Axillary Bud Elongation and Root Initiation in *Milicia excelsa* Welw. (Iroko) in Vitro

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Milicia excelsa is a major tropical forest species and a commercially important timber species in Ghana (Wagner and Cobbinah, 1993). Gall-forming insects (*Phytolyma lata* Scott) attack this species severely, which causes tremendous economic loss. However, some individual trees seem resistant to this pest. To produce true-to-type clones from these resistant genotypes, vegetative propagation techniques for *Milicia excelsa* need to be developed. To date, this species only has been propagated sexually. We conducted a micropropagation trial with 8-month-old seedlings in our laboratory, and some desirable half-sib families rooted poorly. Consequently, micropropagation of this species was investigated, and results of the effects of vitamin and growth regulator levels, half-sib families, and plant aging on axillary bud elongation, growth, and root initiation are reported.

Ten to 15 shoots with axillary buds were removed from greenhouse-grown, 15-month-old seedlings of *Milicia excelsa* half-sib family AA2 (susceptible), AA28 (resistant), and EM1 (resistant), obtained from the Ashanti and eastern regions of Ghana. The shoots were surface-sterilized with 0.5% sodium hypochlorite for 5 min and rinsed with tap water; then six to eight axillary buds were dissected from the shoots, surface-sterilized in 3% H₂O₂ for 3 min, and rinsed with sterile distilled water three times. The buds were soaked in 70% ethanol for 3 min and rinsed with sterile distilled water three times. The surface-sterilized buds were placed on woody plant medium (WPM) (Lloyd and McCown, 1981) containing 2% sucrose and 0.7% Difco Bacto-agar, with a factorial arrangement of growth regula-

tors and vitamins. The pH of all media was adjusted to 5.8 before autoclaving for 24 min at 121°C. Cultures were maintained at 26 ± 1°C with a 16-h photoperiod at 82 µmol·m⁻²·s⁻¹ provided by cool-white fluorescent lamps.

To test the effects of growth regulator and vitamin concentrations on the frequency of axillary bud elongation (ABE) and shoot growth for three half-sib families, we used the following media: basal medium with 0 or half-strength MS vitamins (Murashige and Skoog 1962) combined with (in µM) 4.4 N-phenylmethyl-1H-purin-6-amine (BA) + 0.54 1-naphthaleneacetic acid (NAA) or 2.2 BA + 0.27 NAA, for a total of four treatments. A three-factor randomized complete-block design was used. The frequency of ABE and shoot lengths (>5 mm) were recorded after 4 weeks of culture. One-way analysis of variance (ANOVA) was used to analyze the effect of the four treatments.

There was no significant difference among the four treatments on the frequency of ABE ($P = 0.438$, $df = 3, 15$), but a significant difference was found for axillary shoot length ($P = 0.0079$, $df = 3, 275$). The best treatment for shoot elongation was half-strength MS vitamins and 2.2 µM BA + 0.27 µM NAA (Table 1). To determine if there was any single factor effect and interactions among the factors, we performed a three-way ANOVA; it showed that all three factors significantly influenced shoot growth and no interactions existed between or among each factor (Tables 2 and 3).

To test for age effects on ABE and root initiation, axillary buds of 11-, 15-, and 23-month-old seedlings of half-sib family AA2

Table 2. Percent axillary bud elongation and shoot length of *Milicia excelsa* as affected by MS vitamins, growth regulators, and half-sib families.

Variable	Axillary bud elongation (%) ²	Shoot length (mm) ²
Medium		
No vitamins	71.9 ± 9.4	14.9 ± 0.4
Half-strength vitamins	72.9 ± 9.4	17.1 ± 0.4
4.4 µM BA + 0.54 µM NAA	65.0 ± 9.4	14.5 ± 0.4
2.2 µM BA + 0.27 µM NAA	79.8 ± 9.4	17.5 ± 0.4
Half-sib family		
AA2	81.5 ± 12.7	12.6 ± 0.6
AA28	64.9 ± 12.7	23.8 ± 0.6
EM1	70.7 ± 9.0	11.7 ± 0.4

²Mean ± 1 SE.

Table 3. Three-way analysis of variance for shoot growth response of *Milicia excelsa* on each single factor.

Source of variation	F ratio	P value
Vitamin (V)	15.0	0.0181*
Growth regulators (GR)	26.5	0.0068**
Family (F)	175.6	0.0001**
V × GR	0.3	0.6229 ^{ns}
V × F	6.67	0.0532 ^{ns}
GR × F	3.4	0.1361 ^{ns}
V × GR × F	0.01	0.9101 ^{ns}

^{ns}, *, **Nonsignificant or significant at $P \leq 0.05$ or 0.01, respectively.

were collected. Twenty to 30 axillary buds were cultured on basal medium with half-strength MS vitamins and 4.4 µM benzylamino purine (BAP) + 0.54 µM NAA. After 4 weeks of culture, we recorded the number of explants with shoots >5 mm long and without shoots. Elongated shoots then were transferred to rooting medium (basal medium with full-strength vitamins and 3.24 µM NAA). After 4 weeks, frequency of root initiation was recorded. Chi-square analysis was performed to test the effect of aging on ABE. Donor plant age had a significant impact on ABE ($\chi^2 = 8.04$, $P = 0.018$). The frequency of ABE decreased as the donor plant aged. Explants from 11-, 15-, and 23-month-old donor plants had ABE of 89%, 64%, and 54%, respectively, and rooting of 67%, 12%, and 2%, respectively. Rooted plantlets then were transplanted into a 1 peatmoss : 1 vermiculite : 1 perlite (by volume) medium and maintained for 2 weeks in a growth chamber with the same environmental conditions as in vitro culture, except humidity was 85%. Plantlets then were transferred to a greenhouse and grown for 6 months with 100% survival rate.

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Table 1. Frequency of axillary bud elongation and shoot length of *Milicia excelsa* on four artificial media.

Medium	Axillary bud elongation (%) ²	Shoot length (mm) ²
No vitamins,		
4.4 µM BA + 0.54 µM NAA	62.5 ± 11.7	11.9 ± 1.0 a ³
2.2 µM BA + 0.27 µM NAA	80.3 ± 7.3	14.6 ± 0.9 ab
Half-strength vitamins		
4.4 µM BA + 0.54 µM NAA	67.6 ± 4.6	13.1 ± 1.0 a
2.2 µM BA + 0.27 µM NAA	77.5 ± 8.6	16.3 ± 0.9 c

²Mean ± 1 SE.

³Mean separation by Newman-Keuls procedure.