Clonal Propagation of *Acacia koa*

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Introduction

Koa (*Acacia koa*) is currently the most important commercialized hardwood in Hawai'i's forests. A sustainable supply of koa will depend on reforestation with the best adapted seed sources and perhaps with vegetatively propagated cultivars having unique traits. At present, koa forests are in a serious state of decline, and improvement appears warranted if sustainable supply is to be achieved.

Vegetative (clonal) propagation provides large numbers of genetically identical plants. Conventional methods include cutting and grafting, while in vitro micropropagation is a more recent approach. These methods would be useful to propagate koa trees selected for superior growth and stem form, disease and insect tolerance, and desirable wood characteristics such as curly or fiddleback grain. Clonal propagation could provide opportunities for commercial-scale production of koa. Furthermore, clonal koa trees would be useful for breeders in testing for adaptability and developing elite seed sources.

Conventional vegetative propagation of *A. koa* is not presently viable on an operational scale. In vitro propagation (micropropagation), on the other hand, has been used successfully to obtain large numbers of identical trees in various tree species including the genus *Acacia*. In *Acacia*, micropropagation through shoot multiplication and somatic embryogenesis (induction of seed- like structures in cultures of somatic cells) has been reported in the past 10 years in *A. melanoxylon* (Jones and Smith 1989), *A. nilotica* (Garg et al. 1996), *A. auriculiformis* (Mittal et al. 1989), *A. mangium* (Galiana et al. 1991), *A. saligna* (Barakat et al. 1992). In Hawai'i, Skolmen (1977), of the Institute of Pacific Island Forestry, studied clonal propagation of *A. koa* using both in vitro and conventional methods. Plants were produced through callus culture (Fig. 1), air-layering, and mist rooting from young shoots of juvenile trees. However, development of a successful large-scale clonal propagation method is still needed.

We studied micropropagation, building upon the earlier work of Roger Skolmen. Objectives of our study were: (a) to develop a method for koa micropropagation through shoot multiplication (Fig. 1), (b) to optimize the method for higher efficiency in multiplication, and (c) to induce roots in multiplied shoots.

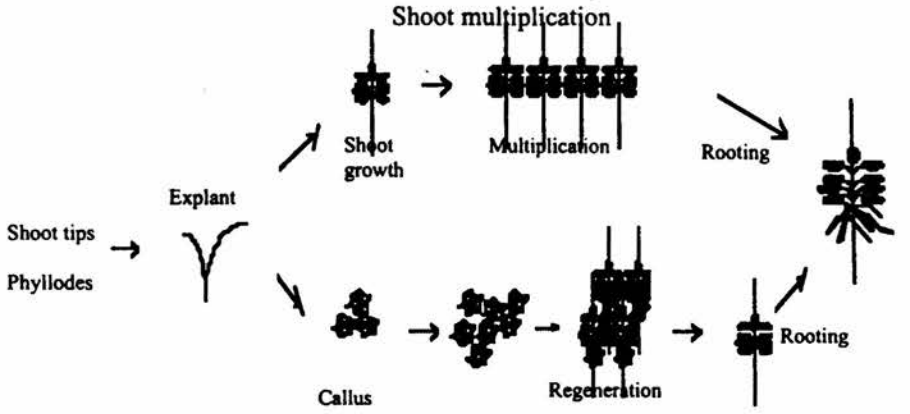
Collection of plant materials

Various tissues of koa including young shoots, phylloid tips, root suckers, and seedlings from the Island of O'ahu were used to initiate tissue culture (Table 1). Plant materials from mature trees were collected from healthy branches with new growth.

Initiation of culture

Shoot tips and young phyllodes were washed with commercial detergent and left under running water for 3–5 hours before use. Plant materials, including shoot tip areas, were cut to 3–5 cm in length, and leaves were removed. They were surface-sterilized with 30% Clorox (sodium hypochrolite 1.5%) and a drop of 0.2% Triton X-100, followed by rinsing in sterile water. Shoots tips were placed on modified MS medium with various amount of cytokinin (kinetin). Lateral shoot growth was observed in culture 3–8 weeks after initiation from young shoots as explants in kinetin media, while no response was found in the culture from a mature tree (Table 1). In a recent experiment (data not shown), in vitro shoots were obtained from a 2.5-year-old tree grown at Maunawili from seed of a selected tree on Maui. Various combinations of auxin (2,4-D and NAA) and cytokinen were also used to follow Skolmen's results for callus induction (Skolmen and Mapes 1976, Skolmen 1977). Various types of callus were derived from explants such as young shoots and phyllodes, but no plants were regenerated from these calluses. Effect of kinetin in culture medium on culture establishment was studied in the phyllode explants from Waimanalo. Green shoots were observed from over 30% of explants cultured on the K8 medium (kinetin 8 mg/L), while only 10% were obtained on E1 medium which contained

Figure 1. Scheme of Acacia koa micropropagation.



Callus-Regeneration (Skolman 1977)

Table 1. Explanting and culture initiation of *Acacia koa*.

Date	Source	Tissue	Age	No. initiated	No. callus	No. multiplied
2/16/94	Waimanalo	phyllode	3 yr	40	6	0
3/3/94	Kunia	young shoots	2 mo	317	25	21 (6.6%)
5/12/94	Kunia	young shoots	2 mo	252	114	35 (13.9%)
6/1/94	Tantalus	phyllode	20 yr	73	-	0
8/30/94	Palolo Valley	phyllode	10 yr	58	-	0
		true leaves		25	-	0
11/24/94	Waimanalo	shoot	3 yr	186	-	7 (3.8%)
to		phyllode		206	-	15 (7.3%)
1/10/95		sucker		60	-	6 (10%)

combination of kinetin and BAP 0.7 mg/L for eucalyptus culture at HARC.

Multiplication

Shoots were further multiplied from the initial shoots in the MS medium with kinetin at 4–6 mg/L. Multiplied shoots were transferred to fresh medium every six weeks. Multiplication rate was low at the rate of multiplication x 1.5 per 6 weeks in the most vigorously growing culture. Callus formation at the base of multiplied shoots was observed in approximately 20% of the cultures on kinetin media. In these cultures, shoots stopped growing, and multiplied shoots were dead in 2–4 weeks after callus formation. This result indicated that prevention of callus growth was important for active shoot multiplication of koa.

Rooting

Roots were induced from multiplied shoots in culture media with auxin, IBA. Several rooted seedlings were transferred to vermiculite medium. They will be transplanted in the greenhouse at HARC's Maunawili Breeding Station.

Future study

We were encouraged by these initial results, and will continue our study of methods to increase the efficiency of shoot multiplication and rooting and to initiate cultures from mature trees with combinations of growth regulators and pre-treatment of explanting materials.

Summary

Tissue culture of A. koa was initiated from shoots and phyllodes of young trees. Shoot culture was most successful in a medium with high kinetin (8 mg/L). Shoots were multiplied in MS medium with kinetin at 4-5 mg/L. Roots were induced on culture medium with IBA.

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References

Barakat, M.N., and El-Lakany, M.H. 1992. Clonal propagation of *A. saligna* by shoot tip culture. Euphytica 59:103–107.

Galiana, A., Tibok, A., and Duhoux, E.1991. In vitro propagation of the nitrogen-fixing tree legume *Acacia mangium* Willd. Plant and Soil.151-159.

Garg, L., Bhandari, N.N., Rani, V., and Bhojwani, S.S. Somatic embryogenesis and regeneration of triploid plants in endosperm cultures of *Acacia nilotica*. Plant Cell Reports 15:11.855–858. Jones, C., and Smith, D. 1989. Effect of 6-benzylaminopurine and 1-naphtylacetic acid on in vitro axullary bud development of mature *Acacia melanoxylon*. Forest Research Inst. combined proceedings-INt. Plant Propagatorsn Soc. 38:389–393.

Mittal, A., Agarwal, R., and Gupta, S.C. 1989. In vitro development of plantlets from axillary buds of *Acacia auriculiformis*, a leguminous tree. Plant cell Tissue and Organ Culture 19:65–70. Nagai, C., and Ibrahim, R. 1995. HSPA Annual Report, 1994. In vitro culture of *Acacia koa*. p. 45.

Skolmen, R.G. 1977. Clonal propagation of *Acacia koa* Gray by tissue culture and conventional methods. PhD. Thesis. University of Hawai'i.

Skolmen, R.G., and Mapes, M.O. 1976. *Acacia koa* Gray plantlets from somatic callus tissue. J. Heredity. 67:114–115.