Title
Regulation of NRT2.1 nitrate transporter by auxin response factor in *Arabidopsis*

Authors
Suzuki, Akinori
Kuromori, Takashi
Shinozaki, Kazuo
et al.

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INTRODUCTION

Uptake of nutrients from the soil environment requires active ion transport systems in roots. Numbers of transporters facilitating acquisition of major nutrients have been identified from *Arabidopsis*. Nitrogen is one of the macro-nutrients required for plant growth. Nitrate and ammonium are the two major forms of nitrogen utilized by various plant species. When supply of nitrogen fertilizers become limited, high-affinity-type transporters facilitating the uptake of nitrate and ammonium are expressed in roots to increase the efficiency of nitrogen acquisition (Orsel et al., 2002; Loqué et al., 2006). Regarding the uptake of nitrate, high-affinity nitrate transporter, *NRT2.1*, plays a central role in roots where its transcripts are induced and accumulated under low-nitrate conditions (Orsel et al., 2002). Besides the fundamental function of NRT2.1 in nitrate uptake, recent studies on root system architecture added intriguing feature to this transporter having inhibitory function in initiating lateral roots under a high-carbon low-nitrogen condition (Little et al., 2005).

In this study, we focused on transcriptional regulation of *NRT2.1* in *Arabidopsis*, aiming to identify transcription factors from a mutant collection. A transposon-tagged collection of transcription factors was screened by RT-PCR of the *NRT2.1* transcripts. Through this method, one can directly identify transcription factors that regulate the expression of the target gene. The method is also useful when mutants show subtle visible phenotypes. In the present study, we isolated *arf7* mutant that showed reduced number of lateral roots and increased levels of *NRT2.1* mRNA. The results suggested interesting connection of auxin signaling and nitrate response, represented by a regulatory scheme consisted of two essential components in lateral root initiation.

RESULTS AND DISCUSSION

In *Arabidopsis*, approximately 2000 genes are predicted to encode transcription factors. To identify transcription factors that may control the expression of *NRT2.1* in *Arabidopsis*, we first collected all available transposon insertion lines containing insertions of *Ds* elements within the coding region or 100 bases upstream of the 5'-untranslational region of known and predicted transcription factors from the RIKEN collection (302 lines in total; Kuromori et al., 2004). Total RNA was prepared from plants grown in liquid culture. For each line, 15 seeds were cultured for 7 days using 12-well plastic plates, and total RNA was extracted from the whole plant tissue. Quantitative real-time RT-PCR of *NRT2.1* was performed using reverse transcribed cDNA and gene specific primers. Values of *NRT2.1* transcripts were normalized with those of ubiquitin (*UBQ2*) as internal control. After statistical tests (log-normal distribution, $p<0.01$), 12 lines were selected as candidates showing significantly increased or decreased levels of *NRT2.1* mRNA from the median of all lines tested. From the secondary screening of 12 initial candidates, one line containing *Ds* element in *ARF7* was shown to express higher level of *NRT2.1* mRNA compared with the wild-type.

The *arf7* mutant isolated in this study contained a *Ds* transposon in the coding region of the *ARF7* gene. ARF proteins have specific DNA binding domains and act as transcription factors in auxin response (Ulmasov et al., 1999). In spite of their functional redundancies, several papers have reported the molecular function of ARF7. *ARF7* was originally identified as a causal gene of the *nph4* mutant which had defects in organ bending in response to auxin (Harper et al., 2000). Additionally, *ARF7* is reported to be essential for lateral root development. According to trascriptome data, *NRT2.1* shows slight increase in *arf7* mutants compared to the wild-type plants (Okushima et al., 2005), which was consistent with our results obtained from the *Ds* insertion

mutant.

Recently, several papers reported that the NRT2.1 may have a sensory function in controlling root system architecture. The evidence was provided by the identification of *lin1* mutant and its causal gene, *NRT2.1* (Little et al., 2005). The *lin1* mutants can overcome the repression of lateral root initiation caused by the combination of high sucrose and low nitrate (Little et al., 2005). On the other hand, lateral root elongation can be promoted by localized supply of nitrate. MADS box transcription factor ANR1 is suggested as a key regulator (Zhang and Forde, 1998), and root-expressed nitrate transporter NRT1.1 likely mediates nitrate signaling in the elongation process (Remans et al., 2006). Both of ARF7 and NRT2.1 control lateral root initiation, but their functions are suggested to be opposite. Although, there is still no direct genetic evidence whether these two components work in parallel or tandem in the same pathway, our results suggested that ARF7 is an upstream regulator of NRT2.1.

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