PLANT SIGNALING & BEHAVIOR
2017, VOL. 12, NO. 10, e1368940 (5 pages)
https://doi.org/10.1080/15592324.2017.1368940

Taylor & Francis
Taylor & Francis Group

SHORT COMMUNICATION

Enhancement of cell wall protein SRPP expression during emergent root hair development in *Arabidopsis*

Hiroshi Uno[a,†], Natsuki Tanaka-Takada[a,b,†], Ryosuke Sato[a], and Masayoshi Maeshima[a]

[a]Laboratory of Cell Dynamics, Department of Biological Mechanisms and Functions, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan; [b]Department of Plant Sciences, University of Oxford, South Parks Road, Oxford, UK

ABSTRACT

SRPP is a protein expressed in seeds and root hairs and is significantly induced in root hairs under phosphate (Pi)-deficient conditions. Root hairs in the knockout mutant *srpp-1* display defects, i.e., suppression of cell growth and cell death. Here, we analyzed the expression profile of *SRPP* during cell elongation of root hairs and compared the transcript levels in several mutants with short root hairs. The mRNA level was increased in wild-type plants and decreased in mutants with short root hairs. Induction of *SRPP* expression by Pi starvation occurred one or two days later than induction of Pi-deficient sensitive genes, such as *PHT1* and *PHF1*. These results indicate that the expression of *SRPP* is coordinated with root hair elongation. We hypothesize that SRPP is essential for structural robustness of the cell walls of root hairs.

ARTICLE HISTORY
Received 28 July 2017
Revised 14 August 2017
Accepted 15 August 2017

KEYWORDS
Arabidopsis thaliana; cell wall; phosphate deficiency; root hair; seed and root hair protective protein; transcription regulation

Root hairs are specialized epidermal cells with large surface areas exposed to the soil, and they exhibit tip growth.[1,2,3] The physiological roles of root hairs have been investigated.[1,4,5,6] Root hairs must maintain water and nutrient absorption capacity and integrity to protect them from injury and pathogen attack. A unique protein, the seed and root hair protective protein (SRPP), was recently identified in the cell walls of root hairs and seeds.[7] The *SRPP* (*SEED AND ROOT HAIR PROTECTIVE PROTEIN*) gene was identified previously as *root hair–specific 13* (*RHS13*) by microarray analysis.[8] SRPP is highly expressed and the protein was detected in root hairs grown under phosphate (Pi)-deficient conditions. SRPP knockout mutants have short, bent root hairs and root hair death as phenotypic properties.[7] SRPP consists of 165 amino-acid residues and is rich in basic residues (pI, 9.21). A protein linked to green fluorescent protein was detected in the cell wall spaces of root hairs. Although the primary sequence of SRPP is similar to those of proline-rich proteins, the protein lacks a proline-rich motif. Molecular and biochemical information on SRPP is currently limited.

Here, we focused our attention on the expression profile of the *SRPP* gene and examined whether enhancement of *SRPP* expression is triggered by Pi depletion or coupled with emergent root hair elongation. The purpose of this study was to determine whether SRPP is involved in cell differentiation or root hair elongation. We determined the transcription levels of *SRPP* in the roots of short-root-hair and no-root-hair mutant seedlings, as well as in wild-type plants. The mRNA levels after Pi depletion treatment were also determined. Furthermore, genes co-expressed with *SRPP* were analyzed in the mutants and under Pi-deficient conditions.

SRPP is expressed in root hairs and seeds. This study focused on characteristics of the *SRPP* gene in root hairs. We investigated the relationship between root hair length and *SRPP* transcription level in wild-type (strain, Columbia-0), NR23#2-1, NR23#4–12, *pip5k3 pip5k4*, and *cpc try* seedlings. The transgenic lines NR23#2–1 and NR23#4–12 express a 23-amino-acid peptide in the N-terminal region of plasma membrane–associated cation-binding protein-2 (PCaP2)[9,10] under the control of the root hair-specific *EXPANSIN A7* promoter.[11] NR23#2–1 has short root hairs and NR23#4–12 has no root hairs.[11] The double mutant *pip5k3 pip5k4*, which has mutations in the phosphatidylinositol-(4,5)-bisphosphate–generating isoenzymes, which are localized in the plasma membrane, has very short root hairs with a normal distribution density.[12] A double mutant of the transcription factors CPC and TRY (*cpc try*) was reported to have abnormal trichomes and no root hairs.[13] The phenotypes of the mutants were observed constantly (Fig. 1B). The transcription levels of SRPP were less than 20% that of Col-0 in NR23#4–12 and negligible in *cpc try* (Fig. 1A). The levels in the NR23#2–1 and *pip5k3 pip5k4* roots were 70% and 50% that of Col-0, respectively, and higher than that of NR23#4–12. The results indicate a correlation between root hair length and *SRPP* expression level.

The number and length of root hairs increase under Pi-deficient conditions.[14] When 14-day-old Col-0 seedlings grown on normal agar plates were transferred to Pi-deficient plates, the distribution density and length of root hairs increased

CONTACT Masayoshi Maeshima maeshima@agr.nagoya-u.ac.jp Laboratory of Cell Dynamics, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, 464-8601, Japan.
[†]These authors contributed equally to this work.
Supplemental data for this article can be accessed on the publisher's website.

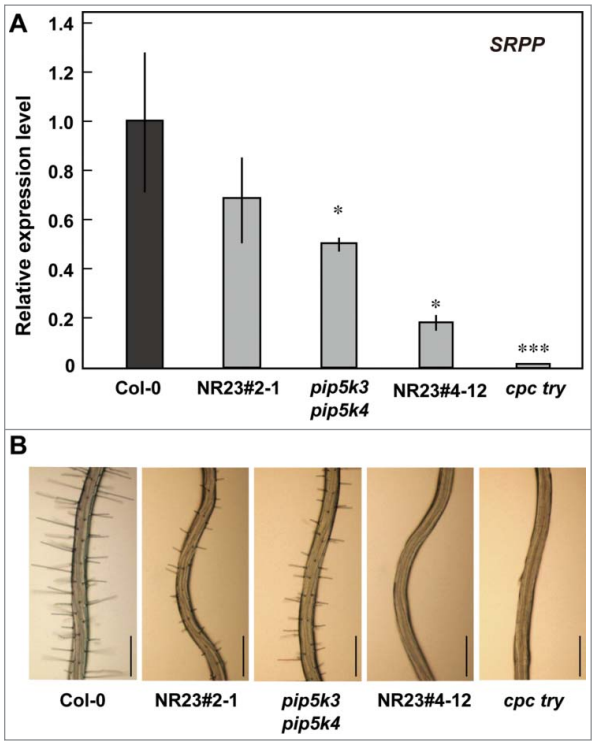

Figure 1. Transcription levels of *SRPP* in roots of Col-0 and mutant seedlings. (A) Expression levels of *SRPP* in Col-0, NR23, *pip5k3 pip5k4*, and *cpc try* grown on agar plates containing half-strength Murashige-Skoog salt mixture (0.5 × MS) medium. Total RNA fractions were prepared from roots of 12-day-old Col-0 and mutant plants using a QIA shredder and RNeasy Mini kit (Qiagen). RNA (0.5 μg) was converted into cDNA using ReverTra Ace qPCR RT Master Mix with gDNA Remover (Toyobo). Then the samples were subjected to real-time RT-PCR analyses of mRNA levels of *SRPP*. Relative mRNA contents were normalized to the *TIP41* transcript. Four replicates of 60 plants were averaged. Values are expressed as means ± SDs. Significant differences are indicated by asterisks ($^{*}P < 0.05$, $^{***}P < 0.005$). (B) Roots of 12-day-old seedlings at a position around 20 mm from the root tip were observed through the microscope. Scale bars = 500 μm.

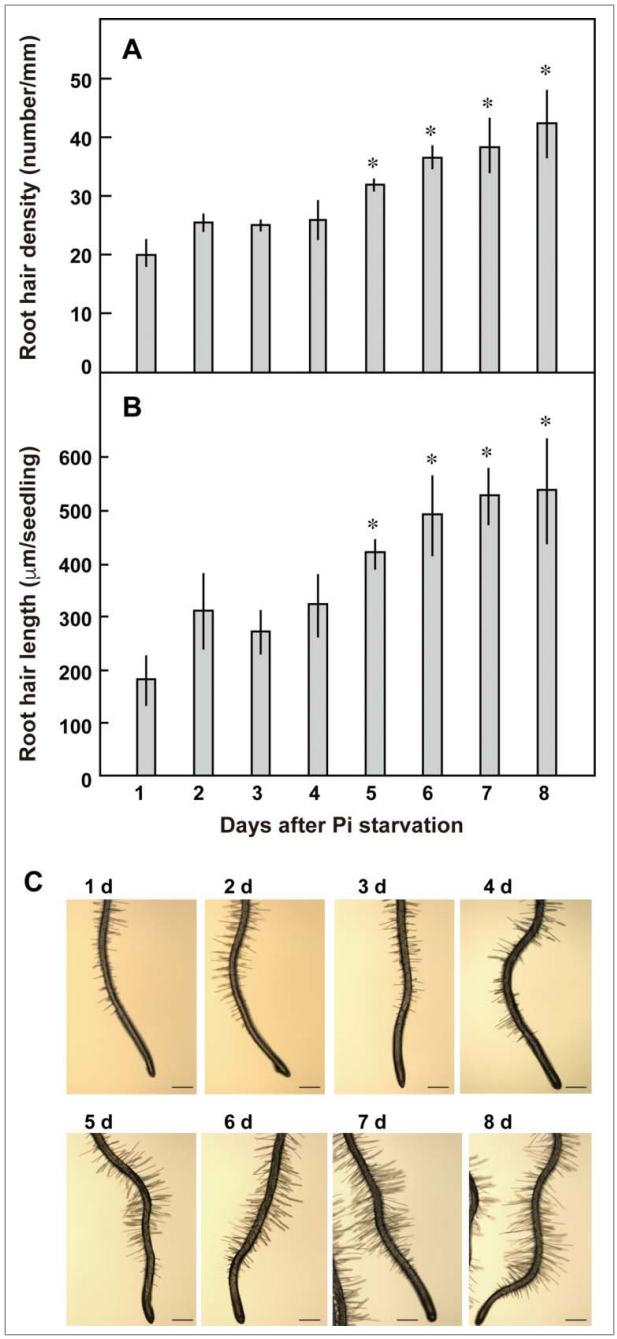

Figure 2. Changes in distribution density and length of root hairs in roots of Col-0 and mutant seedlings under Pi-deficient conditions. Col-0 seedlings grown under normal conditions (0.5 × MS) (Pi, 280 μM) for 14 days were transplanted to Pi-deficient Hoagland medium (Pi, 0 μM). Day 0 is the day of transplantation. Root hair morphology was observed using a BX61 optical microscope (Olympus) equipped with a DP70 CCD camera and an SZ61 stereoscope. Root hair length was measured from photomicrographs. The free software package ImageJ (http://rsbweb.nih.gov/ij/) was used to measure root hair length. (A) The three seedlings were subjected to measurement of root hair number in the region 20–30 mm from the root tip. (B) The three seedlings were used to measure root hair length. Root hairs in the region 30 mm from the root tip (root hair maturation region) were measured. Values are expressed as means ± SDs. Root numbers were 15 to 28 for each measurement. Asterisks indicate significant differences from day 0 at $^{*}P < 0.005$. (C) Typical roots with root hairs in the region 20–30 mm from the root tip were observed every day through a microscope.

significantly after 5 days (Fig. 2A, B). In this experiment, the distribution density and length of root hairs were determined in the region around 30 mm from the root tip. The results show that Col-0 roots require 5 days to induce root hairs.

The SRPP protein was not detected in seedlings grown under normal conditions,[7] but SRPP mRNA was detected before Pi depletion treatment (Fig. 3A). The transcription level of *SRPP* was increased after 5 days, in parallel with the increases in the distribution density and length of root hairs, under Pi-deficient conditions (Fig. 3A). No difference in transcription level was detected on the first day (4, 6, and 8 hours after transplantation; data not shown). We compared the induction of *SRPP* with that of other genes. *Phosphate transporter 1* (*PHT1*) and *phosphate transporter traffic facilitator 1* (*PHF1*) are genes that respond rapidly to Pi deficiency.[6,15] The expression of *PHT1* was clearly induced on day 3 after Pi starvation, and that of *PHF1* was clearly induced on day 4. Therefore, *PHT1* and *PHF1* may be first-phase genes and *SRPP* may be a second-phase gene in response to Pi deficiency. The results suggest that SRPP gene expression is required for the maturation of root hairs, and not for the initial stage of root hair differentiation, under Pi-deficient conditions.

We surveyed genes co-expressed with *SRPP* under normal physiological conditions using the ATTED-II open database (http://atted.jp).[16] Several genes, including peroxidase superfamily proteins, proline-rich proteins, actin depolymerizing factors, and expansins, were listed with mutual ranks lower than 16. Expansins A7 and A18, obtained in this search, are

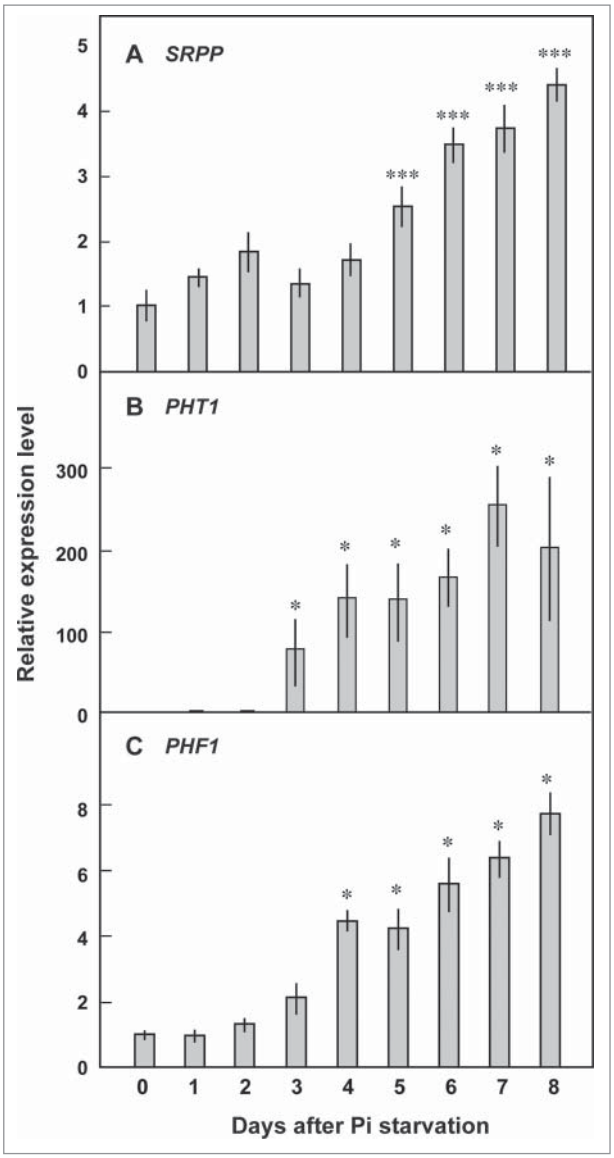

Figure 3. Changes in transcription levels of *SRPP*, *PHT1*, and *PHF1* under Pi-deficient conditions. Col-0 seedlings grown under normal conditions (Pi, 280 μM) for 14 days were transplanted to Pi-deficient medium (Pi, 0 μM). Total RNA fractions were prepared from roots of seedlings each day after transplantation and then subjected to real-time RT-PCR to quantify the mRNA levels of *SRPP* (A), *PHT1* (B), and *PHF1* (C). Four replicates of 21–38 plants were averaged. Values are expressed as means ± SDs. Significant differences from day 0 are indicated by asterisks ($^{*}P < 0.005$).

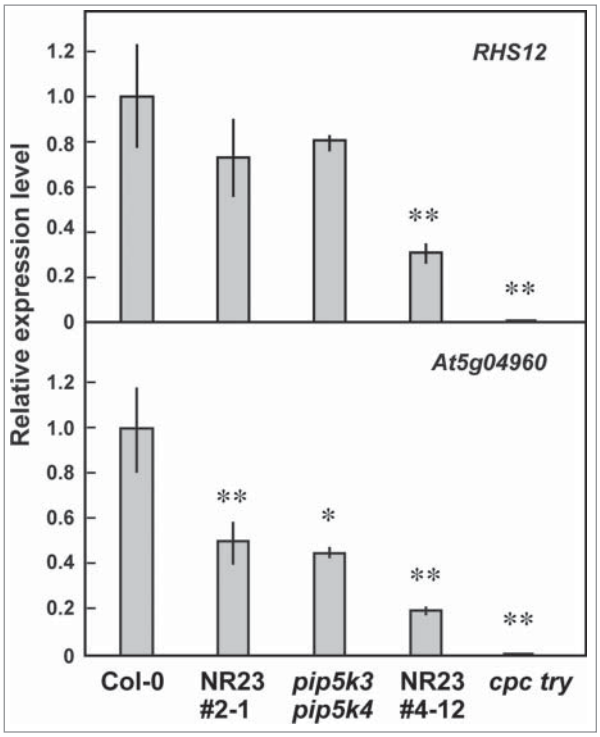

Figure 4. Expression levels of the two *PME* (*pectin methylesterase*) genes in Col-0, NR23, *pip5k3 pip5k4*, and *cpc try* grown on agar plates under normal conditions. Total RNA fractions were prepared from roots of 12-day-old Col-0 and mutant seedlings and then subjected to quantification of mRNA levels of *RHS12* and *At5g04960*. Four replicates of 60 seedlings were averaged. Seedlings were grown on agar plates containing 0.5 × MS medium. Values are expressed as means ± SDs. Significant differences are indicated by asterisks ($^{*}P < 0.01$, $^{**}P < 0.005$).

root hair–specific genes, and their translation products function to loosen the cell wall.[8,17,18] Some peroxidases localized in the cell walls are essential for root hair development.[8] We selected two genes encoding pectin methylesterases, *At5g04960* (pectin methylesterase inhibitor superfamily/pectin methylesterase) and *At3g10710* (*RHS12*; pectin methylesterase). Both genes were reported to be expressed in roots and root hairs in the open databases TAIR and Arabidopsis eFP Browser. We hypothesized that the positively charged *SRPP* (pI, 9.21) would interact with the negatively charged group of pectin (galacturonic acid moiety) in the cell wall. The *RHS12* expression levels were 30% that of Col-0 in NR23#4–1, negligible in *cpc try*, and relatively low in NR23#2–1 and *pip5k3 pip5k4* (Fig. 4). The expression level of *At5g04960* had a similar profile to that of *RHS12*. The results indicate that both gene expression profiles are related to the length and presence/absence of root hairs as well as *SRPP*.

We investigated the responses of the two genes to Pi deficiency in Col-0. The *RHS12* and *At5g04960* genes were induced after 2 and 4 days, respectively, by Pi deficiency, and their relative expression levels reached four and three times, respectively, those on day 0 (Fig. 5). Therefore, these two pectin methylesterase genes tend to be expressed during root hair elongation and in response to Pi deficiency. Their transcriptional properties and cell-wall localization are similar to those of SRPP. However, the inductions of *RHS12* and *At5g04960* are a few days earlier than that of *SRPP*. This may be reflection of their physiological role as pectin methylesterases, which may be required at early stage of root hair development compared with SRPP.

In conclusion, this study revealed the following with regard to *SRPP* gene expression. (1) *SRPP* gene expression was suppressed in mutant roots with short root hairs or no root hairs. Enhancement of *SRPP* did not occur in the early stage of Pi-deficiency response in seedling roots, but in the later stage, after the induction of sensitive genes, such as *PHT1* and *PHF1*. (2) Two pectin methylesterase genes (*At5g04960*; *RHS12*), which were listed as RHS genes co-expressed with *SRPP* in the ATTED-II database, were enhanced by Pi deficiency and suppressed in mutant lines with no or short root hairs. Consistent with a previous report,[7] the present observations suggest that SRPP is involved in the

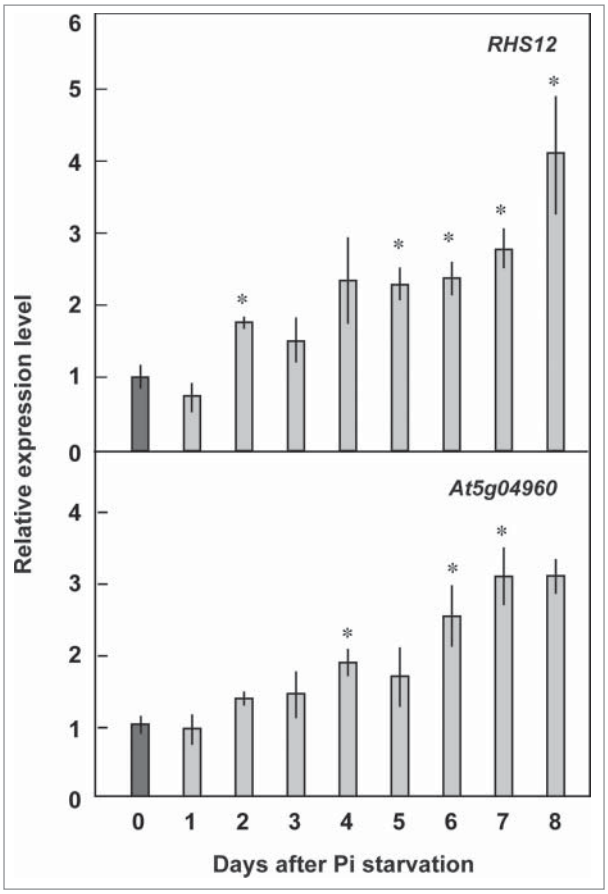

Figure 5. Responses of two PME genes to Pi deficiency. Expression levels of *RHS12* and *At5g04960* in Col-0 in response to Pi-deficient conditions. Col-0 seedlings grown under normal conditions (Pi, 280 μM) for 14 days were transplanted to Pi-deficient medium (Pi, 0 μM). Total RNA fractions were prepared from Col-0 roots every day after transplantation to Pi-deficient medium (day 0, day of transplantation), and then subjected to real-time RT-PCR analyses. Four replicates with 21–38 roots were averaged. Results are means ± SDs. Asterisks, $P < 0.005$.

formation of cell walls during emergent root hair development induced by Pi deficiency, but not in root hair differentiation.

Although SRPP is known to function in the cell wall, further studies may provide more details on cell wall architecture and the regulation of cell wall rigidity and plasticity. Positively charged SRPP molecules are thought to bind electrostatically to negatively charged demethylated pectins. Pectin is a key component of the cell wall and determines its structural rigidity.[19,20] Co-expression of pectin methylesterase genes with SRPP may be involved in the reformation of the cell wall structure. Then, demethylated pectin interacts with SRPP and calcium ions, forming a tertiary network of pectin fibers. Biochemical analyses of the interaction between SRPP and pectin remain to be conducted.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Acknowledgments

We are grateful to Yoichi Nakanishi, Miki Kawachi, and Shoji Segami (Nagoya University, Japan) for their valuable advice and to Takashi Aoyama (Kyoto University, Japan) and Takuji Wada (Hiroshima University, Japan) for sharing with us the double mutants *pip5k3 pip5k4* and *cpc try*, respectively. We would also like to thank the Riken Bioresource Center (Tsukuba, Japan) for delivering seeds of the *srpp-1* mutant (RATM13-5238-1).

Funding

This work was supported by JSPS KAKENHI (grant nos. 26252011 and 26113506 to M.M.) and by a Grant-in-Aid for JSPS Fellows (no. 26002201 to N.T.-T.).

Abbreviations

N23	23-amino-acid peptide in the N-terminal region of PCaP2
NR23	no root hair line that expresses N23
PCaP2	plasma membrane–associated cation-binding protein-2
PHF1	phosphate transporter traffic facilitator 1
PHT1	Phosphate transporter 1
Pi	inorganic phosphate
RHS	root hair specific
SRPP	seed and root hair protective protein

References

1. Datta S, Kim CM, Pernas M, Pires ND, Proust H, Tam T, Vijayakumar P, Dolan L. Root hairs: development, growth and evolution at the plant–soil interface. Plant Soil. 2011;346:1–14. doi:10.1007/s11104-011-0845-4.
2. Grebe M. The patterning of epidermal hairs in *Arabidopsis*—updated. Curr Opin Plant Biol. 2012;15:31–37. doi:10.1016/j.pbi.2011.10.010.
3. Kim CM, Dolan L. ROOT HAIR DEFECTIVE SIX-LIKE class I genes promote root hair development in the grass *Brachypodium distachyon*. PLoS Genet. 2016;12:e1006211. doi:10.1371/journal.pgen.1006211.
4. Gilroy S, Jones DL. Through form to function: root hair development and nutrient uptake. Trends Plant Sci. 2000;5:56–60. doi:10.1016/S1360-1385(99)01551-4.
5. Libault M, Brechenmacher L, Cheng J, Xu D, Stacey G. Root hair systems biology. Trend Plant Sci. 2010;15:641–650. doi:10.1016/j.tplants.2010.08.010.
6. Tanaka N, Kato M, Tomioka R, Kurata R, Fukao Y, Aoyama T, Maeshima M. Characteristics of a root hair-less line of *Arabidopsis thaliana* under physiological stresses. J Exp Bot. 2014;65:1497–1512. doi:10.1093/jxb/eru014.
7. Tanaka N, Uno H, Okuda S, Gunji S, Ferjani A, Aoyama T, Maeshima M. SRPP, a cell-wall protein involved in development and protection of seeds and root hairs in *Arabidopsis thaliana*. Plant Cell Physiol. 2017;58:760–769.
8. Won SK, Lee YJ, Lee HY, Heo YK, Cho M, Cho HT. *Cis*-element- and transcriptome-based screening of root hair-specific genes and their functional characterization in *Arabidopsis*. Plant Physiol. 2009;150:1459–1473. doi:10.1104/pp.109.140905.
9. Ide Y, Nagasaki N, Tomioka R, Suito M, Kamiya T, Maeshima M. Molecular properties of novel, hydrophilic cation-binding proteins associated with the plasma membrane. J Exp Bot. 2007;58:1173–1183. doi:10.1093/jxb/erl284.
10. Kato M, Nagasaki-Takeuchi N, Ide Y, Maeshima M. An *Arabidopsis* hydrophilic Ca^{2+}-binding protein with a PEVK-rich domain, PCaP2, is associated with the plasma membrane and interacts with calmodulin and phosphatidylinositol phosphates. Plant Cell Physiol. 2010;51:366–379. doi:10.1093/pcp/pcq003.
11. Kato M, Aoyama T, Maeshima M. The Ca^{2+}-binding protein PCaP2 located on the plasma membrane is involved in root hair development as a possible signal transducer. Plant J. 2013;74:690–700. doi:10.1111/tpj.12155.
12. Kusano H, Testerink C, Vermeer JE, Tsuge T, Shimada H, Oka A, Munnik T, Aoyama T. The Arabidopsis phosphatidylinositol phosphate 5-kinase

PIP5K3 is a key regulator of root hair tip growth. Plant Cell. 2008;20:367–380. doi:10.1105/tpc.107.056119.
13. Schellmann S, Schnittger A, Kirik V, Wada T, Okada K, Beermann A, Thumfahrt J, Jürgens G, Hülskamp M. TRIPTYCHON and CAPRICE mediate lateral inhibition during trichome and root hair patterning in *Arabidopsis*. EMBO J. 2002;21:5036–5046. doi:10.1093/emboj/cdf524.
14. Péret B, Clément M, Nussaume L, Desnos T. Root developmental adaptation to phosphate starvation: better safe than sorry. Trends Plant Sci. 2011;16:442–450. doi:10.1016/j.tplants.2011.05.006.
15. Heungsop S, Hwa-Soo S, Gary RD, Maria JH. Phosphate transport in *Arabidopsis*: Pht1;1 and Pht1;4 play a major role in phosphate acquisition from both low- and high-phosphate environments. Plant J. 2004;39:629–642. doi:10.1111/j.1365-313X.2004.02161.x.
16. Aoki Y, Okamura Y, Tadaka S, Kinoshita K, Obayashi T. ATTED-II in 2016: a plant coexpression database towards lineage-specific coexpression. Plant Cell Physiol. 2016;57:e5. doi:10.1093/pcp/pcv165.
17. Cosgrove DJ. Loosening of plant cell walls by expansins. Plant Cell. 2000;407:321–326.
18. Cho HT, Cosgrove DJ. Regulation of root hair initiation and expansin gene expression in Arabidopsis. Plant Cell. 2002;14:3237–3253. doi:10.1105/tpc.006437.
19. Wolf S, Greiner S. Growth control by cell wall pectins. Protoplasma. 2012;249:S169–S175. doi:10.1007/s00709-011-0371-5.
20. Daher FB, Braybrook SA. How to let go: pectin and plant cell adhesion. Front Plant Sci. 2015;6:523. doi:10.3389/fpls.2015.00523.