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IN VITRO EFFECT OF SUCROSE FEEDING IN THE DIFFERENTIATION OF WOOD ELEMENTS OF *PLUMERIA*

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Wood with cambium of *Plumeria rubra* Linn. was cultured in modified Schenk and Hildebrandt medium for 75 days. Change in dimension and frequency of cells was compared with that grown *in situ* for 75 days. Feeding of 41% sucrose *in vitro* brought about the same type of differentiation as occurred *in situ*, where non-reducing sugar percentage increased (from 0.0004 before culture to 0.0032 after 75 days' growth *in situ*). Sucrose favoured enlargement of axial elements and numerical increase of all lignified elements.

The cambium consists of two types of initial cells, radial and axial, derived fundamentally from a homogeneous type of meristem, the procambium. This tissue produces a very complex organisation, secondary xylem, consisting of a heterogeneous assemblage of cells, functioning harmoneously. How a genetically homogeneous group of cells cuts off such a heterogeneous assemblage, is a problem to the morphogeneticists.

Culture methods were applied for determining the factors inducing xylem and phloem (WAREING and ROBERTS, 1956; WAREING, 1958; LARSON, 1964; WAISEL and FAHN, 1965). Different hormones were tried for detecting whether the cell types are hormone-specific in differentiation (MAITY, NAG and DATTA, 1976; SEN, BHAUMIK and DATTA, 1975; SARKAR, RAVINDRAN and DATTA, 1976). Effects of acidity levels on the cell types of xylem were also examined (DATTA, CHAKRABARTI and DATTA, 1975). The present attempt is to study the effect of sucrose in different concentrations on the differentiation of cell types of xylem.

MATERIAL AND METHODS

The plant (*Plumeria rubra* Linn.) was growing in the University garden. Seventyfive young branches were selected in a tree, sixty of which were cut and brought to the laboratory. After removing the bark, wood pieces (8 × 4 mm), covered by the cambium zone were cut from those sixty branches at a definite distance (60 cm) from the tip, where their diameter was about 5 to 6 cm. The pieces were placed asceptically with the cambium downwards and attached to the static medium (0.9% agar) of SCHENK and HILDEBRANDT (1972) with 0.1 mg/l of IAA (in place of 2,4-D) with different sucrose (non-reducing sugar) levels (2%, 3%, 4% and 5%) in 50 ml flasks. Replications were 15 for each treatment. The pieces with cambia attached to the media were grown for 75 days at 30°C in normal room light. Portions of wood pieces from the treated ones were preserved in FAA for future study of preculture wood. Fifteen remaining branches were tagged at a distance of 60 cm from the tip (from which distance the wood was cultured) in the same growing plant and were allowed to grow *in situ* for 75 days. During this period the temperature in the open field varied from 23.3–39.3°C (av. 30.9°C). Humidity was 26–90 (av. 37.9). Light intensity varied from 40.000 to 60.000 K Lux. ± 10.000 K Lux. and was 300 to 400 Cal. per cm^2 per day. After this period of growth the tag was left 90 cm behind the tip.

Anatomical characters were studied only of the superficial layer of wood (i.e. the most recently formed

wood) (1) after 75 days' growth *in situ*, (2) after 75 days' growth in different sucrose levels of culture media, and (3) preculture woods (preserved ones). Data were collected from 15 samples of each of the treatments, 15 samples of preculture wood and 15 wood pieces collected from the tagged portions. Mean values, standard errors and standard deviations were calculated from 60 readings (15 X 4) for each character.

Sugar estimation of wood was performed from *in situ* grown wood, 30 cm, 60 cm and 90 cm below the apex. For estimation of sugar the wood was crushed and extracted with 80% alcohol. Interfering colloids were removed by precipitating with basic lead acetate (5%). It was followed by neutralisation with CH_3COOH and then removal of excess lead acetate (if any) by adding saturated Na_2HPO_4. Reducing and total sugars were estimated from two portions, one before hydrolysis and the other after hydrolysis. For both the purpose, Cu-reagent (23 gms of $CuSO_4$, 5 H_2O/litre of H_2O + 35 gms of anhydrous $NaHCO_3$, 28 gms of anhydrous Na_2CO_3, 17 gms of Rochell's salt crystals, 1 gm of KIO_3/litre of water), a solution (10 gms of potassium iodide, 19 gms of potassium oxalate/litre of water) and normal H_2SO_4 were added successively. Usual titration with N/100 $Na_2S_2O_3$ using starch solution as indicator and a blank titration were done side by side.

The thiosulphate values of sugar solution were subtracted from the blank titration values. The remainder gave the value for thiosulphate required to titrate I_2. The factors for these values were then obtained from Vender-Plank caliberation chart. The thiosulphate values were then divided by the factor which gives the amount of sugar in 5 ml solution. The percentages were then calculated.

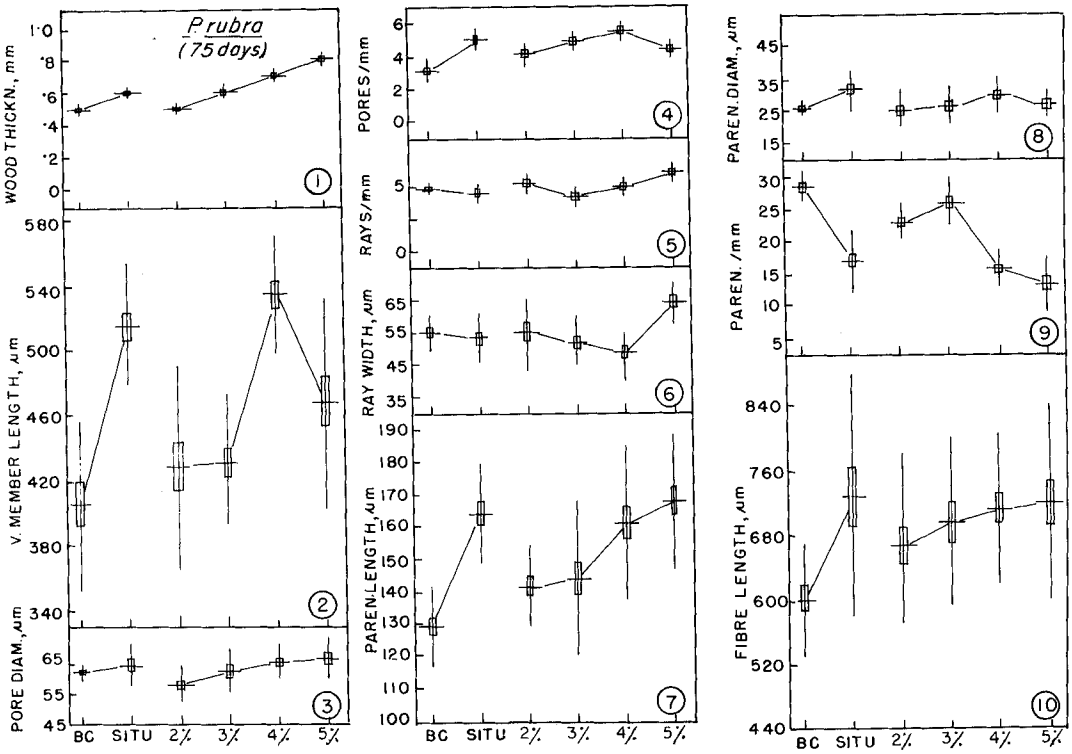
RESULTS AND CONCLUSION

Radial thickness of the wood increased gradually with sucrose concentrations and at 5% level it was much higher (almost triple) than the increase *in situ* during the same period of growth (75 days) (Fig.1). It is apparent in the graph, that the widths at 3% level and *in situ* were almost the same. The width at 4% was highly significantly higher than at 3% or *in situ* ($P < 0.01$) and significantly lower than at 5% ($P < 0.05$).

The length of vessel members also increased in all levels of sucrose concentration and at 4% level the length was highest, slightly higher (insignificant $P > 0.05$) than in the tissue grown *in situ* for 75 days (Figs.2, 11, 12). But the difference between the length at 4% and the nearest value (at 5%) was highly significant ($P < 0.01$). The vessel diameter also increased progressively with rise of concentrations, and was much higher at 5% level than *in situ* (Fig.3). The frequency of vessels responded with the sucrose concentration and probably had an optimum concentration (4%) where the rise was significant ($P < 0.05$). In the *in situ* grown tissue there was a similar rise frequency compared to that before culture ($P < 0.05$) (Fig.4).

Ray frequency change was not remarkable except only a slight decrease *in situ* and at 3% level of sucrose (significant, $P < 0.05$) and a gradual rise with higher concentrations, 4% (highly significant, $P < 0.01$) and 5% (Fig.5). Width of ray decreased *in situ* and at 4% level of sucrose (just significant), which was optimum for the increase of vessel member length and vessel frequency, but was broadest at 5% level (Figs.6, 12, 13).

Length of parenchyma cells increased *in situ* and gradually with concentrations of sucrose (difference between 3% and 4% significant, $P < 0.05$) (Fig.7). Length *in situ* and at 4% were almost similar ($P > 0.05$). Breadth of parenchyma increased *in situ* and at the optimum level of 4% sucrose (Fig.8). The difference between the breadth before culture and at 4% was highly significant ($P < 0.05$). The breadth *in situ* and at 4% were similar ($P > 0.05$). Frequency of parenchyma cells showed a gradual decrease with increase of concentrations as well as *in situ*, (difference between 3% and 4% highly significant, $P < 0.01$) but the medium concentration (3%) favoured an increase of the frequency (Fig.9). The frequency *in situ* and at 4% were almost similar ($P > 0.05$). Fibre length increased *in situ* as well as by increase of sucrose



Figs.1–10: Measurements of the wood and its elements of *Plumeria rubra* Linn. before culturing (BC), after 75 days' growth *in situ* (SITU) and *in vitro* with 2%, 3%, 4% and 5% sucrose.
Indications: Horizontal lines indicate mean values. The ends of vertical lines on their each side are equal to the standard deviation. The distance between the mean line and the upper or lower limit of boxes is equal to standard error.

Fig. 1. Radial thickness of the wood in mm.
Fig. 2. Length of vessel members in µm. The length at 4% was almost similar to the length *in situ*.
Fig. 3. Diameter of vessels (pores) in µm. Pore diameter increased *in situ* as well as by increase of sucrose *in vitro*.
Fig. 4. Frequency of vessels (pores) i.e. number of pores per mm of the circumference of the most recent wood. Pore frequency per mm increased *in situ* as well with rise of sucrose *in vitro*.
Fig. 5. Frequency of ray i.e. number of rays per mm of the circumference of the most recent wood. Ray frequency per mm decreased *in situ* and at 3% sucrose *in vitro*. But the frequency rose with addition of more sucrose.
Fig. 6. Ray width in µm. Ray width was reduced *in situ* and *in vitro* upto 4% level of sucrose.
Fig. 7. Length of parenchyma cells in µm. Parenchyma length *in situ* and with rise of sucrose *in vitro* was much more than in preculture wood.
Fig. 8. Diameter of parenchyma cells in µm. Diameter increased *in situ* as well as with addition of sucrose *in vitro* upto 4%.
Fig. 9. Frequency of parenchyma cells i.e. number of parenchyma cells per mm of the circumference of the most recent wood. Parenchyma decreased *in situ* and *in vitro*.
Fig.10. Fibre cell length in µm. Length increased *in situ* and *in vitro*.

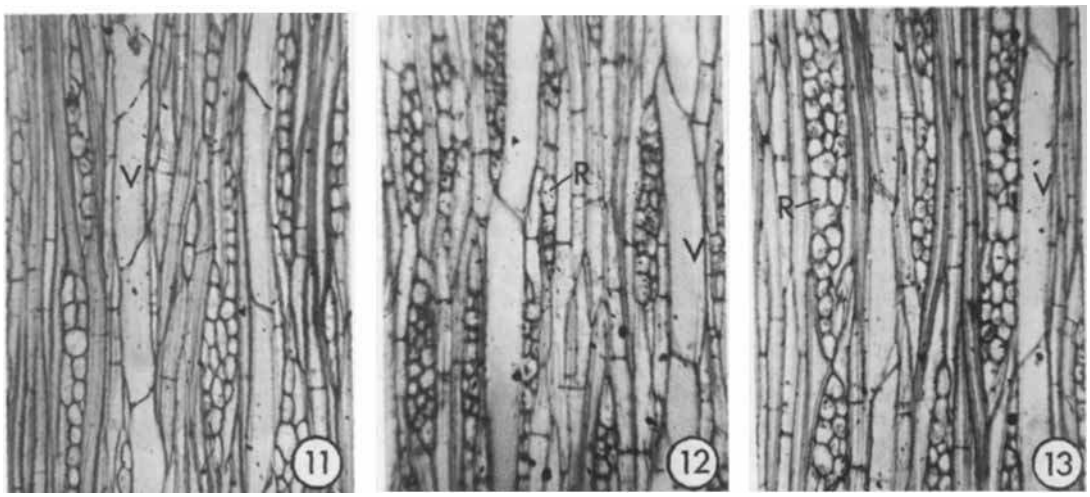
concentration (Fig.10). The difference between lengths before culture and after culture at 4% sucrose or after growth *in situ* was highly significant ($P < 0.01$).

Enlargement of cell types and increase of the frequency of cell types *in situ* almost perfectly correspond to a similar differentiation in optimum sucrose. This suggests a possibility of a gradual increase of non-reducing sugar in the stem with maturity. Result of sucrose estimated in the wood *in situ* collected from different distances from the tip is shown in Table 1.

Table 1. Percentage of sugar in weight in the wood *in situ*.

Distance from the tip	Total sugar	Reducing sugar	Non-reducing sugar
30 cm	0.0244	0.0240	0.0004
60 cm	0.0280	0.0272	0.0008
90 cm	0.0314	0.0272	0.0032

Thus the effect of sucrose feeding coincided almost perfectly with the normal behaviour of *in situ* development of elements. Similar to the *in situ* condition of growth, radial thickness of wood, size of vessel members (in length and breadth), vessel frequency, parenchyma cell size (in length and breadth) and length of fibres in general increased by feeding sucrose, although an optimum concentration in certain cases was evident (Figs.11–13). Increase of vessel frequency roughly corresponded to a decrease of parenchyma cell frequency, both *in situ* and at the sucrose concentration optimum for the growth of other elements. On the whole sucrose favoured the enlargement of all axial elements and increase of the abundance of axial lignified elements, at a certain optimum concentration, but did not favour the enlargement of radial cell types.



Figs.11–13. Photomicrograph showing the shortest vessel members (V) in the preculture wood (Fig.11), longest vessel members (V) and narrowest rays (R) after 75 days' growth *in vitro* in a 4% sucrose medium (Fig.12) and broadest rays and more or less long vessel members after 75 days of growth *in vitro* in a 5% sucrose medium (Fig.13).

The relationships of cambium activity with day length (WAREING and ROBERTS, 1956; LARSON, 1964; GUNNING, PATE and BRIARTY, 1968), with temperature (WAISEL and FAHN, 1965) and with auxin activity (LARSON, 1964), were demonstrated. The relationship of differentiation of individual elements with the different types of auxins (SEN, BHAUMIK and DATTA, 1975; MAITY, NAG and DATTA, 1976; SARKAR, RAVINDRAN and DATTA, 1976) showed that different hormones favour the differentiation of different cell types of secondary xylem. Usually IAA, GA_3 and Kinetin favoured the formation of all soft tissues. IAA increased the frequency of vessels in *Adhatoda* (MAITY, NAG and DATTA, 1976), NAA increased the fibres of *Adhatoda* but effected insignificantly in other plants. In experiments with different pH levels (*in vitro*) an increase of pH reduced the frequency of vessels but increased the total amount of secondary xylem. The other data were similar to that differentiated *in situ*, where the pH level was higher than the preculture xylem.

In the present experiments also, feeding of sucrose *in vitro* brought about a type of differentiation which was almost similar to that occurred *in situ* by a rise of non-reducing sugar percentage from 0.0004 to 0.0032 by 75 days'. Sucrose favoured differentiation of more vessels than parenchymatous cells.

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