Check for updates

A Transcription Factor, OsMADS57, Regulates Long-Distance Nitrate Transport and Root Elongation[1]

Shuangjie Huang,[a,b,2] Zhihao Liang,[a,2] Si Chen,[a] Huwei Sun,[c] Xiaorong Fan,[a,a] Cailin Wang,[d] Guohua Xu,[a] and Yali Zhang[a,3,4]

[a]State Key Laboratory of Crop Genetics and Germplasm Enhancement, Key Laboratory of Plant Nutrition and Fertilization in Low-Middle Reaches of the Yangtze River, Ministry of Agriculture, Nanjing Agricultural University, Nanjing 210095, China

[b]Henan Key Laboratory of Tea Plant Comprehensive Utilization in South Henan, Xinyang Agriculture and Forestry University, Xinyang 464000, China

[c]College of Agronomy, Collaborative Innovation Center of Henan Grain Crops, Key Laboratory of Rice Biology in Henan Province, Henan Agricultural University, Zhengzhou 450002, China

[d]Institute of Food Crops of Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China

ORCID IDs: 0000-0001-7906-0476 (S.C.); 0000-0001-8844-1713 (X.F.); 0000-0002-3283-2392 (G.X.); 0000-0003-1815-0237 (Y.Z.).

Root nitrate uptake adjusts to the plant's nitrogen demand for growth. Here, we report that OsMADS57, a MADS-box transcription factor, modulates nitrate translocation from rice (*Oryza sativa*) roots to shoots under low-nitrate conditions. *OsMADS57* is abundantly expressed in xylem parenchyma cells of root stele and is induced by nitrate. Compared with wild-type rice plants supplied with 0.2 mM nitrate, *osmads57* mutants had 31% less xylem loading of nitrate, while overexpression lines had 2-fold higher levels. Shoot-root ^{15}N content ratios were 40% lower in the mutants and 76% higher in the overexpression lines. Rapid NO_3^- root influx experiments showed that mutation of *OsMADS57* did not affect root nitrate uptake. Reverse transcription quantitative PCR analysis of *OsNRT2* nitrate transporter genes showed that after 5 min in 0.2 mM nitrate, only *OsNRT2.3a* (a vascular-specific high-affinity nitrate transporter) had reduced (by two-thirds) expression levels. At 60 min of nitrate treatment, lower expression levels were also observed for three additional *NRT2* genes (*OsNRT2.1/2.2/2.4*). Conversely, in the overexpression lines, four *NRT2* genes had much higher expression profiles at all time points tested. As previously reported, *OsNRT2.3a* functions in nitrate translocation, indicating the possible interaction between *OsMADS57* and *OsNRT2.3a*. Yeast one-hybrid and transient expression assays demonstrated that OsMADS57 binds to the CArG motif (CATTTTATAG) within the *OsNRT2.3a* promoter. Moreover, seminal root elongation was inhibited in *osmads57* mutants, which may be associated with higher auxin levels in and auxin polar transport to root tips of mutant plants. Taken together, these results suggest that OsMADS57 has a role in regulating nitrate translocation from root to shoot via OsNRT2.3a.

Inorganic nitrogen (N) is available for plants as nitrate in aerobic uplands and as ammonium in flooded wetland or acidic soils. For many plants, nitrate acquired by roots is transported to the shoot before being assimilated (Smirnoff and Stewart, 1985; Xu et al., 2012). By contrast, ammonium derived from nitrate reduction or directly from ammonium uptake is preferentially assimilated in the root and then transported in an organic form to the shoot (Xu et al., 2012). Nitrate levels can vary by 3 to 4 orders of magnitude from micromolar to millimolar concentrations, and nitrate is highly mobile in agricultural soils due to its negative charge and solubility (Crawford and Glass, 1998; Miller et al., 2007). In response to external fluctuations of nitrate, plants have evolved at least three nitrate uptake systems, two high-affinity transport systems (HATS) and one low-affinity transport system (LATS), which are responsible for the acquisition of nitrate (Crawford and Glass, 1998).

The constitutive HATS and nitrate-inducible HATS operate to take up nitrate at low nitrate concentrations in external medium, with saturation in the range of 0.2

[1]This work was supported by the National Key R&D Program of China (2017YFD0200100 and 2018YFD0200503), the National Nature Science Foundation of China (31471936 and 31672225), the 111 Project (12009), the Innovative Research Team Development Plan of the Ministry of Education of China, the PAPD project of the Jiangsu Higher Education Institutions, and the Jiangsu Overseas Research & Training Program for University Prominent Young & Middle-Aged Teachers and Presidents.

[2]These authors contributed equally to the article.

[3]Author for contact: ylzhang@njau.edu.cn.

[4]Senior author.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) is: Yali Zhang (ylzhang@njau.edu.cn).

Y.L.Z. conceived the research plans; Y.L.Z. and G.H.X. supervised the experiments; S.J.H. and Z.H.L. performed the experiments; S.C. and H.W.S. provided technical assistance; X.R.F. gave suggestions on the experiments; Y.L.Z. and S.J.H. analyzed the data and wrote the article; C.L.W. gave suggestions on the writing.

www.plantphysiol.org/cgi/doi/10.1104/pp.19.00142

 © 2019 American Society of Plant Biologists. All Rights Reserved.

to 0.5 mM. In contrast, the LATS predominates at high soil nitrate concentrations. Nitrate LATS and HATS uptake systems have been linked to the NPF (originally named as Nitrate Transporter1/Peptide Transporter [NRT1/PTR]) and NRT2 gene families, respectively (Miller et al., 2007; Léran et al., 2014; O'Brien et al., 2016). In Arabidopsis (*Arabidopsis thaliana*), there are at least 53 and seven members belonging to the NPF (NRT1) and NRT2 families, respectively (Miller et al., 2007; Tsay et al., 2007; O'Brien et al., 2016). The most extensively studied nitrate transporter gene, *AtNPF6.3/AtCHL1/AtNRT1.1*, which is predominantly expressed in the nascent organs, especially the root tip (Guo et al., 2001), is described as a nitrate transceptor (transporter and receptor), playing multiple roles as a dual-affinity nitrate transporter and a sensor of external nitrate concentration (Liu and Tsay, 2003; Ho et al., 2009; Wang et al., 2009; Gojon et al., 2011; Forde, 2014) and auxin transport at low nitrate concentrations (Krouk et al., 2010a). Several nitrate transporters, mainly from the NRT1/NPF family, involved in nitrate transport in the vascular tissue of roots and shoots have been characterized. AtNRT1.5 is involved in nitrate loading into the root stele and translocating from root to shoot, whose expression is regulated by the transcription factor MYB59 (Lin et al., 2008; Li et al., 2017; Du et al., 2019). AtNRT1.7/AtNRT1.8/AtNRT1.11/AtNRT1.12 have been reported to be involved in nitrate unloading from xylem or loading into phloem (Fan et al., 2009; Li et al., 2010a; Hsu and Tsay, 2013).

In submerged rice (*Oryza sativa*), nitrate uptake could be comparable with that of ammonium (Kronzucker et al., 2000; Kirk and Kronzucker, 2005) due to oxygen transported by abundant parenchyma in roots into the rhizosphere, resulting in ammonium nitrification by bacteria on the root surface (Kirk, 2003; Li et al., 2008). A few proteins associated with nitrate uptake or transport have been identified (Cai et al., 2008; Feng et al., 2011; Yan et al., 2011; Tang et al., 2012; Hu et al., 2015, 2016; Li et al., 2015; Xia et al., 2015; Fan et al., 2016; Wang et al., 2018a; Wei et al., 2018). Since the nitrate concentration in the rhizosphere of paddy fields is estimated to be less than 10 μM (Kirk and Kronzucker, 2005), NRT2 family members play a major role in nitrate uptake for rice plants (Araki and Hasegawa, 2006; Feng et al., 2011; Yan et al., 2011). *OsNRT2.1*, *OsNRT2.2*, and *OsNRT2.3a*, belonging to the OsNRT2 family, are transcriptionally up-regulated by nitrate supply and require a partner protein, OsNAR2.1, for nitrate uptake (Feng et al., 2011; Yan et al., 2011). *OsNRT2.3a* is expressed predominantly in xylem parenchyma cells of the root stele and has been demonstrated to play a role in transporting nitrate from the root to the shoot under low-nitrate conditions (Tang et al., 2012). *OsNRT2.3b* is expressed in the phloem and is suggested to be involved in nitrate transport within the shoot (Fan et al., 2016). *OsNRT2.4* encodes a dual-affinity nitrate transporter and functions in nitrate-regulated root growth and nitrate distribution in rice plants (Wei et al., 2018), and several members of the NRT1/NPF family, such as OsNPF2.4, OsNPF2.2, and OsNPF7.2, involved in nitrate transport have also been identified (Li et al., 2015; Xia et al., 2015; Hu et al., 2016).

Knowledge of nitrate transporters has been increasing substantially in the past 30 years. To further illustrate the nitrate uptake mechanism in plants, the regulatory systems controlling the transporter function need to be elucidated (Plett et al., 2018). Among several controlling mechanisms, transcriptional control of nitrate uptake is well documented in which transcription factors (TFs) act as master switches for regulatory networks (Xuan et al., 2017; Kant, 2018; Plett et al., 2018; Wang et al., 2018b). The first TF identified to play a role in N-responsive signaling is the Arabidopsis MADS-box TF (ANR1), which regulates the proliferation of lateral roots in NO_3^--rich patches (Zhang and Forde, 1998; Gan et al., 2005) but also exists in the signaling pathway of the transceptor NRT1.1 (Remans et al., 2006). Thereafter, more s involved in complex regulatory networks and nitrate-dependent signaling pathways, such as LATERAL ORGAN BOUNDARY DOMAIN (LBD) family genes (LBD37/38/39; Rubin et al., 2009), NIN-like protein (NLP) family genes (NLP6/7/NLP8; Castaings et al., 2009; Konishi and Yanagisawa, 2013; Marchive et al., 2013; Yan et al., 2016), SBP-box family gene (SPL9; Krouk et al., 2010b), bZIP transcription factors (TGA1/4; Alvarez et al., 2014), Bric-a-Brac/Tramtrack/Broad family genes (BT1/2; Araus et al., 2016), NITRATE REGULATORY GENE2 (NRG2; Xu et al., 2016), and teosinte branched1/cycloidea/proliferating cell factor1-20 gene (TCP20; Guan et al., 2017), have been identified and intensively investigated in Arabidopsis.

In rice, orthologs of the above TFs (e.g. OsMADS) are also found to be regulated under different N supplies (Puig et al., 2013; Yu et al., 2014a; Yang et al., 2015). However, few of them have been demonstrated to regulate developmental responses to nitrate. For instance, *OsMADS* genes show diverse responses to nitrate supply (Puig et al., 2013; Yu et al., 2014a). Unlike AtANR1, which acts downstream of AtNPF6.3 (Remans et al., 2006), overexpression of nitrate-inducible *OsMADS25* significantly increases the expression of nitrate transporter genes and promotes nitrate accumulation and lateral root and shoot growth, suggesting positive effects of OsMADS25 on nitrate uptake via nitrate transporters (Yu et al., 2015). Interestingly, *OsMADS* genes are targeted by monocot-specific MicroRNA444s (Li et al., 2010b). *OsmiR444a* overexpression reduces *OsMADS* expression and root and shoot growth under nitrate supply (Yan et al., 2014). Thus, OsmiR444a/OsMADS may act as a regulatory module for nitrate-dependent signaling and growth responses in rice. However, the specific gene in the regulatory system controlling nitrate uptake and translocation in rice remains unclear. In this study, we report that a rice MADS-box transcription factor, OsMADS57, is involved in the regulation of long-distance translocation of nitrate from the root to the shoot through regulating *OsNRT2.3a* expression under low nitrate concentration.

RESULTS

OsMADS57 Is Mainly Expressed in Rice Root Stelar Cells and Is Sensitive to Supplies of Nitrate and Auxin

Arabidopsis *AtANR1* was identified as a key gene controlling lateral root growth through nitrate signaling (Zhang and Forde, 1998). To understand the biological functions of the *ANR1*-like-related gene *OsMADS57*, we performed reverse transcription quantitative PCR (RT-qPCR) analysis and GUS assay to examine its expression pattern. A previous study and the Rice Expression Profile Database (http://ricexpro.dna.affrc.go.jp/GGEP/gene-search.php) have shown that *OsMADS57* is abundantly expressed in roots and leaf blades during vegetative stages compared with other stages in rice (Guo et al., 2013). Interestingly, the expression of *OsMADS57* was significantly up-regulated by nitrate treatments, irrespective of nitrate concentrations, rather than by ammonium (Fig. 1A). Transgenic rice plants expressing the *GUS* gene driven by the *OsMADS57* promoter (~3 kb) exhibited GUS activity in the root central cylinder, particularly in the xylem parenchyma cells (Fig. 1, C and D), consistent with previous reports (Puig et al., 2013; Yu et al., 2014a). Stronger GUS staining in the seminal root central cylinder caused by nitrate treatments further demonstrated the above result.

Furthermore, we performed RT-qPCR to test the relative expression of *OsMADS57* in response to external stresses and hormones. The results showed that, compared with the mock treatment, the expression of *OsMADS57* was up-regulated by exogenous application of NAA but not by application of polyethylene glycol (PEG) and NaCl (Fig. 1B). Similarly, NAA-induced expression of GUS staining was also observed in the root central cylinder in transgenic lines (Fig. 1D).

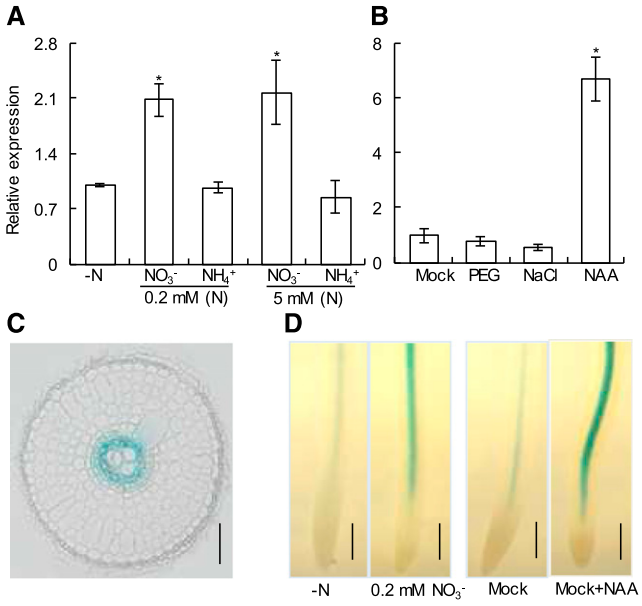

Figure 1. *OsMADS57* gene expression pattern. Rice seedlings were grown in International Rice Research Institute (IRRI) solution supplied by N with/without exogenous 10% (w/v) PEG, 100 mM NaCl, and 10 nM 1-naphthaleneacetic acid (NAA) for 7 d. A, Relative *OsMADS57* expression in response to N supplies. B, Relative *OsMADS57* expression in response to PEG, NaCl, and NAA treatments. Relative mRNA levels of *OsMADS57* were normalized relative to *OsActin*. Data are means of five replications ± SE. *, $P < 0.05$ (Student's *t* test) relative to the −N (0 mM N) treatment (A) or the mock (1.25 mM NH_4NO_3) treatment (B). C, Expression of *MADS57* in a seminal root cross section of a *MADS57pro*::*GUS* transgenic line. Bar = 50 μm. D, Expression of *MADS57* in seminal roots of *MADS57pro*::*GUS* transgenic lines. Bars = 0.5 mm.

OsMADS57 Is Involved in Nitrate Translocation from Roots to Shoots

To determine the specific role of OsMADS57 in rice plants, two independent mutant lines (the transfer-DNA [T-DNA]-inserted *osmads57* mutants *m1* and *m2*) and three independent *35S*::*OsMADS57* overexpression transgenic lines (Ox1–Ox3) were used (Supplemental Fig. S1). Given that *OsMADS57* expression was affected only by NO_3^- supplies, we first analyzed plant growth and N accumulation in rice plants grown under NO_3^- supplies of 0.2 and 5 mM (Supplemental Fig. S2). Surprisingly, less plant dry weight was observed in the two *osmads57* mutants and the three overexpression lines only under low NO_3^- supply in comparison with wild-type plants. Furthermore, under low NO_3^- supply, higher N contents were recorded in roots of the mutants and in roots and shoots of the overexpression lines, with shoot accumulation being most pronounced. Although slight decreases were observed in the shoots of the two mutants, they did not show statistical significance.

Next, time-course ^{15}N-labeled NO_3^--supply experiments were conducted to understand the role of OsMADS57 under low nitrate supply (Fig. 2, A and B). The overexpression lines showed substantially higher ^{15}N accumulation in shoots at all time points (5, 30, and 60 min). The mutant lines showed substantially higher ^{15}N accumulation in roots at 30 and 60 min and lower shoot ^{15}N accumulation at 60 min. These phenotypes resulted in dramatic changes in the shoot-root ^{15}N ratios.

Compared with wild-type plants, the shoot-root ^{15}N ratios were 42% to 76% higher in the three overexpression lines, while shoot-root ^{15}N ratios were approximately 40% lower in the two mutants at 30 and 60 min (Fig. 2C), and shoot-(shoot+root) ^{15}N ratios had the same tendency (Fig. 2D). These data suggest that OsMADS57 might be involved in nitrate distribution in rice plants. To confirm if OsMADS57 plays an important role in long-distance nitrate transport, 1-h xylem exudates of rice plants grown in hydroponics were collected, from the cut surface since 4 cm above the ground level was removed, and analyzed (Fig. 2, E and F). The nitrate concentration and nitrate transport rate decreased by 16% and 31% in xylem sap of the two mutant lines in comparison with wild-type plants; conversely, 20% and 2-fold higher nitrate concentrations were recorded in

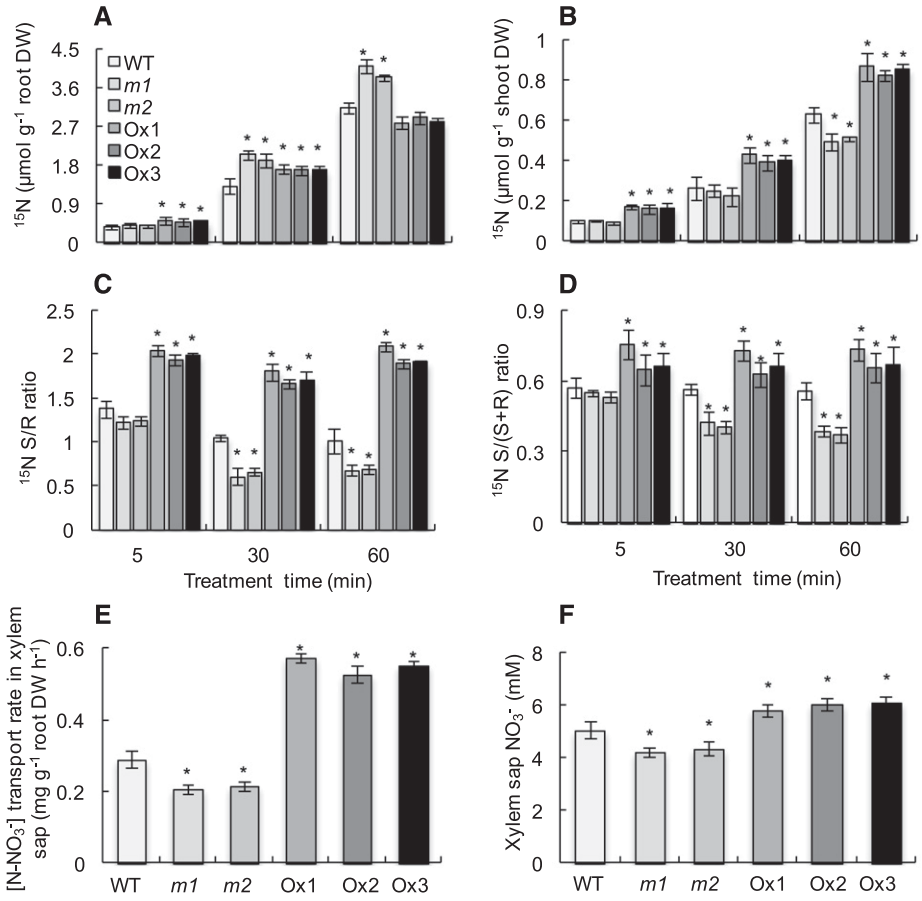

Figure 2. ^{15}N content, ^{15}N distribution ratio, and xylem sap NO_3^- in the wild type (WT), mutants (*m1* and *m2*), and overexpression lines (Ox1–Ox3). Rice seedlings were grown in IRRI nutrient solution containing 0.2 mM NO_3^- for 2 weeks and then transferred to IRRI nutrient solution containing 0.2 mM $[^{15}N]NO_3^-$ for 5, 30, and 60 min. A and B, ^{15}N content in rice roots (A) and shoots (B). C, Shoot-root ratio of ^{15}N content. D, Shoot-(shoot+root) ratio of ^{15}N accumulation. E and F, $[^{15}N]NO_3^-$ transport rate and concentration in xylem sap of rice plants. Rice seedlings were grown in IRRI solution containing 0.2 mM NO_3^- for 4 weeks and then were cut 4 cm above the roots for a 1-h xylem sap collection. Data are means of five replications ± SE. *, $P < 0.05$ (Student's *t* test) comparing the wild type and other lines at the same experimental period. DW, Dry weight.

the three overexpression lines. These results illustrated that OsMADS57 might participate in nitrate transport from roots to shoots.

Five-minute influx experiments of ^{15}N-labeled NO_3^- indicated that NO_3^- influx rates in *OsMADS57* overexpression lines were increased by approximately 80%, whereas no significant difference was recorded between *osmads57* mutant lines and wild-type plants (Fig. 3A). Net NO_3^- fluxes in seminal root tips, using the high-resolution scanning ion-selective electrode technique (SIET), further supported the above results (Fig. 3, B and C). During the entire 11-min experiment, a 38% increase in net nitrate influx was recorded in the overexpression lines in comparison with wild-type plants (Fig. 3C). Thus, higher influx rates by the Ox lines could explain why there is more ^{15}N in the roots of Ox lines.

Root Expression Profiles of Nitrate Reductase and *OsNRT2s* in the Wild Type, *osmads57* Mutants, and Overexpression Lines

As *OsNRT2s*, encoding high-affinity nitrate transporters, play a critical role in nitrate uptake and translocation, we performed time-course expression analyses in rice plants supplied with 0.2 mM NO_3^-. Compared with the wild type, *OsNRT2.3a* transcripts were significantly decreased in the *osmads57* mutants throughout the entire 60-min experimental period (Fig. 4). Decreases in transcript levels were also found for three additional *OsNRT2* genes (*OsNRT2.1*/*OsNRT2.2*/*OsNRT2.4*) in the mutants; however, the time course was delayed: at 5 min, no difference was found between the wild type and mutants (Fig. 4A), but at 30 and 60 min, transcript levels were significantly decreased in the mutants. We also analyzed the expression of *OsNAR2.1*, which interacts with *OsNRT2.1*/*OsNRT2.2*/*OsNRT2.3a* to produce functional transporters (Xu et al., 2012; Liu et al., 2014), and nitrate reductase (*OsNia1*/*2*). The expression of *OsNAR2.1* and *OsNia1*/*2* in roots was not affected in the *osmads57* mutants, but overexpression of *OsMADS57* significantly up-regulated all tested genes (Fig. 4). Because *OsNRT2.3b* was mainly expressed in shoots, we did not detect its expression in rice roots.

OsMADS57 Directly Binds to the *OsNRT2.3a* Promoter

The MADS-box transcription factors can recognize the CArG motif present in the promoters of target genes (Guo et al., 2013). To reveal if OsMADS57 is capable of directly binding to the promoter sequences of *OsNRT2.3a*, we first examined the distribution of CArG elements in the promoter of *OsNRT2.3a*. Two putative

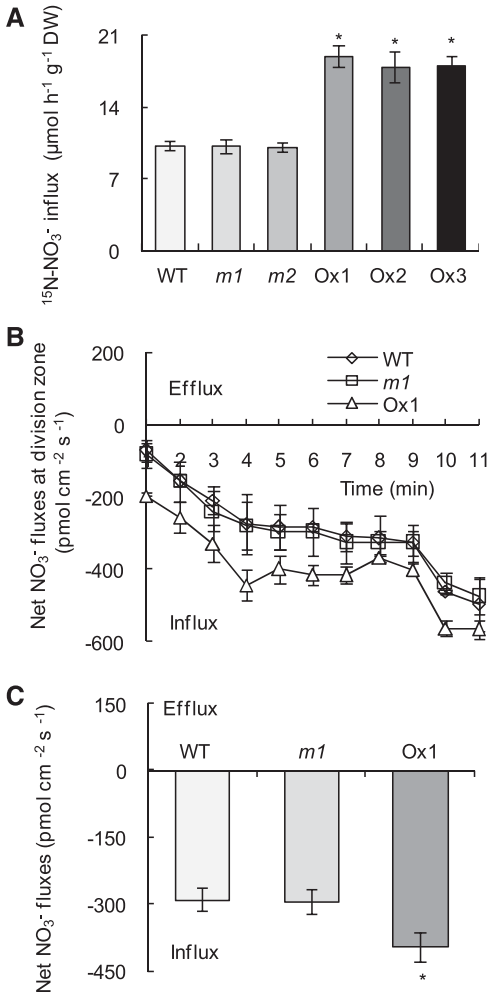

Figure 3. NO_3^- acquisition in the wild type (WT), mutants (*m1* and *m2*), and overexpression lines (Ox1–Ox3). Rice seedlings were grown in IRRI nutrient solution containing 0.2 mM NO_3^- for 2 weeks and then deprived of N for 3 d. The plants were transferred to IRRI nutrient solution containing 0.2 mM [^{15}N]NO_3^- for 5 min. A, Nitrate influx rates. B, Net NO_3^- fluxes in the seminal root meristem of rice plants supplied with 0.2 mM NO_3^- for 11 min. C, Mean rate of NO_3^- fluxes during the entire 11 min. Data are means of five replications ± SE. *, $P < 0.05$ (Student's *t* test) comparing the wild type and other lines. DW, Dry weight.

OsMADS57-binding CArG-box sites, site 1 (CATTTTATAG) and site 2 (CTATTATATG), were found at −2,255 to −2,246 bp and −568 to −559 bp upstream of the translation start site ATG of *OsNRT2.3a*, respectively (Fig. 5A). Thus, we speculated that OsMADS57 may directly bind to the two CArG boxes in the promoter of *OsNRT2.3a*. To verify this hypothesis, we first performed a yeast one-hybrid assay using a full-length coding sequence of *OsMADS57*. One region about 30 bp in length containing the two tandem CArG boxes (P2.3a), an independent mutation of two CArG-box sites in tandem (mP2.3a-1 and mP2.3a-2), and another region about 50 bp in length containing three tandem target DNA sequences of interest designated as P53 were used as the baits for binding assays in the yeast one-hybrid system (Fig. 5B). The interactions between OsMADS57 and the three promoter fragments were tested by growth on medium lacking Ura and Leu. Increasing concentrations of aureobasidin A (AbA) were added to the medium to suppress background activation and assess the strength of the interaction. When the AbA concentration was increased up to 150 nM, the GoldY1H yeast stain containing the P2.3a-AbAi bait and the pGADT7-OsMADS57 prey grew normally on the selective medium, as did the positive control (containing P53-AbAi bait and pGADT7-P53; Fig. 5B), while the construct containing the mP2.3a-1-AbAi bait almost did not grow, similar to the negative control (containing P2.3a-AbAi bait and pGADT7-GFP). However, another mutation of the CArG box (mP2.3a-2) did not affect growth, suggesting that OsMADS57 binds to the CArG box in site 1 rather than in site 2 of the *OsNRT2.3a* promoter.

To confirm the role of OsMADS57 in the regulation of *OsNRT2.3a* expression, we performed transient GUS assays in *Nicotiana benthamiana* as reported by Shim et al. (2013). OsMADS57, with the CaMV35S promoter, was used as the effector. One region about 30 bp in length containing the two tandem CArG boxes (P2.3a) or their mutated versions (mP2.3a-1 and mP2.3a-2) fused to the GUS gene were used as reporters (Fig. 5, A and C). The reporter and effector plasmids were coinfiltrated into *N. benthamiana* leaves. The GUS reporter gene was activated by coexpressing OsMADS57 with the wild-type promoter P2.3a. However, the mutant reporter containing mP2.3a-1 was not activated but mP2.3a-2 was activated (Fig. 5, D and E). These results demonstrate that OsMADS57 can activate promoter transcription by interacting with the CArG box in site 1 rather than in site 2.

Furthermore, we analyzed time-course ^{15}N translocation and the transcript levels of two high-affinity nitrate transporters (*NRT2.1/NRT2.2*) in the two *osnrt2.3a* mutants under IRRI nutrient solution containing 0.2 mM NO_3^- (Supplemental Fig. S3). The two *osnrt2.3a* mutant lines showed substantially higher ^{15}N accumulation in roots at 30 and 60 min and lower shoot ^{15}N accumulation at 60 min (Supplemental Fig. S3, A and B), which was consistent with the phenotype of the *osmads57* mutants. Furthermore, the relative expression of *OsNRT2.1* and *OsNRT2.2* in the *osnrt2.3a* mutants was similar to that of the *osmads57* mutants (Supplemental Fig. S3, C–E).

Knockdown of OsMADS57 Inhibited Elongation of Seminal and Adventitious Roots under Low Nitrate

Because root growth is significantly affected in the *osmads57* mutants supplied with low nitrate (Supplemental Fig. S2), root architecture was further analyzed in the wild type, mutants, and overexpression lines (Supplemental Fig. S4). Compared with the wild-type plants, total root length was markedly reduced in the mutant plants but increased in the overexpression plants. Interestingly, decreased total root length in the *osmads57* mutants was mainly attributed to shorter seminal and adventitious roots rather than to changes

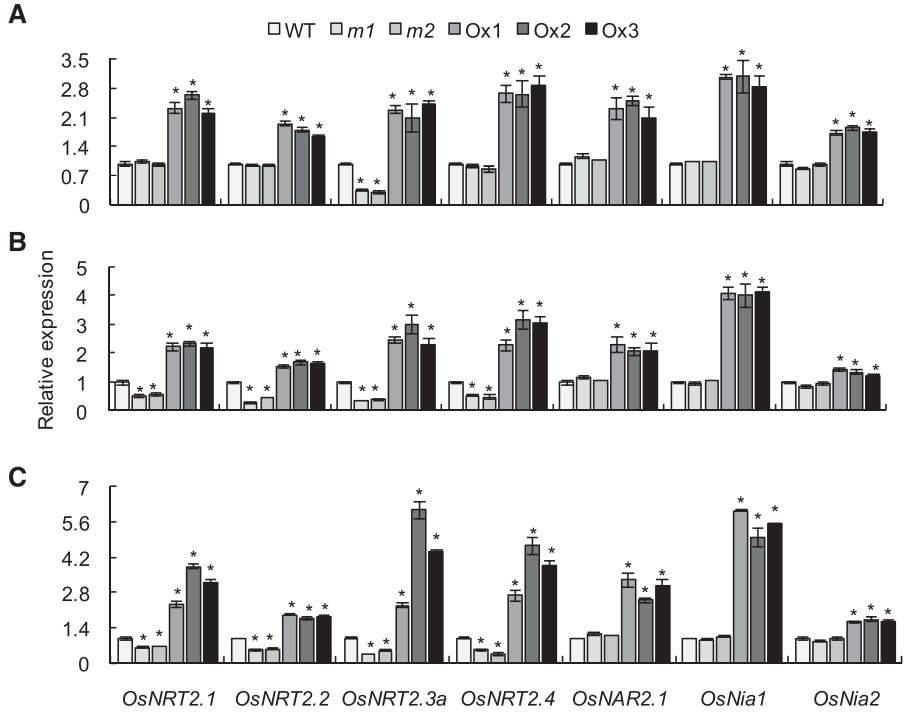

Figure 4. RT-qPCR analysis of root mRNA expression patterns of nitrate transporter genes (*NRT2s*) and nitrate reductase genes (*Nia1-2*) in the wild type (WT), mutants (*m1* and *m2*), and overexpression lines (Ox1–Ox3). Rice seedlings were grown in IRRI nutrient solution containing 0.2 mM NO_3^- for 2 weeks and then deprived of N for 3 d. The plants were transferred to IRRI nutrient solution containing 0.2 mM NO_3^- for 5 min (A), 30 min (B), and 60 min (C) before sampling. Relative mRNA levels for individual genes were normalized relative to *OsActin*. Data are means of five replications ± SE. *, $P < 0.05$ (Student's *t* test) comparing the wild type and other lines at the same gene.

in lateral root lengths. The time-course measurement of seminal root growth is shown in Figure 6A. Treatment of 0.2 mM NO_3^- for 14 d resulted in 20% to 40% shorter seminal roots in the mutant plants compared with the wild-type and overexpression line plants.

Root elongation is determined by two successive processes: cell division in the root apical meristem and elongation of cells outside the root meristem (Beemster et al., 2003). To investigate defects in root elongation at the cellular level, we observed and measured cortical cell length in the maturity zone and cell proliferation in the meristem zone. *osmads57* mutants had little change in the length of cortical cells in the maturity zone (Fig. 6, B and C). The expression of *CYCB1;1*, responsible for meristematic activity of cells, was markedly weaker in *osmads57* mutant plants, whereas in the overexpression lines no difference was observed compared with that in wild-type plants (Fig. 6D). Furthermore, cell numbers in the meristem zone had a similar pattern to that of *CYCB1;1* expression (Fig. 6E), suggesting that *osmads57* mutations inhibit cell division in the root meristem. Consistently, in the transgenic plant *CYCB1;1*::*GUS*, an *osmads57* mutation repressed the GUS activity compared with the wild-type plants (Fig. 6F). Taken together, these data suggest that *osmads57* mutations inhibit seminal root elongation under the condition of 0.2 mM NO_3^- by repressing meristematic cell proliferation.

Induced Auxin Distribution Was Observed in Seminal Root Tips of *osmads57* Mutants

Auxin plays an important role in the regulation of root system architecture and is required for normal cell proliferation and elongation (Grieneisen et al., 2007). Given the root growth defects of the *osmads57* mutants, we hypothesized that auxin may participate in modulating root growth in these mutants. Visualization of the auxin-responsive DR5 promoter (Ulmasov et al., 1997) reporter suggests that auxin levels or responses are elevated in the root tip (0–0.5 cm) of *osmads57* mutant plants and are slightly reduced in the overexpression lines compared with wild-type seedlings (Fig. 7A). Exogenous application of NAA increased *pDR5*::*GFP* expression throughout the roots and decreased seminal root length of wild-type and overexpression plants to a similar level to that in the *osmads57* mutant line (Fig. 7, A and B). These results imply that higher auxin levels in the *osmads57* mutants may play an important role in the inhibition of seminal root elongation. Next, endogenous indole-3-acetic acid (IAA) contents in the root tip and lateral root area were analyzed (Fig. 7C). IAA contents in the root tips (0–0.5 cm) of the osmads57 mutants were elevated by approximately 63% compared with the wild type and overexpression lines; no differences were found in lateral root area (2–8 cm) among the rice plants (Fig. 7C). Next, we performed a [^{3}H]IAA transport assay to investigate whether the auxin distribution in root tips is attributed to auxin polar transport. A significant increase in rootward [^{3}H] IAA transport was observed in root tips of the *osmads57* mutant in comparison with the wild-type and overexpression plants; no difference was observed in shootward transport from the root apex among the rice plants (Fig. 7D). These results indicated that auxin polar transport toward and within the root was induced in *osmads57* mutant plants supplied with 0.2 mM NO_3^-.

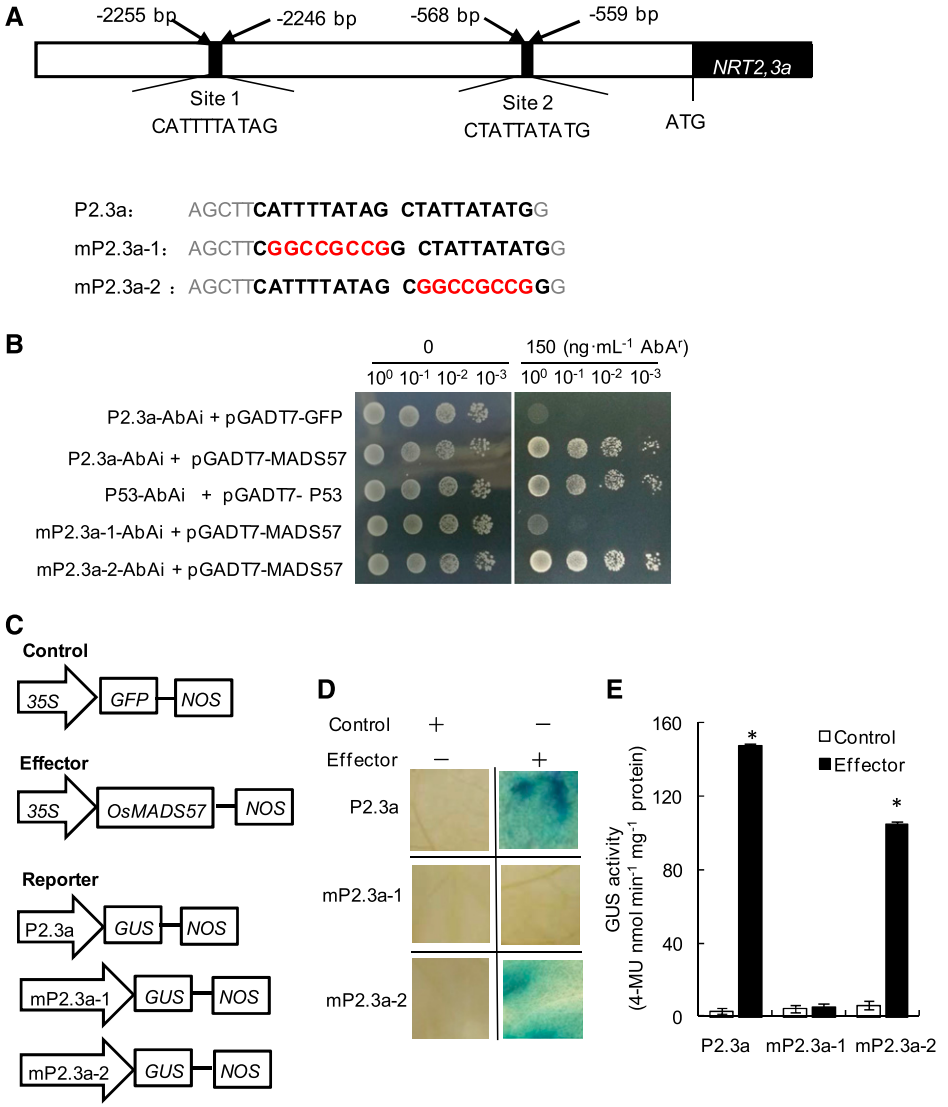

Figure 5. OsMADS57 binds to the *OsNRT2.3a* promoter. A, Two CArG boxes (sites 1 and 2) in the *OsNRT2.3a* promoter. Letters in boldface indicate a tandem repeat of two potential CArG boxes in the promoter of *OsNRT2.3a* (P2.3a), and letters in red are the mutations of site 1 (mP2.3a-1) and site 2 (mP2.3a-2). B, Yeast cells were cotransformed with a bait vector containing a promoter fragment in A fused to an AbAi reporter gene and a prey vector containing MADS57 fused to a GAL4 activation domain. Cells were grown in liquid medium to an OD_{600} of 1 diluted to 0.1 and diluted in a 10× dilution series (from 10^{-1} to 10^{-3}). From each dilution, 6 μL was spotted on medium selecting for both plasmids and selecting for interaction (synthetic dropout, –Ura, –Leu), supplemented with 150 nM AbA to suppress background growth and to test the strength of the interaction. C, Schematic diagram of the effector and reporter used for transactivation studies. The plasmid *35S::OsMADS57* was used as the effector, the plasmid *P2.3a::GUS* and its mutant versions *mP2.3a-1::GUS* and *mP2.3a-2::GUS* were used as the reporter, and *35S::GFP* was used as an internal control. D and E, Transactivation activity was detected by GUS staining (D) and quantitative analysis of the GUS activity (E) after reporter and effector plasmids were coinfiltrated into *N. benthamiana*. Data in E are means of five replications ± SE. *, $P < 0.05$ (Student's *t* test) compared with the control. 4-MU, 4-Methylumbelliferone.

Auxin transport mostly occurs via the polar transport stream, which is facilitated by proteins of the PIN family (Friml et al., 2003). Our RT-qPCR analyses showed that the expression levels of seven *PIN* genes (*PIN1a–PIN1d*, *PIN2*, and *PIN5a* and *PIN5b*) were significantly increased in *osmads57* mutant plants, whereas we observed decreased expression of *PIN10b* in the *osmads57* mutants and *PIN5b*, *PIN10a*, and *PIN10b* in the overexpression lines in comparison with wild-type rice seedlings (Fig. 7E).

DISCUSSION

Plant roots take up nitrate from the external medium and transport a large portion of nitrate from roots to shoots (Kronzucker et al., 2000; Kirk and Kronzucker, 2005). A number of nitrate transporters have been functionally characterized for their specific functions in the acquisition and distribution of nitrate in plants (Huang et al., 1999; Liu and Tsay, 2003; Chiu et al., 2004; Almagro et al., 2008; Lin et al., 2008; Fan et al., 2009, 2016; Wang et al., 2009, 2018a; Li et al., 2010a, 2015; Wang and Tsay, 2011; Tang et al., 2012; Xu et al., 2012; Hu et al., 2015, 2016; Xia et al., 2015; Xuan et al., 2017; Plett et al., 2018). Improvements of nitrate uptake and transport in crops through manipulation of nitrate transporters has recently been successful (Hu et al., 2015; Fan et al., 2016; Wang et al., 2018a); however, it stands to reason that further improvements will require more complete knowledge of the regulatory system to maximize N uptake and utilization. Although increasing evidence suggests that N uptake involves complex gene regulatory networks, and nitrate-dependent signaling pathways have been intensively investigated in Arabidopsis (Xuan et al., 2017; Plett et al., 2018; Wang et al., 2018b), it remains unclear which gene is involved in modulating nitrate uptake and transport in rice. In this study, we demonstrated that OsMADS57, a rice MADS-box transcription factor whose expression is

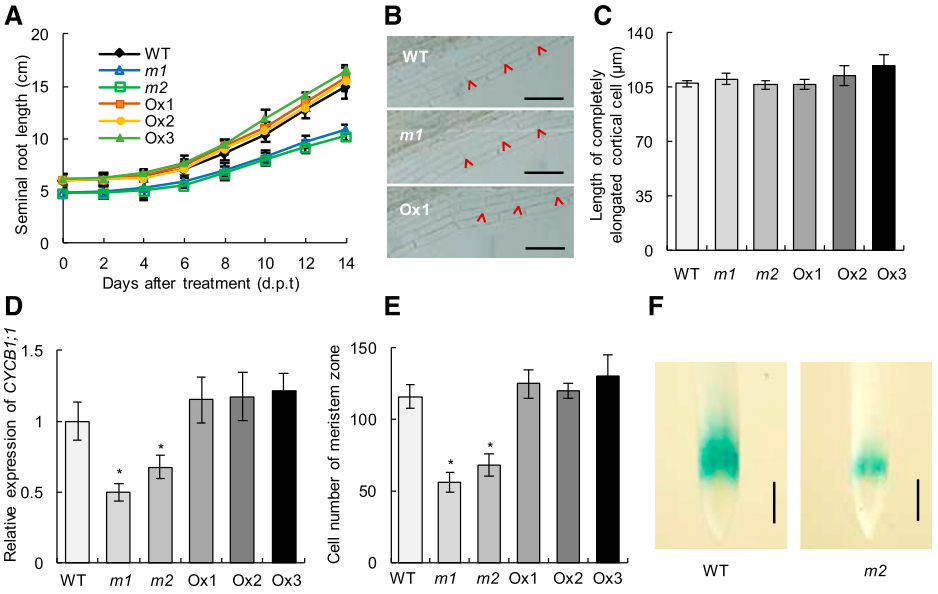

Figure 6. Seminal root elongation of the wild type (WT), mutants (*m1* and *m2*), and overexpression lines (Ox1–Ox3). Rice seedlings were grown in IRRI nutrient solution containing 0.2 mM NO_3^- for 14 d. A, Seminal root elongation. B, Photographs of fully elongated cells in the seminal root (red arrowheads; $n = 15$) at 10 d post treatment. Bars = 100 μm. C, Cell length of fully elongated cells. Measurements were obtained directly by measuring 10 cortical cells from 10 plants. D, *CYCB1;1* expression in roots at 10 d post treatment. E, Cell numbers of the meristem zone in the seminal root. F, Meristem size and root cell cycle activity of wild-type and *m2* plants were measured after treatments for 10 d, as monitored by the *proCycB1;1*::*GUS* reporter. Bars = 500 μm. Data in A and C to E are means of five replications ± SE. *, $P < 0.05$ (Student's *t* test) comparing the wild type and other lines.

induced by nitrate, mediates the transport of root-acquired nitrate to the shoots through modulating the expression of *OsNRT2.3a*.

OsMADS57 Is Involved in Nitrate Transport through Regulating *OsNRT2.3a* Expression

Our data indicate that OsMADS57 functions as a transcriptional regulator of *OsNRT2.3a* to modulate rice nitrate translocation from roots to shoots and are supported by the following evidence: (1) the expression of *OsMADS57* and *OsNRT2.3a* under nitrate treatments was significantly higher than under ammonium treatments in roots; (2) *OsMADS57* and *OsNRT2.3a* expression overlapped spatially; (3) *osmads57* mutants showed a similar phenotype to the *osnrt2.3a* mutant by RNA interference, such as long-distance nitrate translocation but not root influx at low nitrate supply; and (4) OsMADS57 bound to the cis-element in the *OsNRT2.3a* promoter.

The expression of *OsMADS57* can be induced by nitrate as previously reported (Puig et al., 2013; Yu et al., 2014a), suggesting a specific role in the nitrate response. Staining of transgenic GUS plants indicated that *OsMADS57* is preferentially expressed in the stele of rice roots, and the expression increased after nitrate treatments (Fig. 1, A and D). Feng et al. (2011) reported that the expression of *OsNRT2.3a* is significantly induced by nitrate rather than ammonium. Tang et al. (2012) further confirmed that *OsNRT2.3a* is mainly expressed in xylem parenchyma cells in the stele of nitrate-supplied roots. These results indicate that the two proteins may have overlapping localization, mainly in the central cylinder of the root.

Phenotype analyses further showed that *osmads57* mutants exhibited a smaller phenotype under a low-nitrate condition compared with wild-type plants, which is similar to that of *osnrt2.3a*-RNA interference plants (Tang et al., 2012). When plants were exposed to relatively long-term supplies of low nitrate, the two independent *osmads57* mutants accumulated much higher nitrate in roots than wild-type plants (Supplemental Fig. S2E). Analyses of NO_3^- transport rates in xylem sap and the distribution of ^{15}N in the roots and shoots showed a strong decrease in nitrate translocation from roots to shoots in the mutants under low nitrate supply (Fig. 2). Short-term [^{15}N]NO_3^- influx analyses of both whole plants and the root meristem zone showed that NO_3^- influx rates in *OsMADS57* overexpression lines were significantly increased, whereas no significant difference was recorded in *osmads57* mutants compared with wild-type plants. Higher [^{15}N]NO_3^- influx in overexpression lines may be attributed to negative feedback signals from long-distance transport and/or root storage pools at low external supply (Fig. 3). These results demonstrate that OsMADS57 has a major role in nitrate translocation from roots to shoots. Furthermore, we also analyzed time-course [^{15}N]NO_3^- translocation in *osnrt2.3a* mutants and observed higher ^{15}N accumulation in roots at 30 and 60 min and lower shoot ^{15}N

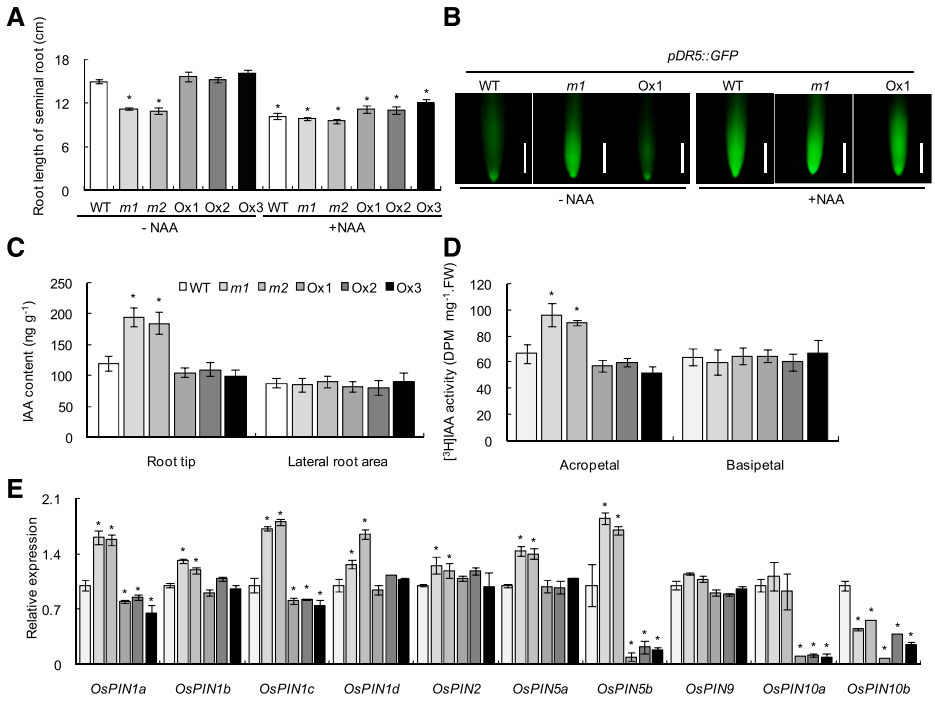

Figure 7. Auxin distribution in root tips of the wild type (WT), mutants (*m1* and *m2*), and overexpression lines (Ox1–Ox3). Seedlings were grown in nutrient solution containing 0.2 mM NO_3^- with or without 10 nM NAA application. A, Seminal root length. Plants were grown hydroponically for 14 d in nutrient solution containing 0.2 mM NO_3^- with/without 10 nM NAA application. B, *pDR5*::*GFP* ($n = 30$) expression in seminal root tips. Seedlings were grown for 7 d in nutrient solution containing 0.2 mM NO_3^- with or without 10 nM NAA application in agar medium. Bars = 1 mm. C to E, Plants were grown hydroponically for 14 d in nutrient solution containing 0.2 mM NO_3^-. C, IAA content in root tips of 0 to 0.5 cm and lateral root area of 2 to 8 cm adventitious root from the root tip ($n = 7$). D, [^{3}H]IAA transport ($n = 7$). E, Relative expression levels of *OsPINs*. Relative mRNA levels for individual genes were normalized relative to *OsActin* ($n = 3$). Data in A and C to E are means ± SE. *, $P < 0.05$ (Student's *t* test) comparing the wild type and other lines. FW, Fresh weight.

accumulation at 60 min (Supplemental Fig. S3, A and B), which was consistent with the phenotype of the *osmads57* mutants.

Interestingly, results from time-course expression analyses showed that, compared with the wild type, *OsNRT2.3a* transcripts were significantly decreased in the *osmads57* mutants throughout the entire 60-min experimental period (Fig. 4); however, the time course was delayed for three additional *OsNRT2* genes (*OsNRT2.1*/*OsNRT2.2*/*OsNRT2.4*) in the mutants: at 5 min supplied by NO_3^-, no difference was found between the wild type and the mutants (Fig. 4A), but at 30 and 60 min, transcript levels were significantly decreased (Fig. 4, B and C). Furthermore, we also performed time-course expression analyses in *osnrt2.3a* mutants. The relative expression of *OsNRT2.1* and *OsNRT2.2* in the *osnrt2.3a* mutants was similar to that of the *osmads57* mutants (Supplemental Fig. S3, C–E). Since *OsNRT2.3a* has been reported to mediate nitrate root-to-shoot translocation at low nitrate concentrations, these further suggest that decreased nitrate translocation from roots to shoots in *mads57* mutants may be due to the decreased expression level of *OsNRT2.3a*. Both the yeast one-hybrid assay and transient expression in *N. benthamiana* suggest that OsMADS57 directly binds to the first specific CArG box in the *OsNRT2.3a* promoter (Fig. 5), confirming that OsMADS57 may be a direct regulator of *OsNRT2.3a*.

Reduced Seminal Root Elongation in the *osmads57* Mutants Might Be Associated with the Increasing Auxin Accumulation in the Root Tip

The MADS-box gene family has been reported to modulate plant root development. In Arabidopsis, *AGL17*/*AGL21* and *ANR1* belong to the *AGL17* clade, which is preferentially expressed in roots (Zhang and Forde, 1998; Gan et al., 2005, 2012; Han et al., 2008; Yu et al., 2014b). Among them, AGL21 has been reported to play a crucial role in lateral root formation and elongation in response to N signals (Yu et al., 2014b), and ANR1 is involved in nitrate-stimulated lateral root development (Zhang and Forde, 1998; Gan et al., 2012). In miR444a-overexpressing rice lines, expression of the target *ANR1*-like genes was down-regulated and lateral root elongation was less responsive to localized nitrate (Yan et al., 2014). And *OsMADS25* has been reported to regulate root development and nitrate accumulation in roots (Yu et al., 2015). These results indicated that

ANR1-like genes might have a similar role in regulating root development, especially lateral root growth, in response to N in Arabidopsis and rice, despite the evolutionary distance between the two species.

Guo et al. (2013) reported that OsMADS57 interacts with OsTB1 to modulate rice tillering via *D14*. Our results showed that *OsMADS57* is exclusively expressed in the xylem parenchyma cells of roots and is significantly induced by nitrate and NAA (Fig. 1), consistent with previous studies (Puig et al., 2013; Yu et al., 2014a), suggesting its potential role in root development in response to external signals. Under the sufficient-nitrate condition, similar root growth and N content were recorded among wild-type plants, *osmads57* mutants, and overexpression lines (Supplemental Fig. S2). Interestingly, under the low-nitrate condition, the mutation of *OsMADS57* results in a decrease of seminal and adventitious root elongation through reducing cell numbers in the meristem zone and the expression level of *CYCB1;1*, but it did not affect lateral root growth (Fig. 6; Supplemental Fig. S4). Although mutants and overexpression plants showed opposite phenotypes in both nitrate translocation (Fig. 2) and transcriptional regulation (Fig. 4), the elongation of seminal and adventitious roots was only affected in mutant plants but not in overexpression plants relative to wild-type plants. Possibly, the increased nitrate uptake and translocation in the overexpression plants can maintain root growth under low-nitrate conditions, but the reduced nitrate uptake and translocation in the mutant plants may result in N deficiency and root growth inhibition. Furthermore, the smaller root diameter in the overexpression lines might result in less dry weight in comparison with wild-type plants (Supplemental Fig. S5).

Plants adjust root growth and development in response to changing environmental conditions through the perception and integration of external signals into the signaling pathways of plant hormones, such as auxin (López-Bucio et al., 2003; Malamy, 2005; Rubio et al., 2009; Krouk et al., 2011; Kazan, 2013; Krouk, 2016). In rice, auxin plays dominant roles in regulating root growth, such as seminal and adventitious root elongation responding to fluctuating N supplies (Sun et al., 2014). This study found that the regulatory role played by auxin in root development of the *osmads57* mutants under low-nitrate conditions likely occurs via modulation of auxin accumulation in the root tip. In our study, under the sufficient-nitrate condition, similar auxin content was found among wild-type plants, *osmads57* mutants, and overexpression lines (Supplemental Fig. S6). Under the 0.2 mM nitrate condition, compared with wild-type plants, (1) elevated IAA content in the mutant root tips and (2) higher activities of [^{3}H]IAA transport from shoots to roots in the mutants (in comparison with wild-type plants) suggested that auxin polar transport was promoted in the *osmads57* mutants. These results were correlated with an increase in the expression of seven *PIN* genes in mutant roots (relative to wild-type plants). Exogenous NAA application restored seminal root elongation and *DR5*::*GFP* expression levels in seminal root tips of wild-type plants to levels similar to those in the *osmads57* mutants. Taken together, these results indicate that more auxin accumulation repressed the cell proliferation activity in the root tip of the *osmads57* mutants.

CONCLUSION

In conclusion, we found that the transcription factor OsMADS57 may be a positive regulator of high-affinity *OsNRT2.3a* expression involved in nitrate translocation from roots to shoots. Moreover, inhibition of seminal root elongation in *osmads57* mutants may be associated with more auxin accumulation in the root tips, thus repressing the cell proliferation activity.

MATERIALS AND METHODS

Vector Construction and Rice Transformation

For the overexpression construct, the full-length complementary DNA of *OsMADS57* was amplified with the primer set MADS57OxF and MADS57OxR (Supplemental Table S1) and then was inserted into the *Bam*HI and *Sac*I sites in the pTCK303 vector. For the fusion construct of the *OsMADS57* promoter and the GUS coding sequence (*pOsMADS57*-GUS), the immediate upstream region of the putative 2.95-kb promoter from the ATG start codon of *OsMADS57* was amplified from rice (*Oryza sativa* 'Dongjin') genomic DNA using the primers MADS57Gus-F and MADS57Gus-R (Supplemental Table S1) and inserted into the pS1aGUS-3 vector at the *Pac*I and *Asc*I sites. These constructs were introduced into *Agrobacterium tumefaciens* (strain EHA105) and transformed into callus derived from mature seeds of cv Dongjin. Selection of transgenic lines (T1 generation) was conducted in the presence of 50 mg L^{-1} hygromycin (Roche), and GUS staining analysis was performed as previously described (Feng et al., 2011).

Identification of *OsMADS57* T-DNA Insertion Mutants and Overexpression Lines

Two independent T-DNA insertion mutants in the *OsMADS57* locus, *m1* (PFG_3A-15619.R) and *m2* (PFG_3A-60459.L), were identified in the SIGnAL database and obtained (http://signal.salk.edu/cgi-bin/RiceGE). Genotyping of the *osmads57* segregating population was performed by PCR (Supplemental Fig. S1, A and B). The insertions were confirmed by qPCR using *OsMADS57*-specific primers (Supplemental Fig. S1C) and T-DNA border primers (LB and RB). The flanking sequences of the T-DNA insertion sites were sequenced by Genescript. The expression levels of *OsMADS57* in *osmads57* mutants were determined by RT-qPCR (Supplemental Fig. S1D). Transcripts of *Actin* were amplified as a control, using the primer set *Actin*-FP and *Actin*-RP. All primer sequences for the PCR and RT-qPCR experiments are listed in Supplemental Table S1. The independent overexpression transgenic lines with one copy, namely Ox-1, Ox-2, and Ox-3, were obtained by Southern-blot analysis (Jia et al., 2011). And the expression levels of *OsMADS57* were determined by RT-qPCR using the primers presented in Supplemental Table S1.

Plant Material and Growth Conditions

Two T-DNA insertion mutant lines (*m1* and *m2*) in the rice (ssp. *japonica*) cv Dongjin background were obtained from RiceGE, the Rice Functional Genomics Express Database. All transgenic lines were developed in the genetic background of cv Dongjin.

Plants were grown in a greenhouse under natural light at day/night temperatures of 30°C/18°C. Rice seeds of the wild type (cv Dongjin), mutants (homologies), and overexpression lines (T2 generation with one copy) were surface sterilized and germinated in one-half-strength Murashige and Skoog (Duchefa) standard medium. Seven-day-old seedlings of uniform size and vigor were selected and then transferred to a tank containing 8 L of IRRI

nutrient solution [(mM) 0.3 KH_2PO_4, 0.35 K_2SO_4, 1 $CaCl_2$, 1 $MgSO_4 \cdot 7H_2O$, 0.5 Na_2SiO_3 and (μM) 20 Fe-EDTA, 9 $MnCl_2$, 0.39 $(NH4)_6Mo_7O_4 \cdot 2H_2O$, 20 H_3BO_3, 0.77 $ZnSO_4$, 0.32 $CuSO_4$, pH 5.5]. The nutrient solution was replaced with fresh solution daily. Nitrate and ammonium were supplied in the nutrient medium as $Ca(NO_3)_2$ and $(NH_4)_2SO_4$. To exclude the potential effects of Ca^{2+} on the treatments, the solutions in the same experimental system were supplemented with Ca^{2+} to the same level as those under the higher NO_3^- conditions using $CaCl_2$. To inhibit nitrification, 7 μM dicyandiamide ($C_2H_4N_4$) was added to each tank to prevent ammonium oxidation.

For analysis of gene expression in response to N, osmotic stress, and auxin, 7-d-old rice seedlings were grown in IRRI solution supplied by N with/without exogenous PEG (10%, w/v), NaCl (100 mM), and NAA (10 nM) for 7 d. The roots were collected and frozen in liquid N.

Determination of Total N Content, $[^{15}N]NO_3^-$ Influx Rate, and ^{15}N Accumulation

Rice seedlings were grown in IRRI solution containing 0.2 or 5 mM NO_3^- for 2 weeks. At each harvest, rice roots and shoots were separated and washed with 0.1 mM $CaSO_4$ for 1 min, placed in an oven at 105°C for 30 min to inactivate the enzymes, and finally dried to a constant weight at 70°C. The dry weight was recorded. Total N content in plants was determined by the Kjeldahl method (Li et al., 2006).

The influx rate of $[^{15}N]NO_3^-$ and ^{15}N accumulation were assayed as previously described (Delhon et al., 1995). Rice seedlings were grown in IRRI nutrient solution containing 0.2 mM NO_3^- for 2 weeks and then deprived of N for 3 d. The plants were transferred first to 0.1 mM $CaSO_4$ for 1 min, then to a complete nutrient solution containing 0.2 mM $[^{15}N]NO_3^-$ (atom % ^{15}N: 99%) for 5, 30, and 60 min, and finally to 0.1 mM $CaSO_4$ for 1 min. After grinding in liquid N, one aliquot of powder was dried to a constant weight at 70°C. Approximately 6 mg of powder from each sample was analyzed using an Isotope Ratio Mass Spectrometer system (Thermo Fisher Scientific).

Xylem Sap Nitrate Content Analysis

Rice seedlings were grown in IRRI nutrient solution containing 0.2 mM NO_3^- for 4 weeks and then were cut 4 cm above the ground level, and the roots were immediately transferred to 0.2 mM NO_3^-. A preweighed absorbent cotton ball was attached to the cut surface and covered with plastic film for 1 h. Next, the collected xylem sap was squeezed from the cotton with a syringe, and the volume of the exudates was calculated from the increase in the weight of the cotton. Nitrate concentration was determined as previously described (Tang et al., 2012).

Measurement of the Net NO_3^- Flux Rate in Rice Plants with the SIET System

Rice seedlings were grown in the IRRI nutrient solution containing 0.2 mM NO_3^- for 2 weeks and then deprived of N for 3 d. Net NO_3^- flux in the root meristem zone was measured using the noninvasive SIET technique as described previously (Tang et al., 2012). The roots of seedlings were equilibrated in the measuring solution for 30 min. The equilibrated seedlings were then transferred to the measuring chamber filled with the solution containing 0.2 mM NO_3^-. Net NO_3^- fluxes were measured under the experimental conditions for 11 min to decrease the variability due to fluctuations. Prior to the flux measurements, the ion-selective electrodes were calibrated using NO_3^- concentrations of 0.05 and 0.5 mM. Each plant was measured once. The final flux values were means of more than five individual plants. The measuring solution was composed of 0.2 mM $CaCl_2$, 0.1 mM NaCl, 0.1 mM $MgSO_4$, and 0.3 mM MES (pH 6, adjusted with 1 M NaOH). The measurements were carried out using the SIET system BIO-003A (Younger USA Science and Technology).

Yeast One-Hybrid Assays

Yeast one-hybrid assays were used to check the binding of OsMADS57 to *OsNRT2.3a* using the Matchmaker Gold Yeast One-Hybrid Library Screening System kit and the Yeastmaker Transformation System 2 kit (Clontech) following the manufacturer's instructions. One region about 30 bp in length containing the two tandem CArG boxes in the *OsNRT2.3a* promoter or a mutation of the CArG boxes was inserted into the vector pAbA. The construct was linearized by *Bst*BI digestion and transformed into a Y1HGold strain to generate a Y1H bait strain. Complementary DNA encoding full-length *OsMADS57* was inserted into the pGADT7AD vector to generate pGADT7AD-*OsMADS57* using primers M57BamHIF and M57SacIR. The *OsMADS57* construct was transformed into the Y1H bait strain and selected on a synthetic dropout-Ura and -Leu plate containing AbA.

Transient Expression in *Nicotiana benthamiana* Leaves

Transient expression was analyzed according to the method of Yang et al. (2000). Four-week-old *N. benthamiana* plants were used for infiltration. The constructs were individually transformed into the *A. tumefaciens* strain EHA105. The *A. tumefaciens* cells were infiltrated onto the abaxial surface of *N. benthamiana* leaves using 2-mL needleless syringes. After infiltration, the *N. benthamiana* plants were grown in a greenhouse under dark conditions for 48 to 60 h.

Histochemical activity of GUS in transgenic plant materials and quantitative analysis of GUS activity in *N. benthamiana* leaves were detected according to the method of Jefferson et al. (1987).

Measurement of Root System Architecture and Histological Observation

The rice root system is composed of seminal and adventitious roots each bearing lateral roots. Rice seedlings were grown in the IRRI nutrient solution containing 0.2 mM NO_3^- for 2 weeks before sampling. The length of seminal and adventitious roots was measured using a ruler, and lateral root density was calculated by dividing the lateral root number by root length. Total root length and lateral root length were measured using the WinRhizo scanner-based image-analysis system (Regent Instruments).

To analyze the length of the cortical cells in the maturity zone and cell number in the meristem zone, the root tips of the seminal roots were treated for 1 h with 1.8 M KOH solution heated to 90°C to clear the tissues and then treated with 1% (v/v) HCl solution for 5 min (Sun et al., 2016). The cortical cells of the root tips were observed with a microscope using a color CCD camera (Olympus Optical). The length of mature cells was measured and cell numbers of the root meristem were determined according to Sun et al. (2016).

The *pCYCB1;1*::*GUS* Construct

The *pCYCB1;1*::*GUS* fusion construct was transformed into wild-type plants (cv Dongjin) and the *osmads57* mutant. The construct was kindly provided by Chuanzao Mao. The root tips were used in histochemical GUS staining analyses. The stained tissues were photographed using an Olympus SZX2-ILLK microscope with a color CCD camera (Olympus; Sun et al., 2016).

Determination of IAA

The content of IAA in the root tips and the lateral root area were determined (Song et al., 2011). Two root zones were sampled: the root tip (0–0.5 cm) and the lateral root zone where lateral root initiation, emergence, and elongation (2–8 cm) occurs. The fresh weight of the samples was measured, after which specimens were immediately frozen in liquid N. We performed sample measurement of free IAA by HPLC. A standard IAA sample was obtained from Sigma-Aldrich.

To detect IAA distribution patterns in plants, the *pDR5*::*GFP* fusion construct was transformed into wild-type plants, *m1* mutants, and Ox-1 lines. The *pDR5*::*GFP* vector was constructed from the *pDR5*::*GUS* construct provided by Chuanzao Mao and was described by Huang et al. (2015). We analyzed the fluorescence of GFP in the cells using 543-nm helium-neon and 488-nm argon lasers using a confocal laser scanning microscope (LSM410; Carl Zeiss).

$[^3H]$IAA Transport Assay

$[^3H]$IAA polar transport was assayed after the 0.2 mM NO_3^- treatment for 2 weeks. Ten replicate roots were sampled. The $[^3H]$IAA solution contained 0.5 mM $[^3H]$IAA (20 Ci $mmol^{-1}$) in 2% (v/v) dimethyl sulfoxide, 25 mM MES (pH 5.2), and 0.25% agar (Song et al., 2013).

Shoot-to-root auxin transport in intact plants was monitored as follows. $[^3H]$IAA solution (20 μL) was applied to the cut surface after rice shoots were removed at 2 cm above the root-shoot junction and then incubated in scintillation solution (4 mL) for 18 h. After an 18-h (overnight) incubation in darkness, rice roots were sampled and weighed. $[^3H]$IAA radioactivity was detected using a multipurpose scintillation counter (LS6500; Beckman-Coulter).

The assay for basipetal auxin transport (transport away from the root tip) was performed using 3-cm-long excised root tip segments. [^{3}H]IAA solution (3 μL) was applied to the root tip placed horizontally on a plastic film. After incubation in a humid, dark environment for 18 h (overnight), root segments were cut into two parts: (1) the distal end 1 cm from the root tip and (2) the remaining 2 cm. [^{3}H]IAA radioactivity was measured in the 2-cm-long segments.

RT-qPCR Analysis

Total RNA was isolated from the roots of rice seedlings. RNA extraction, RT, and qPCR procedures followed the reported procedure (Chen et al., 2012). The primer sets targeting nitrate-related genes and the *PIN* genes are listed in Supplemental Table S1.

Statistical Analysis

Data from the experiments were pooled for calculation of means and SE and analyzed by Student's *t* test at $P \leq 0.05$ to determine the statistical significance of the differences when comparing the wild type and other lines. All statistical evaluations were conducted using the SPSS (version 11.0) statistical software (SPSS).

Accession Numbers

The sequences of rice data in this article can be downloaded from https://blast.ncbi.nlm.nih.gov/Blast.cgi or http://rice.plantbiology.msu.edu: *OsMADS57* (AK108784), *OsNRT2.1* (AB008519), *OsNRT2.2* (AK109733), *OsNRT2.3a* (AK109776), *OsNRT2.4* (NM_193361), *OsNART2.1* (NM_001053852.2), *OsNiA1* (AK102178), *OsNiA2* (AK102363), *OsPIN1a* (AK103208), *OsPIN1b* (AK102343), *OsPIN1c* (AK103181), *OsPIN1d* (LOC_Os12g04000) *OsPIN2* (AK101191), *OsPIN5a* (AK066552), *OsPIN5b* (AK100297), *OsPIN9* (AK05922), *OsPIN10a* (LOC_Os01g45550), *OsPIN10b* (LOC_Os05g50140), and *OsACT* (AK100267).

Supplemental Data

The following supplemental materials are available.

Supplemental Figure S1. Identification of the *osmads57* mutants and overexpression lines.

Supplemental Figure S2. Comparison of growth and total N content of the wild type, *osmads57* mutants, and overexpression lines.

Supplemental Figure S3. ^{15}N content and root mRNA expression pattern of nitrate transporter genes in the wild type and *osnrt2.3a* mutants.

Supplemental Figure S4. Root systems of the wild type, *osmads57* mutants, and overexpression lines.

Supplemental Figure S5. Seminal root diameter of the wild type, *osmads57* mutant, and overexpression line.

Supplemental Figure S6. Auxin content in roots of the wild type, *osmads57* mutants, and overexpression lines.

Supplemental Table S1. Primer sequences used in this study.

ACKNOWLEDGMENTS

We thank Nigel Crawford (University of California, San Diego) for polishing the article and Chuanzao Mao (Zhejiang University) for providing the pCYCB1;1::GUS fusion construct.

Received February 4, 2019; accepted February 25, 2019; published March 18, 2019.

LITERATURE CITED

Almagro A, Lin SH, Tsay YF (2008) Characterization of the Arabidopsis nitrate transporter NRT1.6 reveals a role of nitrate in early embryo development. Plant Cell **20:** 3289–3299

Alvarez JM, Riveras E, Vidal EA, Gras DE, Contreras-López O, Tamayo KP, Aceituno F, Gómez I, Ruffel S, Lejay L, et al (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

Araki R, Hasegawa H (2006) Expression of rice (*Oryza sativa* L.) genes involved in high-affinity nitrate transport during the period of nitrate induction. Breed Sci **56:** 295–302

Araus V, Vidal EA, Puelma T, Alamos S, Mieulet D, Guiderdoni E, Gutiérrez RA (2016) Members of BTB gene family of scaffold proteins suppress nitrate uptake and nitrogen use efficiency. Plant Physiol **171:** 1523–1532

Beemster GTS, Fiorani F, Inzé D (2003) Cell cycle: The key to plant growth control? Trends Plant Sci **8:** 154–158

Cai C, Wang JY, Zhu YG, Shen QR, Li B, Tong YP, Li ZS (2008) Gene structure and expression of the high-affinity nitrate transport system in rice roots. J Integr Plant Biol **50:** 443–451

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

Chen Y, Fan X, Song W, Zhang Y, Xu G (2012) Over-expression of OsPIN2 leads to increased tiller numbers, angle and shorter plant height through suppression of OsLAZY1. Plant Biotechnol J **10:** 139–149

Chiu CC, Lin CS, Hsia AP, Su RC, Lin HL, Tsay YF (2004) Mutation of a nitrate transporter, AtNRT1:4, results in a reduced petiole nitrate content and altered leaf development. Plant Cell Physiol **45:** 1139–1148

Crawford NM, Glass ADM (1998) Molecular and physiological aspects of nitrate uptake in plants. Trends Plant Sci **3:** 389–395

Delhon P, Gojon A, Tillard P, Passama L (1995) Diurnal regulation of NO_3^- uptake in soybean plants. I. Changes in NO_3^- influx, efflux, and N utilization in the plant during the day/night cycle. J Exp Bot **46:** 1585–1594

Du XQ, Wang FL, Li H, Jing S, Yu M, Li J, Wu WH, Kudla J, Wang Y (2019) The transcription factor MYB59 regulates K+/NO_3^- translocation in the Arabidopsis response to low K+ stress. Plant Cell **31:** 699–714

Fan SC, Lin CS, Hsu PK, Lin SH, Tsay YF (2009) The Arabidopsis nitrate transporter NRT1.7, expressed in phloem, is responsible for source-to-sink remobilization of nitrate. Plant Cell **21:** 2750–2761

Fan X, Tang Z, Tan Y, Zhang Y, Luo B, Yang M, Lian X, Shen Q, Miller AJ, Xu G (2016) Overexpression of a pH-sensitive nitrate transporter in rice increases crop yields. Proc Natl Acad Sci USA **113:** 7118–7123

Feng H, Yan M, Fan X, Li B, Shen Q, Miller AJ, Xu G (2011) Spatial expression and regulation of rice high-affinity nitrate transporters by nitrogen and carbon status. J Exp Bot **62:** 2319–2332

Forde BG (2014) Nitrogen signalling pathways shaping root system architecture: An update. Curr Opin Plant Biol **21:** 30–36

Friml J, Vieten A, Sauer M, Weijers D, Schwarz H, Hamann T, Offringa R, Jürgens G (2003) Efflux-dependent auxin gradients establish the apical-basal axis of Arabidopsis. Nature **426:** 147–153

Gan Y, Filleur S, Rahman A, Gotensparre S, Forde BG (2005) Nutritional regulation of ANR1 and other root-expressed MADS-box genes in Arabidopsis thaliana. Planta **222:** 730–742

Gan Y, Bernreiter A, Filleur S, Abram B, Forde BG (2012) Overexpressing the ANR1 MADS-box gene in transgenic plants provides new insights into its role in the nitrate regulation of root development. Plant Cell Physiol **53:** 1003–1016

Gojon A, Krouk G, Perrine-Walker F, Laugier E (2011) Nitrate transceptor(s) in plants. J Exp Bot **62:** 2299–2308

Grieneisen VA, Xu J, Marée AF, Hogeweg P, Scheres B (2007) Auxin transport is sufficient to generate a maximum and gradient guiding root growth. Nature **449:** 1008–1013

Guan P, Ripoll JJ, Wang R, Vuong L, Bailey-Steinitz LJ, Ye D, Crawford NM (2017) Interacting TCP and NLP transcription factors control plant responses to nitrate availability. Proc Natl Acad Sci USA **114:** 2419–2424

Guo FQ, Wang R, Chen M, Crawford NM (2001) The Arabidopsis dual-affinity nitrate transporter gene AtNRT1.1 (CHL1) is activated and functions in nascent organ development during vegetative and reproductive growth. Plant Cell **13:** 1761–1777

Guo S, Xu Y, Liu H, Mao Z, Zhang C, Ma Y, Zhang Q, Meng Z, Chong K (2013) The interaction between OsMADS57 and OsTB1 modulates rice tillering via DWARF14. Nat Commun **4:** 1566

Han P, García-Ponce B, Fonseca-Salazar G, Alvarez-Buylla ER, Yu H (2008) AGAMOUS-LIKE 17, a novel flowering promoter, acts in a FT-independent photoperiod pathway. Plant J **55:** 253–265

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

Hsu PK, Tsay YF (2013) Two phloem nitrate transporters, NRT1.11 and NRT1.12, are important for redistributing xylem-borne nitrate to enhance plant growth. Plant Physiol **163:** 844–856

Hu B, Wang W, Ou S, Tang J, Li H, Che R, Zhang Z, Chai X, Wang H, Wang Y, et al (2015) Variation in NRT1.1B contributes to nitrate-use divergence between rice subspecies. Nat Genet **47:** 834–838

Hu R, Qiu D, Chen Y, Miller AJ, Fan X, Pan X, Zhang M (2016) Knockdown of a tonoplast localized low-affinity nitrate transporter *OsNPF7.2* affects rice growth under high nitrate supply. Front Plant Sci **7:** 1529

Huang NC, Liu KH, Lo HJ, Tsay YF (1999) Cloning and functional characterization of an Arabidopsis nitrate transporter gene that encodes a constitutive component of low-affinity uptake. Plant Cell **11:** 1381–1392

Huang S, Chen S, Liang Z, Zhang C, Yan M, Chen J, Xu G, Fan X, Zhang Y (2015) Knockdown of the partner protein OsNAR2.1 for high-affinity nitrate transport represses lateral root formation in a nitrate-dependent manner. Sci Rep **5:** 18192

Jefferson RA, Kavanagh TA, Bevan MW (1987) GUS fusions: Beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J **6:** 3901–3907

Jia H, Ren H, Gu M, Zhao J, Sun S, Zhang X, Chen J, Wu P, Xu G (2011) The phosphate transporter gene OsPht1;8 is involved in phosphate homeostasis in rice. Plant Physiol **156:** 1164–1175

Kant S (2018) Understanding nitrate uptake, signaling and remobilisation for improving plant nitrogen use efficiency. Semin Cell Dev Biol **74:** 89–96

Kazan K (2013) Auxin and the integration of environmental signals into plant root development. Ann Bot **112:** 1655–1665

Kirk GJD (2003) Rice root properties for internal aeration and efficient nutrient acquisition in submerged soil. New Phytol **159:** 185–194

Kirk GJ, Kronzucker HJ (2005) The potential for nitrification and nitrate uptake in the rhizosphere of wetland plants: A modelling study. Ann Bot **96:** 639–646

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

Kronzucker HJ, Glass ADM, Siddiqi MY, Kirk GJD (2000) Comparative kinetic analysis of ammonium and nitrate acquisition by tropical lowland rice: Implications for rice cultivation and yield potential. New Phytol **145:** 471–476

Krouk G (2016) Hormones and nitrate: A two-way connection. Plant Mol Biol **91:** 599–606

Krouk G, Lacombe B, Bielach A, Perrine-Walker F, Malinska K, Mounier E, Hoyerova K, Tillard P, Leon S, Ljung K, et al (2010a) Nitrate-regulated auxin transport by NRT1.1 defines a mechanism for nutrient sensing in plants. Dev Cell **18:** 927–937

Krouk G, Mirowski P, LeCun Y, Shasha DE, Coruzzi GM (2010b) Predictive network modeling of the high-resolution dynamic plant transcriptome in response to nitrate. Genome Biol **11:** R123

Krouk G, Ruffel S, Gutiérrez RA, Gojon A, Crawford NM, Coruzzi GM, Lacombe B (2011) A framework integrating plant growth with hormones and nutrients. Trends Plant Sci **16:** 178–182

Léran S, Varala K, Boyer JC, Chiurazzi M, Crawford N, Daniel-Vedele F, David L, Dickstein R, Fernandez E, Forde B, et al (2014) A unified nomenclature of NITRATE TRANSPORTER 1/PEPTIDE TRANSPORTER family members in plants. Trends Plant Sci **19:** 5–9

Li B, Xin W, Sun S, Shen Q, Xu G (2006) Physiological and molecular responses of nitrogen-starved rice plants to re-supply of different nitrogen sources. Plant Soil **287:** 145–159

Li H, Yu M, Du XQ, Wang ZF, Wu WH, Quintero FJ, Jin XH, Li HD, Wang Y (2017) NRT1.5/NPF7.3 functions as a proton-coupled H^+/K^+ antiporter for K^+ loading into the xylem in Arabidopsis. Plant Cell **29:** 2016–2026

Li JY, Fu YL, Pike SM, Bao J, Tian W, Zhang Y, Chen CZ, Zhang Y, Li HM, Huang J, et al (2010a) The Arabidopsis nitrate transporter NRT1.8 functions in nitrate removal from the xylem sap and mediates cadmium tolerance. Plant Cell **22:** 1633–1646

Li YL, Fan XR, Shen QR (2008) The relationship between rhizosphere nitrification and nitrogen-use efficiency in rice plants. Plant Cell Environ **31:** 73–85

Li YF, Zheng Y, Addo-Quaye C, Zhang L, Saini A, Jagadeeswaran G, Axtell MJ, Zhang W, Sunkar R (2010b) Transcriptome-wide identification of microRNA targets in rice. Plant J **62:** 742–759

Li Y, Ouyang J, Wang YY, Hu R, Xia K, Duan J, Wang Y, Tsay YF, Zhang M (2015) Disruption of the rice nitrate transporter OsNPF2.2 hinders root-to-shoot nitrate transport and vascular development. Sci Rep **5:** 9635

Lin SH, Kuo HF, Canivenc G, Lin CS, Lepetit M, Hsu PK, Tillard P, Lin HL, Wang YY, Tsai CB, et al (2008) Mutation of the Arabidopsis NRT1.5 nitrate transporter causes defective root-to-shoot nitrate transport. Plant Cell **20:** 2514–2528

Liu KH, Tsay YF (2003) Switching between the two action modes of the dual-affinity nitrate transporter CHL1 by phosphorylation. EMBO J **22:** 1005–1013

Liu X, Huang D, Tao J, Miller AJ, Fan X, Xu G (2014) Identification and functional assay of the interaction motifs in the partner protein OsNAR2.1 of the two-component system for high-affinity nitrate transport. New Phytol **204:** 74–80

López-Bucio J, Cruz-Ramírez A, Herrera-Estrella L (2003) The role of nutrient availability in regulating root architecture. Curr Opin Plant Biol **6:** 280–287

Malamy JE (2005) Intrinsic and environmental response pathways that regulate root system architecture. Plant Cell Environ **28:** 67–77

Marchive C, Roudier F, Castaings L, Bréhaut 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

Miller AJ, Fan X, Orsel M, Smith SJ, Wells DM (2007) Nitrate transport and signalling. J Exp Bot **58:** 2297–2306

O'Brien JA, Vega A, Bouguyon E, Krouk G, Gojon A, Coruzzi G, Gutiérrez RA (2016) Nitrate transport, sensing, and responses in plants. Mol Plant **9:** 837–856

Plett DC, Holtham LR, Okamoto M, Garnett TP (2018) Nitrate uptake and its regulation in relation to improving nitrogen use efficiency in cereals. Semin Cell Dev Biol **74:** 97–104

Puig J, Meynard D, Khong GN, Pauluzzi G, Guiderdoni E, Gantet P (2013) Analysis of the expression of the AGL17-like clade of MADS-box transcription factors in rice. Gene Expr Patterns **13:** 160–170

Remans T, Nacry P, Pervent M, Filleur S, Diatloff E, Mounier E, Tillard P, Forde BG, Gojon A (2006) The Arabidopsis NRT1.1 transporter participates in the signaling pathway triggering root colonization of nitrate-rich patches. Proc Natl Acad Sci USA **103:** 19206–19211

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

Rubio V, Bustos R, Irigoyen ML, Cardona-López X, Rojas-Triana M, Paz-Ares J (2009) Plant hormones and nutrient signaling. Plant Mol Biol **69:** 361–373

Shim JS, Jung C, Lee S, Min K, Lee YW, Choi Y, Lee JS, Song JT, Kim JK, Choi YD (2013) AtMYB44 regulates WRKY70 expression and modulates antagonistic interaction between salicylic acid and jasmonic acid signaling. Plant J **73:** 483–495

Smirnoff N, Stewart GR (1985) Nitrate assimilation and translocation by higher plants: Comparative physiology and ecological consequences. Physiol Plant **64:** 133–140

Song W, Makeen K, Wang D, Zhang C, Xu Y, Zhao H, Tu E, Zhang Y, Shen Q, Xu G (2011) Nitrate supply affects root growth differentially in two rice cultivars differing in nitrogen use efficiency. Plant Soil **343:** 357–368

Song W, Sun H, Li J, Gong X, Huang S, Zhu X, Zhang Y, Xu G (2013) Auxin distribution is differentially affected by nitrate in roots of two rice cultivars differing in responsiveness to nitrogen. Ann Bot **112:** 1383–1393

Sun H, Tao J, Liu S, Huang S, Chen S, Xie X, Yoneyama K, Zhang Y, Xu G (2014) Strigolactones are involved in phosphate- and nitrate-deficiency-induced root development and auxin transport in rice. J Exp Bot **65:** 6735–6746

Sun H, Bi Y, Tao J, Huang S, Hou M, Xue R, Liang Z, Gu P, Yoneyama K, Xie X, et al (2016) Strigolactones are required for nitric oxide to induce root elongation in response to nitrogen and phosphate deficiencies in rice. Plant Cell Environ **39:** 1473–1484

Tang Z, Fan X, Li Q, Feng H, Miller AJ, Shen Q, Xu G (2012) Knockdown of a rice stelar nitrate transporter alters long-distance translocation but not root influx. Plant Physiol **160:** 2052–2063

Tsay YF, Chiu CC, Tsai CB, Ho CH, Hsu PK (2007) Nitrate transporters and peptide transporters. FEBS Lett **581:** 2290–2300

Ulmasov T, Murfett J, Hagen G, Guilfoyle TJ (1997) Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. Plant Cell **9:** 1963–1971

Wang YY, Tsay YF (2011) Arabidopsis nitrate transporter NRT1.9 is important in phloem nitrate transport. Plant Cell **23:** 1945–1957

Wang R, Xing X, Wang Y, Tran A, Crawford NM (2009) A genetic screen for nitrate regulatory mutants captures the nitrate transporter gene NRT1.1. Plant Physiol **151:** 472–478

Wang W, Hu B, Yuan D, Liu Y, Che R, Hu Y, Ou S, Liu Y, Zhang Z, Wang H, et al (2018a) Expression of the nitrate transporter gene *OsNRT1.1A/OsNPF6.3* confers high yield and early maturation in rice. Plant Cell **30:** 638–651

Wang YY, Cheng YH, Chen KE, Tsay YF (2018b) Nitrate transport, signaling, and use efficiency. Annu Rev Plant Biol **69:** 85–122

Wei J, Zheng Y, Feng H, Qu H, Fan X, Yamaji N, Ma JF, Xu G (2018) OsNRT2.4 encodes a dual-affinity nitrate transporter and functions in nitrate-regulated root growth and nitrate distribution in rice. J Exp Bot **69:** 1095–1107

Xia X, Fan X, Wei J, Feng H, Qu H, Xie D, Miller AJ, Xu G (2015) Rice nitrate transporter OsNPF2.4 functions in low-affinity acquisition and long-distance transport. J Exp Bot **66:** 317–331

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

Xu N, Wang R, Zhao L, Zhang C, Li Z, Lei Z, Liu F, Guan P, Chu Z, Crawford NM, et al (2016) The Arabidopsis NRG2 protein mediates nitrate signaling and interacts with and regulates key nitrate regulators. Plant Cell **28:** 485–504

Xuan W, Beeckman T, Xu G (2017) Plant nitrogen nutrition: Sensing and signaling. Curr Opin Plant Biol **39:** 57–65

Yan D, Easwaran V, Chau V, Okamoto M, Ierullo M, Kimura M, Endo A, Yano R, Pasha A, Gong Y, et al (2016) NIN-like protein 8 is a master regulator of nitrate-promoted seed germination in Arabidopsis. Nat Commun **7:** 13179

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

Yan Y, Wang H, Hamera S, Chen X, Fang R (2014) miR444a has multiple functions in the rice nitrate-signaling pathway. Plant J **78:** 44–55

Yang W, Yoon J, Choi H, Fan Y, Chen R, An G (2015) Transcriptome analysis of nitrogen-starvation-responsive genes in rice. BMC Plant Biol **15:** 31

Yang Y, Li R, Qi M (2000) In vivo analysis of plant promoters and transcription factors by agroinfiltration of tobacco leaves. Plant J **22:** 543–551

Yu C, Su S, Xu Y, Zhao Y, Yan A, Huang L, Ali I, Gan Y (2014a) The effects of fluctuations in the nutrient supply on the expression of five members of the AGL17 clade of MADS-box genes in rice. PLoS ONE **9:** e105597

Yu C, Liu Y, Zhang A, Su S, Yan A, Huang L, Ali I, Liu Y, Forde BG, Gan Y (2015) MADS-box transcription factor OsMADS25 regulates root development through affection of nitrate accumulation in rice. PLoS ONE **10:** e0135196

Yu LH, Miao ZQ, Qi GF, Wu J, Cai XT, Mao JL, Xiang CB (2014b) MADS-box transcription factor AGL21 regulates lateral root development and responds to multiple external and physiological signals. Mol Plant **7:** 1653–1669

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