MEMOIRS OF THE FACULTY OF SCIENCE, KYOTO UNIVERSITY, SERIES OF BIOLOGY
Vol. III, pp. 1-12, March 1970

Auxin and Kinetin Interaction during Xylem Differentiation

By

Lorin W. ROBERTS and Sango BABA

Department of Biological Sciences, University of Idaho, Moscow, Idaho, U. S. A., and Department of Botany, Faculty of Science, Kyoto University

(Received November 22, 1969)

ABSTRACT Interfascicular xylem differentiation was experimentally induced in cultured explants from the first internode of *Phaseolus vulgaris* L. (var. Oregon Giant pole bean) seedlings by an indole-3-acetic acid (IAA) treatment (0.2 ppm, 30 hr). Control first internode slices indicated no interfascicular xylem differentiation at the time of explant excision. The minimum time for IAA stimulation of interfascicular xylogenesis, in the presence of 0.01% colchicine as a tracheary element marker, was 18 hr. Treatment of explants with kinetin (0.1 ppm, 30 hr) in the absence of exogenous IAA induced the formation of large numbers of lignified pitted fiber-tracheids and tracheary elements in the interfascicular cambial region. The combined application of kinetin (0.1 ppm) and IAA (0.2 ppm) for 30 hr had a marked synergistic effect on interfascicular scalariform-reticulate vessel member differentiation. The morphology of the differentiated cells from the combined treatment resembled the cells produced by the IAA-alone treatment and not the kinetin-alone treatment. Explants apparently synthesized endogenous auxin during prolonged culture (48-72 hr) on sucrose-agar as indicated by the differentiation of cambial initials and wound vessel member formation. Sequential treatments involving IAA and kinetin indicated that kinetin required a minimum of 24-30 hr of uninterrupted treatment either concomitant with or followed by a minimum of 18 hr IAA treatment. Differentiation of cambial initials demonstrated a strict polarity since stimulation of xylogenesis by a combined treatment of auxin and kinetin was always produced in the morphological basal end of the explant regardless of the application site of the growth substances. Auxin-cytokinin synergism was also given by substituting benzylamino purine (0.1 ppm), or zeatin (0.1 ppm), or kinetin riboside (0.2 ppm) for kinetin. However, treatment of explants with L-proline was ineffective in stimulating xylogenesis under experimental conditions that produced xylogenic responses in *Coleus* explants. Increasing the concentration of IAA from 0.2 ppm to 0.5 ppm and lengthening the incubation period from 30 to 48-72 hr led to extremely large numbers of small isodiametric wound vessel members.

Introduction

The basic requirement for auxin in the initiation of xylem differentiation has been established[1], yet the possible role of cytokinins in xylogenesis has remained a matter of dispute. Although wound vessel member (WVM) formation in *Coleus* is either unaffected or inhibited by the addition of exogenous kinetin in the presence of auxin[2,3], cytokinins are effective in inducing xylogenesis in the prothalli of various ferns and lycopods[1]. Fosket and Torrey[5] have demonstrated the necessity of both exogenous kinetin and auxin for WVM formation in soybean callus cultures, and other workers have reported the stimulation of xylogenesis by the addition of exogenous cytokinins to tissue cultures[6,7,8]. Gautheret[9], on the other hand, was unsuccessful in promoting xylogenesis in Jerusalem artichoke cultures with the addition of kinetin. A dual role for auxin and kinetin was demonstrated by Sorokin, Mathur and Thimann[10] in the differentiation of secondary xylem in the pea epicotyl; a subsequent study by Sorokin and Thimann[11] revealed the promotion of procambial strand formation with combinations of auxin and kinetin. What is the nature of cytokinin-auxin interaction in differentiation of cambial derivatives? Fosket[12] has shown that cell division is a prerequisite for WVM differentiation in *Coleus* stem explants, and he has proposed that xylogenesis originates during some step of the mitotic cycle. Explants of young *Phaseolus vulgaris* L. seedlings are a favorable choice for an investigation of hormone-induced xylogenesis since the undifferentiated interfascicular cambial initials of the first internode show a rapid response to hormonal treatments with the successive cup technique[13,14].

Materials and Methods

Seeds of *Phaseolus vulgaris* L. (var. Oregon Giant pole bean), soaked 12 hr in running tap water, were potted in vermiculite, and placed in a controlled environment chamber where they were given a 12 hr daily photoperiod of combined fluorescent and incandescent light at an intensity of approximately 1500 ft candles, and a constant temperature of 26° ± 2°C. The first internode, measuring 20 mm ± 5 mm in length, was excised from seedlings 10-12 cm in height with a lamina area of approximately 750 mm^2 from a single primary leaf. This stage of growth was not closely correlated with the chronological age of the seedling. The first internode is directly above the cotyledonary node and adjacent to the first pair of primary leaves[10]. Care was taken that explants were not selected from seedlings which had reached a further growth stage exhibiting bolting of the shoot apex. Although the shoot apex, primary leaves, and the cotyledons were excised from some seedlings 48 hr before removing the first internode, in order to remove endogenous auxin sources, this procedure was not followed in the majority of the experiments. Excised first internode segments were surface sterilized by means of a 15 min treatment with 10% "Chlorox", and passed through 4 changes of sterile distilled water. The segments were trimmed by transverse cuts at each end to 12 mm lengths, and were positioned vertically with

their morphological basal ends downward on the surface of a 1.0% agar medium in 10 cm diameter Petri dishes. A 1.0 mm control slice was removed from the basal trimming of the segment and placed in 4.0% NaOH for clearing and subsequent Safranin O staining. This control slice was used to determine if any interfascicular xylem differentiation had occurred, as indicated by Safranin O staining and by the appearance of secondary wall striations, in the seedling internode tissues at the time of explant removal. If interfascicular xylem differentiation was apparent in the control slice, the corresponding explant was then discarded. The explants were carefully handled with sterile forceps which had polyethylene sponge strips cemented to the inside face of the points, since the explant tissues exhibited a wound response when handled with unshielded forceps. The lid of each Petri dish was modified according to the successive cup technique[13,14]. Inside the lid of each Petri dish three shallow glass cups (26 mm inside diameter, 2.0 ml capacity) were cemented. The glass cups were filled with 1.0 % agar plus 2.0% sucrose (SA medium) plus the various treatments as described below. The lid was replaced so that the morphological apical end of the explant was embedded in the agar medium in one of the glass cups. After various time intervals, the lid was raised slightly and rotated so that the adjacent cup was directly over the explant, and then the lid was lowered so that the explant would receive the second treatment. In some cases this maneuver was repeated and the explant was fed from the medium in the third cup. The explants in the modified Petri dishes were placed in a controlled environment chamber where they received the same environmental conditions as the developing seedlings. At the termination of the culture period the explants were softened and cleared by treatment in a 4.0% NaOH solution at 55°C for 7-10 hr. Subsequently the explants were stained by immersion in a 0.03% aqueous solution of Safranin O for 30 min at 55°C, rinsed briefly in distilled water, and destained with 2-3 changes of 1.0 N HCl (55°C) over a period of 2-4 hr. The epidermal, cortical, and pith parenchyma tissues were removed with glass dissecting needles, and the specimens were mounted in glycerin jelly. In some cases, fresh freehand transverse sections of pith-embedded stem internode tissues were prepared with a razor blade, and the tracheary elements were stained with an aqueous solution of 0.05 % Toluidine blue O[15,16]. Cell counts of differentiated xylem elements were made with the aid of an ocular grid. The count was restricted to the total number of tracheary elements in the interfascicular areas between the existing vascular bundles and limited to a horizontal band surrounding the morphological basal end of the segment. This cell count zone commenced 0.26 mm from the basal end of the segment and measured 0.52 mm in width. The Brown and Rickless tissue maceration procedure[17], used to excellent advantage in the cell counting determinations of Fosket and Torrey[5], was inappropriate in the present study because of the fragmentation tendency of the primary tracheary elements. Also, a morphological distinction could not be made between recently-differentiated cambial initials and previously-formed tracheary elements, since the secondary xylem elements frequently had the wall thickenings characteristic of primary xylem.

Results

Cultured explants were induced by auxin treatment to form secondary tracheary elements from the interfascicular cambial initials within 30 hr at the morphological basal end of the explants. Transverse sections taken from the untreated seedling stem directly above the cotyledonary node indicated that cell division had occurred in the interfascicular cambial region (Fig. 1) at the time of explant excision. However, these daughter cells, similar to those found in the basal region of the explant, lacked any visible secondary wall sculpturing indicative of xylogenesis in the initiation of the experiments.

Differentiation of the cambial initials in the explants occurred to some extent in the absence of any exogenous auxin or kinetin. Segments receiving a cup application of 2.0% sucrose exhibited relatively few elements of random number and distribution by the end of 30 hr incubation, but by the end of 72 hr incubation these segments contained a considerable number of tracheary elements (Table 1). It was evident that the explants have the intrinsic capacity to induce xylogenesis, presumably by the synthesis of endogenous auxin, but in insignificant amounts until 48-72 hr of incubation time.

The minimum incubation time for auxin stimulation of interfascicular xylem differentiation was 18 hr. This was demonstrated by employing a cup medium composed of sucrose-agar containing 0.5 ppm IAA and 0.01% colchicine. Tracheary elements induced to form during the auxin-colchicine treatment were readily

Table 1. Cell count of differentiated tracheary elements and fibers in the interfascicular zone at the morphological basal end of the explants following 30 hr incubation[1]

Sucrose-agar medium	5±2
Sucrose-agar medium (after 72 hr)	120±20
Kinetin (0.1 ppm, basal application)	94±24
IAA (0.2 ppm, cup application)	155±97
IAA (0.2 ppm)+kinetin (0.1 ppm)	315±88

[1]The values given represent a mean cell count of 10 segments ± the standard error. The cell count zone was limited to the interfascicular areas between the vascular bundles and commenced 0.26 mm from the basal end of the explants and measured 0.52 mm in width. The cell count zone surrounded the basal end of the explants. Sucrose (2.0%) was incorporated in the cup medium with all IAA and kinetin treatments.

distinguishable from pre-existing xylem elements by the aberrant secondary wall striations produced by the colchicine[13,18] (Fig. 2). The relatively few tracheary elements produced by this treatment were contiguous with the terminuses of the major vascular bundles in the basipetal end of the explant. Aberrant xylogenesis was not observed in any explants after 16 hr incubation with higher auxin levels (2.5, 5, and 10 ppm) under the same cultural conditions.

The extent of secondary xylem differentiation was closely related to both auxin concentration and incubation time. It was thus necessary to select empiri-

cally an appropriate exogenous auxin concentration and incubation time that would yield relatively low tracheary element cell counts in order to determine if the combined treatment of auxin and kinetin was exerting a synergistic effect on xylogenesis. Explants that received 0.2 ppm IAA in sucrose-agar for 30 hr from a cup application produced a suitable response (Fig. 3, Table 1). The tracheary elements induced to form in the interfascicular cambial zone were vessel members with scalariform-reticulate secondary wall thickenings. These IAA-induced cells are morphologically identical with wound vessel members, although the latter cells are frequently small and isodiametric when formed directly following cell division.

Kinetin was introduced into the morphological basal end of the explants by incorporating the reagent in the 1.0% agar medium in the bottom of the Petri dishes. There is evidence that cytokinins may be synthesized by the root systems of higher plants and translocated upward[19]. However, further experiments revealed that the site of kinetin application was immaterial in regard to the auxin-kinetin synergistic response. The addition of kinetin at a concentration of 0.1 ppm for 30 hr, in the absence of exogenous IAA, induced lignification of the cambial initials in the interfascicular zone and the presence of numerous elongated pitted fiber-tracheids and intergrading libriform fibers[20] was observed (Fig. 4, 5, 6, Table 1). Scattered at random in the interfascicular area were infrequent scalariform-reticulate thickened vessel members and vessel strands. These fiber-tracheids were not observed in explants treated with IAA alone or with IAA plus kinetin. Fresh freehand transverse sections from kinetin-treated explants stained more intensely for lignin with phloroglucinol-HCl in the interfascicular cambial zone than staining in comparable sections removed from the untreated seedling. The simultaneous application of 0.2 ppm IAA from the cup and 0.1 ppm kinetin from the basal agar medium produced a synergistic effect in the stimulation of extensive secondary xylem differentiation in the interfascicular region. In the latter case, the induced tracheary elements consisted entirely of scalariform-reticulate thickened vessel members (Fig. 7, Table 1).

Does the IAA-kinetin synergism involved a sequential action whereby kinetin may pre-condition the interfascicular cambial zone for subsequent IAA-induced xylogenesis, or does the enhanced xylogenesis require the presence of both growth regulators simultaneously? Explants were treated for various time intervals with either kinetin or kinetin-IAA followed by an IAA treatment (Table 2). The synergistic effect was not expressed unless the explant received 0.2 ppm IAA and 0.1 ppm kinetin simultaneously for at least 30 hr (treatment 5), or unless the explant received a pre-treatment of 0.1 ppm kinetin for 24 hr followed by 0.2 ppm IAA for 30 hr (treatment 6). Once the physiological functions in xylogenesis performed by IAA and kinetin, respectively, are initiated, the growth regulators are required for minima time intervals of approximately 18 hr and 24 hr, respectively.

The effect of explant polarity and direction of application of IAA and kinetin on xylogenesis was investigated (Table 3). The possible effects of IAA and kinetin separately on xylogenesis were not examined. However, Thompson[21] and

Table 2. Xylogenic differentiation responses resulting from sequential kinetin-IAA treatments of explants with the successive cup technique[1]

Treatment :	Cup A Kinetin(hr)	Cup B Kinetin+IAA(hr)	Cup C IAA(hr)	Differentiation Response
1	10	—	20	weak; same as IAA for 20 hr
2	10	10	10	weak ; same as IAA for 20 hr
3	—	10	10	little or no differentiation
4	24	—	10	same as kinetin alone
5	—	30	—	highly stimulatory ; similar to IAA + kinetin
6	24	—	30	highly stimulatory ; similar to IAA+kinetin

[1]Explants were positioned erect with morphological apical end embedded in cup medium. All cups contained 2.0% sucrose in 1% agar. IAA (0.2 ppm) was fed from cup medium and kinetin (0.1 ppm) was fed from a 1% agar medium in the bottom of the Petri dish. Results given for a total of 10 explants. Differentiation response represents interfascicular xylogenesis in the morphological basal end of the explant.

Table 3. Effect of explant polarity on interfascicular xylogenesis induced by kinetin-auxin treatments

Treatment	Position[1]	Cup medium[2]	Basal medium
1	A	SAIAA	AK
2	A	AK	SAIAA
3	A	SAIAA+K	A
4	A	A	SAIAA+K
5	B	SAIAA	AK
6	B	AK	SAIAA
7	B	SAIAA+K	A
8	B	A	SAIAA+K

[1]Position A indicates morphological basal end in the bottom of Petri dish; position B indicates morphological basal end embedded in cup medium.

[2]Treatments : SAIAA=sucrose (2%) in 1% agar containing 0.2 ppm IAA
AK=1% agar containing 0.1 ppm kinetin
A=1% agar

Note: All of the above treatments resulted in the stimulation of interfascicular xylem differentiation in the morphological basal end of the explant, and approximately to the same extent in all treatments.

Baba[22] have reported that acropetally moving auxin was incapable of stimulating WVM formation in a variety of plants. In the present experiment xylogenesis

was always observed at the morphological basal end of the explants under all possible combinations of polar application of the two growth substances. Although interfascicular tracheary element cell counts were not made, the distribution and quantity of differentiated secondary xylem was consistent with the previously-described experiment involving a combined treatment of IAA and kinetin for 30 hr. The polarity experiments are compatible with the concept that differentiation of cambial initials occurs at the basal end of the explant as a result of basipetal auxin movement regardless of the site of application of kinetin (treatments 1, 3, 6, 8). It is unnecessary to postulate acropetal auxin transport for the additional treatments (2, 4, 5, 7) since in each case the morphological basal end of the explant is embedded in the medium containing IAA. Presumably IAA diffuses readily into the interfascicular cambial zone without entering the polar transport system.

The substitution of other cytokinins for kinetin was effective in producing the synergistic effect on xylogenesis. Explants treated with a cup application of 0.2 ppm IAA with a basal treatment of 0.1 ppm benzylamino purine, or 0.1 ppm zeatin, or 0.2 ppm kinetin riboside were effective in stimulating xylogenesis in the basal end of the explant.

The treatment of explants with (a) 1.0 mM L-proline either with kinetin (0.1 ppm, 24 hr) followed by IAA (0.2 ppm, 30 hr) or (b) 1.0 mM L-proline (24 hr) followed by IAA (0.2 ppm) plus kinetin (0.1 ppm) for 30 hr had no effect in stimulating xylogenesis to a greater extent than that previously described for the IAA-kinetin responses. This was unexpected in view of the previous finding by Roberts and Baba[14] indicating the marked stimulation of WVM formation in *Coleus* explants pre-treated with L-proline.

Increasing the concentration of IAA from 0.2 ppm to 0.5 ppm and lengthening the incubation period from 30 to 48-72 hr leads to extremely large numbers of small isodiametric WVM, formed in massive clusters of cells, and mainly associated with the vascular bundles (Fig. 8).

Discussion

Kinetin in the absence of exogenous auxin induces the interfascicular cambial initials of bean explants to produce a unique xylogenic response in the formation of large numbers of lignified pitted fiber-tracheids and lesser numbers of tracheary elements with conspicuous scalariform-opposite pitting. Explants treated with kinetin plus auxin or auxin alone gave identical qualitative responses, i.e., tracheary elements with scalariform-reticulate secondary wall thickenings with an absence of pitted fiber-tracheids. Different results were reported with etiolated second inter node segments of pea epicotyl treated 6-10 days in auxin and kinetin solutions[10]. In these experiments auxin induced hyperplastic tissue and the occlusion of proto and metaxylem ; both effects reversed by the addition of kinetin. Also kinetin induced a compact tissue of narrow tracheary elements of pitted vessels with alternate pits in the pea epicotyl tissues. In the present experiments similar tracheary elements were frequently observed in the interfascicular zone of kinetin-

treated bean explants. Sorokin, Mathur, and Thimann[10] considered that the presence of endogenous auxin in the excised pea internodes could account for the kinetin-induced xylogenesis. Although the present experiments indicate little or no endogenous xylogenic auxin in the bean explants by the end of 30 hr, we cannot state that the kinetin response occurs in the complete absence of auxin since kinetin may influence the mobilization or synthesis of auxin during the incubation period. Also, there is evidence that kinetin mobility is greatly increased in bean plants in the presence of auxin[23]. We may speculate that two distinct differentiation processes are involved. Kinetin, in the presence of auxin, regulates cell division for subsequent auxin-induced vessel differentiation. Whereas without sufficient auxin kinetin stimulates lignification and wall thickening leading to a characteristic pit development. Both wall sculpturing and pit formation may be reflections of microfibril deposition as influenced by different hormone-directed microtubule patterns. The present results indicate that kinetin may have functions other than that of a cell division stimulant for auxin-induced xylem differentiation. Clutter[6] reported that the ratio of kinetin to IAA was important in regulating the differentiation response in cabbage pith cultures, and the responses were reported independent of cell division.

Colchicine is an excellent reagent for the determination of the minimum length of time a cell requires auxin treatment before aberrant wall striations indicate secondary wall thickening. A minimum time period of 18 hr is required before a visible aberrant wall pattern is discernible, and these cells are at a stage of "incipient xylogenesis" in the fascicular cambium of the major vascular bundles. Other workers have also employed colchicine in studies on xylem differentiation[12,18]. Since these cambial initials have recently undergone cell division the 18 hr may be a requirement for auxin to stimulate protein synthesis for wall metabolism. The time requirement for the cytokinin synergism suggests that this hormone is necessary throughout the mitotic cycle until the daughter cell becomes responsive to xylogenic auxin.

The phenomenon of inherent organ polarity has interested physiologists for many years[24], and experiments were designed to determine if the location of exogenous kinetin would modify auxin transport and the xylogenic response. The application of kinetin may induce localized mobilization of amino acids[25], and the transport of auxins within leaves can be directed by localized cytokinin application[19]. In all cases interfascicular xylem differentiation was observed, typical of IAA-kinetin interaction, at the morphological basal end of the explant. The presence of kinetin had no effect on the basipetal auxin transport, and the effect of kinetin on differentiation was apparent by the synergistic response.

The possible connection between kinetin-induced lignification and xylogenesis merits further investigation, and a discussion of this problem lies beyond the scope of this study. Rubery and Fosket[26] reported that the activity of phenylalanine ammonia lyase (PAL) followed the same time course as the formation of WVM in cultured *Coleus* internode slices. This system deaminates phenylalanine

to *trans*-cinnamic acid which is subsequently converted to the phenolic precursors of lignin[27]. The specific activity of PAL was greater in WVM-producing soybean callus tissue cultured on 5 x 10^{-7}M kinetin than on WVM-free soybean callus grown on a 10^{-8}M kinetin solution. They suggested that PAL may be considered as a biochemical marker of xylem differentiation. Koblitz[28] also reported that cytokinins in the presence of auxins strongly promote the formation of lignin at the expense of polysaccharide synthesis. However, the relationship between lignin synthesis/deposition and xylogenesis is complex since both processes evidently can occur independently[1].

References

1. Roberts, L. W., Bot. Rev. (in press), 1969
2. Earle, E. D., Amer. J. Bot. **55**, 302. 1968
3. Fosket, D. E. & Roberts, L. W. Amer. J. Bot. **51**, 19, 1964
4. DeMaggio, A. E., Amer. J. Bot. **54**, 631, 1967
5. Fosket, D. E. & Torrey, J. G., Plant Physiol. **44**, 871, 1969
6. Clutter, M. E., Plant Physiol. **38** (suppl.), xii, 1963
7. Bergmann, L., Planta **62**, 221, 1964
8. Koblitz, H., Faserforsch Textiltech. **13**, 231, 1962
9. Gautheret, R. J., Compt. Rend. Acad. Sci. (Paris) **253**, 1381, 1961
10. Sorokin, H. P., Mathur, S. N. & Thimann, K. V., Amer. J. Bot. **49**, 444, 1962
11. Sorokin, H. P. & Thimann, K. V., Protoplasma **59**, 326, 1964
12. Fosket, D. E., Proc. Nat. Acad. Sci., U. S. **59**, 1089, 1968
13. Roberts, L. W. & Baba, S., Plant & Cell Physiol. **9**, 315, 1968
14. Roberts, L. W. & Baba, S., Plant & Cell Physiol. **9**, 353, 1968
15. O'Brien, T. P. & McCully, M. E., Plant Structure and Development. A Pictorial and Physiological Approach. 1969, p114, New York : Macmillan
16. Feder, N. & O'Brien, T. P., Amer. J. Bot. **55**, 123, 1968
17. Brown, R. & Rickless, P., Proc. Roy. Soc. (London) Ser. B. **136**, 110, 1949
18. Pickett-Heaps, J. D., Develop. Biol. **15**, 206, 1967
19. Letham, D. S., Bio Science **19**, 309, 1969
20. Esau, K., Plant Anatomy. pp 226, 2nd ed. New York : John Wiley
21. Thompson, N. P., Biochemistry and Physiology of Plant Growth Substances, ed. by F. Wightman & G. Setterfield, p1205, 1968, Ottawa: Runge Press Ltd.
22. Baba, S., unpublished results
23. Seth, A. K., Davies, C. R. & Wareing, P. F., Science **151**, 587, 1966
24. Bloch, R., Encyclopedia of Plant Physiology, ed. by W. Ruhland, **15** (1), 234, 1965. Berlin: Springer-Verlag
25. Mothes, K., Régulateurs Naturels de la Croissance Végétale, ed. by J.P. Nitsch, p131, 1964. Paris : Centre Natl. Rech. Sci.
26. Rubery, P. H. & Fosket, D. E., Planta **87**, 54, 1969
27. Neish, A. C., Phytochem., **1**, 1, 1961
28. Koblitz, H., Qualitas Plant. Mater. Veg., **14**, 37, 1967

Acknowledgement

The authors wish to express their thanks to Mr. Mashouf Shaykh for his technical assistance.

LEGENDS

Fig. 1. Transverse section of base of first internode, taken from bean seedling at time of explant removal, showing interfascicular cambial activity. The daughter cells are living and wall sculpturing is not evident at this stage of development. Freehand section of fresh material stained with toluidine blue O. ×1120.

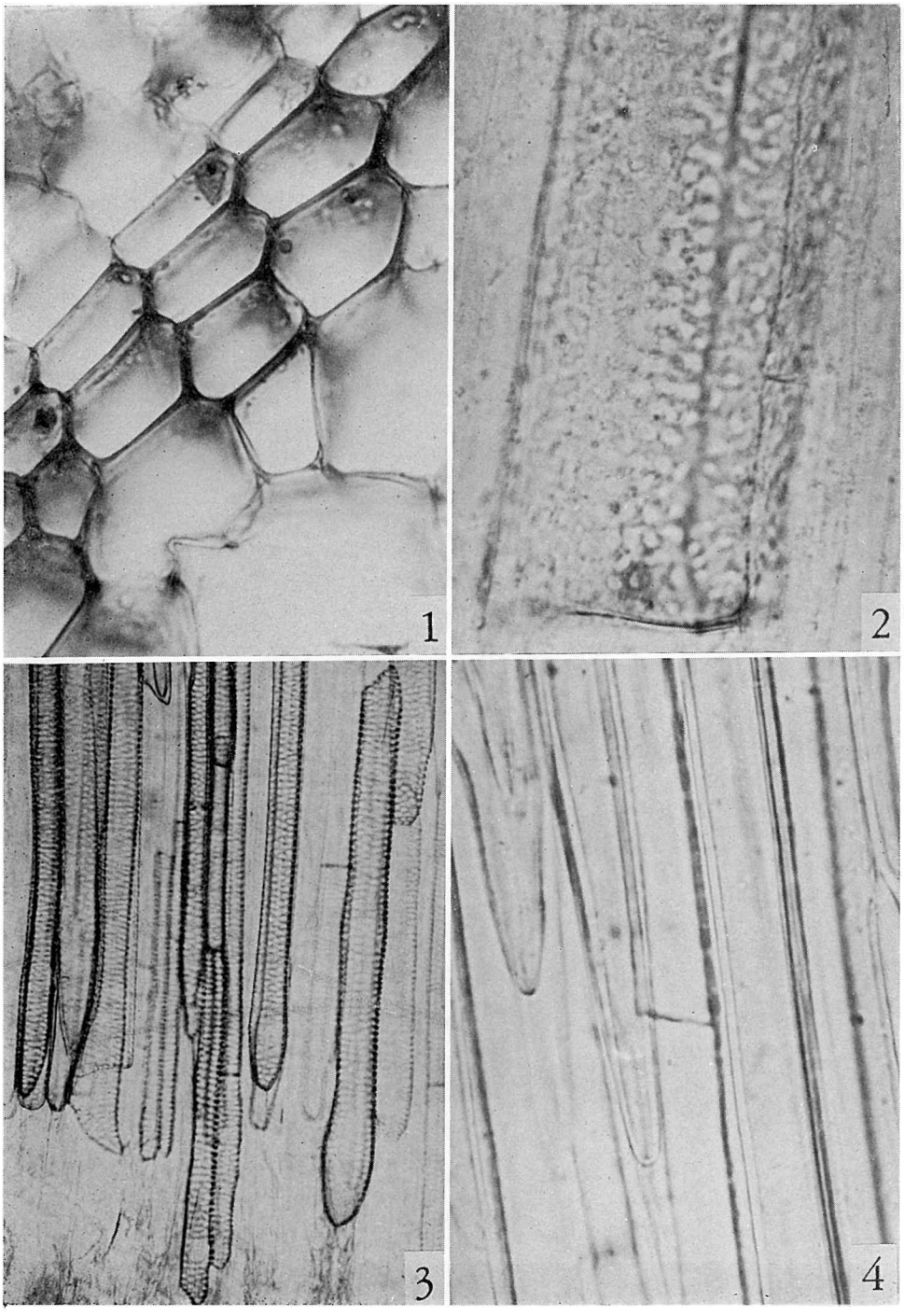
Fig. 2. IAA-induced (0.5 ppm, 18 hr) tracheary element formed in the presence of colchicine (0.01%). The characteristic abnormal secondary wall deposition, resulting from microtubule dissolution. marks this cells as being produced during the 18 hr treatment. Safranin O. ×2400.

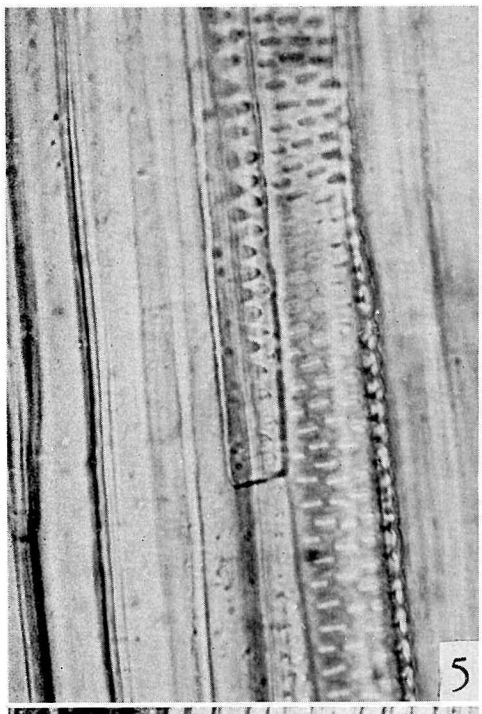
Fig. 3. IAA-induced (0.2 ppm, 30 hr) scalariform-reticulate vessel members formed in the interfascicular cambial region. Safranin O. ×300.

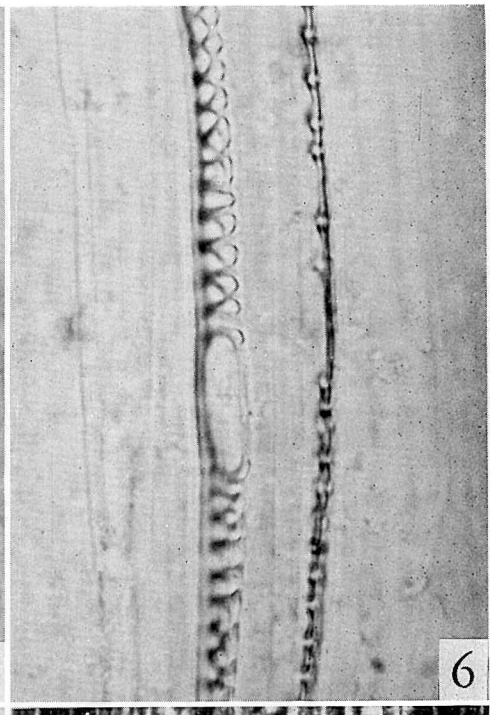
Fig. 4-5-6. Kinetin-induced (0.1 ppm, 30 hr) pitted fiber-tracheids formed in the interfascicular cambial region. Fig. 4. Fiber-tracheids bearing minute pits and lacking additional wall modifications. Fig. 5. Fiber-tracheid with well-developed pits. Fig. 6. Unusual tracheary element with irregular pit arrangement, wall striations, and lateral wall simple perforation plate. Safranin O. ×740.

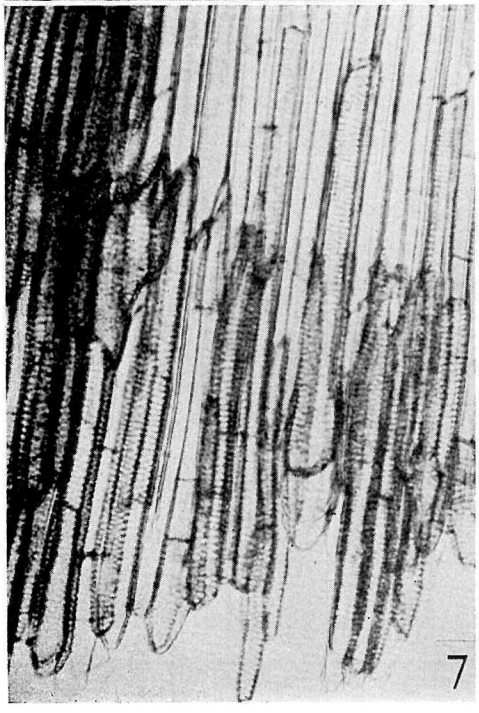
Fig. 7. Scalariform-reticulate vessel members induced to form in interfascicular cambial region following combined IAA (0.2 ppm) and kinetin (0.1 ppm) treatment for 30 hr. Note resemblance in cell wall morphology to Fig. 3. Safranin O. ×300.

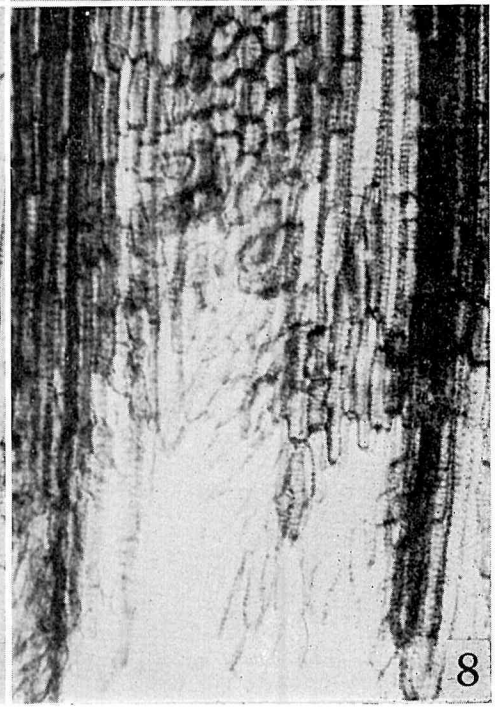
Fig. 8. Masses of small isodiametric wound vessel members formed at the basal end of the explant and associated with the vascular bundles and interfascicular areas. Explant received IAA (0.2 ppm) for 72 hr. Safranin O. ×300.


1
2
3
4


5


6


7


8