Structure of the Vegetative Shoot Apex of *Clethra barbinervis* II. Supplementary Study on Superficial View*

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Abstract

This report is concerned with quantitative treatments for plastochronic changes of the apex in superficial view as a supplement to the first paper⁷⁾ of the present series, which dealt with the anatomical analysis of superficial and transectional views of the vegetative shoot apex of *Clethra barbinervis* Sieb. et Zucc.

The bases of young leaf primordia directly surrounding the apex may be expanding considerably at the expense of the area of the shoot apex. Thus, changes in area of the apex occurring during a plastochron may be intimately correlated with the expansion of the bases of young leaf primordia as well as with that of the apex itself. The growth processes, discussed anatomically in the first paper, are checked quantitatively in the present study.

There have been some papers dealing with quantitative and statistical analyses of morphological and anatomical features of the shoot apex. Denne^{1,2,3)} studied *Trifolium* and *Tradescantia* and Lyndon,^{4,5,6)} *Pisum*. Various data, which were obtained mainly from longisections of the apex, and various processes, which occurred during the growth of the apex, were clearly presented in those papers. Quantitative treatments of growth of the apex are apparently very important, although there are various difficulties as explained in the first paper⁷⁾ of the present series.

A detailed anatomical analysis of plastochronic changes was reported for superficial and transectional views of the vegetative shoot apex of *Clethra barbinervis* Sieb. et Zucc. in the first paper.⁷⁾ In the present study a supplementary analysis of the same material is reported; however, quantitative treatments of plastochronic changes in the apex are restricted to superficial views reconstructed from transections. As explained earlier,⁷⁾ results from quantitative treatments have only supplementary meaning, because there are delicate and complicated problems which cannot be solved by the statistical and quantitative analyses alone.

Material and Methods

The material in the present study is the same as that of the first paper.⁷⁾ For the quantitative study only nine vegetative apices were selected out of many apices sectioned and stained, because they were cut most precisely in the transversal direction.

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Of the nine apices quantitatively treated three are in the minimal area phase of the apex, three in the middle, and the other three in the maximal. These nine apices are the same as those shown in Figs. 14-22 of the first paper.⁷⁾

It is appropriate to consider whether anatomical preciseness obtained from precise sectioning and staining is more essential than statistical significance introduced by a larger number of sections. It is very difficult to obtain precise transverse sections of many apices. If we wish to treat statistically a larger number of apices, we would need to add some apices which are more or less obliquely sectioned. The choice for the present study is relatively small number of apices, which are precisely sectioned.

Areas, cell numbers and lengths of various parts of the apex, angles between two successive leaf primordia and diameters of the apex were measured from the reconstructed figures of the first cell layer of the nine apices, which were shown in Figs. 14-22 of the first papers.⁷⁾ The methods for making a reconstructed figure were described in the study of *Daphne*.⁸⁾

From the reconstructed figure of the first cell layer of an apex (cf., Figs. 1, 2; Figs. 14-22 in the first paper⁷⁾), the areas of the central zone and of the five peripheral zones (subdivided areas of the peripheral meristem) were measured by a planimeter, and thus, the total area of the apex was calculated (cf., Fig. 1). The number of cells in a single row from the center of the apex to the leaf base was counted for each leaf primordium (for example, o to b for sector 0; o to d for sector 1 in Fig. 2). These cell numbers were subdivided into those within the central zone and those of the rest (for example, o to a and a to b for sector 0; o to c and c to d for sector 1 in Fig. 2). At the same time, lengths (distances) were measured for each leaf primordium (for example, o to b, o to a and a to b for sector 0; o to d, o to c and c to d for sector 1 in Fig. 2). The maximum and minimum diameters for each apex were measured also (e to f and g to h in Fig. 2).

Fig. 1. Diagram of superficial view of apex. C: measured area of central zone; 0, 1, 2, 3, 4: measured areas of peripheral zones 0, 1, 2, 3 and 4 respectively; o: center of apex.

Fig. 2. Diagram of superficial view of apex. CZ: central zone; 0: sector 0; 1-4: successive leaf primordia; o: center of apex; detailed explanation of other letters in text.

Results

AREA.—Table 1 shows the areas of the various regions measured by a planimeter, and Fig. 3 is a graph which was made from data of Table 1. The smallest zone is peripheral zone 1 in the minimal area phase of the apex; peripheral zone 1 in this phase is regarded as the youngest in peripheral zone development. The area of sector 0 is the same as that of peripheral zone 0; in the minimal area phase sector 0 is equivalent to the developmental stage which will follow the stage of peripheral zone 4 in the maximal area phase.

We are apt to expect that the area of a peripheral zone will increase steadily during growth from sector 1 to sector 4 or sector 0 as suggested by the total mean areas in Table 1. That this expectation is not actually met can be seen by examining the details in Table 1 and Fig. 3. When the area of peripheral zone n in the maximal area phase is compared with that of peripheral zone $n+1$ in the minimal area phase, the former area is larger than the latter even though it is in a younger stage of development. This correlation is particularly conspicuous when the area of sector 4 in the maximal area phase is compared with that of sector 0 in the minimal area phase.

The area of the base of leaf 1 in the minimal area phase is about $1.00 \times 10^4 \mu^2$. It is interesting that the area of the whole apex in the maximal area phase is larger than the combined areas of the apex and base of leaf 1 in the minimal area phase ($3.54 \times 10^4 \mu^2$ and $2.87 \times 10^4 \mu^2$ respectively in mean value). Although there is no statistical exactitude in the present study, this tendency is corroborated by detailed morphological or anatomical observations on a number of apices which were not measured. The area of the central zone is also largest during the maximal area phase. Thus, the total area of the apex in the maximal area phase is larger than expected.

The area of sector 0 in the middle area phase is very nearly the same as that

Table 1. Areas of apex. ($\times 10^4 \mu^2$). Areas for sectors 1-4 in this table are of peripheral zones 1-4 (area of sector minus area of leaf primordium).

apex	total	sector 1	sector 2	sector 3	sector 4	sector 0	central zone
minimal a	1.60	0.05	0.08	0.18	0.48	0.39	0.41
area b	1.70	0.05	0.11	0.19	0.40	0.58	0.36
phase c	2.31	0.07	0.22	0.35	0.54	0.73	0.41
mean	1.87	0.06	0.14	0.24	0.48	0.57	0.40
middle d	2.39	0.09	0.13	0.17	0.52	0.87	0.60
area e	2.48	0.09	0.18	0.32	0.58	0.78	0.53
phase f	2.82	0.14	0.16	0.30	0.47	1.19	0.57
mean	2.56	0.11	0.16	0.26	0.52	0.95	0.57
maximal g	2.97	0.13	0.19	0.47	0.66	0.84	0.68
area h	3.31	0.22	0.22	0.61	0.71	0.89	0.64
phase i	4.35	0.21	0.41	0.62	0.86	1.24	1.00
mean	3.54	0.19	0.28	0.57	0.74	0.99	0.77
total mean	2.66	0.12	0.19	0.36	0.58	0.83	0.58

Fig. 3. Comparison between areas of peripheral zones. ($\times 10^4 \mu^2$). 1: sector 1 (peripheral zone 1 is considered to be in youngest stage of peripheral zone development); 2, 3, 4: sectors 2, 3, 4; 0: sector 0 (peripheral zone 0 is considered to be in oldest stage of development, although it is in youngest stage as a sector); i: minimal area phase; m: middle area phase; a: maximal area phase; x: mean value for each sector; each point shows mean value for three apices, as well as in Figs. 4-6.

Fig. 4. Comparison between cell numbers in various parts of apex. A: value from center of apex to leaf base; C: value within central zone; B: difference between A and C; 0-4, i, m, a, x: the same as in Fig. 3. Detailed explanation in text.

in the maximal. If the mean values are considered, the area increases rather slowly from sector 1 to sector 2 and more or less rapidly after the sector 2 stage.

CELL NUMBER (Fig. 4).—The mean value for the cell number between the center of the apex and the leaf base increases slowly from sector 1 to sector 3, and very rapidly from sector 3 to sector 0. Values for all sectors in the minimal area phase are relatively small, and those in the maximal area phase are relatively large. The cell number between the center of the apex and the leaf base is divided into the number within the central zone and that within the sector. The mean value for the cell number within the central zone related to each sector increases slightly from sector 1 to sector 0. The number in the minimal area phase is small, related to the small size of the central zone in this phase; in the maximal area phase it is relatively large. The mean value for the cell number within the sector increases gradually. Changes in cell number within the sector almost parallel the changes in cell number between the center of the apex and the leaf base, since changes in the central zone are only slight.

LENGTH (Fig. 5).—Changes in cell number mentioned above almost parallel changes in lengths (distances); the length from the center of the apex to the leaf base for each sector and the subdivided lengths within the central zone and within the sector.

Fig. 5. Comparison between lengths of various parts of apex. (μ). A, B, C, 0-4, i, m, a, x: the same as in Fig. 4. Detailed explanation in text.

Fig. 6. Angles between two successive leaves (sector 0 is actually presumptive position for next leaf primordium). 0-1, 1-2, 2-3, 3-4: angles between sector 0 and leaf 1, between leaves 1 and 2, 2 and 3, 3 and 4 respectively; i, m, a, x: the same as in Fig. 3.

ANGLES BETWEEN TWO SUCCESSIVE LEAVES (Fig. 6).—The minimum angle (less than 140°) observed occurs between leaf 1 and leaf 2 in the minimal area phase. The largest angle observed is between sector 0 and leaf 1, although this is not a divergence angle in the strict sense because sector 0 does not yet have a leaf primordium.

DIAMETER.—Since the outline of the apex area in the superficial view is asymmetrical, the maximum and minimum diameters are measured for each apex. For the minimal area phase the mean values are 212μ in maximum diameter and 95μ in minimum, for the maximal area phase 301μ in maximum diameter and 145μ in minimum. The total mean values of the diameter are 253μ in maximum diameter and 121μ in minimum. The largest diameter measured was 339μ and the smallest 78μ .

Discussion

Hara⁹⁾ already reported that both the area of the central zone and the width of the shoot apex were greater in the maximal area phase of a plastochron than in the minimal area phase. This fact was corroborated in the present study. The area of the largest apex in the maximal area phase is more than twice that of the smallest one in the minimal area phase; even when mean values are compared, the area in the maximal area phase is nearly twice that in the minimal area phase.

It is difficult to explain why the area of the apex in the maximal area phase is larger than the sum of the areas of the apex and of the base of leaf 1 in the minimal area phase. This observation is more or less paralleled by results obtained from measurements of cell number and length.

The following possible explanations are considered:

- 1) These results may not be reliable because of lack of statistical treatment.
- 2) The quantitative data may not be correct because of microtome compression occurring at the time of sectioning of paraffine blocks.
- 3) The apex is not flat in the strict sense but is very slightly domed. The

data for the area as well as length, therefore, may not be precise, because the area measured is not the actual surface area but a projection of it.

4) The time interval from the maximal area phase to the minimal area phase is very small, so the area of the apex scarcely increases during this period.

5) Some apices retard a new leaf primordium to initiate, for a while, continuing expansion of the apex area in a prolonged maximal area phase, while others hasten a new leaf primordium to initiate. In the former type of the apex the new primordium appears very rapidly, while in the latter type of the apex it appears gradually. Thus, the minimal area phase is readily observable only in the case of the second type of the apex.

6) Simultaneously with the expansion of the apex area, the bases of leaf primordia, which directly surround the apex, expand more or less progressively using up the area of the apex. Thus, between the maximal and the minimal area phases, the net area of the apex actually decreases or increases only slightly, because the area of the base of the newly initiated leaf primordium, the most actively expanding area in the apical region of the shoot, is measured as the area of the leaf after this phase of growth, but not as a part of the area of the apex. After this phase, sector 0 actively expands and since this is measured as a part of the apex, the area of the apex again shows a net increase.

7) Between the maximal and the minimal area phases, the area increase activity of the apex itself decreases, while the leaf base expansion and leaf primordium elevation increases using a part of the area of the apex. Thus, the area of the apex in the minimal area phase is extremely small. After this phase, the area increase activity of the apex increases and the leaf base expansion and leaf primordium elevation decrease.

The first explanation is reasonable from the standpoint of statistics. Statistic significance, however, is not taken as a serious view in the present study as was explained already. Microtome compression pointed out in the second explanation gives somewhat bad effect on results of angle and length measurements.

The third explanation should be examined also. It is very probable that the true surface area is somewhat larger than the measured area. It would seem that the error does not exceed 10% of the measured value, because height from the level of axil of the fifth leaf primordium up to the center of the apex does not exceed $30\ \mu$, while the radius is over $100\ \mu$. The apex in the maximal area phase is relatively lower than in the minimal area phase. Even if, as an extreme case, the apex in the maximal area phase is strictly flat (such apex does not actually exist in *Clethra*) and that in the minimal area phase has a low dome with the measured area showing a 10% error, this amount of error is not critical about the present question; in the *Clethra* apex the mean value for the apex area in the maximal area phase is $3.54 \times 10^4\ \mu^2$ and that of the apex plus the base of leaf 1 in the minimal area phase is $2.87 \times 10^4\ \mu^2$; if a 10% error is assumed, the latter becomes $(2.87 + 0.287) \times 10^4\ \mu^2$. Even if corrected value is used, the area in the minimal area phase still does not exceed the area in the maximal area phase. It is important that the data for the cell number are parallel to those for the area; comparisons of graphs show that cell number decreases when the area decreases. Therefore the third explanation is not thought to be important in the explanation of area differences.

Any one of the fourth to seventh explanations is possible on the basis of detailed comparison of apices. The fourth and sixth are particularly reasonable ones.

Lyndon^{5,6)} concluded, from his study of cell divisions at the shoot apex of *Pisum*, that the formation of a primordium resulted primarily from a change in the plane of growth, rather than a change in the rate of growth in the apical dome. If Lyndon's conclusion is applicable to the apex of *Clethra*, the seventh explanation is also worthy of consideration.

The graphs for cell number and length (Figs. 4, 5) show similar curves. This suggests us that changes in area of the apex result from changes in cell number rather than from those in cell size of the apex.

The angle between sectors 1 and 2 in the minimal area phase is less than 140°. Leaf 1 in the minimal area phase is of the youngest stage of development among the leaves already initiated. The angle prior to this stage, i.e., the angle between sectors 0 and 1, is larger. This means that, for the growth of sector 0 files of cells in peripheral zone 3 are utilized more than those in peripheral zone 2. Conclusions as to the phyllotaxis of *Clethra* cannot be reached from the present study, because there were some questions on the statistical significance and artifacts of the preparation.

In conclusion, it is sure that the bases of leaf primordia directly surrounding the apex are expanding considerably using up part of the area of the shoot apex. Thus, area changes of the apex occurring during a plastochron probably are intimately correlated with expansion of young leaf primordia as well as with that of the apex itself. This conclusion is deduced not only from quantitative treatments but from detailed observations of a large number of shoot apices.

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