ANATOMICAL STUDY ON ROOT FORMATION IN *ACACIA MANGIUM* STEM CUTTINGS

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DARUS HAJI AHMAD. 1989. Anatomical study on root formation in *Acacia mangium* stem cuttings. The root formation in 1-*y*-old *Acacia mangium* stem cuttings was first detected in the phloem region, very near to the cambial layer and in between the actively elongated medullary rays. The cortical cells were the first to divide and then the medullary rays broadened. Subsequently groups of smaller cells which had meristimatic capability appeared in the phloem region. These smaller cells then developed into root initials. After further cell division, the root initial together with the newly developed vascular elements, formed the compact spherical root primordium, which grew outward through outer layers to become adventitious roots.

Key words: *Acacia mangium* - root initial- root primordium - adventitious roots

Introduction

It is reported that the root primordia of stem cuttings are initiated from a wide range of stem tissues, all of which contain cells capable of differentiation. Haissig (1974) reported that the cambium, phloem and pericycle have most often been observed as the seats of root primordial initiation in stem cuttings whereas the cortex, pith and xylem have been noticed as being less important for root formation. Root primordia also originate from the callus tissue which develop at the cut surface of stem cuttings. The callus is considered important for some woody species in the root formation because of the capability of its cells to divide and to redifferentiate. This experiment was aimed at investigating the origin of adventitious roots in stem cuttings of 1-*y*-old seedlings of *Acacia mangium.*

Materials and methods

The cutting materials were taken from the middle portion of seedling stems. They were treated with a hormone rooting powder (Seradix 3) and planted into humidified rooting chambers containing a mixture of Irish

sphagnum peat and sand in equal proportions. The air temperature in rooting chambers was maintained on average at 21.2°*C* during the day and 16.4°*C* during the night which was about 4°*C* - 12°*C* less than the temperature of the rooting medium. Intermittent mist sprayers maintained the air humidity inside rooting chambers at 73.4% during the day and 77.4% during the night. Cuttings were also given an 18 *h* photoperiod at the light intensity of about 20,000 - 22,000 *lux* using white fluorescent tubes.

Two cuttings were taken out every three days and carefully washed to remove the growing medium. They were cut into smaller pieces, approximately 30 to 50 *mm* long, and fixed using FAA solution (Berlyn & Miksche 1975). The samples were then dehydrated, solvent infiltrated, wax impregnated, embedded in paraffin wax and finally sectioned to 15 - 20 μm thick using a rotary microtome and mounted on glass slides using Haupts adhesive with 3% formalin and left on a warm plate for 24 *h*. Finally they were stained with Safranin and Fast Green Method. Microscopic examination was carried out using a 'Wild' stereo microscope.

Results

Anatomical characteristics of 1-y-old stems

Figures 1 and 2 are the longitudinal and transverse sections of *A. mangium* stem cuttings taken from the middle portion of a stem of 1-y-old greenhouse-grown seedlings. They show a continuous ring of thick-walled fibers (sclerenchymatous sheath), encircling the vascular bundles. Outside this sheath lies a zone of cortex with larger cells and stained green. Between the phloem and xylem is a cambium zone, stained very dark green. The medullary rays pass through the xylem, cambium and phloem zones.

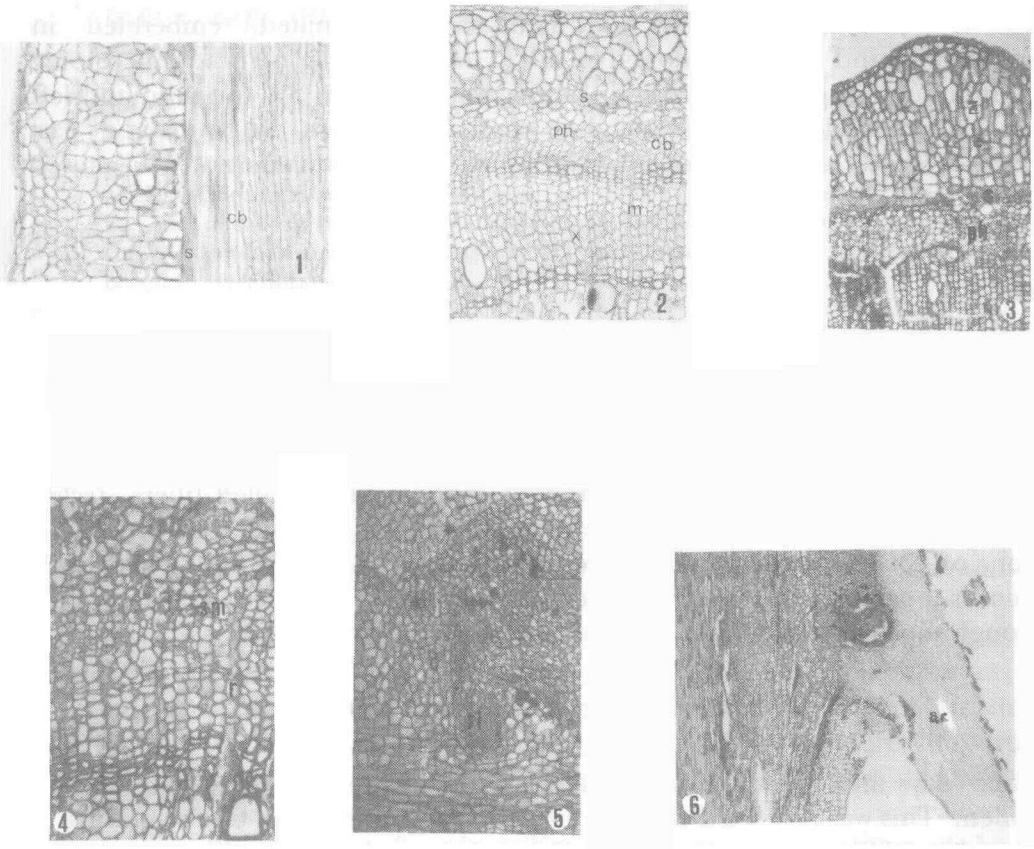
Formation of the root initials and primordia

Six days after planting, the basal zone of the stem cutting was already swollen. This was restricted to the area from the cut surface of the cutting to the surface of the rooting medium. Figure 3 shows that the swelling was caused by the cell division and the enlargement of cortical cells. No callus formation was observed.

At the same time, medullary rays elongated and formed slightly bigger cells in the phloem region, close to the sclerenchymatous tissues (Figures 3 & 4). The cambium cells also actively divided and produced many new cells. Groups of smaller cells with dense cytoplasm appeared between the elongated medullary rays (Figure 4). These meristimatic cells continuously divided in the phloem region and then developed into root initials (Figure 5). Three days later (day 9), new vascular elements, both phloem and xylem developed. After further cell

divisions, the meristimatic cells together with vascular elements formed the compact spherical root primordium in the phloem region (Figure 5).

The root primordia then extended outwards through the outer phloem, into the sclerenchymatous sheath, cortical tissues and the epidermis in order to appear as adventitious roots (Figure 6) after having undergone the cell division, elongation and differentiation to form the root apex.



Figures 1 - 6 (clockwise). Root formation in *Acacia mangium* stem cuttings: **1**. longitudinal section (66×) of a 1-y-old stem; **2**. cross section (66×) of a 1-y-old stem; **3**. cross section of a stem (33×) showing the swollen stem caused by the cell division and enlarged cortical cells; **4**. cross section of a stem (66×) showing elongated medullary rays; **5**. longitudinal section of a stem (33×) showing the root initials; **6**. longitudinal section of a stem showing an early stage of adventitious root development (ar= adventitous root, c = cortex, cb= cambium, e = epidermis, m = medullary ray, ph = phloem, r = elongated medullary ray, ri=root initial, s = sclerenchyma, sm = small meristimatic cells, x = xylem, z = enlarged cortical cells)

Discussion and conclusion

The present study shows that the anatomical characteristics of 1-y-old *A. mangium* stems are similar to the stem structure of 1-y-old *Acacia mearnsii* (Beakbane 1961) and other juvenile leguminous genera, such as *Cassia, Delonix* and *Castanea* (Nanda *et al.* 1969). Nanda *et al.* (1969) and Komissarov (1964) reported that the main cause of poor rooting in stem cuttings of many tree species was the presence of a thick-walled sclerenchymatous tissue surrounding the vascular cylinder which acted as a barrier to the penetration of water as well as mechanically obstructing the initiation and elongation of adventitious roots. However, in this study although a ring of sclerenchymatous tissues was present, surrounding the vascular cylinder but this layer did not obstruct the initiation and elongation of adventitious roots in *A. mangium* stem cuttings.

A. mangium stem cuttings passed through many changes during the rooting process. First, the base of cuttings became larger due to the enlargement of cortical tissues. This was reported by several researchers: for example, Haeman and Owen (1972) stated that cuttings of *Pseudotsuga menziesii* were swollen due to the proliferation of callus tissues 24 days after being planted in humidified rooting chambers.

Second, the medullary rays were activated and produced bigger cells in the phloem region. It seems that these medullary rays play a very important role in the formation of root initials in *A. mangium* stem cuttings. In fact, Nanda *et al.* (1969) have mentioned that in most "easy-to-root" genera, for instance, *Buddelia, Hibiscus, Rosa, Platanus, Prunus* and *Salix,* the cortical ends of the medullary rays broadened to form funnel-like structures which possessed meristimatic activity. Fahn (1982) also reported that adventitious roots in most woody plant stem cuttings were initiated by meristematic cells that were mostly situated in the cambial zone and sometimes in between the region of elongated medullary rays.

Third, groups of smaller cells with very dense cytoplasm suddenly appeared and actively divided in the phloem area, near to the cambial cells and in between the active medullary rays. After further division, these active meristimatic cells and newly developed vascular elements formed a compact spherical meristem of the root primordia. Komissarov (1964) also stated that the initiation of root premordia in *Osmanthus heterophylla* stem cuttings started in the area very near to cambial tissues and then grew through the sclerenchymatous ring to emerge from the stem.

This study shows that the root initiation occurred in the phloem region, very near to the cambial layers and in between the actively elongated medullary rays. It appears that cortical cells divided first and then medullary ray cells broadened followed by development of groups of smaller cells which had meristimatic activity. These cells later developed into root primordia and grew outward through outer layers to form adventitious roots. The

presence of a cylinder of sclerenchymatous cells within the stem of 1-y-old *A. mangium* seedlings was not a serious barrier to root formation and development.

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References

BEAKBANE, A.B. 1961. Structure of plant stem in relation to adventitious rooting. *Nature* 192: 954-955.

BERLYN, C.P. & MIKSCHE, J.P. 1976. *Botanical microtechnique and cytochemistry.* The Iowa State University Press, Ames, Iowa.

FAHN, A. 1982. *Plant anatomy.* Pergamon Press, Oxford, England.

HAEMAN, J.C. & OWEN, J.N. 1972. Callus formation and root initiation in stem cuttings of Douglas-fir (*Psuedotsuga menziesii* Mirb. Franco). *Canadian Journal of Forestry Research* 2: 121-133.

HAISSIG, B. E. 1974. Metabolism during adventitious root primordium initiation and development. *New Zealand Journal of Forestry Science* 4: 324-337.

KOMISSAROV, D. A. 1964. *Biological basic for propagation of woody plants by cuttings* (Translated by Shapido, Z. 1969). Israel Program for Scientific Translations, Jerusalem.

NANDA, K. K., ANAND, V.K. & Kumar, P. 1969. Rooting ability of cuttings of forest tree species and its relationship with tissue characteristics, nutrition and auxins. Paper presented at *Second World Consultation on Forest Tree Breeding,* Washington, August 7 - 16, 1969.