

The putative high-affinity nitrate transporter NRT2.1 represses lateral root initiation in response to nutritional cues

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Edited by Robert Haselkorn, University of Chicago, Chicago, IL, and approved August 10, 2005 (received for review May 20, 2005)

Lateral root initiation is strongly repressed in *Arabidopsis* by the combination of high external sucrose and low external nitrate. A previously isolated mutant, *lin1*, can overcome this repression. Here, we show that *lin1* carries a missense mutation in the *NRT2.1* gene. Several allelic mutants, including one in which the *NRT2.1* gene is completely deleted, show similar phenotypes to *lin1* and fail to complement *lin1*. *NRT2.1* encodes a putative high-affinity nitrate transporter that functions at low external nitrate concentrations. Direct measurement of nitrate uptake and nitrate content in the *lin1* mutant seedlings established that both are indeed reduced. Because repression of lateral root initiation in WT plants can be relieved by increased concentrations of external nitrate, it is surprising to find that repression is also relieved by a defect in a component of the high-affinity nitrate uptake system. Furthermore, lateral root initiation is increased in *lin1* relative to WT even when seedlings are grown on nitrate-free media, suggesting that the mutant phenotype is nitrate-independent. These results indicate that NRT2.1 is a repressor of lateral root initiation and that this role is independent of nitrate uptake. We propose that *Arabidopsis* NRT2.1 acts either as a nitrate sensor or signal transducer to coordinate the development of the root system with nutritional cues.

carbon | nitrogen | nutrition | *lin1*

The plant root system develops through the formation of lateral roots along the length of the primary root. As these lateral roots grow, they also give rise to new lateral roots. Unlike the preordained placement of most organs in animals, the number and distribution of lateral roots are strongly influenced by environmental conditions (1–3). The plasticity of lateral root formation serves an important function, as it allows the architecture of the root system to be optimized to the unique distribution of water and nutrients in the soil. Little is known about how nutrient signals are sensed and transduced to regulate plant development.

We are studying lateral root initiation to determine how environmental cues can modulate plant developmental processes. Lateral roots originate from an internal layer of cells called the pericycle. Lateral root initiation involves the stimulation of mature, quiescent pericycle cells to divide and become founder cells for a new lateral root (1, 2). Only pericycle cells situated at the protoxylem poles are competent to become founder cells, and only a small percentage of these cells ever participate in lateral root initiation. We have little understanding about how specific competent pericycle cells are stimulated to initiate lateral root formation or how environmental cues modulate the number and location of lateral root initiations in the root system. This process must require sensing of external conditions and a signal transduction system that terminates in targeted pericycle cell division.

In a previous paper (4) it was demonstrated that lateral root initiation is repressed in seedlings grown on high ratios of sucrose to nitrogen. To further understand the coordination of

nutrient cues and lateral root initiation, a mutant that is able to initiate large numbers of lateral roots when grown on these conditions was isolated (4). The existence of the lateral root initiation mutant phenotype (*lin1*) indicates that the low rate of lateral root initiation in WT plants under repressive conditions is not caused by inadequate nutrients to complete the essential cell divisions. Instead, the high sucrose/low nitrogen condition must trigger a signal that negatively regulates the lateral root initiation program, and this negative regulation must require LIN1. Lateral root initiation in the *lin1* mutant only differs from WT under specific growth conditions. Therefore, LIN1 is a component of a signaling pathway connecting specific nutritional cues with lateral root initiation.

Based on the mutant phenotype, we can postulate two alternative models for LIN1 function. In the first model, LIN1 represses lateral root initiation by sensing sucrose and/or nitrogen or by transducing a signal from the actual sensor. In this case, *lin1* would fail to perceive the repressive nutritional condition and therefore would fail to repress lateral root initiation. In the second model, LIN1 modifies nutrient uptake or assimilation. For example, if the *lin1* mutant obtains less sucrose or more nitrogen from the media, the lateral root repression pathway would not be triggered.

In this article, we report that a change in a conserved amino acid of NRT2.1 is responsible for the *lin1* phenotype. NRT2 proteins are members of the major facilitator superfamily (MFS) of transporter and transporter-like proteins (5). Higher plant NRT2 proteins are predicted to be nitrate transporters based on their homology to the fungal nitrate transporter CRNA and on the changes in nitrate uptake kinetics observed in *Arabidopsis* plants with mutations in *NRT2* genes (6–8). Direct studies of nitrate uptake kinetics in higher plants have revealed two distinct systems: the low-affinity transport system that is responsible for uptake when nitrate is plentiful (>1 mM) and the high-affinity transport system (HATS) that is able to scavenge nitrate from the soil at concentrations between 1 μ M and 1 mM. Molecular and physiological studies in a number of plant species indicate that the NRT2 proteins are required for the HATS (6–8).

In *Arabidopsis*, there are seven *NRT2* genes (6–10). Of these, the NRT2.1 protein appears to be the most critical for high-affinity nitrate uptake based on the following findings: (i) the activity of the HATS is strongly reduced ($V_{\max} = 27\%$ of WT) in an *NRT2.1/NRT2.2* deletion mutant (11); (ii) *NRT2.1* is strongly expressed in external cell layers of the root, the main site for nitrate uptake from the soil (9, 10, 12, 13); and (iii) the

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: MFS, major facilitator superfamily; HATS, high-affinity transport system; MS, Murashige and Skoog.

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expression profile of *NRT2.1* is consistent with studies of HATS activity. For example, HATS-dependent nitrate uptake is stimulated by low external nitrate and inhibited in response to downstream products of nitrate assimilation such as ammonium and certain amino acids. Similarly, low levels of external nitrate induce *NRT2.1* expression, whereas expression is repressed by downstream nitrate metabolites (6–14).

The cloning and identification of the *LIN1* gene as *NRT2.1* indicates that this component of the high-affinity nitrate uptake system also plays a role in repressing lateral root initiation in response to high sucrose/low nitrogen. Because repression of lateral root initiation can be relieved by increased concentrations of external nitrate, it is surprising to find that it is also relieved by a defect in the HATS, which would be expected to decrease nitrate uptake. However, in this article we demonstrate that the role of *NRT2.1* in repressing lateral root initiation is independent of its role in nitrate uptake. Therefore, we propose that the putative nitrate transporter *NRT2.1* acts as a nitrate sensor or a downstream signal transducer in a pathway that coordinates nitrate availability with the development of the root system.

Materials and Methods

Plant Materials. Both T-DNA insertion mutants [SALK_035429 (*nrt2.1-2*) and SALK_008253 (*nrt2.1-3*)] were obtained from the *Arabidopsis* Biological Resource Center (Ohio State University, Columbus).

Plant Growth Conditions. All seeds were sterilized in 100% bleach containing Tween 20 (three drops per 10 ml) for 3 min and then rinsed four times with sterile distilled water. The seeds were then stratified in water at 4°C for 2–4 days. Seeds were sown on modified Murashige and Skoog (MS) media (described below) and grown on vertically oriented plates in a growth chamber set at 22°C, 16-h light, 8-h dark.

Nitrogen-free MS basal medium (GIBCO/BRL, custom ordered) was supplemented with 0.5 g/liter 2-(*N*-morpholino)ethanesulfonic acid and 75 g/liter sucrose. Nitrogen was added in the form of NH_4Cl , KNO_3 , and/or NH_4NO_3 . KCl was used to equalize the total salt concentration among the treatments within an experiment. The pH was adjusted to 5.7 with 1 M KOH. Agar (7 g/liter; Sigma A-7921) was added before autoclaving.

Microscopic Analysis of Lateral Root Initiation. The number of lateral root initiation events was determined in cleared roots as described (4). All lateral roots and lateral root primordia were counted to obtain the total number of lateral root initiations.

Cloning of the *LIN1* Gene. The *lin1* mutant was crossed to a WT *Arabidopsis* var. WS to fine-map the gene. Polymorphic markers on chromosome 1 were obtained from the public chromosome maps (www.arabidopsis.org) or identified by the Malamy laboratory. Breakpoint analysis of recombinant F_2 plants delimited the mutation to an ≈ 110 -kb region flanked by two simple sequence length polymorphism markers, PHY4 and HY6. The PCR primers used to identify the PHY4 polymorphism were: forward, 5'-ttgtaccagtcgaagggtgg-3'; and reverse, 5'-agttccatcaacattgtgg-3'. The primers for the HY6 polymorphism were: forward, 5'-aagatagagacatataacccc-3'; and reverse, 5'-tcaaacacagagaaaaacc-3'.

Determination of Nitrate Uptake and Accumulation. Influx rates of $^{15}\text{NO}_3^-$ were tested for WT and *lin1* plants grown for 14 days on MS media (as described). The seedlings were transferred to 0.1 mM CaSO_4 for 1 min, to a nutrient solution containing 7.5% sucrose, 0.1 mM $^{15}\text{NO}_3^-$ (atom% ^{15}N : 99% in the form of NH_4NO_3) for 5 min, and finally to 0.1 mM CaSO_4 for 1 min. Roots were separated from the shoots immediately after the final

transfer to CaSO_4 , dried for 48 h at 70°C, and analyzed by using the ANCA-MS system (PDZ Europa, Crewe, U.K.). Influx of $^{15}\text{NO}_3^-$ was calculated from the ^{15}N content of the roots ($\mu\text{mol/hr}$ per g dry weight). For determination of nitrate accumulation ($\mu\text{mol/g}$ fresh weight), ethanol extracts, made as described (15), were evaporated and diluted in water before analysis by ion exchange chromatography with a DX-120 column (Dionex, Sunnyvale, CA).

Results

Lateral Root Initiation Is Repressed by a High Sucrose/Nitrate Ratio.

When WT plants are grown on standard growth media, lateral root initiation can always be seen along the length of the primary root. In contrast, we previously showed that WT plants initiate few, if any, lateral roots when grown with high sucrose (4.5%) and low nitrogen (0.01 mM NH_4NO_3). Decreasing the sucrose concentration to 0.5% or increasing the nitrogen to 60 mM restores lateral root initiation (4), indicating that neither the high sucrose nor the limited nitrogen alone is responsible for the repression of lateral root initiation. Therefore, we hypothesized that the repressive element in these conditions is the coincidence of limiting nitrogen supplies and high sucrose concentration, creating a high ratio of sucrose/nitrogen.

If the high sucrose/low nitrogen condition is indeed the critical parameter in repressing lateral root initiation, we would expect to be able to increase sucrose and nitrogen concentrations together and maintain repression of lateral root initiation. Indeed, when WT seedlings were grown with 7.5% sucrose, 0.1 mM NH_4Cl , and 0.1 mM KNO_3 , initiation was significantly repressed compared with seedlings grown with higher concentrations of KNO_3 ($P < 0.001$) (Fig. 1, first two bars). This finding provides further evidence that the coincidence of high sucrose and low nitrogen concentrations is a signal for repression of lateral root initiation. For all further experiments, we used 7.5% sucrose, 0.1 mM NH_4Cl , and 0.1 mM KNO_3 as our lateral root initiation assay condition. This condition was preferred to our previous assay because of the higher concentration of nitrogen salts. This improvement relieved our concern that trace amounts of contaminating nitrate could contribute significantly to the final nitrate concentration in our experiments. The higher concentrations of nitrogen salts used in this assay also showed that severe nitrogen limitation is not essential for repression of lateral root initiation. Finally, the use of NH_4Cl and KNO_3 , as opposed to NH_4NO_3 , allowed us to assess the affects of nitrate while holding ammonia concentrations constant. Our results demonstrate that, within the context of this assay, lateral root initiation is directly correlated with exogenous nitrate concentrations.

The *lin1* mutant was isolated based on its ability to overcome the repression of lateral root initiation under high sucrose/low nitrate conditions (4). The *lin1* mutant also showed significantly increased lateral root initiation when compared with WT seedlings under the new assay conditions ($P < 0.001$) (Fig. 1 *A* and *B*). In contrast, lateral root initiation levels in *lin1* and WT plants were similar when the external nitrate concentration was increased ($P > 0.75$) (Fig. 1 *A* and *C*). Hence, the role of the *LIN1* gene in repressing lateral root initiation is specific to the high sucrose/low nitrate conditions. This finding confirms our previous prediction that *LIN1* is a component of a signaling mechanism that coordinates lateral root initiation with external nutritional cues.

The *LIN1* Gene Encodes a Putative High-Affinity Nitrate Transporter, *NRT2.1*.

We previously demonstrated that the *lin1* mutation is recessive and mapped the mutation to the top of chromosome I (4). To identify the gene associated with the mutation, the *lin1* mutant (in a Columbia background) was crossed to a WT *Arabidopsis* var. WS-0 plant. F_2 progeny showing the *lin1* phenotype were analyzed by using cleaved amplified polymorphic sequences and simple sequence length polymorphism markers in

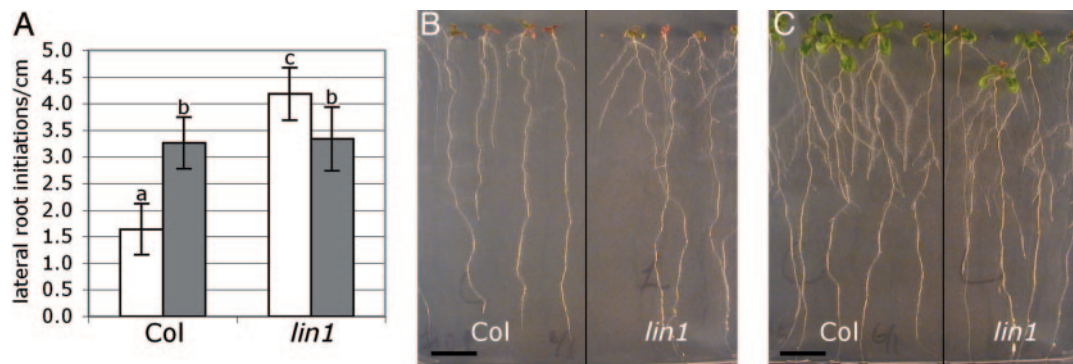


Fig. 1. High sucrose/low nitrate represses lateral root initiation in WT but not *lin1*. (A) WT (Col) and *lin1* seedlings were grown for 14 days on MS media with high sucrose/low nitrate (7.5% sucrose, 0.1 mM NH_4Cl , and 0.1 mM KNO_3 , empty bars) or high sucrose/high nitrate (7.5% sucrose, 0.1 mM NH_4Cl , and 5 mM KNO_3 , filled bars). KCl was used to balance total salt concentrations between media. Shown are means of the number of initiation events per cm of primary root length. Error bars = SD. Different letters indicate means that are significantly different based on Student's *t* test ($P < 0.005$). $n = 8-10$. (B) *lin1* is more highly branched than WT under high sucrose/low nitrate conditions. Shown are representative images of seedlings from A. (C) *lin1* is similar to WT under high sucrose/high nitrate conditions. Shown are representative images of seedlings from A. (Scale bar: 1 cm.)

the region (www.arabidopsis.org). Mapping of chromosomal breakpoints in the F_2 recombinants delimited the mutation to an ≈ 110 -kb region between 2,456 and 2,567 kb on chromosome I (see *Materials and Methods*). This region contains two putative high-affinity nitrate transporters, *NRT2.1* (At1g08090) and *NRT2.2* (At1g08100), which occur in tandem in a tail-to-tail arrangement. By comparing the sequences of the coding regions between WT Columbia and the *lin1* mutant, we discovered a base-pair difference in the *NRT2.1* gene (Fig. 2). The G at position 355 is changed to an A in *lin1*, which is predicted to change Gly-119 to arginine in the NRT2.1 protein. NRT2 proteins are predicted to have 12 membrane spanning domains; Gly-119 is predicted to be located either at the end of transmembrane region 2 or between transmembrane regions 2 and 3 on the cytoplasmic side (7). Gly-119 is part of a signature sequence of proteins in the MFS (5, 7) and is conserved in the nitrate/nitrite porter family of proteins to which the NRT2 family belongs (7). Therefore, it is likely that Gly-119 is critical for protein function.

To further confirm that the mutation in *NRT2.1* is responsible for the phenotype of *lin1*, we characterized several additional mutants with defects in this gene. Two T-DNA insertion alleles are available with inserts located in the first intron and 3' UTR of *NRT2.1* (Salk_035429 and Salk_008278, respectively) (Fig. 2). An additional mutant, *atnrt2a*, has a deletion that covers the entire *NRT2.1* gene and a portion of the adjacent *NRT2.2* gene

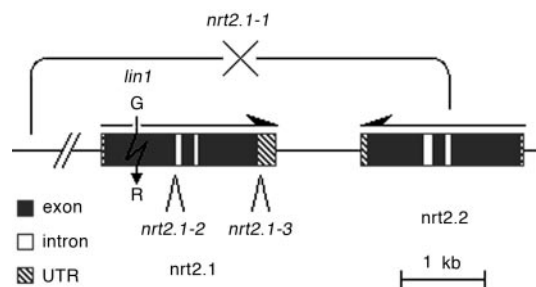


Fig. 2. Representation of genomic region of chromosome I containing *NRT2.1* and *NRT2.2*. Arrows indicate orientation of transcript. Gene structure predictions are based on annotation from The *Arabidopsis* Information Resource (www.arabidopsis.org). *nrt2.1-1*: deletion of indicated region. *nrt2.1-2*: T-DNA insertion in first intron of *NRT2.1* (Salk_035429). *nrt2.1-3*: T-DNA insertion in 3' UTR of *NRT2.1* (Salk_008278). *lin1* (*nrt2.1-4*): Gly-119 missense mutation in *NRT2.1*.

(Fig. 2); this mutant is null for both gene transcripts (11). For simplicity, we refer to the alleles in the order in which they were isolated. The deletion line is *nrt2.1-1* and the T-DNA insert lines are *nrt2.1-2* and *nrt2.1-3*. When the three *nrt2.1* mutants were grown under repressive conditions for 13–14 days (with 7.5% sucrose, 0.1 mM NH_4Cl , and 0.1 mM KNO_3) they all showed significantly increased initiation of lateral roots compared with their respective WT controls, and thus resembled the *lin1* mutant (Table 1). Furthermore, when each of these mutants was crossed to *lin1*, no complementation was seen in the F_1 generation (see Table 2, which is published as supporting information on the PNAS web site). Hence, we conclude that the *lin1* mutant phenotype is caused by a loss of function of NRT2.1 and rename the *lin1* mutant *nrt2.1-4*. We further conclude that *NRT2.1* represses lateral root initiation under specific nutritional conditions.

***lin1* (*nrt2.1-4*) Has Decreased Nitrate Uptake and Accumulation.** The discovery that *lin1* (*nrt2.1-4*) carries a mutation in a predicted nitrate transporter, known to play a key role in high-affinity nitrate uptake, immediately suggested that the phenotype is related to changes in nitrate uptake. Indeed, mutation of the same conserved glycine in the *Aspergillus nidulans* high-affinity nitrate transporter CRNA as in *lin1* (*nrt2.1-4*), and mutation of other amino acids in this signature sequence in several prokaryotic MFS proteins, lead to reduced transport activity (5, 7, 16–19). To directly assess the effect of the *lin1* (*nrt2.1-4*) mutation on nitrate uptake, seedlings were grown under the high sucrose/low nitrate conditions that repress lateral root initiation

Table 1. Three mutants in *NRT2.1* phenocopy the *lin1* mutant

Genotype	Initiations per cm	SD	<i>P</i> value
WT (Col)	2.1	0.89	
WT (WS)	3.12	1.46	
<i>nrt2.1-1</i>	5.98	0.78	<0.001
<i>nrt2.1-2</i>	4.01	1.06	<0.001
<i>nrt2.1-3</i>	4.08	1.13	0.001
<i>lin1</i>	4.62	0.97	<0.001

The *nrt2.1-1* mutant is in the WS background; all other mutants are in the Col background. Seedlings were grown for 14 days on media containing 7.5% sucrose, 0.1 mM NH_4Cl , and 0.1 mM KNO_3 . The number of lateral root initiations per cm primary root were scored as described in *Materials and Methods*. *P* values (Student's *t* test) for each genotype compared with WT control.

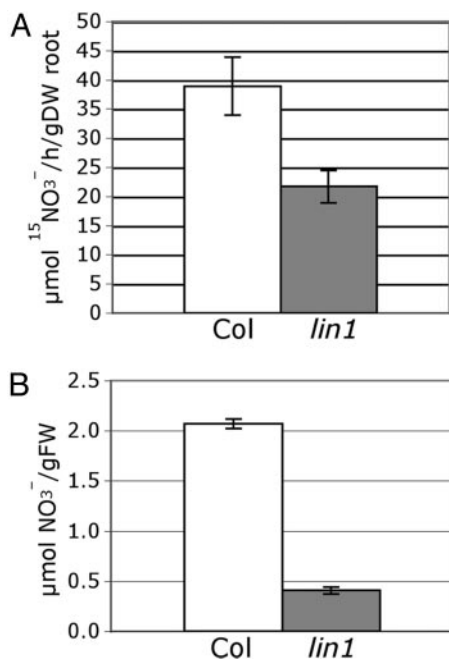


Fig. 3. *lin1* mutants are impaired in NO_3^- uptake and accumulation. WT (Col) and *lin1(nrt2.1-4)* seedlings were grown on media containing 7.5% sucrose and 0.1 mM NH_4NO_3 for 14 days. (A) $^{15}\text{NO}_3^-$ uptake was measured after 5 min in growth media containing 0.1 mM $^{15}\text{NO}_3^-$. Uptake in root tissues is shown. The values are means of five replicates of three plants each. gDW, gram dry weight. (B) Total NO_3^- content of roots. The values are means of five replicates of three plants each. gFW, gram fresh weight. Error bars = SD.

in WT, and then briefly exposed to $^{15}\text{NO}_3^-$. Nitrate influx rates were then measured (see *Materials and Methods*). The *lin1(nrt2.1-4)* mutant had a significantly reduced rate of nitrate influx compared with WT (Fig. 3A), indicating that the mutation of Gly-119 compromises the function of NRT2.1 in nitrate uptake. Total nitrate accumulation was also measured in roots of plants grown for 14 days under repressive conditions. This experiment revealed a reduction in nitrate content in the *lin1(nrt2.1-4)* mutant compared with WT (Fig. 3B). Nitrate accumulation in shoot tissues, although much lower, was also slightly reduced in the *lin1(nrt2.1-4)* mutant compared with WT, and total amino acid content in *lin1(nrt2.1-4)* was similar or slightly reduced compared with WT (data not shown). Together, these results indicate that the mutation in *lin1(nrt2.1-4)* indeed reduces nitrate uptake activity and that there are no compensatory mechanisms in the plant that increase nitrate uptake or accumulation.

The Decrease in Nitrate Uptake Does Not Explain the Derepression of Lateral Root Initiation in *lin1(nrt2.1-4)*.

We next tested whether a reduction in nitrate uptake and content in the specific range of our assay conditions could cause increased lateral root initiation. To test this possibility, we made the assumption that if we decrease external nitrate, this will result in a decrease in nitrate uptake by the plant, and therefore simulate the effects of a defective transporter. The correlation of external nitrate concentrations with nitrate uptake has been confirmed many times in studies of nitrate transport kinetics (i.e., ref. 11). An increase in lateral root initiation with decreasing nitrate uptake would be unexpected given our previous observation that low levels of external nitrate repress lateral root initiation in our assay. However, it was possible that analysis of lateral root initiation over a range of nitrate concentrations would reveal a more

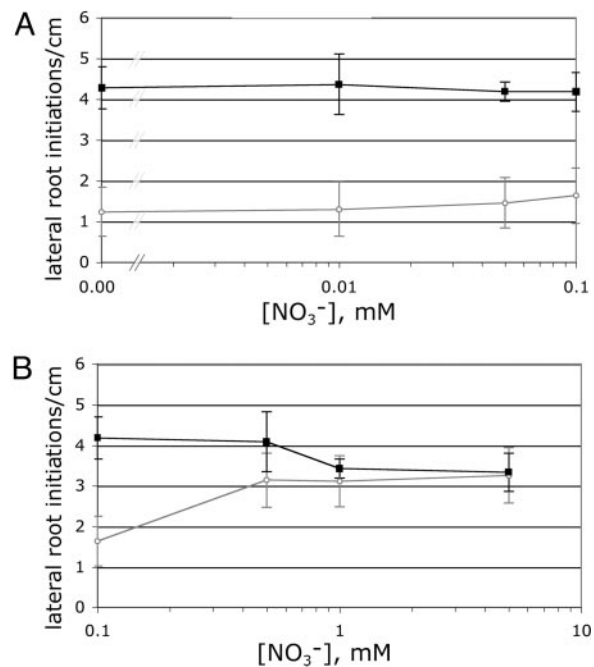


Fig. 4. The phenotype of the *lin1* mutant is not explained by its decreased nitrate uptake. Lateral root initiation in WT (Col, \circ) and *lin1(nrt2.1-4)* (\blacksquare) seedlings at various NO_3^- concentrations are shown. Seedlings were grown on media containing 7.5% sucrose, 0.1 mM NH_4Cl , and various concentrations of KNO_3 (0, 0.01, 0.05, 0.1, 0.5, 1, or 5 mM) for 13 days. Total salt concentrations were balanced with KCl. Shown are means of the number of initiation events per cm of primary root length. Error bars = SD. $n = 10$.

complex relationship between nitrate and initiation, with a “spike” in initiation at some nitrate concentration.

WT and mutant seedlings were grown on media containing 7.5% sucrose, 0.1 mM NH_4Cl , and KNO_3 concentrations equal to (0.1 mM) or less than our original assay conditions (0.05, 0.01, or 0 mM) (Fig. 4A). Lateral root initiation in WT plants either stayed constant or gradually decreased with decreasing external nitrate in this concentration range (Fig. 4A). None of the nitrate concentrations tested caused the WT seedlings to phenocopy *lin1(nrt2.1-4)*, providing no support for the initiation spike model.

As another approach to determine whether decreases in nitrate uptake could cause increased lateral root initiation, we tested whether the *lin1(nrt2.1-4)* mutant phenotype could be reversed by exogenous nitrate. Although uptake rates are compromised in the mutant, it still shows the capacity to maintain a nitrate influx (Fig. 3A). Therefore, if increases in lateral root initiation in *lin1(nrt2.1-4)* are the result of decreased nitrate uptake, we should be able to reduce lateral root initiation to the levels of repressed WT seedlings by increasing exogenous nitrate. WT and mutant seedlings were therefore grown on media containing 7.5% sucrose, 0.1 mM NH_4Cl , and KNO_3 at concentrations equal to (0.1 mM) or greater than our original assay conditions (0.5, 1, or 5 mM) (Fig. 4B). However, exogenous nitrate was unable to repress lateral root initiation in the *lin1(nrt2.1-4)* mutant to the levels seen in WT under repressive conditions (Fig. 4B). Indeed, *lin1(nrt2.1-4)* showed higher lateral root initiation rates than WT until sufficient nitrate was added to eliminate the high sucrose/low nitrate repression in WT (1 mM) (Fig. 4B).

Together, these results indicate that the reduction in nitrate uptake observed in *lin1(nrt2.1-4)* does not explain the increase in lateral root initiation. Instead, these data support the model that mutations in NRT2.1 increase lateral root initiation by

status. *NRT2.1* transcription is induced by external nitrate levels as low as 10 μM but repressed when products of nitrogen assimilation accumulate in the plant (6–10). Furthermore, sucrose and light increase *NRT2.1* transcription under low nitrate conditions, suggesting that photosynthate levels contribute to the regulation of *NRT2.1* expression (14).

It is unclear how the internal and external nutritional cues are sensed or how *NRT2.1* expression is regulated in response to these cues, but a precise mechanism clearly exists. The efficiency of exploiting *NRT2.1* expression levels to coordinate nutritional cues with both nitrate uptake and development would be extremely elegant. It also provides a model for our experimental results. In this model, an unknown sensor and signal transduction pathway would activate *NRT2.1* expression in response to high sucrose/low nitrate conditions. (Sensing may involve the *NRT2.1* protein itself, which is expressed at some basal level under all conditions examined.) Increased *NRT2.1* gene expression would result in increased *NRT2.1* protein accumulation. *NRT2.1* protein would serve as a signal transducer to activate nitrate uptake and repress lateral root initiation. The activation of nitrate uptake may simply be a result of increased amount of the *NRT2.1* protein, if this protein is actually a transporter. In contrast, both activation of nitrate uptake and repression of lateral root initiation may require a signal transduction pathway that is triggered by *NRT2.1*.

Transcriptional regulation of the *NRT2.1* gene could explain why repression of lateral root initiation is seen only under certain conditions: (i) lateral root repression is specific to high sucrose/low nitrate conditions (Fig. 1), consistent with the expectation that these conditions induce transcription of the *NRT2.1* gene (6–10, 12–14); and (ii) increases in exogenous nitrate levels relieve the repression of lateral root initiation (Figs. 1 and 4B), consistent with a reported decrease in *NRT2.1* expression in response to high nitrate (6–10, 12–14). Regulation of *NRT2.1* expression could also explain why the phenotype of the *lin1(nrt2.1-4)* mutant is condition-specific; WT plants may only differ phenotypically from a *nrt2.1* mutant under conditions where *NRT2.1* is expressed above a certain threshold level.

Developmental Plasticity and Nutrient Sensing in the Plant Root System. The number and location of lateral roots in the plant root system determine its depth, breadth, and shape. Decisions about

root system architecture are made throughout the lifetime of the plant, allowing each plant to continually optimize its structure in accordance with growth conditions. External nitrate, sulfate, and phosphate all have been shown to have dramatic effects on root system architecture (3). For example, localized patches of nitrate lead to localized increases in lateral root elongation and effective capture of the nutrient in several plants studied (3). In another example, *Arabidopsis* root systems sensing phosphate limitation increase lateral root formation at the expense of primary root elongation, perhaps to maximize the acquisition of phosphate in shallow soil layers (3). It is more difficult to rationalize why plants would repress both primary root growth (4) and lateral root initiation in response to high sucrose/low nitrate. We can speculate that our assay conditions may simulate a stress situation, where it would be in the best interest of the plant to conserve energy and limit growth. Alternatively, our high sucrose/low nitrate assay conditions may simulate a situation that would normally occur only in a small portion of the root system, where a photosynthetically active plant is providing carbon to lateral roots that are not acquiring nitrate. In this case, it might be advantageous to limit growth and root proliferation of that portion of the root system and preferentially sustain other tissues.

The mechanisms for nutrient sensing and developmental responses remain poorly understood in plants. The idea that transporter or transporter-like proteins can modulate downstream events in response to nutrient cues has been substantiated in bacteria and yeast. *NRT2.1*-mediated repression of lateral root initiation now provides a paradigm for a similar strategy in plants. The ability to modify root system morphology in response to nutrients has been shown to confer a selective advantage in many plants (3). Therefore, it is likely that, as in yeast and bacteria, the nutrient transporters have diversified to take on additional roles in nutrient signaling. In the case of lateral root initiation, the signaling pathway culminates in the regulation of specific cell divisions that give rise to lateral root founder cells in the pericycle. Understanding the mechanism by which the putative transporter *NRT2.1* regulates pericycle cell divisions in response to nutritional cues would represent a significant step forward in understanding plant developmental plasticity.

This work was supported by National Science Foundation Grant 0131690 (to J.E.M.).

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