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

by

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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.

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, Izahki 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-ZIP III*) gene family plays a key role to make the cell identity adaxial. Loss-of-function of *HD-ZIP III* 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, Izahki and Bowman 2007, Kerstetter et al. 2001), *AUXIN RESPONSE 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 and Eshed et al 2004). In addition to transcription factor genes, small regulatory RNAs are also localized in an adaxial- or abaxial-specific manner (Chitwood et al. 2009; Kinder et al. 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). Waits 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 (Waits 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 Waits 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 Waits 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.

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 modelled 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 ($\Delta t=0.001$).

Cell-based morphogenesis (Vertex Model)

Vector $\mathbf{r}_i$ 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 $\nabla_i = \left(\frac{\partial}{\partial x_i}, \frac{\partial}{\partial y_i} \right)$.

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

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

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.

Gene expression

As for the dynamics of mutual repression system, we refer to Tameshige (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:

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

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 expression levels of both adaxial (AD_i) and abaxial (AB_i) gene are over threshold K ($=0.15$). A cell division is implemented by adding new vertices on the middle point of two non-adjointed edges of the dividing cell and by connecting them by a new edge. After adding the new edge, the size and shape of the daughter cells will spontaneously come close to the optimum through the dynamics of vertex model. Depending on the ways of choosing the pair of edges for division, we modeled three different types of cell division; random, periclinal and anticlinal division. The details of choosing the pair of edge of dividing cell are shown in Appendix. There are two ways for distribution of adaxial and abaxial factors with cell division: (1) half

quantities of concentration of dividing cell are inherited into two daughter cells, and (2) same quantities are inherited. The first model is appropriate when cell volume recovery is sufficiently rapid after cell division. Regardless of ways of distribution, we obtained qualitatively the same results from both ways. Each cell at margin of a leaf is examined every 0.3 (0.33, 0.47, 0.5 or 1.0) unit time whether it should divide or not from the condition of gene expression levels above. If the conditions above are not satisfied, cell division does not occur. The values of parameters are fixed as follows: $\sigma_L=2.0$, $\sigma_O=3.4$, $\kappa_S=1.0$, $S_{std}=3.5$, $p_{AD}, p_{AB}=0.1$, $r_{AD}, r_{AB}=2.0$, $d_{AD}, d_{AB}=0.1$, $c_{AD}, c_{AB}=2.0$, $D_{AD}, D_{AB}=0.075$. With these fixed parameters, we did numerical calculation until number of cell become 900.

Initial condition

We use the initial state of leaf primordia as an ellipse composed of twenty four cells. We assume that an initial leaves already has adaxial-abaxial polarity, which is reasonable from experimental results (Sussex, 1951, 1954). Upper side (adaxial side) cells which are near in SAM has nine cells whose AD and AB values are $AD=0.9$ and $AB=0.1$, respectively. Six cells in middle area of the primordia has $AD=0.5$ and $AB=0.5$. Nine cells of lower side (adaxial side), far from SAM, have $AD=0.1$ and $AB=0.9$, respectively.

RESULTS

Random cell division

We assume that the direction of the cell division in each cell is randomly chosen. In other words, cells divide by new cell wall which is randomly oriented to the organ surface. For convenience, we here name this cell division manner “Random cell division”. For the details of cell division rule, see Appendix. We repeated the calculation of the dynamics of morphogenesis and gene expression numerically for 250 times by changing the random seeds and observed the results.

Examples of the successful growth of the leaf are shown in Fig. 2(a). The leaves have a flat structure. The boundary between adaxial and abaxial domains is continuously maintained at the

center of the leaf on the way of development. Several failure examples, where non-flat leaves are obtained, are shown in Fig. 2(b). In one example, the adaxial domain occupies the larger area than the abaxial does (Fig. 2(b) top-right). In other one example, the adaxial domain is included by the abaxial domain (Fig. 2(b) top-middle).

To evaluate the obtained leaf shapes, we introduce the characteristic parameters (CPs) which express the results of numerical calculation quantitatively. We introduce four CPs as follows: $CP1 = \frac{C}{2\sqrt{\pi A}}$ measuring the degree of flatness, $CP2 = \frac{B}{C}$ measuring how boundary is winded, $CP3 = \frac{|C_{AD}-C_{AB}|}{C}$ measuring adaxial-abaxial domain bias. CP4 has one or zero integer value, where the integer value 1 means that the obtained leaf has just one adaxial-abaxial domain. Each value represents that C ; Circumference of leaf, A ; Area of cross section, B ; Domain boundary length, C_{AD} ; Adaxial domain surface length and C_{AB} ; Abaxial domain surface length.

To determine criteria of success leaves, we prepared a sample set of 750 patterns by numerical simulation with cell division length 1.0, and classified it artificially by human-eye observation into two: success set of 497 patterns and failure set of the others. Fig. 3 shows the scatter plots of the CPs of the sample patterns and exemplifies that the CP values express feature of leaves obtained from numerical calculation. The examples of defect patterns and their CPs are explained in Appendix. The range of CP values for “successful patterns” is determined so that all of the members of success set are included in it. In other words, a pattern is “successful” if the CPs of it satisfy the following: $Th1 < CP1$, $Th2 < CP2 < Thu2$, $CP3 < Thu3$, where the thresholds are given as: $Th1 \equiv \min[spCP1_i]$, $Th2 \equiv \min[spCP2_i]$, $Thu2 \equiv \max[spCP2_i]$, $Thu3 \equiv \max[spCP3_i]$ ($spCP_i$; successful pattern CP (i , successful pattern number)). Here, our choice from the success set are: $Th1=1.8$, $Th2=0.58$, $Thu2=0.72$, $Thu3=0.29$.

By using these CP values as the criteria we evaluate obtained patterns and judge automatically whether they are “normal leaf” or not. In the following we call “successful leaves” as the ones satisfying the criteria. For example, the leaves satisfying these criteria appear to be flat (Fig. 3 #2). Outside the normal leaf condition, numerical calculations produce many leaves whose shapes

are diverse (Fig. 3).

We obtained at most about 60 % success rate with CPs' criteria even if we search successful conditions by changing important parameters, like cell cycle length, which controls the balance between growth and shape relaxation of tissue (Fig. 4 Green). We set the parameter values in reaction term of gene expression for cell-type specification so that the system has symmetric bi-stable stationary points from the biological observations that cells in a leaf express either adaxial or abaxial genes exclusively. After choosing one of such parameter set in reaction term, we examined effect of two parameters, diffusion constants (D_{AD} , D_{AB}) and threshold for cell division (K). Both of these parameters influence the number of cells competent to divide. We did numerical calculations in several diffusion constants (D_{AD} , D_{AB}) and threshold of cell division (K) with random cell division and the cell division length 1.0 (Fig 5(a)). The results show that the large (small) diffusion constants and large (small) threshold reduce the success rate of leaf growth. There is an optimum parameter region for normal leaf development, although the success rate in this parameter examination does not exceed 75 % (Fig 5(b)). In the case where diffusion constant (D) is small and the threshold (K) is small, each cell has strong cell-autonomous feature because of small D . This results in winded boundary between AD and AB (Fig. 5(c)). In the region that the diffusion constants are small and the thresholds are large, there are no cells that can be divided and the leaf growth is stopped because no cells are satisfied with the condition of cell division (Fig. 5(d)). In the case of large diffusion constants, instability of the boundary between AD and AB is easily induced by the cell division. This instability results in traveling waves of AD or AB factors. Thus, the tissue of leaf is occupied by AD or AB only and leaf developments stop (Fig. 5(e)). These results show that the basic model based on the hypothesis by Waits and Hudson is incomplete to realize high success rate. We need to modify the model.

We give intuitive explanation for the reason why abnormal shapes are obtained frequently with random cell division. The adaxial-abaxial boundary in leaf margin frequently deviates from the direction of growing axis in the dynamics. Once a small deviation occurs by chance, the deviation would not be diminished autonomously because there is no mechanism to reduce the deviation, i.e.

straight growth is not a stable but neutral solution in the dynamics. For flat leaf formation, the growing axis at the leaf margin should be kept straight. However, this straight growth is easily disturbed and will not recover autonomously.

Polarized cell divisions

To resolve the problem of instability of growing axis, we introduced two cell division rules. There are two types (Fig. 6) as follows: one is periclinal division, where a new cell wall forms parallel to the organ surface (Fig. 6(a)). The other one is anticlinal division, where a new wall forms vertical to the organ surface (Fig. 6(b)). We name these cell division manners “polarized cell division”. For the details of cell division rules, see Appendix. The examples of results for the time evolution of leaf growth in numerical calculation under the periclinal division rules are shown in Fig. 7.

Fig. 4 shows the success rate (%) of flat leaves against cell cycle length for each division rule. When cell cycle length is 0.5, the success rate of random cell division is less than 50 %. The rate of anticlinal cell division is even lower than that of the random division. On the other hand, the rate of periclinal cell division is close to 90 % under the same value of the cell cycle length. As the cell cycle length increases, the success rates of random and anticlinal division increase, and that of periclinal division decreases. The differences of success rates between these three roughly decrease with cell cycle. However, the rate of periclinal cell division is the highest even if the cell cycle length is twice from original. We confirmed that the periclinal division always gives the highest success rate for flat leaf formation under the broad range of the cell cycle length. In addition, the success rates of the other two division rules are strongly influenced from cell cycle length, although that of periclinal division gives almost constant values irrespective of the cell cycle length.

Here, we provide a possible explanation that such a simple modification of cell division strongly reduces fluctuation of growth direction in developmental process in Fig. 8. The deviation of growing axis at the leaf margin was induced when cell divisions occur. Among two daughter cells of a divided cell, both are possibly selected as the next cell to divide because of the inheritance

of gene expression. In the case of periclinal division, one located at the margin of the leaf will be always selected as the next cells for division among two daughter cells. This process will not change the direction of growing axis of leaf margin. Fig. 9(a) shows the change of shape in a normal growth with the periclinal division at peripheral region. On the contrary, in the cases of random division or anticlinal division, either of two daughter cells will be the next-dividing cell with almost equivalent probability. We cannot specify which daughter cell will divide from their positions. This is the possible origin of fluctuation of growing axis in random cell division and anticlinal division. Fig. 9(b) shows the change of shape in an abnormal growth with the anticlinal division in the developmental process.

DISCUSSION

In this study we examined the plausibility of classically believed hypothesis for flat leaf development by mathematical modeling. We found that the coupled dynamics between gene regulation and cell proliferation, which is the essence of the hypothesis, is not sufficient for generating flat leaves with high probability. Under random cell division, the boundary between adaxial and abaxial domains at the leaf margin easily deviates by fluctuations in cell proliferation on the way of dynamical process. (The deviation of the growing axis will not be reduced autonomously, i.e. the straight growth is not stable.) The deviation of growing axis results in non-flat, rugged leaves. To solve the problem, we searched possible mechanisms including gene regulations which enhance the ability of flat-leaf formation. After some examinations, we found a simple plausible mechanism which strongly improves the success rate for flat-leaf formation. It is a control of division axis. There are two types of regulations, anticlinal and periclinal division. These two different regulations have the inverse effect. The periclinal division strongly improves the success rate for flat-leaf formation. On the other hand, the anticlinal division rather decreases the success rate. Another positive result of the periclinal division is robustly high success rate for the broad range of the cell cycle length. We predict the control of cell division axis as another important mechanism for realizing flat leaf development in real plants. This prediction would be verified

easily by experimental observation. In actual facts, it was reported that the periclinal divisions occur at the periphery of the SAM in the leaf primordia, which is consistent with our prediction (Cunninghame and Lyndon, 1986; Tepfer, 1960).

Unlike animal cells, plant cells do not change their relative position among cells because of the constraint of cell walls. Plant morphogenesis is the splendid process that the accumulation of cell divisions makes up plant individual. From this, it is expected that the regulation of cell division could be one of the main factors for plant morphogenesis. At the same time this view provides us another biological question. What is the mechanism by which each cell knows the global direction to divide in a plant body? Cell division control requires existence of such a mechanism. In the case of the bifacial leaf formation, the spatial pattern of adaxial-abaxial gene expression can be a candidate for directional information. In addition to the bifacial leaf, the research for unifacial leaf formation will be a key to unclear the mechanism for polarization of cell division. Unlike the bifacial leaves, this type of leaf does not have adaxial-abaxial pattern formation. Some of the unifacial leaf species can make flat leaf (Yamaguchi et al., 2010). It means that cells in this type of leaf have to know their positions and directions without adaxial-abaxial spatial information. Fukushima and coworkers showed that the polarized cell division is an essential factor to produce intricate shaped leaf like carnivorous pitcher leaves of *Sarracenia purpurea* (Fukushima et al., 2015).

The morphogenic and mechanical signals are candidates for the trigger of polarized cell division. It was reported that auxin works in regulating polarization of cell divisions in embryonic development (Yoshida et al., 2014). In animal morphogenesis, mechanical signal is one of the important factors for the polarized cell division (Fink et al. 2011). As well as the animal cells, this kind of signal may also work in plant morphogenesis. In *yabby* mutants, regardless of the establishment of the adaxial-abaxial pattern, the lamina outgrowth is lost (Sarojam et al., 2010). It is expected that *YABBY* activity may works as an output of these signals for polarized cell division.

In this study we found that the bifacial leaf morphogenesis is dependent on not only adaxial-abaxial pattern but also polarization of the cell division. Recent molecular genetics have

found the regulatory network of determination of adaxial and abaxial side in detail. However, the mechanism which determines the polarization of cell division is still unknown. The result of our study insists that elucidating the division-rule mechanism leads to comprehensive understanding of general plant morphogenesis.

APPENDIX

Implementation of cell division

A cell division is implemented by adding new vertices on the middle point of two non-adjointed edges of the dividing cell and by connecting them by a new edge. The choice of two dividing edges in N-sided cell is as below. In case of Random cell division, first, one edge of a dividing cell is chosen randomly and a new vertex is added on the center of the edge. Subsequently the $(N/2)$ -th edge next to the left of the first one is chosen and a new vertex is added on the center of the edge. Two newly added vertexes are connected with new edge. In case of Periclinal cell division first, two edges next to both right and left to the surface one of a dividing cell are chosen and two new vertexes on the centers of the edges are added. Two newly added vertexes are connected with a new edge. In case of Anticlinal cell division first, surface edge of a dividing cell is chosen and a new vertex on the center of the edge is added. Subsequently the $(N/2)$ -th edge next to the left of the first one is chosen and a new vertex on the center of the edge is added. Two newly added vertexes are connected with new edge.

Calculation for characteristic parameters

We adjusted the results of numerical calculations as a preprocessing for smoothing leaf shapes for calculating CPs. We calculated the dynamics with $D_{AD}=0$ and $D_{AB}=0$ and $\sigma_0=5.2$ using the results of numerical calculation as an initial condition. After this treatment, topological and geometric changes do not affect essential results. Then, we calculated CPs according to their definitions.

Characterizing patterns by CP criteria

The CP values characterize the features of leaf patterns, which diverse in the numerical simulation with parameter changes. The CPs are based on the following measurements: C ; circumference of leaf, A ; area of cross section, B ; domain boundary length, C_{AD} ; adaxial domain surface length and C_{AB} ; abaxial domain surface length. $CP1 = \frac{C}{2\sqrt{\pi A}}$, which measures the degree of flatness. CP1 becomes closer to 1.0 if the shape is like circle (Fig. 3 #1), and larger when the shape of leaf becomes flatter (Fig. 3 #2). $CP2 = \frac{B}{C}$, which measures how boundary is winded. A value of CP2 reflects the adaxial-abaxial boundary shape. If the boundary is winded in tissue, CP2 becomes much larger than 0.5 (Fig. 3 #3). If the boundary is positioned, not winded, at the center of a leaf tissue, the value is expected to be around 0.5. In the numerical calculation, the circumference of the tissue is smooth. On the other hand, the boundary is zigzag shape. It causes the boundary length is longer than expected. This results in that the value of CP2 is shift to a bit larger than expected. $CP3 = \frac{|C_{AD}-C_{AB}|}{C}$, which measures adaxial-abaxial domain bias. If the domain areas which the adaxial and abaxial sides occupy are not biased, the value of CP3 becomes near zero (Fig. 3 #2). If biased, it is apart from zero (Fig. 3 #4). CP4, which has one or zero integer value. The integer value 1 means that the obtained leaf has just one adaxial-abaxial domain.

The method to evaluate the numerically obtained patterns by CP values has the risk of the “false positive” fundamentally, because the degree of freedom of leaf shapes is much higher than the dimension of CPs. For example, Fig. 10 shows abnormal shaped leaves. They have a swelling-like structure. CPs we defined in this study cannot reject them. With periclinal division, these structures are seldom observed. On the other hand, compared to periclinal division, these abnormalities are observed many times with anticlinal division. Thus, the differences of success rates between these division rules are much bigger in fact. However, characterizing such high-dimensional patterns by small number of statistic values should be useful from practical meaning. It also helps our understanding for complex patterns.

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REFERENCE

Byrne, M.E., Barley, R., Curtis, M., Arroyo, J.M., Dunham, M., Hudson, A., Martienssen, R.A., 2000. Asymmetric leaves1 mediates leaf patterning and stem cell function in Arabidopsis. *Nature* 408:967–971.

Carlsbecker, A., Lee, J.Y., Roberts, C.J., Dettmer, J., Lehesranta, S., Zhou, J., Lindgren, O., Moreno-Risueno, M.A., Vatén, A., Thitamadee, S., Campilho, A., Sebastian, J., Bowman, J.L., Helariutta, Y., Benfey, P.N., 2010. Cell signalling by microRNA165/6 directs gene dose-dependent root cell fate. *Nature* 465:316–321.

Chitwood, D.H., Nogueira, F.T., Howell, M.D., Montgomery, T.A., Carrington, J.C., Timmermans, M.C.P., 2009. Pattern formation via small RNA mobility. *Genes Dev.* 23: 549–554.

Cunninghame, M.E., Lyndon, R.F., 1986. The relationship between the distribution of periclinal cell divisions in the shoot apex and leaf initiation. *Ann Bot* 57:737–746.

Emery, J.F., Floyd, S.K., Alvarez, J., Eshed, Y., Hawker, N.P., Izhaki, A., Baum, S.F., Bowman, J.L., 2003. Radial patterning of Arabidopsis shoots by class III HD-ZIP and KANADI genes. *Curr. Biol.* 13: 1768–1774.

Esau, K., *Anatomy of Seed Plants* (Wiley, 1977).

Eshed, Y., Baum, S.F., Perea, J.V., Bowman, J.L. 2001. Establishment of polarity in lateral organs of plants. *Curr. Biol.* 11: 1251–1260.

Eshed, Y., Izhaki, A., Baum, S.F., Floyd, S.K., Bowman, J.L., 2004. Asymmetric leaf development and blade expansion in *Arabidopsis* are mediated by KANADI and YABBY activities. *Development* 131:2997–3006.

Fink, J., Carpi, N., Betz, T., Bétard, A., Chebah, M., Azioune, A., Bornens, M., Sykes, C., Fetler, L., Cuvelier, D., Piel, M., 2011. External forces control mitotic spindle positioning. *Nat. Cell Biol.* 13, 771–778.

Fukushima, K., Hasebe, M., 2014. Adaxial–abaxial polarity: the developmental basis of leaf shape diversity. *Genesis* 52:1–18.

Fukushima, K., Fujita, H., Yamaguchi, T., Kawaguchi, M., Tsukaya, H., Hasebe, H., 2015. Oriented cell division shapes carnivorous pitcher leaves of *Sarracenia purpurea*. *Nat Commun.* 6:6450

Garcia, D., Collier, S.A., Byrne, M.E., Martienssen, R.A., 2006. Specification of leaf polarity in *Arabidopsis* via the trans-acting siRNA pathway. *Curr Biol* 16: 933–938.

Husbands, A.Y., Chitwood, D.H., Plavskin, Y., Timmermans, M.C.P., 2009. Signals and prepatterns: new insights into organ polarity in plants. *Genes Dev.* 23: 1986–1997.

Izhaki, A., Bowman, J.L., 2007. KANADI and class III HD-Zip gene families regulate embryo patterning and modulate auxin flow during embryogenesis in *Arabidopsis*. *Plant Cell* 19: 495–508.

Kerstetter, R.A., Bollman, K., Taylor, R.A., Bomblies, K., Poethig, R.S., 2001. KANADI regulates organ polarity in Arabidopsis. *Nature* 411:706–709.

Kidner, C.A., Martienssen, R.A., 2004. Spatially restricted microRNA directs leaf polarity through ARGONAUTE1. *Nature* 428: 81–84.

Kidner, C.A., Timmermans, M.C., 2010. Signaling sides adaxial–abaxial patterning in leaves. *Curr. Top. Dev. Biol.* 91: 141–168.

Miyashima, S., Koi, S., Hashimoto, T., Nakajima, K. 2011. Non-cell-autonomous microRNA165 acts in a dosedependent manner to regulate multiple differentiation status in the Arabidopsis root. *Development* 138:2303–2313.

Nagai, T., Honda, H., 2001. A dynamic cell model for the formation of epithelial tissue. *Phylos. Mag.*, B81, 699-719.

Pekker, I., Alvarez, J.P., Eshed, Y., 2005. Auxin response factors mediate Arabidopsis organ asymmetry via modulation of KANADI activity. *Plant Cell* 17: 2899–2910.

Prigge, M.J., Otsuga, D., Alonso, J.M., Ecker, J.R., Drews, G.N., Clark, S.E., 2005. Class III homeodomain-leucine zipper gene family members have overlapping, antagonistic, and distinct roles in Arabidopsis development. *Plant Cell* 17: 61–76.

Sarojam, R., Sappl, P.G., Goldshmidt, A., Efroni, I., Floyd, S.K., Eshed, Y., Bowman, J.L., 2010. Differentiating Arabidopsis shoots from leaves by combined YABBY activities. *Plant Cell* 22:2113–2130.

Sussex, I.M., 1951. Experiments on the cause of dorsiventrality in leaves. *Nature* 167:651–652.

Sussex, I.M., 1954. Experiments on the cause of dorsiventrality in leaves. *Nature* 174:351–352.

Tameshige, T., Fujita, H., Watanabe, K., Toyokura, K., Kondo, M., Tatematsu, K., Matsumoto, N., Tsugeki, R., Kawaguchi, M., Nishimura, M., Okada, K., 2013. Pattern Dynamics in Adaxial-Abaxial Specific Gene Expression Are Modulated by a Plastid Retrograde Signal during *Arabidopsis thaliana* Leaf Development. *PLoS Genet* 9(7): e1003655.

Tepfer, S.S., 1960. The shoot apex and early leaf development in *Clematis*. *Am J Bot* 47:655–664.

Vatén, A., Dettmer, J., Wu, S., Stierhof, Y.D., Miyashima, S., Yadav, S.R., Roberts, C.J., Campilho, A., Bulone, V., Lichtenberger, R., Lehesranta, S., Mähönen, A.P., Kim, J.Y., Jokitalo, E., Sauer, N., Scheres, B., Nakajima, K., Carlsbecker, A., Gallagher, K.L., Helariutta, Y., 2011. Callose biosynthesis regulates symplastic trafficking during root development. *Dev Cell* 21:1144–1155.

Waites, R., Hudson, A., 1995. *phantastica*: A gene required for dorsoventrality of leaves in *Antirrhinum majus*. *Development* 121:2143–2154.

Waites, R., Selvadurai, H.R., Oliver, I.R., Hudson, A., 1998. The PHANTASTICA gene encodes a MYB transcription factor involved in growth and dorsoventrality of lateral organs in *Antirrhinum*. *Cell* 93:779–789.

Yamaguchi, T., Yano, S., Tsukaya, H., 2010. Genetic framework for flattened leaf blade formation in unifacial leaves of *Juncus prismatocarpus*. *Plant Cell* 22:2141–2155.

Yamaguchi, T., Nukazuka, A., Tsukaya, H., 2012. Leaf adaxial–abaxial polarity specification and lamina outgrowth: evolution and development. *Plant Cell Physiol.* 53(7):1180-1194.

Yoshida, S., Barbier de Reuille, P., Lane, B., Bassel, G.W., Prusinkiewicz, P., Smith R.S., Weijers, D., 2014. Genetic control of plant development by overriding a geometric division rule. *Dev. Cell* 29:75–87.

FIGURE LEGENDS

Figure 1

Schematic view of the Waits 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.

Figure 2

Examples of leaves obtained by numerical calculation

Leaves are classified as flat (a) and abnormal (b) based on the criteria of CPs. Shades of red or green represent concentration of adaxial or abaxial factors, respectively.

Figure 3

Scatter plots of the characteristic parameters (CPs) for the result of random cell division model with the cell cycle length 1.0. The red shaded areas surrounded by dashed lines show region of successful leaves of CPs ($CP1 > 1.8$, $0.58 < CP2 < 0.72$, $CP3 < 0.29$ and $CP4 = 1$). #1-#4 indicate examples of obtained leaf patterns and their corresponding CPs.

Figure 4

The success rate (%) of flat leaves against cell cycle length for each division rules. Red; periclinal, green; random, blue; anticlinal.

Fig. 5

(a) Success rate (%) for flat leaf formation in several diffusion constants of adaxial/abaxial factors (D_{AD} (D_{AB})) and thresholds of cell division (K) with random cell division and cell cycle length 1.0. (b)-(d) Result examples of numerical calculations. (b) $D=0.075$, $K=0.1$. (c) $D=0.0375$, $K=0.1$. (d) $D=0.0375$, $K=0.3$. (e) $D=0.3$, $K=0.2$.

Figure 6

Polarized cell division; (a) anticlinal division, (b) periclinal division.

Figure 7

The examples of results for the time evolution of leaf growth in numerical calculation with periclinal division rule.

Figure 8

Direction of cell division is likely to be the same as the direction of growing axis under the periclinal division. Leaf margin is magnified. Yellow asterisk cell is a daughter cell of white asterisk cell.

Figure 9

(a) The shape change of a normal growth in developmental process with periclinal division. (b) The shape change of abnormal growth in developmental process with anticlinal division. Once the boundary is deviated, the deviation likely increases and increases due to anticlinal division.

Figure 10

Examples of abnormal shaped leaves which cannot be rejected by characteristic parameters (CPs) defined in this study.

Figure 1

Figure 2

(a)

(b)

Figure 3

Figure 4

Figure 5

Figure 6

(a)

(b)

Figure 7

Figure 8

Figure 9

(a)

b)

Figure 10