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STRUCTURAL AND HISTOCHEMICAL STUDIES OF THE CAMBIUM AND SHOOT MERISTEMS OF SOYBEAN TREATED WITH 2,3,5-TRIIODOBENZOIC ACID

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STRUCTURAL AND HISTOCHEMICAL STUDIES OF THE CAMBIUM AND SHOOT MERISTEMS OF SOYBEAN TREATED WITH 2,3,5-TRIIODOBENZOIC ACID

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TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

STRUCTURAL AND HISTOCHEMICAL STUDIES OF THE CAMBIUM AND SHOOT MERISTEMS OF SOYBEAN TREATED WITH 2,3,5-TRIIODOBENZOIC ACID

CHAPTER I

INTRODUCTION

Several workers (Galston, 1947; Whiting and Murray, 1948; Ishihara, 1956; Anderson and Greer, 1965) found that the plant growth regulator 2,3,5-triiodobenzoic acid (TIBA) may induce a rapid change from the vegetative to the reproductive stage of development. TIBA-treated tomato meristems exhibit an altered form which (Gorter, 1949) correlated with a decrease in leaf production and an increase in flower production. Heslop-Harrison and Heslop-Harrison (1957) suggest that the enhancement of the total flowering response in TIBA-treated Cannabis sativa is a direct outcome of the inhibition of apical dominance, hence more sites for flower production then become active.

Greer (1965) found that TIBA caused a more rapid accumulation of dry matter in the reproductive parts of Glycine max. This increase in weight was a result of both earlier pods and more pods, and the seeds matured more rapidly on the plants treated with TIBA than in the

controls. Galston (1947) suggested that low auxin levels at the meristem may stimulate the production of flower primordia in the soybean treated with TIBA.

In view of the above findings, it appears that TIBA directly or indirectly enhances the flowering response in soybean, as well as in several other plants. Generally there are several known structural and chemical indicators of floral activity observed in the cambium and main and lateral shoot meristems of photo-induced plants (Wilton and Roberts, 1936; Wetmore, Gifford, and Green, 1959; Gifford and Tepper, 1962; Sadik and Ozbun, 1962; Gifford, 1964; Nougarède, Gifford, and Rondet, 1965). This investigation was undertaken to study some of the structural and histochmical changes which accompany floral production in TIBA-treated soybean plants.

CHAPTER II

MATERIALS AND METHODS

Soybeans, Glycine max (L.) Merrill 'Harosoy', were grown in the greenhouse in 10 in. pots containing number two grade vermiculite. Two uniform plants were allowed to develop in each pot. Hoagland's mineral solution (one-half strength) was added to the pots each day (Hoagland, 1948). The vermiculite was leached with distilled water once a week to prevent the accumulation of salts.

Test solutions were prepared from a 2.5% stock solution of TIBA as the dimethylamine salt in water. Tween 20 (polyoxyethylene (20)-sorbitan monolaurate) was used as a wetting agent for spray application. Five ml of 50 ppm TIBA-wetting agent solution were placed in a low-pressure hand sprayer and then sprayed on the upper surface of the two youngest trifoliate leaves, including the apical tip, of flowering plants 44 days old, i.e., with 6 trifoliate leaves nearly expanded and 1 or 2 flowers open.

The oldest node, excluding the cotyledonary node, is called the 1st; succeeding nodes are referred to as the 2nd, 3rd, etc. The internode between the cotyledonary node and the 1st node is called the 1st or oldest;

succeeding internodes are referred to as the 2nd, 3rd, etc. Three weeks after treatment, whole nodes and 5 mm sections of internodes of internodes of treated and control plants were fixed in formalin-acetic acid-50% alcohol (Johnasen, 1940). An estimate of starch content in these nodes was made by studying one-half sections of the whole node stained in a dilute aqueous solution of IKI (Sass, 1958) for 24 hr, followed by a distilled water rinse for 1 hr. After fixation, internodes of these plants were dehydrated in tertiary butyl alcohol (Johansen, 1940) and embedded in paraffin (Paraplast 56-57C). Trnasverse sections of these internodes were cut at 8 u and then stained with quadruple stain (Conant, 1950).

Every week for 4 weeks, main and lateral shoot meristems of control and treated plants were fixed in Craf V (Sass, 1958) and prepared for sectioning according to the previously mentioned methods or with tetrahydrofuran (Leuty, 1964). Median or near-median sections were cut at 8 u and then stained in one of three ways: (a) quadruple stain (Conant, 1950); (b) mercuric-bromophenol-blue alcoholic solution for total protein (Mazia, et al., 1953); (c) aqueous solution of dilute iodine-potassium iodide (IKI) for starch (Sass, 1958).

CHAPTER III

OBSERVATIONS

Treated soybean plants exhibited responses to TIBA treatment similar to those found by Galston (1947), i.e., reduction in height, shortening of internodes, loss of apical dominance, epinasty of young leaves, thickening and wrinkling of young leaves, and occasional premature abscission of apical leaves and buds. The above responses were especially pronounced 3 wks after treatment.

Structural Changes in the Internodes

Distinct structural and developmental changes in the internodes of treated plants became pronounced 2-3 wks after treatment. Three weeks after treatment, plants had 10 nodes fully established while control plants had 14 nodes. There was noticeable decrease in cambial activity in internodes 5 to 9 of these treated plants as compared to corresponding internodes of control plants (Fig. 1-6). Specifically, treated plants showed approximately 1-2 layers of cells in the cambial zone in internodes 5 and 7 (Fig. 1,3), while corresponding control internodes showed approximately 4-6 layers of cambial cells (Fig. 2,4).

The secondary xylem lying adjacent to the cambium in

the treated plants consisted mainly of small, thick walled elements as shown in Fig. 1,3,5, whereas control internodes (Fig. 2,4,6) had thin-walled parenchyma cells. Smaller vessels and thicker-walled protophloem cells occurred in internodes 8 and 9 of the treated plants than in corresponding internodes of control plants (Fig. 5,6). The internode immediately behind the main apex (internode 10) showed an increase in procambial activity with smaller vessels and thicker-walled protophloem cells, while the corresponding internode of the control plant (internode 14) showed a less active cambium with larger vessels and few thick-walled protophloem cells (Fig. 7,8).

Morphological and Histological Changes in the Meristems

At each node on the main axis of the untreated soybean plant, flowers are produced in one of three ways (Fig. 9): (a) an axillary bud may produce an inflorescence containing usually 2 flowers; (b) an axillary bud may form a lateral branch on which the arrangement in (a) is repeated; (c) supernumerary buds may form in the axils of leaves borne on the main and lateral stems and give rise to an inflorescence with 2 flowers. Inflorescence apices as well as lateral branches first appear in the axils of the lower nodes (nodes 3 and 4), then progressively toward the tip.

One week after treatment, opened flowers appeared in

short inflorescences in the upper nodes (1-3 nodes away from the main apex) in the treated plants, while corresponding nodes in controls had short inflorescences but no opened flowers (Fig. 10,11). I found that TIBA-treated soybeans produced more lateral branches than control plants which was in agreement with several investigators (Anderson, Greer, and Tanner, 1965; Ghorashy, 1967; Galston, 1947).

The main, as well as lateral, shoot apices of the control plants were approximately 100-200 μ in diam and had a distinct tunica-corpus type of organization. These apices had a two-layered tunica, a corpus, and a rib meristem with highly vacuolated enlarged cells (Fig. 12). Two weeks after treatment, the main apex of treated plants developed into a rounded cylinder with associated, enlarged axillary buds 1-2 nodes from it (Fig. 13). This cylindrical apex consisted of highly vacuolated enlarged parenchyma cells with no apparent tunica-corpus arrangement. This alteration also marked the termination of inteterminate growth in the main shoot apex 3-4 wks earlier than in control plants. Figure 14 illustrates cessation of indeterminate growth in a 70-day-old control plant, as indicated by vacuolated distal cells of the apex. Lateral branch meristems of both treated and control plants exhibited this type of growth later in development.

Two weeks after treatment several morphological and

histochemical changes occurred in lateral apices at nodes nearest the main shoot apex (nodes 8 and 9). A single conical apex sometimes developed at these upper nodes (Fig. 15), while a somewhat dome-shaped meristem, similar to that of the main shoot apex, developed in lateral meristems of the control plant. Cells of the first tunica layer in lateral meristems of treated plants were narrower on the flanks of the meristem than at the summit, indicating a greater rate of cell division on the periphery of the meristem (Fig. 15).

Lateral shoot apices formed in the axils of lower nodes on the main axis (i.e., nodes 4-7) of both treated and control plants developed a less rounded, broader, and flatter apical dome than the main shoot meristem (Fig. 16). A noticeable enlargement of nucleoli in the distal part of the second layer of tunica cells occurred in both of these lateral meristems; however, a conspicuous, clear, unstained zone (quadruple stain) surrounding the nucleoli appeared more frequently in meristems of treated plants (Fig. 16).

In both treated and control plants, axillary buds arising from the lateral branches usually produced an inflorescence apex or a floral apex directly. Three weeks after treatment, axillary buds two nodes behind the lateral shoot apices of treated plants were frequently conical and about 95 μ in height, while corresponding buds in the

control plants were more or less dome-like and about 80 μ in height (Fig. 17,18). Axillary buds in treated and control plants stained with Conant's quadruple stain had small densely-stained nucleoli in the cells of the peripheral layer as well as small, moderately-stained nuclei (Fig. 17,18). Remaining cells in the developing buds of the treated plants had large, weakly-stained nuclei especially in the cells of the distal zone of the bud apex (Fig. 17), while these cells in the control plants had enlarged nuclei which were more densely stained (Fig. 18).

Associated with the above alterations in the lateral meristems of treated plants, cells in the rib meristem appeared to have undergone plasmolysis, leaving unstained spaces between their protoplasts and inner wall surfaces (Fig. 19). Several cells in the developing pith of both treated and control plants had thickened primary walls, although this occurred more often in treated plants, especially in the pith cells of developing nodes within an inflorescence (Fig. 20). These cell wall thickenings are probably cellulose since they were stained by the methyl orange in the quadruple stain rather than by safranin. Figure 20 also shows large pith cells adjacent to these smaller, thick-walled cells.

Protein in the Meristems

The use of mercuric bromophenol blue to demonstrate protein produced a uniformly dense stain in cells of the

lateral shoot apices and conical axillary buds of treated plants 3 wks after treatment (Fig. 21). Cells in corresponding sites of control plants were less densely stained (Fig. 22). However, nuclei in several cells of the first tunica layer in control plants were densely-stained (Fig. 22).

Three weeks after treatment, nuclei and cytoplasm of distal cells in the main shoot meristem of treated plants showed less protein than those of control plants of comparable age. Less protein in cells of the main shoot meristem may be correlated with the inability of the apical cells to produce more leaf and bud primordia (Fig. 23). When main and lateral shoot apices in both treated and control plants ceased to initiate flower primordia, the distal cells showed distinctly less protein than those of apices developing flowers (Fig. 23). One to three weeks after treatment, floral apices in the inflorescences of treated plants stained for protein showed 6-7 layers of densely-stained distal cells, while only 2-3 layers of cells in control plants were densely stained (Fig. 24,25). Two to three weeks after treatment, apices of supernumerary buds in both treated and control plants showed a dense protein stain.

Starch in the Nodes and Meristems

Soybean starch is stored almost exclusively as relatively large, spherical grains with concentric layering

and a central hilum. Both treated and control plants had abundant starch grains in the mature xylem elements and in the pith cells immediately adjacent to the primary xylem, as illustrated for the lower internodes (Fig. 3). Two to three weeks after treatment, upper nodes (7,9,10) of treated plants stained with aqueous dilute IKI showed a larger area lacking starch at sites of lateral branches, inflorescences, and supernumerary buds (Fig. 26,27,28) than in corresponding sites (9,11,13,14) of control plants (Fig. 31,32,33,34). Lower nodes in both treated and control plants showed similar amounts of storage starch (Fig. 29,30,35,36,37).

Starch accumulated in very small grains in meristematic cells of the tunica, corpus, and procambium of the main and lateral shoot apices. These small grains make relative comparisons of starch content in these cells difficult. Although large starch grains occurred in the rib meristem and young pith cells of lateral shoot apices of flowering soybean, there were no apparent differences in these areas between treated and control plants. Figure 38 shows large dark-stained starch grains in the cells of the rib meristem, young pith, and axillary bud; on the other hand, note the virtual absence of these starch grains in the tunica, corpus, and procambium.

Two to three weeks after treatment, floral apices of both treated and control plants contained large quantities

of starch grains in the pith, but there were very few in distal cells of the apex (Fig. 39). Abundant large, starch grains occurred in pith cells throughout the lateral shoot meristems in conjunction with developing floral apices (Fig. 39). Figure 40 shows a developing flower near anthesis with many starch grains in the floral parts (excluding the vascular tissue). Curiously, many nuclei in the rib meristem cells of shoot apices in both treated and control plants were surrounded by large starch grains. In fact, some starch grains were intimately associated with these nuclei during mitosis (Fig. 41).

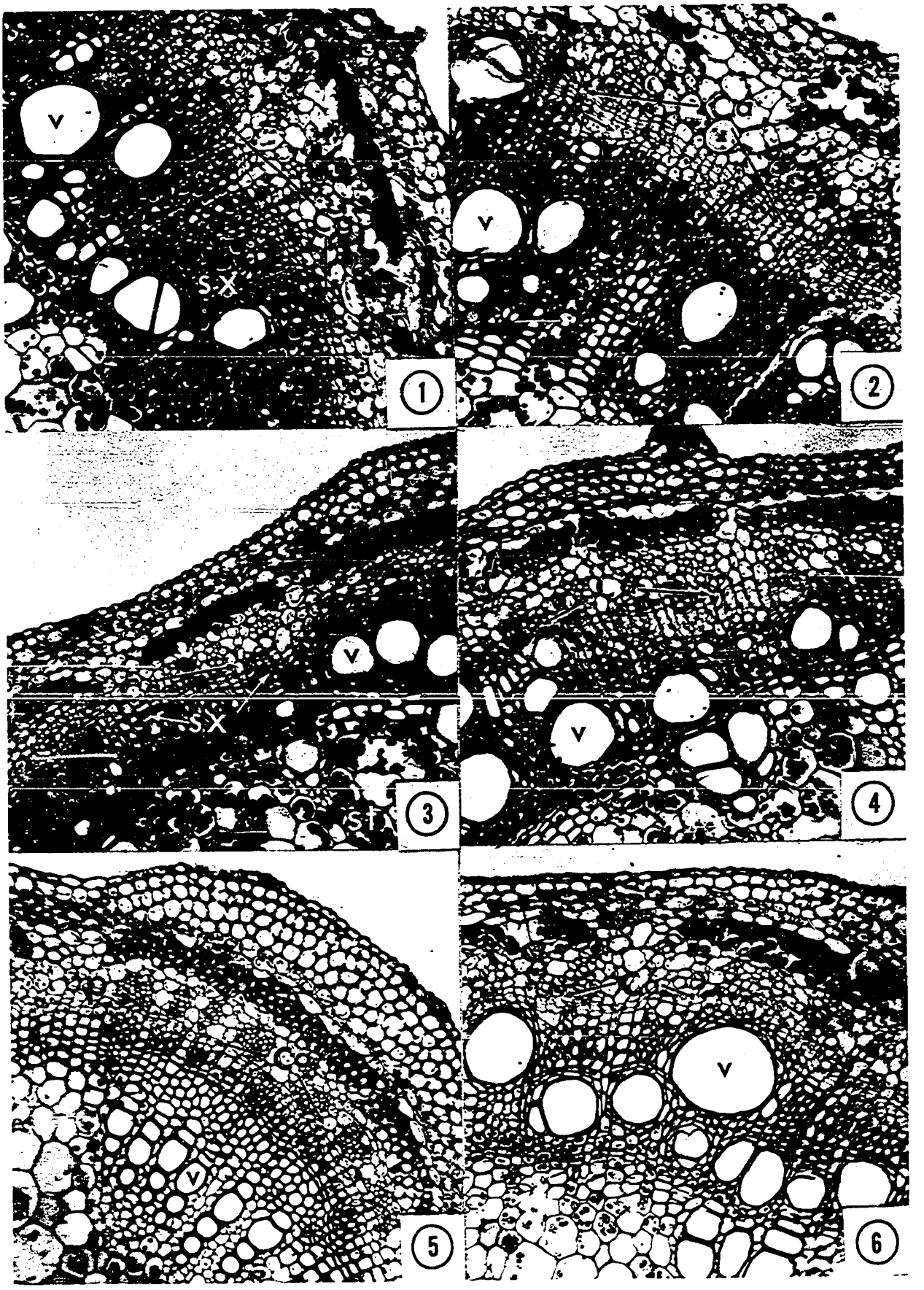
Two weeks after treatment, there were a few small starch grains in the apical cylinder and pith of the main shoot apex; in control plants there were numerous starch grains in the pith and a few small ones in the tunica and corpus (Fig. 13). The limited accumulation of starch in the main shoot apex of treated plants may be correlated with the inability of the apical cells to produce more leaf and bud primordia. When main shoot apices of control plants ceased to initiate flower primordia, the distal and rib meristem cells in the apex contained small starch grains (Fig. 14). Lateral shoot and supernumerary bud apices of both treated and control plants contained these small starch grains at the close of indeterminate growth.

ILLUSTRATIONS 1-6

1. Transection of internode 9 of a treated plant, showing 1 - 2 layers of cambial cells. Note starch grains in pith and xylem elements. X 130
2. Transection of internode 9 of a control plant, showing 4 - 6 layers of cambial cells. Note thin-walled cells of secondary xylem adjacent to cambium. X 130
3. Transection of internode 7 of a treated plant, showing 1 - 2 layers of cambial cells. Note small thick-walled elements in the secondary xylem adjacent to the cambium. X 130
4. Transection of an internode of a control plant, showing 4 - 6 layers of cambial cells. Note thin-walled elements in the secondary xylem adjacent to the cambium. X 130
5. Transection of internode 9 of a treated plant showing 1 - 2 layers of cambial cells. Note thick-walled protophloem elements and small thick-walled vessels. X 130
6. Transection of internode 9 of a control plant showing 3 - 4 layers of cambial cells. Note large, lightly stained protophloem and large vessels. X 130

Key to Abbreviations

ab	axillary bud	p	phloem
at	apical tip	pc	procambium
c	corpus	pe	pedicel
Ca	cambium	r	rib meristem
fa	floral apex	sb	supernumerary bud
fl	flower	sp	sepal primordia
l	leaf	st	starch
lb	lateral branch	sx	secondary xylem
lp	leaf primordia	t	tunica
n	nucleus	v	vessel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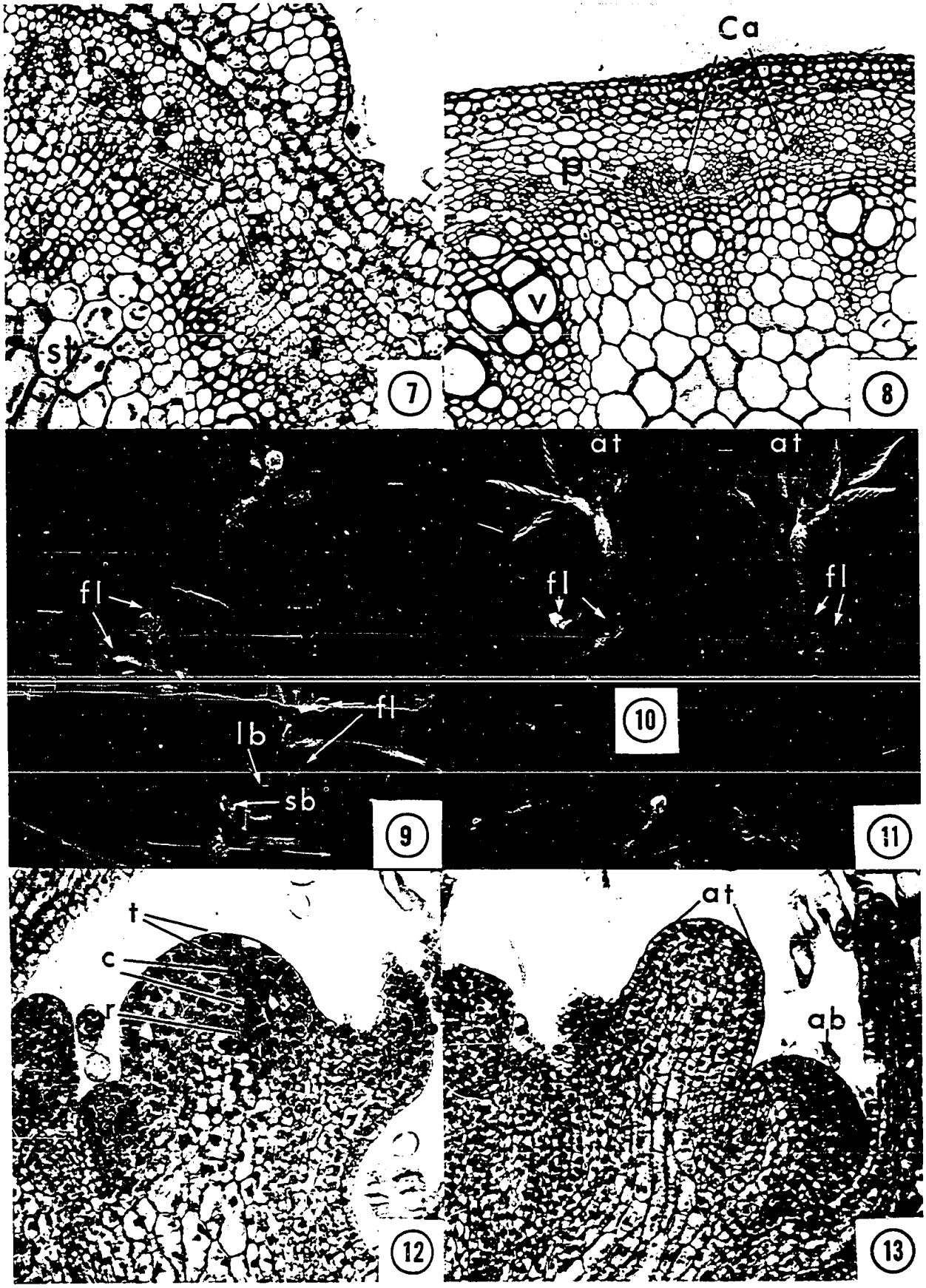
ILLUSTRATIONS 7-13

7. Transection of internode 10, immediately behind the main apex, of a treated plant. Note 3 - 4 layers of procambial cells, small vessels, thick-walled protophloem cells, and starch grains in pith. X 130
8. Transection of internode 14, immediately behind the main apex, of a control plant. Note weak cambium development, large vessels, and few thick-walled protophloem cells. X 130
9. Control flowering soybean, showing the following: (a) an axillary bud with two flowers; (b) a lateral branch with 2 flowers in one of its axils; (c) a supernumerary bud at the axil of the main stem. X 1
10. Plant one week after treatment, showing opened flowers three nodes away from the main apex. X 1
11. Control plant 51 days old, showing unopened flowers three nodes away from the main apex. X 1
12. Median section of the main apex of a control plant, showing a tunica-corpus arrangement with highly vacuolated cells in the rib meristem. Note developing axillary bud and leaf primordia. X 280
13. Median section of the main apex of a treated plant two weeks after treatment. Note the main apex has developed into a rounded cylinder consisting of highly vacuolated parenchyma cells with no apparent

tunica-corpus arrangement. Note also the enlarged axillary bud two nodes away from the main apex. X 280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ILLUSTRATIONS 14-19

14. Median section of the main apex of a 70 day old control plant showing vacuolated distal cells of the apex containing small starch grains. Note poorly developed axillary buds. X 280

15. Median section of a conical upper lateral shoot meristem of a treated plant (i.e., at node 8). Note narrower cells on the flanks of the meristem than at the summit. Note also the enlarged axillary bud immediately next to the apex. X 280

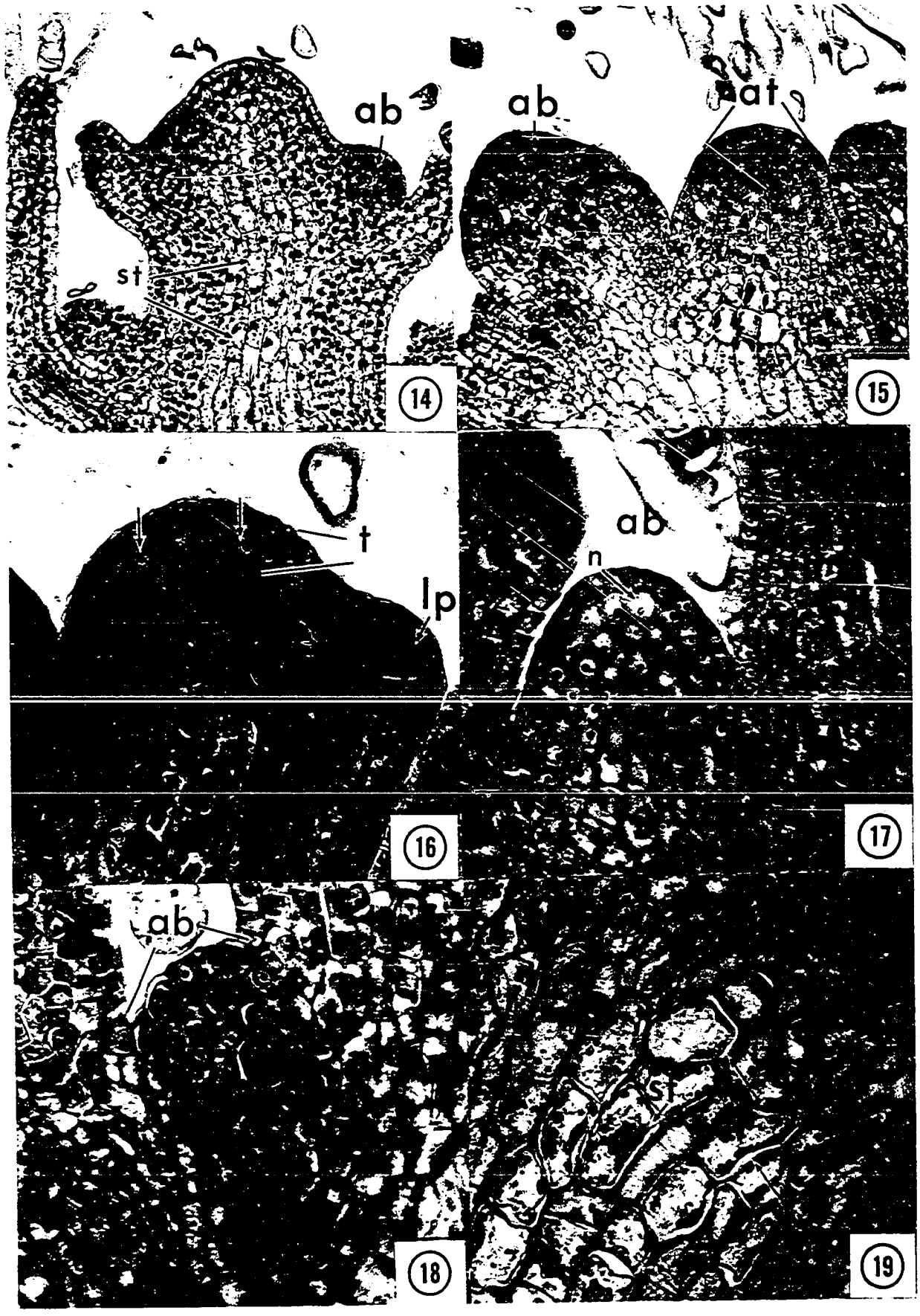
16. Median section of a lateral shoot meristem formed in the axil of a lower node (i.e., node 7) showing enlarged nucleoli in the distal cells of the second tunica layer. Arrows point to clear, unstained zones in the nuclei adjacent to the nucleoli. X 380

17. Median section of a conical axillary bud located two nodes behind the apex of a treated plant. Note that the quadruple stain reveals large and lightly stained nuclei in cells beneath the peripheral layer. Note also large nucleoli. X 380

18. Median section of an axillary bud located two nodes behind the apex of a control plant. Note densely stained nuclei in the cells throughout the apex. X 380

19. Longitudinal section through the rib meristem of the lateral shoot apex of a treated plant showing slightly

plasmolyzed pith cells. Note storage starch grains in the cytoplasm. X 570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ILLUSTRATIONS 20-25

20. Longitudinal section through node 11 of a treated plant showing thick-walled pith cells (arrows) and large thin-walled cells adjacent to them. X 570

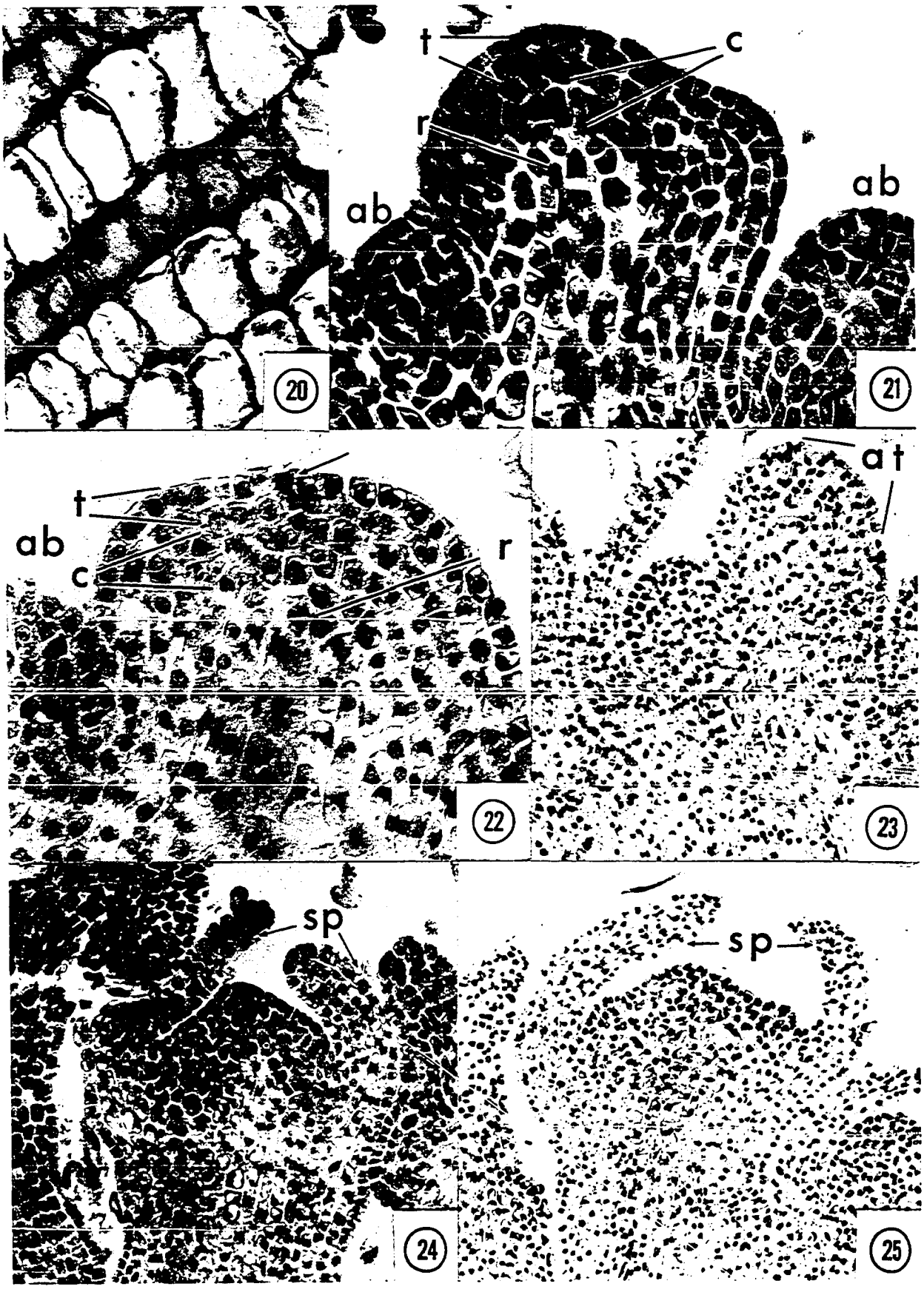
21. Median section of a lateral shoot meristem of a treated plant stained for protein. Note dense-stain in axillary buds. X 310

22. Median section of a lateral shoot meristem of a control plant stained for protein. Note dense staining nuclei in some cells of the first tunica layer. X 310

23. Median section of the main apex of plant three weeks after treatment, stained for protein. Note weakly-stained cytoplasm throughout the apex. X 280

24. Median section of a floral apex in an upper lateral branch of a treated plant stained for protein. Note dense stain in the distal cells. X 280

25. Median section of a floral apex in an upper lateral branch of a control plant stained for protein. Note the weak stain, especially in the cytoplasm of the distal cells. X 280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ILLUSTRATIONS 26-41

Illustrations 25-37 are one-half sections of nodes of treated and control plants (58 days old), stained with IKI.

26. Node 10 and part of the main apex of a treated plant, showing large unstained area at sites of flower pedicels. X 1

27. Node 9 of a treated plant showing large, unstained area at the site of a lateral branch and a supernumerary bud. X 1

28. Node 7 of a treated plant showing a small, unstained area at the site of a lateral branch and a supernumerary bud. X 1

29. Node 5 of a treated plant showing a small, unstained area at the site of a lateral branch. X 1

30. Node 3 of a treated plant showing starch throughout the area of the node and lateral branch. X 1

31. Node 14 and part of the main apex of a control plant showing a completely stained area at the site of a flower pedicel. X 1

32. Node 13 of a control plant showing a very small, unstained area at the site of a flower pedicel. X 1

33. Node 11 of a control plant showing a completely stained area at the site of a flower pedicel. X 1

34. Node 9 of a control plant showing a small, unstained area at the site of a flower pedicel. X 1

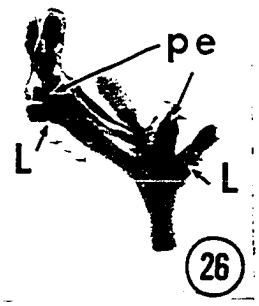
35-37. Nodes 7,5, and 3 respectively, showing little, if any, unstained areas at the sites of lateral branches. X 1

38. Median section of a lateral branch apex (at node 2) of a treated plant stained with IKI. Note large dark-staining starch grains in the pith, axillary bud, and developing rib meristem. Large dark-staining starch grains are virtually absent in the tunica, corpus, and procambium cells. X 200

39. Median section of a lateral branch apex (at node 5) of a treated plant stained with IKI. Note large amount of dark-staining starch grains in the pith cells, axillary bud, floral apex, and rib meristem. A few dark-staining, small starch grains can be seen in the tunica, corpus, and procambium cells. X 100

40. Longitudinal section of a developing flower in the axil of a lateral branch (at node 5) of a treated plant stained with IKI. Note the large, dark-staining starch grains in cells throughout the floral parts, except in the developing vascular tissue. X 100

41. Longitudinal section of a young, dividing pith cell in the lateral branch of a treated plant. Note the telophase nucleus, cell plate (arrow), and adjacent starch grains. X 900



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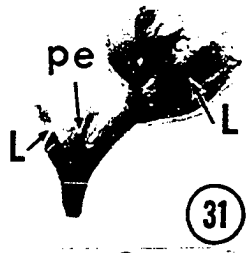

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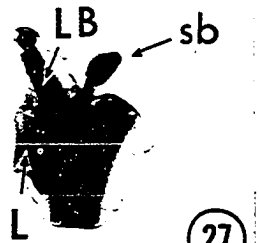

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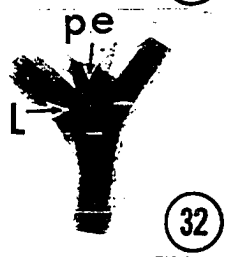

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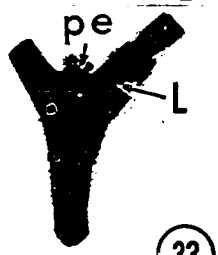

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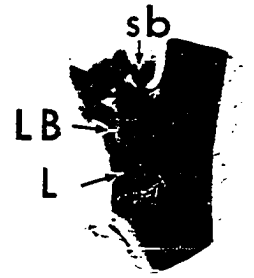

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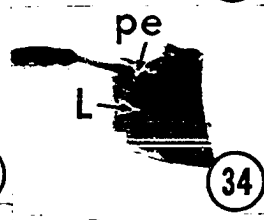

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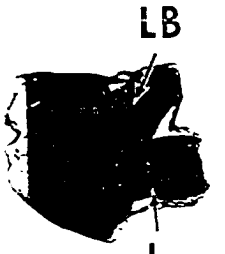

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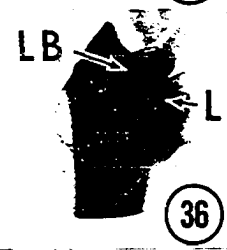

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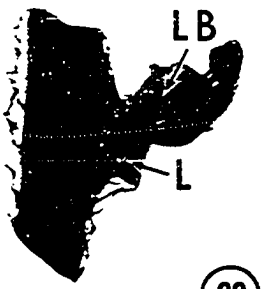

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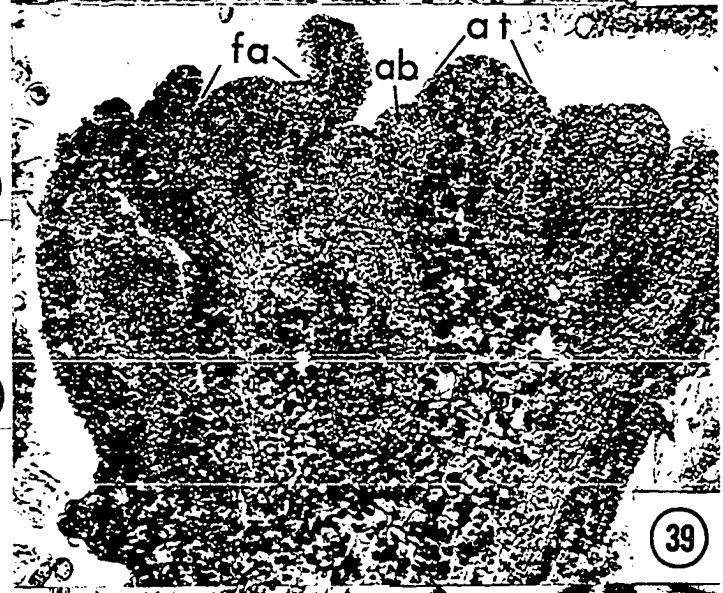

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41

CHAPTER IV

DISCUSSION

According to Heslop-Harrison and Heslop-Harrison (1957), young hemp plants on the verge of flower formation, which were treated with TIBA or had their apices removed, produced flowers at upper nodes (2-3 nodes below the apex). In control plants, however, flowers seldom occurred at these sites because of the continued dominance of the main apex. Gorashy (1967) found a heavy concentration of pods in the upper nodes of TIBA-treated soybean. Although flowers are produced at the upper nodes of untreated soybean, earlier flower production in these nodes occurred with TIBA-treatment. Cumming (1959) and Audus and Thresh (1956) found that TIBA lowers the amount of diffusable auxin within a plant. Furthermore, since low auxin concentration are associated with initiation of flower production, enhancement of flowering can be correlated directly or indirectly with inhibition of apical dominance by TIBA.

Several noticeable changes occurred in the upper and middle internodes during early flower production in TIBA-treated soybeans. Increased procambial activity, in

conjunction with rapidly differentiated thick-walled protophloem cells, and small vessels in the youngest two internodes might be explained as a synergistic effect of TIBA on auxin activity. TIBA does cause a small amount of auxin to bring about a disproportionate amount of growth (Thimann and Bonner, 1947). Cambial activity decreased, however, in the middle internodes of treated plants. This suggests a close correlation with increased flowering in the plant.

After studying internodal anatomy of 14 families of plants in the vegetative and reproductive state, Wilton and Roberts (1936) concluded that cambial activity seems to decrease in plants that have mostly reproductive primordia. They also found that reduction in size of vessels and the production of thick-walled xylem and phloem elements accompanied this decreased cambial activity. The middle internodes of TIBA treated flowering soybean showed similar structural changes. Moreover, Gorashy (1967) found a reduction in the size of vessels in the stem of TIBA-treated soybean.

Heslop-Harrison and Heslop-Harrison (1957) suggest that TIBA tends to prevent localization of growth activity, substituting instead, generalized growth throughout a larger area or volume of tissue. I have found that changes in main and lateral shoot meristems of flowering soybeans treated with TIBA, lend some support to this

hypothesis. These apices exhibited alterations, which somewhat paralleled those of Bedesem's (1958) findings with apices of TIBA-treated tomato plants. According to Bedesem, tomatoes treated with TIBA exhibited the following alterations in the main and lateral apices: (a) development of cylindrical apices; (b) development of conical apices with small stack-of-brick-like peripheral cells; (c) shrinkage of protoplasmic contents in some meristematic cells.

Two weeks after treatment, the main shoot apex of treated soybean plants developed into a rounded cylinder with associated enlarged axillary buds near the apex. This apex consisted of highly-vacuolated parenchyma cells with little starch and protein, as evidenced by small starch grains and light-staining cytoplasm (mercuric bromophenol blue). It no longer produced new leaf and bud primordia; hence, indeterminate growth ceased at the apex about 2-3 wks before it did in control plants.

Shoot apices in the reproductive state are known to have larger nuclei and nucleoli in the distal cells of the apex than in the flanks (Nougarède, Gifford, and Rondet, 1965; Gifford and Tepper, 1962). According to Buvat et al. (1952), only rapidly dividing cells or cells engaged in organogenetic processes possess large nucleoli. More recently Brown (1966) working with embryonic cells of

Xenopus laevis (South African clawed toad) suggests that an enlarged nucleolus in a developing cell can, to some extent, be correlated with an increased rate of ribosomal RNA synthesis. In agreement with the above findings, similar conditions occur in lateral shoot apices of both treated and control plants. However, two weeks after treatment, some lateral shoot apices in treated plants became a conical structure consisting of small peripheral cells with frequent mitoses, indicating more cell division than occurred in corresponding apices of control plants. Further structural and cytological evidence of enhanced cell division and metabolism in apices of TIBA-treated plants was exhibited three weeks after treatment in the conical axillary buds of lateral branches, which consisted of the following features: (a) larger overall structure than in corresponding buds of control plants; (b) many enlarged, densely-stained nucleoli (Conant's stain); and (c) many enlarged, weakly-stained nuclei (Conant's stain), possibly indicating the occurrence of high enzymatic activity. Moreover, plasmolysis of protoplasm and frequent occurrence of thickened primary walls in young pith cells of lateral shoot meristems of treated plants, further suggest rapid biosynthesis of cell material (Fig. 19 and 20).

It is generally accepted that RNA and protein synthesis increase in shoot apices with the onset of floral

initiation. Three weeks after TIBA-treatment cells of lateral shoot meristems with conical axillary buds contained more protein, especially in the cytoplasm, than did cells of corresponding meristems of control plants (Fig. 21,22). Floral apices borne on lateral shoots of treated plants also contained more protein than did cells of corresponding meristems of control plants. This noticeable increase in protein content may be directly correlated with enhanced floral development. Gifford and Tepper (1962) found that apices of Chenopodium album stained with mercuric bromophenol blue for protein had a greater concentration of protein after four short-day light exposures (4 SD) than in controls or after 2 SD. Osborne and Mullins (1969), working with garden beans, found that TIBA does not inhibit protein synthesis but, in reality, increases the extent of incorporation of radioactive leucine into protein.

During early flower production in the upper nodes of treated plants, little starch accumulated, suggesting that the products of starch degradation are closely associated with flower production. Thorpe and Muroshige (1968), working with starch accumulation in shoot forming tobacco callus cultures, suggest that starch possesses a distinct advantage over other sources of energy. Its degradation results in high yields of gluclose 1-phosphate, the subsequent catabolism of which, through glycolysis,

produces adenosine triphosphate without the expenditure of existing high-energy phosphates. Furthermore, Nanda and Dhindsa (1967) working with soybean, found that starch hydolysis increases with the extension of internodes. They also believe that the products of starch hydrolysis are translocated from elongated internodes to places where they are needed. Although shortening of the internodes in soybean occurs with TIBA-treatment, it is probable that high-energy products of starch hydrolysis in the upper nodes of these plants could be shunted into the production of lateral branches and inflorescences, instead of being utilized for extension of the internodes.

The presence of starch in the apical regions of both treated and control plants warrants further attention. According to Gifford and Tepper (1962), apices of Chenopodium album subjected to 2-4 shoot days have numerous starch grains in the cells of the tunica, peripheral zone, rib meristem, and young leaf primordia. Few occur in the central portion of the corpus. However, with additional inductive cycles, starch grains tend to disappear from the apex, although they persist in the rib meristem and leaf primordia. I found that lateral shoot apices of both treated and control plants had numerous starch grains in the rib meristem, young pith, leaf primordia, and axillary buds, although few appeared in the tunica, corpus, and procambium.

Sadik and Ozbun (1967), working with cold-temperature floral induction of cauliflower, observed the following in cold treated plants: (a) starch accumulation occurred especially in sub-apical zones; (b) starch grains usually surrounded the nucleus in these cells; (c) floral primordia which developed into functional flowers were glutted with starch grains. Lateral shoot meristems of both treated and control plants showed similar conditions. The presence of starch in such unusually large amounts in these areas suggests a significant role in the flowering process.

CHAPTER V

SUMMARY

Flowering soybeans were sprayed at the tips with 50 ppm TIBA. Microscopic and macroscopic observations were made of the nodes, internodes, and shoot meristems every week for four weeks after TIBA-treatment. TIBA-treated plants produced opened flowers at the upper nodes one week earlier than did control plants of comparable age. Accompanying this early flower development, the following changes occurred in the upper internodes (1-2 internodes behind the main apex), as compared to control plants: (a) increased activity of the procambium; (b) rapid development of thick-walled protophloem cells; (c) production of small vessels. The above changes were especially pronounced three weeks after treatment.

Three weeks after treatment, middle internodes of treated plants showed less cambial activity than did corresponding internodes of control plants. These internodes had smaller vessels and thicker-walled xylem and phloem elements than did the controls. Changes in the middle internodes of treated plants suggest close correlation with an increased degree of flowering.

Two weeks after treatment the main shoot apex of treated plants developed into a rounded cylinder consisting of highly vacuolated parenchyma cells with little starch (IKI) and protein as evidenced by small starch grains and light-staining cytoplasm (mercuric bromophenol blue). Consequently this apex no longer produced new leaf and bud primordia, while in control plants the main apex with a typical tunica-corpus arrangement continued to produce these structures for another 2-3 wks. Two weeks after treatment some lateral shoot apices at nodes nearest the main shoot apex exhibited the following changes, in contrast with control plants: (a) development of conical apices with stack-of-brick-like peripheral cells; (b) shrinkage of protoplasmic contents in some rib meristem cells and young pith cells; (c) frequent thickening of primary walls in young pith cells.

Further structural and cytological evidence of enhanced cell division and metabolism in apices of TIBA-treated plants was exhibited three weeks after treatment in the conical axillary buds of lateral branches. These buds, as opposed to those of control plants, were generally larger with enlarged, densely stained nucleoli and enlarged, weakly stained nuclei (quadruple stain). Three weeks after treatment, cells of lateral shoot meristems with conical axillary buds showed a denser stain for protein, especially in the cytoplasm, than did cells of corresponding meristems

of control plants. Floral apices in these meristems also stained more densely for protein than did similar apices in controls. This increased protein content may be attributed to the accumulation of a protein carrier for auxin transport and related directly to enhanced floral development or both.

Together with early flower production in the upper nodes of treated plants, less starch occurred 2-3 wks after treatment than in corresponding nodes of control plants. This suggests the possible role of the high-energy products of starch degradation in the enhancement of floral development. Two to three weeks after treatment, lateral shoot apices of both treated and control plants had numerous, large starch grains in the rib meristem, young pith, leaf and bud primordia, and developing flowers but few starch grains appeared in the tunica, corpus, and procambium. Starch grains in such large amounts in developing flowers may provide a convenient reserve of high-energy products essential for rapid cell division and metabolism.

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