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IN VITRO EFFECT OF HORMONES ON THE BARK OF *PLUMERIA RUBRA* LINN. VAR. *ACTUIFOLIA* BAILEY

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Young bark (with cambium) of *Plumeria rubra* Linn. var. *acutifolia* Bailey was cultured in solid media (i) without hormone, (ii) Kinetin (K), (iii) with GA_3 and (iv) with IAA at concentrations of 0.05, 0.10, 0.15 and 0.20 mg per litre. The nutrients of the media were fed laterally through the cambium zone. The amount of the phloem zone was increased considerably by GA_3, less by K and IAA. The lignified secondary wall of the pericyclic living fibres was dissolved by each of the hormones. Sieve tube member length decreased in all treatments, more in higher concentrations. K decreased the frequency of sieve tubes (most at 0.10 mg/ℓ) and increased the frequency of parenchyma cells (optimum at 0.10 mg/ℓ). GA_3 also favoured formation of parenchyma cells and decrease of the frequency of sieve tubes, progressively with the increase of concentration. IAA also increased the parenchyma cell frequency progressively with concentration and decreased slightly the sieve tube frequency. IAA and GA_3 increased ray frequency and decreased parenchyma cell diameter, much at high concentration. But K had less effect on ray frequency and increased parenchyma cell diameter progressively with concentrations.

Bark (pharmacognostic) of *P. rubra* Linn. var. *acutifolia* Bailey has medicinal properties. It is used in diarrhoea and is employed as rubifacient in rheumatism and as purgative (CHOPRA, NAYAR and CHOPRA, 1956). The main tissues of *Plumeria* bark are periderm, cortex, pericyclic fibres, phloem tissues and a few layers of the cambium zone.

Auxins are reported to have profound effect on the differentiation of sclerenchymatous cells (AL-TALIB and TORREY, 1959, 1961; SIRCAR and CHAKRAVERTY, 1960; ATAL, 1961; STANT, 1963). Control of phloem differentiation by auxins is also reported (ESCHRICH, 1953; WALKER, 1960; LA MOTTE and JACOBS, 1963). But questions regarding the specificity of hormone types, time of supply and concentrations required for the differentiation of different tissue elements of the bark have not yet been well attended. The present paper is an attempt in this direction.

MATERIAL AND METHODS

The tree was growing in the University garden. The bark from growing branches was

collected at a distance of about 2 to 3 cm from the tip, where the diameter of the branch was 2.3 to 2.5 cm. The bark pieces, approximately 8 X 4 mm, were placed aseptically on solid culture media with the cambium downwards. Media contained ingredients suggested by SCHENK & HILDEBRANDT (1972), excepting 2,4-D, without hormone and with three different hormones, Kinetin (6-furfuryl amino purine), IAA and GA_3, at concentrations of 0.05, 0.10, 0.15 and 0.20 mg per litre. To the media 3% sucrose was added and pH was adjusted at 5.8. 0.9% agar solidified the media.

Ten bark pieces from the same level of the treated pieces were preserved in FAA, without treatment. The anatomical characters of these pieces would be considered as before culture characteris for comparison with the developments in cultured pieces.

Fibre characters were studied every fifteenth day of growth. Other records were made after 45 days. Data was collected from about ten samples of each treatment and from ten bark pieces preserved before each treatment.

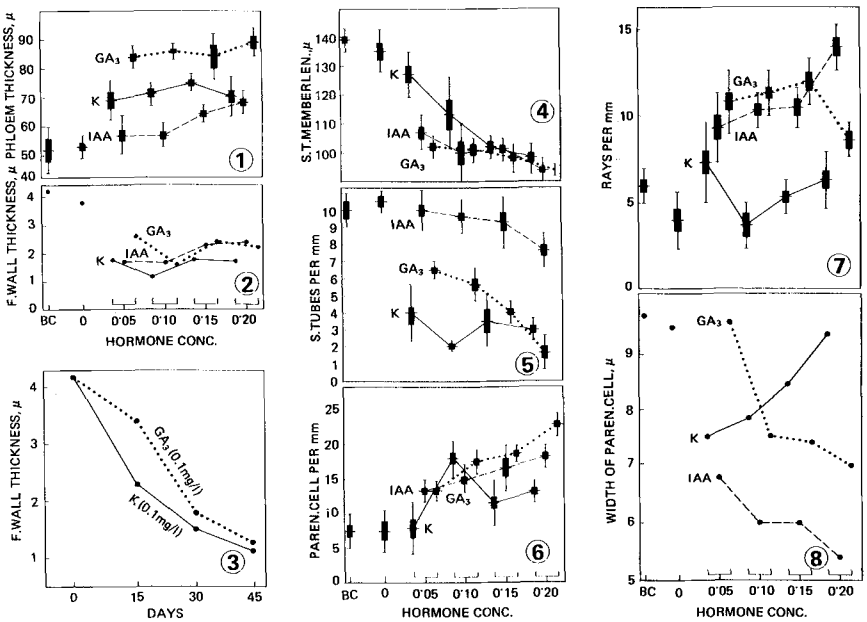
RESULTS

Inclusion of GA_3 in the medium resulted in considerable increase in radial dimension of the secondary phloem (zone thickness) in comparison to the before culture condition. The increase was progressively higher with higher concentration (Fig.1). Kinetin and GA_3 showed less increase of secondary phloem zone thickness, the former having an optimum level at 0.15 mg/ℓ and the latter increasing gradually (Fig.1). The medium without hormone showed no significant increase.

One interesting change was noticed in the fibres of the pericyclic patches. Before culturing, these cells had considerably thick walls and living protoplasts. In comparison to the wall thickness before culture, all hormonal media decreased it considerably after 45 days of culture, the most effect being at 0.10 mg per litre level. The medium without hormone had no remarkable effect (Fig.2). This reduction was marked when the media contained GA_3 or K (Fig.2). After 45 days, the secondary wall was almost totally removed (compare Fig.9 with Figs.10, 11 and 12). By observing at seven days' intervals, this wall thickness was found reducing gradually with all hormones, particularly with GA_3 and K (Fig.3). Thus in culture media, the activity of the protoplast brought about partial dedifferentiation, and the change was towards parenchymatization. After culturing with Kinetin for 45 days only about 10% of the fibre cells had a thin secondary wall, rest had a primary wall only (Fig.10). Before dissolution, the secondary wall was often separated from the primary layer and it formed inward folds at places (Fig.10).

Sieve tube members were shorter when cultured with hormones, but not without it (Fig.4). This decrease of length was very marked in samples cultured in media having GA_3 or IAA. These hormones probably favoured pseudo-transverse division of fusiform initials. The effect of concentration was gradual.

Number of sieve tubes (per mm of the innermost phloem attached to cambium, in tangential direction) was considerably lowered by the K and GA_3 (Fig.5). K



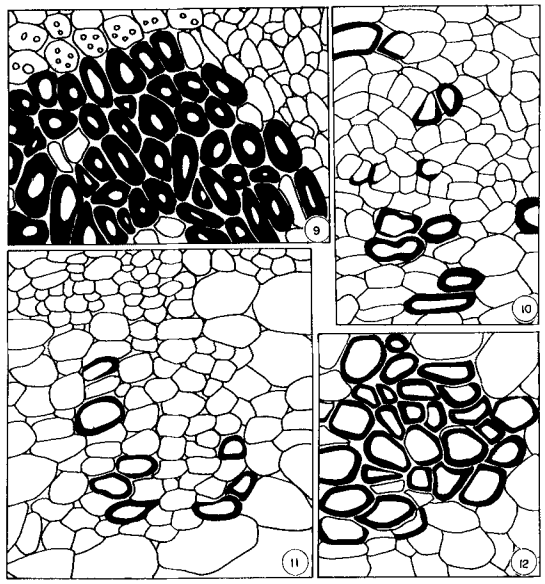
Figs.1, 2. Graph showing effects of different concentrations of IAA, Kinetin (K), GA_3 on radial thickness of secondary phloem zone (Fig.1) and thickness of pericyclic fibres (Fig.2) of barks as compared with the before culture thickness (BC) and the thickness after culturing without hormone (0).

Fig.3. Graph showing the gradual dissolution of fibre wall after every 15 days in GA_3 & K.

Figs.4–8. Comparison of the before culture conditions (BC) with the effects of culturing for 45 days in media containing different concentrations of K, GA_3 & IAA and without hormones (0). Fig.4, sieve tube member length (μ); Fig.5, frequency of sieve tubes per mm (tangentially); Fig.6, frequency of parenchyma cells per mm (tangentially); Fig.7, frequency of rays (all uniseriate) formed per mm tangentially in the innermost layers of secondary phloem; Fig.8, tangential width of parenchyma cells.

showed a most effective concentration (0.10 mg/ℓ) while GA_3 showed a gradual decrease. The medium without hormone showed no such change, and that with IAA decreased sieve tube frequency slightly at high concentration.

This decrease of sieve tube frequency corresponded to the increase of parenchyma cells (per mm) with Kinetin and GA_3 (Fig.6). The optimal level of parenchyma cell frequency (0.10 mg/ℓ) corresponded to the optimal level of sieve tube frequency for Kinetin. This process of parenchymatization, probably a change prior to callus formation, was evident in all cultured barks (except without hormone). Number of rays per mm (in tangential direction) also increased in barks cultured with hormones, but decreased without hormones. This was significantly high with IAA and GA_3. IAA showed gradual increase with concentration. GA_3 showed an



Figs.9–12. Transections through pericyclic fibre patches of barks before culturing (Fig.9) and after culturing for 45 days in K (Fig.10), GA_3 (Fig.11) and IAA (Fig.12).

optimum level (0.15 mg/ℓ) (Fig.7). Width of parenchyma cells was reduced in barks cultured with hormones, probably due to rapid longitudinal division (Fig.8). IAA was most effective even at low concentration. GA_3 effected at high concentrations. But K affected gradually less at high concentrations.

DISCUSSION

Culture of bark and the study of the activity of cambium in relationship to the differentiation of phloem is one of the least attended field of research. Regarding the effect of hormones on the formation of phloem from cambium, experiments (WAREING, 1958; WAREING, HANNEY and DIGBY, 1964; CUTTER, 1971) showed that a combination of IAA and GA_3 applied *in vivo* produced normal secondary phloem. In other experiments (BALATINECZ and FARRAR, 1966; DIGBY and WAREING, 1966), IAA favoured wood formation and GA_3 favoured differentiation of secondary phloem. In case of *Plumeria* also IAA was found to favour wood formation (SEN, BHAUMIK and DATTA, in press). In the present study GA_3 increased phloem quantity. Thus these two types of vascular tissues appear hormone-specific.

Now, the natural question is, what are the factors that control differentiation of different "elements" within these tissues from cambial initials. Practically little light on this question is available in literature. The present preliminary work revealed the following facts:

1. GA_3 IAA and Kinetin favoured parenchymatization, all probably having

optimum levels.

2. In experiments of LA MOTTE and JACOBS (1963) IAA favoured formation of sieve elements. In the present experiment, IAA produced almost normal quantity of sieve tubes, while other hormones decreased considerably.
3. All these three hormones (significantly GA_3 and IAA at low concentration, K at high concentration) decreased the length of sieve elements, probably by increasing the frequency of pseudo-transverse division in initials (commonly encountered in axial initials).
4. Decrease of parenchyma cell diameter with GA_3 and IAA corresponded to the increase of cell frequency, particularly at higher concentration. K had no such relation and increased cell size with increase of concentration.
5. IAA and GA_3 increased ray frequency probably by inducing divisions in fusiform initials.

According to WETMORE and RIER (1963), a balance between sucrose and auxin may be critical in the differentiation of phloem elements.

All hormone treatments in the present experiment favoured dedifferentiation. The medium without hormone did not support the process. Direction of supply of hormones (which was lateral in this case) might be an important factor for differentiation of axial elements.

Decrease of axial parenchyma cell length (probably by increased pseudo-transverse division) and increase of rays (probably by increased transverse division of fusiform initials) are related phenomena (increase of transverse division) favoured by GA_3 and IAA, supplied laterally. But longitudinal division of axial parenchyma cells (Figs. 10 and 12) was also favoured by GA_3 and IAA supplies laterally.

Experiments with radio-active isotopes showed that protein synthesized in the cytoplasm are regularly transported into the cell wall and is probably involved in orientation of microfibrils (MÜHLENTHALER, 1967). This synthesis is no doubt an enzymatic process, which is controlled by hormones.

Experiments on the differentiation of sclerenchyma cells suggested that hormonal factors affect sclerenchyma cell formation, play a role in determining where and when sclereid initials will develop and may inhibit sclereid formation at certain concentrations (AL-TALIB and TORREY, 1959, 1961). In experiments on jute by SIRCAR and CHAKRAVERTY (1960), GA increased the quantity of fibre. Individual fibers of GA-treated plants of jute and hemp were longer and wider (ATAL, 1961, STANT, 1963). The length of fibers increased considerably in GA-treated plants (STANT, 1961). Thus hormones either inhibited or promoted differentiation of sclerenchyma.

Dedifferentiation of pericyclic fibres by dissolution of differentiated thick lignified secondary wall in the present experiment with nutrient media having GA or IAA, is an interesting effect of hormones and is probably contradictory to the

observations of SIRCAR and CHAKRAVERTY (1960), ATAL (1961) and STANT (1961, 1963). Results of AL-TALIB and TORREY (1959, 1961) are probably at par with the present observations. Two possibilities may explain these contradictory evidences:

(1) An optimum concentration of hormones (specific for a tissue or a plant) might favour sclereid or fibre formation, below or above which the differentiation might be checked or reverted. The present data showed an optimum for dedifferentiation, not for differentiation.

(2) At certain stages of development hormones might be helpful in differentiation, prior to or after which some physiological mechanism might render the hormones helpful, or useless or inhibitory to differentiation.

According to BONNER (1965) hormones may turn off or on individual or whole sets of genes in appropriate cells eliciting the production of characteristic enzyme molecules and in appropriate instances setting a cell or cells on a new pathway of development. The differential synthesis of enzymes are basic processes of cell differentiation (STANGE, 1965).

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