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Gene Note

Phenome analysis of root development in *Arabidopsis*

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Abstract Mutant lines covering all *Arabidopsis* genes allow us to pursue systematic functional genomics. A comprehensive phenotypic description, called phenome, is highly sought after in the profiling of –omics. We previously selected 4,000 transposon-insertional lines with transpson insertion in their gene-coding regions, systematically observed and recorded the visible phenotype of aerial portion of each line (ecotype Nossen) (Kuromori et al. 2006). In this paper, we tried to observe root phenotypes of seedling in these transposon-insertional lines with transpson insertion in their gene-coding regions. For example, we measured the length of main root and the length of the root-hair. We totally observed root-phenotypes of 1817 lines, and identified seven phenotypes in roots. We also ordered alleles from Salk-knock-out lines (ecotype Col-0) to make sure that one line showed the similar phenotype among them.

Key words: *Arabidopsis*, phenome, root.

Functional genomics of many model organisms is progressing rapidly after completion of the sequencing of their genomes. A direct method of investigating gene function is to assess the phenotype of knockout mutants of genes. Large-scale collection of gene knock-out or knockdown mutants have been generated in experimental organisms (Fraser et al. 2000; Maeda et al. 2001; Ross-Macdonald et al. 1999; Winzeler et al. 1999). Several recent reports of phenotyping analysis have used a variety of mutant resources for model organism. For example, in *Saccharomyces cerevisiae*, all predicted genes have been deleted individually, and quantitative phenotypic analysis has been done (Giaever et al. 2002). In *Caenorhabditis elegans* and *Drosophila*, large-scale analysis of gene function has been achieved through the use of RNA interference (RNAi) techniques (Boutros et al. 2004; Kamath et al. 2003). In mammals, large scale mutagenesis and phenotyping projects are progressing (Berns et al. 2004; Paddison et al. 2004)

Previously, Kuromori et al. and Ito et al. constructed 1817 transoposon-insertional lines of *Arabidopsis* (ecotype: Nossen) by using the *Activator* (*Ac*)/ *Dissociation* (*Ds*) system, to collect insertional mutanats as resources for functional genomics (Ito et al. 2002; Ito et al. 2005; Kuromori et al. 2004). In our previous work, we selected about 40,000 transoposon-insertional lines, each of which had *Ds* transpson in a gene-coding region, and examined visible aerial phenotypes systematically at each stage (Kuromori et al. 2006).

In this paper, we selected about 1,817 transoposon-insertional lines and examined visible phenotypes of root systematically at each stage. We identified seven phenotypes in seedlings.

For example, the main root of Ds 16-0310-1 showed a short-root. Ds insertion was found in the third exson of At1g27070 at Ds 16-0310-1 plant. Ds insertion was also found in the first exson of At1g27070 at Ds 53-2093-1 plant (Figure 1; Ito et al. 2002).

The length of the main root of 11-2956-1, in which At1g01940 was disrupted by Ds insertion, was short. We obtained the allele of At1g01940 from Salk knock out line, which was SALK_061120 (Figure 2; Alonso et al. 2003).

We collected data by visual observation of seedlings of 1,817 transposon-insertion lines. We concentrated on visible phenotypes in this trial as a first step in root phenome analysis of *Arabidopsis*. We found seven mutants out of 1,817 lines (0.7% lines grown). Table 1 lists mutants. However, visible phenotypes are only part of the total phenome. Finer evaluation by digital treatment provides another type of physical analysis of the phenome. For example, Boyes et al. reported a monitoring system for digital evaluation of *Arabidopsis* growth (Boyes et al. 2001). Our results provide basic

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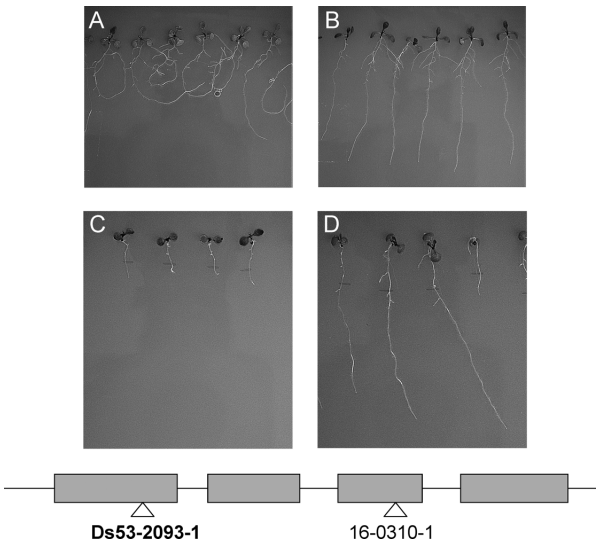

Figure 1. Root phenotype of At1g27070. (A)16-0310-1 (B) Parental line (C) Ds53-2093-1 (D) Ds53. Parental line Short root was observed in (A) and (C). For the observation of the seedlings, plants were grown on the surface of agar plates at vertically (Okada and Shimura 1990). Plants were grown at 22°C by continuous-light. For observation of the seedling images were obtained with a three-dimensional digital fine scope (VC4500-PC; Omron, Tokyo, Japan).

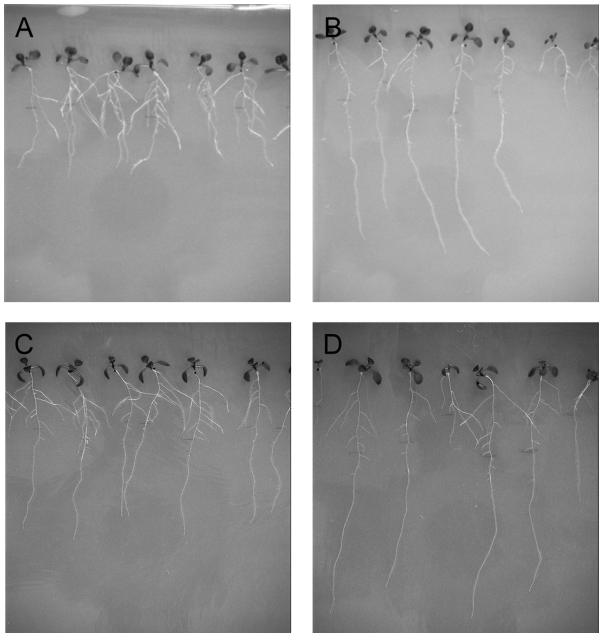

Figure 2. Root phenotype of At1g01940. (A)11-2956-1 (B) Parental line (C) Salk_061120 (D) Col-0 wild type. Short root is observed in (A) and (C). For each line, at least ten individual seedlings were observed. To establish homozygote line from Salk T-DNA insertional line, we used the primer LBa1(TGGTTCACGTAGTGGGCCATG) and each gene-specific primer for PCR (Kuromori et al. 2006).

Table 1. List of root mutants

Ds number	Mips code	Phenotype	Gene product	Salk line number
11-2956-1	At1g01940	short root	peptidyl-prolyl cis-trans isomerase cyclopphillin-type family proteine	SALK_061120
13-3223-1	At3g18170	short root hair	expressed protein	SALK_004788
12-4212-1	At1g64960	short root	expressed protein	SALK_049790
12-4701-1	At1g61410	abnormal root-hair	tolA potein-related	SALK_093939
13-4796-1	At1g10990	few root-hair	expressed protien	SALK_073189
15-1096-1	At4g26690	few root-hair	glycerophosphoryl diester phosphodiestrate family protein	SALK_008071

data for expanding finer phenotyping, and will help improve digital phenotyping by automated monitoring for use in systems biology in future.

Bouche and Bouchez reported that fewer than 2% of T-DNA lines display significant alternations (Bouche and Bouchez 2001). Kuromori et al. find 3% visible phenotype on the aerial portion (Kuromori et al. 2006). One reason for our lower frequency might be that it is difficult to detect the phenotype of the root, because the structure of the root is relatively simple comparing to the aerial portion, which has leaf, stem, and flower.

We selected about 1,817 transoposon-insertional lines and examined visible phenotypes of root systematically at each stage, and identified seven phenotypes in root. We also ordered alleles from Salk-knock-out lines (ecotype Col-0) to make sure that some lines show the similar phenotype among them. One line showed the similar phenotype between Nossen and Col-0. Rest of them in Col-0 background did not show phenotypes, which were seen in Nossen background (Alonso et al. 2003). This might be due to allele difference or point mutations were occurred in other genes of these mutants.

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