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Histological Study on Adventitious Root Formation in Stem Cuttings of Young Durian (*Durio zibenthinus* Murr.) Seedlings

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ABSTRAK

*Keratan yang diperolehi dari anak pokok durian (*Durio zibethinus *Murr.) berakar dengan mudah. Kajian histologi yang dijalankan ke atas zon perakaran keratan-keratan ini mendapati bahawa dinding sel-sel penghasil adalah nipis. Keadaan ini amat sesuai bagi sel-sel ini untuk berubah kembali dengan mudah kepada bentuk yang tidak khusus dan akhirnya bersedia membentuk primordia akar. Kajian ini juga menunjukkan bahawa ranting-ranting dari stok muda belum lagi membentuk sebarang halangan mekanikal yang dapat menyusahkan pembentukan akar 'adventisi'.*

ABSTRACT

*Cuttings obtained from young durian (*Durio zibenthinus *Murr.) seedlings rooted very readily. A histological study carried out on the rooting zone of these cuttings showed that cell walls of the derivative cells appeared relatively thin which probably is desirable for these cells to readily revert back to the undifferentiated state and ultimately become root primordia. It also showed that the stem had yet to develop any form of mechanical barrier that may restrict the development of adventitious roots.*

INTRODUCTION

The formation of an adventitious root begins with the differentiation of derivative cells in the stem to form meristematic foci. Inside each meristematic focus the root initial is formed which later develops into a root primordium and finally emerges as a rootlet.

In most plants, parenchyma cells are the ones capable of reverting to meristematic activity and it is generally these cells which start to divide to form root initials (Tukey 1979). There are cases, however, where root initials arise from the cambium and medulla (Tarasenko 1965., Bose *et al* 1975). Likewise, not all parenchyma cells become meristematic and produce roots. Usually in the stem, parenchyma cells near or just outside the phloem, in the *phloem rays* or in the interfascicular region between vascular bundles, most often produce root initials (Tukey 1979).

Following the differentiation of derivative tissues, active meristematic foci appear within them. Inside these foci, the root initials, conical in shape, begin to form. Then differentiation of phloem and xylem of the root initial takes place and subsequently becomes structurally associated with the closest vascular bundle of the parent stem. About this time, a root growing tip is formed which aids the embryo rootlet to emerge through the bark and epidermis (Tarasenko 1965).

In case of rooting, plants are often classified as 'easy-to-root' (ETR) or as 'difficult-to-root' (DTR) species. There are situations, however, when cuttings of DTR species root easily, such as, when collected from juvenile or rejuvenated stocks. The main difference between the two types is that, in ETR species and perhaps juvenile stocks of DTR species, the thin-walled derivative cells show signs of recent

division and they contain cytoplasm and organelles of which nuclei are clearly visible. In contrast, the derivative cells of DTR plants are usually found between fibres, elongated periclinally and are in the process of being transformed into thick-walled sclereids lacking living contents (Beakbane 1969). Furthermore, in ETR plants, the root initials arise in the secondary phloem usually in association with rays. Soon, rays make contact at their distal ends with living cells by means of cytoplasmic strands. In cuttings of DTR plants, the root initials attach to the ends of fibres, sclereids or other elements without living protoplast (Beakbane 1969). Other possible factors that may delay the formation of root initials are i) the presence of a greater extent of tissue sclerification approaching an uninterrupted layer of sclerenchyma in the pericycle, and ii) when derivative tissues are less conspicuous (Bose *et al* 1975).

MATERIALS AND METHODS

The stock plants used in this study were unknown seedlings obtained from Malaysia and grown at Wye College. They were grown individually in 25 cm black plastic pots containing a peat based compost, kept in a heated glasshouse maintained at a minimum temperature of 25°C, 12-hour day length at an intensity of 150 to 160 $\mu E/m^2$,/second. These seedlings were used as stock plants when they were about two years of age. Between 8 to 10 cm long, healthy shoots were detached from the stock plants using a sharp scalpel. Following their detachment, leaves were removed leaving only two or three near the shoot tip. If the remaining leaves were too large they were trimmed to about one half their length. Bases of these cuttings were individually dipped for 15 seconds in a solution of 50% alcohol containing 5000 ppm indolebutyric acid (IBA), to a depth of 1.0 cm and surface-dried for five to ten minutes. Prior to planting, cuttings were quickly dipped in a solution of 0.4% 'nimrod' fungicide up to about 4 to 5 cm from the base, to reduce rotting.

Various cutting pieces, including unrooted stem segments and calli from cuttings were collected for histological studies to study the anatomical changes that take place during the rooting process. These samples were collected at weekly intervals.

Samples were kept in vials containing excess FAA and fixed under vacuum for at least 24 hours. The subsequent processes that is dehydration in a series of solutions with varying proportions of water, alcohol and tertiary butanol, infiltration with wax, embedding in wax, and mounting were done in the normal way as described by Purvis *et al* (1966).

Transverse sections 15 µm thick were made using a sledge microtome and temporarily mounted on a glass slide with Haupt's adhesive. Prior to staining with Safranin 0 and counter staining with fast green, wax from the sections was removed by immersing the slides completely in low-sulphur xylene until the wax had fully dissolved. The subsequent staining procedures that is rehydration in descending strengths of alcohol, staining with Safranin 0, dehydration in ascending strengths of alcohol, immersion in a 50:50 mixture of alcohol/clove oil, counter staining with fast green, and permanent mounting in euparal, were done in the usual way as described by Purvis et al (1966).

From each section, representative 'fields of view' showing the rooting process were examined and photomicrographs of these 'fields' were taken with an Orthomat microscope camera, attached to a Leitz microscope.

RESULTS AND DISCUSSION

Anatomically, softwood cuttings obtained from very juvenile stock plants have yet to develop any form of mechanical barriers, such as thick layers of cork tissue outside the cortex, or a sclerenchymatic sheath peripheral to the primary phloem. Cell wall of the parenchymatous cells, especially the cortex, appeared relatively thin (Plates I to V). The relationship between stem anatomy and adventitious rooting may have an important bearing on the capacity of these stems to form adventitious roots.

From the histological examinations made on the samples taken immediately after cuttings were made (week 0) (Plate I) and one week after propagation (Plate II), no pre-formed root initials could be traced. However, after the cuttings had been propagated for two weeks, root primordia became conspicuous (Plate III)

and by the third week, some roots emerged (Plate IV); others were already fully initiated but had yet to develop into roots.

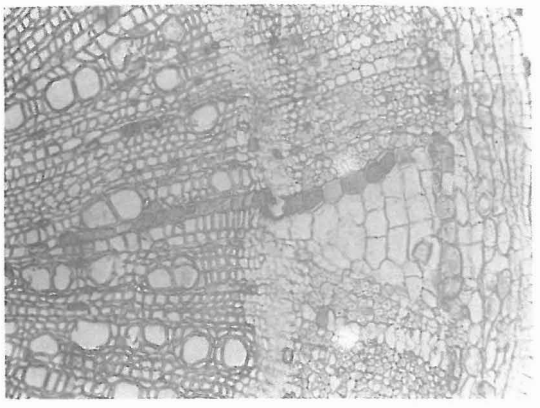

Plate I Transverse section of the stem immediately after cutting was made (× 40)

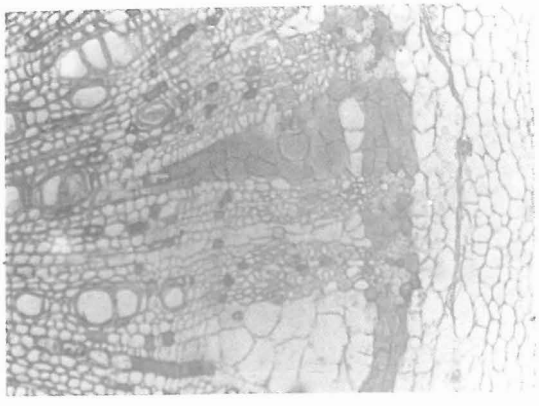

Plate II One week after cutting was made (× 40)

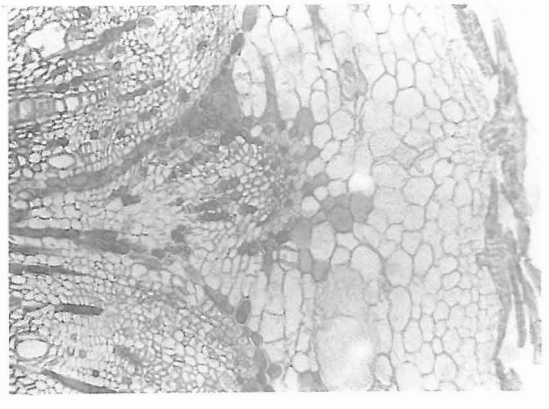

Plate III Root primordium from cells in the pericyclic zone (× 100)

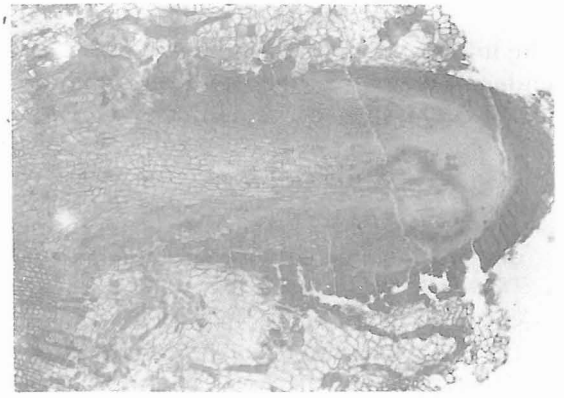

Plate IV Adventitious root (× 40)

These roots were believed to be initiated from the living cells in the pericylic zone, near the fibre groups and in association with ray parechyma and cortical cells. When callus was present, root could also be initiated from the callus cells (Plates V and VI).

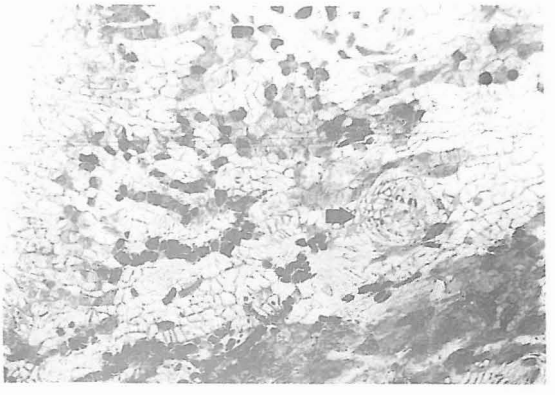

Plate V (× 40)

Plate VI Root initial (arrow) from the callus cells formed at the base of softwood stem cutting

CONCLUSION

The information from this study forms a useful understanding for further studies on the rooting of cuttings from less juvenile materials.

REFERENCES

BERKBANE A.B. 1969. Relationship between Structure and Adventitions Rooting. *Comb. Proc. Int. Plant Prop. Soc.* **19:** 192-201.

BOSE, T.K., T.P. MUKHERJEE, and T. ROY. 1975. Standardization of Propagation from Cutting under Mist. I. Effect of Type of Wood and Size of Cutting on Root formation. *Punjab Hortic J.*, **15:** 139-143.

PURVIS M.J., D.C. COLLIER, and D. WALLS. 1966. Laboratory Techniques in Botany, 2nd edn, London: Butterworth 439 pp.

TARASENKO, M.T. 1965. Morphological and Anatomical Features of the differentiation of Tissues and Organs in Vegetative Propagation of Higher Plants. I*zvestiya Timir. Sel'skok. Akad.* **3:** 58-70.

TUKEY, H.B. 1979. Back to the Basics of Rooting. *Comb. Proc. Int. Plant Prop. Soc.* **29:** 422-428.

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