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The leaf is the fundamental unit of the shoot system, which is composed of the leaf and stem. The diversity of plant forms is mostly attributable to variation of leaf and floral organs, which are modified leaves. Moreover, leaf shape is sensitive to environmental stimuli. The leaf is therefore the key organ for a full understanding of plant morphogenesis. The genetic control of the development of leaf shapes, however, has remained unclear. Recently, studies of leaf morphogenesis reached a turning point after our successful application of the techniques of developmental and molecular genetics using the model plant *Arabidopsis thaliana* (L.) Heynh. (Tsukaya 2008).

I. Mechanisms of leaf development

Focusing on the mechanisms that govern the polarized growth of leaves in *Arabidopsis thaliana,* we have identified four genes for polar-dependent growth of leaf lamina: the *ANGUSTIFOLIA* (*AN*) and *AN3* genes, which regulate the width of leaves, and the *ROTUNDIFOLIA3* (*ROT3*) and *ROT4* genes, which regulate the length of leaves. *AN* and *ROT3* genes control cell shape while *AN3* and *ROT4* genes regulate cell numbers in leaves. In addition to polar-dependent leaf shape control, we have focused on the mechanisms of organ-wide control of leaf size, which are reflected in the ‘compensation’ phenomenon (reviewed in Tsukaya 2008). Additionally, the accumulation of knowledge on the basic mechanisms of leaf shape control has enabled us to conduct Evo/Devo studies of the mechanisms behind leaf-shape diversity. Below is an overview of our research activities and achievements during 2010.

1-1 Polar growth of leaves in *A. thaliana*

ROT4 is a member of the *RTFL/DVL* gene family that encodes peptides. Overexpression of ROT4 is known to cause stunted leaves due to decrease of number of cells in the lamina. In addition, we found that pedicels and secondary inflorescences bend abnormally by the constitutive over-expression of ROT4 peptide in Arabidopsis. Detailed examination of the morphology revealed that the bending was caused by an abnormal protrusion of the inflorescence stem, suggesting that ROT4 might be somehow involved in positional value determination (Ikeuchi et al. 2011). This idea is further supported by analyses of chimeric expression of ROT4 in the lamina: sectors over-expressing ROT4 showed altered positioning of leaf blade/leaf petiole boundary. We also identified an important domain in the ROT4 peptide by a series of deletion experiments (Ikeuchi et al. 2011).

1-2 Evolution of establishment mechanisms of leaf polarities in monocots

We have recently started to attempt an understanding of the genetic basis of the development of unifacial leaves that are known only from monocot clades. Our analyses indicated that the unifacial character might be due to overall changes in all polarities. Understanding the differences in the genetic mechanisms for the establishment of unifacial and normal bifacial leaves will provide good clues as to how leaf-shape is diversified.

For such purposes, comparative molecular-genetic and anatomical analyses between unifacial and bifacial leaf development have been undertaken using members of the genus *Juncus* (Yamaguchi and Tsukaya, 2010). Interestingly, molecular characterization of unifacial leaves of *Juncus* revealed that they have only abaxial identity in the leaf blades, lack leaf margins, and possess flattened leaf lamina. This finding is very surprising, because laminar growth occurs at the adaxial-abaxial junction in bifacial leaves such as Arabidopsis and snap dragon.

Detailed analyses of *Juncus* species revealed that the flattened leaf lamina in the unifacial leaves in *Juncus* is, at least in part, dependent on function of the *DL* gene that acts to thicken the lamina (Yamaguchi et al. 2010). We also isolated several interesting mutants of *Juncus* that exhibit abnormalities in leaf polarity have already been isolated.

1-3 Size control of leaves and mechanisms of compensation

We recently showed that the meristematic region in the leaf primordia is spatially and temporally regulated (Kazama et al. 2010) and *SPT* controls the size of the meristematic region (Ichihashi et al., 2010). Leaf size depends on not only meristematic activity but also the cell enlargement process that follows. How are cell proliferation and cell enlargement coordinated in leaf morphogenesis?

To answer this, a new tool for studies of the coordination system, heat-shock-inducible, chimeric expression systems of *KRP2* or *AN3,* was established. Using this (Figure 1), we found that *an3*-dependent compensation is a non-cell-autonomous process, and that *an3* cells seem to generate and transmit an intercellular signal that enhances post-mitotic cell expansion. This is the first proof of an organ-level, cell non-autonomous integration system between cell proliferation and cell expansion.

In addition, we revealed genetic pathways of leaf serration (Kawamura et al. 2010); an unexpected role of *AN3* on dorsoventral identification in leaves (Horiguchi et al. 2011); and brassinosteroid- and auxin- related regulation of shade avoidance syndrome in leaves (Kozuka et al. 2010). We also

†: This laboratory was closed on 31 March, 2010.

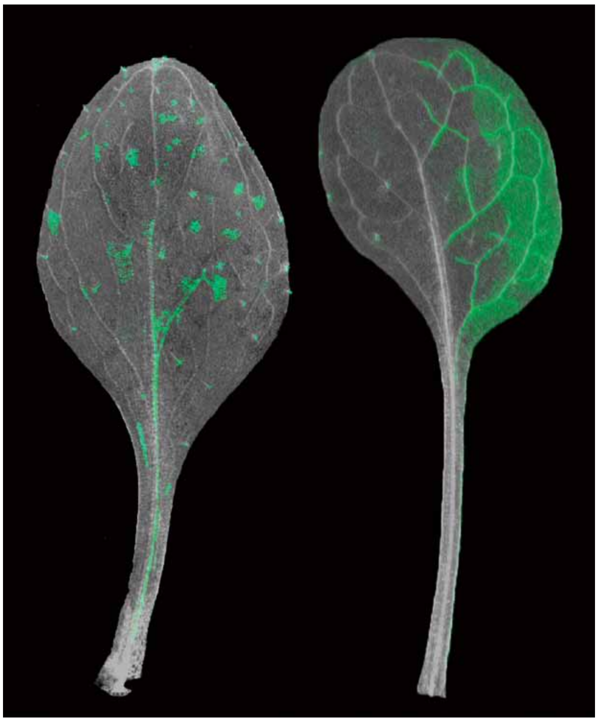

Figure 1. AN3 leaf chimera. Green signal represents *AN3::3xGFP* cells while the other areas are composed of *an3* mutant cells. Modified from Kawade et al. (2010).

found that plant Elongator regulates auxin-related genes during RNA polymerase II transcription elongation (Nelissen et al., 2010).

II. Biodiversity in plants

This year we reported two new species from Asian countries: *Pipthospata repens* and *Phaius hekouensis* (Okada and Tsukaya 2010; Tsukaya et al. 2010).

Publication List

〔Original papers〕

- Ichihashi, Y., Horiguchi, G., Gleissberg, S., and Tsukaya, H. (2010). The bHLH transcription factor SPATULA controls final leaf size in *Arabidopsis thaliana*. Plant Cell Physiol. *51*, 252-261.
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- Kazama, T., Ichihashi, Y., Murata, S., and Tsukaya, H. (2010). The mechanism of cell cycle arrest front progression explained by a KLUH/CYP78A5-dependent mobile growth factor in developing leaves of *Arabidopsis thaliana*. Plant Cell Physiol. *51*, 1046–1054.
- Kozuka, T., Kobayashi, J., Horiguchi, G., Demura, T., Sakakibara, H., Tsukaya, H., and Nagatani, A. (2010). Involvement of auxin and brassinosteroid in the regulation of petiole elongation under the shade. Plant Physiol. *153*, 1608–1618.
- Nakayama, H., Yamaguchi, T., and Tsukaya, H. (2010). Expression patterns of *AaDL*, a *CRABS CLAW* ortholog in *Asparagus asparagoides* (Asparagaceae), demonstrate a stepwise evolution of *CRC/DL* subfamily of *YABBY* genes. Amer. J. Bot. *97*, 591-600.
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- Toriba, T., Suzaki, T., Yamaguchi, T., Ohmori, Y., Tsukaya, H., and Hirano, H. (2010). Distinct regulation of adaxial-abaxial polarity in anther patterning in rice. Plant Cell *22*, 1452-1462.
- Tsukaya, H., Nakajima, M., and Wu, S.-G. (2010). A new species of *Phaius* (Orchidaceae) from Yunnan, China. Curtis's Bot. Mag. *27*, 339-347
- Yamaguchi, T., Yano, S., and Tsukaya, H. (2010). Genetic framework for flattened leaf blade formation in unifacial leaves of *Juncus prismatocarpus*. Plant Cell *22*, 2141–2155.

〔Original papers (E-publication ahead of print)〕

- Horiguchi, G., Nakayama, H., Ishikawa, N., Kubo, M., Demura, T., Fukuda, H., and Tsukaya, H. ANGUSTIFOLIA3 plays roles in adaxial/abaxial patterning and growth in leaf morphogenesis. Plant Cell Physiol. 2010 Nov 21.
- Ikeuchi, M., Yamaguchi, T., Kazama, T., Ito, T., Horiguchi, G., and Tsukaya, H. ROTUNDIFOLIA4 regulates cell proliferation along the body axis in Arabidopsis shoot. Plant Cell Physiol. 2010 Sep 8.

〔Review articles〕

- Ferjani, A., Horiguchi, G., and Tsukaya, H. (2010). Organ size control in Arabidopsis: Insights from compensation studies. Plant Morph. *22*, 65-71.
- Tsukaya, H. (2010). Leaf development and Evolution. J. Plant Res. *123*, 3-6.
- Yamaguchi, T., and Tsukaya, H. (2010). Evolutionary and developmental studies of unifacial leaves in monocots: *Juncus* as a model system. J. Plant Res. *123*, 35-41.