The Fourteenth International Symposium on Artificial Life and Robotics 2009 (AROB 14th '09),
B-Con Plaza, Beppu, Oita, Japan, February 5 - 7, 2009

Mathematical modeling for morphogenesis of leaf with employing cell automata and reaction-diffusion equation

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Abstract: The phenome analysis is used for an elucidation of the leaf morphogenesis. However, it is difficult to identify a complicated biochemical system which exists between genome and phenome. Designing a mathematical model for the morphogenesis has a possibility to infer the complicated system by using the observed data on phenome analysis. We attempted to design a novel mathematical model in order to analyze the effects of the interaction between the expansion of leaf blade and elongation of leaf venation in the development of leaf shape, and discussed an availability of the proposed model on the numerical analysis of development of leaf. The expansion of leaf blade and the elongation of leaf venation were designed by employing the reaction-diffusion equation and cellular automata (CA) theory, respectively. As for the numerical simulation for development of *gingko*'s leaf primordium, the proposed model could realize several well-known biological findings such as a reconnection of leaf venation. Moreover, the numerical analysis demonstrated that the interaction between the elongation of leaf venation and the expansion of leaf blade was indispensable for the complicated pattern formation of leaf venation. These results agreed with biological findings, which implied that the proposed model was useful for elucidating a development of leaf.

Keywords: Morphogenesis, Cellular automata, Reaction-diffusion equation, Pattern formation, Leaf shape, Leaf venation**.**

I. INTRODUCTION

Morphogenesis of organisms is important process related to a determination of size, shape and mutual arrangement of tissues, and is regulated by a complicated biochemical system which exists between the genotype and the phenotype. The phenome analysis is used for an elucidation of relationship between genotype and phenotype by comparing wild type with mutant strain with using genetic engineering. In particular, the phenome analysis for higher plants such as *Arabidopsis thaliana* was implemented in world wide, and contributed to clarify a mechanism of morphogenesis. Although the analyses identified the genome related to the morphogenesis, it was difficult to infer the complicated biochemical system. Designing of the mathematical model which infers the complicated biochemical system is useful for elucidating a mechanism of morphogenesis in detail. With employing a reaction-diffusion equation, Turing [1] demonstrated that the unstable diffusive dynamics on activator-inhibitor system affected the pattern formation of fish's skin (Turing model). Moreover, Meinhardt *et al.* [2] represented a differentiation of plant cells with applying Turing model, and realized a branching pattern of leaf venation (Meinhardt's model). On the other hand, by employing an automata theory, Lindenmayer [3] designed a mathematical model for a branching pattern formation of tree, and demonstrated that the interactions between tissues were indispensable for the development of organisms. Furthermore, Markus *et al.* [4] designed a mathematical model (Markus's model) for a development of leaf venation based on gene regulation with employing a cellular automata (CA) theory, and realized a pattern formation of leaf venation as same as that seen in Meinhardt's model. Thus, the numerical analysis based on the phenome analysis is useful for elucidating a mechanism of morphogenesis. However, since these conventional models had not considered an expansion of leaf blade, it was difficult to realize several complicated pattern formations of leaf venation such as reconnection phenomena. The mathematical model which considers interaction between the expansion of leaf blade and elongation of leaf venation could elucidate a mechanism of the complicated pattern formation of leaf venation in more detail. In this paper, we attempted to design a novel mathematical model (proposed model) for which interaction between the expansion of leaf blade and elongation of leaf venation

affects the development of leaf shape. The expansion of leaf blade and the elongation of leaf venation are designed by employing a reaction-diffusion equation and a CA theory, respectively. Next, we shall numerically analyze a development of *gingko*'s leaf primordium, and verify an effect of the expansion of leaf blade in the pattern formation of leaf venation with changing a kinetic parameter. Finally, we shall discuss a validity of the proposed model on the numerical analysis of development of leaf.

II. EXPERIMENTAL PROCEDURES

1. The proposed model

This study assumed that the mesophyll cell of leaf differentiates into either venation cell or blade cell. With using both a set of reaction-diffusion equations and CA theory, the proposed model considered an interaction between an elongation of leaf venation and an expansion of leaf blade.

A. The growth of blade tissue

The growth of blade tissue is depended on a dynamic behavior of Mesophyll Morphogenesis Factor (MMF) such as water and nutrient salts. MMF is supplied from the venation tissue, and is propagated on the blade tissue.

$$\frac{\partial U}{\partial t} = D_x \frac{\partial^2 U}{\partial x^2} + D_y \frac{\partial^2 U}{\partial y^2} - \alpha \qquad (1)$$

where U is level of MMF. Both Dx and Dy are diffusion coefficient to x-axes and y-axes, respectively. The α is consumption rate of MMF on both the blade cell and mesophyll cell. This mathematical model is presumed that the size of the blade tissue is nearly equivalent to the area on which MMF can propagate.

B. The elongation of venation tissue

Venation forming factor (VCF) such as phytohormone is regulated by transport factor, propagated and accumulated on whole cell. With stimulating by VCF, mesophyll cell differentiates into venation cell. Markus et al. [4] designed the mathematical model to represent the differentiation of mesophyll cell into venation cell, in which VCF, transport factor of VCF and gene for venation formation are set to Substrate, Activator and Inhibitor, and Gene, respectively (Markus model). The proposed model is integrated algorism of the Markus model with our algorism (Fig.1). The u, v and s in Fig.1 represent the level of Activator, Inhibitor and Substrate, respectively. According to the procedure, the levels of these factors at the next state are dependent on the current state of these factors. The c, d, γ, δ_1, δ_2, δ_3, δ_4, β_0, β_1 and η represent kinetic parameters for each transient. The ω is the amount of supply of VCF, ε is the threshold for Activator, u_{max} is the upper limit of Activator and g takes the two state, 1 or 2, which determines whether the mesophyll cell differentiates into the venation cell (g=1) or the blade cell (g=0). Step (0) determines whether the procedures from (I) to (VIII) are executed or not at the mesophyll, the venation and the blade cells. Step (VIII) shows the calculation of the moving-average for u,v and s. The initial conditions of u, v and s for the formed mesophyll cell are set to 0.0, 0.0 and 1.0, respectively.

$u_t \rightarrow u^{\mathrm{I}} \rightarrow u^{\mathrm{II}} \rightarrow u^{\mathrm{III}} \rightarrow u^{\mathrm{IV}} \rightarrow u^{\mathrm{V}} \rightarrow u_{t+1}$
$v_t \rightarrow v^{\mathrm{I}} \rightarrow v^{\mathrm{II}} \rightarrow v^{\mathrm{III}} \rightarrow v_{t+1}$
$s_t \rightarrow s^{\mathrm{I}} \rightarrow s_{t+1}$

(0) if U>0, then goto (I)
(I) if u_t>d・v_t, then u_t =c・u_t・s_t, else $u^{\mathrm{I}} = u_t$
(Ia) if $u^{\mathrm{I}} > u_{max}$, then $u^{\mathrm{II}} = u_{max}$, else $u^{\mathrm{II}} = u^{\mathrm{I}}$
(II) $u^{\mathrm{III}} = \delta_1 \cdot u^{\mathrm{II}} - \delta_2$
(IIa) $u^{\mathrm{III}} < 0$, then $u^{\mathrm{IV}} = 0$, else $u^{\mathrm{IV}} = u^{\mathrm{III}}$
(III) $v^{\mathrm{I}} = \delta_3 \cdot v_t - \delta_4$
(IIIa) if $v^{\mathrm{I}} < 0$,then $v^{\mathrm{II}} = 0$, else $v^{\mathrm{II}} = v^{\mathrm{I}}$
(IV) $v^{\mathrm{III}} = \gamma \cdot u^{\mathrm{IV}} + v^{\mathrm{II}}$
(V) $u^{\mathrm{IV}} > \varepsilon$, then g_{t+1}=1, else g_{t+1}=0
(VI) if g_{t+1}=1,then $s^{\mathrm{I}} = s_t + \omega - \beta 1 \cdot s_t$,
else $s^{\mathrm{I}} = s_t + \omega - \beta_0 \cdot s_t$
(VII) if g_{t+1}=1,then uV = $u^{\mathrm{IV}} + \eta$,else $u^{\mathrm{V}} = u^{\mathrm{IV}}$
(VIII) $u_{t+1} = < u^{\mathrm{V}} >$, $v_{t+1} = < v^{\mathrm{III}} >$, $s_{t+1} = < s^{\mathrm{I}} >$

Fig. 1. Procedure of the mathematical model with cellular automata theory.

2. Numerical analysis

In this study, the numerical simulations of the proposed model for the spational pattern formation of *gingko*'s leaf primordium are implemented in arbitrary time on two dimensional surfaces (300 × 300 cells) simultaneously. Results are visualized at every 100 time step interval. The number of iterations is 500. Initial conditions of U, u, v and s are set to 1.0, 300.0, 0.0 and 0.2, respectively in only a mesophyll cell at the arbitrary corner (petiole). Numerical simulation result

with standard kinetic parameter (Table 1) is set to reference pattern. And we examine several effect of variation of diffusion coefficient on the pattern formation of leaf venation. Furthermore we evaluate the time course of both the number of whole cells (*Nwc*) and the development of leaf venation (*Ocp*) to validate the proposed model for simulating the spatiotemporal pattern formation of *gingko*'s leaf primordium. The *Ocp* is the ratio of the number of venation cells (*Nvc*) to *Nwc*, represented by,

$$Ocp = \frac{Nvc}{Nwc} \times 100 \quad (2)$$

The spatiotemporal development of leaf venation was evaluated by using *Ocp*, which was observed at every 100 time step interval.

Table 1. The value of the standard kinetic parameters for *gingko*'s leaf

D_x	100.0	D_y	100.0
α	0.0001	c	2.7
d	2.0	γ	0.5
u_{max}	250.0	δ_1	0.9
δ_2	1.0	δ_3	0.5
δ_4	2.0	ω	0.3
ε	70.0	η	0.0
β_0	0.2	β_1	1.0

III. RESULTS AND DISCUSSION

To validate adequacy of the proposed model, numerical analysis for the development of *gingko*'s leaf primordium was implemented by using a set of standard kinetic parameter shown in Table 1. Fig.2 shows the time courses of development of *gingko*'s leaf primordium. The venation tissue elongated with the expansion of blade tissue, and generated both the branching and filament pattern formation (Fig.2a: reference pattern). Moreover, blade tissue expanded in the form of fan shape. These results were qualitatively in good agreement with typical pattern formation shown in development of *gingko*'s leaf. When the elongation of venation tissue reached to the tip of leaf blade, the reconnection of leaf venation was generated between existing venation tissues. Berleth *et al.* reported that the reconnection occurred when the venation tissue fully elongated on the leaf blade [5]. This biological finding supported the numerical simulation result, which implied that the proposed model is useful for numerical analysis of the elongation of leaf venation in comparison with the conventional models. With setting *Dx* and *Dy* to 2000.0, since the blade tissue slightly expanded than that seen in the reference pattern, the pattern formation of venation showed a discrepancy with that of reference pattern (Fig.2b). On the other hand, when *Dx* and *Dy* were set to 5, the elongation of venation tissue was inhibited due to a deficiency of an expansion of blade tissue as seen in malnutrition (Fig.2c). These results demonstrated that the expansion of leaf blade affected the pattern formation of leaf venation. Fig.3 shows the time-course of the number of whole cells; both *Dx* and *Dy* increased the number of whole cells. In addition to this, the time-course of the number of whole cells showed an exponential behavior, which corresponded to the typical dynamic behavior of leaf primordium. Furthermore, when Dx and Dy were

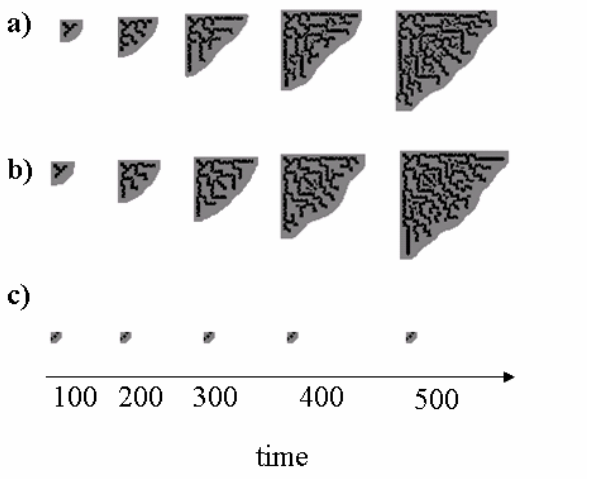

Fig. 2. Effect of kinetic parameters *Dx* and *Dy* on the spatiotemporal development of leaf shape.
a)standard parameters (Table1) b)*Dx* = *Dy*=2000.0 c) *Dx* =*Dy* = 5.0. Black fields and gray fields show venation tissue and blade tissue, respectively.

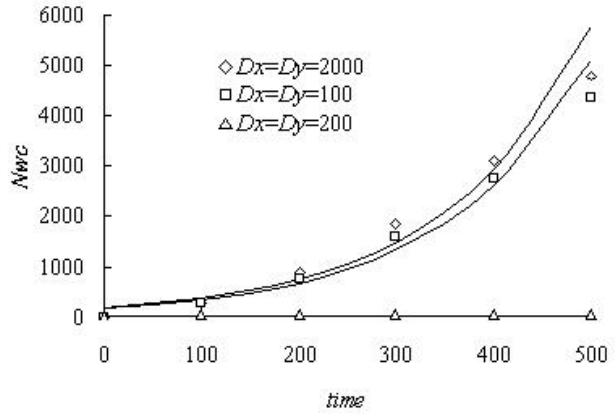

Fig. 3. Time course of the number of whole cells (*Nwc*).

This figure showed effect of *Dx*, *Dy* on the number of *Nwc*. Numerical calculations were executed with *Dx*=*Dy*=5, 100, 2000, respectively.

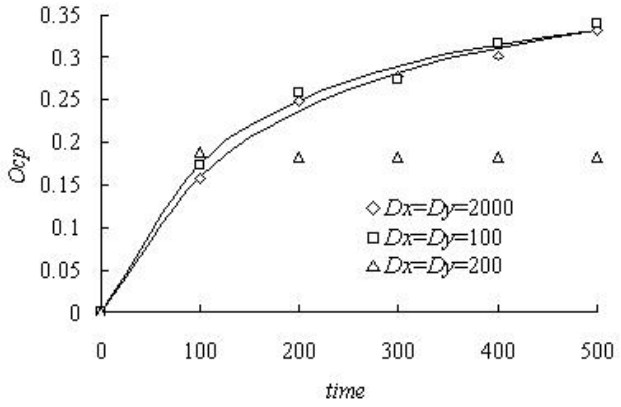

Fig. 4. Time course of the ratio of venation cell to whole cells (*Ocp*).

This figure showed effect of *Dx*, *Dy* on the value of *Ocp*. Numerical calculations were executed with *Dx*=*Dy*=5, 100, 2000, respectively.

set to either 100.0 or 2000.0, as shown in Figs. 3and 4, the number of whole cells is growing with time, however, the ratio of venation cell to whole cells shows saturable with time. Aloni *et al.* reported [6] that the basic skeleton of leaf venation was almost determined in the development process of the leaf primordium. The saturable behavior shown in Fig.4 implied the completion of formation of the basic skeleton of leaf venation, which was in good agreement with the biological finding. Thus, the interaction between the elongation of leaf venation and the expansion of leaf blade is indispensable for the complicated pattern formation of leaf venation. The proposed model with considering the interaction is useful in the numerical analysis of development of leaf in comparison with conventional model.

IV. CONCLUSION

With employing a set of reaction-diffusion equations and CA theory, we designed a novel mathematical model for which an interaction between the elongation of leaf venation and the expansion of leaf blade affects a development of leaf. The proposed model could realize several salient features of development of *gingko*'s leaf primordium such as the reconnection of venation tissues, the branching pattern formation and the fan shape formation of blade tissue, which implied that the interaction between the elongation of leaf venation and the expansion of leaf blade is indispensable for the complicated pattern formation of leaf venation. Therefore the proposed model was useful for the numerical analysis of development of leaf in comparison with the conventional models.

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