

## THE EFFECT OF APPLIED GROWTH HORMONES ON XYLEM DIFFERENTIATION IN *COLEUS BLUMEI* STEM TISSUE

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### Abstract

Xylem differentiation was observed as a response to IAA alone, interaction of IAA and GA and combined effect of IAA, GA and Kinetin. There was no significant difference in the amount of xylem differentiation when 0.1 ppm Kinetin was added to IAA and GA. Xylem differentiation varied with the concentration of the hormones and duration of treatment.

### Introduction

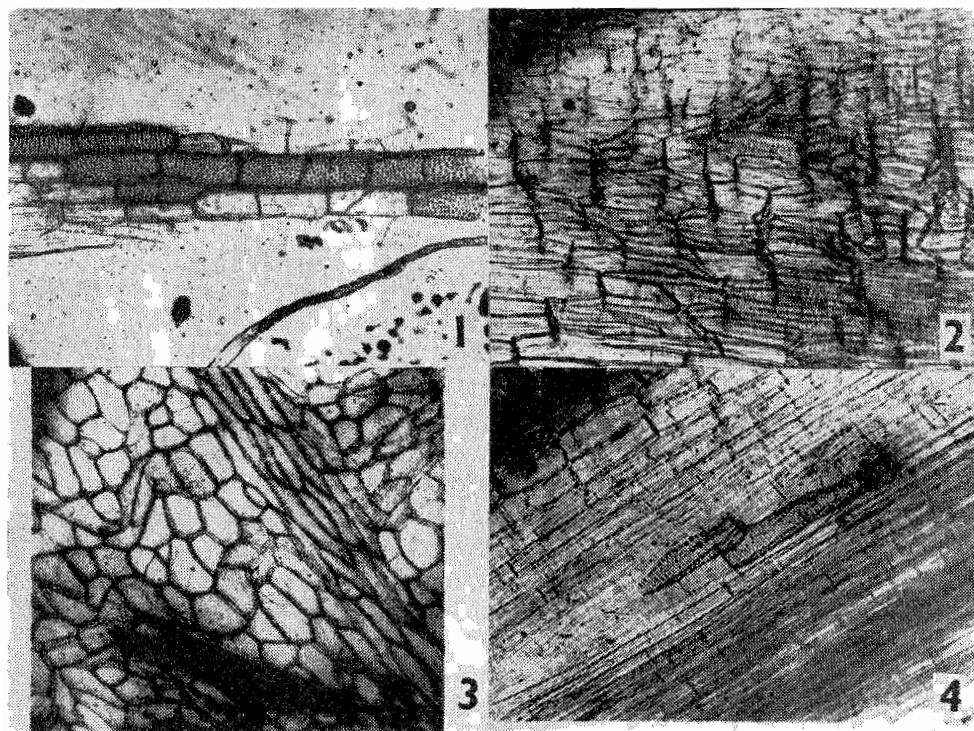
Induction of vascular elements in response to auxin treatment has been reported by a number of investigators. Jacobs & Morrow (1957) and Earle (1968) have demonstrated the effect of IAA on xylem differentiation in plant parts. Bradley & Crane (1957) and Wareing (1958b) have reported that GA results in the stimulation of cambium and formation of secondary xylem in some woody plants. Fosket & Roberts (1966) have shown that interaction between IAA and GA resulted in xylem formation in the cortical tissue of excised *Coleus blumei* internodes.

Xylem regeneration in response to IAA and Kinetin interaction has been reported by (Schaeffer & Smith, 1963; Earle & Torrey, 1965; Torrey & Loomis, 1967). But according to Fosket & Roberts (1966) IAA and Kinetin inhibited the differentiation of wound vessel member (WVM).

Role of growth substances in tissue differentiation has two aspects. The first is their effect on cell division and the second is their effect on tissue differentiation. In the present investigation effect of IAA, interaction of IAA and GA and combined effect of IAA, GA and Kinetin on xylem differentiation has been studied. No attempt has been made to study their effect on cell division.

### Materials and Methods

Growth substances mixed in lanolin were applied in the V-notch made across a major strand of vascular tissue in the fifth internode of *Coleus blumei*. The portion of the stem above fifth internode was cut. Position of fifth internode was determined following Earle (1968). Three concentrations of IAA viz. 2.18, 4.37 and 8.75 ppm were used. Three combinations of IAA and GA were also prepared by adding 0.034 ppm GA to each of the above mentioned concentration of IAA. Three combinations of IAA, GA and Kinetin were also used. To each of the three above stated combinations of IAA and GA, 0.1 ppm of Kinetin was mixed.



- Fig. 1. Longitudinal hand section of *Coleus blumei* stem. Macerated with 25% alcoholic HCl followed by 0.5% ammonium oxalate and mounted in glycerine. Strands of WVM with reticulate thickenings can be seen. X 100.
- Fig. 2. Longitudinal section of *Coleus blumei* stem treated with 8.75 ppm IAA for 24 days. Section shows pith region in which xylem cells are differentiated. WVM show reticulate thickenings and have developed, in strands. x 100.
- Fig. 3. Longitudinal section of *Coleus blumei* stem treated with 4.37 ppm IAA for 24 days. 30  $\mu$ m thick sections were cut on a freezing microtome. Sections were dehydrated through ethanol series, stained with safranin and light green and mounted in canada balsam. WVM are differentiated in pith region in strands. They show reticulate thickenings. Differentiated xylem elements are like ordinary parenchyma cells of the pith in shape. x 100.
- Fig. 4. Longitudinal hand section of *Coleus blumei* stem treated with 2.18 ppm IAA. The section was cut on a freezing microtome about 30  $\mu$ m thick and mounted in glycerine. WVM are differentiated in the cortex and show reticulate thickenings. x 100.

After an interval of 12 and 24 days explants were fixed in F.A.A. (3:6:91 by volume). Sections were cut by clamping explants on a freezing microtome holder. Free hand sections were also cut. Sections for permanent mount were dehydrated through ethanol series, stained with safranin and light green and mounted in canada balsam. Temporary sections were mounted in glycerine.

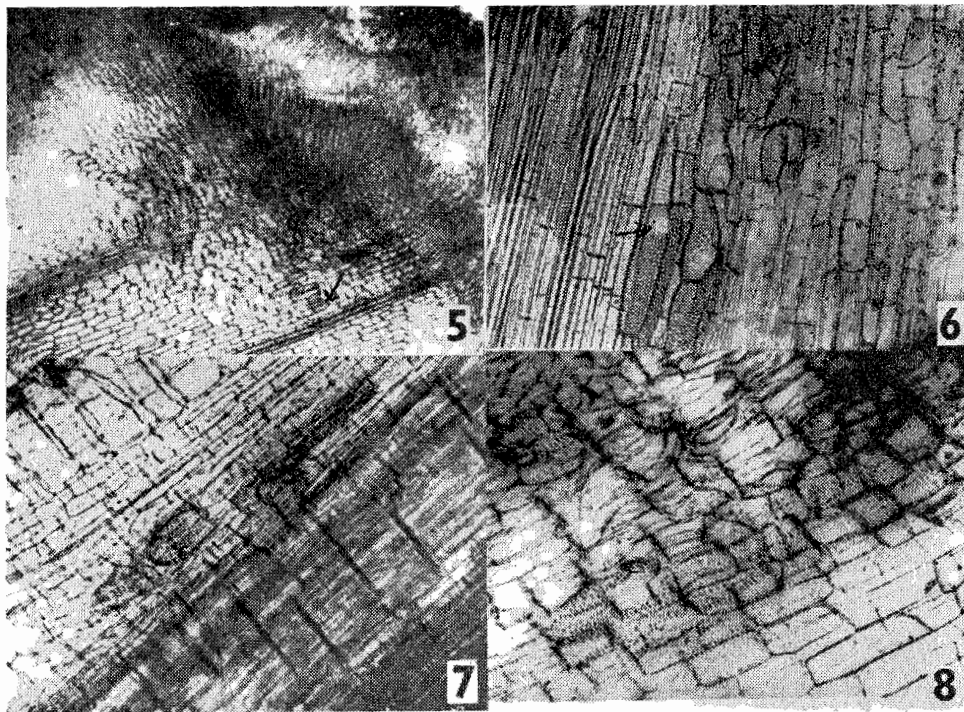


Fig. 5. Longitudinal hand section of *Coleus blumei* stem treated with 8.75 ppm IAA & 0.034 ppm GA. The section was mounted in glycerine. Section shows the region where IAA & GA were applied. A zone of small actively dividing cells is visible just below the place of application of growth substances. Below this zone a zone of newly differentiated xylem elements can be seen (see arrow) x 25.

Fig. 6. Longitudinal section of *Coleus blumei* stem cut on freezing microtome and mounted in glycerine. The stem was treated with 2.18 ppm IAA & 0.034 ppm GA. WVM show reticulate thickenings. Simple perforation plates are clearly visible in regenerated elements (please see arrow) x 100.

Fig. 7. Longitudinal hand section of *Coleus blumei* stem treated with 4.37 ppm IAA and 0.034 ppm GA. The section was mounted in glycerine. WVM with reticulate thickenings can be seen in the cortex. x 100.

Fig. 8. Longitudinal hand section of *Coleus blumei* stem treated with 8.75 ppm IAA, 0.34 ppm GA and 0.1 ppm Kinetin. The section was mounted in glycerine. WVM are seen in Pith region with reticulate thickenings. x 100.

## Observations

### 1. Effect of IAA on xylem differentiation:

WVM\* were observed in all experiments after 12 days but optimum differentiation was noted at 8.75 ppm. Xylem elements were rectangular in shape and showed reticulate thickenings (Fig. 1). In 24 days experiments, regenerated xylem elements developed in strands in cortex and pith. They also showed reticulate thickenings (Figs. 2, 3 and 4).

### 2. Effect of IAA & GA on xylem differentiation:

Xylem differentiation was not observed after 12 days with 2.18 ppm IAA and 0.034 ppm GA but it could be observed in the other two combinations of IAA and GA. Regenerated elements were observed in cortex and pith. In fig. 6, reticulate thickenings can be seen. Simple perforation plates are clearly visible in these elements. Optimum differentiation was at 8.75 ppm IAA and 0.034 ppm GA.

### 3. Effect of IAA, GA and Kinetin on xylem differentiation:

Xylem elements were not observed in 2.18 ppm IAA, 0.034 ppm GA and 0.1 ppm Kinetin in 12 days experiments but they could be seen in 24 days experiments (Fig. 7). Xylem elements could be observed in the other two combinations of IAA, GA and Kinetin even after 12 days.

## Discussion

Roberts & Fosket (1966) have shown that a wide range of IAA concentrations induce WVM formation, in cortical parenchyma of excised *Coleus* internodes but a level of 5 ppm gave consistent results. Earle (1968) found that high auxin level such as  $5 \times 10^{-5}$  IAA induced more xylem elements than low levels of IAA such as below  $10^{-6}$ . Wetmore & Rier (1963) found that higher concentration of auxin is required for the induction of cell division than tissue differentiation. Wetmore & Rier (1963) and Fosket & Roberts (1964) have reported that higher concentrations of auxin inhibit tissue differentiation. Shininger (1971) has shown that IAA treatment for 14 days at concentration of 0.1 to 10.0 mg/litre had no effect on xylem regeneration or cambial division in *Xanthium*. From this discussion it can be said that optimum level of IAA for initiation of xylem regeneration varies with different plants. The time required for xylem—regeneration also varies in various plants. Torrey & Loomis (1967) observed xylem differentiation after 7 days of IAA treatment in the isolated first transfer roots of

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\*The term WVM (wound vessel member) employed in this text denotes lignified cells with scalariform—reticulate secondary wall striations having either simple or scalariform plates which are experimentally produced in the presence of auxin (after Roberts, 1969).

*Raphanus sativus* whereas Earle (1968) observed xylem differentiation in *Coleus* pith explants within 11 days of culture as a response to IAA treatment.

The interaction of IAA and GA in xylem regeneration has been reported by a number of workers. Wareing (1958b) and Wareing, Hanney & Digby (1964) observed fully developed normal xylem cells in the presence of IAA and GA. Digby & Wareing (1966a) observed higher number of differentiated xylem elements at high IAA and low GA concentrations, in *Populus robusta*. Shininger (1971) has reported that GA treatment for 14 days did not induce differentiation of xylem fibres in *Xanthium*, but GA enhanced production of cambial derivatives in this period. He has further stated that simultaneous application of GA and IAA did not increase the formation of cambial derivatives above that induced by GA alone and did not induce xylem fibre differentiation.

In the present study 2.18 ppm IAA with 0.034 ppm GA did not induce xylem regeneration but higher concentrations of IAA with the same concentration of GA induced more xylem elements than any single concentration of IAA alone. Failure of 2.18 ppm IAA and 0.034 ppm GA to produce WVM may be explained on the grounds that GA with a low level of IAA such as 2.18 ppm would have favoured cell division than tissue differentiation. Optimum differentiation was recorded with 8.75 ppm IAA and 0.034 ppm GA (Fig. 5), though differentiation also occurred with 4.37 ppm IAA and 0.034 ppm GA. More xylem elements were differentiated in 24 days experiments than in 12 days experiments. Digby & Wareing (1966a) have said that in *Acer pseudoplatanus* effect of GA is additive to that of IAA. They have also pointed out in their experiments that IAA alone promoted cell expansion whereas interaction of IAA with GA increased cell division and decreased cell size. Xylem elements differentiated as a response to IAA and GA interaction were like normal xylem elements. Normal differentiation of xylem elements in presence of IAA and GA has been shown by Wareing *et al* (1964) and Fosket & Roberts (1966).

Xylem elements were not observed in 12 days experiments when 2.18 ppm IAA, 0.034 ppm GA and 0.1 ppm Kinetin were used but they could be observed in 24 days experiments (Fig. 8). In the presence of IAA, GA and Kinetin no significant difference in the amount of xylem differentiated could be observed than as a response to IAA and GA interaction. The role of Kinetin in tissue differentiation has been a debatable question. Schaeffer and Smith, 1963; Earle & Torrey, 1965; and Torrey & Loomis, 1967; have reported xylem regeneration in presence of IAA and Kinetin, whereas Fosket & Roberts (1966) have reported that IAA and Kinetin inhibited WVM differentiation. In the present investigation no significant effect of the addition of Kinetin to IAA and GA on xylem differentiation could be observed. However, it may be pointed out that amount of Kinetin used in present study was low, and therefore it is difficult to comment upon the exact role of Kinetin in xylem differentiation.

The regenerated xylem elements in some places are like parenchyma cells of the region in which differentiation has occurred (see Figs. 3 & 8). It seems as non-elongated parenchyma cells of the cortex and pith have been directly converted to xylem elements. Such observations have already been made by Earle (1968) and Beslow & Rier (1969) in *Coleus*.

In conclusion it may be said that IAA plays major role in the differentiation of wound vessel members. Effect of higher concentration of GA is additive to IAA. Addition of 0.1 ppm Kinetin to IAA and GA did not produce any significant effect on the differentiation of WVM.

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