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Autoreproductive cells and plant meristem construction: the case of the tomato cap meristem

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Dedicated to Professor Brian E. S. Gunning on the occasion of his 65th birthday

Summary. Root apical meristems are composed of two zones in which either formative or proliferative cell divisions occur. Within the formative zone, autoreproductive initial cells (a-cells) occupy distinctive locations. By means of graph-L-systems, the behavior of one such type of a-cells has been investigated, with particular reference to root caps within the developing primordia of lateral roots of *Lycopersicon esculentum* cultivated in vitro. Here, the a-cells constitute the "protoderm initials", cells which are found also in the root cap of many angiosperm species. A set of cuboidal (i.e., six-sided) a-cells develops early in the ontogeny of a lateral-root primordium. Then, according to both anatomical observations and theoretical simulations obtained by the application of graph-L-systems, sequential production of descendents from each a-cell leads to the formation of a new autoreproductive cell (a), a cap columella initial (c), and two mother cells (e and f) whose respective descendents differentiate as root epidermis and cap flank cells. In this graph-L-system, there is specification of the location of sister cells with respect to the three orthogonal directions of a cuboidal. In the early stage of root cap formation, only a few rounds of these formative cell divisions by each a-cell and its four types of descendents are required to provide the basic set of cells necessary for full cap development. After the lateral root emerges from the parent root, there may be a temporary cessation of the formative divisions of the a-cells which give rise to columella initials. Columella production is then supported entirely by its own independent set of autoreproductive c-initials. At the same time, division of the autoreproductive protoderm initial cell is directed towards maintaining the cap flank and the epidermal cell files. The regulation of the types of formative division by the a-cell may be represented by means of a division counter which may be specific for a given species.

Keywords: Autoreproductive cell; Epidermis; Lateral-root primordium; L-system; Root cap; Tomato.

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Introduction

Many aspects of plant growth and development appear to be so regular that they can be codified by a set of deterministic rules. L-systems (Rozenberg and Salomaa 1980) are composed of such sets of rules and these may apply at different levels of plant organization. They are especially relevant to the description of branching events which play such an important part in plant growth.

Generally, L-systems are composed of algorithms that reproduce, within an ordered time-frame, recurrent events in the development of constructions composed of elements. In the case where the elements are walls, or wall segments, the corresponding constructions are cells; where the elements are cells, the constructions are organs; and where the elements are organs and their meristems, the constructions are organisms. If growth and development at each organizational level is to realize its full potential, the constructions at each level must participate in branching events which increase the number of constructions and, hence, their scope for further differentiation. Sets of branching events within a hierarchy of constructions are thus natural and highly regulated accompaniments to plant growth. Branching is accomplished by the insertion of new elements which results in the construction being separated into two independent portions. L-system algorithms can precisely specify the location of the inserted elements, as well as the growth and development of the constructions prior to their

branching. Some of the more evident branching events in plants are those associated with the generation of new leaves, buds, and shoots.

In the present paper, branching events at the organizational level of the cell will be considered: namely, the sets of cell divisions which accompany tissue and organ construction. However, the precise orientation of elements in three dimensions is not given by L-systems, even those systems that apply to filaments which eventually branch. Therefore, the nonrandom placement of successive generations of cell division walls needs further definition. This is generally furnished by consideration either of cellular graph productions with cell gluing rules, as used in Graph Grammars (e.g., Lück and Lück 1979), or by wall matching rules, as used in Cellworks (e.g., Lück and Lück 1996). Using the three orthogonal directions of cuboidals for cellular location in a three-dimensional space, we propose here much simpler systems than have been described previously. We call these graph-L-systems. The outcome of their application is an ordered series of organogenetic cell divisions which, in turn, is accompanied by an ordered pattern of cells.

On the basis of the regularity of cellular arrangements resulting from cell divisions within plant tissues, it is reasonable to assume that such arrangements have relevance not only to the branching and self-maintenance of the organ within which these tissues reside, but also to the functions of the tissues themselves. The particular construction which we have explored, from the point of view of establishing the rules for its assemblage, is that which is composed of the cap and epidermis of tomato roots. Cells contained within the pericycle of maturing root tips are taken as the starting point for this construction. Algorithms have been developed for the tomato root cap which utilize cells with specific orientations associated with their division walls. These are the elements from which the cellular patterns of the cap and epidermal tissues are generated by simulation, a methodology which plays an important part in validating the algorithms used in this type of analysis.

Material and methods

Cap tissue development was analyzed in lateral-root primordia, as well as in emerging and recently emerged roots, of *Lycopersicon esculentum* Mill. (cv. Moneymaker). The source root material was grown in vitro in modified White's solution supplemented with sucrose, as described in Barlow (1992). Root segments, 5 cm long and known to contain primordia, were excised from behind the root tip, fixed in formaldehyde-ethanol-acetic acid and embedded in paraffin wax. Using a rotary microtome, transverse sections, 10 μm thick, were prepared from the whole length of each segment. All the sections were placed serially on microscope slides, stained with safranin-tannic acid-orange G (Sharman 1943), dehydrated, and finally mounted in Canada Balsam under a coverslip. Sections prepared from a number of roots containing lateral root primordia at all stages of development were examined with the ×25 and ×40 objective lenses of a Zeiss Photomicroscope. Because the growth axes of both the primordia and the young roots are perpendicular to that of their parent root axis, these younger roots were always cut longitudinally during the sectioning procedure. Their median sections were located and photographed on Pan F film (50 ASA; Ilford). The exposed film was then developed in Acutol (Paterson, Telford). Boundaries of the cell packets were traced on glossy prints prepared from the negatives. Numbers of cells mentioned in reference to packets of cells apply only to those which have been seen in a single median longitudinal section of the lateral root.

Results

The objective of this study of young roots of tomato grown in vitro was to arrive at a formal model of root cap construction. Graph-L-system methodology was proposed because this class of L-systems, besides having recurrent growth functions which predict the number of cells produced by an initial mother cell, also specifies the position of the daughter cells in several spatial directions. Although the final outcome of the L-system model for the root cap is sketched in Fig. 6, it will be useful to refer to this figure in the various sections that follow. It should be noted, however, that negative states in Fig. 6 refer to observed or extrapolated states which have not been simulated by the L-system.

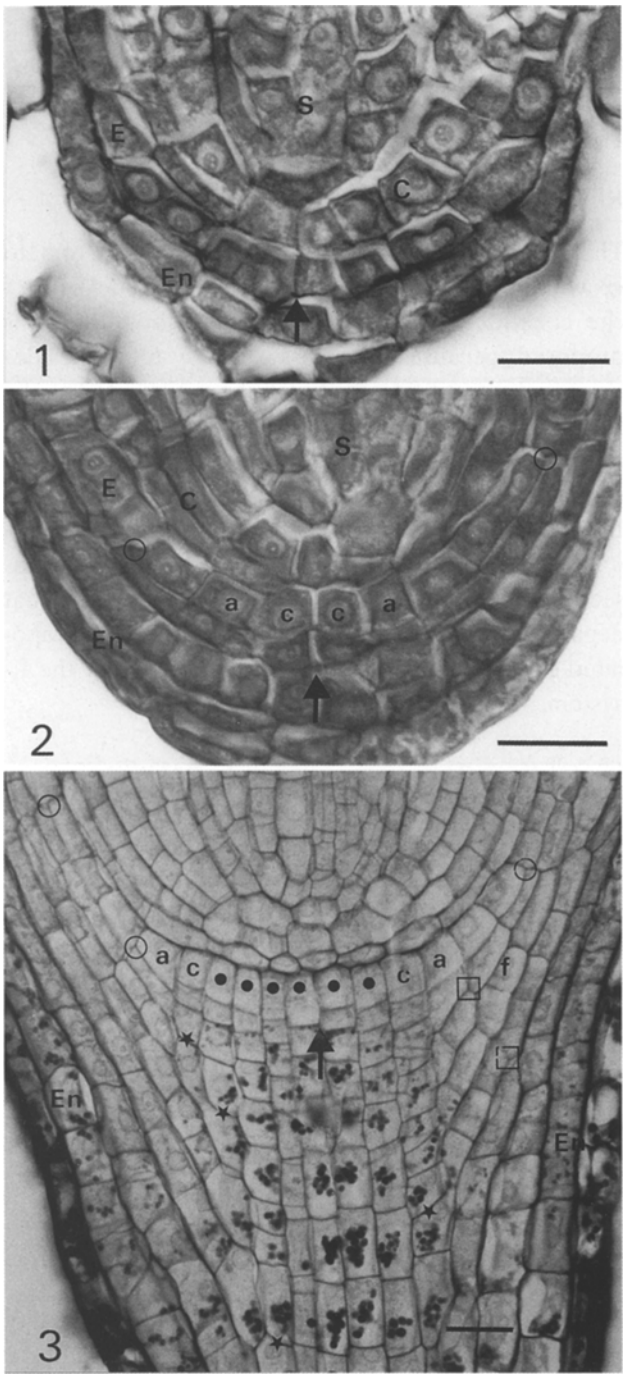
Summary of primordium construction and root cap development in young lateral roots

There is a progression of development – less to more advanced – along the distal-proximal axis of the parent root. Thus, cross sections of in vitro grown tomato roots (Figs. 1–3), cut from tip to base, revealed all stages of lateral-root development, from the primordial stage up to the emergence of young lateral roots from the confines of the parent root tissue.

The earliest primordial stages could be identified in the pericycle (Fig. 6, step –4) and adjacent endodermis by the relatively strong affinity of some cells for cytoplasmic stains (that is, relative to the weaker staining of neighboring, nonprimordial cells). Whether these cells were dividing anticlinally, as is the case in the earliest stages of primordium development (Lloret et al. 1989, Malamy and Benfey 1997), was not determined.

Closer towards the base of the parent root, cells of the pericycle which had divided periclinally were observed. The inner layer of daughter cells of these divisions serve as progenitors for the vascular cylinder of the lateral root (Fig. 6, step –3). The outer layer of daughter cells undergo another periclinal division (Fig. 6, step –2). Their descendents serve as progenitors for the epidermis (this tissue being derived from the outer layer of daughters of this second periclinal division) and for the cortex (derived from the inner layer of daughters) which is sandwiched between the newly forming epidermis and vascular cylinder (Fig. 1).

Following their production by means of a second periclinal division in the pericycle complex, a row of eight epidermal cells is present, six of which divide transversely (Fig. 6, step –1) (i.e., transverse with respect to the axis of growth of this new group of cells, but radially with respect to the parent root axis). Within this group of cells (Fig. 1) there are presumably also radial (longitudinal) divisions which accommodate the increasing width of the young primordium whose growth is in a direction perpendicular to the parent root axis. After about 14 more cells have been produced along the length of the epidermal file, a third periclinal division occurs (Fig. 6, step 0). However, these last-mentioned divisions are not found in all cells of the file but are confined to a few cells at the tip of the root. Both the inner and outer daughters of these periclinally divided cells then undergo transverse division. This third round of periclinal divisions (Fig. 2) creates the first T wall junction (T_1), in which the new division wall, forming the stem of the T, abuts the basally facing anticlinal wall of the mother cell, which forms the capital of the T. (For a complete description of T wall junctions and their significance for root



Figs. 1–3. Median sections through a lateral-root primordium and two young roots. Tissues are: endodermis (*En*), epidermis (*E*), cortex (*C*), and stele (*S*). The arrows mark the mid-lines between the two lateral halves of the developing root caps. Bars: 20 μm

Fig. 1. A lateral-root primordium. The epidermis is complete. It lies beneath a skin of endodermis and overlies the cortex. The stele makes up the core of the primordium

Fig. 2. A newly emerged young lateral root. At the root apex, cells of the epidermal layer have undergone their first periclinal division and thereby have created the first primary T wall junction (circles), T_1. Identifiable on either side of the mid-line are a columella cell (*c*) and an autoreproductive protoderm initial cell (*a*)

Fig. 3. An emerged lateral root showing autoreproductive cells (*a*) and a series of successively formed columella initials (solid circles and *c*, the most recent) on either side of the mid-line. The distal ends of some of the recently inserted files of columella are indicated by a star. Two primary T junctions (open circles) (T_3, basal, and T_4, apical) are evident on the left side of the section, and another (T_4) is on the right. Two secondary T junctions (squares) are present within the cap periphery (*f*) on the right. The cap is covered by two layers of endodermis. The arrangement of columella files is asymmetric in this particular section: there are three columella files on the right side of the mid-line, but there are five files on the left side

meristem development, see Schüepp 1926, Wagner 1939.) As a result of subsequent transverse divisions, the root tip grows away from the T junction (Fig. 6, step 1). The inner layer of cells produced by the third periclinal division maintains its state as an epidermal layer, whereas the outer cells begin to differentiate as root cap.

The cells at the tip which have undergone the third periclinal division give rise to an autoreproductive a-cell and a cap columella initial, c (Fig. 2). The a-cells assume responsibility for the construction of the epidermis-cap complex (Fig. 3). In median section, a pair of a-cells lies on either side of the mid-line of the developing root (Fig. 2). One other such cell pair would be found in an adjacent section, making in all a set of four a-cells at the summit of the new root. Step 0 in Fig. 6 shows a- and c-cells in one of the four 90° sectors of the primordial cap complex. At this stage, there is then a further division – radial – to produce two a-cells per sector. Thus, in total, there would be four central c-cells surrounded by a ring of eight a-cells. Following a further transverse division (Fig. 6, step 1), the a-cells again divide periclinally (Fig. 6, step 2). This latter division initiates the flank layer (f) of cap tissue located to the outside of the first layer. This division is also accompanied by the formation of the second T wall junction, T_2.

These early steps of root cap and epidermis development, which have already been described in simplified form by Barlow (1993), serve as the basis for a theoretical scheme of cap tissue morphogenesis. The crucial element at these early stages is the autoreproductive behavior of the a-cells.

Theoretical scheme of autoreproductive cell behavior

Cap growth and development fall into two main phases: addition of columella files, which widens the cap, and cap maintenance, in which no further columella widening occurs. During both phases, preexisting columella cells continue to divide and produce new columella cells. Graph-L-systems are now proposed as a means of analyzing these phases of cap development, as described below.

Cell productions with columella widening

A theoretical scheme was devised to account for the generation of the two regions of the root cap, the internal columella and the peripheral portion, as well as the epidermis of the root. The starting point for the theoretical scheme is an autoreproductive cuboidal cell occupying state a_1 and a columella initial c_1. (In Fig. 6, step 0, c_1 lies to the left of the a_1-cell; the same configuration, a_1 c_1, is repeated at step 8.) Each a-cell participates in four types of division according to its subscript numeral, which may be 1, 2, 3, or 4. Divisions of the a-cell occur in a definite sequence: $a_1 \rightarrow a_2 \rightarrow a_3 \rightarrow a_4 \rightarrow a_1 \ldots$ Because the a-state is autoreproductive, one daughter of each a-cell always adopts the a-state (though taking the next subscript in the sequence), whereas the other daughter adopts a non-a state, except for the products of the cell in state a_4, where both daughters of the radial division adopt the a-state. This behavior defines the autoreproductive property of the a-cell.

There are three orthogonal planes, periclinal (p), transverse (t) and radial (r), available for the division of a cuboidal cell. Following the deposition of a division wall in one of these planes, the non-a daughter cell receives one of the two different cell walls of the divided mother cell (i.e., one of the two end walls which are opposite and facing the new division wall). Therefore, the production of daughter cells can be described as being in accordance with one of a pair of "directions", arbitrarily described as N–S, E–W, F–B, to signify productions of pairs of daughter cells in north–south, east–west, and front–back directions, respectively. These three directed productions occur in the p-, t-, and r-planes (Fig. 4).

Each of the three types of cell division orientation and their associated cell productions can be specified by a correspondingly labelled directed arc ($\longrightarrow$) in the following graph-L-system algorithm:

$$\mathrm{A1} = \left\{ \begin{array}{l} a_1 \rightarrow a_2 \xrightarrow{t} e \\ a_2 \rightarrow a_3 \xrightarrow{p} f \\ a_3 \rightarrow c \xrightarrow{t} a_4 \\ a_4 \rightarrow a_1 \xrightarrow{r} a_1 \end{array} \right\}$$

Each directed arc relates two daughter cells and indicates, firstly, by its label, the orientation (p, t, and r) of the completed division and, secondly, the relative position of each daughter cell according to whether it is on the left or right side of the arc. For example, in the productions from a_1 and a_2 the new a-cells are on the left, whereas the new cells from a_3 and a_4 are on the right. Also specified in algorithm A1 is the state held by the non-a daughter cell. These states are either c, e, or f. They are outputs of the a-state mother cell in accor-

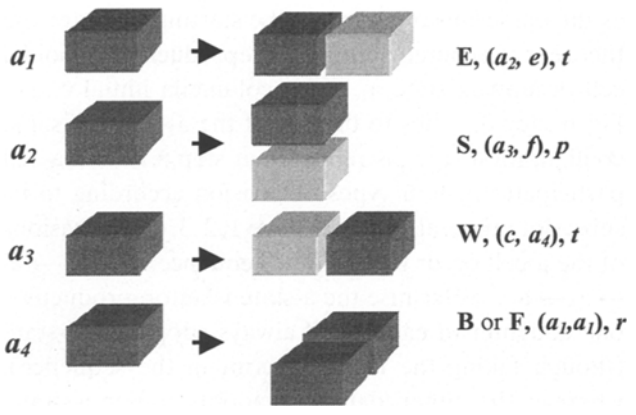


Fig. 4. Representation of the variously oriented divisions of the four classes of a-state cells (dark shading) and three classes of non-a cells (light shading) according to the graph-L-system algorithm A1. At the right-hand side of the figure the direction (E, S, W, B, or F) of production of each a-state daughter cell (a_1–a_4) is indicated and also the corresponding state of the non-a daughter. The directions of cell production are also related to whether the divisions are transversal (t), periclinal (p), and radial (r)

dance with its subscript numeral. The four graph production rules of A1 thus concern the a-cell states and use, in this context, a set of seven cell-state labels.

Algorithm A1 is presented in diagrammatic form in Fig. 4 as a series of directed cell productions. In this scheme it will be noted that a periclinal division (p) never produces a non-a daughter in the N direction. From the point of view of the constructional endpoint, B and F directions are considered to be equivalent, though it is possible that there could be some handedness (chirality) associated with these radial divisions, in which case either B or F productions might be favored. In further discussion, the F production is arbitrarily selected. Where there are initially four or eight a-cells in the developing tissue, particular sequences of cell production could be due to differences in the sequence in which divisions commence in cells occupying any one of the four a-states (at step –2 or step –1, Fig. 6). Some initial sequences of a-cell activity could even produce one or more spirals of cell packets within the cap; that is, the succession of T wall junctions ($T_1, T_2, \ldots$) produced by periclinal divisions of a_2-cells would appear to wind into the initial zone, as shown by Baum and Rost (1996). Whether the winding of the T wall junctions is in either a dextrorse or a sinistrorse fashion may depend upon whether the productions of cell a_4 are B or F.

The representations in Fig. 4 are used in the simulation in Fig. 6, which starts with an initial state composed of two cells, $\omega_0 = c_1\ a_1$ (Fig. 6, step 0). The successive developmental states of the simulation denote the approximate relative position of the three rows of merophytes derived from non-a daughter cells of an a-state mother cell. It shows, for example, that after the production of each c-cell the position of the daughter a-cell is displaced outwards (E-wards, if the center of the root is at the left) (Fig. 6, step 11). The divisions undertaken by the original eight a-cells maintain those eight cells within the developing cap until r-divisions result in a ring of 16 a-cells (Fig. 6, step 12).

The system is now able to generate a root cap with unlimited productions of columella (c), cap flank (f), and epidermal initials (e). Daughter cells e, f, and c, each derived from an a-cell, are all merophyte mother cells which subsequently contribute to different parts of the cap. The mother cells develop as one-rowed cell packets, but with variable rates for the increase of their cell numbers. At each timestep, a new merophyte of one of the three types of non-a cell is added to the total complement of cap and epidermal cells. The sequence of cell and/or merophyte productions by the autoreproductive a-cell is e, f, c, and a, as specified by algorithm A1.

For completeness, a third representation of the algorithm A1 is introduced. It is a cell-state derivation graph (Fig. 5a) which ignores the orientations of the cell productions but emphasizes the cell relationships (as in a simple L-system). The loop for columella formation specified by algorithm A1 and the consequent widening of the cap is represented in the center of this graph.

Cell productions for cap maintenance

During the growth of the tomato root in vitro, both the number of columella cell files and the number of cells they contain eventually cease to increase. Median longitudinal sections of lateral roots prepared over a 20-day period following their emergence from the parent root revealed that the number of such c-files increased from two to eight. That is, in addition to the first pair of c-cell files, three more pairs of c-cells were produced by a succession of three a_3-cells. (The median longitudinal sections reveal the past activity of two a-cells on opposite sides of the cap so that $4c + 4c = 8c$.) Therefore, in a 90° root sector, three c_2-cells were produced before c-cell production ceased and the columella became committed to maintain a constant size. Continuance of this maintenance phase naturally requires

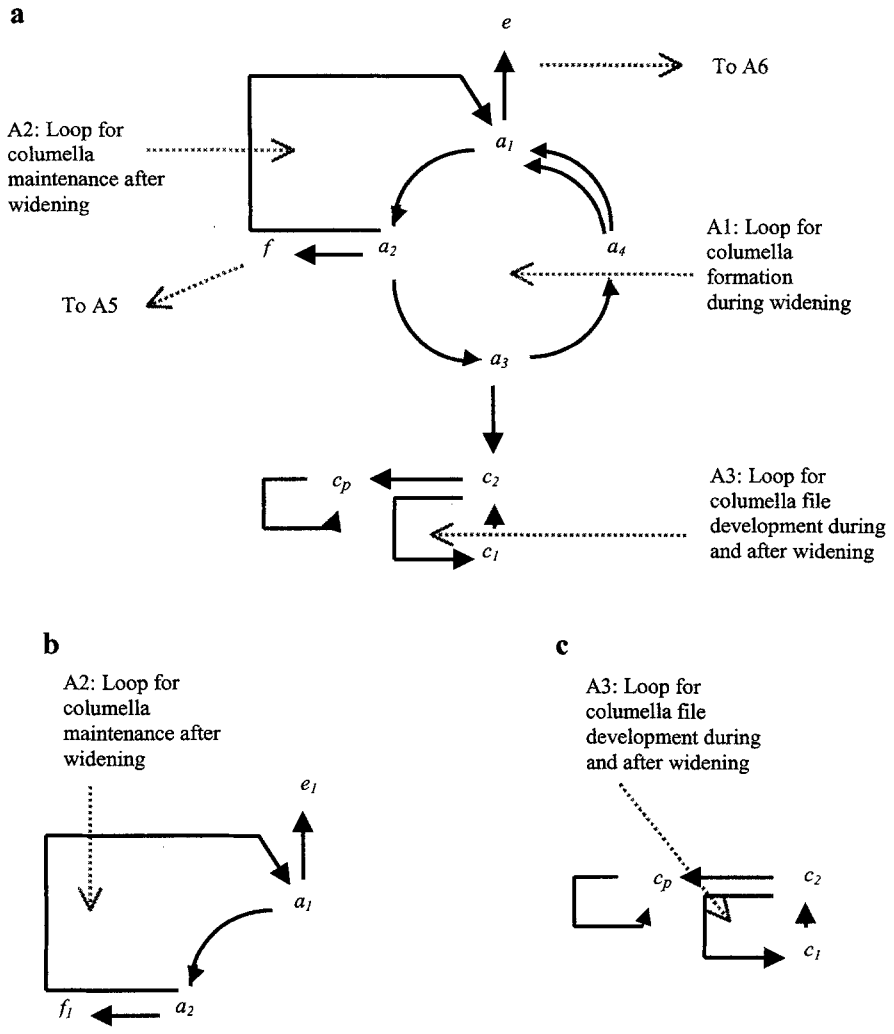


Fig. 5. **a** Representation of the division of a-state cells as specified by a cell state derivation graph based initially upon algorithm A1. The cell derivation graph also shows two supplementary loops which are related to algorithms A2 and A3, which determine cap columella maintenance and widening. The specifications of algorithms A5 and A6 enable development of cap flank (f) and epidermis (e), respectively. **b** and **c** Algorithms A2 and A3 can apply as disconnected subgraphs and thus relate to the independent productions of a- and c-cells, respectively, in the established cap

the cessation of a_3-cell productions. The a_4-state is also no longer required because, with the cessation of columella widening, the ring of a-cells remains constant in number and maintains a constant distance from the center of the root cap. Thus, should a-cells loop only through a_1- and a_2-states, then c-cell production would be curtailed. A counter (λ) of the number of a_1–a_4-cycles is suggested as a means of specifying when this loop is put into effect. Whenever widening of the cap and columella is curtailed, algorithm A1 is modified at the first two of its steps (A2) as follows:

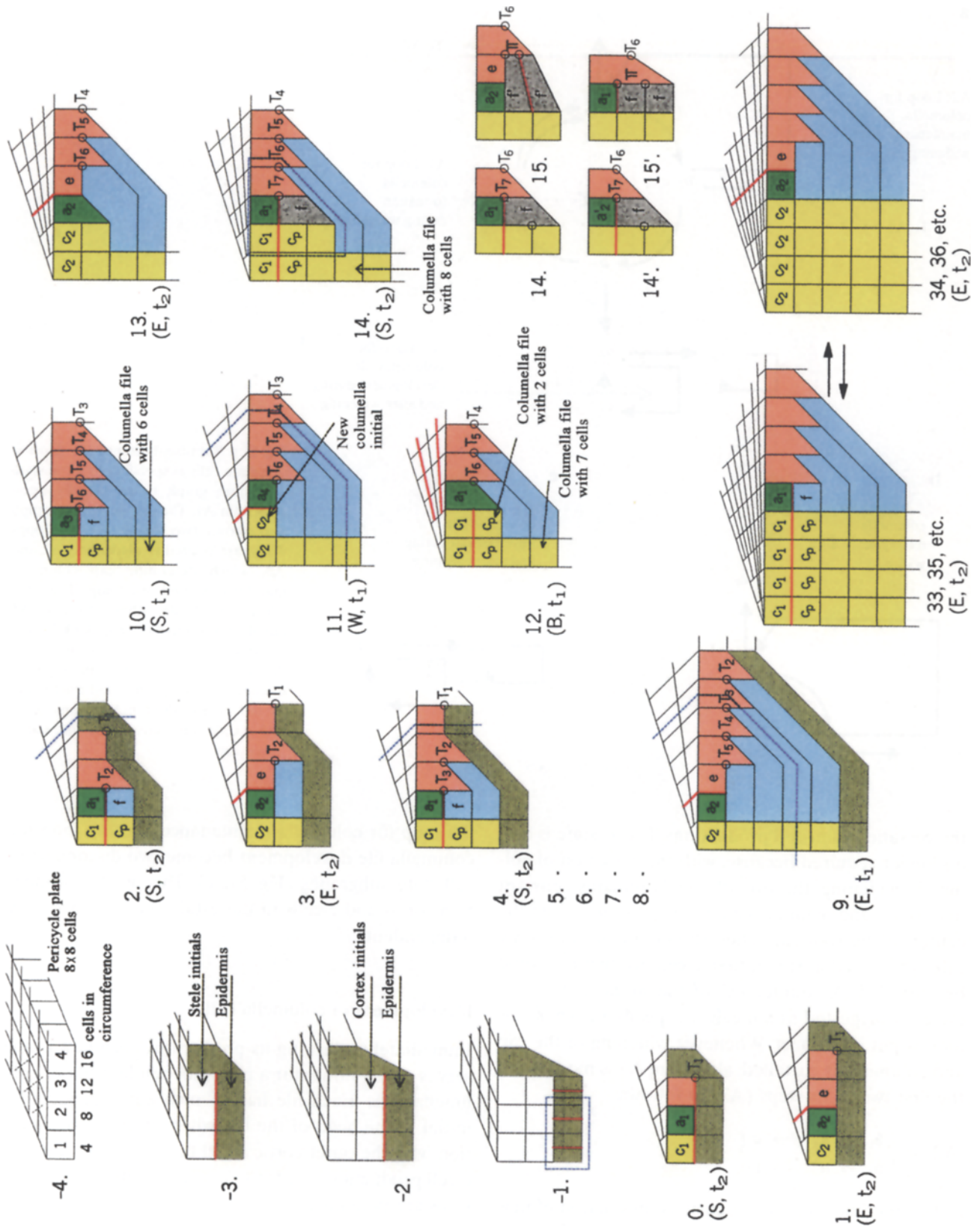
$$A2 = \left\{ \begin{array}{l} a_1 \rightarrow a_2 \xrightarrow{1} e \\ a_2 \rightarrow a_1 \xrightarrow{P} f \end{array} \right\}$$

This modification, which curtails production of new columella cell files, appears in Fig. 5a as the loop for lateral cap maintenance after the cessation of columella widening. As a consequence of algorithm A2, the loop for columella maintenance and the loop for columella file development become two disconnected cell-state subgraphs (Fig. 5b, c). That is, the productions of a- and c-cells in the established cap are now independent.

Development of columella cells

Immediately following its production, each new c-cell serves as the initial for a new, independent file of columella cells. Each file has its own autoreproductive initial at the head of the file where it abuts the junction with the outer cortical cell file at the root tip. All c-cell productions are from this initial cell, and all divisions are periclinal.

The behavior of c-cells can be defined in more detail and integrated into the previously mentioned system. Following its birth from an a_3-cell, the new c-daughter


-4.
Pericycle plate
8x8 cells
4 8 12 16 cells in
circumference
1 2 3 4
-3.
Stele initials
Epidermis
-2.
Cortex initials
Epidermis
-1.
0.
(S, t2)
1.
(E, t2)
2.
(S, t2)
3.
(E, t2)
4.
(S, t2)
5. .
6. .
7. .
8. .
9.
(E, t1)
10.
(S, t1)
Columella file
with 6 cells
11.
(W, t1)
New
columella
initial
12.
(B, t1)
Columella file
with 2 cells
Columella file
with 7 cells
13.
(E, t2)
14.
(S, t2)
Columella file
with 8 cells
14.
15.
14'.
15'.
33, 35, etc.
(E, t2)
34, 36, etc.
(E, t2)

cell is at state c_2. It then divides periclinally to produce an autoreproductive cell, c_1, and a cell in a final state, c_p (Fig. 6, steps 33–36). Thus, there are only three cell states to be considered. In graph-L-system notation, this is:

$$A3 = \left\{ \begin{array}{l} c_1 \rightarrow c_2 \\ c_2 \rightarrow c_1 \xrightarrow{p} c_p \\ c_p \rightarrow c_p \end{array} \right\}$$

This set of productions holds irrespective of whether the cap is in its widening phase or its maintenance phase. The cell-state derivation graph accommodates this aspect of cap development, as shown in Fig. 5 a by the loop for columella file development.

During the phase of columella widening, a new c-cell appears at each fourth timestep but, according to algorithm A3, divides at each second step after its formation. Therefore, there will be one more c-cell in the columella file than the number of lateral cap layers (Fig. 6, step 14). This circumstance could be related to the initiation of the secondary periclinal divisions (Fig. 3) occurring in f-cells close to the external cell files of the columella (Fig. 6, step 15).

Formation of additional files of peripheral cap cells

Inspection of the peripheral cell files shows that not every T wall junction within the tomato root cap is associated with the production of an epidermal cell (i.e., not every T is a primary T wall junction; these latter are labelled T_1–T_6 in Fig. 6). To account for this, and at the same time harmonize both the observed numbers of primary Ts associated with epidermal files and the number of columella cells within each of their files, it is sufficient to propose a transitory state a_2' in the behavior of the a-cells as follows:

$$A4 = \left\{ \begin{array}{l} a_1 \rightarrow a_2 \xrightarrow{t} e \\ a_2 \rightarrow a_2' \xrightarrow{p} f \\ a_2' \rightarrow a_1 \xrightarrow{p} f \end{array} \right\}$$

This supplementary step generates a double-T, or Π, wall junction (see Fig. 6, steps 15 and 15′).

A second type of Π junction is present in older cap layers and is located close to the columella files. It could arise in two ways. The first is a natural consequence of differential division times, as shown in the previous section, occurring when algorithm A1 is switched to A2. The second is due to the separate productions of columella cells and cap periphery cells. Files of c- and f-cells are bound together along their longitudinal walls. The outermost c-cells at the perimeter of the columella are contiguous with the a-cells. Symplastic cell growth would require that the derivative c_p-cells (derived from the c_2-cells) be bound to the peripheral f-state cells. Theoretically, however, this is not a necessary requirement for these cap tissues. The longitudinal files of the columella may be able to grow quicker than the adjacent, obliquely oriented files of f-state cells, and hence, the outer columella cell-files would glide over the inner surface of the peripheral cell files. If, however, sliding growth within the cap does not take place, and symplastic growth prevails, then the strain imposed on the flank by the columella files may have something to do with the induction of the additional periclinal divisions in f-state cells or their descendents. Instead of the following usual set of divisions:

$$A5 = \left\{ \begin{array}{ll} a_2 \rightarrow a_1 \xrightarrow{p} f_1 & \\ f_1 \rightarrow f_1' & \\ f_1' \rightarrow f_2 \xrightarrow{t} f_2 & \\ f_2 \rightarrow f_2' & \\ f_2' \rightarrow f_3 \xrightarrow{t} f_3 & \\ \ldots & \\ f_{p-1}' \rightarrow f_p \xrightarrow{t} f_p & \\ f_p \rightarrow f_p & \end{array} \right\}$$

there is a supplementary periclinal division

$$f_1 \rightarrow f_1' \xrightarrow{p} f_1'$$

Fig. 6. Steps in the construction of a theoretical epidermis and root cap (based on histological analysis of the tissues in tomato roots) according to the graph-L-systems described in the text. The development shown considers only a 90° sector of the primordium or root cap. This sector arises from one of the four groups of initiating cells in the pericycle. *–4* to *–1* Initial steps for constructing the primordium. Present at step –1 are cells homologous to those in the tissue layers shown in Fig. 1. *0–14* Cellular behavior during the phase of columella widening with the creation of new columella initials and primary T wall junctions. Surrounded by broken dark blue lines are those cells which are shown developing at the subsequent step in this figure. A red line (cell wall) within the group of cells indicates the new division wall. A double T wall junction (Π junction) is produced at step 15. *33–36* Cap maintenance, when no columella initials are generated. Step numbers are noted at the left of the diagram. Beneath the step number are given both the direction (E, S, W or B) of cell production and the algorithmic table (t_1 or t_2 of Fig. 8) that is appropriate to the step

This latter division results in a Π wall junction similar to that of Fig. 6, steps 14 and 15, but is located deeper within the layers of cap cells than was the double division of the f-cell immediately derived from an a-cell (see algorithm A4 and Fig. 6, steps 14 and 15). Additional files in the cap periphery, revealed by Π wall junctions, seem to have arisen in this way.

Cell packets of epidermal and peripheral cap tissues

Epidermal merophyte mother cells (e-cells) are produced by the division of a_1-cells. The number of transverse divisions which each e-cell undergoes is indicated by a state-associated subscript numeral, *p*. For example, an e-cell is born in state e_1 (e.g., Fig. 5b). Then, at each second timestep, it divides by a transverse division to give cell e_2. Divisions continue until a final state, e_p, is reached. So:

$$A6 = \left\{ \begin{array}{lll} a_1 & \rightarrow a_2 \xrightarrow{t} & e_1 \\ e_1 & \rightarrow e_1' & \\ e_1' & \rightarrow e_2 \xrightarrow{t} & e_2 \\ e_2 & \rightarrow e_2' & \\ e_2' & \rightarrow e_3 \xrightarrow{t} & e_3 \\ \cdots & & \\ e_{p-1}' & \rightarrow e_p \xrightarrow{t} & e_p \\ e_p & \rightarrow e_p & \end{array} \right\}$$

The subscript *p* indicates the number of cells finally present within the merophyte derived from cell e_1. In the example where $p = 4$ (i.e., e_p is equivalent to e_4), the final packet size is 2^{p-1} ($2^3 = 8$) cells. Because the final size of these packets relates to the proximal limit of meristematic activity in the epidermal file, the value of *p* is significant for simulating the construction of the root meristem. The value of *p* can be deduced from the number of radial divisions which bring about a splitting of the files in the epidermal cell packets. For example, where eight cells develop along a file, but where there have been two radial divisions, the subscript numeral of e will be increased from 1 to 4 + 2, i.e., up to a final state of $p = 6$. A wide range of root meristem behavior can be contained in the numeral associated with e since it relates, on the one hand, to the length of the meristem and, on the other hand, to the number of file-splittings.

A similar reasoning applies to the f-merophyte mother cells at the origin of the lateral cap layers. The subscript numeral associated with f indicates the number of peripheral cap cell layers generated by the f-merophyte mother cell. Where an f-merophyte mother cell produces three cap layers by periclinal division, it takes the state f_3 before producing the final state, f_p. The f_p-cells are, at this stage, members of a packet of eight cells. More and more layers of f_p-cells are added to the cap flank. As a result, the outer layer of cells becomes stretched until, at last, the whole layer peels off and is shed from the cap.

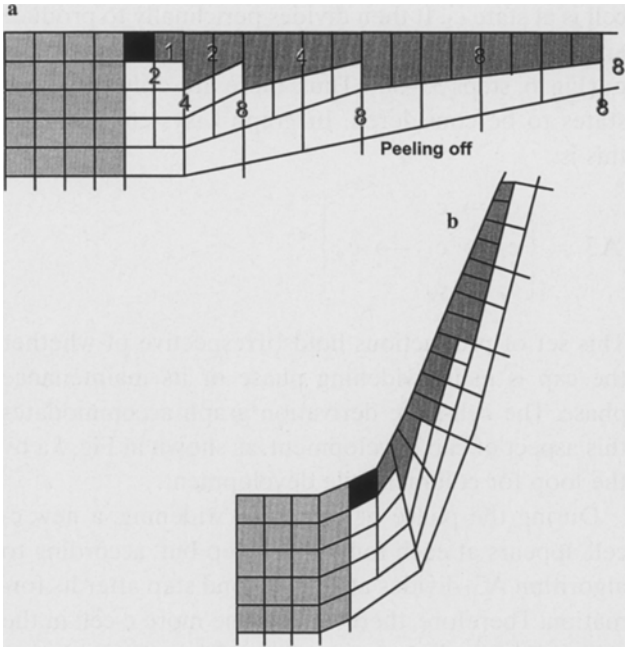


Fig. 7a, b. One of the concluding steps of root cap formation is the shedding of the outer layer of cap cells. **a** A final number of eight cells has been achieved along the length of the more distal peripheral and epidermal merophytes. **b** The same group of cells as depicted in **a** but with the application of tissue deformation. This procedure produces a more realistic depiction of the two-dimensional shape of the cap tissue and its cellular pattern

Simulations should be able to show the implications of different values of *p* for the epidermal and peripheral cap cells. In the tomato root, there are epidermal cell packets with up to 33 cells along their length. This indicates that there have been slightly more than five transverse cell divisions. During the same phase of development, epidermal cell numbers around the circumference increase from 31 to 80 cells as a result of one or two radial divisions. Consequently, there are six to seven cap layers (without counting supplementary layers added by Π junctions) plus two outer layers before peeling off occurs (Fig. 7).

Theoretical doubling times of cells

At each timestep during the widening phase of cap development an a-cell divides (with a doubling time

t_1

Loop for root cap formation, including widening of the columella

Algorithm A1:

$a_1 \rightarrow a_2 \xrightarrow{t} e_1$

$a_2 \rightarrow a_3 \xrightarrow{p} f_1$

$a_3 \rightarrow c_2 \xrightarrow{t} a_4$

$a_4 \rightarrow a_1 \xrightarrow{r} a_1$

plus algorithms A3, A5 and A6.

t_2

Loop for root cap maintenance after the cessation of columella widening

Algorithm A2:

$a_1 \rightarrow a_2 \xrightarrow{t} e_1$

$a_2 \rightarrow a_1 \xrightarrow{p} f_1$

$a_3 \rightarrow a_2 \xrightarrow{t} e$

$a_4 \rightarrow a_1 \xrightarrow{p} f$

plus algorithms A3, A5 and A6.

Fig. 8. A table-graph-L-system with two sets of cell-state transitions, t_1 and t_2, that incorporate the algorithms (A1, A2, A3, A5, and A6), and from which development of tomato root cap can be simulated. The productions in t_2 which are enclosed in the box may be used only at the moment of re-entering this loop from t_1

equaling 1 timestep) to produce a non-a daughter cell in one of the four available directions (E, S, W, and B). During the maintenance phase, a-cell production is restricted to two directions (E and S). At each second timestep, c-cells divide (doubling time equaling 2 timesteps). One of their divisions coincides with the periclinal divisions of the a-cells. Therefore, during the widening period, there are two divisions of columella initials for each periclinal division of an a-cell. Division of e- and f-cells also occurs at each second timestep. Estimates of cell doubling time (Barlow 1992) showed that lateral cap cells and columella initials have about the same generation time. Moreover, counts of cell numbers (P. W. Barlow unpubl.) show that the successive e-cell packets in the epidermis are members of 2^x, where x is the number of cell generations within the e-merophyte. If the doubling time is set to one timestep, then the merophytes would be members of generation 2^{2x} or 2^{2x+1}.

General model and summary

An economical way of formulating the two stages of development and maintenance of the tomato root cap and epidermis is by means of a table-graph-L-system (for an introduction to table-L-systems, see Herman and Rozenberg 1975). A table-system with two sets of cell-state transitions (see Fig. 8; t_1, integrating algorithms A1, A3, A5, and A6, and t_2, integrating algorithms A2, A3, A5, and A6) that differ in their respective outcomes can account not only for the actual numbers of cap columella cell files and the number of cells in each file but also for the number of primary T divisions and the number of peripheral cap files with or without Π junctions. According to t_1, columella widening is permitted, whereas according to t_2, it is not and the cap maintains its structure with continuing division of its cells.

Commencing from an initial condition $\omega_0 = a_1$, the switching between the state transitions of t_1 and t_2 of Fig. 8 has to be defined before the system can be regarded as being complete. It is effected by loop counters, λ, instead of timesteps (Table 1). During each phase of columella widening, t_1 is applied for one loop (i.e., $\lambda_2 = 1$; loop duration, 4 timesteps), and is then followed by two loops ($\lambda_3 = 2$; loop duration, 2 timesteps) of state transitions specified by t_2. The sequence $\lambda_2\lambda_3$ is repeated three times, signified by $\Lambda = 3$. As a result, three columella files are produced. Following this period, during which formative and maintenance activities alternate, maintenance activities prevail and t_2 applies for an unspecified number (λ_4) of loops. These loops continue until conditions no longer permit this mode of growth. In biological terms, the switching from the processes inherent to t_1 to those of t_2, and then the switching back to t_1, are not necessarily properties of the system itself but may depend on information coming from outside the system.

A final point concerns the entry of cells of the pericycle into the phase of columella widening. This occurs by means of events specified by t_2 which are applied to the ω_0 initial cell, a_1, through four loops ($\lambda_1 = 4$; loop duration, 2 timesteps).

The sequence of events composing the table-graph-L-system is summarized in Table 1, which is also a plan of the constructional events shown in Fig. 6. From the

Table 1. Summary of primordium and young lateral-root cap development in in vitro grown tomato roots[a]

Duration[b]	Number of timesteps	Developmental steps	Developmental scheme[c]		Outcome[d]
	≥4	–3 to 0	first pericycle divisions		primordium initiation
$\lambda_1 = 4$	≥8	1–8 or more	t_2 loops		initial cap formation
$\lambda_2 = 1$	4	9–12	t_1 loop	One columella	$\Lambda = 3$ columella widening period, three files added
$\lambda_3 = 2$	4	13–16	t_2 loop	file added	
$\lambda_2 = 1$	4	17–24	t_1 loop	One columella	
$\lambda_3 = 2$	4		t_2 loop	file added	
$\lambda_2 = 1$	4	25–32	t_1 loop	One columella	
$\lambda_3 = 2$	4		t_2 loop	file added	
$\lambda_4 = 1$ to ∞	unlimited	33 to ∞	t_2 loop		cap maintenance

[a] The information herein provides the instructions for the growth of the 3-dimensional cellular complexes shown in Fig. 6
[b] λ_1 to λ_4, the number of times cells loop through the various algorithms specified in Fig. 8
[c] t_1 and t_2, the algorithms specified in Fig. 8
[d] Λ, number of looping operations

above description, the system can be completely formulated as: $t_2^{\lambda_1}\,[t_1^{\lambda_2}\,t_2^{\lambda_3}]^{\Lambda} t_2^{\lambda_4}$.

Discussion

Behind the present use of L-systems lies an attempt to extract the rules, or laws, for the development of a particular group of cells. These rules are not arbitrary. On the contrary, they are already embedded within the cells and pertain to the sequence and spatial pattern of cell division within the root cap. In the present case, the graph-L-system algorithms which emerge make comprehensible many of the varied cellular patterns that are typical of root cap and epidermis of different species (see Clowes 1994, for a summary of these patterns). Moreover, in connection with plant organs, it is probably true to say that wherever there is a set of initial cells with its particular set of formative divisions and T wall junctions a corresponding graph-L-system can be recovered.

The theoretical rules by which a system is defined are obtained following the close analysis of actual cellular patterns. They should therefore be considered as the counterpart of the biological algorithms inherent to the cells. The L-system algorithms accordingly represent the biological system in the objective languages of logic and mathematics. Although the cytological observations necessarily deal with the past behavior of the cells, the uncovering of the corresponding division rules from which a given pattern is derived can also anticipate future cellular behavior.

A consequence of using deterministic L-systems is that a given set of elements must always show identical transformations. Clearly, this is the case of the actual plant systems: otherwise it would be impossible to distinguish between, as evidently one can, the pattern of cells in, say, root caps of different species. It follows that, in the general case, L-systems provide a rigorous means of seeing whether the chosen aspects of cellular behavior really do obey laws and whether the system deals with the facts of observations supplied by different observers and sources of plant material. In the case of the root cap, use of L-systems also leads to the exploration of mechanisms involved in the behaviors of different systems, such as cell pattern generation and maintenance. Nondeterministic L-systems would certainly allow the derivation of cell patterns more in conformity with those which have been observed but would give less access to the underlying laws. Moreover, where system behavior is shown to have an underlying basis in a few mathematical laws, conclusions pertaining to its behavior are much more powerful than deductive statements.

The complex of epidermal and cap cells of the tomato root apex is typical of the closed type of meristems of dicot species. Epidermal cell files are derived

from autoreproductive a-cells which are also progenitors of the cap tissue. The modelling and simulation procedures have made explicit the steps involved in developing these two zones of the root apex and reveal the underlying regularity of the cellular production process. A similar though more general presentation of the model has been used to analyze the cellular construction of the root cap of *Arabidopsis thaliana* (Lück et al. 1999), whose constructional basis was described by Baum and Rost (1996). Lateral-root primordium development of *A. thaliana* was also described by Malamy and Benfey (1997). Although their images of the cap are longitudinal-radial and are therefore in a plane at right angles to ours, they are complementary and confirm the existence of a central group of four cells (see Fig. 2). With regard to their mature structure, one major difference between *A. thaliana* and tomato root cap constructions is in the number of columella and flanks cell files due to the different numbers of division loops, λ_2 and λ_3. Cap construction in both species nevertheless uses the same repertoire of cells – autoreproductive protoderm initial a-cells and derivative c-, e- and f-cells – as well as the sequential utilization of division planes p, r, and t.

There are a number of important questions relating to the behavior of the autoreproductive a-cell early in cap development. The first relates to the means by which divisions occur regularly in different orientations so that all three available planes (p, r, and t) are used in sequence. A second question relates to the directionality of a-cell productions. Given that there is such a phenomenon as directional production, then it is noticeable that, although productions in E, S, and W directions occur in the tomato root cap, there are no productions in the N direction. Directionality is an expression of the asymmetry, or polarization, of either the dividing a-cells themselves or the differentiation status of the daughter cells which is acquired soon after the mother a-cell has divided. Interestingly, the cell pattern of the root cap of the *gib-1* mutant of tomato (Barlow 1992), where a new cap develops beneath the proximal border of a preexisting cap, suggests that there can sometimes be cell production in the N direction by the initial cells. This could establish new sets of a- and c-cells which supersede those of the original cap. The division of an a_4-state cell is symmetrical, however, and involves cell productions in either B or F direction. Both daughters continue as a-cells in state a_1. It is noticeable that here the cell productions are in the same plane (circumferential) as the ring of a-cells.

To answer some of the points above, it is noteworthy that rotating sequences of division are well known – they occur in early embryos of both plants and animals and in the tetrahedral apical cell of leptosporangiate fern roots. However, the basis for the rotation of the division plane is unknown. One possibility is that, in cells exhibiting such highly regulated division patterns (see Barlow et al. 2000, 2001), there is a macromolecular or physical connection between the cell walls, the associated peripheral components of the cytoplasm, and the cell division apparatus, whose seat of constructional activity is at the nuclear surface (Baluška et al. 2000). The effectiveness of the wall-nucleus association is related to the state of the wall, "state" here being considered in terms of the age of the wall (that is, the number of timesteps since its formation) (Barlow et al. 2000). It may be that changes in wall state, regulated over a defined number of timesteps, underlie the directed nature of cell division. Plant cell walls can impart information for cellular development. Hence, certain wall states may forbid certain division directions. For example, the state of the columella–cortex boundary on its acroscopic face may normally preclude division in the N direction. However, in the present modelling of tomato cap construction, the system is delineated at the organ level, with cells, not their walls, as the constructional elements. In the present simulations, wall states are not considered as being relevant, except in so far as the states may be implicated in the internal polarity of cells and hence in the direction of cell production.

Further questions concern not only how the a-cell maintains its autoreproductive status but also its commitment to division at a faster rate than any of its derivatives. The kinetics of the theoretical pattern of cell division suggest that the a-cells have a division rate which is at least double that of all other dividing cells in the root cap. Such a situation is not unprecedented: the autoreproductive apical cell (a-cell) of *Azolla filiculoides* roots has a division rate that is higher by a factor of two or three than that of the proliferative cells in its neighboring merophytes (Gunning et al. 1978). The same is probably true of secondary vascular cambial initials, which resemble the a-state of the root cap in that they, too, possess alternative directions of cell production (P. W. Barlow unpubl.). It should be possible to look for direct evidence of the more rapid cell division of a-cells of cap tissues by using markers for nuclear DNA synthesis.

The present study of the root cap predicts a switching between a set of divisions which generates the cap columella and another set which maintains cap structure (Fig. 8). Both sets of division are formative in the sense that, in each case, the outcome is new files of cells. The two sets of algorithms used in this switching (t_1 and t_2 in Fig. 8) reflect the complex pattern of behavior of the a-cells. The sequence of their states, until maintenance divisions are finally established, is: $a_1, a_2, a_3, a_4, a_1, a_2, a_1, a_2, a_1, a_2, a_1, a_2, a_3, a_4, \ldots$, and the corresponding sequence of directed cell productions is: E, S, W, F, E, S, E, S, E, S, E, S, W, F, ... It is possible that certain walls, following their participation in a division, require different numbers of timesteps to recover the state(s) that permits participation in the next cell division. Another explanation could relate to the changes in shape of the autoreproductive a-cells; it has been shown theoretically that cell shape influences the number and distribution of merophytes within developing apices (Lück and Lück 2000). The repeated sequence of E- and S-directed cell productions found in t_2 of Fig. 8 can be generated by one type of autoreproductive cell (type III in the family called "apical meristems", Lück and Lück 2000), whereas the repeated sequence of S-directed productions in the cap columella can be independently generated by another cell type (type II cells in the same family). Each type of autoreproductive cell leads to its own special type of merophyte with a unique spatial pattern of cell division. This simple observation opens the wider question concerning the relationship between the global aspect of organ growth – the deformation of organs, famously illustrated by Thompson (1917) and Schüepp (1926) for plant meristems – and the more localized cell divisions contained within such growth zones. Given a system in which there is continuous cytoplasmic growth, two major principles, organ shaping and cell division, are then sufficient to account for all cellular patterning during plant organogenesis. However, at present, it is hard to know whether one principle regulates the other or whether there is a mutual interaction between them. As a result, it is possible that organ shaping, as shown in Fig. 7 b, could eventually be coupled with L-systems for cell division to produce a more comprehensive formalization of plant development (see also Lück and Lück 2000). It is even possible that such an interaction might touch upon patterns of gene activity and hence provide a link to patterns of tissue differentiation.

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