Cell Research (2009) :1309-1311.

www.nature.com/cr

LETTER TO THE EDITOR

A transcription factor with a bHLH domain regulates root hair development in rice

Cell Research **19**:1309-1311. doi: 10.1038/cr.2009.109; published online 15 Spetember 2009

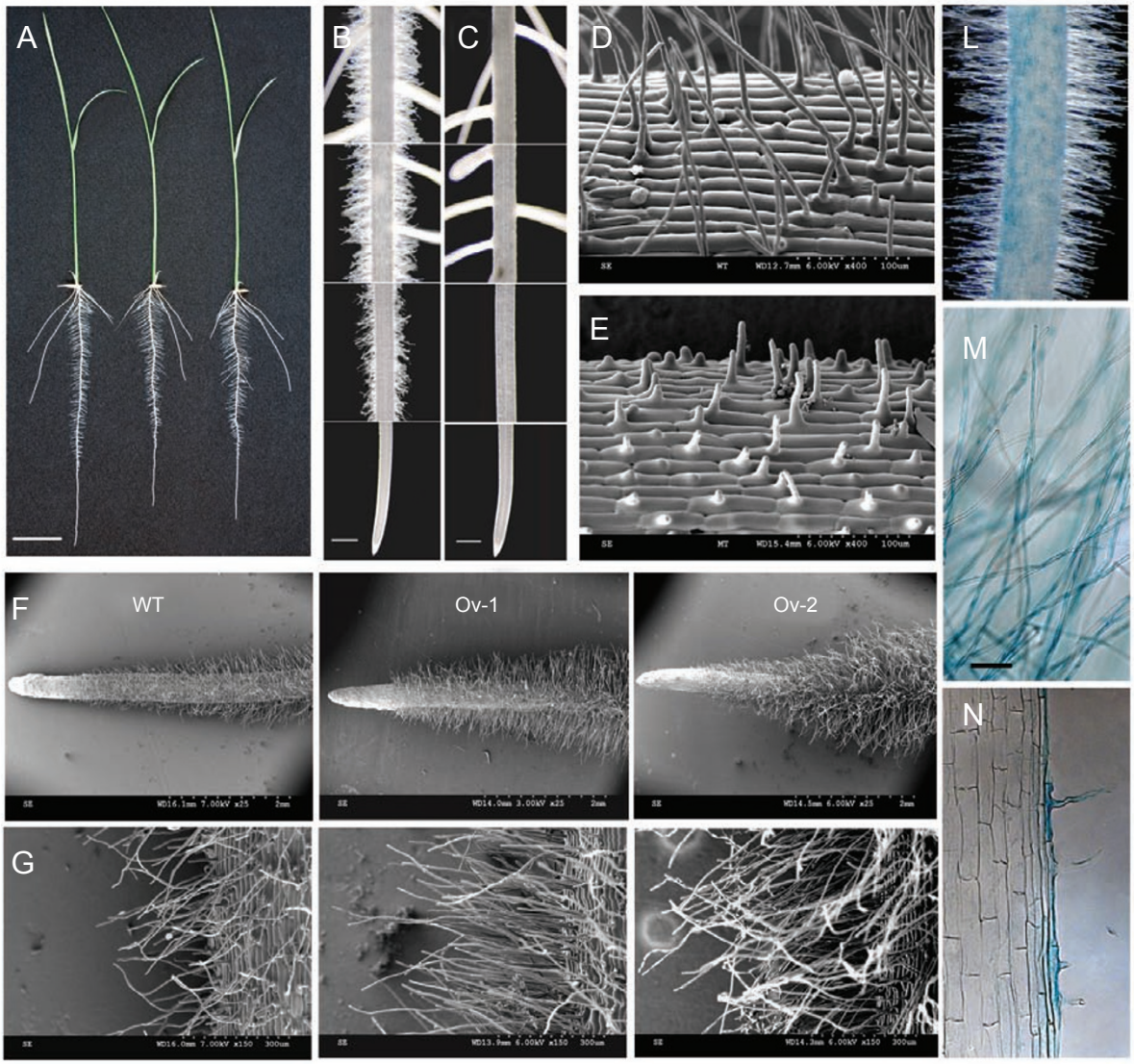
Dear Editor,

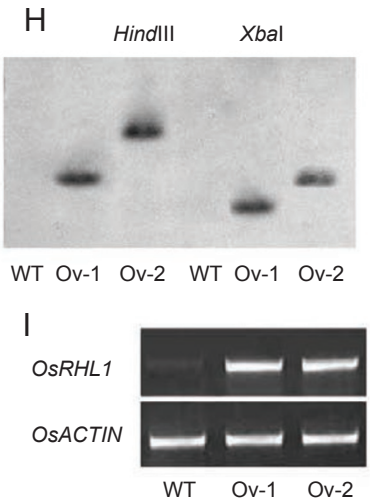
In plants, root hairs are important organs for the uptake of nutrients and water from the rhizosphere and serve as sites of interaction with soil microorganisms [1]. A root hair grows as an extension of a single epidermal cell and is a simple system, making it an elegant model for studying higher plant cell differentiation and cell fate determination. Three types of root hair pattern have been suggested [2, 3]. *Arabidopsis* has the striped pattern (Type 3) of root hairs, which has been extensively reported [4, 5]. Type 2 patterns depend on asymmetrical cell division, which is found in rice (*Oryza sativa L.*), Barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*). Hair-forming cells (trichoblasts) in Type 2 roots derive from a late, unequal transverse cell division in the epidermal stem cell [2]. These different patterns of root hair development in different plant species imply the existence of different genetic and molecular mechanisms controlling epidermal cell behaviors, while the knowledge of the molecular mechanisms of Type 2 root hair pattern formation in monocot crops is still limited.

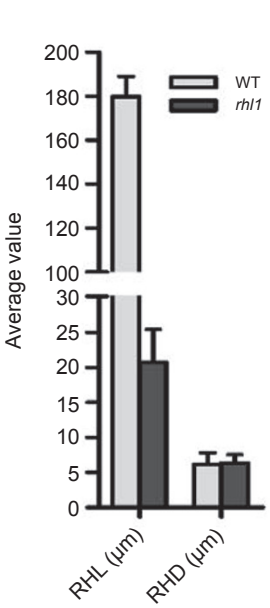
Here, we report the identification and characterization of a novel basic helix-loop-helix (bHLH) transcription factor that regulates root hair development in rice (*O. sativa* L.). To isolate mutants with defects in root hair growth, an ethyl methanesulfonate-generated rice (*O. indica*, Kasalath) mutant library was screened in solution culture. Two mutants defective in root hair elongation were isolated by examining root hair using stereomicroscope, and designated as *Osrhl1-1* and *Osrhl1-2*. The mutant plants showed no significant differences in root length, number of lateral roots and adventitious roots (Figure 1A), except for very short root hair (Figure 1B and 1C). To examine the morphology of the epidermal cells of the mutants in more detail, seedlings were grown on Murashige and Skoog media for 3 days after germination. Roots from wild-type (WT) and *Osrhl1-1* plants were compared using cryo-scanning electron microscopy (Cryo-SEM) (see Supplementary information, Data S1). Root hairs located 2-3 mm from the apex were examined, and their length and diameter were determined (Figure 1D and 1E). The length of root hairs of *Osrhl1-1* was reduced remarkably relative to WT, while no difference in root hair diameter was found (Figure 1J). The image of Cryo-SEM also showed that the WT epidermal cell pattern as Type 2 formed by unequal transverse cell division in the epidermal stem cell [2] may be remediated. In the mutant, no clear shorter and longer cells were observed as in the WT (Figure 1D and 1E), suggesting that RHL1 may control the root hair elongation as well as epidermal cell patterning in rice.

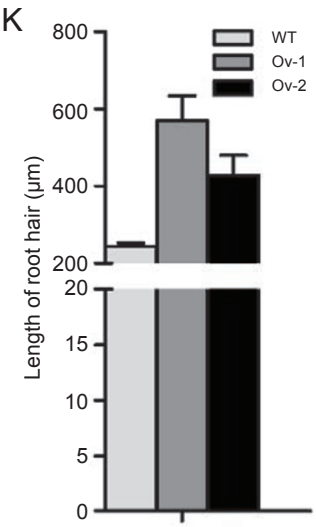
Genetic analysis of 700 F_2 progenies derived from a cross between a homozygous *rhl1-1* line and the *Japonica* cultivar Nipponbare revealed that *rhl1-1* possessed a recessive mutation at a single nuclear locus. The *OsRHL1-1* locus was mapped to chromosome 6 between STS1 and STS2 in a PAC clone P0554A06 (Supplementary Information, Figure S1A). Within the mapped region, one gene with a bHLH domain (GenBank accession No. BAD72512) was found. Sequencing analysis indicated that the point mutation in *Osrhl1-1* disrupted the splicing site between exon 2 and intron 2, resulting in the addition of intron 2 to the mRNA of *OsRHL1* and a shift in the reading frame. In *Osrhl1-2*, two base pairs (GC) at position 179 were deleted (Supplementary information, Figure S1B and S1C).

To determine the function of RHL1, complementation analysis was performed in the *Osrhl1-1* mutant line using *Agrobacterium tumefaciens*-mediated transformation. The 1 263-bp coding region of *OsRHL1* was cloned into the pCAMBIA 1301 vector and expression was driven by the 35S promoter. Ten independent transgenic lines were obtained, with 10-20 sibling plants in each line. Insertion and expression of the transgene were confirmed by RT-PCR and Southern analysis (Figure 1H and 1I). Cryo-SEM images of primary roots of WT and two independent *Osrhl1-1* mutant transgenic lines with overexpression of *OsRHL1* showed that the transgenic lines had longer root hairs on primary roots than WT plants


A
B
C
D
L
E
M
F
WT
Ov-1
Ov-2
G
N


H
HindIII
XbaI
WT Ov-1 Ov-2 WT Ov-1 Ov-2
I
OsRHL1
OsACTIN
WT Ov-1 Ov-2


J
WT
rhl1
Average value
RHL (μm)
RHD (μm)


K
WT
Ov-1
Ov-2
Length of root hair (μm)

(Figure 1F, 1G and 1K). To examine the subcellular localization of OsRHL1, *OsRHL1* was fused in-frame to the amino-terminus of *mGFP4* and transiently expressed in onion epidermal cells. The green fluorescence signal of OsRHL1-GFP was detected only in nuclei, suggesting that OsRHL1 is a nuclear protein (Supplementary information, Figure S2A and S2B). To evaluate the expression pattern of *OsRHL1*, the 2 442 bps upstream of the coding region of *OsRHL1* was fused to the GUS reporter gene. This chimeric gene cassette was introduced into WT plants via agrobacterium-mediated transformation. Histochemical staining for GUS activity in T_2 plants showed that the reporter gene was expressed in root hair cells (Figure 1L and 1M). To investigate detailed expression pattern in roots, we examined the GUS staining pattern in longitudinal section from the root hair zone. It was shown that the gene was expressed specifically in root hair cells at root hair development sites (Figure 1N). In the above ground tissues, the GUS staining was observed in hull and base of panicle branches, but not in leaf, stem and flower (data not shown).

OsRHL1 belongs to subfamily C of the rice bHLH family and is highly homologous to members of subfamily 17 of the bHLH family in *Arabidopsis* [6, 7] (Supplementary information, Figure S1D). No member of this subfamily was found to be involved in root hair development in *Arabidopsis* so far. In light of our findings, we suggest that OsRHL1 is a novel bHLH transcription factor involved in the regulation of plant root hair development. Data from this report provide new information about the molecular mechanisms controlling plant root hair development and increase our understanding of the regulation of root hair development in cereal crops.

Acknowledgments

We are grateful to Dr Liam Dolan (John Innes Center, UK) for constructive suggestion to this work and instruction on the Cryo-SEM analysis. This research is supported by the National Basic Research and Development Program of China (grant 2005CB120901), the Special Program of Rice Functional Genomics of China (2006AA10A102), the National High-Tech Research and Development Program (863) of China (2007AA10Z190), and Science and Technology Department of Zhejiang Province, China.

Wona Ding[1], Zhiming Yu[1], Yanli Tong[1], Wei Huang[1], Hanmin Chen[1], Ping Wu[1]

[1]*State Key Laboratory of Plant Physiology and Biochemistry, College of Life Science, Zhejiang University, Zi Jin Harbor Campus, Yu Hang Tang Road 388, Hangzhou 310058, China*
Correspondence: Ping Wu
Tel: 86-571-88208589; Fax: 86-571-88206613
E-mail: clspwu@zju.edu.cn

References

1 Gilroy S, Jones DL. Through form to function: root hair development and nutrient uptake. *Trends Plant Sci* 2000; **5**:56-60.
2 Clowes FAL. Pattern in root meristem development in angiosperms. *New Phytol* 2000; **146**:83-94.
3 Dolan L, Costa S. Evolution and genetics of root hair stripes in the root epidermis. *J Exp Bot* 2001; **52**:413-417.
4 Kwak SH, Schiefelbein J. The role of the SCRAMBLED receptor-like kinase in patterning the *Arabidopsis* root epidermis. *Dev Biol* 2007; **302**:118-131.
5 Horn R, Yi KK, Menand B, *et al*. Root epidermal development in *Arabidopsis*. *Annu Plant Rev* 2009; **37**:64-82.
6 Toledo-Ortiz G, Huq E, Quail PH. The *Arabidopsis* basic/helix-loop-helix transcription factor family. *Plant Cell* 2003; **15**:1749-1770.
7 Li XX, Duan XP, Jiang HX, *et al*. Genome-wide analysis of basic/helix-loop-helix transcription factor family in rice and Arabidopsis. *Plant Physiol* 2006; **141**:1167-1184.

(**Supplementary information** is linked to the online version of the paper on the *Cell Research* website.)

Figure 1 Phenotypic and complementation analyses of the *rhl1-1* mutant. **(A)** Seedlings (7-day-old) of WT and two allelic *rhl1* mutants (from left to right). Bar = 2 cm. **(B** and **C)** Stereomicroscope images of roots of WT and *rhl1* mutant (only *rhl1-1* is shown). Bar = 0.5 mm. **(D** and **E)** Cryo-SEM images of root hairs at 3 mm from root tip of WT **(D)** and *rhl1-1* mutant **(E)**. Seedlings were grown vertically for 3 days on MS media. **(F)** Cryo-SEM images of the root tips of the wild type and two lines with overexpression of *RHL1* (Ov1 and Ov2). **(G)** Cryo-SEM images of 2 to 3 mm from the root apex of the wild type and two lines with overexpression of *RHL1* (Ov1 and Ov2). **(H** and **I)** Southern blotting analysis **(H)** and RT-PCR analysis **(I)** for two lines of transgenic plants. **(J)** Average values of root hair length (RHL) and root hair diameter (RHD). Seedlings were grown vertically for 3 days on MS media (only *rhl1-1* is examined). Error bars indicate the SD (n = 100). Root hairs examined were in the 2 to 3 mm range from the root apex in a 100×100-μm^2 region. **(K)** Comparison of the length of root hairs (RHL) on primary roots of 3-day-old seedlings grown on agar medium between wild type and the two transgenic lines (Ov1 and Ov2). Error bars indicate the SD (n = 100). Root hairs examined were in the 2 to 3 mm range from the root apex in a 100×100-μm^2 region. **(L-N)** *RHL1* promoter-driven GUS expression in roots. **L**, root hair region; **M**, root hairs; **N**, longitudinal section of root hair region from **L**.