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Regulation of Leaf Polarity during Leaf Development

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Leaves are indeterminate organs and possess a lot of genes which is involved in establishing leaf polarities. These polarities are regulated relatively early during leaf development and defined relative to the factors intrinsic to the primordia and interactions with the shoot apical meristem (SAM). Recently, several genes that control the polarity of lateral organs have been identified. Our genetic study of *deformed root and leaf1* (*drl1*) mutant, which produces narrow, filament like leaves and defective meristems, revealed that DRL1 is involved in the regulation of SAM activity and leaf polarity. The *DRL1* gene was found to encode a novel protein showing homology to Elongator associate protein (EAP) of yeast KTI12. The amino acid sequence of DRL1 is universally conserved in prokaryotes and eukaryotes. DRL1 and the plant DRL1 homologs clearly formed a monophyletic clade, suggesting the evolutionary conservation of DRL1 homologs was maintained in the genomes of all land plants.

Key words: dorsoventrality, *drl1* mutant, leaf polarity, shoot apical meristem

The leaf is a major component of the shoot; it is the organ that is the key to a full understanding of plant morphogenesis and plant biodiversity. Leaves are the primary photosynthetic organs, and their shapes are adapted to the natural environment for efficient photosynthesis. Recent studies of the genetic control of leaf morphogenesis have provided an understanding of the underlying complexity of leaf shape control. The early

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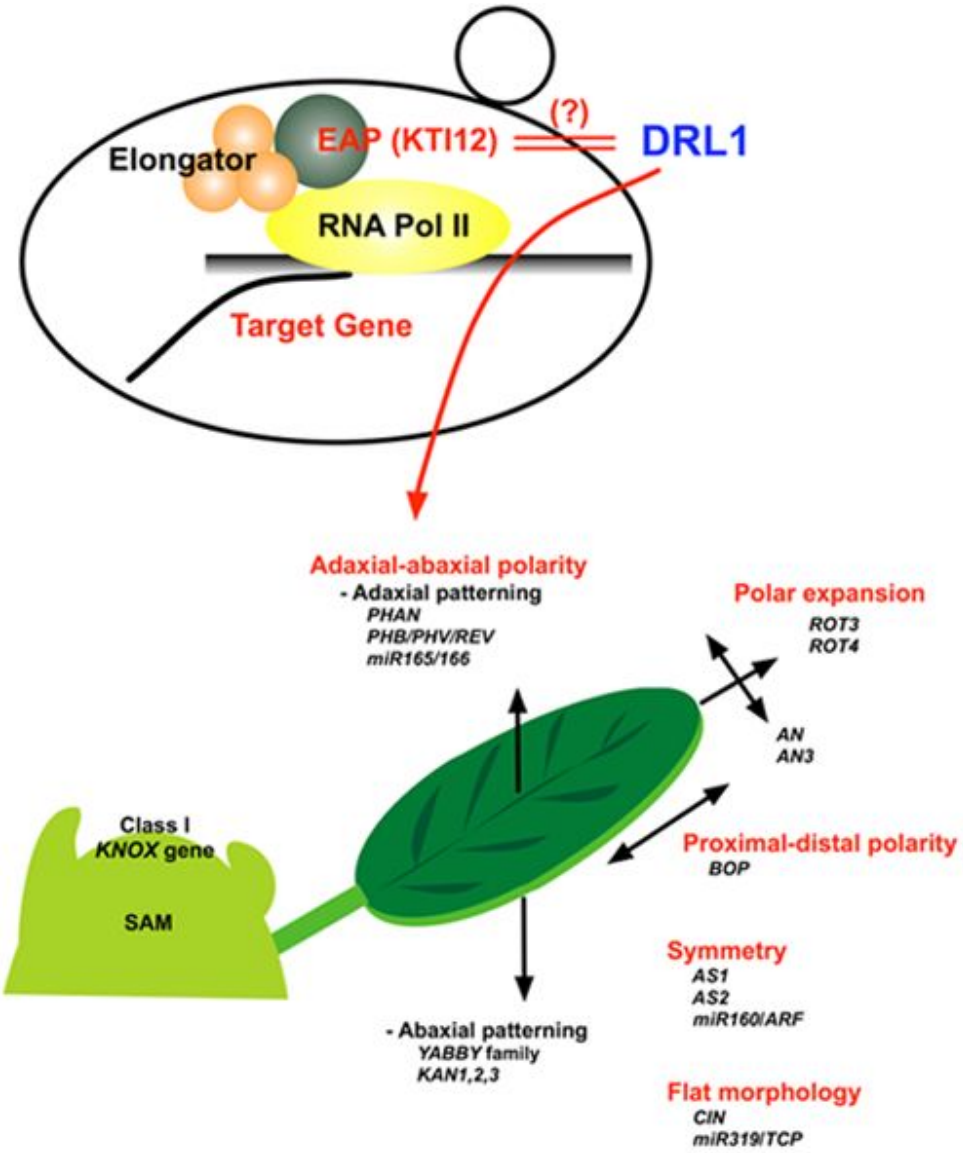

Fig. 1. A simple model representing several polarities in leaf development and putative role of DRL1 protein in leaf polarity. Yeast KTI12 which is a homologous protein of DRL1 regulates the transcription as an associated protein of Elongator complex. Several genes that control the initial stages of leaf formation, polarity (e.g., adaxial - abaxial polarity, proximal distal polarity, symmetry, and flat morphology), and expansion are summarized.

control of leaf shape relies on controlling leaf initiation at the shoot apical meristem (SAM), changes in the rates and planes of cell division, and polarity dependent differentiation of leaf cells (Steeves and Sussex, 1989).

Lateral organs contain two primary axes of polarity, a proximal - distal axis and an adaxial - abaxial axis (Fig. 1). In addition, leaves get the polarity of symmetry and flatness (Fig. 1). These polarities are established relatively early during leaf development and are defined relative to the SAM (Steeves and Sussex, 1989). Subsequent shaping of leaves in later development also involves the process of leaf expansion. The correlation between cell division and elongation plays an important role in establishing leaf morphology (Kim and Cho, 2006). The rates of cell division and elongation at each stage contribute to the final shape of a leaf (Steeves and Sussex, 1989, Tsukaya, 2003) and play important roles throughout leaf development.

Several recent molecular genetic studies of the polarity of lateral organs, including studies of adaxial-abaxial patterning, revealed that mechanism of polarity of leaves will helped us to understand the complexity of leaf shape (Siegfried *et al.*, 1999, Kerstetter *et al.*, 2001, McConnell *et al.*, 2001, Otsuga *et al.*, 2001, Emery *et al.*, 2003).

In this study, we isolated and characterized novel mutant that is involved in adaxial abaxial patterning of leaves. We will report and discuss about the roles of the *DRL1* gene in adaxial abaxial polarity of leaf development.

Materials and Methods

Plant materials and histological analysis

drl1-101 mutant allele was originally isolated from a library of *Ds* transposon containing *Arabidopsis* mutants (background ecotype Nossen-0) created using a local transposition system (Ito *et al.*, 1999). Plants were grown MS agar medium or in small plastic pots with vermiculite at 22°C as described previously (Kim *et al.* 2002). For anatomical analysis, samples were fixed in FAA solution under a vacuum as described by Kim *et al.* (1998). After dehydration, by applying a graded ethanol series, samples were embedded in Technovit 7100 resin and examined as described previously (Kim *et al.*, 1998).

Cloning and phylogenetic analysis

A full length coding sequence of DRL1 was isolated by using RT-PCR method with specific primers as described previously (Cho *et al.*, 2007). A phylogenetic tree was

generated using PROTPARS, a maximum parsimony algorithm that is included in the PHYLIP version 3.5 software package, as described in Kim *et al.* (2002, 2005). Topological robustness was assessed by bootstrap analysis with 100 replicates using simple taxon addition. Several short sequences in the N- and C-terminal regions that could not be aligned unambiguously were excluded from the analysis.

Results and Discussion

Genes involved in abaxial adaxial polarity control leaf shape during early development

Leaves initiate post embryonically at the periphery of the SAM in a radial pattern (Reinhardt *et al.*, 2000). The repression of the class-I *KNOX* gene *SHOOT MERISTEMLESS* (*STM*) and the activation of the myb-domain transcription factor gene *ASYMMETRIC LEAVES1* (*AS1*) are essential for leaf initiation (Long *et al.*, 1996, Byrne *et al.*, 2000). Shortly after the initiation of the leaf initial, the radially symmetrical primordia flatten in a plane parallel to the meristem periphery, and soon display dorsal ventral or abaxial adaxial ("abaxial" is away from the meristem; "adaxial" is adjacent to the meristem, see Fig. 1) polarity due to the asymmetrical distribution of cell types in the mature organ (Kim and Cho, 2006). Recently, the *BLADE-ON-PETIOLE1* gene of *Arabidopsis*, which encodes a BTB/POZ-domain protein, was shown to promote proximodistal identity (Ha *et al.*, 2004). Genes that regulate adaxial abaxial polarity in *Arabidopsis* are *PHABULOSA* (*PHB*), *PHAVOLUTA* (*PHV*), and *REVOLUTA* (*REV*), which encode class-III homeodomain/Leu zipper transcription factors (McConnell *et al.*, 2001, Otsuga *et al.*, 2001). Semi-dominant gain-of-function mutations in *PHB* and *PHV* result in the formation of adaxialized leaves (McConnell *et al.*, 2001). In addition, members of the *YABBY* and *KANADI* gene families regulate adaxial abaxial polarity by specifying abaxial cell fate (Siegfried *et al.*, 1999, Kerstetter *et al.*, 2001, Emery *et al.*, 2003).

Because of the formation of adaxial-abaxial polarity in leaf primordia, leaves are dorsoventrally flattened. Thus, the pathways that control organ polarity are pivotal in regulating leaf shape and growth. Although many genes that regulate dorsoventral patterning have been identified, detailed mechanisms of the genetic interactions between SAM activity and the differentiation of leaf organs remain unknown.

To elucidate the regulation of the differentiation of leaf cells by signals from the

SAM, we isolated mutations in genes that might be involved in the early steps of leaf development. To this end, we screened a collection of *Arabidopsis thaliana Ds* transposon insertion lines. Here, we report the isolation of the novel recessive mutant *356-2*, a new *drl1* mutant allele, and the function of DRL1 in SAM activity and leaf development.

The *drl1* mutant defective in the adaxial patterning of leaves

We isolated the mutant *356-2*, which forms abnormal leaves (Fig. 2), from a library of *Ds* transposon containing *Arabidopsis* mutants (background ecotype Nossen 0) created using a local transposition system, as previously reported (Ito *et al.*, 1999, Cho *et al.*, 2007). The phenotype of *356-2* was very similar to that of *drl1* mutant (Bancroft *et al.*, 1993). Previous our genetic analysis revealed that *356-2* mutant was a new allele of *drl1* mutant (Cho *et al.*, 2007). We named this mutant *drl1-101* and characterized this allele for further study. The leaves of the mutant are narrower than those of the wild type (No-0) at the fully expanded stage (Fig. 2a). Interestingly, the mutant produces trumpet like leaves and filamentous leaves (Fig. 2b, c). Several mutants, such as *phb 1d*, *kan* and *yab* of Arabidopsis, showed similar phenotype of filamentous leaves (Siegfried *et al.*, 1999; Kerstetter *et al.*, 2001, McConnell *et al.*, 2001). Mutations at the PHAN locus of Antirrhinum lead to the loss of dorsoventrality in leaves and floral organs and resulted in filamentous shaped organs (Waites *et al.*, 1998). However, the roots of the *drl1-101* mutant are slightly shorter than those of the wild (Fig. 2a).

The young leaves of the *drl1-101* mutant are narrower than those of the wild type (Fig. 2). To dissect this morphological difference at the cellular level, we observed the palisade mesophyll cells of the first leaf using whole mount preparations as described previously (Kim *et al.*, 1999). Wild type leaves have distinct, dense palisade mesophyll cells on the adaxial side (Fig. 2d). The sizes of the abaxial spongy mesophyll cells are more varied, and intercellular air spaces gradually became more prevalent in this region. In contrast, *drl1-101* mutant leaf blades contain many enlarged and irregularly sized palisade cells and intercellular spaces (Fig. 2e). Leaves of the *drl1-101* mutant contain fewer palisade cells than wild type leaves (Fig. 2e). The larger and fewer leaf cells in the *drl1-101* mutant, as compared to the wild type, strongly suggest that cell division activity during leaf development is significantly altered in the mutant. These results strongly suggest that the adaxial patterning of the *drl1-101* mutant is altered, and that it has, instead, the patterning of the normal abaxial structure of the leaf blade.

The trumpet like structure of leaves of the *drl1-101* mutant resulted from the attachment of the petiole to the middle part of the leaf blade (Fig. 2b, c). In transverse sections of trumpet like leaves of the *drl1-101* mutant, the middle side of the leaf blade

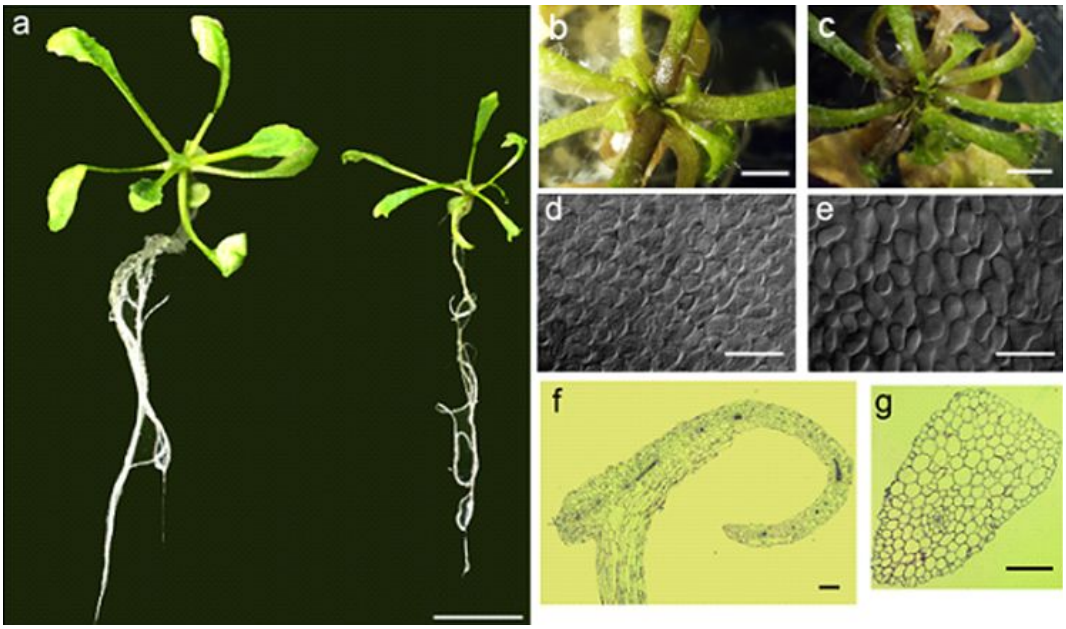

Fig. 2. Phenotypes of the wild type (No-0) and the *drl1 101*-mutant. (a) Leaf and root phenotypes of the wild type (left) and *drl1-101*. Bars, 1 cm. (b, c) Phenotype of trumpetlike and filamentous leaves of *drl1-101*. (d, e) Paradermal images of round palisade cells of the wild type (d) and of irregularly sized palisade cells of *drl1-101*. (f, g) Transverse sections of trumpet like leaves (f) and filamentous leaves of *drl1-101* (g).

was fused with the petiole at the cellular level (Fig. 2f). Some palisade tissue in these trumpet like leaves also had a sponge like structure (Fig. 2f). Filamentous leaves of the *drl1-101* mutant were nearly radial at the initiation stage, but these leaves still displayed some polarity, as a flat structure was present on the adaxial sides of the leaves (Fig. 2g). Anatomical studies of other mutants which had filamentous leaves also revealed that mutants had a defective adaxial abaxial patterning. For instance, transverse sections of *Antirrhinum* leaves *phantastica* (*phan*) plants revealed that *phan* leaf was radial and composed of abaxial parenchyma surrounded by abaxial epidermis, compare to wild type (Waites *et al*., 1998). This vascular arrangement was also altered in the filamentous leaves of the *drl1-101* mutant (Fig. 2g), as it is in the abaxialized radial leaves of the *phan* mutants of *Antirrhinum* (Waites *et al*., 1998) and the abaxialized cotyledons of the *phb phv rev* triple mutant (Emery *et al*., 2003). Taken together, these results strongly

suggest that the adaxial patterning of the *drl1-101* mutant is altered, and that it has an abaxial identity.

DRL1 encoding a homologous gene of Elongator associate protein has evolutionally conserved sequence

We previously isolated *DRL1* gene by using TAIL-PCR method (Cho *et al*., 2007). Transposed *Ds* element is inserted in an exon of the At1g13870 locus in *drl1-101* mutant (data not shown). The gene encodes a ORF of 34 kDa protein with 302 amino acids. The predicted amino acid sequence contains two characteristic motifs: a putative ATP/GTP binding motif near the N terminus and a CaM binding motif near the C terminus. The *Arabidopsis* database indicates that *DRL1* is a single copy gene in the *Arabidopsis* genome. The DRL1 protein is similar to the yeast KTI12 protein, which associates with the Elongator complex (Frohloff *et al*., 2001). KTI12 belongs to a family of chromatin associated proteins that interact with the Elongator complex, a component of the elongating form of RNA polymerase II that also has histone acetyltransferase activity (Frohloff *et al*., 2001, Petrakis *et al*., 2005). DRL1 homologs are present in organisms from archaebacteria to mammals, including *M. kandleri*, *S. pombe*, *C. elegans*, *D. melanogaster*, *M. musculus*, and *H. sapiens*. DRL1 protein exhibited the strongest homology to a rice protein AK099835 (58% identity) from Arabidopsis, homology to a *Xenopus* protein AAH99247 (35% identity), and to the yeast KTI12 (28% identity).

To examine the evolutionary history of the *DRL1* genes, we compared the DRL1 sequence to available sequences of other KTI12 homologs. The phylogenetic tree revealed that DRL1 and the KTI12 homologs can be divided into several subfamilies (Fig. 3): one each for fungal, plant, and animal proteins. The analysis also indicated that these amino acid sequences are evolutionally conserved from prokaryotes to eukaryotes. Moreover, DRL1 and the plant DRL1 homologs clearly formed a monophyletic clade, suggesting the evolutionary conservation of DRL1 homologs in the genomes of all land plants (Fig. 3). The biochemical function of DRL1 associated with chromatin remains still unknown, but its elucidation will provide an important clue for understanding plant development. Further elucidation of the target processes of these transcription factors will allow us to understand the role of temporal and spatial coordination of differential growth during the formation of adaxial - abaxial polarity in early leaf development and SAM activity.

The genes controlling leaf shape that have been isolated and characterized to date offer considerable promise for the improvement of crops and horticultural plants. It should be possible to engineer plants using these genes to design horticultural novelties.

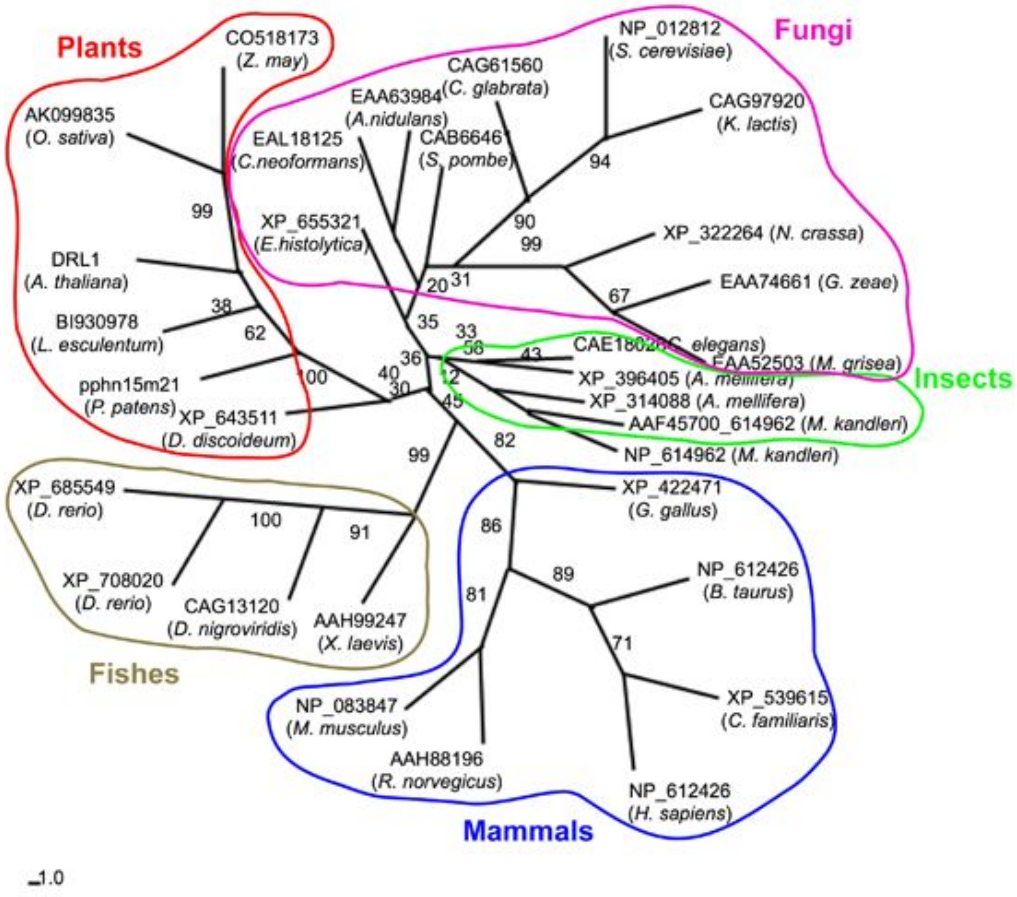

Fig. 3. Phylogenetic tree of amino acid sequences of DRL1 and related proteins. The phylogenetic tree was generated from the deduced amino acid sequences of DRL1 of *Arabidopsis* and its homologs in 30 other organisms, including archaebacteria, protozoa, *Dictyostelium*, fishes, insects, and mammals. Numbers at nodes indicate bootstrap values.

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잎의 발생과정에 있어서의 극성제어

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잎은 무한생장기관으로 잎의 극성제어에 많은 유전적인 요소가 필요하다. 이들 극성은 잎의 초기발생과정에서 제어되기 시작하고, 정단분열조직과 잎기관의 원기와의 제어를 담당하는 인자들에 의해서 결정이 된다. 본 연구에서는 가늘고 바늘처럼 생긴 잎을 가진 *deformed root and leaf1* (*drl1*) 돌연변이체를 유전학적 해석하였고, 그 결과 *DRL1* 유전자는 정단분열조직과 잎의 극성축을 제어하고 있는 것으로 판명되었다. 이 *DRL1* 유전자는 효모의 *KTI12* 유전자 산물과 유사한 단백질인 Elongator associate protein을 만들어 내는 것으로 판명되었다. 또한, 이 단백질의 아미노산 서열이 원핵생물에서부터 진핵생물까지 광범위하게 진화적으로 보존되고 있는 것으로 밝혀졌다. 특히, DRL1 단백질과 유사한 식물의 단백질은 계통해석 결과 단일계통을 나타내고 있는 것으로 나타났고, 이는 이 단백질들이 육상식물의 진화과정에서 잘 보존되고 있음을 시사하고 있다.

주요어: *drl1* 돌연변이체, 배복성, 잎의 극성, 정단분열조직

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