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The sequence of cell divisions in the I tunic layer of *Actinidia arguta* Planch in light of the development of twin cell complexes

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Abstract

In *Actinidia arguta*, the I tunc layer is formed by four cell complexes which descend from single initials. These initials are positioned in a corner of their complex, around the meristem axis. The meristematic activity of the I tunic layer depends on the formative divisions of the initials; the entire I tunic layer above the youngest leaf primordia is formed during the time the initials undergo only 4-8 divisions. In light of the development of the twin cell complexes, it is impossible for cells to be displaced from the I tunic layer into the meristem. The supposition is set forth that the impermanent, mericlinal sectors on variegated perinclinal chimeras develop due to periclinal cleavages within the subcomplexes which derive from tissue mother cells. Whereas, the cell initials do not undergo periclinal divisions and are not displaced.

Key words: twin cell complexes, initial cells, tunica, chimeras

INTRODUCTION

THE NUMBER AND POSITION OF INITIALS IN THE I TUNIC LAYER

Guttenberg (1960) holds that there is one, apically positioned initial in the I tunic layer. According to him, this cell permanently retains its identity and is the mother cell of the closest neighboring cells. By anticlinal divisions in all directions, it forms the first „necklace" of cells. In turn, the cells of this necklace, by horizontal anticlinal divisions, almost simultaneously from a second necklace. Thanks to this, the meristem axis is permanently located in the center of the same initial. It is hard to argue

in support of this view, because a given cell is replaced by two cells after division, and one cannot assume that one of them arose from the other (Prat 1945 according to Newman 1965).

Schüepp (1966) avoids these difficulties by assuming that the center of the intial is moved to the side in respect to the meristem axis. If the center of the cell was on the meristem axis, then after its first crosswise division — indispensable for the growth of the tunic in all directions — a cell wall would be on the axis. Then, all four cells would be initials. According to Schüepp (1966), the four initials exist only temporarily. Only the cell through the center of which the meristem axis passes, remains permanently on the axis. In this way, only one cell is in the meristem axis all of the time, although it is not always the same cell. However, Newman (1965) thinks that there can be two initials in the individual tunic layers and these cells would have a common wall on the axis; there could also be three or more cells with a common corner on the axis — the function, not the cell, would be permanent.

On the basis of studies on variegated periclinal chimeras with mericlinal sectors, Barteles (1960) thinks that there are four initials surrounding the meristem axis in the I tunic layer of *Epilobium hirsutum*. Stewart and Dermen (1970), studied chimeras in several species and also think that the initials, 3-4 in number, are positioned in the I tunic layer around the axis.

It is imposible to determine directly and definitely, what the situation is in each individual case. It is impossible to show and justify, that certain, and no other cells, are intials. In order to do so, it is necessary to discover the sequence of cell divisions in the first tunic layer. Such a possibility is presented in this paper.

CELL LINEAGE, TWIN CELLS, TWIN CELL COMPLEXES

The term daughter cells, applied to the two cells which arise as the result of cell division, does not adequately convey the nature of their relationship. Daughters can be of the same or different ages. The two cells which arise in the place of one are the same age. They are twins. Twin cells, depending on what they are destined for, can be of equal or unequal value. In connection with this, the divisions leading to their formation can also be termed equal or unequal cell division. Gunning et al. (1978) call equal division — proliferative cell division, unequal division — formative cell division. As the result of further divisions, equal twin cell complexes arise from equal twin cells. Unequal twin cell complexes arise as the result of continuing divisions of unequal twin cells. A characteristic trait of initial cells is formative cell division. After such a division, one

cell is a renewed cell initial, the other — a tissue mother cell. The population of cells descending from the cell initial extends the life-span of the meristem and is formed by twin, unequal cell subcomplexes. Whereas, the population of cells descending from the tissue mother cell is formed by equal, twin cell subcomplexes and, in its entirety, becomes part of a given tissue.

When examining the development of the I tunic layer from one cell, it should be accepted, as does Schüepp (1966), that this cell divides crosswise. After such a division, twin cells can be identified under the following conditions. After the division of a cell, twin cells are formed: *ab-ac* (Fig. 1a and b). Next, the twin cells divide, nosimultaneously, in

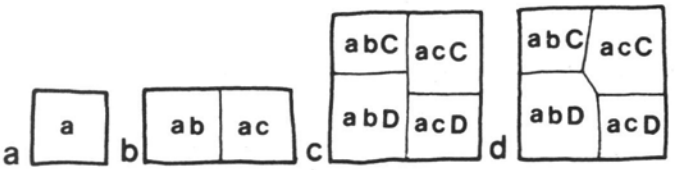

Fig. 1. The principle of the cell walls passing each other. a — a cell before division; b — first generation twin cells, *ab-ac*; c — second generation twin cells, *abC-abD*, *acC-acD* before the walls are kinked; d — after the walls kinked. Twin cells and twin complexes are labeled according to the following rules: to the symbol denoting the starting cell, in this case the letter *a*, the letters *b* or *c* are added to denote the first generation twin cells. The second generation twin cells have a third letter identifying them, added to their identical two first symbols: *abC-abD*, *acC-acD*. The symbols *ab-ac* denote then, first generation twin complexes

a plane perpendicular to that of the first division. The cell wall started later does not meet the earlier one in one point, but is shifted in respect to it by a variable distance. Second generation twin cells arise: *abC-abD* and *acC-acD*. Between cells *abC* and *abD*, the single, youngest partitioning wall is found. Whereas the wall which separates cell *abC* and *acC* is part of the partitioning wall between the twin complexes, *ab-ac*. Thanks to this, it is possible to precisely identify twin pairs among the four cells (Fig. 1c and d).

If the four cells which have arisen in this way are found in the I tunic layer, then all of them should remain on the apex of the meristem and fulfill the role of initials. None of the four complexes started by cells *abC*, *abD*, *acC* and *acD* will be able to be dislocated from the meristem apex without the remaining three being taken along. The corners of the four complexes, joined by common walls, should permanently remain on the apex of the meristem. Checking this supposition is the aim of this study.

MATERIAL AND METHODS

Actinidia arguta Planch is a climber which grows wild in eastern Asia. The material used in this study came from specimens from the Botanical

Garden of the Wrocław University. CrAF (0.5:0.5:20) was used to fix the apices. A series of cross sections through the apex of the meristem of samples embedded in paraffin was made by with a microtome. The sections were stained with hematoxylin, safranine and fast green and closed in balsam.

The apical meristem of *Actinidia arguta* Planch is almost flat (Fig. 2). Due to this, the area of almost the entire meristem above the youngest leaf primordia could be found on two sections through the apex. The leaves are arranged spirally with a limit divergence of 137.5° (Puławska 1965).

RESULTS

Figure 3a presents the arrangement of cells in the I tunic layer of the shoot apical meristem of *Actinidia arguta* as seen from above. Pairs of twin cells are clearly seen. Two-celled, twin complexes (Fig. 3a) are also

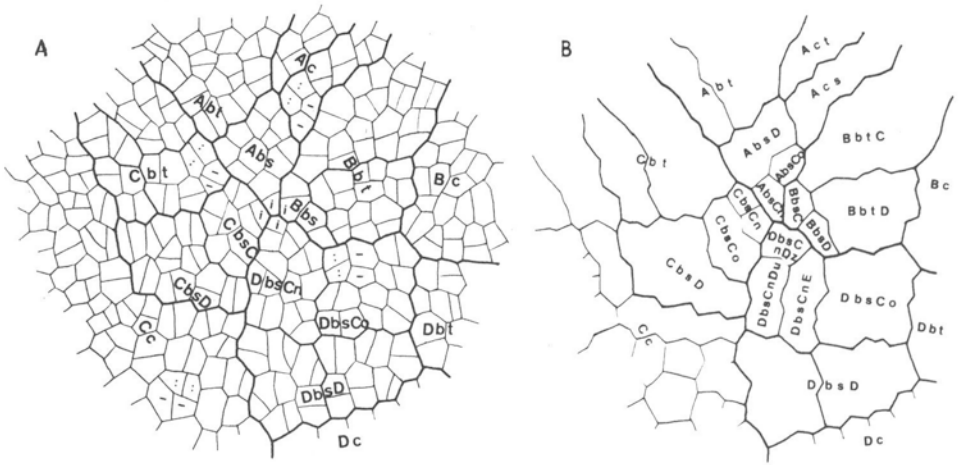

Fig. 3. *Actinidia arguta* Planch. The I tunic layer, seen from above. 600 x. a — Arrangement of cells and complexes. Some twin cells are marked by dots and dashes. They are part of two-celled twin complexes. i — initials. The borders between complexes *A*, *B*, *C* and *D* are marked by heavier lines. The borders of the oldest subcomplexes within them are also marked. The subcomplexes are marked according to these rules: The oldest, twin, unequal subcomplexes have, in addition to their complex letter, one symbol identifying it, e.g. *Ab-Ac*. Younger, twin unequal subcomplexes, e.g. those within complex *Ab*, have a second latter added, e.g. *Abs-Abt*, etc. *b* — Borders and labels of some subcomplexes (wthout delineation of cells)

easy to identify. After exact analysis, it became evident that the entire area of the meristem above the youngest leaf primordia is formed by four complexes: *A*, *B*, *C* and *D*. The corners of these complexes, with four initials, are found on the apex of the meristem (Fig. 3a).

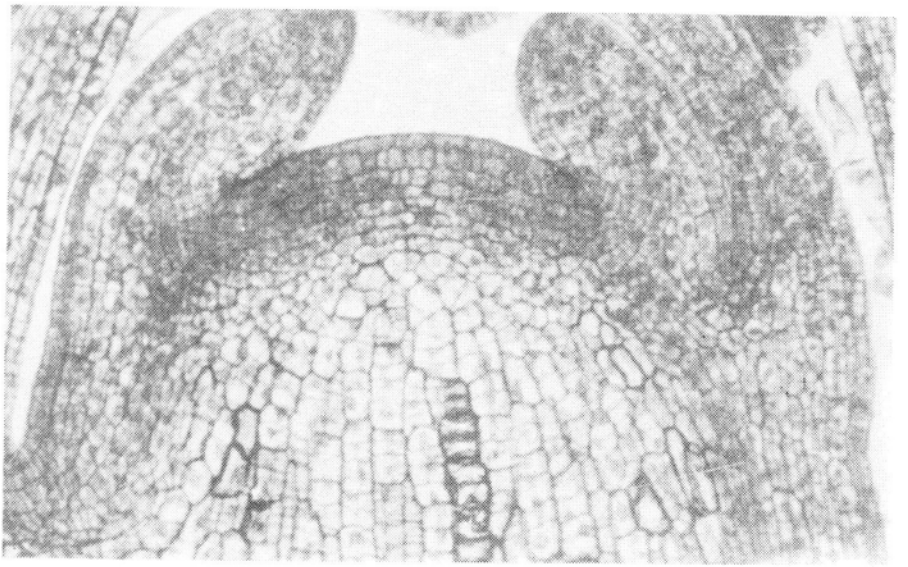

Fig. 2. *Actinidia arguta* Planch. Longitudinal section through the shoot apical meristem. × 190

After each division of the initial, unequal twin cells are formed. One of them is the continuing cell initial, the other — the protoderm mother cell. Because of this, every complex which contains an initial cell is formed by unequal, twin subcomplexes. In complex *A*, for example, subcomplex *Ab* derives from the initial, its twin, *Ac*, from the protoderm mother cell. In complex *Ab* in turn, subcomplex *Abs* is derived from the initial, *Abt*, from the protoderm mother cell. In complex *Abs*, subcomplex *Abs*C derives from the initial and *Abs*D from the protoderm mother cell (Fig. 3a and b). And finally, complex *AbsC* contains the youngest, unequal, twin subcomplexes. Namely, subcomplex *AbsCo* is the subpopulation of the protoderm and is made up of equal, twin cells. Whereas, subcomplex *AbsCn* is formed by the intial and the protoderm mother cell (Fig. 3a an b). The number of formative divisions of the initial cell is equivalent to the number of unequal, twin subcomplexes. In complex *A*, there are four pairs of increasingly younger, unequal subcomplexes: *Ab-Ac*, *Abs-Abt*, *AbsC-AbsD*, *AbsCn-AbsCo*. They arose as the result of four formative divisions of the initial cell. This can be expressed in one formula as follows: «[(*AbsCn-AbsCo*)*AbsD*]*Abt*»*Ac* (Fig. 3b). The present intial was formed by the fifth division as was the mother cell of the next protoderm cell subcomplex (Fig. 3a and b).

The initial cell renewed itself four times in complex *B*. The formula for three pairs of unequal, twin subcomplexes is as follows: [(*BbsC-BbsD*)*Bbt*]*Bc* (Fig. 3a and b).

In complex *C*, the initial renewed itself six times. The formula for four pairs of unequal twin subcomplexes is: {[(*CbsCn-CbsCo*)*CbsD*]*Cbt*}*Cc* (Fig. 3b). Complex *CbsCn* contains the youngest, unequal subcomplexes. One of them is the protoderm subcomplex and is formed by unequal, twin cells. The other is formed by the initial cell and mother cell of the next protoderm subcomplex (Fig. 3a and b).

The initial cell renewed itself eight times in complex *D*. The formula for six pairs of unequal twin subcomplexes is: {[{[(*DbsCnDz-DbsCnDu*) *DbsCnE*]*DbsCo*]*DbsD*]*Dbt*}*Dc*. Complex *DbsCnDz* contains the youngest, unequal subcomplexes. One of them is the protoderm subcomplex, the other contains the current initial (Fig. 3a and b).

The entire I tunic layer can then be shown to derive from one cell which underwent crosswise division. The first, eldest division wall marks the border between complexes *A-B* and complexes *C-D*. The shifted walls formed in the second division, which do not come in contact with each other, mark the borders between complexes *A* and *B*, *C* and *D*. The arrangement of the four complexes is the same as of the four initial cells at the beginning, from which the complexes derive. The wall segment between the shifted walls of the second division is a peculiar place. No new wall has rested on it, and it has therefore not expanded. At the same time, it is the wall section

which keeps the corners of the four complexes in the meristem axis.

Thus, the meristematic activity of the I tunic layer is upheld by the formative divisions of four initials. This, however, does not mean that the initials divide more frequently than other cells. In *Actinidia arguta*, the entire multicellular I tunic layer above the youngest leaf primordia, arose in the time during which the initials renewed themselves only several times.

DISCUSSION

NUMBER, POSITION AND FREQUENCY OF DIVISION OF INITIAL CELLS IN THE I TUNIC LAYER, IN LIGHT OF THE DEVELOPMENT OF TWIN COMPLEXES

In *Actinidia arguta*, the I tunic layer is formed by 4 cell complexes. Each of them derived from one initial cell. The initial is located in the corner of its complex. The wall which holds together the corners of the four complexes with initial cells marks the meristem axis.

If one initial existed, common for the protoderm, in the I tunic layer, it would then have to be in the center of only one complex. In addition, the cell would have to constantly retain its identity, as presented by Guttenberg (1960). However, there is no evidence that the mechanism of division of the initial is any different from that of other cells. Every cell ceases to exist after division and is replaced by twin cells, which are surrounded by a common wall.

Two different views on the activity of the apical part of the meristem can be explained and reconciled in the light of the development of twin cell complexes. A bit of truth lies in the view about the "initial ring" and fairly inactive apical part (Buvat 1955, Langenawer and Davis 1973, Davis and Steeves 1977). The initial ring cannot, however, be viewed as a self-continuing part of the meristem.

The view on the fundamental formative role of the apical part of the meristem, and the initial cells located there, should be accepted. In the opinion of this author, the often used term "descendent of the initial" is somewhat misleading. If some cells are the descendants of other cells, this means that those other cells are always the same. If there are no permanent initials, then their descendants also do not exist. There are only twin cells and twin complexes derived from them.

CHIMERAS IN LIGHT OF THE DEVELOPMENT OF TWIN COMPLEXES

Stewart and Derman (1970) described the development of mericlinal sectors on periclinal chimeras. For example, on *Ligustrum ovalifolium* Hassk., on a white-green periclinal chimera of type *G-W-G*, a *G-G-W* mericlinal

sector developed. It encompassed one-half of the stem diameter and extended through 8 internodes. Above, the branch was again *G-W-G*. According to Stewart and Dermen (1970), the *G-G-W* sector developed due to the periclinal division of an initial from the I layer and shifting of the lower cell to the II layer, and also by the substitution of the initial from the III layer by the initial from the II layer. The disappearance of the sector was determined by the substitution of one type of cell by another within the II and III layers.

The substitution of initial cells between or within layers is, in light of the development of twin complexes, impossible. The twin cells and the twin complexes deriving from them are permanently fused by common walls. After the periclinal division of a cell from the I tunic layer, the lower cell is as much fused with the undivided cell in its layer as is the upper cell

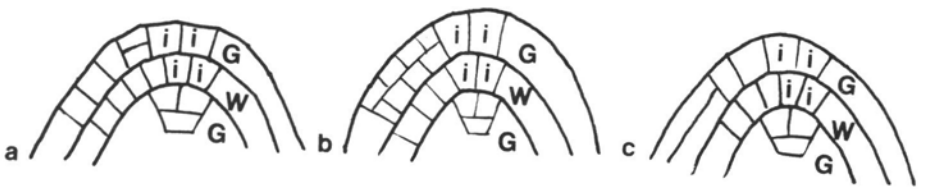

Fig. 4. A diagram of divisions which lead to the development of the mericlinal sector in a periclinal chimera with three layers of initial cells of the *G-W-G* type. ii — initials. a — Periclinal division of a tissue mother cell in the green I tunic layer. b — Two twin layers, deriving from a cell which underwent periclinal division in the I tunic layer. c — Start of the elimination of the periclinal cleavage. After the formative division of the initial, the tissue mother cell underwent anticlinal division

(Fig. 4a). The development of a mericlinal sector is possible presuming that in the I green layer, the tissue mother cell, not the initial, underwent periclinal division. The subsequent twin cells, undergoing anticlinal division many times, initiated the two twin layers. Both layers remained fused at the apex with the initials of the I layer which did not undergo periclinal division (Fig. 4b). In the II layer, no shifting of initials had place either. The mother cells of this layer were only covered by two, deriving from the first layer, tiers (Fig. 4b). As a result, they initiated those tissues which previously were derived from the III layer. Also, the initials of the III layer did not move, only the complexes deriving from them were included in the developing leaf.

The disappearance of the mericlinal sector and return to the initial form is connected with the fact that in the I layer, after renewal of the initial by anticlinal division, cell in the protoderm complex underwent only anticlinal division. Two green layers were again replaced by one layer (Fig. 4c). The cells derived from the II layer found themselves once again under the surface layer, and the complexes derived from the III layer were included in the developing leaf.

The impermanent mericlinal sectors would then be developing on permanent periclinal chimeras due to periclinal cleavages within the subcomplexes derived from tissue mother cells. The number of layers and position of initials would remain unchanged.

Stewart and Dermen (1979) think that in both mono- and dicotyledons, there are three layers of initial cells. For instance, the striped species, *Tradescantia albiflora* cv. albovittata, in the opinion of these authors, is a type *G-W-G* periclinal chimera. The white sectors would develop in this plant due to the substitution of the initial from the III layer by the initial from the II layer. The green sectors, by the substitution of the initial from the II layer by that of the first. Whereas Thielke (1954) thinks that there are two layers, *G-W*, in this plant. The green sectors would develop due to periclinal fission in the I layer. In light of the development of twin complexes, only the interpretation by Thielke (1954) is acceptable. It also remains in agreement with the presence of the one-layered tunic in the apical meristem of *Tradescantia albiflora*.

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Sekwencje podziałów w I warstwie tuniki u Actinidia arguta Planch w świetle rozwoju bliźniaczych zespołów

Streszczenie

U *Actinidia arguta* I warstwa tuniki składa się z czterech zespołów, które pochodzą od pojedynczych komórek inicjalnych. Te ostatnie mieszczą się w narożach swoich zespołów wokół osi merystemu. Merystematyczna aktywność I warstwy tuniki zależy od twórczych podziałów komórek inicjalnych; przy czym cała I warstwa tuniki powyżej najmłodszych zawiązków liści powstaje w czasie, gdy komórki inicjalne dzielą się twórczo zaledwie 4-8 krotnie. Po każdorazowym podziale komórki inicjalnej powstają nierównocenne, bliźniacze komórki. Jedna z nich jest odnowioną komórką inicjalną, a druga komórką macierzystą praskórki. W związku z tym każdy zespół, w skład którego wchodzi komórka inicjalna składa się z nierównocennych, coraz młodszych bliźniaczych podzespołów, aż do bliźniaczych nierównocennych komórek.

W świetle rozwoju bliźniaczych zespołów niemożliwe są przesunięcia komórek z I wartwy tuniki w głąb merystemu. Wysunięto przypuszczenie, że nietrwałe, meryklinalne sektory na różnobarwnych peryklinalnych chimerach rozwijają się dzięki peryklinalnym rozszczepieniom w obrębie podzespołów, które wywodzą się od komórek macierzystych tkanki.