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Primary root growth, tissue expression and co-expression analysis of a receptor kinase mutant in *Arabidopsis*

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Abstract

There is no functional annotation for the majority of the several hundreds of receptor-like kinases in plants. A direct way of inferring the function of these proteins is to study the phenotype that results from loss of function mutants such as T-DNA mutant lines. In this research a function (phenotype) to At2g37050 gene that encodes a receptor like kinase in *Arabidopsis* T-DNA line was assigned. This phenotype has a shorter primary root length at later stages of development. Transcription study of the gene showed some tissue specificity with more expression level in the root in comparison with other tissues. To study genes co-expressed with At2g37050, ATTED-II web tool was used. It was found that the *CLASP* gene is co-expressed with At2g37050 with a Pearson correlation > 0.6. In kinematic analysis of the difference in root growth, the length between the root tip and the first epidermal cell with a visible root hair bulge for 8 day-old seedlings of wild type plants was 1327± 76.50 µm (n=6) and for the mutant plants, was 1109± 72.28. This parameter of the wild type and the mutant plants shows that loose of function of At2g37050 gene, reduce cell elongation in the elongation zone of root.

Key words: *Arabidopsis*, Mutant, Receptor Like Kinase, Root.

Introduction

The root system in plants is a fundamental organ. The primary functions of root are anchoring, water uptake and nutrient uptake. *Arabidopsis* has a relatively simple root structure that can easily be studied for the effects of mutations and physiological changes. The *Arabidopsis* root system can be characterized as a single primary root of which lateral roots are branching off (Benfey and Schiefelbein 1994). Stem cells at the tip of the root generate all root cell types by continuous stereotyped divisions and coordinate them by cell differentiation. After cell division in the tip, further root growth is achieved by cell expansion in the elongation zone. The mechanism of cell expansion in this zone has been studied extensively (Hauser *et al.* 1995; Verbelen *et al.* 2001). Cell elongation in roots is sensitive to various endogenous and exogenous factors such as ethylene (Le *et al.* 2001), auxin (Laskowski *et al.* 2006), ABA (Deak and Malamy 2005)

and nutrient availability (Lopez-Bucio *et al.* 2003).

Root system architecture (RSA) is also important for plant to deal with their changing conditions. RSA can be modulated through promotion or inhibition of primary root growth, induction of lateral roots (LRs), the formation of adventitious roots, and an increase in root hairs (Osmont *et al.* 2007). RSA is the result of the interplay between the genetic background of the plant, the abiotic and biotic environment of the root as well as the physiological status of the plant (Malamy 2005).

Receptor-like kinases (RLKs) in plant play important roles in perceiving the extracellular ligands and in activating the downstream pathway via phosphorylation of intracellular serine/threonine kinase domains (Osakabe *et al.* 2013). There are indications of the involvement of RLKs in root growth and development. Several classical brassinosteroid (BR) synthesis or perception mutants show a reduction in root growth as compared to wild type. For example, *bri1-5* (*Brassinosteroid insensitive 1-5*) mutant of the gene encoding BRI1, (A Leucine Rich Repeat RLK (LRR-RLK) for perception of brassinosteroids), shows an approximate 50% reduction in primary root growth (Osmont *et al.* 2007). Another indication is the severe inhibition of root growth upon the application of the CLAVATA3 (CLV3) peptide. CLV3 is a peptide ligand of the receptor complex of CLV1 and CLV2 and restricts the stem cell population in the Shoot Apical Meristem (SAM). Miwa *et al.* (2008) suggested that an RLK, together with CLV2, plays an important role in the regulation of root meristem development. More recently, de Lorenzo *et al.* (2009) reported a role of a LRR- RLK gene, *Srlk1*, from the legume *Medicago truncatula* in root growth under salinity stress.

A direct way of inferring the function of these proteins is to study the phenotype that results from the loss of function mutants. For this, the available T-DNA insertion lines of the respective genes can be screened for altered phenotypes.

In this study, the phenotype of an LRR-RLK which encode by At2g37050 in *Arabidopsis* was investigated and characterized.

Materials and Methods

Identification and analysis of T-DNA insertion mutants

The database at the SALK Institute Genome Analysis Laboratory (SIGnAL, England) was searched to identify confirmed homozygous T-DNA insertion mutants. The lines were obtained from the Nottingham *Arabidopsis* Stock Center (NASC, England). Correct insertion of T-DNA in these lines was assessed with PCR according to the protocol described by the SIGnAL website.

Plant growth conditions

Plants were grew in green house at 21°C during the16h day period and 19°C during the night period at 72% relative humidity with supplemental light when necessary. For in vitro growth of *Arabidopsis*, seeds were surface-sterilized with gaseous chlorine and transferred to half-strength MS medium, supplemented with 1% sucrose, 2.5 mM MES (2-(N-

morpholino) ethane-sulfonic acid) (Sigma) 1% agar and pH was set at 5.7 by KOH. After sowing, the plates were incubated at 4°C in the dark for three days, subsequently transferred to the growth chamber and placed vertically at 22°C, 72% relative humidity and a 16h photoperiod.

Kinematic analysis and the length of primary root

For kinematic analysis and measuring the length of primary root, from tip to the first epidermal cell with a visible root bulge, pictures of 8 day-old roots were made by an optical microscope on which a digital camera (Nikon, coolpix 990) was installed. Nine consecutive pictures were made of 3 independent roots at 10x and 20x magnification with 20s intervals. The obtained image stacks were used for calculation of velocity and strain rate profiles using RootflowRT software (Van der Weele *et al.* 2003).

The primary root length from tip to the first epidermal cell with a visible root bulge was measured.

Reverse transcript PCR analysis

After harvesting, tissues were rapidly frozen with liquid nitrogen. Total RNA was extracted with the trizol method (Chomczynski, 1997). First strand cDNAs were synthesized from total RNA with superscript II reverse transcripts (Fermentas) and Oligo (dT) primer. The cDNA amplification reactions were performed under the following conditions: 94°C for 2min, followed by 35 cycles consisting of 94°C for 20s, 55°C for 30s, and 72°C for 1min, followed by a 5min incubation at 72°C. For 35 cycles with At2g37050 primers (5'- GGTCCTTAACTTACAGAA-TGAACC-3' and 5'- CCATCAAGCC-ATAACTCA ACC-3') and tubulin primers (5'- GAGCCTTACAACGCTACTCTGTCT GTC-3' and 5'- ACACCAGACATAGA-GCAGAAATCAAG-3') for a control product.

Bioinformatics analysis

Data for characterization of T-DNA line were retrieved from TAIR (www.arabidopsis.org) and Plantsp (http://plantsp.genomics.purdue.edu/html/) web sites. Microarray data in different organs and at different ages was retrieved from Genevestigator database (https://www.genevestigator.ethz.ch/gv/index.jsp) (Zimmermann *et al.* 2004). Co-expression analysis was performed by ATTED-II database (http://atted.jp).

Results and Discussion

Characterization of the T-DNA insertion lines

The phenotypes of the T-DNA insertion lines were monitored for growth- and development-related characteristics on agar plate. According to the results the knock-out of gene At2g37050 showed a distinct phenotype with decrease in primary root length. Because of the decrease in primary root length and the increase in the number of lateral roots the gene was named branching inhibiting receptor1, (*BIR1*) and the allele with the T-DNA was named as *bri1-1*.

Tissue expression and co-expression with BIR1

Reverse transcription showed that *BIR1* transcripts could be detected in all

tissues examined (Figure 1a and b). However, they showed some tissue specificity with the highest level in the root and the lowest level in the rosette stage relatively.

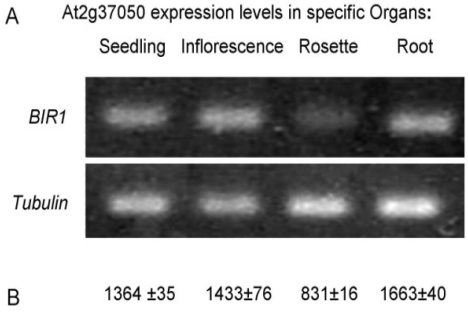

Figure 1. Tissue specific expression of the BIR1 gene using total RNA from seedlings, inflorescences, rosette leaves, and roots. A: RT-PCR of BIR1 with β-tubulin as a control. B: Expression levels (±SE) produced from Genevestigator (Zimmermann *et al.* 2004).

The ATTED-II web tool provides co-expressed gene networks for *Arabidopsis* (Obayashi 2009). In study of genes co-expressed with *BIR1*; ATTED-II web tool (http://atted.jp) the following genes were found to be co-expressed with *BIR1* with a Pearson correlation > 0.6: *CLASP* (encodes a microtubule-associated protein involved in cell expansion and division), RTV1 (related to vernalization) and *RGA1* (responsor of *GA1*-3 1) (Figure 2).

CLASP encodes a microtubule-associated protein that is located in plasma membrane and is involved in both cell division and cell expansion. Plants with the *clasp-1* allele are dwarf, with significantly reduced cell numbers in the root division zone, and defects in directional cell expansion (Ambrose *et al.* 2007).

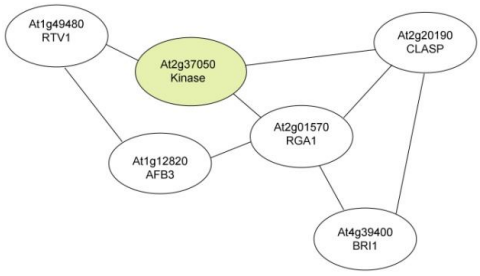

Figure 2. The co-expression network around *BIR1*. Lines are only drawn between genes when the Pearson correlation between the genes is larger than 0.6, (see http://www. atted .bio.titech.ac.jp/data/locus/AT2G37050.html).

The RLK encoded by BIR1 has a role in root architecture

The *bir1-1* mutant showed a shorter primary root length compared to wild type. The difference between mutant and wild type was not obvious during the first 7 days of growth and became more distinct after 2 weeks. Although primary root length was significantly different between wild type and mutant but root fresh weight, root dry weight and the root to shoot ratio were not significantly different. Apparently, the reduction in primary root length in the mutant is compensated by an increase in the number and the total length of the secondary roots (15 ± 3 in *bir1* and 11±2 in wild type plants).

Kinematic analysis of the difference in root growth

For a kinematic analysis (Beemster and Baskin 1998), of the difference in root growth between *bir1* and wild type plants, RootflowRT program was used. RootflowRT program identifies the rate of expansion of the different zones along the root (Van der Weele *et al.* 2003). Figure 3 shows the results of this

kinematic analysis. This figure shows the rate with which different root segments at indicated distances move in relation to the quiescent center. The slope of the line indicates the rate of movement; a horizontal line means no elongation and a positive slope means elongation. Distal to the elongation zone, the growth rates of the *bir1* mutant and wild type are similar, but in the elongation zone the rate of elongation of wild type roots is higher.

Processes that determine root architecture and functioning are also essential for agriculturally important parameters, such as pathogen sensitivity and nutrient uptake efficiency. Several genes that are involve in signal perception and transduction in root growth and root patterning, have been identified by molecular and physiological techniques, (Scheres *et al.* 2002).

The length between the root tip and the first epidermal cell with a visible root hair bulge (RTL), is a parameter to quantify effects on cell elongation (Le *et al.* 2001). Result showed that RTL for 8 days old seedlings of wild type plants growing under controlled conditions was 1327± 76.50 µm (n=6). For the *bir1-1* mutant plants the RTL was 1109± 72.28 (Figure 3a). According to kinematic analysis, the RTL of both the wild type plant and the mutant coincided with the point that the elongation ceased (Figure 3b).

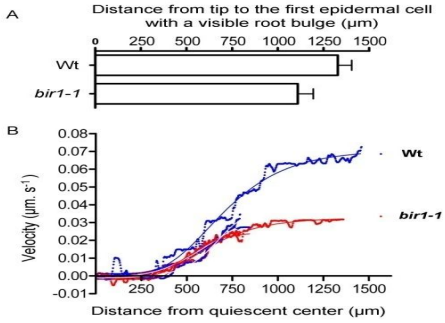

Figure 3. Root growth analysis. A: Distance from root tip to the first epidermal root with a root hair (Data is the average of 8 plants ±SD). B: Kinematic analysis of root growth in wild type and *bir1-1* plants. (Data is the average of 3 plants).

Conclusion

The RLK genes form one of the largest gene families in plants genomes. Because extracellular domains are highly diverse, RLKs potentially can recognize a wide array of ligand types. According to this study, the silences of the At2g37050 gene (*BIR1*) results in a reduction of root growth like results from the silences of the CLASP gene. However, in *bir1* mutants; shoot growth is normal and there is no obvious effect of the mutant on the shoot. This phenotype might suggest that BIR1 functions upstream of CLASP and only in the root. From the detailed kinematic analysis it becomes clear that mutations of *BIR1* caused a reduction in the rate of elongation in the elongation zone.

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آنالیز رشد ریشه اولیه؛ بیان ژن و هم‌بیانی ژن در یک موتان گیرنده کینازی در آرابیدوپسیس

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چکیده:

صدها شبه گیرنده کینازی در گیاهان وجود دارد که نقش و عملکرد اکثر انها هنوز نا شناخته است. روش مسقیم برای بررسی نقش این پروتئین‌ها، مطالعه فنوتیپ گیاهان حاصل از خاموشی ژن مربوطه از جمله استفاده از لاین‌های موتان T-DNA است. در این تحقیق نقش ژن At2g37050 که یک شبه گیرنده کینازی غنی از لوسین را در گیاه آرابیدوپسیس کد می‌کند، مطالعه گردید. با بررسی لاین‌های موتان T-DNA مربوطه، فنوتیپ کاهش در رشد ریشه چه اولیه مشاهده شد. بیان ژن در گیاهان نرمال نشان داد که میزان بیان At2g37050 در ریشه نسبت به دیگر بافت‌ها بیشتر است. برای بررسی ژنهای مرتبط با At2g37050 از پایگاه ATTED-II استفاده گردید و مشخص شد که این ژن با ژن *CLASP* که از ژن‌های مرتبط در رشد و نمو ریشه است با ضریب همبستگی پیرسون بزرگتر از ۰/۶ هم‌بیانی دارد. میزان رشد در ناحیه رشد (Elongation zone) ریشه در گیاهان نرمال و گیاهان موتان ژن At2g37050 بررسی شد. بر اساس نتایج این مقدار برای گیاهان نرمال برابر (n=6) 76.50 µm ±1327 و برای گیاهان موتان برابر 72.28 µm ±1109 بود. این اطلاعات برای اولین بار نشان می‌دهد که ژن At2g37050 که یک شبه گیرنده کینازی را کد می‌کند، در رشد ریشه اولیه در ناحیه رشد نقش دارد.

کلمات کلیدی: آرابیدوپسیس، ریشه، شبه گیرنده کینازی، موتان.