GAF domain is essential for nitrate-dependent AtNLP7 function

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Abstract

Nitrate is an essential nutrient and an important signaling molecule in plants. However, the molecular mechanisms by which plants perceive nitrate deficiency signaling are still not well understood. Here we report that AtNLP7 protein transport from the nucleus to the cytoplasm in response to nitrate deficiency is dependent on the N-terminal GAF domain. With the deletion of the GAF domain, AtNLP7ΔGAF always remains in the nucleus regardless of nitrate availability. AtNLP7 ΔGAF also shows reduced activation of nitrate-induced genes due to its impaired binding to the nitrate-responsive cis-element (NRE) as well as decreased growth like nlp7-1 mutant. In addition, AtNLP7ΔGAF is unable to mediate the reduction of reactive oxygen species (ROS) accumulation upon nitrate treatment. Our investigation shows that the GAF domain of AtNLP7 plays a critical role in the sensing of nitrate deficiency signal and in the nitrate-triggered ROS signaling process.

Keywords: AtNLP7, Nitrate deficiency signaling, Nuclear localization, GAF domain, ROS

Introduction

Nitrate is not only an essential mineral element for plant growth, but also a signal molecule involved in many important developmental processes. Plants have evolved sophisticated mechanisms to respond to nitrate triggering a cascade of consequent reactions [1–4]. Recent studies have shown that NIN-like proteins (NLPs) function as key transcription factors of primary nitrate responses [5–11]. Nitrate provision signaling is known to promote nuclear localization of Arabidopsis AtNLP7 through Ca2+ sensor CPKs [12]. Nevertheless, how nitrate deficiency signaling is relayed to AtNLP7 remains unclear.

Members of the NLP family all contain an amino-terminal GAF domain, an intermediate RWP-RK domain, and a carboxy-terminal Phox and Bem1 (PB1) domain [13]. Additionally, a nuclear export signal domain is predicted in the N-terminus [6, 14]. The RWP-RK domain is characterized by the conserved five-amino acid sequence Arg-Trp-Pro-X-Arg-Lys, which binds nitrate-responsive cis-elements (NREs) [15, 16]. The PB1 domain is mainly involved in the interaction between proteins [17], such as the interactions of NLP-NLP and NLP-TEOSINTE BRANCHED1/CYCLOIDEA/PROLIFERATING CELL FACTOR1-20 (TCP20) [18]. Furthermore, the PB1 domain of NLP transcription factors may mediate homo- and hetero-oligomerization, thereby regulating the expression of target genes in the presence of nitrate [19].

The GAF domain is widely present in different types of proteins and was named after cGMP-regulated phosphodiesterase, certain adenyl cyclases and FhlA were found to contain this domain [20]. Most GAF domains can bind multiple small molecular ligands and participate in various signal transduction pathways throughout the life

Footnotes:

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cycle [20–23], such as the GAF domain of NreA in *Staphylococcus* can form hydrophobic pocket that directly bind nitrate [20–23]. In higher plants, the proteins containing GAF domains are usually involved in light absorption and ethylene signal transduction [24, 25]. Whereas, the role of the GAF domain in NLPs remains unclear.

It is known that AtNLP7 responds to nitrate through a nuclear retention mechanism [6]. AtNLP7 is present in both the nucleus and cytoplasm under normal conditions. When nitrate is deficient, AtNLP7 locates in the cytoplasm, then relocates to the nucleus after a few minutes of re-supply of nitrate [6]. Studies have shown that the N-terminal region of AtNLP6 is responsible for receiving nitrate signals [7]. In addition, the phosphorylation of Ser205 at the N-terminus of AtNLP7 is necessary for AtNLP7 to translocate to the nucleus in response to nitrate signals [12]. It is known that AtNLP7 senses the nitrate signal, then moves into the nucleus and play its regulatory role. However, the underlying mechanism by which AtNLP7 senses the nitrate deficiency signal and then moves out of the nucleus remains unknown.

In this study, we revealed that the GAF domain is critical for the transport of AtNLP7 from the nucleus to the cytoplasm. With the loss of the GAF domain, AtNLP7ΔGAF was retained in the nucleus, while the protein lost its ability to bind to NRE or to increase plant nitrogen (N) use efficiency. In addition, AtNLP7ΔGAF lost its ability to mediate the reduction of ROS accumulation upon nitrate treatment. Our investigation shows that the N-terminal GAF domain is required for AtNLP7 protein translocation from the nucleus to the cytoplasm in response to nitrate deficiency.

**Results**

**GAF domain is required for AtNLP7 nuclear exportation in response to nitrate deficiency**

To investigate which domain is responsible for the nitrate deficiency-triggered AtNLP7 relocation from the nucleus to cytoplasm, we generated transgenic lines expressing a series of truncated AtNLP7 domains fused with the green fluorescence protein (GFP) in the *nlp7-1* background. The nuclear export signal (NES), GAF domain and PB1 domain of AtNLP7 were deleted respectively as shown in Fig. 1A. The expression of AtNLP7 was significantly increased in all complementary lines compared to *nlp7-1* and wild-type (WT) plants (Fig. S1).

In the presence of nitrate, AtNLP7-GFP was mainly located in the nucleus. Removing nitrate within hours led to the relocation of AtNLP7-GFP into the cytoplasm (Fig. 1B). Interestingly, we found that when the NES of AtNLP7 was deleted (NLP7ΔNES), the protein could still be transported between the nucleus and cytoplasm, indicating that the NES pathway is not the only way for AtNLP7 to be transported out of the nucleus. Similarly, when the PB1 domain was deleted (NLP7ΔPB1), the protein could also be transported between the nucleus and cytoplasm (Fig. 1B).

Surprisingly, two AtNLP7 deletion variants that did not contain the GAF domain (NLP7ΔGAF−1 and NLP7ΔGAF−2) remained in the nucleus regardless of nitrate presence in the surrounding environment, suggesting that these variants could be imported into the nucleus but could not be exported out of it (Fig. 1B). Taken together, these results indicate that the GAF domain, but not the NES and the PB1 domain, is responsible for sensing the nitrate deficiency signal that mediates AtNLP7 nuclear exportation.

**NLP7ΔGAF variants fail to complement *nlp7-1* mutant**

In order to explore the function of different domains of AtNLP7, the generated lines were grown on MS medium with nitrate as the only N source. The *nlp7-1* mutants exhibited N-deficient phenotype regardless nitrate level, and the phenotype has been restored by expressing 35S:AtNLP7-GFP construct (NLP7) (Fig. 1C). Compared with *nlp7-1* plants, overexpression of NLP7ΔNES could increase shoot biomass regardless nitrate level, as well as the overexpression of NLP7ΔPB1 (Fig. 1E). However, the transgenic lines overexpressing NLP7ΔGAF−1 and NLP7ΔGAF−2 exhibited impaired growth even under nitrate-rich conditions (Fig. 1C, E). When grown in soil, NLP7ΔGAF−1 and NLP7ΔGAF−2 plants showed N-stressed phenotypes with lower biomass whereas NLP7ΔNES and NLP7ΔPB1 plants exhibited a normal phenotype (Fig. 1D, H). We also found that chlorophyll contents increased significantly in NLP7ΔNES and NLP7ΔPB1 plants while reduced dramatically in NLP7ΔGAF−1 and NLP7ΔGAF−2 plants compared to the WT under both low and high nitrate conditions (Fig. 1E, G). All these results indicate that the mutated forms of AtNLP7 without GAF domain lost its ability to restore the phenotype of the *nlp7-1* plant.

**NLP7ΔGAF plants show downregulated expression of nitrate-responsive genes and impaired nitrate assimilation**

To assess the ability of truncated AtNLP7 to affect nitrate-responsive gene expression, we examined the transcriptional levels of these genes without nitrate and with re-supply of nitrate for one hour. The expression of typical nitrate-responsive genes, containing nitrate transporter gene *NITRATE TRANSPORTER 2.1 (NRT2.1)*, nitrate assimilation genes *NITRATE REDUCTASE 1 (NIA1)* and *NITRITE REDUCTASE 1 (NIR1)*, glutamine synthetase gene *GLUTAMINE SYNTHETASE 2 (GS2)* and two transcription factor genes *LOB DOMAIN-CONTAINING PROTEIN 37 (LBD37) and LBD39* was...
analyzed. Induction of these genes in nlp7-1 is approximately half the levels seen in WT seedlings (Fig. 2A-F). However, expressing wild-type AtNLP7 with the CaMV-35S promoter in nlp7-1 (NLP7 plants) strongly restored the expressive levels of these genes in response to nitrate. Nitrate-induced expression of all genes analyzed also showed a similar increase in NLP7ΔNES plants (Fig. 2A-F). Nevertheless, transcription levels of these genes were basically the same in nlp7-1 and NLP7ΔGAF seedlings after nitrate addition (Fig. 2A-F). In addition, the expression of these genes in the NLP7ΔPB1 plants were similar to those in the WT plants under either N-free or nitrate-supplied conditions (Fig. 2A-F). These results suggest that the GAF domain plays an essential role for AtNLP7 regulation of nitrate-inducible genes.

We further investigated N absorption and assimilation by analyzing 15 N-nitrate absorption and found that 15 N accumulation in NLP7, NLP7ΔNES and NLP7ΔPB1 plants was observably more than that in WT while much less in NLP7ΔGAF−1 and NLP7ΔGAF−2 plants (Fig. 2G). We next analyzed the nitrate content of different lines under low and high nitrate conditions. As expected, the nlp7-1 mutant accumulated more nitrate due to the weak nitrate reductase (NR) activity [5]. The nitrate content in NLP7ΔGAF−1 and NLP7ΔGAF−2 plants also increased significantly while decreased dramatically in NLP7ΔNES and NLP7ΔPB1 plants (Fig. 2H). We then compared the NR activity of the transgenic lines with WT and discovered that NR activities were lower in NLP7ΔGAF−1 and NLP7ΔGAF−2 plants while higher in NLP7, NLP7ΔNES and NLP7ΔPB1 plants under different nitrate conditions (Fig. 2I). Overall, our analysis suggests that the GAF domain is required for the function of AtNLP7.

**NLP7ΔGAF fails to bind its targets in vivo and activate their expression**

To reveal the function of the GAF domain in AtNLP7 target gene activation, we conducted chromatin
immunoprecipitation (ChIP) quantitative PCR and identified the association of AtNLP7 with NREs from the AtNIR1 and AtNIA1 promoters in vivo. Compared with NLP7, the binding ability of NLP7ΔNES to NRE element was not significantly changed, but NLP7ΔPB1 was slightly impaired. In contrast, NLP7ΔGAF−1 and NLP7ΔGAF−2 almost lost the function to combine NRE element (Fig. 3A, B). This result was further verified with transient transactivation assay, in which AtNLPL7ΔGAF weakened the activation of the AtNIR1 promoter (Fig. 3C, D).

The GAF domain of AtNLPL7 is required for the nitrate-induced reduction of ROS accumulation

ROS has recently been found to play an important role in nitrate signal transduction in Arabidopsis thaliana [26–32]. In order to confirm whether nitrate modulates ROS contents through AtNLPL7, we used nitroblue tetrazolium (NBT) and 3,3’-diaminobenzidine tetrahydrochloride (DAB) staining to detect the level of ROS in WT and different AtNLPL7 truncated mutants with or without nitrate application. The results showed that nitrate treatment significantly reduced ROS levels in the primary root of NLP, NLP7ΔNES and NLP7ΔPB1 plants compared with the WT, but there was no obvious change in the primary root

![Graphs showing expression levels of different genes under various conditions](image-url)
of NLP7ΔGAF−1, NLP7ΔGAF−2 and nlp7-1 plants (Fig. 4A-D), suggesting that nitrate-triggered ROS reduction is dependent on GAF domain.

To confirm the above results, we analyzed truncated AtNLP7-GFP proteins in the primary root of transgenic plants to determine whether H2O2 addition influenced the localization of AtNLP7. Nitrate treatment induced the nuclear localization of AtNLP7-GFP. Whereas, addition of H2O2 dramatically inhibited nitrate-induced translocation of NLP7-GFP and various truncated AtNLP7-GFP from the cytoplasm to the nucleus except NLP7ΔGAF variants (Fig. 4E). These results indicate that the GAF domain is required for the H2O2 treatment-reduced movement of AtNLP7 from cytoplasm into nucleus.

**Discussion**

AtNLP7 has been shown to play a crucial role in the primary nitrate response [6, 9]. The subcellular localization of AtNLP7 is exclusively and quickly induced by nitrate [6]. Phosphorylation of AtNLP7 at Ser205 by Ca2+ receptor kinase AtCPK10/30/32 is required for nitrate-induced migration of AtNLP7 from the cytoplasm to the nucleus [12]. Whereas, the underlying mechanism by which AtNLP7 senses the nitrate deficiency signal and thus relocate from the nucleus to cytoplasm remains unclear. In this study, we demonstrated that AtNLP7ΔGAF remained in the nucleus under nitrate-starvation conditions and lost its function as a transcriptional activator. Therefore, the GAF domain is responsible for sensing nitrate deficiency signal that mediates AtNLP7 nuclear exportation.
Most of the nucleocytoplasmic transport is a signal dependent process, requiring a sequence motif in the transported protein [14]. The presence of a predicted leucine-rich NES within AtNLP7 led us to examine whether this NES was responsible for the cytoplasmic localization of AtNLP7 during nitrate starvation. Leptomycin B (an inhibitor of Exportin1) treatment have been reported to inhibit the transport of NLP7–GFP from the nucleus to the cytoplasm when the seedlings are transferred from the nitrate-rich condition to the N-starvation [6]. Unexpectedly, we found that NLP7ΔNES could still be transported between the nucleus and cytoplasm (Fig. 1B),
indicating that the NES is dispensable for AtNLP7 to be transported out of the nucleus in response to nitrate deficiency signal.

The RWP-RK domain is required for AtNLP7 binding to NRE cis-elements of target genes but not for nitrate signaling [7, 15, 33, 34]. In the present study, reduced enrichment of NRE cis-elements in chromosome immunoprecipitation assays and attenuated levels of transcription in protoplast transient tests (Fig. 3), indicating that the GAF domain is also crucial for AtNLP7 binding to NRE. NLP7Δ GAF significantly weakens its binding to the promoter of target genes and thereby its transcription-activating activity compared with wild-type NLP7. Because the GAF domain can bind low-molecular-weight ligands such as nitrate or form homodimer [20–23], it is possible that the specific spatial conformation of AtNLP7 ligands such as nitrate or form homodimer [20–23], it is

The PB1 domain plays an important role in protein–protein interaction [17, 35]. Regulation of nitrate-dependent target gene expression by NLP transcription factors requires protein–protein interactions caused by the PB1 domain in plants [19]. Indeed, we showed that the deletion of the PB1 domain (NLP7ΔPB1) had a significant effect on the expression level of nitrate-induced genes compared with wild-type AtNLP7 (NLP7) (Fig. 2A–F), consistent with its growth phenotype (Fig. 1C–H) and other results (Figs. 3 and 4), although there was no impairment of nuclear export capacity in response to nitrate starvation (Fig. 1B).

H2O2 is produced during N deficiency and act as a potential messenger in the nitrogen starvation response [26, 27, 30, 31]. Recently, it has been reported that nitrate application decreases the accumulation of H2O2, while H2O2 suppresses nitrate signaling through modulating the nucleocytoplasmic shuttling of AtNLP7 [28]. We found that the GAF domain plays an important role in this process and reached similar conclusions (Fig. 4). The results of NBT and DAB staining showed that the addition of nitrate significantly reduced the content of ROS in WT, NLP7, NLP7ΔNES and NLP7ΔPB1 seedlings compared with the nitrate starvation treatment. However, due to the deletion of GAF domain in AtNLP7, ROS accumulated in the absence of nitrate and could not be effectively reduced after nitrate addition, consistent with those in nlp7-1 mutant (Fig. 4A–D). These results indicate that the GAF domain of AtNLP7 is required for nitrate-induced reduction of ROS. In addition, we also found that H2O2 addition decreases the nuclear localization of AtNLP7 triggered by nitrate in a GAF domain-dependent manner (Fig. 4E). H2O2 usually modulates the activity of the target proteins by oxidative modification of cysteine [36]. AtNLP7 GAF domain contains 2 cysteine residues (Cys273 and Cys296) that can be oxidized by H2O2, thereby sensing H2O2 signal to regulate AtNLP7 subcellular localization and subsequent events.

**Methods**

**Plant materials and growth conditions**

*Arabidopsis thaliana* ecotype Col-0 and nlp7-1 (SALK_26134C) seeds were obtained from *Arabidopsis* Biological Resource Center with permission and used for the genetic transformation of the AtNLP7 gene. 35S:NLP7ΔNES-GFP, 35S:NLP7ΔGAF−1-GFP, 35S:NLP7ΔGAF−2-GFP and 35S:NLP7ΔPB1-GFP constructs were made by inserting the different coding regions of AtNLP7 into pGWBS through GATEWAY cloning system. In the construction of 35S:NLP7−GFP, the fragment containing the complete coding sequence of AtNLP7 was cloned into pGWBS to fuse with GFP. All transgenic lines were generated and screened as previously described [5].

The sterilization of the seeds and the growth conditions of the seedlings were as previously described [5–8, 11]. Seeds were sterilized with 15% bleach for 12 min, and then washed five times with sterile water. Sterilized seeds stratified at 4 °C for 2 days, and plated on solid medium containing 1% (w/v) sucrose and 0.6% (w/v) agar. Growth medium was modified on MS medium with KNO3 as sole N source: 1 mM (LN) nitrate medium (similar to MS except 20 mM KNO3 and 20 mM NH4NO3 was replaced with 19 mM KCl and 1 mM KNO3), 10 mM (HN) nitrate medium (similar to MS except 20 mM KNO3 and 20 mM NH4NO3 was replaced with 10 mM KCl and 10 mM KNO3), N-free medium (similar to MS except 20 mM KNO3 and 20 mM NH4NO3 was replaced with 20 mM KCl).

The seedlings for phenotypic analysis in Fig. 1 were grown on 1 mM (LN) and 10 mM (HN) medium for 10 days or in soil for 3 weeks, respectively. To analyze expression of nitrate-induced genes, seedlings were grown on N-free medium for 7 days and then treated with 10 mM KCl or KNO3 for 1 h.

To investigate different growth rates under different N conditions, seeds were germinated and grown on medium containing different concentrations of nitrate at 22 °C under 16-h light/8-h dark photoperiod. For evaluation the phenotype of soil-grown plants, seeds were germinated and grew in soil at 22 °C under 16-h light/8-h dark photoperiod.

**Subcellular localization assay**

To investigate the nuclear-cytoplasmic shuttling of AtNLP7 with different domains, seedlings grown for 5 days on 10 mM KNO3 medium were transferred to N-free medium for 2 days and then treated with 10 mM KNO3 or KCl for 1 h. To analyze truncated AtNLP7-GFP
proteins nuclear retention triggered by nitrate and H$_2$O$_2$, seedlings growing on medium with 10 mM KNO$_3$ for 5 days were transferred to N-free medium for two days then treated with 10 mM KNO$_3$ or 10 mM KNO$_3$+0.5 mM H$_2$O$_2$ for 1 h. Laser scanning confocal imaging used Zeiss 880 microscope with argon laser (488 nm for green fluorescent protein (GFP) excitation).

RNA extraction and qRT-PCR
RNA extraction, reverse transcription, and qRT-PCR were performed as described previously [5, 7]. Briefly, reverse transcription was performed using total RNA extracted with Trizol reagent (Invitrogen, Carlsbad, California, USA). qRT-PCR was conducted with StepOne Plus Real Time PCR System by using TaKaRa SYBR Premix Ex Taq II reagent kit. UBQ5 was used as the internal control. The primers used are listed in Supplementary table. All materials used for RNA extraction were whole plants.

Nitrate content and enzyme activity analysis
Whole 14-day-old seedlings grown on agar medium with 1 mM (LN) or 10 mM (HN) KNO$_3$ were used for analysis. Nitrate was extracted in 50 mM HEPES–KOH (pH 7.4), and measured as described previously [37]. NR activity was analyzed with an enzyme-coupled spectrophotometer assay kit (SKBC, China) according to the manufacturer's guidelines.

Uptake of $^{15}$N-nitrate
$^{15}$N-uptake assay was carried out with $^{15}$N-labeled KNO$_3$ (99 atom % $^{15}$N, Sigma-Aldrich, no. 335134). For $^{15}$N-nitrate uptake experiment, 10-day-old seedlings grown in MS medium were pretreated with MS solution for 1 h and then transferred to 0.1 mM CaSO$_4$ for 1 min. Then it was cultured in the modified MS solution with 5 mM K$^{15}$NO$_3$ as the only N source for 1 h, and finally returned to 0.1 mM CaSO$_4$ for 1 min. The whole seedlings were dried to constant weight at 70°C and ground. Used continuous flow isotope ratio mass spectrometer (DELTAV Advantage) and elemental analyzer (EA-HT, Thermo Fisher Scientific, Inc., Bremen, Germany) to analyze $^{15}$N content.

Chromatin immunoprecipitation
Seedlings were grown on MS medium for 10 days. The ChIP experiment was conducted as previously described [38]. The nlp7-1 mutant, 35S:NLPtr$^{\text{NES}-GFP}$, 35S:NLPtr$^{\text{GAF}-1}$-GFP, 35S:NLPtr$^{\text{GAF}-2}$-GFP, 35S:NLPtr$^{\text{APB1}}$-GFP and pNLPtr:NLPtr–GFP transgenic plants, anti-GFP antibodies (Abmart), and salmon sperm DNA/protein A agarose beads (Millipore, USA) were used for ChIP assay. The DNA was purified and precipitated with phenol/chloroform (1:1, v/v). Used qRT-PCR to detect the degree of enrichment of DNA fragments. Enrichment values were calibrated with input DNA levels.

Transient transactivation assay
Protoplast preparation and PEG transformation was performed as described before [39]. Different coding regions of AtNLPtr derivatives were recombined into the pGreenII 62-SK vector as effectors, the entire promoter or the binding cis-element of target genes were recombined into pGreenII 0800-LUC vector as reporters. The dual luciferase reporter gene system was used for transient transactivation detection. Determined the relative activity of firefly luciferase (LUC) and renilla luciferase (REN), and computed the LUC/REN ratio. REN luciferase activity was used as an internal reference.

NBT and DAB staining
The seedlings used for NBT and DAB staining were grown on 10 mM KNO$_3$ for 5 days, transferred to nitrogen-free medium for 2 days and then treated with 10 mM KCl or KNO$_3$ for 1 h. Plants were incubated with 1 mg/mL NBT solution or 1 mg/ml DAB solution at room temperature for 2 h and 6 h respectively, and then soaked in 80% (v/v) ethanol for 20 min before photographing to stop staining [28]. The signal intensity was quantified by Image J.

Statistical analysis
Statistical analyses of the data were performed using analysis of variance (ANOVA). For multiple comparisons, one-way ANOVA with a Duncan post hoc test and Tukey’s honest significant difference test were used. For all statistical analyses, the difference was considered statistically significant when the $P$ value < 0.05.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12870-022-03755-x.

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Author’s contribution
J.W. and C.-B.X. designed the experiments. J.W., Y.S., Z.-S.Z., and J.-X.W., X.Z., J.-Y.Z., M.-Y.B., L.-H.Y. performed the experiments. J.W. wrote the manuscript.
C.-B.X. and J.W. revised the manuscript. C.-B.X. supervised the project. All authors read and approved the final manuscript.

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Availability of data and materials
All data and materials generated or analyzed during this study are included in this published article and its supplementary file. The sequence data used in this study can be found in the Arabidopsis Information Resource (https://www.arabidopsis.org/). The accession number of genes involved in the manuscript: At4g24040 (AtNL4), At5g98900 (AtNL7), At1g77760 (AtNL1), At2g15620 (AtNL14), At1g55630 (AtG52), At5g67420 (AtLB37), At4g37540 (AtLB39). The datasets and materials used during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Arabidopsis thaliana ecotype Col-0 and NL7-1 (SALK_26134C) seeds were obtained from Arabidopsis Biological Resource Center with permission. All plant materials involved in this manuscript were generated and screened by Prof. Jie Wu and Dr. Zisheng Zhang (University of Science and Technology of China), except for those purchased from ABRC. All plant seeds are kept in our laboratory (School of Life Sciences, University of Science and Technology of China) and no specimens are kept in public herbariums. The study complies with the institutional/national guidelines.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interest.

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References
1. Crawford NM. Nitrates: Nutrient and Signal for Plant Growth. Plant Cell. 1995;7:859–68.
2. Crawford N, Glass A. Molecular and physiological aspects of nitrate uptake in plants. Trends Plant Sci. 1998;3(10):389–95.
3. Daniel-Vedele F, Filleur S, Caboche M. Nitrate transport: a key step in nitrate assimilation. Curr Opin Plant Biol. 1998;1:235–9.
4. Forde BG. Nitrate transporters in plants: structure, function and regulation. Biochem Biophys Acta. 2000;1465:219–35.
5. Yu LH, Wu J, Tang H, Yuan Y, Wang SM, Wang YP, Zhu QS, Li SG, Xiang CB. Overexpression of Arabidopsis NL7 improves plant growth under both nitrogen-limiting and sufficient conditions by enhancing nitrogen and carbon assimilation. Sci Rep. 2016;6:27795.
6. Marchive C, Roudier F, Castaings I, Brehatu V, Blonder E, Colot V, Meyer C, Krapp A. Nuclear retention of the transcription factor NL7 orchestrates the early response to nitrate in plants. Nat Commun. 2013;4:1713.
7. Konishi M, Yanagisawa S. Arabidopsis Nin-like transcription factors have a central role in nitrate signalling. Nat Commun. 2013;4:1617.
8. Castaings I, Camargo A, Pocholle D, Gaudon V, Texier Y, Boutet-Mercet S, Taconnat L, Renou J, Daniel-Vedele F, Fernandez E, et al. The nodule inception-like protein 7 modulates nitrate sensing and metabolism in Arabidopsis. Plant J. 2009;57(3):426–35.
9. Alvarez J, Schinke A, Brooks M, Pasquino A, Leonelli L, Varala S, Safi A, Krouk G, Krapp A, Coruzzi G. Transient genome-wide interactions of the master transcription factor NL7 initiate a rapid nitrogen-response cascade. Nat Commun. 2020;11(1):1157.
10. Alfath A, Wu J, Zhang ZS, Xia JQ, Jan SU, Yu LH, Xiang CB. Rice Nin-LIKE PROTEIN 1 rapidly responds to nitric oxide deficiency and improves yield and nitrogen use efficiency. J Exp Bot. 2020;71(19):6032–42.
11. Wu J, Zhang ZS, Xia JQ, Alfath A, Song Y, Huang YJ, Wan GY, Sun LQ, Tang H, Liu Y, et al. Rice NIN-LIKE PROTEIN 4 plays a pivotal role in nitrogen use efficiency. Plant Biotechnol J. 2021;19(3):448–61.
12. Liu KH, Niu Y, Konishi M, Wu Y, Du H, Sun Chung H, Li L, Boudsocq M, McCormack M, Maekawa S, et al. Discovery of nitrate-CPK-NLP signalling in central nutrient-growth networks. Nature. 2017;545(7645):311–6.
13. Chardin C, Girin T, Roudier F, Meyer C, Krapp A. The plant RWP-RK transcription factors: key regulators of nitrogen responses and of gametophyte development. J Exp Bot. 2014;65(19):5577–87.
14. Ila Cout T, Gupta R, Rapacki K, Skriver K, Brunak S. NESbase version 1.0: a database of nuclear export signals. Nucleic Acids Res. 2003;31(1):393–6.
15. Konishi M, Yanagisawa S. Identification of a nitrate-responsive cis-element in the Arabidopsis NRI1 promoter defines the presence of multiple cis-regulatory elements for nitrogen response. Plant J. 2010;63(2):269–82.
16. Sato T, Maekawa S, Konishi M, Yoshiko N, Sasaki I, Maeda H, Ishida T, Kato Y, Yamaguchi J, Yanagisawa S. Direct transcriptional activation of BT genes by NLP transcription factors is a key component of the nitrate response in Arabidopsis. Biochem Biophys Res Commun. 2017;483(1):380–6.
17. Sumimoto H, Kamakura S, Ito T. Structure and function of the NTH domain, a protein interaction module conserved in animals, fungi, amoebas, and plants. Sci Signal. 2007;2007(401).n6.
18. Guan P, Ripoll JJ, Wang R, Vuong L, Bailey-Steinzeit LJ, Ye D, Crawford NM. Interacting TCP and NLP transcription factors control plant responses to nitrate availability. Proc Natl Acad Sci USA. 2017;114(9):2419–24.
19. Konishi M, Yanagisawa S. The role of protein-protein interactions mediated by the NTH1 domain of NLP transcription factors in nitrate-inducible gene expression. BMC Plant Biol. 2019;19(1).n90.
20. Su YS, Lagarias JC. Light-Independent Phytochrome Signaling Mediated by Dominant GAF Domain Tyrosine Mutants of Arabidopsis Phytochromes in Transgenic Plants. Plant Cell. 2007;19:2124–39.
21. Grefen C, Stadele K, Ruzicka K, Obrdlik F, Harter K, Horak J. Subcellular localization and in vivo interactions of the Arabidopsis thaliana ethylene receptor family members. Mol Plant. 2008;1(2):308–20.
22. Jung YJ, Ahn JH, Schachtman DP. CC-type glutaredoxins mediate plant response and signaling under nitrate starvation in Arabidopsis. BMC Plant Biol. 2018;18(1).n281.
23. Safi A, Medicci A, Szponarski W, Marshall-Colton A, Ruffell S, Gaymard F, Coruzzi G, Lacombe B, Krouk G. HR1/HPS1/GARP transcription factors and reactive oxygen species are regulators of Arabidopsis nitrogen starvation response. BioRxiv. 2018:164277. http://hdl.handle.net/1854/LU-8634978.
24. Chu XQ, Wang JG, Li MZ, Zhang SJ, Gao YY, Fan M, Han C, Xiang FN, Li GY, Wang Y, et al. HBl Transcription Factor-Mediated ROS Homeostasis Regulates Nitrate Signal Transduction. Plant Cell. 2021;33(9):3004–21.
25. Zang L, Morere-Le Paver MC, Clochard T, Porcher A, Savoie P, Mojovic M, Vidovic M, Lamami AM, Montrichard F. Nitrate inhibits primary root...
growth by reducing accumulation of reactive oxygen species in the root tip in Medicago truncatula. Plant Physiol Biochem. 2020;146:363–73.
30. Safi A, Medici A, Szponarski W, Martin F, Clement-Vidal A, Marshall-Colon A, Ruffel S, Gaynard F, Rouached H, Leclercq J, et al. GARP transcription factors repress Arabidopsis nitrogen starvation response via ROS-dependent and independent pathways. J Exp Bot. 2021;72(10):3881–901.
31. Wany A, Foyer CH, Gupta KJ. Nitrate, NO and ROS Signaling in Stem Cell Homeostasis. Trends Plant Sci. 2018;23(12):1041–4.
32. Chaput V, Martin A, Lejay L. Redox metabolism: the hidden player in carbon and nitrogen signaling? J Exp Bot. 2020;71(13):3816–26.
33. Schauser L, Wieloch W, Stougard J. Evolution of NIN-like proteins in Arabidopsis, rice, and Lotus japonicus. J Mol Evol. 2005;60(2):229–37.
34. Soyano T, Shimoda Y, Hayashi M. NODULE INCEPTION antagonistically regulates gene expression with nitrate in Lotus japonicus. Plant Cell Physiology. 2015;56(2):368–76.
35. Mutte SK, Weijers D. Deep Evolutionary History of the Phox and Bem1 (PB1) Domain Across Eukaryotes. Sci Rep. 2020;10(1):3797.
36. Tian Y, Fan M, Qin Z, Lv H, Wang M, Zhang Z, Zhou W, Zhao N, Li X, Han C, et al. Hydrogen peroxide positively regulates brassinosteroid signaling through oxidation of the BRASSINAZOLE-RESISTANT1 transcription factor. Nat Commun. 2018;9(1):1063.
37. Cataldo DA, Maroon M, Schrader LE, Youngs VL. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Commun Soil Sci Plant Anal. 1975;6(1):71–80.
38. Cai KT, Xu P, Zhao PX, Liu R, Yu LH, Xiang CB. Arabidopsis ERF109 mediates cross-talk between jasmonic acid and auxin biosynthesis during lateral root formation. Nat Commun. 2014;5:833.
39. Yoo SD, Cho YH, Sheen J. Arabidopsis mesophyll protoplasts: a versatile cell system for transient gene expression analysis. Nat Protoc. 2007;2(7):1565–72.

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