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LeNRT2.3 functions in nitrate acquisition and long-distance transport in tomato

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ABSTRACT

Nitrogen plays an important role in plant growth and development. Nitrate transporters have been extensively studied in *Arabidopsis*, but in tomato they have not been functionally characterized. In this study, we report the functions of *LeNRT2.3* in nitrate transport in tomato. Our results show that *LeNRT2.3* is induced by nitrate, and mainly localizes to the plasma membranes of rhizodermal and pericycle cells in roots. Further analysis in *Xenopus* oocytes showed that LeNRT2.3 mediates low-affinity nitrate transport. *35S:LeNRT2.3* increased nitrate uptake in root and transport from root to shoot. More interestingly, *35S:LeNRT2.3* showed high biomass and fruit weight. Taken together, these results suggest that *LeNRT2.3* plays a double role in nitrate uptake and long-distance transport in tomato.

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1. Introduction

Nitrate (NO_3^-) concentration varies drastically in the soil, hence plants adopt two types of transport systems to take up NO_3^-, including low-affinity transport systems (LATS) and high-affinity transport systems (HATS) [1–4]. When external nitrate concentration is high (>1 mM), LATS contributes substantially to nitrate uptake, while HATS is activated at low NO_3^- concentration [1,4–6].

Two families of nitrate transporters, NPF/NRT1 and NRT2, have been identified in *Arabidopsis*, which are responsible for LATS and HATS, respectively [6]. Among the 53 members of the NPF/NRT1 family, AtNPF6.3/NRT1.1 was identified as a dual-affinity transporter, and the dual-affinity uptake is realized by phosphorylation and dephosphorylation [7–9]. Other characterized NPF/NRT1 transporters showed a broad range of substrate selectivity [10].

The *NRT2* family consists of 7 members in *Arabidopsis*. AtNRT2.1, AtNRT2.2, AtNRT2.4 and AtNRT2.5 are involved in high-affinity nitrate uptake [11–13]. AtNRT2.4 plays a double role in nitrate uptake in roots and phloem NO_3^- transport in shoots [12]. AtNRT2.5 takes part in nitrate uptake in roots and loading into the phloem during nitrate remobilization [13]. In rice, five NRT2 members have been identified [14–16]. OsNRT2.1, OsNRT2.2, and OsNRT2.3a affect nitrate transport interact with OsNAR2.1 [16]. In barley, four members of NRT2 family have been isolated [17,18]. HvNRT2.1 transports nitrate with HvNAR2.3 [19]. In *Chlamydomonas reinhardtii*, CnNRT2.1 and CnNRT2.2 act in high affinity nitrate transport [20].

Once transported into roots, nitrate is either stored in vacuoles, or assimilated to organic nitrogen and partitioned to plastids [21]. Alternatively, nitrate is loaded into xylem vessels and transported to the aerial parts [22]. AtNPF7.3/NRT1.5, AtNPF7.2/NRT1.8 and AtNPF2.9/NRT1.9 participate in the step of nitrate long-distance transport. NRT1.5 is expressed in pericycle cells, and loads nitrate into xylem [23]. AtNPF7.2/NRT1.8 is expressed in xylem parenchyma cells, and unloads nitrate from xylem [24]. AtNPF2.9/NRT1.9 is expressed in phloem companion cells, removes nitrate from the xylem sap and acts in shoot-to-root transport of nitrate [25].

Tomato is one of the most economically important vegetable crops in the world. As the major nitrogen resource, nitrate plays

Abbreviations: LATS, low-affinity transport systems; HATS, high-affinity transport systems; NRT, nitrate transporter

Authors contributions: Yan-lei Fu and Ji-ming Gong designed the research. Yan-lei Fu, Hong-ying Yi and Juan Bao performed the research and analyzed the data. And Yan-lei Fu wrote the article.

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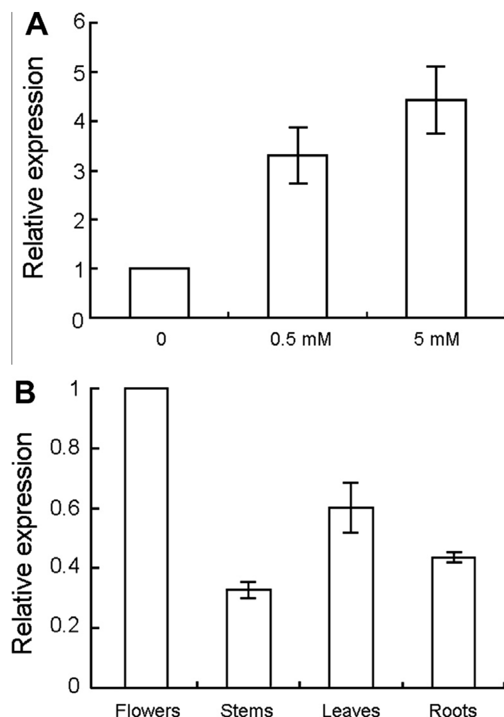

Fig. 1. *LeNRT2.3* is nitrate responsive in tomato. (A) 24-day-old plants grown hydroponically were treated with 0, 0.5 and 5 mM nitrate for 4 d. The expression levels of *LeNRT2.3* were determined by quantitative realtime-RT-PCR. (B) Relative *LeNRT2.3* mRNA expression levels in different tissues. *GAPDH* was used as an internal control. Values are mean ± S.E., *n* = 3.

an important role in plants growth and development. However, the molecular mechanisms of nitrate uptake in roots and long-distance transport are poorly understood. So far, only five genes, *LeNRT1.1*, *LeNRT1.2*, *LeNRT2.1*, *LeNRT2.2* and *LeNRT2.3* are identified. The five genes are all expressed in roots and induced by nitrate [26–28], but none of the genes are functionally studied.

In this study, we showed that LeNRT2.3 is a plasma membrane localized nitrate transporter implicated in two processes, uptake of nitrate in roots and transport of nitrate from root to shoot. This dual role of LeNRT2.3 possibly allows tomato to utilize nitrate more efficiently.

2. Materials and methods

2.1. Plant materials and growth conditions

Tomato (*Solanum lycopersicum*) ecotype Micro-Tom was used as the wild-type controls. The seeds were germinated and grown on half-strength MS plates for 7 d before being transferred to hydroponics under long-day conditions (16-h light/8-h dark) at 22 °C. Plants were grown in half-strength MS hydroponics to 4 weeks of age, and exposed to nitrate treatments as indicated.

2.2. DNA constructs and plant transformation

The *LeNRT2.3* cDNA was amplified by RT-PCR. The two restriction sites for *Bam*HI and *Spe*I were introduced using *LeNRT2.3*-1 primers (forward, 5′-ggatccatgggtgatattgaaggat-3′; reverse, 5′-acta gtcagacgcgatttggtgtta-3′). The resulting fragments were confirmed by sequencing and then subcloned into the binary vector pBI121 (predigested with *Bam*HI and *Spe*I). Tomato cotyledon explants were transformed with agrosuspension essentially as described [29]. Transgenic lines were used to further screen homozygotes and strong alleles with a segregation rate of 3:1 grown on kanamycin plates.

2.3. Quantitative RT-PCR

Total RNA was isolated from plants grown under the indicated conditions using TRIzol reagent. First-strand cDNA synthesis, quantitative RT-PCR were performed as previously described [24]. The primers used were as follows: *GAPDH* (forward, 5′-ctgctct ctcagtagccaacac-3′; reverse, 5′-cttcctccaatagcagaggttt-3′) and *LeNRT2.3*–2 (forward, 5′-tgtacacttccagtaatgttagtt-3′; reverse, 5′-gg tacccagacgcgatttggtgtta-3′).

2.4. In situ hybridization

One-week-old tomato seedlings were transferred to nitrogen-depleted medium for 3 d from half-strength MS medium. Then they were subjected to nitrate induction as indicated for 4 d. Tissue sectioning, digoxigenin labeling of RNA probe, and in situ hybridization were performed as described [24,30]. A gene-specific fragment containing the 1596-bp (1–1596) coding region of *LeNRT2.3* was amplified by PCR and cloned into pGEM T Easy vector (Promega). Sense and antisense probes were

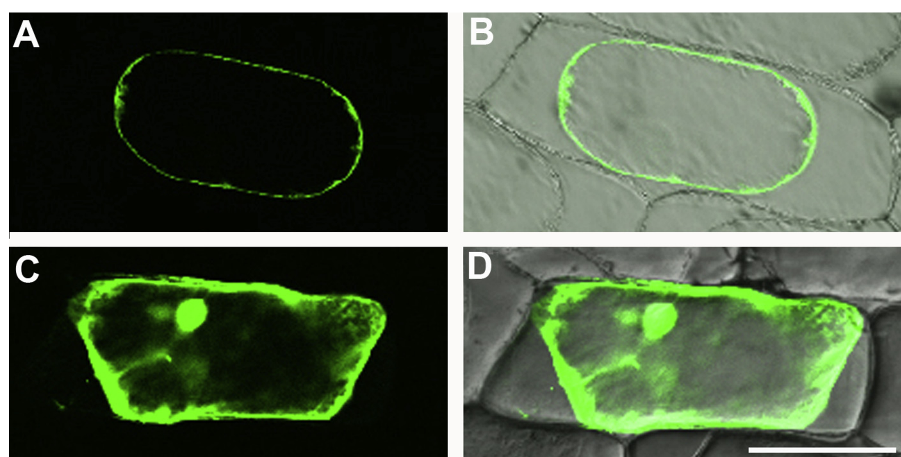

Fig. 2. *LeNRT2.3* is subcellular located to the plasma membrane. (A) Fluorescence image of epidermal cell expressing the EYFP:LeNRT2.3 fusion protein. (B) Merged control EYFP fluorescence and bright-field image. (C) Fluorescence image of epidermal cell expressing EYFP as a control. (D) Merged EYFP fluorescence and bright-field image. Bars = 100 μm in A–D.

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