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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 2006a).

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, 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 (reviewed in Tsukaya 2006a). 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 (Tsukaya 2006a). Furthermore, we have isolated and analyzed mutants with leaf polarity defects (*drl*: Cho et al. 2007) and leaf epidermis formation defects (*ale2*: Tanaka et al. 2007). Additionally, the accumulation of knowledge on the basic mechanisms of leaf shape control have 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 2007.

1-1 Polar growth of leaves in A. thaliana

AN is a member of *CtBP-BARS* gene family reported from animal genomes (reviewed in Tsukaya 2006b). Gene products of this gene family are very curious: they act as co-repressors in nuclei as a form of CtBP and also as Golgi regulators as BARS. Is AN a functional homolog of CtBP or of BARS? Our swapping experiments and two-hybrid analysis clearly showed that AN does not have any of the molecular functions of CtBP in *Drosophila melanogaster* (Stern et al. 2007). On the other hand, our detailed analysis of intracellular localization suggested that AN have a unique role (or roles) in Golgi-related function. Further analyses of AN functions are ongoing.

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

We have recently started to try to understand the genetic basis of the development of unifacial leaves that are known only 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 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 recently undertaken using members of the genus *Juncus*.

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* show that a compensatory system (or systems) acts in leaf morphogenesis and an increase in cell volume might be triggered by a decrease in cell number (Tsukaya 2006a). 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 specific mutants for number or size of leaf cells (Fujikura et al. 2007a, b). Moreover, we have isolated a number of new mutants that exhibit typical compensation syndrome, namely, decreased number of cells and increased cell volume: *fugu* (Ferjani et al. 2007). Combining these mutants as a resource, we have revealed that: (1) compensation syndrome results from the enhancement of a particular set of cell-expansion pathways which is required for normal leaf expansion; (2) compensation does not depend on the enhancement of endoreduplication; (3) compensation involves at least three different expansion pathways; and (4) compensation is not caused by the uncoupling of cell division from cell expansion at all. 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? The isolation of mutants with endoreduplication defects supplied us with a good clue for understanding the linkage mechanisms between the ploidy level and cell/organ size. *bin4* mutants cannot make their leaf cells higher than 8C and show severe dwarfism. If this dwarfism - and smaller leaves - are the direct result of lower ploidy level, tetraploidization of this mutant should suppress the dwarfism. We found that the tetraploid *bin4* showed partial recovery in cell/organ size, and this fact showed that ploidy level is a key factor for the determination of cell/organ size (Breuer et al. 2007). In addition, we found that the endoreduplication mutational defects were responsible for a curious enhancement of the effects of tetraploidization in terms of cell-size increase. Further studies of tetraploids of the other mutants are now ongoing to determine the precise relationship between the ploidy level and cell/organ size.

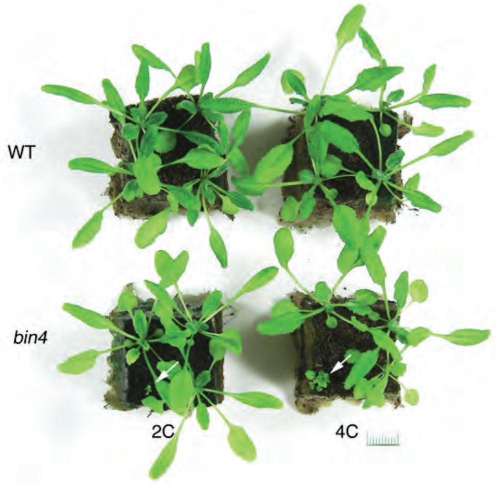

Figure 1. Severe dwarfism exhibited by the *bin4* mutant is partially recovered by tetraploidization. Arrows: homozygous individuals of the mutations. Bar, 1 cm.

Ⅱ. Biodiversity of leaf form

We are also interested in the biodiversity of wild plants. *Ainsliaea apiculata* is a perennial weed that is widely distributed in Japan. Some endemic varieties of this species, which are defined by characteristic leaf shapes, have been reported from Yakushima Island, Kyushu, Japan. We have re-examined the variations in the leaf shape of this species from Yakushima Island, in comparison with other localities in Japan, and found that the variations in the leaf shape were continuous with the other forms. Thus, these variants cannot be treated as distinct varieties. Yakushima Island was proved, however, to be a center locality of morphological variation in this species (Tsukaya et al. 2007a).

On the other hand, we have revealed that *Cayratia tenuifolia*, characterized by its leaf shape and flower/fruit characters, is a distinct species from the closely-related *C. japonica* (Okada et al. 2007). Cytological and molecular phylogenetic analyses of *Oxygyne shinzatoi* were also carried out (Tsukaya et al. 2007b; Yokoyama et al. 2008).

Publication List

〔Original papers〕

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