

**DIVISION OF PLANT DEVELOPMENTAL
GENETICS (ADJUNCT)**

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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. However, the genetic control of development of these shapes 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 model plants, *Arabidopsis thaliana* (L.) Heynh. (reviewed in Kim *et al.* 2005, Tsukaya 2003).

I. Polarized growth of leaf blades

Focusing on the mechanisms that govern polarized growth of leaves in *Arabidopsis thaliana* we have identified genes for polar-dependent growth of leaf lamina: the *ANGUSTIFOLIA* (*AN*) gene regulates width of leaves and the *ROTUNDIFOLIA3* (*ROT3*) gene regulates length of leaves. Both *AN* and *ROT3* genes control leaf cell shape. In addition to these, we added *ROT4* gene to the list of the genes responsible for the polarity-dependent control of leaf shape. This year, we found that the *ROT3* is involved in biosynthesis of brassinosteroid, a steroid phytohormone (Kim *et al.* 2005). In relation to the function of the *ROT3*, we also found that the different growth responses of the *A. thaliana* leaf blade and the petiole during shade avoidance are regulated by photoreceptors and sugar. Moreover, we identified the *AN3* gene that regulates number of leaf cells (Horiguchi *et al.* 2005) and a homolog of *AN* from *Ipomoea nil* (Cho *et al.* 2005).

1-1 *ROT3* gene function

We found that the *ROT3* gene encodes a cytochrome P450 that catalyzes the conversion of typhasterol to castasterone, an activation step in the biosynthesis pathway of brassinosteroids (BRs) (Kim *et al.* 2005). Differed from already known mutants of genes for biosynthesis of BRs, the loss-of-function mutant of *ROT3* has a specific defect in the length of leaves, suggesting the importance of fine tuning the levels of BRs on the

polarized growth of leaves. Interestingly, CYP90D1, the most closely related cytochrome P450 to the ROT3/CYP90C1 enzyme, was suggested to catalyze the other conversion steps of BR biosynthesis (Kim *et al.* 2005). Double mutant for the *ROT3/CYP90C1* and for the *CYP90D1* exhibited extreme dwarf that is observed for the other known mutants of genes for biosynthesis of BRs. Since the loss-of-function mutant of *ROT3* has defect in response of petioles to dark, the *ROT3* might have specific role(s) in the shade-avoidance syndrome. In relation to this topic, the adaptive responses of arabidopsis leaves against gravity and other environmental factors were also analyzed and the interaction between light signal and gravity-response in leaves was suggested (Kim *et al.* 2005, Tsukaya 2005).

1-2 Shade avoidance syndrome

As mentioned above, we also focused on the effects of environmental factors on leaf morphogenesis. In darkness, the expansion of leaf lamina is inhibited, while at the same time petiole elongation is enhanced. This phenomenon is termed the shade-avoidance syndrome. We analyzed the nature of the shade-avoidance syndrome and found that phytochromes and cryptochromes specifically regulate the contrasting growth patterns of the leaf blade and petiole in shade (Kozuka *et al.* 2005). Differed from photomorphogenesis of hypocotyl, cell elongation was stimulated in the petiole in dark conditions without an increase in the ploidy level. By examining the effects of sucrose on the growth of the leaf blade and petiole, we revealed that the growth promotional effects of sucrose are highly dependent on the light conditions.

1-3 *AN3* gene regulates leaf cell numbers

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 2003). Thus, leaf size is, at least to some extent, uncoupled from the size and number of cells by the compensatory system(s). Recently, we have revealed that *ANGUSTIFOLIA3* (*AN3*) gene is involved in the maintenance/establishment of activity of cell proliferation in leaf primordia. *AN3* encodes a co-activator, and is found to control cell cycling in leaf primordia by interacting with a transcription activator, *AtGRF5* (Horiguchi *et al.* 2005). Interestingly, the *an3* shows clear "compensation", namely, accelerated cell expansion in relation to a decrease in the number of leaf cells (Figure 1) whereas overexpression of the *AN3* caused simple increase of leaf area in association with an increased number of leaf cells. By using various mutants with altered number and/or size of leaf cells, we are currently analyzing the genetic system of the compensation.

Kalimantan, Borneo, Indonesia (Tsukaya and Okada 2005, Tsukaya *et al.* 2005).

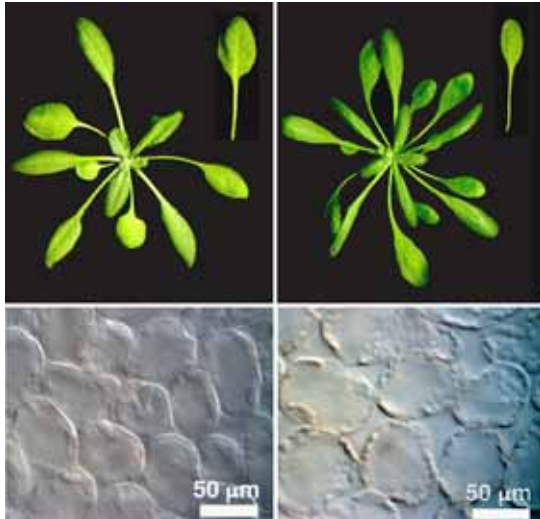


Figure 1. Left, Columbia wild type; right, *an3* mutant. Lower panels show paradermal views of palisade tissue. Note enlarged palisade cells in the *an3* mutant that has narrow and small leaves.

1-4 Evolution of AN gene family

How have these genes evolved the function of leaf-shape control? The *AN* is a homolog of animal *CtBP/BARS* gene family which has varied roles in morphogenesis and organelle control. To understand the common role(s) of *AN* subfamily in the plant kingdom, we analyzed a homolog of *AN* from *Ipomoea nil*, *IAN*, and showed that the *IAN* exhibits the same function with the *AN* on the control of leaf shape when introduced to *A. thaliana* (Cho *et al.* 2005).

II. Biodiversity of plant form

2-1 Biodiversity in leaf size

We are also interested in biodiversity in wild plants. This year, *Spiranthes sinensis* (Orchidaceae) in Japan was analyzed (Tsukaya 2005). *S. sinensis* shows a wide range of morphological diversity and a dwarf form has been known on Yakushima Island. Analysis on molecular variation of this species revealed that the dwarf form has not yet gained any specific variations in *ITS* and *trnL-F* loci, suggesting recent evolution of the dwarf form.

2-2 Genetic analyses of plant diversity

The so-called "Evo/Devo" study of leaf morphogenesis is also one of our research projects. In the course of field research of natural evolution of leaf shape/size, we have revealed some aspects of the biodiversity of plants (Ikeda *et al.* 2005, Okada *et al.* 2005, Tsukaya and Okada 2005, Tsukaya *et al.* 2005, Yokoyama *et al.* 2005). *Thismia mullerensis* and *Didymoplexiella cinnabarina* are new plant species discovered in our botanical survey in central

Publication List:

Original papers

- Cho, K.-H., Shindo, T., Kim, G.-T., Nitasaka, E., and Tsukaya, H. (2005). Characterization of a member of the *AN* subfamily, *IAN*, from *Ipomoea nil*. *Plant Cell Physiol.* **46**, 250-255.
- Horiguchi, G., Kim, G.-T., and Tsukaya, H. (2005). The transcription factor *AtGRF5* and the transcription coactivator *AN3* regulate cell proliferation in leaf primordia of *Arabidopsis thaliana*. *Plant J.* **43**, 68-78.
- Ikeda, H., Akiyama, S., Tsukaya, H., Mohamed, M., and Darnaedi, D. (2005). A cytotoxic study of *Impatiens* (Balsaminaceae) in Java and Borneo, Malesia. *J. Jap. Bot.* **80**, 271-277.
- Kim, G.-T., Fujioka, S., Kozuka, T., Tax, F.E., Takatsuto, S., Yoshida, S., and Tsukaya, H. (2005). The biosynthesis of active forms of brassinosteroids in *Arabidopsis*. *Plant J.* **41**, 710-721.
- Kozuka, T., Horiguchi, G., Kim, G.-T., Ohgishi, M., Sakai, T., and Tsukaya, H. (2005). The different growth responses of the *Arabidopsis thaliana* leaf blade and the petiole during shade avoidance are regulated by photoreceptors and sugar. *Plant Cell Physiol.* **46**, 213-223.
- Okada, H., Tsukaya, H., and Mohamed, M. (2005). Chromosome observations in species of *Cayratia* (Vitaceae). II. Intraspecific polyploidy in *C. trifolia*. *Acta Phytotax. Geobot.* **56**, 203-206.
- Tsukaya, H. (2005). Molecular variation of *Spiranthes sinensis* (Orchidaceae) in Japan, with special reference to systematic treatment of seasonally differentiated groups and a dwarf form, f. *gracilis*, from Yakushima Island. *J. Plant Res.* **118**, 13-18.
- Tsukaya, H., Iokawa, Y., Kondo, M., and Ohba, H. (2005). Large-scale general collection of DNA of wild plants in Mustang, Nepal. *J. Plant Res.* **118**, 57-60.
- Tsukaya, H., Nakajima, M., and Okada, H. (2005). *Didymoplexiella cinnabarina* (Orchidaceae): a new species from Muller Range, Central Kalimantan, Indonesia. *Acta Phytotax. Geobot.* **56**, 206-212.
- Tsukaya, H., and Okada, H. (2005). *Thismia mullerensis* (Burmanniaceae), a new species from Muller Range, Central Kalimantan. *Acta Phytotax. Geobot.* **56**, 129-133.
- Yokoyama, J., Fukuda, T., and Tsukaya, H. (2005). Molecular identification of the mycorrhizal fungi of the epiparasitic plant *Monotropastrum humile* var. *glaberrimum* (Ericaceae). *J. Plant Res.* **118**, 53-56.

Review articles

- Kim, G.-T., Yano, S., Kozuka, T., and Tsukaya, H. (2005). Photomorphogenesis of leaf : Shade-avoidance syndrome and differentiation of sun/shade leaves. *Photochem. Photobiol. Sci.* **4**, 770-774.
- Tsukaya, H. (2005). Leaf shape: genetic controls and environmental factors. *Int. J. Dev. Biol.* **49**, 547-555.