DIVISION OF PLANT DEVELOPMENTAL GENETICS (ADJUNCT)

Professor (Adjunct)
TSUKAYA, Hirokazu

Assistant Professor	YAMAGUCHI, Takahiro
Postdoctoral Fellows	ISHIKAWA, Naoko
	USAMI, Takeshi
	YANO, Satoshi
Visiting Scientists	GOTOH, Ayako
	ICHIHASHI, Yasunori
	NAKAYAMA, Hokuto
	TAKASE, Masahide
Technical Assistants	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 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 the 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 2008.

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

AN is a member of the *CtBP-BARS* gene family reported from animal genomes; last year, however, we showed that AN does not have any of the molecular functions of CtBP in *Drosophila melanogaster* (Stern et al. 2007). If so, how widely is the AN function conserved in plants? We have isolated a homolog of AN from *Larix gmelinii*, a gymnosperm, and named it *LgAN*. *LgAN* fully complemented all known morphological phenotypes caused by *an-1* mutation in Arabidopsis, suggesting that the AN function is conserved between angiosperms and gymnosperms (Li et al. 2008). Furthermore, our detailed analysis of intracellular localization suggested that AN have a unique role (or roles) in Golgi-related functions. Further analyses of AN functions are ongoing.

On the other hand, constitutive over-expression of deletion series of ROT4 revealed that a 32-amino-acid core region is enough to exhibit the ROT4 function when over-expressed.

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 around leaves (*i.e.* adaxial-abaxial, distal-proximal, and central-lateral polarities). Moreover, the genetic controls of leaf polarities were revealed to differ, at least in part, between eudicot and rice, a monocot model species. 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*. 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. Taken together, our data strongly suggests the presence of unknown mechanisms for flat leaf organogenesis that were not previously suspected from studies of model plants. We also established mutational and transgenic approaches to analyze the unifacial leaf formation; several interesting mutants of *Juncus* that exhibit abnormalities in leaf polarity were already isolated.

1-3 Size control of leaves and mechanisms of compensation

We have recently noticed that leaf organogenesis depends on "leaf meristem" that is seen only in the border region between leaf blade and leaf petiole. All cells required for leaf formation seem to be supplied from this leaf meristem. How are cell proliferation and cell enlargement coordinated in leaf morphogenesis? In a determinate organ - such as a leaf - the number of leaf cells is not necessarily reflected in leaf shape or, more particularly, in leaf size. Genetic analyses of leaf development in arabidopsis showed that a compensatory system (or systems) acts in leaf morphogenesis in a way that an increase in cell volume might be triggered by a decrease in cell number (reviewed in Tsukaya 2008). Thus, leaf size is, at least to some extent, regulated at the organ level by the compensatory system or systems. 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.

As a result, we have succeeded in isolating *oli* mutants

which have a specific defect in the number of leaf cells, *fugu* mutants that exhibit typical compensation syndrome, namely, decreased number of cells and increased cell volume, and *msc* mutants that exhibit an "opposite-type" compensation syndrome, namely an increased number of cells and decreased cell volumes.

This year we have revealed that: (1) *fugu5* phenotype is cancelled by supplying sucrose to the growth medium; (2) several *oli* mutations are loss-of-function mutations of ribosome biogenesis genes; (3) "opposite-type" compensation syndrome in *msc* mutants is attributed to accelerated heteroblasty (Usami et al., 2009); detailed analyses of this phenomenon strongly suggested that traits of heteroblasty are regulated by several different pathways. Furthermore, a new tool for studies of the mechanisms of compensation, chimeric expression system of *KRP2* or *AN3*, was established, and several candidate genes responsible for the compensation were selected from microarray analysis of *fugu2* and *an3*.

In addition, in the course of studies of *AN3* function, we found that *an3* mutation phenotype is drastically changed when combined with ribosome-biogenesis mutations and/or #2047 mutation (Figure 1). These facts suggest that AN3 is involved in various key aspects of organogenesis in Arabidopsis. Further analyses of the mechanisms of compensation are in progress.

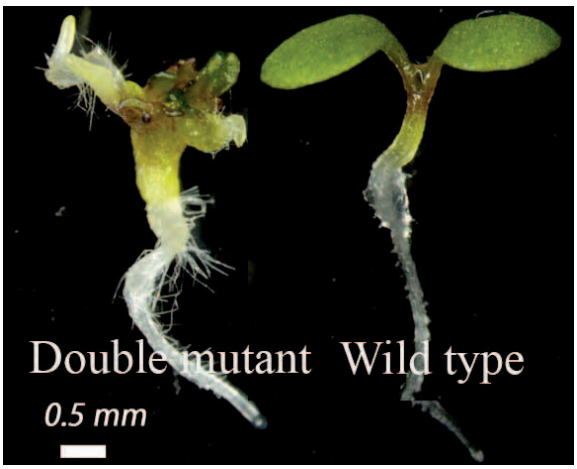

Figure 1. Ectopic root formation is seen on cotyledon region in *an3-#2047* double mutant. Bar, 0.5 mm.

1-4 Size control of leaves and ploidy level

Why does a high-ploidy level cause increased cell/leaf size? In other words, why are tetraploid leaf cells twice as large in volume as diploid leaf cells? The reasons are not yet perfectly understood. Curiously, plants with high-ploidy syndrome have more than eight sets of homologous chromosomes (8C), resulting in an increase in cell volume, but have smaller leaves (Tsukaya 2008).

The construction of a series of tetraploids of leaf shape/size mutants supplied us with a good clue for understanding the linkage mechanisms between the ploidy level and cell/organ size. We found that mutational defects in the endoreduplication were responsible for a curious enhancement of the effects of tetraploidization in terms of cell-size increase, suggesting that some unknown mechanisms (*e.g.* feedback systems) are hidden behind the relationship between the ploidy level and cell/organ size. We also found the ratio of cell size between diploid and tetraploid varied among the mutants examined, suggesting that an increase of cell size due to tetraploidization is not direct or automatic. Further construction and analyses of tetraploid mutants are in progress.

Ⅱ. Biodiversity of leaf form

We are also interested in the biodiversity of wild plants. This year we analyzed several achlorophyllous mycoheterotrophs. Mophological and molecular phylogenetic analyses revealed that *Monotropastrum humile* var. *glaberrimum* must not be conspecific to *M. humile* (Tsukaya et al. 2008). Molecular phylogenetic analysis of *Oxygyne shinzatoi* showed that this very rare genus would be basal taxon of tribe Thismieae (Yokoyama et al. 2008). Moreover, we have found a new species of the genus *Oxygyne*, *O. yamashitae* from Yakushima island (Yahara and Tsukaya 2008). This is the third species of this genus reported from Japan. Yakushima Island was proven once again to be a hot spot for plant biodiversity in Japan.

Publication List

〔Original papers〕

- Cho, K.-H., Tsukaya, H., and Kim, G.-T. (2008). Characterization of a dehydrogenase motif and an uORF in Arabidopsis *ANGUSTIFOLIA* gene. Plant Biotech. *25*, 365-368.
- Lin, X., Minamisawa, N., Takechi, K., Zhang, W., Sato, H., Takio, S., Tsukaya, H., and Takano, H. (2008). Isolation and characterization of the *Larix gmelini ANGUSTIFOLIA* (*LgAN*) gene. Planta *228*, 601-608.
- Nagano, A., Fukazawa, M., Hayashi, M., Ikeuchi, M., Tsukaya, H., Nishimura, M., and Hara-Nishimura, I. (2008). AtMap1: a DNA Microarray for Genomic Deletion Mapping in *Arabidopsis thaliana*. Plant J. *56*, 1058-1065.
- Tsukaya, H., Yokoyama, J., Imaichi, R., and Ohba, H. (2008). Taxonomic status of *Monotropastrum humile*, with special reference to *M. humile var. glaberrimum* (Ericaceae, Monotropoideae). J. Plant Res. *121*, 271-278.
- Yahara T., and Tsukaya H. (2008). *Oxygyne yamashitae*, a new species of Thismiaceae from Yaku Island, Japan. Acta Phytotax. Geobot. *59*, 97-104.
- Yokoyama, J., Koizumi, Y., Yokota, M., and Tsukaya, H. (2008). Phylogenetic position of *Oxygyne shinzatoi* (Burmanniaceae) inferred from 18S rDNA sequences. J. Plant Res. 121, 27-32.

〔Review article〕

- Tsukaya H. (2008). Controlling Size in Multicellular Organs: Focus on the Leaf. PLoS Biol. *6*, 1373-1376.