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Flat leaf formation realized by cell-division control and mutual recessive gene regulation

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HIGHLIGHTS

- We study mechanism for flat leaf formation by using mathematical model.
- Coupling of cell proliferation and gene expression is not enough for flat leaf.
- Polarized cell division increases the success rate for flat leaf formation.

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ABSTRACT

Most of the land plants generally have dorsoventrally flat leaves, maximizing the surface area of both upper (adaxial) side and lower (abaxial) side. The former is specialized for light capturing for photosynthesis and the latter is specialized for gas exchange. From findings of molecular genetics, it has been considered that the coupled dynamics between tissue morphogenesis and gene regulation for cell identity is responsible for making flat leaves. The hypothesis claims that a flat leaf is generated under two assumptions, (i) two mutually recessive groups of genes specify adaxial and abaxial sides of a leaf, (ii) cell divisions are induced at the limited region in the leaf margin where both of two groups are expressed. We examined the plausibility and possibility of this hypothesis from the dynamical point of view. We studied a mathematical model where two processes are coupled, tissue morphogenesis induced by cell division and deformation, and dynamics of gene regulations. From the analysis of the model we found that the classically believed hypothesis is not sufficient to generate flat leaves with high probability. We examined several different modifications and revision of the model. Then we found that a simple additional rule of polarized cell division facilitates flat leaf formation. The result of our analysis gives prediction of possible mechanism, which can be easily verified in experiments.

1. Introduction

Most of the plant species develop dorsoventrally flat leaves. The flat shape increases the surface area of both upper and lower sides, which have different specialized properties, called bifacial properties. The upper or adaxial side characterizes light capturing for photosynthesis. The lower or abaxial side characterizes gas exchange through stomata.

It was firstly shown that morphogenesis of flat leaves and formation of adaxial–abaxial identity are strongly linked from examination of the loss-of-function mutant *phantastica* (*phan*) in *Antirrhinum majus* (snapdragon) (Byrne et al., 2000; Waites et al., 1998; Waites and Hudson, 1995). In the model plant Arabidopsis, several genes are shown to be expressed in an adaxial- or abaxial-specific manner by recent molecular genetics studies (Yamaguchi et al. 2012; Fukushima and Hasebe, 2014). Adaxial–abaxial genes intricately repress each other directly by transcription factors or indirectly by way of small RNA (Eshed et al., 2001; Izhaki and Bowman, 2007; Kerstetter et al., 2001; Husbands et al., 2009; Kidner and Timmermans, 2010). It has been thought that this mutual repression relation produces adaxial–abaxial complement patterns in leaf tissue. Several families of transcription factors play key roles in the establishment of leaf adaxial–abaxial polarity with polar expression patterns. *The class III HOMEODOMAIN-LEUCINE ZIPPER* (*HD-ZIPIII*) gene family plays a key role to make the cell identity adaxial. Loss-of-function of *HD-ZIPIII* genes results in abaxialized cotyledons (Emery et al., 2003; Prigge et al., 2005). On the other hand, members of *KANADI* (*KAN*) (Eshed et al., 2001;

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Izhaki and Bowman, 2007; Kerstetter et al., 2001), *AUXIN RESPOSE FACTORS3/ETTIN* (*ARF3/ETT*) and *ARF4* gene families (Pekker et al., 2005) are expressed in the abaxial domain of leaf primordia and promote abaxial cell fate specification. Loss-of-function *kan* mutants shows adaxialized leaves such as loss of lamina flattening, fully radialized leaves and narrow leaves with ectopic adaxial domains on the abaxial side (Eshed et al., 2001, 2004). In addition to transcription factor genes, small regulatory RNAs are also localized in an adaxial- or abaxial-specific manner (Chitwood et al., 2009; Kidner and Martienssen, 2004; Garcia et al., 2006) and have an activity of intercellular mobility through plasmodesmata (Carlsbecker et al., 2010; Miyashima et al., 2011; Vatén et al., 2011). These experimental results imply that abnormal expressions of genes for adaxial–abaxial identity directly result in abnormal shapes of leaf.

Histological observation showed, in the early stage of leaf blade development, cell proliferation is observed only at the marginal region (Esau, 1977). Waites and Hudson observed detailed distribution of outgrowth region of the leaf lamina and found that it is limited at the boundary between the adaxial and abaxial sides. From these observations they proposed a hypothesis claiming that a regulation of cell proliferation by adaxial–abaxial gene polarity realizes the morphogenesis of flat leaves (Waites and Hudson, 1995). The hypothesis is now supported by two assumptions that (i) regulations between two groups of genes specifying adaxial and abaxial sides are mutually recessive, and that (ii) cell divisions are induced at the limited region in the boundary where both of two groups are expressed in the leaf margin. From these two mechanisms, many researchers expected that formation of adaxial–abaxial polarity and flat leaf formation are realized at the same time. However, it is unclear whether the dynamics based on this hypothesis can realize stable growth of flat leaves. It is neither obvious nor trivial whether the growing tip induced at the boundary of gene expressions will keep its shape with time even under the perturbation of cell proliferations. In this study we examine the relevance of the Waites and Hudson's hypothesis from the dynamical point of view using mathematical modeling. We are standing on the basic understanding that the mechanism proposed by Waites and Hudson is a major driving force for making flat leaves (Fig. 1). Our examination focuses on whether the basic understanding is sufficient for producing the bifacial, flat leaf. In conclusion we will show that only the mechanism based on the hypothesis cannot produce flat leaves with high probability. Taking this result, moreover, we propose additional important mechanism, cell division rule, which stabilizes the morphogenesis of flat leaves.

1.1. Model

Leaf primordia protrude from sites of auxin maxima in the shoot apical meristem (SAM) and grow flat like a sheet by cell divisions and tissue deformation. We consider a cross section of a leaf primordium perpendicular to the distal-proximal axis and model the cell group in the section on two-dimensional space. A real leaf primordium is a three-dimensional object. Here, in this study, we assume that the growth along the distal-proximal axis is uniform and focus on only the pattern formation on the two-dimensional space perpendicular to the distal-proximal axis.

We model the leaf growth by coupling two processes, cell-based morphogenesis and gene expression. We used vertex dynamics for modeling cell-based morphogenesis (Nagai and Honda, 2001). In this mathematical modeling, the dynamics of shape of polygonal cell is modeled by a set of ordinary differential equations for position of vertices of the polygon. For the second process of gene expression, we used an ODE for the dynamics of activity of each gene in each cell under the assumption that gene activities are uniform inside of each cell. In real plants, many genes are involved in the dynamics of gene expression of adaxial–abaxial polarity. To simplify this system, we consider a system where two genes (adaxial and abaxial genes) mutually inhibit each other. We used Euler's method for numerical calculations with sufficiently small time step ($\Delta t=0.001$).

1.2. Cell-based morphogenesis (Vertex Model)

Vector $\boldsymbol{r}_i$ indicates the position of i-th vertex. Each vertex moves to the direction to decrease the potential energy U as defined below. ∇_i is defined as $\nabla_i=\left(\frac{\partial}{\partial x_i}, \frac{\partial}{\partial y_i}\right)$.

$$\frac{d\boldsymbol{r}_i}{dt} = -\nabla_i U + \boldsymbol{\eta}_i$$

$$U = \sigma_L \sum_{k\in IT} L_k + \sigma_O \sum_{k'\in TS} L_{k'} + \kappa_S \sum_{\alpha} (S_\alpha - S_{std})^2$$

IT; Internal tissue, TS; Tissue surface

Variables and parameters represent L_k; length of edge k, $L_{k'}$; length of edge k' at tissue surface, S_α; area of cell α and S_{std}; standard cell area. σ_L and σ_O denotes the interface energy per unit length between two neighboring cells and against the outside, respectively. κ_S is the elastic constant.

1.3. Gene expression

As for the dynamics of mutual repression system, we refer to Tameshige et al. (2013). The cell type is determined by the level of gene expression. Our focal cell types here are adaxial and abaxial. If the level of adaxial factor is larger than that of abaxial, the cell type is adaxial, and vice versa. The dynamics of the adaxial (AD) and abaxial (AB) factors are expressed by ODEs as follows:

$$\frac{dAD_i}{dt} = p_{AD} + r_{AD}\frac{1}{1+AB_i^2} - \left(d_{AD} + c_{AD}\frac{AB_i}{1+AB_i}\right)AD_i + D_{AD}\sum_k \frac{l_k}{S_i}(AD_k - AD_i)$$

$$\frac{dAB_i}{dt} = p_{AB} + r_{AB}\frac{1}{1+AD_i^2} - \left(d_{AB} + c_{AB}\frac{AD_i}{1+AD_i}\right)AB_i + D_{AB}\sum_k \frac{l_k}{S_i}(AB_k - AB_i)$$

Each value and parameter represent, AD_i; adaxial factor concentration of i-th cell, AB_i; abaxial factor concentration of i-th cell, p_{AD}, p_{AB}; production rate, r_{AD}, r_{AB}; repression of transcription, d_{AD}, d_{AB}; decay rate, c_{AD}, c_{AB}; the level of post-translational repression by small RNAs, D_{AD}, D_{AB}; diffusion constant, S_i; cell area of i-th cell, l_k; contact area against neighbor cells.

To couple dynamics of cell-based morphogenesis and gene expression, we assume that cells divide at leaf margin when the

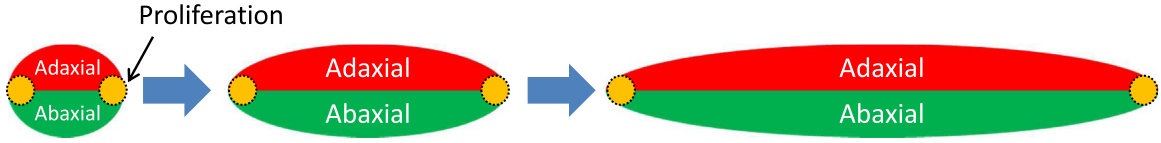

Fig. 1. Schematic view of the Waites and Hudson's hypothesis for flat leaf formation.
The hypothesis for flat leaf formation is supported by two assumptions that (i) regulations between two groups of genes specifying adaxial and abaxial sides are mutually recessive, and (ii) cell divisions are induced at the limited region at the boundary where both of two groups are expressed in the leaf margin.