

## DIVISION OF PLANT DEVELOPMENTAL GENETICS (ADJUNCT)

Professor (Adjunct): TSUKAYA, Hirokazu  
 Research Associates: HORIGUCHI, Gorou  
 YAMAGUCHI, Takahiro  
 NIBB Research Fellow: ISHIKAWA, Naoko  
 Postdoctoral Fellows: FERJANI, Ali  
 USAMI, Takeshi  
 YANO, Satoshi  
 Graduate Student: FUJIKURA, Ushio  
 Technical Assistants: TAKABE, Eriko  
 YAMAGUCHI, Chinami  
 NAGURA, Masako  
 KADOWAKI, Tamaka  
 Secretary: KOJIMA, Yoko

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 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 2006).

### I. Mechanisms of leaf development

Focusing on the mechanisms that govern 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 regulate width of leaves and the *ROTUNDIFOLIA3* (*ROT3*) and *ROT4* genes regulate length of leaves. *AN* and *ROT3* genes control cell shape while *AN3* and *ROT4* gene regulate cell number in leaves (reviewed in Tsukaya 2006a, b; Yamaguchi and Tsukaya 2006). In addition to the polar-dependent leaf shape control, we have focused on the mechanisms of organ-wide control of leaf size, reflected in the phenomenon "compensation" (Tsukaya 2006). On the other hand, accumulation of knowledge on basic mechanisms of leaf shape control have enabled us to conduct Evo/Devo studies of mechanisms behind leaf-shape diversity. Here we overview achievements during this year on the above research themes.

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

The genome of *A. thaliana* encodes 23 members of RTFL peptides that regulate the number of cells along the proximo-distal polarity in lateral organs (Yamaguchi and Tsukaya 2006). Homology search revealed that rice genome has a number of RTFL homologs. Therefore, we constructed transgenic *Arabidopsis* that overexpresses one of such rice homologs. Our result clearly showed that the rice homolog also regulated the number of leaf cells along the proximo-distal polarity, suggesting that the basic

function of RTFL is conserved among monocot and eudicot (Yamaguchi and Tsukaya 2006). Further mutation analyses have been carried out (Horiguchi et al. 2006a) and will provide us with more clues to understand how the polar-dependent growth of leaf lamina is controlled.

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

On the other hand, we have recently started to try to understand the genetic basis of the development of unifacial leaves that are known from monocot clades. Our preliminary analyses suggested that the unifacial character might be due to overall changes in all polarities around leaves (*i.e.* adaxial-abaxial, distal-proximal, and central-lateral polarities). Moreover, genetic controls of leaf polarities were revealed to differ, at least in part, between eudicot and rice, a monocot species. Understanding the differences in the genetic mechanisms of establishment of leaf polarities in eudicot and monocot will provide good clues to how leaf shape is diversified from the Evo/Devo view point. For such purposes, comparative molecular-genetic and anatomical analyses between unifacial and normal bifacial leaf development have been recently undertaken using a number of monocot families such as Juncaceae, Liliaceae, Alliaceae, and Iridaceae.

#### 1-3 Size control of leaves and mechanisms of compensation

How are cell proliferation and cell enlargement coordinated in leaf morphogenesis? In a determinate organ- a leaf- the number of leaf cells is not necessarily reflected in leaf shape or, in particular, in leaf size. Genetic analyses of leaf development in *A. thaliana* shows that a compensatory system(s) acts in leaf morphogenesis and an increase in cell volume might be triggered by a decrease in cell number (Tsukaya 2006; Horiguchi et al. 2006b). Thus, leaf size is, at least to some extent, regulated at an organ level by the compensatory system(s). To understand the details of such totally unknown regulatory mechanisms, we have conducted a large scale screening of leaf-size and/or leaf-shape mutants and have categorized these mutants in terms of number and size of leaf cells (Horiguchi et al. 2006a, 2006b). For this purpose, we have developed easy methods to visualize the precise number and size of leaf cells, namely, the centrifugation method and the dried-gel method (Fig. 1; Horiguchi et al. 2006 a).

As a result, we have succeeded in isolating specific mutants for number or size of leaf cells (Horiguchi et al. 2006a, b). Since our re-examination revealed that all known plant hormone-related mutants do not have specific defects in number or size of leaf cells, differing from previous reports (Horiguchi et al. 2006a), this is the first report on the isolation of mutants that have specific defects in the number or size of leaf cells. Moreover, we have isolated a number of new mutants that exhibit typical compensation syndrome, namely, decreased number of cells and increased cell volume. Combining

these mutants as a resource, mechanisms of compensation are now underway.

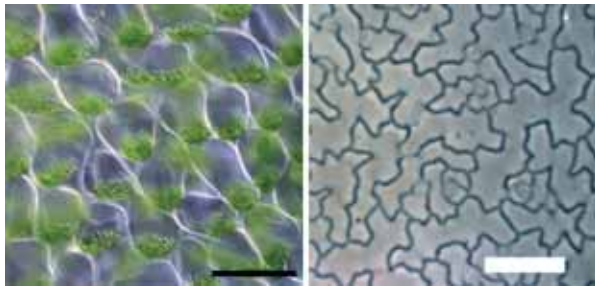


Figure 1. Easy methods to visualize size and shape of leaf cells in *Arabidopsis thaliana* (Horiguchi et al. 2006a). Left, Living palisade cells are visualized by the centrifugation method; right, contour of epidermal cells are shown by the dried gel method. Bars, 100  $\mu$ m.

## II. Environment adaptation of leaves

### 2-1 Gravitropism of leaves

How is leaf positioning controlled? Curiously, until now, no one had elucidated how rosette leaves are so elegantly arranged on the ground surface. Moreover, no one had elucidated whether or not leaves have gravitropism. Our analysis of *A. thaliana* revealed that leaves not only have negative gravitropism but hyponasty as well, both of which are suppressed by light (Mano et al. 2006). The finding of these curious features of leaf movement satisfactorily explained the positioning control of rosette leaves.

## III. Biodiversity of leaf form

### 3-1 Biodiversity in leaf size

We are also interested in the biodiversity in wild plants. This year, we analyzed *Plantago asiatica* (Plantaginaceae) in Japan (Ishikawa et al., 2006). *P. asiatica* shows a wide range of size diversity and dwarf forms have been taken from temples and shrines, from deer habitats, and from Yakushima Island. Analysis of the molecular variations of this species revealed that the dwarf forms have evolved several times independently.

Similar dwarfism has also been seen in *Paederia foetida* (Rubiaceae). We re-examined the morphological and anatomical features of a small narrow leaf form of this species from Miyajima Island. We found that this form should be defined by the smallness of leaves but not by the leaf blade proportion, and that this variation in leaf size is attributable to the altered number of leaf cells without any change in the shape and size (Tsukaya et al. 2006).

### 3-2 Domatia formation on leaves

Some trees are known to make domatia on leaves that are thought to function as living rooms for mites. To understand how such additional structures are formed on

leaf blades, we examined the developmental course of four types of domatia in *Cinnamomum camphora* (Lauraceae). The results clearly showed that the domatia are derived from ectopic, co-ordinated growths of parenchymatous cells after completion of the basic organogenesis of leaf blades (Nishida et al. 2006).

## Publication List:

### Original papers

- Horiguchi, G., Fujikura, U., Ferjani, A., Ishikawa, N., and Tsukaya, H. (2006a). Large-scale histological analysis of leaf mutants using two simple leaf observation methods: identification of novel genetic pathways governing the size and shape of leaves. *Plant J.* 48, 638-644.
- Horiguchi, G., Ferjani, A., Fujikura, U., and Tsukaya, H. (2006b). Coordination of cell proliferation and cell expansion in the control of leaf size in *Arabidopsis thaliana*. *J. Plant Res.* 119, 37-42.
- Ishikawa, N., Yokoyama, J., Ikeda, H., Takabe, E., and Tsukaya, H. (2006). Evaluation of morphological and molecular variation in *Plantago asiatica* var. *densiuscula*, with special reference to the systematic treatment of *Plantago asiatica* var. *yakusimensis*. *J. Plant Res.* 119, 385-395.
- Mano, E., Horiguchi, G., and Tsukaya, H. (2006). Gravitropism in leaves of *Arabidopsis thaliana* (L.) Heynh. *Plant Cell Physiol.* 47, 217-223.
- Nishida, S., Tsukaya, H., Nagamasu, H., and Nozaki, M. (2006). A comparative study on the anatomy and development of different shapes of domatia in *Cinnamomum camphora* (Lauraceae). *Ann. Bot.* 97, 601-610.
- Niwa, Y., Goto, S., Nakano, T., Sakaiya, M., Hirano, T., Tsukaya, H., Komeda, Y., and Kobayashi, H. (2006). *Arabidopsis* mutants by activation tagging in which photosynthesis genes are expressed in dedifferentiated calli. *Plant Cell Physiol.* 47, 319-331.
- Tsukaya, H., Imaichi, R., and Yokoyama, J. (2006). Leaf-shape variation of *Paederia foetida* L. in Japan: reexamination of the small, narrow leaf form from Miyajima Island. *J. Plant Res.* 119, 303-308.

### Review articles

- Tsukaya, H. (2006a). Mechanism of leaf shape determination. *Ann. Rev. Plant Biol.* 57, 477-496.
- Tsukaya, H. (2006b). A new member of the CtBP/BARS family from plants: *Angustifolia*. In *CtBP Family Proteins*, G. Chinnadurai, ed. (Landes Bioscience, Georgetown, Texas, USA) Chapter 12.
- Tsukaya, H., and Beemster, G. (2006). Genetics, cell cycle and cell expansion in organogenesis in plants. *J. Plant Res.* 119, 1-4.
- Yamaguchi, T., and Tsukaya, H. (2006). ROTUNDIFOLIA4, a plant-specific small peptide involved in the polar cell proliferation of leaves in *Arabidopsis thaliana*. In *Handbook of Biologically Active Peptides*, A. J. Kastin, ed. (Elsevier, San Diego, USA), pp. 37-40.