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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 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 2009.

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, we showed that homologs of AN from *Larix gmelinii,* a gymnosperm, *Marchantia polymorpha* and *Physcomitrella patens,* mosses, all fully complemented all known morphological phenotypes caused by *an-1* mutation in Arabidopsis, suggesting that the AN function is conserved among land plants. Furthermore, our detailed analysis of intracellular localization suggested that AN has a unique role (or roles) in Golgi-related functions. Further analyses of AN functions are ongoing.

On the other hand, we found that constitutive over-expression of ROT4 peptide in Arabidopsis caused abnormal protrusion of the inflorescence stem, suggesting that ROT might be somehow involved in positional value determination.

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 eudicots 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* (Yamaguchi and Tsukaya, J. Plant Res. 123, 35-41, 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. 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 unifacial leaf formation; 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 have recently noticed that leaf organogenesis depends on the "leaf meristem" that is seen just in the border region between leaf blade and leaf petiole. All cells required for leaf formation seem to be supplied from this leaf meristem. We also identified that *SPT* controls the size of the leaf meristem (Ichihashi et al., Plant Cell Physiol. 51, 252-261, 2010). 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 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 2006; 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 leaf-cell numbers. Using these mutants we revealed that the compensation is triggered only when decrease of cell numbers in leaf primordia exceeds some threshold level; and several *oli* mutations are loss-of-function mutations of ribosome biogenesis genes (Fujikura et al. 2009).

In addition, we have revealed that: (1) *fugu5* phenotype is cancelled by supplying sucrose to the growth medium; (2) large-leaved *gra* mutations are caused by translocation-dependent duplication of a segment of a chromosome (Horiguchi et al. 2009). This enlargement of leaves due to the duplication of the chromosome segment can be explained only partially by the increase of the gene dosage of known gene functions in the chromosome region, suggesting presence of some unknown genes for leaf size control; (3) "opposite-type" compensation syndrome in *msc* mutants was attributed to accelerated heteroblasty (Usami et al., 2009: Fig. 1). Detailed analyses of this phenomenon strongly suggested that traits of heteroblasty are regulated by at least two different pathways governed by small RNAs; (4) a new tool for studies of mechanisms of compensation, inducible, chimeric expression systems of *KRP2* or *AN3*, was established: and (5) several candidate genes responsible for the compensation were selected from microarray analysis of *fugu2* and *an3*.

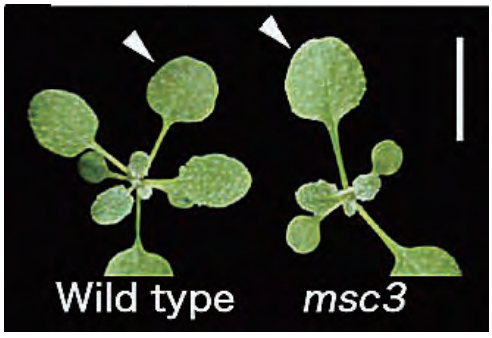

Figure 1. The *msc3* mutant has an altered heteroblasty pattern and develops larger leaf blades that contain a larger number and smaller size of cells, due to disturbance of miR156 function. Modified from Usami et al. (2009).

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. These facts suggest that AN3 is involved in various key aspects of organogenesis in Arabidopsis. We also found that plant Elongator regulates auxin-related genes during RNA polymerase II transcription elongation (Nelissen et al., PNAS *107*, 1678-1683, 2010). Further analyses on the mechanisms of compensation are in progress.

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 (Tsukaya 2009). 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. We found that *P. asiatica* is a cryptic allotetraploid from the molecular data, and pursued detailed molecular phylogenetic analyses of members of the subgenus *Plantago* of the genus *Plantago*. As a result, we found that most known species in this section are derived from inter-species hybridization. Surprisingly, whole species in some sections are found to be derived from inter-section hybridization, showing that subgenus *Plantago* is derived from extensive reticulate evolution (Ishikawa et al., 2009).

Publication List

〔Original papers〕

- Fujikura, U., Horiguchi, G., Ponce, M.R., Micol, J.L., and Tsukaya, H. (2009). Coordination of cell proliferation and cell expansion mediated by ribosome-related processes in the leaves of *Arabidopsis thaliana*. Plant J. *59*, 499–508.
- Horiguchi, G., Gonzalez, N., Beemster, G.T.S., Inzé, D., and Tsukaya, H. (2009). Impact of segmental chromosomal duplications on leaf size in the *grandifolia-D* mutants of *Arabidopsis thaliana*. Plant J. *60*, 122-133.
- Ishikawa, N., Yokoyama, J., and Tsukaya, H. (2009). Molecular evidence of reticulate evolution in the subgenus Plantago (Plantaginaceae). Amer. J. Bot. *96*, 1627–1635.
- Usami, T., Horiguchi, G., Yano, S. and Tsukaya, H. (2009). The more and smaller cells mutants of *Arabidopsis thaliana* identify novel roles for *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE* genes in the control of heteroblasty. Development *136*, 955-964.

〔Review article〕

- Tsukaya, H. (2009). Size regulation of plant body via control of cell volume by endoreduplication. Reg. Plant Growth Dev. *44*, 135-141.