IN VITRO PROPAGATION OF *KHAYA IVORENSIS* FROM COPPICED SHOOTS

I Haliza*, MS Ahmad Fauzi, AR Siti Suhaila, H Nor Hasnida, A Nazirah & Y Muhd Fuad

Forest Research Institute Malaysia, 52109 Kepong, Selangor Darul Ehsan, Malaysia

Received January 2013

Khaya ivorensis, commonly known as African mahogany and belonging to the Meliaceae family is a medium hardwood tree. In Malaysia, this exotic species is planted along roadsides and in plantations. It is well known for its high quality wood. It is also used for traditional medicine against malaria and also for the prevention of the disease in Cameroon (Tepongning et al. 2011). *Khaya ivorensis* grows fast and produces large quantities of seeds but of limited germination capacity. Therefore, tissue culture technique, preferably using trees with good traits, can be an alternative method for germplasm conservation and also clonal propagation of this species. In this regard, coppicing shoots from hedged stumps are more recommendable as they are more juvenile and easier to be micropropagated compared with shoots taken directly from tree crown. The purpose of this paper is to provide preliminary information regarding the utilisation of coppiced shoots for in vitro micropropagating of *K. ivorensis.*

Figure 1 Coppiced shoots from hedged back stems of 3-year-old selected trees

Three-year-old selected *K. ivorensis* trees planted at the Forest Research Institute Malaysia were felled at approximately 1 m above ground level for stimulating the production of epicormic shoot from the remaining stem (Figure 1). Coppiced shoots, which were approximately 30–40 cm long after two months were harvested and cut into 2-cm long segments with at least one axillary bud after the leaves had been removed. Plant material manipulations from this step onwards were done under laminar flow hood in contamination-free conditions. Apical and nodal segments were first soaked in 0.1% (w/v) of Benlate solution for 15 min, then in 70% (v/v) ethanol added with three drops of Tween 20 for 30 s followed by immersion in 50% (v/v) Chlorox (2.63% sodium hypochlorite) for 15 min and finally rinsed three times in sterile distilled water. After further trimming into approximately 1.5-cm long, the explants were immersed again in 10% (v/v) Chlorox (0.53% sodium hypochlorite) for 5 min then rinsed thoroughly with sterile distilled water.

Three different basal culture media were selected for this study, i.e. Murashige and Skoog medium (MS) (Murashige & Skoog 1962), Lloyd and McCown woody plant medium (WPM) (Lloyd & McCown 1980) and Gamborg B5 medium (Gamborg et al. 1968) each supplemented with 30 g L^{-1} sucrose and 0, 1, 10, and 100 µM (0, 0.22, 2.25 and 22.5 mg L^{-1}) of 6-benzylaminopurine (BAP). After pH adjustment to 5.7 with HCl and NaOH, and addition of 10 g L^{-1} Difco powder agar, the media were heated to about 80 °C then poured into 25 mm × 150 mm test tubes, each receiving 10 ml of medium and covered with polypropylene cap prior to autoclaving at 121 °C and 103 kPa for 15 min. The cultures were

*haliza@frim.gov.my

© Forest Research Institute Malaysia

grown at 23 ± 2° C under 16-hour photoperiod with light intensity of 22.22 mmol m^{-2} s^{-1} supplied by white fluorescent lights. Immediately after disinfection, the explants were inoculated (one explant per culture tube) onto different basal media without any plant hormone for two weeks before being transferred onto the same media with the four concentrations of BAP. They were maintained in this condition for six weeks. A total of 30 explants were used per treatment. Shoots induced were subcultured every 6-week cycle for three cycles in MS medium incorporated with 4.44 µM of BAP. The shoots reached about 2–3 cm in height within 6 weeks in this medium and were transferred onto a rooting media consisting of MS medium enriched with 4.92 µM indolebutyric acid (IBA) in test tubes. Rooted plantlets were brought to the nursery and planted on washed sand for acclimatisation in the greenhouse under approximately 90% relative humidity maintained by an automatically-controlled misting system. Shoot production data were analysed using statistical analysis system package (ANOVA). Further mean separation tests were evaluated using the least significant difference (LSD) test. In all analyses, means differing at probability of ≤ 0.05 were considered to be significantly different.

Different basal media significantly affected shoot induction (Figure 2). MS medium was found to be the best medium for induction of multiple shoots. The LSD test showed significant differences in mean number of shoots and leaves produced from different basal media and BAP concentrations (Figures 3 and 4). MS basal medium incorporated with 10 µM BAP was found to be the most appropriate concentration for shoot initiation with mean number of shoots 3.4 and mean number of leaves 5.7 after 1 month in culture. Further

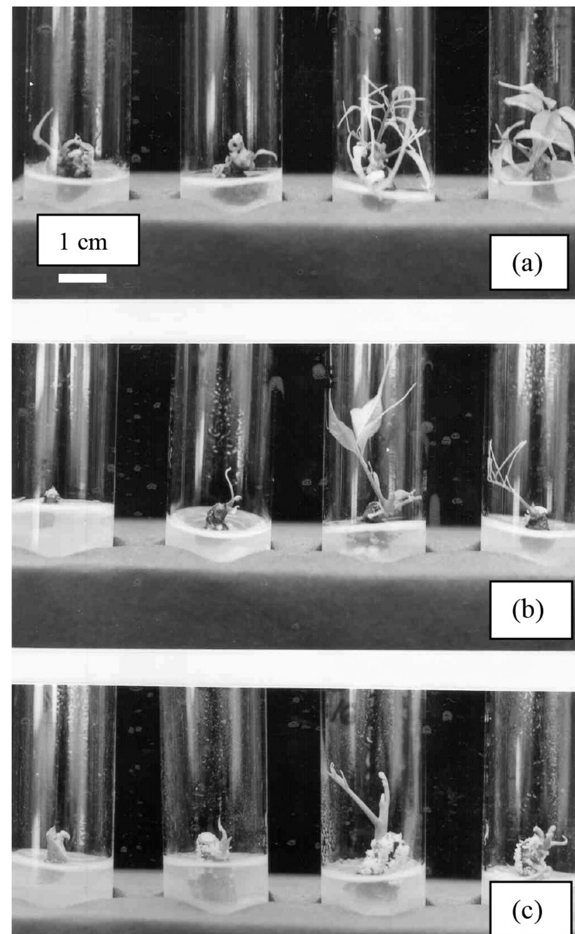

Figure 2 Shoot initiation on different type of media: (a) MS, (b) WPM and (c) B5 and different concentrations of BAP (left to right: 0, 100, 10 and 1 µM)

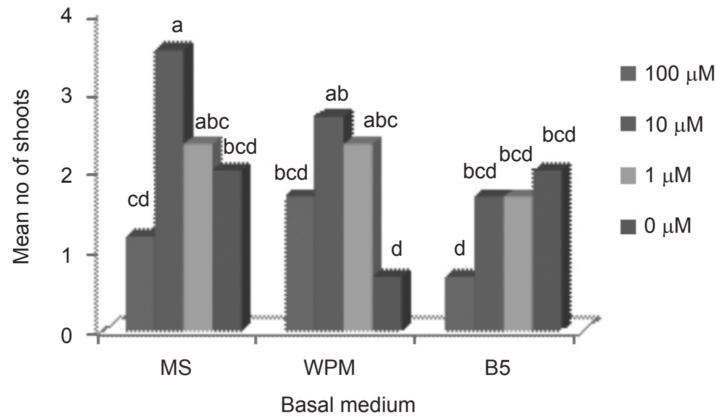

Figure 3 Mean number of shoots (more than 5 mm in length) produced after 1 month for the three culture media tested: MS, WPM and B5, each supplemented with 100, 10, 1 and 0 µM of BAP; bars with the same letters are not significantly different at 5% level, n = 30

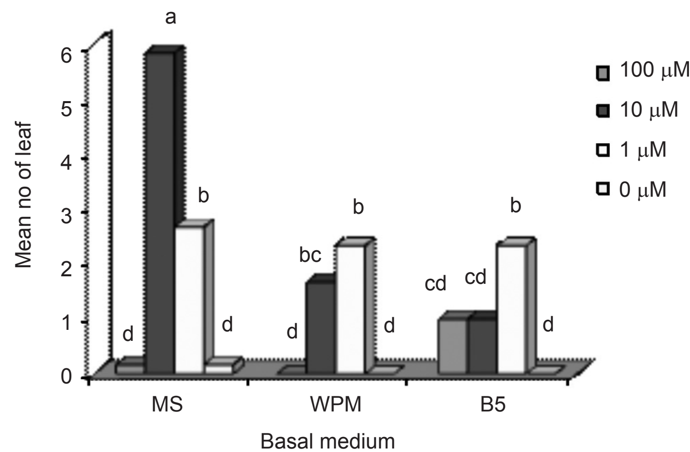

Figure 4 Mean number of leaves produced after 1 month from three different basal media: MS, WPM and B5, each supplemented with 100, 10, 1 and 0 µM BAP; Note: bars with the same letters are not significantly different at 5% level, n = 30

subculturing of 6 week-interval in MS medium incorporated with lower BAP concentration of 4.44 µM resulted in production of multiple shoots (Figure 5). The multiplication rate in the multiplication medium was 3.5. It has been reported that 4.44 µM BAP was the best concentration for shoot multiplication in *Khaya senegalensis* cultures (Cao & Trueman 2011). The same result was obtained in micropropagation of the medicinal African mahogany timber, *Khaya grandifolia* whereby 4.44 µM BAP in combination with 0.01 mg L^{-1} NAA (l-naftaleneacetic acid) achieved the optimum number of nodes and shoot length (Okere & Adegeye 2011). Eighty per cent of 30 elongated *K. ivorensis* shoots were rooted in the rooting medium (MS incorporated with 4.92 µM IBA). Ninety per cent of plantlets were successfully acclimatised in the washed sand in the greenhouse (Figure 6). Plantlets successfully survived in the nursery. Coppiced stumps provide a valuable source of juvenile shoots especially useful when starting clones from selected trees (Longman & Wilson 1993). This study showed that it was possible to micropropagate selected trees of *K. ivorensis* using coppiced shoots from stump hedge.

ACKNOWLEDGEMENTS

We thank the Director-General of Forest Research Institute Malaysia and the tissue culture staff especially M Sharifah for support in this study.

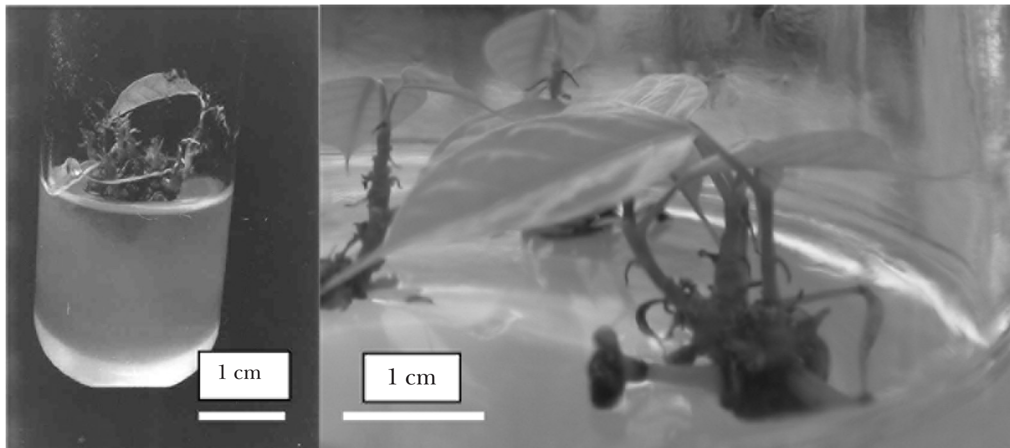

Figure 5 Multiple shoots after further subculture in MS medium with 4.44 μM BAP in (a) test tube and (b) bottle

Figure 6 Plantlets acclimatised in the greenhouse after 2 weeks on washed sand

REFERENCES

Cao DH & Trueman SJ. 2011. *In vitro* propagation of the African mahogany *Khaya senegalensis. New Forests* 42: 117–130.

Gamborg OL, Miller RA & Ojima K. 1968. Nutrient requirements of suspension cultures of soybean root cells. *Experiment Cell Research* 50: 151–158.

Lloyd G & McCown B. 1980. Commercially feasible micropropagation of mountain laurel *Kalmia latifolia* by use of shoot tip culture. *Journal Combined Proceedings, International Plant Propagators' Society 1980*. 30: 421–427.

Longman KA & Wilson RHF. 1993. *Rooting Cuttings of Tropical Trees. Tropical Trees: Propagation and Planting Manuals*. Volume 1. Commonwealth Science Council, London.

Murashige T & Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* 15: 473–497.

Okere AU & Ayegere A. 2011. *In vitro* propagation of an endangered medicinal timber species *Khaya grandifolia* C. Dc. *African Journal of Biotechnology* 10: 3335–3339.

Tepongning RN, Lucantoni L, Nasuti CC, Dori GU, Yerbanga SR, Lupidi G, Marini C, Rossi G, Esposito F & Habluetzel A. 2011. Potential of a *Khaya ivorensis–Alstonia boonei* extract combination as antimalarial prophylactic remedy. *Journal of Ethnopharmacology* 137: 743–751.