutive N starvation phenotypes with a biomass (fresh weight) decrease by ~60% in the shoot and ~50% in the root compared to the wild

Figure 3. OsNLP3 affects seedling growth in nitrate-sufficient conditions. A-D. Seedling growth response to nitrate. Wild type (*ZH11*), mutant (*osnlp3*) and overexpression (OE) plants grown in hydroponic solution with varying amounts of nitrate for 25 days after germination. Bars = 5 cm. E-J. Seedling growth parameters. Shoot fresh weight (E), root fresh weight (F), shoot fresh weight/root fresh weight ratio (G), stem diameter (H), shoot length (I), and leaf chlorophyll content (J) of 25-day-old seedlings were measured. Values are the mean $\pm$ SD of 3 independent replications each containing 16 plants per genotype. Different letters denote significant differences evaluated by one-way ANOVA with Tukey's test ($P < 0.05$). FW, fresh weight.

190 type (Figure 3C-F and H-I). The chlorophyll content of leaves and shoot/root ratio
 191 also decreased in *osnlp3* mutants (Figure 3C-D, G and J). The OE lines did not
 192 display clear phenotypic difference from the wild type except a slightly higher
 193 shoot/root ratio (Figure 3G). Notably, the OE lines were much more green than wild
 194 type which support by much higher chlorophyll content under these nitrate conditions
 195 (Figure J).

196 To test the growth response to ammonium, we hydroponically grew different
 197 genotypes in culture medium with ammonium as the sole N source. Surprisingly, we
 198 did not observe obvious differences among the *osnlp3* mutants, OE lines, and the wild
 199 type under low (0.2 mM) or high ammonium (2 mM) conditions (Figure 4A and B,

Figure 4. OsNLP3 does not influence seedling growth in response to ammonium.

A-C. Seedling growth response to ammonium. Wild type (ZH11), mutant (*osnlp3*) and overexpression (OE) plants grown in hydroponic solution with varying amounts of ammonium for 25 days after germination. Bars = 5 cm.
D-I. Seedling growth parameters. Shoot fresh weight (D), root fresh weight (E), shoot fresh weight/root fresh weight ratio (F), stem diameter (G), shoot length (H), and leaf chlorophyll content (I) of 25-day-old seedlings were measured. Values are the mean $\pm$ SD of 3 independent replications each containing 16 plants per genotype. Different letters denote significant differences evaluated by one-way ANOVA with Tukey's test ($P < 0.05$). FW, fresh weight.

D-I). However, *osnlp3* mutants grown in medium with 1 mM nitrate plus 1 mM

ammonium showed a visible retarded growth phenotype compared with wild type

(Figure 4C, D-I). These results implicate that OsNLP3 plays a less important role in

ammonium utilization.

OsNLP3 positively modulates nitrate uptake and assimilation

The above results of growth response led us to test whether OsNLP3 positively

modulates nitrate uptake and assimilation. As expected, the chlorate-sensitivity assay

showed that *osnlp3* mutants grew better under hydroponic culture with chlorate, a

Figure 5. *OsNLP3* modulates nitrate uptake and assimilation. A. Chlorate sensitivity assay. Wild type (ZH11), mutant (*osnlp3*) and overexpression (OE) seedlings treated with chlorate as described in Materials and Methods. Bar = 5 cm. B. Chlorate sensitivity. Chlorate sensitivity was evaluated by the death ratio after a chlorate-sensitivity test. Values are the mean $\pm$ SD of 3 independent replications each containing 48 plants per genotype. Different letters denote significant differences evaluated by one-way ANOVA with Tukey's test ($P < 0.05$). C and D. N uptake assay with ¹⁵N-nitrate or ¹⁵N-ammonium. 12-day-old wild type (ZH11), mutant (*osnlp3*) and overexpression (OE) plants were used for N uptake assays as described in Materials and Methods. Values are the mean $\pm$ SD of 3 independent replications each containing 6 plants per genotype. Different letters denote significant differences evaluated by one-way ANOVA with Tukey's test ($P < 0.05$). DW, dry weight. E and F. Total nitrogen content. 12-day-old wild type (ZH11), mutant (*osnlp3*) and overexpression (OE) seedlings grown in hydroponic solution with 5 mM nitrate or 2 mM ammonium were sampled for total N content analyses as described in Materials and Methods. Different letters denote significant differences evaluated by one-way ANOVA with Tukey's test ($P < 0.05$). DW, dry weight. G and H. Free nitrate content and NR activity. Free nitrate content (G) and NR activity (H) in the shoots of 15-day-old wild type (ZH11), mutant (*osnlp3*) and overexpression (OE) plants grown in hydroponic solution with 0.2 mM and 2 mM nitrate were assayed as described in Materials and Methods. Values are the mean $\pm$ SD of 4 independent replications each contained 3 plants per genotype. Different letters denote significant differences evaluated by one-way ANOVA with Tukey's test ($P < 0.05$). FW, fresh weight.

209 toxic analog of nitrate, compared with wild type. Inversely, *OsNLP3* OE seedlings
210 exhibited the highest chlorate sensitivity with significantly higher mortality than wild
211 type (Figure 5A and B). Moreover, ¹⁵N-nitrate or ¹⁵N-ammonium uptake assays also
212 indicated that nitrate acquisition was reduced in the *osnlp3* mutants while enhanced in
213 *OsNLP3* OE lines, but there was no obvious difference for ammonium absorption
214 (Figure 5C and D). Consistently, the total nitrogen content was decreased in the
215 *osnlp3* mutants and increased in *OsNLP3* OE lines with nitrate as sole nitrogen source,
216 but there was no difference under ammonium conditions (Figure 5E and F). We also
217 measured the free nitrate content and NR activity in the leaves of seedlings
218 hydroponically grown under low (0.2 mM) and normal (2 mM) nitrate conditions. The
219 free nitrate content was higher in *osnlp3* mutants and lower in *OsNLP3* OE lines

under normal nitrate, but no obvious difference under low nitrate solution (Figure 5G), indicating that nitrate assimilation is impaired in *osnlp3*, which is supported by significantly reduced NR activity in *osnlp3* mutants and enhanced NR activity in *OsNLP3* OE plants under normal nitrate conditions (Figure 5H). These results indicate that OsNLP3 may directly modulates the uptake and assimilation of nitrate but not ammonium to improve both NUpE and NUtE.

OsNLP3 regulates the expression of nitrate transport, assimilation, and signaling related genes

To further investigate the molecular mechanism of OsNLP3 coordinating nitrate transport, assimilation, and signaling, we measured the expression of genes known to be associated with these processes. Under normal hydroponic cultivation, the genes for primary nitrate assimilation such as *OsNIA1*, *OsNIA2*, *OsNIA3*, and *OsNIR1*, were significantly downregulated in the *osnlp3* mutants and upregulated in *OsNLP3* OE plants (Figure 6A-D). Consistently, the expression of genes related to nitrate uptake and transport such as *OsNRT1.1B*, *OsNRT1.1C*, *OsNRT2.1*, *OsNRT2.3a* and *OsNRT2.4* were down-regulated in the *osnlp3* mutants, and up-regulated in OE plants except *OsNRT1.1C* (Figure 6E-I). Moreover, OsGRF4 was also down regulated in the *osnlp3* mutants and upregulated in OE plants (Figure 6J). These results suggest that OsNLP3, as a transcription factor, may orchestrate the expression of nitrate transport, assimilation, and signaling related genes.

Figure 6. OsNLP3 regulates the expression of nitrate utilization and signaling related genes. A-J. RT-qPCR-based expression analyses of genes involved in nitrate utilization and signaling in wild type (ZH11), mutant (*osnlp3*) and overexpression (OE) plants. 18-day-old plants grown in normal nitrogen medium were used for the analyses. Nitrate reductase genes: *OsNIA1*, *OsNIA2*, and *OsNIA3*; Nitrite reductase gene: *OsNIR1*; Nitrate transporter genes: *OsNRT1.1B*, *OsNRT1.1C*, *OsNRT2.1*, *OsNRT2.3a*, *OsNRT2.4*; Growth-regulating factor gene: *OsGRF4*. Values are the mean $\pm$ SD (n = 3). Different letters denote significant differences evaluated by one-way ANOVA with Tukey's test (P < 0.05).

OsNLP3 regulates its target genes by directly binding to their promoters

To demonstrate whether OsNLP3 directly modulates the expression of nitrate transport and assimilation related genes, we searched putative NRE-like *cis*-elements in the promoters of the putative target genes regulated by OsNLP3 (Table S1). Those promoters contain one or more NRE-like *cis*-elements. To evaluate whether OsNLP3 directly binds to these NREs, we performed chromatin immunoprecipitation (ChIP) qPCR experiments. ChIP-qPCR results showed that OsNLP3 bound at least one NRE-like *cis*-element in the promoter of N related genes including *OsNIA1*, *OsNIA3*, *OsNRT1.1B*, *OsNRT2.4*, and *OsGRF4* (Figure 7A-E). Yeast-one-hybrid assay (Y1H) further verified these interactions (Figure 7F). These data show that OsNLP3 can directly bind to the promoters of its target genes and potentially regulate their expression.

OsNLP3 overexpression improves NUE and grain yield

Figure 7. OsNLP3 directly binds to promoters of N-related genes. A-E. ChIP-qPCR assay. HA-OsNLP3 mediated ChIP-qPCR enrichment (relative to wild type) of NRE-like fragments from promoters of *OsNIA1*, *OsNIA3*, *OsNRT1.1B*, *OsNRT2.4*, *OsGRF4* was performed as described in Materials and methods. Values are the mean $\pm$ SD ($n = 3$). Different letters denote significant differences evaluated by one-way ANOVA with Tukey's test ($P < 0.05$). F. Yeast one-hybrid assays. pAD-GAL4-OsNLP3 (AD-NLP3) fusion protein activate the expression of pHIS2 (BD) reporter gene driven by the NRE-like of *OsNIA1*, *OsNIA3*, *OsNRT1.1B*, *OsNRT2.4*, *OsGRF4*. The empty vector pAD-GAL4 (AD) and pHIS2 (BD) were used as a negative control.

256 To evaluate NUE and grain yield of *osnlp3* mutants and *OsNLP3* OE lines in the
 257 field, we designed field trials at two locations (Chengdu, E104°, N30°; Hefei, E117°,
 258 N31°). In Chengdu, three nitrogen levels (low nitrogen, LN; normal nitrogen, NN;
 259 and high nitrogen, HN) were used to evaluate the performance of each genotype as
 260 described in Materials and Methods. Plant height was decreased in *osnlp3* mutants
 261 and improved in *OsNLP3* OE plants at all N fertilizer levels compared with wild type
 262 (Figure 8A and C).

263 More importantly, *OsNLP3* OE plants improved grain yield per plant and the
 264 NUE by ~13% and ~14% under LN, ~11% and ~15% under NN and ~16% and ~20%
 265 under HN conditions, respectively. In contrast, the grain yield per plant and the NUE
 266 of *osnlp3* mutants exhibited a significant decrease by ~14% and ~15% under LN, ~16%
 267 and ~17% under NN and ~30% and ~32% under HN compared to that of the wild

Figure 8. *OsNLP3* overexpression improves NUE and grain yield under different nitrogen levels. A. Morphological phenotypes of wild type (ZH11), mutant (*osnlp3*) and overexpression (OE) grown in the field under low N (LN), normal N (NN) and high N (HN) conditions. A representative plant was dug out from the field and potted for photograph. Bars = 20 cm. B. Total grains per plant of wild type (ZH11), mutant (*osnlp3*) and overexpression (OE) grown in the field under LN, NN and HN conditions. Bars = 5 cm. C-E. Agronomic traits. Plant height (C), grain yield per plant (D) and NUE (E) of wild type (ZH11), mutant (*osnlp3*) and overexpression (OE) plants grown in the field under LN, NN and HN were statistically analyzed. Values are the means $\pm$ SD (n=20). Different letters denote significant differences evaluated by one-way ANOVA with Tukey's test (P < 0.05).

type, respectively (Figure 8A-E).

The results of the field tests with normal N level in Hefei were similar to those in Chengdu. Compared with the wild type, plant height was decreased ~8% to 15% in *osnlp3* mutants and increased ~3% to 5% in *OsNLP3* OE plants (Figure 9A and C). Moreover, the grain yield per plant and NUE of *osnlp3* mutants decreased by ~14% - 18% and 19% - 24% while increased by ~13 % - 15% and 16% - 20% in *OsNLP3* OE plants, respectively (Figure 9A-E).

Taken together, these results clearly demonstrate that *OsNLP3* improves NUE and grain yield in the field. In addition, the differences in yield and NUE between different genotypes appears to be less significant under LN conditions but significantly augmented under HN conditions, suggesting that *OsNLP3* plays a more

Figure 9. *OsNLP3* overexpression confers to improve NUE and grain yield under normal nitrogen level. A. Morphological phenotypes of wild type (ZH11), mutant (*osnlp3*) and overexpression (OE) grown in the field with normal nitrogen. A representative plant was dug out from the field and potted for photograph. The senescent leaves were removed. Bar = 15 cm. B. Total grains per plant of wild type (ZH11), mutant (*osnlp3*) and overexpression (OE) plants grown in the field with normal nitrogen. Bar = 5 cm. C-E. Agronomic traits. Plant height (C), grain yield per plant (D), and NUE (E) of wild type (ZH11), mutant (*osnlp3*) and overexpression (OE) plants grown in the field with normal nitrogen were statistically analyzed. Values are the means $\pm$ SD (n=20). Different letters denote significant differences evaluated by one-way ANOVA with Tukey's test ($P < 0.05$).

279 important role under HN than LN conditions.

280

281 ***OsNLP3* can rescue Arabidopsis *nlp7* and increase biomass**

282 As the most homologous gene to Arabidopsis *NLP7*, we predicted that *OsNLP3*

283 could functionally complement Arabidopsis *nlp7* mutant. The results showed that

284 *OsNLP3* overexpression in Arabidopsis *nlp7* mutant not only rescued the N starvation

285 phenotypes of *nlp7*, but increased biomass compared with wild type under normal (3

286 mM) and rich (10 mM) nitrate conditions, while no obvious improvement was seen

287 under poor (1 mM) nitrate conditions (Figure S2), consistent with the observations in

288 rice OE lines.

289

290

291 Discussion

292 Nitrogen fertilizers are applied excessively in the field to achieve higher yields,
 293 especially for green revolution varieties, which is not sustainable and generate serious
 294 environmental problems (Han et al., 2015). It has become an urgent issue and a major
 295 challenge to reduce fertilizer overuse to protect the environment and increase crop
 296 yields to feed the world population . Breeding smart crops that produce acceptable
 297 yield with minimum fertilizer input would certainly alleviate the issue. Therefore,
 298 improving crop NUE is one of the most economic solutions.

299 We recently reported *OsNLP1* and *OsNLP4* that improve N utilization and
 300 enhance yield and NUE in rice (Alfatih et al., 2020; Wu et al., 2020). In this study, we
 301 unveil that *OsNLP3* also plays a crucial role in regulating nitrate utilization and NUE
 302 in rice. Different from *OsNLP1* and *OsNLP4*, *OsNLP3* shows a different expression
 303 pattern in response to N availability in shoot and root. *OsNLP3* nuclear localization
 304 was specifically and rapidly regulated by nitrate via nuclear retention mechanism but
 305 not by ammonium. Moreover, multiple N assimilation genes were significantly
 306 down-regulated in *osnlp3* mutants while upregulated in OE lines. More importantly,
 307 field tests of the loss-of-function mutants and OE lines clearly demonstrated that
 308 *OsNLP3* plays a crucial role in NUE and grain yield, particularly in high N
 309 conditions.

310 Why do shoots and roots show different response pattern to N availability?

311 *OsNLP3* displays an interesting expression pattern with strong induction by
 312 N-starvation and repressed by either nitrate or ammonium resupply in the shoot,

313 whereas in the root, it is induced by nitrate or ammonium (Figure 1A-B). This pattern
314 is different from that of *OsNLP1* and *OsNLP4*, which exhibit similar pattern in shoot
315 and root in response to N availability (Alfatih et al., 2020; Wu et al., 2020). It is also
316 different from *AtNLP7* that is not responsive to nitrate (Marchive et al., 2013). The
317 different response pattern to nitrogen in the shoot and root implies that *OsNLP3* may
318 play a more exquisite role to mediate N use and facilitate NUtE in the shoot under N
319 deficiency conditions. In contrast, the induction of *OsNLP3* in the root could rapidly
320 accelerate NUtE when N availability becomes high. This point is supported by higher
321 nitrate absorption (Figure 5) and expression levels of nitrate transport genes (Figure 6)
322 in *OsNLP3* overexpression lines.

323 ***OsNLP3* nucleocytoplasmic shuttle is only responsive to nitrate.**

324 *OsNLP3* nuclear localization is regulated specifically by nitrate but not by
325 ammonium possibly via the nuclear retention mechanism (Figure 2). This is quite
326 different from *OsNLP1*, which is localized in the nucleus regardless of the availability
327 of nitrate or ammonium (Alfatih et al., 2020), and different from *OsNLP4*, whose
328 nuclear localization is responsive to both nitrate and ammonium although much
329 slower and weaker to ammonium (Wu et al., 2020). Considering that *AtNLP7*
330 activates the genes involved in PNR via nuclear retention in Arabidopsis (Marchive et
331 al., 2013), we speculate that the transcription factor *OsNLP3* maybe also activate
332 PNR and allowing a rapid *OsNLP3*-dependent genes expression by nuclear retention
333 mechanism in rice. Taken together, given that the nitrate is most readily leached form
334 and spatiotemporal distribution disproportion in field, the swift regulation of *OsNLP3*

by nitrate via nuclear retention (Figure 2) together with different expression pattern in shoot and root (Figure 1) highlights the crucial role of OsNLP3 in the response to nitrate availability and nitrate signaling pathway.

OsNLP3 is less important in ammonium utilization

Although *OsNLP3* expression responds to ammonium similarly to nitrate (Figure 1A and B), no obvious phenotypical difference was detected among the wild type, *OsNLP3* OE and mutant lines when grown in medium with ammonium as sole N source (Figure 4). This inconsistency maybe due to the OsNLP3 protein localization in response to ammonium (Figure 2D). Nuclear retention of OsNLP3 only responds to nitrate but not ammonium. OsNLP3-GFP proteins were mainly found in cytosol with hardly any signal in nucleus in plants grown in ammonium medium (Figure 2). Thus, OsNLP3 is unlikely to modulate N assimilation in conditions with ammonium as sole N source. Consistently, chlorate sensitivity assay and ¹⁵N-ammonium uptake assay revealed that OsNLP3 only affects nitrate uptake rather than ammonium uptake (Figure 5). Similar to *OsNLP3*, *OsNLP1* and *OsNLP4* also respond to both nitrate and ammonium, however, unlike OsNLP3, OsNLP1 proteins constitutively localize in nucleus and OsNLP4 nuclear retention is induced by both nitrate and ammonium (Alfatih et al., 2020; Wu et al., 2020). Consequently, both OsNLP1 and OsNLP4 regulate both nitrate and ammonium assimilation, whereas OsNLP3 mainly functions in nitrate rather than in ammonium utilization.

OsNLP3 plays more important roles in nitrate-sufficient condition to promote N

357 utilization and improve NUE and yield

358 Notably, functional characterization of the loss-of-function mutants and OE rice
 359 at seedling stage in hydroponic culture demonstrated that OsNLP3 appears to function
 360 in promoting nitrate utilization, particularly in sufficient nitrate conditions, but not
 361 ammonium (Figure 3, Figure 4). This is supported by chlorate sensitivity assay,
 362 ¹⁵N-uptake assay, total nitrogen content, free nitrate content and NR activity (Figure
 363 5). In contrast, OsNLP1 and OsNLP4 functions in both nitrate and ammonium
 364 utilization (Alfatih et al., 2020; Wu et al., 2020). Field test show that grain yield and
 365 NUE in mutants decreased ~14% and ~15% under LN, ~30% and ~32% under HN
 366 respectively, whereas OE plants increased ~13% and ~14% under LN, ~16% and ~20%
 367 under HN, respectively. These results further support that OsNLP3 exhibits stronger
 368 functions under N-sufficient conditions than under N-deficient conditions. Inversely,
 369 OsNLP1 and OsNLP4 field phenotype is more evident under deficient N conditions
 370 than under sufficient N conditions (Alfatih et al., 2020; Wu et al., 2020). The
 371 functional divergence between OsNLP3 and OsNLP1/4 in high-N or N-limited
 372 conditions suggests that multiple strategies be integrated by OsNLP family members
 373 to enhance plant growth under the full range of N statuses.

374 In conclusion, our study demonstrate that *OsNLP3* plays a crucial role in nitrate
 375 utilization and NUE and is a promising candidate to improve NUE and grain yield in
 376 rice for sustainable agriculture.

377

378 Materials and Methods

379

380 **Plant materials and growth conditions**

381 The rice (*Oryza sativa* L.) *japonica* variety Zhonghua11 (ZH11) was used in this
382 study. Three loss-of-function mutants *osnlp3-1*, *osnlp3-2*, *osnlp3-3* were obtained
383 through CRISPR-Cas9 technology (Lu et al., 2017) via service provided by Hangzhou
384 Biogle Co., Ltd (Hangzhou, China) and verified by sequencing (Figure S1A). Two
385 *OsNLP3* OE lines (OE-1, OE-10) were generated by inserting the full CDS of
386 *OsNLP3* into the vector of pCB2006 via GATEWAY cloning system (Lei et al., 2007),
387 introduced into ZH11 via *Agrobacterium*-mediated transformation, and verified by
388 RT-PCR analyses (Figure S1B).

389 For hydroponic culture, seeds were submerged in water at room temperature for
390 48 h, followed by germination at 37°C. Seeds were then sown in a bottomless 96-well
391 plate that was placed in modified Kimura B solution in a growth chamber with a
392 14-hour light (30°C)/10-hour dark (28°C) photoperiod, ~300 $\mu\text{mol}/\text{m}^2/\text{s}$ light intensity,
393 and ~70% humidity. The modified Kimura B solution contains the following
394 macronutrients (mM): $(\text{NH}_4)_2\text{SO}_4$ (0.5), KNO_3 (1), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.54), CaCl_2 (0.36),
395 K_2SO_4 (0.09), KH_2PO_4 (0.18), and $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$ (0.7); and micronutrients (μM):
396 $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (9.14), H_3BO_3 (46.2), $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ (0.56), $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (0.76),
397 $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.32), and Fe(II)-EDTA (40), with the pH adjusted to 5.8.

398 **Growth response of hydroponically grown seedlings to nitrate and ammonium**

399 For growth response assay of *osnlp3* mutants and *OsNLP3* OE lines (T3 and T4
400 generation), seedlings were growth in hydroponic solutions with nitrate or ammonium

at different concentrations. For 0 N solution, all nitrogen was removed. For 0.2 mM, 2 mM, 5 mM nitrate solutions, all nitrogen was removed and KNO₃ was added to the indicated concentrations. For 0.2 mM and 2 mM ammonium solutions, all nitrogen was removed and NH₄Cl was added to the indicated concentrations. For nitrate and ammonium mixed solution, all nitrogen was removed and 1 mM NH₄Cl plus 1 mM KNO₃ were added. The nutrient solution for hydroponic culture was renewed every other day. The plants were grown in a growth chamber with a 14-hour light (30°C)/10-hour dark (28°C) photoperiod, ~300 µmol/m²/s light intensity, and ~70% humidity for 25 days before sample collection.

Complementation of Arabidopsis *nlp7-1* with *OsNLP3*

The *Arabidopsis thaliana* ecotype Columbia (Col), *nlp7-1* (SALK_26134C) mutant, and *AtNLP7* OE lines were previously described (Yu et al., 2016). Two *OsNLP3* OE Arabidopsis lines in *nlp7-1* background (*OsNLP3*-OE-4, *OsNLP3*-OE-8) were obtained by *Agrobacterium*-mediated floral-dip method with *p35S::OsNLP3* construct. Plant growth conditions were previously described (Yu et al., 2016). Nitrate medium was modified on Murashige and Skoog (MS) medium with KNO₃ as sole N source: 10 mM nitrate medium (similar to MS except all nitrogen source was replaced with 9 mM KCl and 10 mM KNO₃), 3 mM nitrate medium (similar to MS except all nitrogen source was replaced with 16 mM KCl and 3 mM KNO₃), 1 mM nitrate medium (similar to MS except all nitrogen source was replaced with 18 mM KCl and 1 mM KNO₃).

RNA extraction, cDNA preparation, and RT-qPCR

423 Total RNA was extracted using Trizol reagent (TransGen, Beijing, China, Cat. No.
424 ET101-01) from the indicated tissues of rice plants. One microgram of total RNA was
425 used to synthesize cDNA using Easyscript One-step gDNA Removal and cDNA
426 Synthesis Super Mix reagent kit (TransGen, Beijing, China, Cat. No. AE311-01).
427 qRT-PCR was performed with a StepOne Plus Real Time PCR System by using a
428 SYBR Premix Ex Taq II reagent kit (Vazyme, Nanjing, China, Cat. No. Q311-02).
429 Rice *Actin1* was used as the internal reference. All the primers used are shown in
430 Supplementary Table S2.

431 **Subcellular localization of OsNLP3-GFP**

432 To investigate the subcellular localization of OsNLP3, the
433 *pActin1::OsNLP3-GFP* fusion constructs was constructed by inserting the full CDS of
434 *OsNLP3* into the pCB2006-*Actin1::GFP* vector via GATEWAY cloning system and
435 transformed into ZH11 (Lei et al., 2007). Transgenic rice seedlings were grown on
436 normal modified Kimura B solution medium or N-free Kimura B solution medium for
437 18 days.

438 To observe the nuclear-cytoplasmic shuttling of OsNLP3, we designed nitrate
439 addition and depletion kinetics experiments. Seedlings grown on N-free modified
440 Kimura B solution medium for 18 days, then treated with 50 mM KNO₃ for 5 min and
441 15 min, and finally transferred to N-free medium for 50 min and 120 min. Roots were
442 imaged for GFP signals during the time course. For ammonium or KCl (as the control)
443 addition and depletion kinetics experiment, 50 mM NH₄Cl or 50 mM KCl were used
444 instead of 50 mM KNO₃ and the rest was the same. Laser scanning confocal imaging

of roots was performed using the Zeiss 880 microscope equipped with an argon laser (488nm for green fluorescent protein excitation).

Response of *OsNLP3* expression to N availability

Rice seedlings of ZH11 were cultured in modified Kimura B solution (NN) for 18 days in growth chamber with a 14-hour light (30°C)/10-hour dark (28°C) photoperiod, ~300 $\mu\text{mol}/\text{m}^2/\text{s}$ photon density, and ~70% humidity. For N starvation, seedlings were transferred to N-free modified Kimura B solution (0 N) for 1, 3, 6, 12 and 24 hours, and then transferred to modified Kimura B solution containing 5 mM KNO_3 or NH_4Cl (re-N) (5 mM KCl as the control) and grew for 0.5, 1, 2, 4, 8 and 16 hours. Roots and shoots were separately collected at indicated time points for the analyses of *OsNLP3* expression by RT-qPCR.

Chlorate sensitivity assay

Seedlings were first cultured in modified Kimura B solution containing 2 mM KNO_3 for 4 days after germination. Seedlings were subsequently treated with 2 mM chlorate for 2 days and allowed to recover in modified Kimura B solution (2 mM KNO_3) for 5 days. Chlorate sensitivity was calculated as the percent of death. Three replicates were used for chlorate sensitivity assays and each replicate contains 48 seedlings.

N uptake assay using ^{15}N -nitrate or ^{15}N -ammonium

^{15}N -labeled KNO_3 (99 atom % ^{15}N ; Sigma-Aldrich; Cat. No. 335134) or ^{15}N -labeled NH_4Cl (98 atom % ^{15}N ; Sigma-Aldrich; Cat. No. 299251) were used for N uptake assay. For ^{15}N -nitrate uptake assay, rice seedlings were cultured in the

modified Kimura B solution with 5 mM KNO₃ for 12 days. Next, the seedlings were pretreated with the fresh modified Kimura B solution with 5 mM KNO₃ for 2 hours and then transferred to modified Kimura B solution containing 5 mM ¹⁵N-KNO₃ for 24 h. At the end of labeling, the roots were washed for 1 min to remove the ¹⁵N-KNO₃ on the root surface. Then the seedlings were dried at 70 °C to a constant weight and ground to powder. For ¹⁵N-ammonium uptake assays, we followed the same procedure as for nitrate uptake except that 5 mM KNO₃ and 5 mM ¹⁵N-KNO₃ was replaced with 2 mM NH₄Cl and 2 mM ¹⁵N-NH₄Cl, respectively. Finally, the samples were ground and the ¹⁵N was analyzed using a continuous-flow isotope ratio mass spectrometer (DELTA V Advantage) coupled with an elemental analyzer (EA-HT, Thermo Fisher Scientific, Inc., Bremen, Germany). For each sample, 6 seedlings were collected as a sample, and three biological replicates were used as described (Hu et al., 2015).

Metabolite analyses and NR activity assay

For chlorophyll content measurement, 25-day-old seedlings grown hydroponically with different N sources and concentrations as phenotyping assays were used. Leaf chlorophyll was extracted by using 80% acetone, and measured by spectrophotometric method as described previously (Yu et al., 2016).

For total N content assay, 12-day-old seedlings cultured hydroponically with 5 mM KNO₃ or 2mM NH₄Cl as N sources were collected and dried at 70 °C to a constant weight and ground. Samples were analyzed with an NC analyzer (Vario EL III model, Elementar, Hanau, Germany) according to the manufacturer's instructions.

489 For free nitrate content and NR activity assay, 15-day-old seedlings were cultured
490 hydroponically with 0.2 mM /2 mM KNO₃ as N sources. Free nitrate was extracted in
491 50 mM HEPES–KOH (pH 7.4), and measured as described previously (Cataldo et al.,
492 1975). The maximum *in vitro* activity of NR was assayed as previously described
493 (Sylvie et al., 1998).

494 **Chromatin immunoprecipitation-qPCR**

495 A chromatin immunoprecipitation (ChIP) assay was carried out according to the
496 protocol described previously (Cai et al., 2014). The leaves of one-month-old
497 *pActin1::OsNLP3-HA* transgenic rice plants grown in soil, anti-HA antibodies
498 (Abmart, Shanghai, China), and salmon sperm DNA/protein A agarose beads
499 (Millipore, USA) were used for ChIP experiment. DNA was purified using phenol/
500 chloroform (1:1, v/v) and precipitated. The enrichments of DNA fragments were
501 quantified by qPCR using specific primers (Table S2). Enriched values were
502 normalized with the level of input DNA.

503 **Yeast one-hybrid assay**

504 The putative NRE from the flanking sequences of N-relates genes and full CDS
505 of *OsNLP3* were cloned into BD vector (pHIS2) and AD vector (pAD-GAL4-2.1),
506 respectively. A yeast one-hybrid assay was conducted according to the procedure
507 described previously (Cai et al., 2014).

508 **Field evaluation of rice agronomic traits**

509 To investigate the field performance of different *OsNLP3* genotypes, field tests
510 using ZH11, T4 generation of *osnlp3* mutants and *OsNLP3* OE lines were conducted

in the paddy field under natural growth conditions during the regular rice growing season in 2019 at two experimental locations: Chengdu (E104°, N30°) and Hefei (E117°, N31°). In Chengdu, three different N concentrations were designed and urea was used as the N source with 80 kg N/hm² for low N, 200 kg N/hm² for normal N and 500 kg N/hm² for high N. The plants were transplanted in 8 rows × 10 plants for each plot and 4 replicate plots were used for each N condition. In Hefei, urea was used as the N source with 100 kg N/hm². The plants were transplanted in 15 rows × 10 plants for each plot and 3 replicate plots were used. For field data collection, the edge lines of each plot were excluded to avoid margin effects.

Analyses of yield and NUE

All the agronomic traits including plant height, grain yield per plant were collected on a single-plant basis. Detailed methods for measurement of these agronomic traits were described previously (Hu et al., 2015). NUE was defined as the total amount of yield in the form of grain achieved per unit of available N (actual yield per plot / total N application per plot) (Xu et al., 2012).

Statistical analyses

Statistically significant differences were computed based on the one-way ANOVA.

Accession Numbers

Sequence data from this article can be found in the Arabidopsis TAIR database (<https://www.arabidopsis.org>) or Rice Genome Annotation Project (<http://rice.plantbiology.msu.edu/>) under the following accession numbers: *AtNLP7*, *AT4G24020*; *OsNLP3*, *LOC_Os01g13540*; *OsNIA1*, *LOC_Os08g36480*; *OsNIA2*,

533 *LOC_Os08g36500; OsNIA3, LOC_Os02g53130; OsNIR1, LOC_Os01g25484;*
 534 *OsNRT1.1B, LOC_Os10g40600; OsNRT1.1C, LOC_Os03g01290; OsNRT2.1,*
 535 *LOC_Os02g02170; OsNRT2.3a, LOC_Os01g50820; OsNRT2.4, LOC_Os01g36720;*
 536 *OsGRF4, LOC_Os02g47280.*

537

538 **Acknowledgements**

539 This work was supported by grants from the National Natural Science
 540 Foundation of China (grant no. 31572183), the National Key R & D Program of
 541 China (grant no. 2016YFD0100701), the Fundamental Research Funds for the Central
 542 Universities (grant no. WK6030000122), National Natural Science Foundation of
 543 China (grant no. 3157110003), and Ministry of Science and Technology of China
 544 (grant no. 2016ZX08005-004-003). Alamin Alfatih is a recipient of CAS-TWAS
 545 President's Fellowship and CAS International Postdoctoral Fellowship.

546

547 **Author's contribution**

548 ZSZ, JW, LHY and CBX designed the experiments. ZSZ. performed experiments
 549 and data analysis, and wrote the manuscript. JW, JQX, AA, YJH, YS, LQS, and GYW
 550 contributed to performing part of the experiments. SMW, YPW, BHH, GHZ, PQ, SGL
 551 contributed to field trials. CBX, JW and LHY revised the manuscript. CBX supervised
 552 the project.

553

554 **Supporting Information**

555 Figure S1. Verification of the *osnlp3* mutants and *OsNLP3* overexpression lines.

556 Figure S2. *OsNLP3* overexpression in Arabidopsis *nlp7* increases biomass under

557 nitrate-rich condition.

558 Table S1. Predicted NRE cis elements in the promoters of N related genes.

559 Table S2. Primers used in this study.

560

561 **Conflict of interests**

562 The authors declare that they have no conflict of interests.

563

564

565

566

567 **Figure Legends**

568 **Figure 1. *OsNLP3* expression responds to nitrogen availability.**

569 A. Response of *OsNLP3* expression to N availability in shoots of wild type (ZH11).

570 Seedlings were hydroponically grown and treated as described in Materials and

571 Methods (NN, normal nitrogen; 0N, nitrogen starvation; re-N, N resupply). KCl was

572 used as a negative control. Shoots were sampled at indicated time points for RNA

573 analyses with RT-qPCR. Values are the mean $\pm$ SD (n = 3).

574 B. Response of *OsNLP3* expression to N availability in root of wild type (ZH11).

575 Roots of the same seedlings as in A were sampled at indicated time points for RNA

576 analyses with RT-qPCR. KCl used as a negative control. Values are the mean $\pm$ SD (n

577 = 3).

578 C. Expression of *OsNLP3* in different tissues at flowering stage of wild type (ZH11).

579 RNA was extracted from tissues as indicated and subjected to RT-qPCR analyses.

580 Values are the mean $\pm$ SD (n = 3).

581

582 **Figure 2. *OsNLP3* nuclear retention is only induced by nitrate.**

583 A and B. Subcellular localization of *OsNLP3*-GFP in *pActin1::OsNLP3-GFP*

584 transgenic rice seedlings grown on normal nitrogen medium (A) and N-free medium

585 (B) for 18 days. Bars = 20 μ m.

586 C-E. Localization of *OsNLP3*-GFP in response to nitrate and ammonium. 18-day-old

587 *pActin1::OsNLP3-GFP* seedlings grown on N-free medium (0 N) were transferred to

588 medium with 50 mM KNO₃ (C) or NH₄Cl (D) or KCl (E, as a negative control) for 5

min and 15 min, and then transferred to N-free medium for 50 min and 120 min. The images of root cells were captured with a confocal microscope at indicated time points. Bars = 20 μ m.

Figure 3. *OsNLP3* affects seedling growth in nitrate-sufficient conditions.

A-D. Seedling growth response to nitrate. Wild type (ZH11), mutant (*osnlp3*) and overexpression (OE) plants grown in hydroponic solution with varying amounts of nitrate for 25 days after germination. Bars = 5 cm.

E-J. Seedling growth parameters. Shoot fresh weight (E), root fresh weight (F), shoot fresh weight/root fresh weight ratio (G), stem diameter (H), shoot length (I), and leaf chlorophyll content (J) of 25-day-old seedlings were measured. Values are the mean $\pm$ SD of 3 independent replications each containing 16 plants per genotype. Different letters denote significant differences evaluated by one-way ANOVA with Tukey's test ($P < 0.05$). FW, fresh weight.

Figure 4. *OsNLP3* does not influence seedling growth in response to ammonium.

A-C. Seedling growth response to ammonium. Wild type (ZH11), mutant (*osnlp3*) and overexpression (OE) plants grown in hydroponic solution with varying amounts of ammonium for 25 days after germination. Bars = 5 cm.

D-I. Seedling growth parameters. Shoot fresh weight (D), root fresh weight (E), shoot fresh weight/root fresh weight ratio (F), stem diameter (G), shoot length (H), and leaf chlorophyll content (I) of 25-day-old seedlings were measured. Values are the mean $\pm$

SD of 3 independent replications each containing 16 plants per genotype. Different letters denote significant differences evaluated by one-way ANOVA with Tukey's test ($P < 0.05$). FW, fresh weight.

614

Figure 5. OsNLP3 modulates nitrate uptake and assimilation.

A. Chlorate sensitivity assay. Wild type (ZH11), mutant (*osnlp3*) and overexpression (OE) seedlings treated with chlorate as described in Materials and Methods. Bar = 5 cm.

B. Chlorate sensitivity. Chlorate sensitivity was evaluated by the death ratio after a chlorate-sensitivity test. Values are the mean $\pm$ SD of 3 independent replications each containing 48 plants per genotype. Different letters denote significant differences evaluated by one-way ANOVA with Tukey's test ($P < 0.05$).

C and D. N uptake assay with ^{15}N -nitrate or ^{15}N -ammonium. 12-day-old wild type (ZH11), mutant (*osnlp3*) and overexpression (OE) plants were used for N uptake assays as described in Materials and Methods. Values are the mean $\pm$ SD of 3 independent replications each containing 6 plants per genotype. Different letters denote significant differences evaluated by one-way ANOVA with Tukey's test ($P < 0.05$). DW, dry weight.

E and F. Total nitrogen content. 12-day-old wild type (ZH11), mutant (*osnlp3*) and overexpression (OE) seedlings grown in hydroponic solution with 5 mM nitrate or 2 mM ammonium were sampled for total N content analyses as described in Materials and Methods. Different letters denote significant differences evaluated by one-way

ANOVA with Tukey's test ($P < 0.05$). DW, dry weight.

G and H. Free nitrate content and NR activity. Free nitrate content (G) and NR activity (H) in the shoots of 15-day-old wild type (ZH11), mutant (*osnlp3*) and overexpression (OE) plants grown in hydroponic solution with 0.2 mM and 2 mM nitrate were assayed as described in Materials and Methods. Values are the mean $\pm$ SD of 4 independent replications each contained 3 plants per genotype. Different letters denote significant differences evaluated by one-way ANOVA with Tukey's test ($P < 0.05$). FW, fresh weight.

Figure 6. OsNLP3 regulates the expression of nitrate utilization and signaling related genes.

A-J. RT-qPCR-based expression analyses of genes involved in nitrate utilization and signaling in wild type (ZH11), mutant (*osnlp3*) and overexpression (OE) plants. 18-day-old plants grown in normal nitrogen medium were used for the analyses. Nitrate reductase genes: *OsNIA1*, *OsNIA2*, and *OsNIA3*; Nitrite reductase gene: *OsNIR1*; Nitrate transporter genes: *OsNRT1.1B*, *OsNRT1.1C*, *OsNRT2.1*, *OsNRT2.3a*, *OsNRT2.4*; Growth-regulating factor gene: *OsGRF4*. Values are the mean $\pm$ SD ($n = 3$). Different letters denote significant differences evaluated by one-way ANOVA with Tukey's test ($P < 0.05$).

Figure 7. OsNLP3 directly binds to promoters of N-related genes.

A-E. ChIP-qPCR assay. HA-OsNLP3 mediated ChIP-qPCR enrichment (relative to

wild type) of NRE-like fragments from promoters of *OsNIA1*, *OsNIA3*, *OsNRT1.1B*, *OsNRT2.4*, *OsGRF4* was performed as described in Materials and methods. Values are the mean $\pm$ SD ($n = 3$). Different letters denote significant differences evaluated by one-way ANOVA with Tukey's test ($P < 0.05$).

F. Yeast-one-hybrid assays. pAD-GAL4-*OsNLP3* (AD-NLP3) fusion protein activate the expression of pHIS2 (BD) reporter gene driven by the NRE-like of *OsNIA1*, *OsNIA3*, *OsNRT1.1B*, *OsNRT2.4*, *OsGRF4*. The empty vector pAD-GAL4 (AD) and pHIS2 (BD) were used as a negative control.

Figure 8. *OsNLP3* overexpression improves NUE and grain yield under different nitrogen levels.

A. Morphological phenotypes of wild type (ZH11), mutant (*osnlp3*) and overexpression (OE) grown in the field under low N (LN), normal N (NN) and high N (HN) conditions. A representative plant was dug out from the field and potted for photograph. Bars = 20 cm.

B. Total grains per plant of wild type (ZH11), mutant (*osnlp3*) and overexpression (OE) grown in the field under LN, NN and HN conditions. Bars = 5 cm.

C-E. Agronomic traits. Plant height (C), grain yield per plant (D) and NUE (E) of wild type (ZH11), mutant (*osnlp3*) and overexpression (OE) plants grown in the field under LN, NN and HN were statistically analyzed. Values are the means $\pm$ SD ($n=20$).

Different letters denote significant differences evaluated by one-way ANOVA with Tukey's test ($P < 0.05$).

677

678 **Figure 9. *OsNLP3* overexpression confer to improve NUE and grain yield under**
 679 **normal nitrogen level.**

680 A. Morphological phenotypes of wild type (ZH11), mutant (*osnlp3*) and
 681 overexpression (OE) grown in the field with normal nitrogen. A representative plant
 682 was dug out from the field and potted for photograph. The senescent leaves were
 683 removed. Bar = 15 cm.

684 B. Total grains per plant of wild type (ZH11), mutant (*osnlp3*) and overexpression
 685 (OE) plants grown in the field with normal nitrogen. Bar = 5 cm.

686 C-E. Agronomic traits. Plant height (C), grain yield per plant (D), and NUE (E) of
 687 wild type (ZH11), mutant (*osnlp3*) and overexpression (OE) plants grown in the field
 688 with normal nitrogen were statistically analyzed. Values are the means $\pm$ SD (n=20).
 689 Different letters denote significant differences evaluated by one-way ANOVA with
 690 Tukey's test ($P < 0.05$).

691

692

Parsed Citations

Alfatih A, Wu J, Zhang ZS, Xia JQ, Jan SU, Yu LH, Xiang CB (2020) Rice NIN-LIKE PROTEIN 1 rapidly responds to nitrogen deficiency and improves yield and nitrogen use efficiency. J Exp Bot 71: 6032-6042

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Alvarez JM, Riveras E, Vidal EA, Gras DE, Contreras-Lopez O, Tamayo KP, Aceituno F, Gomez I, Ruffel S, Lejay L, Jordana X, Gutierrez RA (2014) Systems approach identifies TGA1 and TGA4 transcription factors as important regulatory components of the nitrate response of Arabidopsis thaliana roots. Plant J 80: 1-13

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Alvarez JM, Schinke AL, Brooks MD, Pasquino A, Leonelli L, Varala K, Safi A, Krouk G, Krapp A, Coruzzi GM (2020) Transient genome-wide interactions of the master transcription factor NLP7 initiate a rapid nitrogen-response cascade. Nat Commun 11: 1157

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Cai XT, Xu P, Zhao PX, Liu R, Yu LH, Xiang CB (2014) Arabidopsis ERF109 mediates cross-talk between jasmonic acid and auxin biosynthesis during lateral root formation. Nat Commun 5: 5833

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Camargo A, Llamas A, Schnell RA, Higuera JJ, Gonzalez-Ballester D, Lefebvre PA, Fernandez E, Galvan A (2007) Nitrate signaling by the regulatory gene NIT2 in Chlamydomonas. Plant Cell 19: 3491-3503

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Cao H, Qi S, Sun M, Li Z, Yang Y, Crawford NM, Wang Y (2017) Overexpression of the Maize ZmNLP6 and ZmNLP8 Can Complement the Arabidopsis Nitrate Regulatory Mutant nlp7 by Restoring Nitrate Signaling and Assimilation. Front Plant Sci 8: 1703

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Castaigns L, Camargo A, Pocholle D, Gaudon V, Texier Y, Boutet-Mercey S, Taconnat L, Renou JP, Daniel-Vedele F, Fernandez E, Meyer C, Krapp A (2009) The nodule inception-like protein 7 modulates nitrate sensing and metabolism in Arabidopsis. Plant J 57: 426-435

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Cataldo DA, Maroon M, Schrader LE, Youngs VL (1975) Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Commun Soil Sci Plant Anal 6: 71-80

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Chardin C, Girin T, Roudier F, Meyer C, Krapp A (2014) The plant RWP-RK transcription factors: key regulators of nitrogen responses and of gametophyte development. J Exp Bot 65: 5577-5587

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Crawford NM (1995) Nitrate: Nutrient and Signal for Plant Growth. Plant Cell 7: 859-868,

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Crawford NM, Forde BG (2002) Molecular and developmental biology of inorganic nitrogen nutrition. Arabidopsis Book 1: e0011

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Fang Z, Xia K, Yang X, Grotemeyer MS, Meier S, Rentsch D, Xu X, Zhang M (2013) Altered expression of the PTR/NRT1 homologue OsPTR9 affects nitrogen utilization efficiency, growth and grain yield in rice. Plant Biotechnol J 11: 446-458

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Gao Z, Wang Y, Chen G, Zhang A, Yang S, Shang L, Wang D, Ruan B, Liu C, Jiang H, Dong G, Zhu L, Hu J, Zhang G, Zeng D, Guo L, Xu G, Teng S, Harberd NP, Qian Q (2019) The indica nitrate reductase gene OsNR2 allele enhances rice yield potential and nitrogen use efficiency. Nat Commun 10: 5207

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Garnett T, Conn V, Kaiser BN (2009) Root based approaches to improving nitrogen use efficiency in plants. Plant Cell Environ 32: 1272-1283

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Ge M, Wang Y, Liu Y, Jiang L, He B, Ning L, Du H, Lv Y, Zhou L, Lin F, Zhang T, Liang S, Lu H, Zhao H (2020) The NIN-like protein 5 (ZmNLP5) transcription factor is involved in modulating the nitrogen response in maize. Plant J 102: 353-368

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Good AG, Shrawat AK, Muench DG (2004) Can less yield more? Is reducing nutrient input into the environment compatible with maintaining crop production? Trends Plant Sci 9: 597-605

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Han M, Okamoto M, Beatty PH, Rothstein SJ, Good AG (2015) The Genetics of Nitrogen Use Efficiency in Crop Plants. Annu Rev Genet 49: 269-289

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Hirel B, Tétu T, Lea PJ, Dubois F (2011) Improving Nitrogen Use Efficiency in Crops for Sustainable Agriculture. Sustainability 3: 1452-1485

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Ho CH, Lin SH, Hu HC, Tsay YF (2009) CHL1 functions as a nitrate sensor in plants. Cell 138: 1184-1194

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Ho CH, Tsay YF (2010) Nitrate, ammonium, and potassium sensing and signaling. Curr Opin Plant Biol 13: 604-610

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Hu B, Jiang Z, Wang W, Qiu Y, Zhang Z, Liu Y, Li A, Gao X, Liu L, Qian Y, Huang X, Yu F, Kang S, Wang Y, Xie J, Cao S, Zhang L, Wang Y, Xie Q, Kopriva S, Chu C (2019) Nitrate-NRT1.1B-SPX4 cascade integrates nitrogen and phosphorus signalling networks in plants. Nat Plants 5: 401-413

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Hu B, Wang W, Ou S, Tang J, Li H, Che R, Zhang Z, Chai X, Wang H, Wang Y, Liang C, Liu L, Piao Z, Deng Q, Deng K, Xu C, Liang Y, Zhang L, Li L, Chu C (2015) Variation in NRT1.1B contributes to nitrate-use divergence between rice subspecies. Nat Genet 47: 834-838

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Hu HC, Wang YY, Tsay YF (2009) AtCIPK8, a CBL-interacting protein kinase, regulates the low-affinity phase of the primary nitrate response. Plant J 57: 264-278

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Kirk GJ, Kronzucker HJ (2005) The potential for nitrification and nitrate uptake in the rhizosphere of wetland plants: a modelling study. Annals Botany 96: 639-646

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Konishi M, Yanagisawa S (2013) Arabidopsis NIN-like transcription factors have a central role in nitrate signalling. Nat Commun 4: 1617

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Krapp A, David LC, Chardin C, Girin T, Marmagne A, Leprince AS, Chaillou S, Ferrario-Mery S, Meyer C, Daniel-Vedele F (2014) Nitrate transport and signalling in Arabidopsis. J Exp Bot 65: 789-798

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Lei Z-Y, Zhao P, Cao M-J, Cui R, Chen X, Xiong L-Z, Zhang Q-F, Oliver DJ, Xiang C-B (2007) High-throughput Binary Vectors for Plant Gene Function Analysis. J Integr Plant Biol 49 (4): 556-567

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Li S, Tian Y, Wu K, Ye Y, Yu J, Zhang J, Liu Q, Hu M, Li H, Tong Y, Harberd NP, Fu X (2018) Modulating plant growth-metabolism coordination for sustainable agriculture. Nature 560: 595-600

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Liu K-H, Huang C-Y, Tsay Y-F (1999) CHL1 is a dual-affinity nitrate transporter of Arabidopsis involved in multiple phases of nitrate uptake. Plant Cell 11: 865-874

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Liu KH, Niu Y, Konishi M, Wu Y, Du H, Sun Chung H, Li L, Boudsocq M, McCormack M, Maekawa S, Ishida T, Zhang C, Shokat K, Yanagisawa S, Sheen J (2017) Discovery of nitrate-CPK-NLP signalling in central nutrient-growth networks. Nature 545: 311-316

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Lu Y, Ye X, Guo R, Huang J, Wang W, Tang J, Tan L, Zhu JK, Chu C, Qian Y (2017) Genome-wide Targeted Mutagenesis in Rice Using the CRISPR/Cas9 System. Mol Plant 10: 1242-1245

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Mandal VK, Sharma N, Raghuram N (2018) Molecular Targets for Improvement of Crop Nitrogen Use Efficiency: Current and Emerging Options. In A Shrawat, A Zayed, DA Lightfoot, eds, Engineering Nitrogen Utilization in Crop Plants. Springer New York, pp 77-93

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Marche C, Roudier F, Castaigns L, Brehaut V, Blondet E, Colot V, Meyer C, Krapp A (2013) Nuclear retention of the transcription factor NLP7 orchestrates the early response to nitrate in plants. Nat Commun 4: 1713

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Mu X, Luo J (2019) Evolutionary analyses of NIN-like proteins in plants and their roles in nitrate signaling. Cellular Mol Life Sci 76: 3753-3764

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Mueller ND, West PC, Gerber JS, MacDonald GK, Polasky S, Foley JA (2014) A tradeoff frontier for global nitrogen use and cereal production. Environ Res Lett 9: 054002

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Oldroyd GED, Leyser O (2020) A plant's diet, surviving in a variable nutrient environment. Science 368

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Para A, Li Y, Marshall-Colon A, Varala K, Francoeur NJ, Moran TM, Edwards MB, Hackley C, Bargmann BO, Birnbaum KD, McCombie WR, Krouk G, Coruzzi GM (2014) Hit-and-run transcriptional control by bZIP1 mediates rapid nutrient signaling in Arabidopsis. Proc

Natl Acad Sci USA 111: 10371-10376

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Robertson GP, Vitousek PM, resources (2009) Nitrogen in agriculture: balancing the cost of an essential resource. Annu Rev Environ Resour 34: 97-125

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Rubin G, Tohge T, Matsuda F, Saito K, Scheible WR (2009) Members of the LBD family of transcription factors repress anthocyanin synthesis and affect additional nitrogen responses in Arabidopsis. Plant Cell 21: 3567-3584

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Sylvie FM, Valadier MH, Foyer CH (1998) Overexpression of nitrate reductase in tobacco delays drought-induced decreases in nitrate reductase activity and mRNA. Plant Physiol 117: 293-302

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Tang W, Ye J, Yao X, Zhao P, Xuan W, Tian Y, Zhang Y, Xu S, An H, Chen G, Yu J, Wu W, Ge Y, Liu X, Li J, Zhang H, Zhao Y, Yang B, Jiang X, Peng C, Zhou C, Terzaghi W, Wang C, Wan J (2019) Genome-wide associated study identifies NAC42-activated nitrate transporter conferring high nitrogen use efficiency in rice. Nat Commun 10: 5279

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Undurraga SF, Ibarra-Henríquez C, Fredes I, Álvarez JM, Gutiérrez R (2017) Nitrate signaling and early responses in Arabidopsis roots. J Exp Bot 68: 2541-2551

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Vidal EA, Araus V, Lu C, Parry G, Green PJ, Coruzzi GM, Gutierrez RA (2010) Nitrate-responsive miR393/AFB3 regulatory module controls root system architecture in Arabidopsis thaliana. Proc Natl Acad Sci USA 107: 4477-4482

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Wang W, Hu B, Yuan D, Liu Y, Che R, Hu Y, Ou S, Liu Y, Zhang Z, Wang H, Li H, Jiang Z, Zhang Z, Gao X, Qiu Y, Meng X, Liu Y, Bai Y, Liang Y, Wang Y, Zhang L, Li L, Sodmergen, Jing H, Li J, Chu C (2018) Expression of the Nitrate Transporter Gene OsNRT1.1A/OsNPF6.3 Confers High Yield and Early Maturation in Rice. Plant Cell 30: 638-651

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Wang YY, Cheng YH, Chen KE, Tsay YF (2018) Nitrate Transport, Signaling, and Use Efficiency. Annu Rev Plant Biol 69: 85-122

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Wu J, Zhang ZS, Xia JQ, Alfatih A, Song Y, Huang YJ, Wan GY, Sun LQ, Tang H, Liu Y, Wang SM, Zhu QS, Qin P, Wang YP, Li SG, Mao CZ, Zhang GQ, Chu C, Yu LH, Xiang CB (2020) Rice NIN-LIKE PROTEIN 4 plays a pivotal role in nitrogen use efficiency. Plant Biotechnol J

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Wu K, Wang S, Song W, Zhang J, Wang Y, Liu Q, Yu J, Ye Y, Li S, Chen J, Zhao Y, Wang J, Wu X, Wang M, Zhang Y, Liu B, Wu Y, Harberd NP, Fu X (2020) Enhanced sustainable green revolution yield via nitrogen-responsive chromatin modulation in rice. Science 367

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Xu G, Fan X, Miller AJ (2012) Plant nitrogen assimilation and use efficiency. Annu Rev Plant Biol 63: 153-182

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Xu N, Wang R, Zhao L, Zhang C, Li Z, Lei Z, Liu F, Guan P, Chu Z, Crawford NM, Wang Y (2016) The Arabidopsis NRG2 Protein Mediates Nitrate Signaling and Interacts with and Regulates Key Nitrate Regulators. Plant Cell 28: 485-504

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Yan D, Easwaran V, Chau V, Okamoto M, Ierullo M, Kimura M, Endo A, Yano R, Pasha A, Gong Y, Bi YM, Provart N, Guttman D, Krapp A, Rothstein SJ, Nambara E (2016) NIN-like protein 8 is a master regulator of nitrate-promoted seed germination in Arabidopsis. Nat Commun 7: 13179

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Yan M, Fan X, Feng H, Miller AJ, Shen Q, Xu G (2011) Rice OsNAR2.1 interacts with OsNRT2.1, OsNRT2.2 and OsNRT2.3a nitrate transporters to provide uptake over high and low concentration ranges. Plant Cell Environ 34: 1360-1372

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Yu LH, Wu J, Tang H, Yuan Y, Wang SM, Wang YP, Zhu QS, Li SG, Xiang CB (2016) Overexpression of Arabidopsis NLP7 improves plant growth under both nitrogen-limiting and -sufficient conditions by enhancing nitrogen and carbon assimilation. Scientific Rep 6: 27795

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Zhang H, Forde BG (1998) An Arabidopsis MADS box gene that controls nutrient-induced changes in root architecture. Science 279: 407-409

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)