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In vitro Culture of the Wood of *Adhatoda vasica*

By

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With 16 Figures (1 Plate)

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Summary

Wood pieces from young branches of *Adhatoda vasica* Nees (*Acanthaceae*) of 4—5 mm ⌀ and cut 5—6 cm behind the tips, were cultured in Agar SH-media (Schenk & Hildebrandt) containing IAA, NAA, GA_3 and Kinetin for 45 days. *In situ* growth incresed the tangential diameter of procumbent cells, and the number of the vessels and fibres in tangential direction. IAA increased the length of vessel members, the diameter of axial parenchyma and the number of vessels, parenchyma and rays. GA_3 incresed the amount of secondary xylem, the length of the fibres and the diameter of parenchyma. NAA incresed the number and the lenght of the fibres and the number of parenchyma. Kinetin incresed the amount of the secondary xylem and the length of the fibres.

Zusammenfassung

Proben von jungen, ca. 4—5 mm dicken Zweigen von *Adhatoda vasica* Nees (*Acanthaceae*), 5—6 cm hinter der Sproßspitze entnommen, wurden auf Schenk und Hildebrandt — Agar mit Zusätzen von IAA, NAA, GA_3 und Kinetin 45 Tage lang kultiviert. *In situ* nahmen in dieser Zeit der tangentiale Durchmesser der liegenden Zellen sowie die Zahl der Gefäße und Fasern in tangentialer Richtung zu. In IAA nahmen die Länge der Gefäßglieder, der Durchmesser des axialen Parenchyms und die Zahl der Gefäße, der Parenchymzellen und der Holzstrahlen zu. Durch GA_3 wurden der Anteil des sekundären Xylems, die Faserlänge und die Durchmesser der Parenchymzellen vergrößert, in NAA die Zahl und die Länge der Faserzellen und der Anteil des Holzparenchyms. In Kinetin nahmen das sekundäre Xylem und die Faserlänge zu.

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Introduction

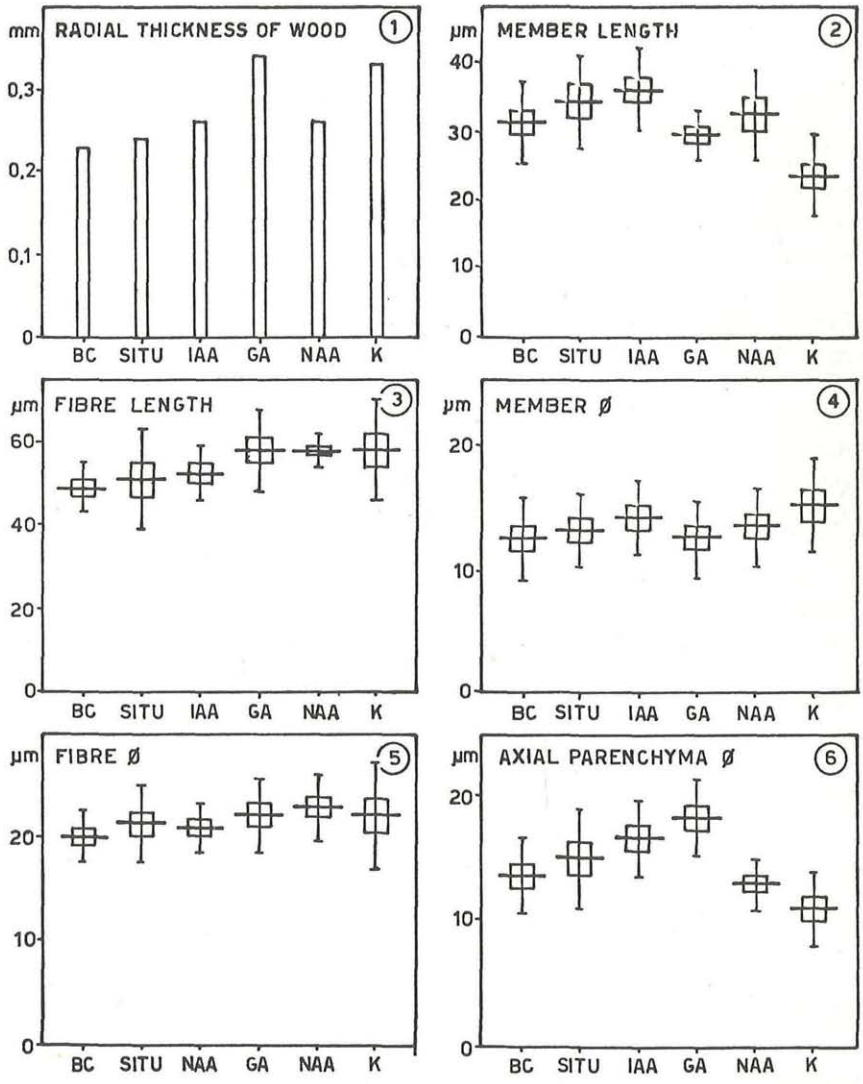
Factors affecting differentiation of secondary xylem from cambium has been studied by different authors. Hormonal effects are in records. In a previous paper by SEN, BHOWMIK & DATTA (1975) the specificity of hormones on the differentiation of cell types of secondary xylem in a species of *Plumeria* was observed. The present attempt is with a comparatively harder wood to see whether such specificity is a constant feature or variable with species.

Adhatoda vasica NEES (*Acanthaceae*) is a medicinal shrub used in cough, chronic bronchitis, asthma, phthisis, rheumatism and as an insecticide (CHOPRA, NAYAR & CHOPRA, 1956). The plant is a native of tropical Asia and is distributed throughout the plains of India and in the Sub-Himalayan tracts. The plant has been selected for its availability, well developed wood consisting of all elements, prolific growth and ease of handling.

Material and Methods

The plant used in the investigations reported below was growing in the University garden. Young branches (about six months after initiation) were collected from the tree during October. Small pieces were cut at a distance of 5—6 cm from the tip. After removing the bark, wood pieces with cambium and pith portion (10 mm long, 4 mm broad and 5 mm thick of which the wood portion was radially 250 μm on average) were cut from the young branch. The diameter of the branch at that region was 4—5 mm. Four different types of media were prepared each, basically that of SCHENK and HILDEBRANDT (1972) but without 2,4-D, contained any one of the hormones: (1) IAA, (2) GA_3, (3) NAA and (4) Kinetin (6-Furfurylaminopurine, K), each 0.1 mg per litre. pH was adjusted at 5.8. Wood pieces (with cambium and pith) were inoculated aseptically with the cambium attached to the medium. About 10 wood pieces from the mentioned region of the branch were kept untreated and preserved in FPA (JOHANSEN 1940) for future comparison of the wood differentiated before sampling with the cultured segments (25 in each medium). Ten branches were marked at the same region (5—6 cm from the tip) and were allowed to grow normally on the tree, for future comparison of the *in situ* growth of wood during culturing period with that of the cultured ones. The marked portions were cut from the tree after 45 days (the culture period) and examined for the purpose.

Anatomical characters of cultured wood pieces were studied after 45 days of growth from thin sections (approximately 15 μm). Informations were recorded from samples of cultured lot, the samples preserved without treatment (i. e., before culture) and the wood which grew for 45 days *in situ* on the tree. Mean measurement mentioned in the text were calculated from 10 wood samples from each medium (after 45 days of growth), 10 samples of preculture condition and 10 wood samples grown *in situ* for 45 days.



Figs. 1—6. Wood characters of *Adhatoda vasica* before culture (BC), and after growing *in situ* (SITU) and *in vitro* with IAA, GA_3, NAA and Kinetin (K). 1, radial thickness of wood (mm); 2, length of vessel members (µm); 3, length of fibres (µm); 4, diameter of vessel members (µm); 5, diameter of fibre cells (µm); 6, diameter of axial parenchyma (µm). (Transverse lines indicate mean values, the vertical lines, the standard deviations and the upper and lower limits of boxes the standard errors)

Results and Conclusions

Different scientists studied the different factors affecting the activity of the cambium, such as, effect of day length (Wareing & Roberts 1956, Gunning, Pate & Briarty 1968), effect of temperature (Waisel & Fahn, 1965) and response to auxins (Waisel & Fahn 1965).

In the experiments of Digby & Wareing (1966) high IAA and low GA produced maximum xylem. Maximum wood was produced in *Plumeria* by IAA (Sen, Bhowmik and Datta 1975). The present study gave different results.

The cambium continued to cut off new wood elements adding to the already differentiated wood. *In situ* growth of the wood for 45 days by cambial activity increased naturally the amount of wood (compare BC and SITU in Fig. 1). After 45 days of culture, considerable amount of wood (Fig. 1) was formed by GA_3 and K., slightly by IAA. From statistical analysis the radial thickness of the wood formed both by GA_3 and K was found significantly higher than the pre-cultured condition (P 0.001 in both). The increase was also significantly higher than the *in situ* growth for 45 days (P 0.001 in case of GA_3 and P 0.05 in case of K).

Length of vessel members (Fig. 2), on the other hand, slightly increased *in situ* on the tree and quite significantly with IAA. With NAA the length did not change but decreased significantly with K (P 0.05). This decrease of the vessel member length might be due to pseudo-transverse division of the fusiform initials or vessel member mother cells (Philipson, Ward & Butterfield 1971).

The fibre length did not increase much *in situ* and with IAA, but with GA_3, NAA and K the length increased significantly (P 0.01) (Fig. 3).

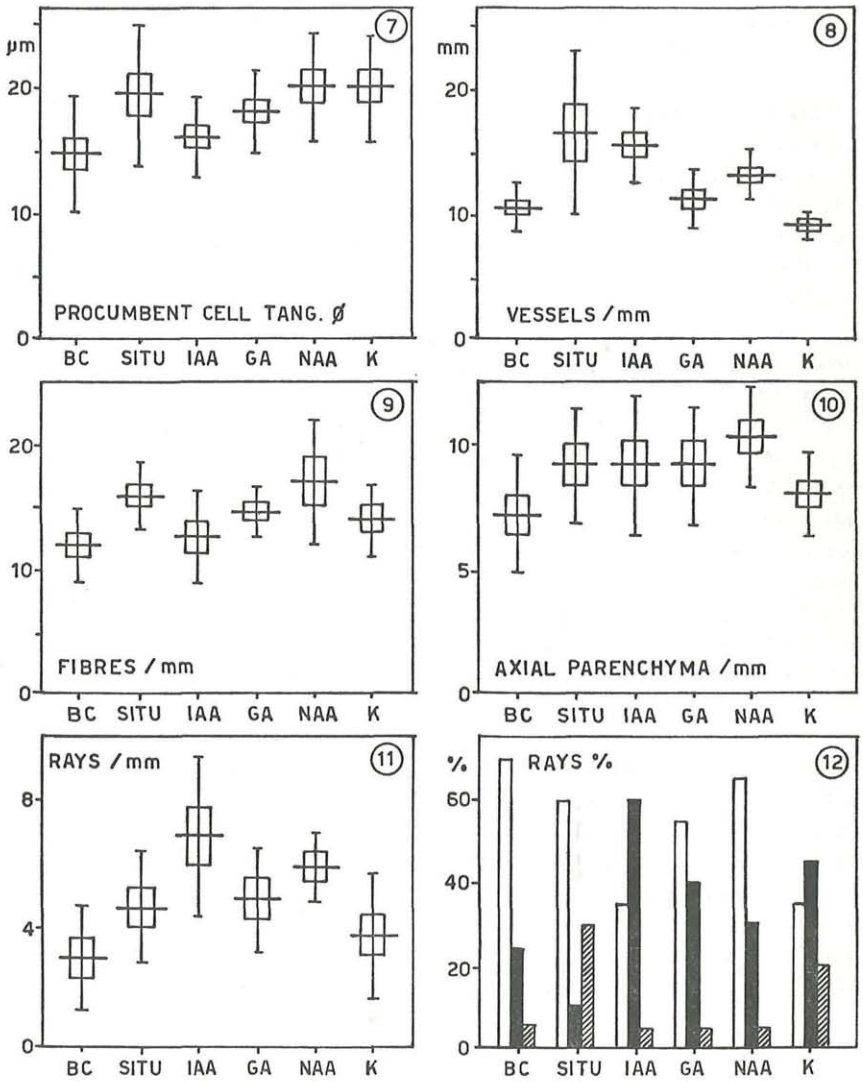
In previous experiments with auxin (Waisel & Fahn 1965), IAA increased the diameter of vessels in Robinia and diameter of tracheids in *Pinus* but reduced in *Plumeria* (Sen, Bhowmik & Datta 1975). In the present experiment IAA and Kinetin increased the diameter (Fig. 4).

Fibre diameter did not significantly change in any condition (Fig. 5).

Diameter of axial parenchyma (Fig. 6) increased considerably with GA_3 (P 0.01) similar to *Plumeria* and decreased significantly with K (P 0.05).

Procumbent cells of the ray (Fig. 7) became remarkably broader (i. e. of larger tangential diameter) after *in situ* growth (P 0.05) and after culturing, particularly with NAA and K (P 0.01).

Number of vessels per mm as counted in tangential direction on the outermost secondary xylem formed during the 45 days was significantly higher *in situ* (P 0.05) and with IAA (P 0.01) (Fig. 8). Fibres per mm, counted similarly, increased significantly after *in situ* growth (P 0.01) and with NAA (P 0.01) (Fig. 9). Number of parenchyma per mm counted as above, increased significantly with NAA (P 0.01). The other results differed insignificantly (Fig. 10). Number of rays per mm, counted similarly, in-



Figs. 7—12. Wood characters (continued). 7, tangential diameter of procumbent ray-cells (µm); 8, number of vessels per mm in tangential direction of the outermost xylem formed in each treatment; 9, number of fibres per mm in the outermost xylem; 10, number of axial parenchyma per mm in the outermost xylem; 11, number of rays per mm of outermost xylem; 12, percentage of raytypes, uniseriate outlined, biseriate solid and 3 to 4- seriate striated. Signatures see Figs. 1—6

creased significantly with IAA (P 0.05). The other values of ray number differed insignificantly (Fig. 11).

It was interesting to note that most of the rays were uniseriate before culture and after culture with NAA (Fig. 13). After growth *in situ* (Fig. 14) the number of 3—4 seriate rays increased considerably but biseriate rays decreased. The percentage of biseriate rays increased remarkably with IAA (Fig. 15) and fairly with GA and K (Fig. 16). 3 to 4 seriate rays were very few in the wood formed by IAA, GA, NAA and before culture (Fig. 12).

From these observations it was evident that after 45 days of *in situ* growth the tangential diameter of the procumbent cells, number of vessels per mm in tangential direction and number of fibres per mm in tangential direction increased significantly. The other characters were almost similar to the preculture condition. *In vitro*, IAA increased the length of vessel members, diameter of axial parenchyma, number of vessels per mm, number of parenchyma per mm, number of rays per mm and percentage of biseriate rays. GA_3 increased the amount of secondary xylem, fibre length, diameter and number of axial parenchyma and percentage of biseriate rays. NAA increased the fibre lenght, number of fibres per mm and number of parenchyma per mm. Types of rays formed by NAA was almost similar to that found before culturing. Kinetin increased the amount of secondary xylem, length of fibre, and the percentage of biseriate and other multi-seriate rays, but decreased the member length, and the number of vessels per mm in tangential direction.

Increase of the number of rays by IAA, NAA etc. seems to be an unexpected phenomenon. But according to Barghoorn (1940a, b, 1941) and Steevs & Sussex (1972) a new ray arise from fusiform initial (1) by the cutting off of a new cell from the tip, which then functions as a ray initial; (2) by septation of an entire fusiform initial to form a vertical series of ray initials and (3) by the division of existing rays by intrusion of elongated fusiform initials. Further experiments with hormone combinations at different pH and carbohydrate levels are expected to provide us with informations on the requisite for differentiation of cell types of xylem.

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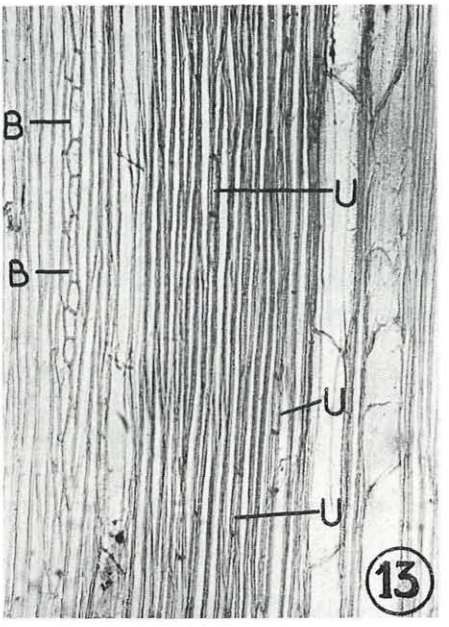

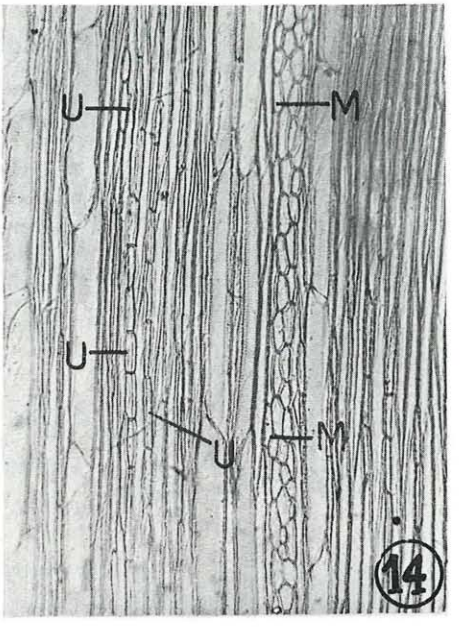

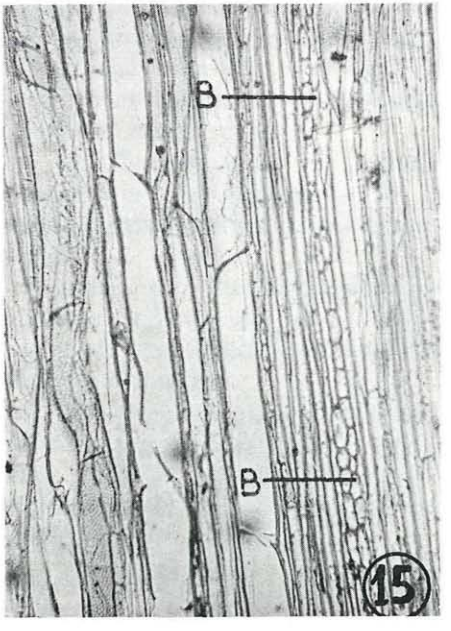

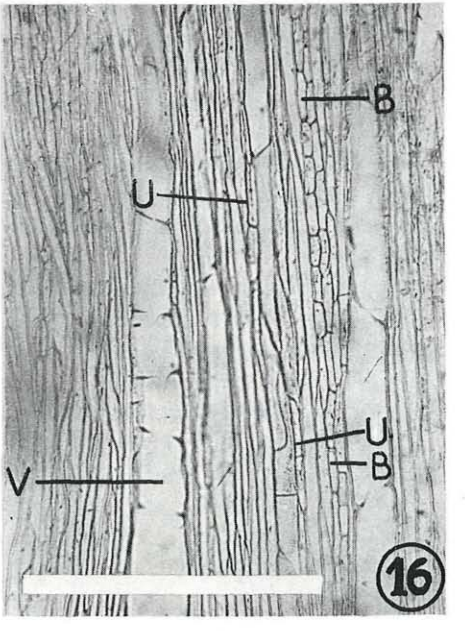

Figs. 13—16. Photomicrograph showing rays and vessels after *in situ* and *in vitro* growth for 45 days. 13, wood formed in NAA, mostly uniseriate rays (U) and biseriate rays (B); 14, wood formed *in situ*, showing mostly uniseriate (U) and 3 to 4-seriate rays (M); 15, wood formed in IAA showing mostly biseriate rays (B) and the longest vessel members; 16, wood formed in Kinetin, showing mostly uni- and biseriate rays (U, B) and the shortest and broadest vessel members (V). The length of the white mark represents 100 μm.