Distribution Pattern of Cell Division Centers on the Epidermis of Stem Segments of Torenia fournieri during de Novo Bud Formation

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ABSTRACT

The stem epidermis in Torenia fournieri, which has budding potentialities, is composed of one cell layer which can be easily separated from the rest of the stem segment at different stages of bud formation. As the buds are formed directly from the epidermis, without intermediate callus formation, it is possible to observe simultaneously the cell division centers over the entire excised epidermal surface. The quantitative analysis at the 6-day stage of bud formation showed that the cell division centers do not have a random distribution on the epidermal surface. With respect to the length of the stem segment, the frequency of cell division centers increases toward the base which is also the direction of auxin transport. With respect to the width, the maximum number of division centers is observed on either side of the median zone. The median zone and the lateral zones have few division centers. An anatomical study showed that the zones with few division centers are the closest to underlying vascular tissue. A more uniform distribution of division centers can be obtained by addition of auxin to the medium.

Classical studies of morphogenesis in entire plants (8) or organ fragments and tissue cultures (1, 6, 9, 15, 20) remain essentially descriptive in spite of numerous biochemical and molecular studies, as long as the morphogenetic events are not precisely localized and controlled at the cellular level. In effect, in order to make a quantitative investigation of morphogenetic processes, a maximum number of cells must be involved in the same events at the same time. In entire plants, only a few specific loci such as bud meristems are engaged in morphogenetic processes induced by various hormonal, nutritional, and environmental factors. In organ fragments, the polar circulation of endogenous factors and intertissue correlations produce a similar result—only a small number of cells situated at one pole react. However, since a simpler experimental system was defined for Nautilocalyx (20)—epidermis with one to three subepidermal layers—quantitative studies could be carried out.

Since these first results, others obtained in our research group show that it is possible to control the type of organogenesis from thin layers of epidermal and subepidermal cells (10, 18, 19, 21, 22) and to demonstrate organogenetic potentialities in excised parenchymatous subepidermal layers normally masked by intertissue correlations existing in organ fragments (5).

On a material in which earlier physiological and histological studies (2, 3) determined the experimental conditions necessary for maximum reactivity in the epidermal layer, the formation in this same layer of cell division centers has been studied cytologically (4). The goal of the present article is to determine, using statistical methods, the distribution pattern of these division centers.

Apart from a few statistical studies of cell division in entire plant meristems (11), no quantitative studies have been made, to our knowledge, on cell divisions leading to de novo meristem formation in one tissue. This study was difficult because of limitations of classical cytological techniques.

MATERIALS AND METHODS

The stem epidermis of Torenia fournieri, capable of bud formation (3), is composed of a single cell layer (Fig. 1). It can easily be separated without any subjacent tissue at different stages of bud formation from 1-cm stem segments grown in vitro on a defined medium (2). Since bud formation occurs directly from the epidermis, without any intermediate callus formation, it is possible to observe simultaneously all the cell division centers formed over the entire surface of the segments (Figs. 2 and 3).

The epidermal strip, which measures about 10 × 2.5 mm, is fixed and then stained with nuclear stains such as aceticarmine 2% and mounted in glycerine for observation.

The direct microscopic observation of the epidermal strip made possible (a) the description of the succession of cell divisions leading to the formation of cell division centers (4) and (b) the statistical study of the distribution pattern of cell division centers over the epidermis as a whole at the 6-day stage. It is this second point which is treated herein.

**Fig. 1.** Control epidermal strip; st: stomata.

**Fig. 2.** Formation of several cell division centers each one around a single epidermal cell (arrow).

**Fig. 3.** Formation of a bud meristem (arrow) from a single epidermal cell.
the chi square test for the results of the count presented in Table I. The degrees of freedom, for the number of rectangles in the table, is 5. At a probability of 0.95, the distribution table gives chi square = 1.15 < 90.768. Thus, for this example, the chi square test rejects the hypothesis of the distribution of the frequencies of division centers according to Poisson's law. Thus the division centers are not distributed at random over the epidermal surface. We then attempted to establish the distribution pattern. For this, the number of centers per band (horizontally aligned rectangles) and per column (vertically aligned rectangles) were totalled (Table I).

The differences in the distribution of cell division centers in bands and columns is statistically highly significant. Cell divisions are thus not uniformly distributed on the epidermal surface. A study of the distribution of division centers in relation to length and width parameters on an epidermal strip about 10 × 2.5 mm was then made.

**Distribution of Division Centers on Epidermal Strip Considered in Length.** The epidermis is divided into horizontal bands from the apical side (first band) to the basal side (last band). The number of division centers increases in the apical-basal direction, from the first to the second-last band (Table I). The reduced number of division centers observed in the last band may be due to wounding at the cut edge of the explant. Figure 5 shows the total number of division centers per band (mean established for 10 samples) in the apical-basal direction. Figure 6 shows the total number of centers on an entire internode cut into four segments. At each cut, on the apical side of each segment, a sharp reduction in the number of centers is observed.

| Table I. Number of Cell Division Centers per Rectangle of Epidermal Strip from Wide Face of Stem Segment |
|---------------------------------------------------|---|---|
| Dimensions were 10 × 2.5 mm. | Width of Epidermis | Total |
| Apical side | 0 0 0 0 1 1 0 0 0 0 0 0 1 0 0 2 2 1 1 0 | 9 |
| | 0 0 0 0 1 2 0 0 0 0 0 0 0 1 3 1 3 2 0 | 10 |
| | 0 0 0 0 0 0 2 2 0 0 0 0 1 1 2 2 1 1 1 0 | 13 |
| | 0 0 0 0 0 0 2 4 1 0 0 0 0 0 2 1 1 1 1 | 13 |
| | 0 0 0 1 2 2 1 0 0 0 0 0 0 2 2 2 2 2 2 | 17 |
| | 0 2 2 2 3 4 3 0 0 0 0 1 2 1 3 5 3 2 2 | 31 |
| | 1 1 3 3 4 3 4 3 1 1 0 0 1 2 4 5 4 2 0 | 39 |
| | 1 2 3 6 4 4 0 0 0 0 0 1 2 4 4 4 4 4 1 | 44 |
| | 4 6 4 6 6 5 1 1 2 0 1 0 2 6 6 4 3 1 | 58 |
| Basal side | 1 1 3 4 6 3 1 1 0 0 0 0 1 5 4 3 2 1 | 36 |
| Total | 7 1 2 1 5 2 2 9 2 8 1 9 5 3 0 1 5 | 270 |

| Table II. Chi Square Test: Adjustment to Poisson’s Law |
|---------------------------------------------------|---|---|---|---|
| No. of Centers per Rectangle | Observed Frequencies (ο) | Theoretical Frequencies (t) | χ² = (ο - t)²/t |
| 0 | 69 | 40 | 21.025 |
| 1 | 40 | 60 | 6.666 |
| 2 | 31 | 45 | 3.755 |
| 3 | 12 | 22 | 4.545 |
| 4 | 16 | 9 | 5.444 |
| 5 | 4 | 3 | 0.333 |
| 6 | 8 | 1 | 4.9 |

(ο - t)²/t = 90.768
The frequency of cell divisions increases in the apical-basal direction over a stem segment. One of the physiological interpretations of this distribution is that the intensity of divisions reflects the endogenous auxin gradient (which augments toward the base in a stem fragment). Thus, a hormonal gradient could regulate the distribution in length of cell division centers on a stem segment. Even within a single initial cell, often the number of divisions also increased in the apical-basal direction.

**Distribution of Cell Division Centers in Width on Epidermal Surface of Stem Segment.** The stem segment is rectangular in section (Fig. 10), and there are therefore two wide and two narrow faces. We studied both faces. 

**Wide Face.** The number of division centers per column is very small in the lateral zones; it increases and reaches a maximum before decreasing again in the central column (Table I; Fig. 7). Thus, two maxima on either side of the central zone are observed. Figure 8 shows a schematic distribution of all the division centers over the epidermal surface of a wide face of a stem segment. Figure 9 shows buds formed on either side of the median zone (mz).

**Narrow Face.** On the narrow face, the maximum number of centers is observed in the median zone. The number of centers decreases on either side of this zone. Little or no decrease is observed in the central columns (Fig. 7).

**Effect of Correlations within Stem Segment on Distribution of Cell Division Centers.** The difference observed in the distribution of division centers on the two stem faces coincides with an anatomical difference within the stem. The stem has a rectangular section with four major vascular bundles. However, on the wide face, between the two major bundles, a third smaller one is situated (Fig. 10). Thus, on the wide face, the number of division centers increases progressively with the distance from the vascular tissues. The maximum number of centers is observed on either side of the median zone between the vascular bundles (Table I, Fig. 7).

Our results seem to indicate that the distribution of division centers is regulated by the intertissue correlations within the stem segment. Endogenous hormone and trophic levels are no doubt important factors in this regulation. The influence of the auxin- cytokinin-sugar balance on in vitro organogenesis has been shown on a wide range of materials including *Torenia* (2). Skoog and Miller (16) showed that the auxin- cytokinin equilibrium determines the type of organ formed; the sugar concentration can modify or reverse the effect of these substances (1, 7). Little research has been done on the effect of endogenous substances. Whereas the direction of cytokinin transport is still not clear, the basipetal movement of auxin is well established. This basipetal transport could bring about localized inequalities in the tissues (13, 14). In the case of *Torenia*, one could suppose that the median zone of the wide face of the stem which generates few meristematic zones and which is situated in line with the median vascular bundle, would have a lower endogenous auxin content than that required for bud formation. This assumption would be reasonable if the lateral diffusion of auxin, as shown in *Zea mays* coleoptiles (12), could be applied to the *Torenia* stem segment. If auxin is diffused from the median and two lateral vascular bundles as shown by the arrows in Figure 12, the median part and the lateral parts would receive a smaller quantity of auxin than the intermediate zones.

A bioassay of endogenous auxin content of the different zones would be necessary to verify the hypothesis but is difficult to undertake because of the very small dimensions of the zones involved. However, if the median epidermal zone does not form buds because of a lack of auxin, an exogenous auxin addition should bring about this formation. In effect, 0.1 μM NAA brought about homogeneous bud formation over the epidermis in 20 to 30% of the cultures (Fig. 11). Although this percentage is relatively low, it still indicates that endogenous
Fig. 9. Bud formation (b) on either side of the median zone (mz) of the wide face of a stem segment.

Fig. 10. Transverse section of a stem segment and the four principal vascular bundles (VB), two smaller ones (vb) are located in the median subjacent zone of the wide face (WF). Such small vascular bundles are not observed in the median subjacent zone of the narrow face (NF).

sc: sclerenchyma; e: epidermis.

Fig. 11. Homogeneous distribution of buds (b) on the wide face of a stem segment after an auxin treatment.
auxin content constitutes one of the factors controlling cell division and meristem distribution during bud formation.

CONCLUSIONS AND DISCUSSION

Several conclusions can be drawn from this study. (a) Division centers are not distributed at random on the epidermal surface. (b) If the epidermal layer is arbitrarily divided into horizontal bands and vertical columns, the effects of bands or columns on the division center distribution are highly significant, and the cell divisions are distributed unequally over the epidermal surface. (c) With respect to the stem segment length, the number of cell division centers increases in the apical-basal direction which is also the direction of endogenous auxin transport; the distribution in this direction seems then to depend on a hormonal gradient. (d) With respect to the segment width, the appearance of cell division centers is reduced in the neighborhood of vascular tissues; the maximum number of division centers is observed in the epidermal regions the furthest from vascular tissue. Thus, the distribution of cell division centers on the width of the segment is regulated by the order of succession of different tissues. These tissues (parenchymatous, vascular, and collenchymatous) make up a series of filtering dynamics which regulate the speed and the distribution of metabolites. This study has thus shown the influence of intertissue correlations on the formation of bud meristems from an organ fragment grown in vitro.

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LITERATURE CITED