Empirical investigations into the tunica structuring point of the shoot apex of *Pelargonium zonale*

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Abstract

A tunica structuring point of 3.85 cell units was formally predicted for the shoot apex. This point is decisive for the genesis of the tunica corpus structure as such and for the number of tunica layers in an apex at any given moment. Here, a method to determine, in empirical investigations, the theoretical number of tunica layers in real apices is demonstrated. Furthermore, results of an empirical investigation into the postulated tunica structuring point are presented and causes for deviations of the observed values from the theoretical values are discussed. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Most of the higher plants are characterized by a multi-layered structure of their hemispherical shoot apex. This cell layer arrangement known as tunica corpus structure is based upon a specific way of cell division. While cells of the tunica divide in anticlinal direction (= at right angle to the surface) only, cells of the corpus divide anticlinal as well as periclinal (= parallel to the surface).

It could be shown that there is no primarily genetic fixation of this tunica corpus structure but apex-internal stress causes its development. This apex-internal stress is caused by a high accumulation of meristematic cells. For the shoot apex, a tunica structuring point of 3.85 cell units was predicted saying that every cell layer beyond the radius of 3.85 cell units behaves like a tunica. Furthermore, a mathematical formula was deduced to calculate the theoretical number of tunica layers in a shoot apex at any given moment [1]. Here, a method to determine in empirical investigations the theoretical number of tunica layers in real apices is demonstrated. Besides that, results of an empirical investigation into the postulated tunica structuring point are presented and causes for deviations of observed values from calculated values are discussed.

2. Material and methods

For investigations *Pelargonium zonale* (L.) L’Hérit. ex Ait. ‘Kleiner Liebling’ was used. Preserved slides of the shoot apices of these plants were made. To judge the layered structure, median longitudinal sections through the shoot apex are necessary which can be found in the series of microcuttings. The preparation process was carried out following Romeis [2] and modified by the following:

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1. De-aerate and fixing: Carnoy’s solution (six parts 96% ethanol, three parts chloroform, one part acetic acid) for 2 h
2. Dehydration: 96% ethanol (1 day), propanol (1 day), dyeing the plasma in a 1:1 propanol:eosin mixture (2 h), xylol (2 days)
3. Embedding: 1:1 xylol:paraffin mixture (at 60°C in the incubator for 2 days), allow the xylol to evaporate out of the mixture (at 60°C for 3 days), embedding in paraffin
4. Slicing: 8 µm thick at the microtome stretching the sections with some drops of water (drying the preparations for 1 day)
5. Dyeing: xylol (10 min); propanol, two times 96% ethanol, 70% ethanol, water (always dipping shortly), dyeing in haemalum (1 min, rinse of excess dye with running water), dyeing in eosin (20 min, removing excess dye by short dipping in water)
6. Conservation: 70% ethanol, 96% ethanol, two times propanol, xylol (always dipping shortly), conservation in Canada balsam

3. Results

To decide the theoretical number of tunica layers, it is necessary to determine the radius of the shoot apex. However, since apices are practically never absolutely equal to a complete hemisphere one cannot simply measure straight across the picture.

The deviation of the real apex section from a half circle results in more than one central point and more than one radius. Therefore, for every apex several ‘central points’ and ‘radiuses’ were determined (compare Fig. 1, below). First, the contours were drawn using photographs of apex longitudinal sections. Then, five strings were drawn in each apex outline. The strings were cut in the middle at right angles by a straight line (= the central perpendicular). In a half circle (or arc of a circle), all the central perpendiculars would cross at exactly one point (= the central point of the circle). In the investigated longitudinal sections, however, three to ten points of intersection were found. At the contours of the apex (that is analogous to the arc of the circle), seven points were set up. Five of them were the points of intersection of the central perpendicular and the apex contours. The other two points were the outermost intersections of the strings and the apex outline. By this procedure, the measuring points are equally distributed over the entire apex contours.

The Fig. 1 (above) shows the median longitudinal section through a shoot apex of *Pelargonium zonale* ‘Kleiner Liebling’ (above); construction to find the central point in the contours of this apex (below). In this example the ‘central points’ A, B,..., F were found from which the distances to the points T, U,..., Z were measured as the ‘radiuses’ of the apex. Further explanations are given in the text.
Table 1
Quantitative distribution of the deviations of the theoretical tunica numbers from their observed values (764 pairs of values)

<table>
<thead>
<tr>
<th>Measurements with higher observed values than calculated values</th>
<th>Absolute numbers</th>
<th>Relative numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 tunica layers</td>
<td>4</td>
<td>0.52</td>
</tr>
<tr>
<td>1 tunica layer</td>
<td>52</td>
<td>6.81</td>
</tr>
<tr>
<td>±0 tunica layers</td>
<td>121</td>
<td>15.84</td>
</tr>
<tr>
<td>Measurements with observed values in accordance to their calculated values</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 tunica layer</td>
<td>140</td>
<td>18.32</td>
</tr>
<tr>
<td>2 tunica layers</td>
<td>174</td>
<td>22.77</td>
</tr>
<tr>
<td>3 tunica layers</td>
<td>162</td>
<td>21.20</td>
</tr>
<tr>
<td>4 tunica layers</td>
<td>62</td>
<td>8.12</td>
</tr>
<tr>
<td>5 tunica layers</td>
<td>30</td>
<td>3.93</td>
</tr>
<tr>
<td>6 tunica layers</td>
<td>5</td>
<td>0.65</td>
</tr>
<tr>
<td>7 tunica layers</td>
<td>4</td>
<td>0.52</td>
</tr>
<tr>
<td>8 tunica layers</td>
<td>4</td>
<td>0.52</td>
</tr>
</tbody>
</table>

cluded in these measurements; that means they had not started expanding yet. (The meristematic state of these cells was recognized through their very small size and through their very high plasma concentration resulting in a very intensive eosin dyeing during preparation.) The theoretical number of tunica layers which exists when assuming the tunica structuring point of 3.85 cell units is calculated from the average cell diameter and the radius of the apex. The calculation of these theoretical numbers followed the formula:

\[ n_T = \frac{r_A - 3.85d_{\text{Cell}}}{d_{\text{Cell}}} \]

where \( n_T \) = number of tunica layers; \( r_A \) = radius of the apex; \( d_{\text{Cell}} \) = cell diameter (measured in direction of the radius).

A total of 764 calculated values were compared to their corresponding observed values.

In these investigations two to six tunica layers were observed and, by contrast, theoretical tunica numbers ranging from 2 to 12 were calculated. Table 1 shows the distribution of deviation of the theoretical values from the observed values. It is especially emphasized that the observed values refer to a specific radius and therefore incomplete tunica layers are included. Counting incomplete tunica layers is in contrast to Schmidt [3] and other authors who regarded only those layers as tunicas which were entirely free from periclinal divisions. It has been explained in a previous paper why their view must be objected [1].

The results show that generally fewer tunica layers were observed than theoretically predicted.

On average, 1.85 tunica layers were calculated more than observed.

4. Discussion

The calculations of the theoretical numbers of tunica layers are based on the assumption that the apex is equal to a hemisphere. Casting a quick glance over the preparations of \( P. \) zonale one will find that the sections match a half circle quite well. Furthermore, in all apices a higher number of tunica layers can be found so that it is never just a yes/no decision when comparing real apices to the theoretical model. In that regard, \( P. \) zonale is a quite suitable object for empirical investigations into the tunica structuring point.

The three to ten ‘central points’ found in the constructions for the apex central point reveal the deviations of real apices from their model. Greater divergences of the apex outline from a half circle resulted in points of intersection being in particular far outside of the plot which therefore gave particularly small or large radiuses. Scattered points emerged in this way, however; they resulted more often in too high than in too low theoretical numbers of tunica layers.

A further reason for the generally too high theoretical tunica numbers might be that tunicas were not recognized as such simply because indirectly neighboring cells underwent periclinal division by which the impression of seeing a layer got lost. Beside that, it seems possible that tunicas were not recognized because their cells were not
arranged evenly enough — for the genesis of such very even cell rows, particularly high pressure from the corpus and strong tension inside the tunica, are necessary.

The major source of error in verifying the tunica structuring point is probably the beginning expansion of apical cells which can be seen in the longitudinal section by a lighter staining and larger diameter of the more central cells (Fig. 1, above). The differences in staining intensity reflect the reduced plasma density in growing cells. The presented photograph can be regarded as representative of the sampling. For the calculations equal size and the same frequency of division of all apical cells were assumed. Even though these cells keep dividing it must be assumed that along with the beginning expansion and differentiation the direction of cell division follows a genetically given purpose.

The calculation of the theoretical number of tunica layers is affected by the cell size. For the determination of the average cell size only entirely meristematic (meaning the smallest) cells were considered. Since tunica cells come under increasingly strong tangential tension along with their increasingly outward position they will appear thinner and so the average cell size is distorted downwards. A too small cell size results in calculating too high a number of theoretical tunica numbers. By contrast, the too large cell diameters of expanding cells distort the theoretical tunica numbers below the observed numbers.

When calculating the theoretical numbers of tunica layers the three dimension of the apex are considered. When analyzing longitudinal sections, however, only two dimensions are looked at meaning that no volumes but areas are investigated. Outer factors which might have an influence on the apex shape, e.g. lateral pressure from attached leaves, cannot be seen in a longitudinal section if these factors lay before or behind the sectional view.

At this point, some investigations by Thielke are worth quoting. She examined apices of different species of Saccharum [4–9] and Erianthus [10]. Her investigations lead her to the conclusion that the development of a tunica is clone specific. For unlayered apices she assumed an abnormal genetic constitution [4]. In her investigations, she recognized that unlayered apices consist of only few but large cells. In subsequent investigations she reported variations in the number of tunica layers at different developmental stages and she saw the connection of apex size and the development of a tunica layer [5,7–10]. However, in her reports she never established a connection between cell size and the apex radius. Apical cells were just described as ‘to be of large size’. Even though Thielke does not give any suitable measured values to recognize that the ratio of apex to apical cells is the cause for the development of a layered structure in the shoot tip, her observations can be very well regarded as a lump verification of the existence of the tunica structuring point as postulated earlier [1].

With this paper a method to determine the number of tunica layers in real apices is demonstrated with the purpose of clarifying the developmental nature of the tunica corpus structure of the shoot apex. Furthermore, results of an empirical verification of the tunica structuring point are presented and causes for deviations of observed values from theoretical values are discussed.

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References

