Genetic Variations of *Acacia koa* Seed, Seedling, and Early Growth Traits

W. Sun[1], J. L. Brewbaker[2], and M. T. Austin[1]
[1]Hawai'i Agriculture Research Center, [2]Department of Horticulture, CTAHR, University of Hawai'i at Manoa

Introduction

Koa (*Acacia koa* Gray) is a native Hawaiian legume tree with 2n=52 chromosomes (Atchison 1948). It is endemic throughout Hawai'i's main islands from sea level to 2300 m elevation. Koa is prized for its beautiful wood and is used for variety of wood products (Whitesell 1990). Koa provides a sustainable economic resource for Hawai'i's forest industries and an ecosystem for many endanger'ed Hawaiian species. Koa forests have diminished significantly owing to the establishment of ranches and sugarcane plantations in the last two centuries (Metcalf et al. 1978).

Previous studies suggest koa has a diverse genetic base. Morphological differences including phyllode size, tree form, seed size, and flower characteristics are found among natural koa stands throughout the Hawaiian islands. These differences have led to the classification of three species within Hawaiian *Acacia*, namely, those of *A. koa* Gray, *A. koaia* and *A. kauaiensis* (Hillebrand 1888, Rock 1920, Lamoureux 1971, St. John 1979). Brewbaker (1977) reported isozyme polymorphism and variation of phyllode size and shape among koa populations from the islands. Attempts of koa reforestation have also shown variable growth performance (Judd 1919, Whitesell and Isherwood 1971, Ching 1981, Scowcroft and Adee 1991). Conrad et al. (1995) reported differences for plant growth among eight koa provenances from four Hawaiian islands. Genetic variation of koa wood quality and other traits have also been observed. Simmons et al. (1991) reported variation in wood color, grain, and specific gravity. Skolmen (1990) observed differences in koa response to volcanic fumes. Plants from regions distant to an area of volcanic activity were more prone to fume damage than plants proximal to volcanic activity. Further evidence of the genetic variation in koa is suggested by its highly diverse ecological range.

The objectives of this study were to quantify seed sources for reforestation and to select superior koa as the basis for long-term genetic improvement.

Materials and methods

Acacia koa germplasm was collected from 1991 to 1996 from diverse ecosystems around the state. Most accessions were collected as families from single trees. Accession seed sources were documented and seeds were stored at the University of Hawai'i Foundation Seed Facility at 15°C. Average seed weight (based on a random sample size of 100 seeds), width, and length (based on a random sample of 10 seeds) were measured in the laboratory. Seedlings were raised in the greenhouse, and growth rates were measured until transplanting at the CTAHR Waimanalo Research Station, O'ahu.

Three-and-one-half-month-old seedlings were planted at the CTAHR Hamakua Research Station, Hawai'i (650 m. a.s.l.) on May 25, 1991. The station's annual rainfall averages 2500 mm and annual mean temperature 19°C. The soil series is the Maile silty clay loam with a pH of about 5.0. An augmented design of the randomized complete block (RCB) with two replications was used to test 48 accessions. Each plot consisted of 10 trees in two rows. Plant spacing was 1 x 1.5 m, or 6667 trees ha^{-1}.

Individual tree height and DBH (diameter at breast height) were measured at five- and seven-month intervals. Phyllode development rate based on the percentage of phyllode coverage in the tree was scored one year after transplanting. Survival percentage of each plot was also recorded yearly. Analysis of variance was done on these data using a Quattro Pro spreadsheet (Brewbaker 1993) and PROC GLM of SAS (SAS 1990). Individual tree and family heritability estimates for the tree growth rates were according to Zobel and Talbert (1984) with modification. Individual-tree narrow-sense heritability is calculated as $h^2=3*\sigma^2_F/(\sigma^2_W+\sigma^2_{RF}+\sigma^2_F)$; family heritability is calculated as $h^2_F= \sigma^2_F/(\sigma^2_W/TR+\sigma^2_{RF}/T+\sigma^2_F)$. σ^2_W, σ^2_{RF}, and σ^2_F are the within-plot, replication X family, and family variance components, respectively. T and R refer to trees per family-replication plot and replications. Predicted family genetic gain is calculated as $G=S*h^2_F$, where S is the selection differential between mean of the selected families and mean of all families.

Figure 1. ***Acacia koa*** **seeds collected from the Hawaiian Islands.** 1M2-1: Makiki, Oahu; 1N2-1: Nuuanu, Oahu; 2PH1-2: Puu Hinahina, Kauai; 2PK1-2: Puu Ka Pele, Kauai; 2W2-2: Waimea Canyon Drive, Kauai; 5KO1-1: Kokomo Rd., Maui; 5MA1-2: Hamana Rd., Maui; 6KMC2C: Kilauea Military Camp., Hawaii; 6MLR1-1 and 6MLR7-1: Mauna loa Rd., Hawaii.

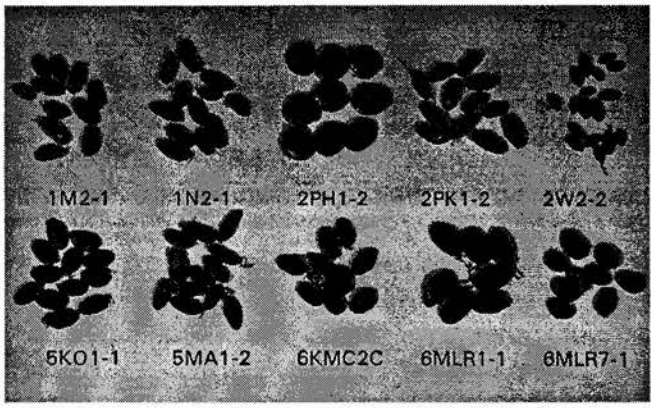


Table 1. Traits observed in two distinct *Acacia* populations from Kaua'i.

Trait	*A. koa*	*A. kauaiensis*
Seed shape	Oblong	Round
Seed width (W)	4.8 mm	7.5 mm
Seed length (L)	10.0 mm	9.8 mm
L/W ratio	2.1	1.3
Seedling color	purple	green-yellow
Seedling height		
3 mo	35 cm	16 cm
12 mo	250 cm	110 cm
Phyllode size	Narrow to medium	Variable
Flowering seasons	winter	summer
Location	Along highways	Widely distributed

Results and discussion

To date, a total of about 400 koa accessions has been collected and documented. This collection includes 116 accessions collected by Brewbaker during the 1970s and 44 accessions contributed by the USDA Forest Service in Hilo. These collections were mainly from Kaua'i, O'ahu, Maui, and Hawai'i. A wide range of ecological conditions are represented in these collections, with elevation ranging from 100 to 2300 meters above sea level and mean annual precipitation from 600 to 5000 mm.

Variation in seed weight, size, and morphology from different koa collections was observed (Fig. 1). Seed weight of *Acacia koa* from 294 accession averaged 8.5 g per 100 seeds and ranged from 1.7 g (about 58,800 seeds/kg) to 17.7 g (about 5650 seeds/kg). Seeds from Kaua'i and Hawai'i were significantly ($P<0.05$) heavier than those from O'ahu, Lana'i, and Maui. Koa seed width from 95 accessions averaged 6.4 mm and ranged from 3.4 to 9.3 mm, while seed length averaged 10.2 mm and ranged from 6.7 to 13.4 mm. Two distinct seed types, round and oblong seeds, were found among 49 Kaua'i collections. The round seeds averaged 7.5 mm in width, and were significantly ($P<0.05$) wider than the oblong seeds. However, no difference was found for seed length between these two seed types.

Distribution of trees with oblong seeds on west Kaua'i are limited to certain areas compared to trees with round seeds. Trees with round seeds are widely distributed in west Kaua'i. Trees with oblong seeds could only be found along Waimea Canyon Drive, Koke'e Road, Ka'aweiki Ridge, and Kumuwela Trail, and are presumed to trace to reforestation in the 1930s.

Significant ($P<0.05$) differences for seedling height two weeks after sowing were found among 80 accessions from Kaua'i, O'ahu, Maui, and Hawai'i. Average seedling growth rates from the different islands are presented in Figure 2. Accessions with the round seeds from Kaua'i grew significantly ($P<0.01$) slower than those from Kaua'i with the oblong seeds from O'ahu, Maui, and Hawai'i. The result is in agreement with a similar study using different koa seed sources (Sun et al. 1996).

Three groups of seedlings were observed among these collections based on the different color of the seedling stem, the leaf rib, and the leaflets. Group I is from Kaua'i and has round seeds; green stems, leaf rib, and leaflets; and slow growth. Group II is distributed on O'ahu, Maui, and some areas of Kaua'i and has oblong seeds, purple stems and leaf ribs, dark green leaflets, and normal seedling growth. Group III is from Hawai'i and has both round and oblong seeds, green leaflets, reddish stems, and normal growth. Two koa groups

Table 2. Estimated variance, heritability, and predicted family selection gain for tree height and DBH at various growth stages.

	Height (m) in month				DBH (mm) in montth			
	6	14	26	31	26	37	48	60
Mean	1.7	3.0	5.0	5.9	57.0	85.0	99.0	109.0
Variance								
Between family(s^2_F)	5.10	0.13	0.17	39.60	100.10	2.64	3.59	3.63
Rep/family(s^2_{RF})	2.90	0.05	0.15	10.30	0.00	0.41	0.35	0.15
Within family(s^2_W)	22.60	0.60	1.11	70.00	620.00	10.50	12.90	18.01
†Heritability								
Individual tree(h^2)	0.50	0.50	0.36	0.99	0.42	0.58	0.64	0.50
family(h^2_F)	0.69	0.74	0.50	0.80	0.79	0.74	0.75	0.68
‡Predicted family selection gain	0.5	0.7	0.8	1.3	26.1	25.8	30.1	34.8
Gain%	28.6	24.6	15.0	21.8	45.8	30.4	31.2	31.9

†Individual-tree narrow-sense heritability is $3*s^2_F/(s^2_W+s^2_{RF}+s^2_F)$; family heritability is $s^2_F/(s^2_W/TR+s^2_{RF}/T+s^2_F)$.
‡Predicted family genetic gain is $S*h^2_F$ where S is selection differential from the selected best performance family.

found on Kaua'i can be easily distingished and should be treated as separate species (Table 1). Trees with round seeds from Kaua'i were previously described as *A. kauaiensis* (Lamoureux 1971). Koa groupings suggested in this study seem to have some geographic partitionings in addition to morphological similarities. This suggests there may be some evolutionary significance to the groups. For this reason, it may be expected that other traits such as wood characteristics, disease resistance, and even molecular markers may also tend to be classified according to these groups.

Overall koa seedling survival at two months was 95.5 percent and declined to 84 percent two years after planting. The lowest survival rate was from accessions of 6-1288C (Hawai'i), 2DT2-1, 2MA2-1, 2MA3-1, and 2OV1-1 (Kaua'i), with 30, 0, 20, 60, and 60 percent survival, respectively. After three years, all four Kaua'i accessions were dead. All these accessions were the round-seed phenotypes.

Significant ($P<0.05$) difference for phyllode development was found among the 22 replicated accessions. One year after planting, phyllode coverage averaged 64 percent and ranged from 0 percent for 2DT2-1 to 100 percent for 1M7-1, 1SL3-1, 5K1-1, and NFTA891C. Accessions from Hawai'i showed earlier ($P<0.01$) phyllode development than those from the other islands.

Differences ($P<0.05$) in height and DBH were found among these accessions. Overall, average tree height attained 3 m in the first year and continued to add two meter every year thereafter. After two-and-one-half years, heights of these accessions averaged 5.9 m and ranged from 2.0 to 7.6 m. After five years, DBH averaged 109 mm and ranged from 55 to 166 mm. Significant differences for DBH among accessions from the same area were also observed after five years. DBH of 1M6-1 and 1M8-4 from Makiki, O'ahu, was significantly different after two and half years. This was not the cause for height. Similar results were observed for 2PH1-1 and 2PH2-1 families from Kaua'i and 5K1-2 and 5K1-6 families from Maui.

Estimated variance, heritability, and expected family selection gain for tree height and DBH at early growth stages are presented in Table 2. Family heritablity estimates for height and DBH were about 0.7. Predicted genetic gain for one cycle of family selection was about 1.3 m for height at two and a half years and 35 mm for DBH at five years.

Various disease symptoms of koa rust and sooty black mold were observed in this trial. Variation of disease symptoms among families was noticed. Overall, the progenies from Hawai'i were more susceptible to rust compared with the progenies from the other islands.

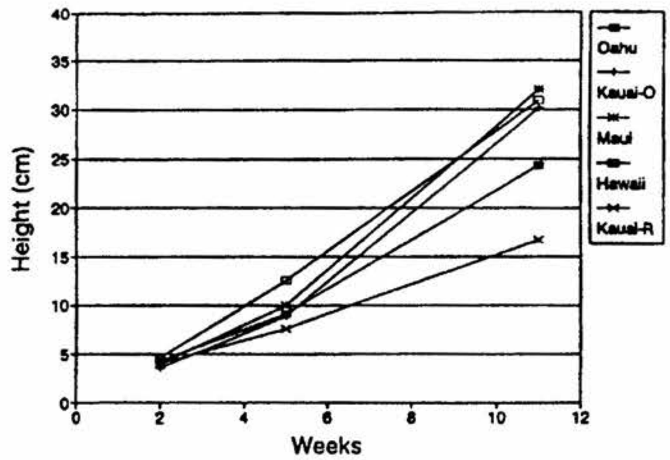
After five years, the fastest growing families in terms of DBH (>140 mm) were 1M6-1 and 1N2-5 from O'ahu, 2PH1-1 and 2PK3-1 from Kaua'i, and 5K1-2 from Maui. Some of these families flowered and set seed in year four, and seeds were collected from these outstanding families. Testing of these advanced progenies will be carried out to study the potential genetic gain from selection.

It is evident that *Acacia koa* is a fast growing tree in the juvenile stage. This finding is in contrast with the general assumption that koa is a slow growing tropical leguminous tree (NAS 1979). This conclusion may have derived from the observation of koa trees growing in degraded forest lands. The slow growing trees may also be due to poor seed source. However, an early study did record that koa was a fast growing tree in juvenile stages, in which it reached 9 m in 5 years (Judd 1919). Skolmen (1990) observed that one progeny grew straight and tall while the other was defoliated and died shortly thereafter at Volcano, Hawai'i. Conrad et al., (1995) reported koa provenances from Kaua'i and O'ahu grew faster than koa from Maui and Hawai'i when grown at Wahiawa, O'ahu. Progenies with round seeds from Kaua'i always grew slower than progenies with oblong seeds (Sun 1996).

There appears to be genetic differences for early plant growth, survival rate, and development among the koa collections from the different islands. Consistent and significant differences for these traits over five years were largely due to seed source. The presence of genetic variability in tree growth is very important and can be used immediately for rapid genetic advance at relatively low cost through individual tree or family selection. The results of estimated individual tree and family heritability for height and DBH clearly suggest that there is potential for genetic improvement of koa, especially, the fast growing koa populations from some areas of Kaua'i, O'ahu, and Maui.

Skolmen (1990) reported that only the Big Island koa with wide phyllodes would make long, branch-free logs for timber production. However, most of the fast growing koa progenies with a single main stem identified from this trial were from Kaua'i, O'ahu, and Maui. These koa populations with unique seedling characteristics, described as Group II koa, are different from koa with round seed character on Kaua'i and koa on the Island of Hawai'i. The present findings suggest that more attention should be paid to selection and silviculture of these koa populations for the state reforestation programs.

Figure 2. Average seedling growth rate of *Acacia koa* collections from the Hawaiian Islands. Kaua'i-O is from Kaua'i with the oblong seed; Kaua'i-R is from Kaua'i with the round seed.



Acknowledgements

This study was supported by USDA-McIntire Stennis Forestry Grants to Dr. Brewbaker.

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Questions to the Panel

Q: You put up a measure related to the seeds; I assume it was weight, size, etc. You had the unit of measure as mm, which I take as millimeter.
A: That should be grams.

Q: For example, I noted that the weight for Big Island was 6.4, that should be 6.4 grams. What is the quality seed source that you identified, is it the Maui seed?
A: We identified some from Kaua'i, some from Maui, some from this island, Oahu.

Steve Smith: [to J. Brewbaker] In your abstract, which you didn't really talk about, you made a comment that "there are superb tropical hardwoods such as *Acacia melanoxylon*, that will simply wipe out koa in the future, unless koa is genetically improved." Is this because they've done a lot of work already on the melanoxylon, or because we haven't seen a lot of good stuff here in Hawai'i?
Jim Brewbaker: Well, they don't have exactly the 80-year head start that Bill was talking about, but melanoxylon ranges in Australia all the way from central Queensland down to Tasmania. There's tremendous genetic diversity through that range. They have cloned it quite easily using root-sprout cloning. Otherwise, they're not a heck of a long way into commericialization of blackwood, but it is a beautiful wood, and it does have curly wood, and so forth. Our foresters have grown it here through the years and there is a very good reason to keep it in the arsenal of species that could be researched in Hawai'i. After all, there are 1200 other acacia species out there, and many of them are quite striking. One that has both extensive recent research and is a good hardwood is *Acacia mangium*, and also its related species like *auriculiformis*. Of course, there's the historic work on *Acacia mearnsii*, and now in South Africa

extensive breeding for hardwood rather than gum.

Q: [to Conkle] The research from Dr. Sun concentrated on half of the equation, which was the quantity side and how fast can we grow them. I know Dr. Brewbaker and I have talked about the quality side, and we have to make sure we select seed that gives us both fast growing seeds but that are worth something when it comes time to harvest them. Dr. Conkle, you showed a dendrogram that showed some sort of relationship between trees from different islands. It seemed like Maui and Kaua'i were more closely associated than O'ahu and Kaua'i. I was curious if that was significant, if it was an anomaly; why do trees in those two islands, which are further apart, have more closely related genetics?
Tom Conkle: I think those are tempting dendrograms. My collection from Maui was more limited than from Kaua'i and O'ahu, and I have less confidence in the Maui genotypes and characteristics of that geographic location. I would enjoy having more seed, and I'm really working on techinques using those phyllodes as the source of enzymes to do genetic trials. It would put these numbers up into the thousands of trees that we look at, rather than the hundreds that we looked at in this collection. The answer is that the Maui collection was rather small and I wouldn't put a lot of confidence in the exact location of those three. My take-home message is that the three island populations are relatively similar in comparison to the Hawai'i collections.
Jim Brewbaker: May I add a point to this? Wei Guo did show a PDR, phyllode development rate. Big Island material develops phyllodes . . . you might comment on that.
Wei Guo Sun: On Kaua'i, it's very interesting, if you work along the highway in Waimea Canyon, you will see two kinds of koa trees. One has zigzag pods and this one flowers in the summer. Another one has a straight pod and flowers in the wintertime. When you go into the ridges and hike, you will only see the zigzag pod with round seeds, none of the oblong-seed koa. We assume that along the highway and a couple of ridges, including Koke'e, the seed is from another island. The round seed occurs all over the rest of west Kaua'i.

Q: I'm curious to see if any of the panelists knows anything about the genetic variance with the Maui koa, why it is this particular color, why it's always darker, and more of a red leaf, especially when they're young.
Wei Guo Sun: Through evolution the Maui situation perhaps [evolved] through some difficiency.
Jim Brewbaker: Is there no exception to that? Are all the Maui red?
Wei Guo Sun: To a certain degree. Some are much darker, some are almost the same as O'ahu.

Q: It seemed that there are two ways that you could improve the trees that we have in the field. One was a vegetative approach that Chifumi Nagai talked about. The other was sexual reproduction, cross pollination. What are the relative advantages and disadvanteages? When would you use one as opposed to the other?
Jim Brewbaker: From the evidence that we have, one would argue on the basis of the heritability values you've just seen that an initial step, replicating what's been done with every other important tree species, using sexually derived material from elite trees, would make a substantial improvement in form, in growth rate. Something on the order of the estimates that have been given here, we could go into the 1991 planting, which is starting to seed, and rogue it down to what we would consider elite parental trees, and derive seed from that. One of the biggest questions in my mind, still unanswered, is to what extent that seed will be derived by hybridization within the population and to what extent it will be selfed seed. If, in my view, there is a high degree of self-fertilization occurring, then we can ask the question, why not take selfed seed from individual superior trees. Then you have several options. You certainly don't want to plant entire plantations from progenies from a single tree. It' s difficult to show pictures that illustrate this, but many of the single-family progenies are like peas in a pod. They are extraordinarily uniform. Wei Guo did show a picture, for example, from Maunawili of such a family. All ten trees were lovely in form. I'll let Chifumi address the question of clonal propagation, but anyone with the experience of Bill Libby would certainly say that's the direction we'll head in ultimately if we want to capitalize on the genetic opportunities in a cross-pollinated polyploid, in particular to improve quality, color, fiddleback, and so forth.
Chifumi Nagai: I want to add that for clonal propagation you can speed up, as a supplement to the sexual cycle of selection and breeding, when you must wait years to get the results. For example, if the method works to clonally propagate a certain seedling, we can propagate hundreds of each one. Meanwhile, if longer-term selection is going on and breeders decide here is the elite tree, there's already propagules available.