Ontogeny of the Vegetative and the Reproductive Apices in *Pharbitis nil* Chois. II. Development of the Terminal Flower Bud

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The ontogeny of the vegetative apex in *Pharbitis nil* was reported in the previous paper¹⁾. In this paper, changes from the juvenile apex to the floral one in the main shoots of seedlings will be described.

In *Pharbitis*, plant form and position of flower primordia differ somewhat with environmental conditions, especially when comparison is made between plants grown in a field under natural conditions and those grown under 8-hr. short day conditions in a green house. When sown in May in the field, the apices of the main and long shoots give rise to inflorescences which bear one to three flowers, at the axillary meristem. On the the other hand, when seedlings with fully expanded cotyledons are subjected to the short day conditions, they initiate a terminal flower bud after having produced 4 to 5 leaves with a single flower bud in their axils, and further growth ceases²⁾. In this paper, changes which take place at the apices of seedlings in initiation of terminal flower primordia will be described. Those of axillarybuds will be presented in the next paper.

Material and Methods

Seedlings of *Pharbitis nil*, strain Violet, were grown in the same way as mentioned in the previous paper¹⁾. Five days after swelling, seedlings with fully expanded cotyledons were transferred to short day conditions consisting of 8-hr. light and 16-hr. dark period. The seedlings were sampled every day. The experimental methods employed were similar to those reported in the previous paper¹⁾.

Observation

In the floral apex of *Pharbitis*, five sepals with the arrangement of 2/5 are initiated very closely in time and in vertical distance after initiation of about two bracteal leaves. Subsequently, a whorl of five petals and a whorl of five stamens differentiate almost at the same time. Two whorls alternate and lie very closely in vertical distance. Finally three carpels are initiated in a whorl. Though the growth rate of flower primordia varies in some degree with environmental conditions, especially with temperature and light, the primordia of sepal, petal, stamen and pistil are formed during about 10 days after transferring plants to the short day conditions.

At the start of short day treatment, five days old seedlings have the juvenile apex (Fig. 4-a, b and Photo. 3 in the previous paper¹⁾). During the first three days of short day cycles, the cytohistological configuration of shoot apices hardly differs from those of the same age under long day conditions (compare Fig. 1-a, b; Photo. 1

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with Fig. 5-a, b; Photo. 4 in the previous paper¹⁾). Cells of the central zone are large and have large light-staining nuclei. The peripheral zone contains small cells with relatively dark-staining nuclei. In the rib meristem, cells have less dense cytoplasm and dark-staining nuclei. The cytohistological features are characteristic of the juvenile type, and the initiation of floral organs cannot be discerned morphologically, though leaf primordia that have been initiated at this time differentiate into bracteal leaves later.

After 4 to 5 photoinductive cycles, the first symptom of morphological changes appears (Fig. 2-a, b; Photo. 2 and Fig. 3; Photo. 3). The cytohistological zonation characteristic of the juvenile apex is gradually replaced by more uniform cell configuration of the floral apex. The cells of the peripheral zone begin to manifest large light-staining nuclei and their nucleoli become large and dark-staining. The cells of the central zone are cut up into somewhat smaller units and the nucleoli become distinct. It seems likely that the modified central zone extends to the flanks and that the peripheral zone disappears gradually. After 5 inductive cycles, the initiation of bracteal leaves has already completed. Fig. 3 and Photo. 3 show the shoot apex between the end of bract initiation and the commencement of sepal initiation.

After 6 inductive cycles, sepals are initiated. Figs. 4 and 5, and Photo. 4 show the early stage of sepal initiation. Sepal primordia are initiated in close succession, so that the apex does not return to dome-shape in contrast to the case in initiation of foliage leaves. As the differentiation of sepals proceeds, the apex increases in diameter and decreases in height (Figs. 6 and 7; Photo. 5). At the outer portion of the corpus, active anticlinal divisions are observed, and the tunica-like second row of cells and even the parts of a regular third layer can be followed easily. In the rib meristem, increasing cell divisions in various planes and lateral extension of the rib meristem can be observed. Thus the outer zone containing cells with dense cytoplasm and large light-staining nuclei, and the inner zone containing cells with thin cytoplasm and relatively dark-staining nuclei, become distinguishable. Floral organs are initiated in the outer zone into which the central and the peripheral zones have been transformed. The inner zone into which the rib meristem has been transformed forms the pith-like parenchyma of the receptacle. The transitional portion is seen in the lower part of the outer zone as in the case of the vegetative apex, but it is not apparent whether cells of this portion resemble the inner zone

Fig. 1. (cf. Photo. 1) Apices after 3 short day cycles. Organization of juvenile apex remains, and they hardly differ from those at the same age under long day conditions (compare with Fig. 5 and Photo. 4 in the previous paper¹⁾). Cells containing large light-staining nuclei are stippled; cells containing relatively large, relatively dark-staining nuclei are dotted around; small cells containing dense cytoplasm and dark-staining nuclei are indicated by open circles; cells containing thin cytoplasm and dark-staining nuclei are indicated by single dots. Upper scale (100 μ) is for high-power views, and lower scale (500 μ) is for low-power views throughout the figures in the present report.

Figs. 2-3. (cf. Photos. 2-3) Transition from juvenile to floral apex. Cells in central zone divide into smaller units, and in peripheral zone nuclei become large and light-staining, gradually. Fig. 2 (cf. Photo. 2), apices after 4 short day cycles; Fig. 3 (cf. Photo. 3), apices after 5 short day cycles.

Figs. 4-7. (cf. Photos. 4-5) Sepal initiation in apices after 6 short day cycles. In later stage, active anticlinal division in surface layers (Figs. 6, 7), and lateral extension of rib meristem (Fig. 7) establish outer and inner zone of floral apex. Se, sepal.

under its influences or whether they are the initials of the inner zone. Although size or shape of the floral apex fluctuates considerably during the initiation of various floral organs, this cytohistological configuration remains remarkably constant.

After sepal initiation, the floral apex enlarges rapidly by increasing cell number

Photo. 1. (cf. Fig. 1-a) Apex after 3 short day cycles.

Photo. 2. (cf. Fig. 2-b) Apex after 4 short day cycles. First sign of floral apex becomes appearing.

Photo. 3. (cf. Fig. 3) Apex after 5 short day cycles. Prior to sepal initiation, apical configuration of juvenile stage is replaced by more uniform one of floral apex.

Photo. 4. (cf. Fig. 4) Sepal is about to be initiated in apex after 6 short day cycles.

Photo. 5. (cf. Fig. 7) Outer and inner zone of floral apex becomes established. Two sepal primordia are in this plane. $\times 315$.

Figs. 8-9. Prior to petal and stamen initiation, floral apex increases its diameter.

Figs. 10-12. (cf. Photo. 6) Petal and stamen primordia are initiated almost simultaneously, but stamens develop faster than petals. The apex reduces its diameter for their development. P, petal; S, stamen.

Fig. 13. (cf. Photo. 7) Prior to carpel initiation apex increases its diameter.

Fig. 14. In outer zone, cells of submarginal portion, especially in 3 to 4 cell layers' depth from surface, divide at faster rate than those of center.

Figs. 15-18. (cf. Photo. 8) Initiation and development of carpel primordia. Outer zone becomes thin, and apex is almost used up. C, carpel.

Photo. 6. (cf. Fig. 11) Petal (on left side) and stamen (on right side) are about to be initiated.

Photo. 7. (cf. Fig. 13) Prior to carpel initiation, apex increases its diameter. Stamen primordium (on left side) develops faster than petal (on right side).

Photo. 8. (cf. Fig. 16) Three carpels are initiated as circular ridge.

Photo. 9. (cf. Fig. 20) During extension of central depression of carpel ring, infolded carpel margin is elevated (on right side).

Photo. 10. (cf. Fig. 23) Floral apex is used up for development of carpel margin with placenta formation. $\times 315$.

Fig. 19. Central depression of apex again extends.

Fig. 20. (cf. Photo. 9) During extension of area, infolded carpel margin is elevated (on right side).

Fig. 21. Central part of carpel (on left side) is taller than marginal (on right side). Apex extends and carpel margin develops.

Fig. 22. Placenta initiated from ventral part of carpel margin is elevated (a). b, carpel margin.

Fig. 23. (cf. Photo. 10) Floral apex is completely used up for development of carpel margin with placenta formation. Outer zone is losing its identity, but inner zone carries on active cell division. C, carpel; CM, carpel margin; S, stamen; P, petal; Se, sepal; B, bract.

within about one day, and the apex becomes flat (Figs. 8 and 9). At this stage, nucleoli in the outer zone gradually become small and light-staining.

The apex in Fig. 10 is about to initiate a petal on the left and a stamen on the right. The apex has enlarged more rapidly, and its summit has become flat. Cells in the portion in which stamen and petal primordia are initiated have large dark-staining nucleoli and dense cytoplasm. All the five petals and all the five stamens are initiated almost simultaneously at the edge of the apex (Fig. 11; Photo. 6). Once initiated, however, stamen primordia develop more rapidly than petals (Fig. 12). Stamen primordia are situated at a somewhat higher position than petals, and alternate with petal primordia. So the median longitudinal section of young flowers shows a petal primordium on one side and a stamen on the other. The flower of *Pharbitis nil* is polypetalous in the early stage of development. The petals are completely free for some time after initiation and there is a conspicuous space between petal margins. A corolla tube is produced and stamens are shifted to the lower portion of corolla tube at the later stage. During this initiation and development, the diameter of the apex is reduced remarkably, and the apex is still flat (Fig. 12). Cell size in the outer zone is relatively uniform for this period.

Prior to carpel initiation, this flat apex enlarges in diameter (Fig. 13; Photo. 7 and Fig. 14). Then in the outer zone, cells of the submarginal portion, especially 3 to 4 cell layers' depth from the surface, divide at a faster rate than those in the center (Figs. 13, 14; Photo. 7). The result is a low circular ridge which surrounds a central depression (Figs. 15, 16; Photo. 8). Continuity of these carpel primordia was established by observation of fresh flower primordia with a binocular microscope at low magnification. It is noted that cells of this meristematic ring have distinct nucleoli. As the carpel primordia develop, the remnant apex seems to be used up (Figs. 17, 18, 19). The outer zone becomes somewhat thin and its nuclei stain relatively dark as compared with those in the previous stage. However, cells of this central depression divide again, mainly in the anticlinal plane. During extension of the area the infolded carpel margin is elevated from the margin of the central depression (right side of the apex in Fig. 20 and Photo. 9).

At the stage shown in Fig. 21, the central part of carpel (on the left side) is taller than the marginal part (on the right side), and the carpel margin develops still further. The inner zone seems to take part in this development in addition to development of the receptacle. The outer zone gradually comes to contain cells with relatively dark-staining nuclei and resembles the transitional portion in cytological feature. The outer zone loses its identity. In Fig. 22, the placenta is initiated on the right side (a), and this protuberance was born from the ventral part of the carpel margin (b). The floral apex is completely used up for the development of the carpel margin with placenta (Fig. 23; Photo. 10), and cells in a small remnant of the depression vacuolate later. The inner zone carries on active cell divisions and the receptacle develops still further.

Discussion

Three types of apical meristem in shoot of *Pharbitis nil* are distinguishable; (1) the juvenile apex, (2) the vegetative apex of the adult type, and (3) the floral apex. The juvenile apex differentiates into the adult vegetative or the floral apex. The adult vegetative apex can hardly differentiate into the floral one.

In *Vinca*^{3,4)}, the configuration of the vegetative, the reproductive, and the floral apices are all similar. The apical meristem of *Michelia*⁵⁾ changes in shape but not in configuration at different stages in its life cycle. In contrast to the plants above mentioned, the vegetative and the reproductive apices are very dissimilar in many plants reported till now. The zonate pattern characteristic of the vegetative apex, the central zone in particular, gradually disappears when the vegetative apex is transformed into the reproductive one. As a result of this transformation, the reproductive apex is composed of two zones: the mantle-like outer zone composed of small intensively staining cells with dense protoplast, and the core-like inner zone of highly vacuolated, weakly staining cells⁶⁻¹¹⁾. The massive core characteristic of the floral apex is, however, lacking in *Chenopodium*¹²⁾. In *Pharbitis nil*, a similar transformation of apical configuration is seen when the juvenile apex differentiates into the floral one. Prior to the sepal initiation, nuclei of the peripheral zone become large and light-staining and cells of the central zone divide into smaller units. An anticlinal division in the outer portion of corpus becomes active and the formation of the outer zone is completed in the later stage of sepal initiation. The rib meristem is transformed into the inner zone which has an irregular cell arrangement in the later stage of sepal initiation. In the early stage of this transformation, it seems from the nuclear feature that the peripheral zone disappears and the modified central zone extends downwards. However, the differences in this point between *Pharbitis* and other species such as *Xanthium*, *Chenopodium*, etc. in which the central zone disappears in accordance with the transformation from the vegetative apex into the reproductive one might be a matter of details or personal interpretation only.

Considerable attention has been paid to nucleolar size of the vegetative and the reproductive apices¹²⁻¹⁶⁾. In *Pharbitis*, large nucleoli appear frequently at different stages in the ontogeny of the shoot apex. As the central and peripheral zones of the juvenile apex transform into the outer zone of the floral apex, large nucleoli in these portions become conspicuous. In the floral apex, the portion in which floral leaves are about to initiate and the initiated primordia have large nucleoli. However, when the shoot apex increases its diameter prior to the initiation of petal and stamen, or carpel primordia, nucleoli become indistinct in spite of active cell division. In the vegetative apex, changes of nucleolar appearance accompanying changes of plastochronic stage cannot be observed.

Summary

In the transition from the juvenile to the floral apex, the following changes occur. Prior to the sepal initiation, cells in the central zone divide into somewhat smaller units, nuclei of the peripheral zone become large and light-staining, and active anticlinal cell divisions in the outer portion of the corpus take place in the later stage of sepal initiation. These changes result in the mantle-like outer zone consisting of uniform cells with dense cytoplasm and large light-staining nuclei. Active cell divisions in various planes in the rib meristem extend its diameter and change the cell arrangement in the later stage of sepal initiation. These changes result in the formation of the inner zone consisting of cells with thin cytoplasm and relatively dark-staining nuclei. In further development of flower primordia, the outer zone produces floral organs and the inner zone is concerned with the development of the receptacle. During this period, the shape of the floral apex

changes, but this apical configuration remains unchanged until the floral apex has been used up.

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摘 要

丸重靖子: アサガオの茎と花の生長点の発達過程 II. 花芽生長点

幼形の生長点が主軸の花芽生長点に分化する時には次のような変化がみられる。がくの分化に先だって中央分裂組織 (central zone) の細胞は少し小さくなり、側方分裂組織 (peripheral zone) の細胞は、核が大きくなりうすく染まるようになって、この二層は一樣になる。次にくがく分化の後期に生長点の外側の部分で交層分裂が盛んになって、花芽生長点の外層 (outer zone) が形成される。外層の細胞は濃い細胞質と大きなうすく染まる核を持っている。またがく分化の後期に中肋分裂組織 (rib meristem) で種々の方向の細胞分裂が盛んになって直径が拡がり、花芽生長点の内層 (inner zone) ができる。内層の細胞は薄い細胞質と比較的濃く染まる核をもっている。花芽原基の発達に際して、外層からは花葉が分化し、内層は花托をつくる。この期間を通じて生長点の形は変わるが、この生長点構造は生長点が使いつくされてしまうまで変わらない。(京都大学農学部応用植物学研究室)