DIVISION OF PLANT ENVIRONMENTAL RESPONSES

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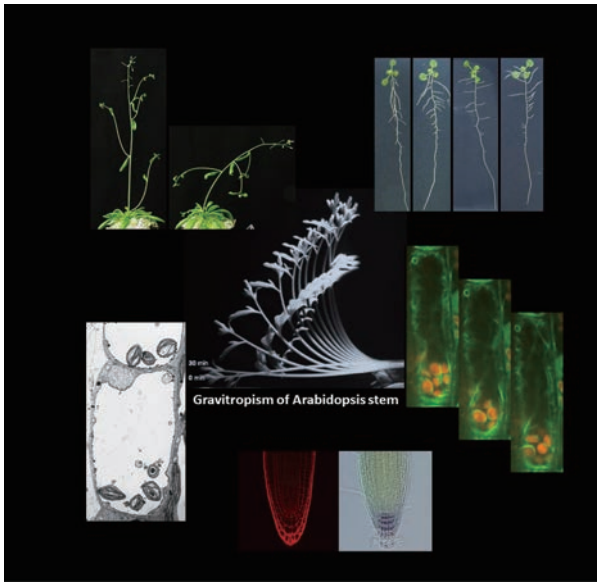
Plant organs have the ability to sense various vectorial stimuli such as light, humidity, touch and gravity as well as reorient their growth direction so as to be in a suitable position to survive and acclimate to their environment. Our aim is to understand the molecular mechanism of environmental responses as they pertain to gravity in the main.



Visual overview of this lab's work.

I. Molecular mechanisms of gravity sensing and signaling in gravitropism

Gravitropism is a major determinant in directing plant organ growth angles. In gravity sensing cells (statocytes), plastids accumulating starch in high-densities relocate toward the direction of gravity. Amyloplast relocation serves as a physical signal trigger for biochemical signal transduction, which in turn leads to the regulation of the polar auxin transport necessary to change the direction that a given plant is growing. We are investigating the detailed molecular mechanism of gravity sensing and signaling by using the model plant *Arabidopsis thaliana*.

LAZY1 family genes have been shown to be involved in gravitropic responses in a variety of plants. *LAZY1-LIKE* (*LZY)2, LZY3,* and *LZY4* are involved in root gravitropism of Arabidopsis. Previously, we have found that LZY3-mCherry is polarly localized on the basal side of the plasma membrane in the columella cells (root statocytes) in response to inclination. At that time, we utilized fixed and cleared root samples because LZY3-mCherry is barely detected in live cell imaging; probably due to low expression levels and the tissue depth of its root cap. We subsequently generated *LZY4p:LZY4-mScarlet* transgenic lines which complemented the *lzy4* mutant. We then succeeded in live cell imaging of LZY4-mScarlet in the columella cells by using vertical-stage confocal microscope equipped with a high sensitivity EMCCD camera. LZY4-mScarlet was polarly localized on the basal side of the plasma membrane, in a similar way to that which LZY3-mCherry was localized. In addition, LZY4-mScarlet was found to be localized at the periphery of amyloplasts. We are now focusing on the dynamics of LZY4-mScarlet in response to gravistimulation and also on the mechanism of localization to the plasma membrane and amyloplast periphery.

II. Determination mechanism of gravitropic setpoint angle

Growth angles affected by gravity are known as the gravitropic setpoint angles (GSA). Many gravitropic mutants show abnormal GSA in lateral branches; meaning they produce wider growth angle phenotypes due to the likelihood of reduced gravitropism. We are trying to understand how roots' and shoots' lateral branches maintain inclined growth angles with respect to gravity.

2-1 Determination of GSA in roots

It has been reported that primary and lateral roots of *lzy2;3;4* triple mutants showed negative gravitropism. We refer to the phenotype of the reversed growth direction in the *lzy* triple mutant as "anti-gravitropic". It has been proposed that GSA is determined by balancing two opposing growth components: gravitropism and "anti-gravitropic" offsets (AGO). We assumed that the balance between gravitropism and AGO was disrupted in *lzy2;3;4* mutants, and hypothesized that AGO would be manifested as an "anti-gravitropic" phenotype. To investigate the mechanism of GSA control, we performed genetic analyses with *lzy2;3;4* mutants and *pgm* (Figure 1). Since *pgm* is a starch-less mutant, gravity sensing ability was greatly reduced in this type of mutant, resulting in varied growth direction (Figure 1D and H). The *pgm* mutation suppressed the "anti-gravitropic" phenotype of *lzy2;3;4* triple mutants (Figure 1B, C, F, and G). This finding indicates that gravitropic and "anti-gravitropic" growth is likely to share a similar gravity-sensing mechanism in primary roots.

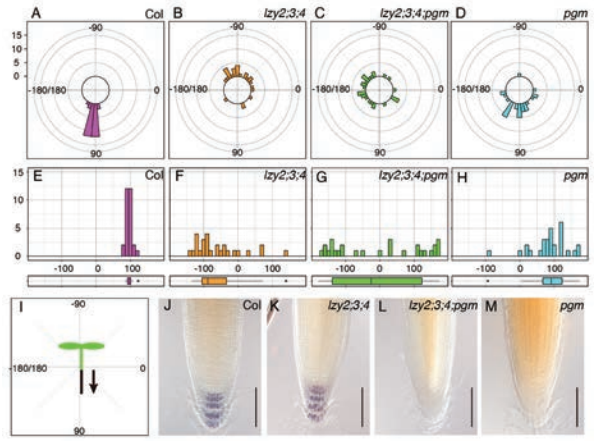


Figure 1. Starchless mutation affects "anti-gravitropic" response in roots. Polar distribution of the growth direction of primary root in wild-type Col (**A**), *lzy2;3;4* (**B**), *lzy2;3;4;pgm* (**C**), and *pgm* (**D**). Growth angles were plotted on histograms and distributions were evaluated with boxplots from wild-type Col (**E**), *lzy2;3;4* (**F**), *lzy2;3;4;pgm* (G), and *pgm* (**H**). Schematic representation of growth angle quantification (I). Each bar indicates the number of roots. Lugoal's staining of primary roots of wild-type Col (**J**), *lzy2;3;4* (**K**), *lzy2;3;4;pgm* (**L**), and *pgm* (M). Bars indicate 100 µm.

2-2 Determination of GSA in shoots

The primary shoots of *lzy1;2;3* triple mutants exhibited non-responsiveness to gravistimulation, so it was expected that the lateral branches of the triple mutant would grow horizontally like those of *sgr* mutants. However, they unexpectedly grew downward and showed positive gravitropism upon gravistimulation (Figure 2). As amyloplast sedimentation is thought to be important for gravity sensing in shoot gravitropism as well as root gravitropism, we examined whether starch-accumulated amyloplasts are required for the "anti-gravitropic" phenotype of lateral branches in the *lzy1;2;3* mutants. Although no clear difference was observed in the growth angles of lateral branches by adding *pgm* mutation in *lzy1;2;3* mutants, the *pgm* mutation delayed the "anti-gravitropic" response in *lzy1;2;3* plants upon gravi-stimulation (Figure 2C, D and E).

We then investigated the relationship between the gravity sensing tissues for shoot gravitropism and the "anti-gravitropic" phenotype of lateral branches in *lzy1;2;3* plants. The endodermis is the gravity-sensing tissue in shoots, and *eal1* is classified as an agravitropic mutant due to defect in its endodermis differentiation and because it's lateral branches grow horizontally. The *eal1* mutation clearly suppressed

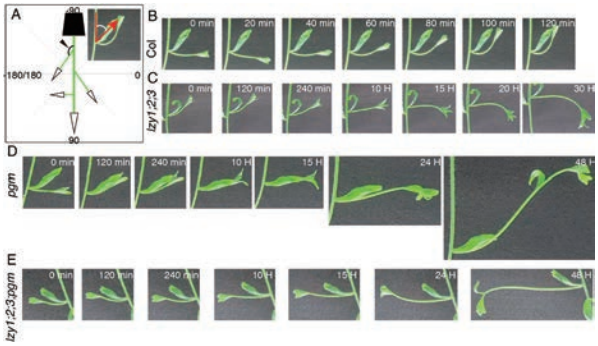


Figure 2. The gravitropic responses of lateral branches. **A.** Graphical image of the quantification of growth angles of lateral branches. Time-lapse images of lateral branches from wild-type Col (**B**), *lzy1;2;3* (**C**), *pgm* (**D**), l*zy1;2;3;pgm* (**E**) plants after inversion.

"anti-gravitropic" phenotype of *lzy1;2;3* triple mutants. In other words, the lateral branches of *eal1 lzy1;2;3* quadruple mutants grew horizontally. Overall, the endodermal tissue appears crucial for the normal gravitropism and "anti-gravitropic" phenotypes of lateral branches in *lzy1;2;3* triple mutants, but it remains unclear whether gravitropism and "anti-gravitropic" phenotypes share a similar gravity-sensing mechanism in shoots.

III. Functional analysis of RLD (RCC1-like domain) proteins

RLD proteins were isolated as interactors of LZY. RLD1 and RLD4 function in gravitropism of primary roots and the GSA control of lateral roots. We have also shown that *RLD1*, *RLD2*, *RLD3*, and *RLD4* redundantly function in plant morphogenesis, the morphological abnormality gradually becomes more severe by multiplying *rld* mutations (Figure 3). Indeed, *rld1;2;3;4* quadruple mutants displayed severe embryonic development defects due to a reduced amount and abnormal localization of PIN proteins (auxin efflux carriers) in the plasma membrane. Defects in root gravitropism and morphogenesis of *rld* multiple mutants are associated with defective auxin transport. It had been reported that the RLD1 fragment containing RCC1-like domain show guanine nucleotide exchange activity in Rab8 and Rab11. Thus, it's likely that RLD proteins regulate auxin transport through regulating membrane trafficking during root gravitropism and plant development.

In columella cells, RLD1 is polarly recruited to the plasma membrane by interacting with LZY3 and regulates the direction of auxin transport. The interaction between RLD1 and LZY3 occurs through the binding of the BRX domain of RLD1 to the CCL domain of LZY3. While *LZY1*, *LZY2*, and *LZY3*, has been shown to play a key role in the gravity signaling process in shoot endodermis, the role of RLDs in shoot gravitropism remains unclear because evaluating shoot gravitropism in *rld* multiple mutants is difficult due to their abnormal morphology and retarded growth.

To investigate the importance of the BRX domain for RLD function, we examined whether *RLD3p:RLD3ΔBRX-mClover3* could complement the morphological phenotype of *rld2;3;4*. Unexpectedly, RLD3ΔBRX-mClover3 recovered the morphological abnormality of *rld2;3;4* (Figure 3). Interestingly, a wider GSA phenotype possessing lateral branches was observed in *RLD3p:RLD3ΔBRX-mClover3/rld2;3;4*. Both of these indicate that RLD3 lacking the BRX domain retains the function for morphogenesis but not for the GSA phenotype. Moreover, when RLD3 was expressed under the control of the endodermis-specific ADF9 promoter in *RLD3p:RLD3ΔBRX-mClover3/rld2;3;4*, the GSA phenotype was significantly restored, thus suggesting that RLD is involved in gravity signaling in the endodermal cells in shoot gravitropism in a manner similar to LZYs. Furthermore, these results suggest that RLD3 without BRX domain retain general functions in membrane trafficking required for development, and that LZYs regulates the function of RLD via the BRX domain to conduct gravity signaling in the gravity sensing cells.

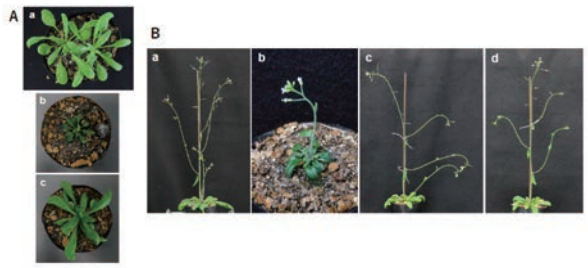


Figure 3. **A.** Phenotype of rosette leaves of wild-type, *rld2;3;4* (b) and *RLD3p:RLD3ΔBRX-mClover3*/*rld2;3;4*. **B.** The growth angle of shoot in wild-type, *rld2;3;4* (b), *RLD3p:RLD3ΔBRX-mClover3*/*rld2;3;4* (c), *ADF9p:RLD3*/*RLD3p:RLD3ΔBRX-mClover3*/*rld2;3;4* (d).

IV. Re-orientation mechanism of the polarized growing root hair cell

Root hairs, thin tubular cells grown outward from the root surface, increase root surface area and the uptake of nutrients and water from soils. They are typical polarized growing cells and grow straight and long in a cell-autonomous manner when they are grown in open air or within a liquid medium (Figure 4A). However soils are filled with soil particles like grains of sand of various sizes or silt, which may interfere with the growth of root hairs (Figure 4B). We have developed an imaging system based on microfluidics to observe how root hairs grow in soils, since soils are not transparent enough to conduct observations within them through the use of microscopes (Figure 4C-E). Applying this system, paths or objects (2~250 μm) are formed in a transparent silicone-rubber to mimic the condition of soil particles (Figure 4D, E). It enables us to visualize Arabidopsis roots including root hairs that come into contact with obstacles in the microfluidic device. We found that root hairs grow along the surface of the obstacles, which supports the role of root hairs on uptake of nutrients and water from soil particles (Figure 4E). To further get insight into the mechanism of root hair growth in soils, we have been analyzing various regulators that are important for normal root hair growth with the imaging system. We found that two protein kinases are regulators for re-orientation of root hair growth.

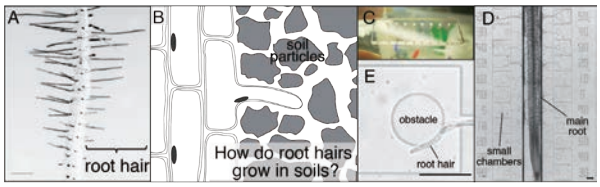


Figure 4. Live-imaging of root hairs. **A**. An Arabidopsis root grown on the Agar medium. **B**. Illustration of root hairs in soils. **C**. A micro-fluidic device for imaging of Arabidopsis root hairs. **D**. Main roots grow in a main path within the device, and root hairs elongate into small chambers aligned beside the main path. **E**. A root hair elongating along an obstacle set in a small chamber. Bars indicate 200 μm or 100 μm in (A) or (D, E), respectively.

Publication List:

〔Original papers〕

- Abe, Y., Meguriya, K., Matsuzaki, T., Sugiyama, T., Yoshikawa, H.Y., Morita, M.T., and Toyota, M. (2020). Micromanipulation of amyloplasts with optical tweezers in *Arabidopsis* stems. Plant Biotechnol. *37*, 405–415. DOI: 10.5511/plantbiotechnology.20.1201a
- Kawamoto, N., Kanbe, Y., Nakamura, M., Mori A., Terao Morita, M.T. (2020). Gravity-sensing tissues for gravitropism are required for "anti-gravitropic'' phenotypes of *lzy* multiple mutants in Arabidopsis. Plants *9*. DOI: 10.3390/plants9050615
- Shindo, M., Makigawa, S., Kodama, K., Sugiyama, H., Matsumoto, K., Iwata, T., Wasano, N., Kano, A., Morita, M.T., and Fujii, Y. (2020). Design and chemical synthesis of root gravitropism inhibitors: Bridged analogues of ku-76 have more potent activity. Phytochemistry *179*. DOI: 10.1016/j.phytochem.2020.112508
- Shindo, M., Makigawa, S., Matsumoto, K., Iwata, T., Wasano, N., Kano, A., Morita, M.T., and Fujii, Y. (2020). Essential structural features of (2Z,4E)-5-phenylpenta-2,4-dienoic acid for inhibition of root gravitropism. Phytochemistry *172*. DOI: 10.1016/j.phytochem.2020.112287
- Tsugawa, S., Sano, T.G., Shima, H., Morita, M.T., and Demura, T. (2020). A mathematical model explores the contributions of bending and stretching forces to shoot gravitropism in Arabidopsis. Quant. Plant Biol. *1*, e4. DOI: 10.1017/qpb.2020.5