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Ploidy Variation in *Acacia koa*

NFGEL Project #186

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INTRODUCTION

Currently there is interest in understanding and managing for resistance to koa wilt in *Acacia koa* (koa). Toward this end, there is a need to verify ploidy levels within koa populations.

Koa is endemic to Hawaii, and is the only species in its 'group' of *Acacia*'s (the Australian and Pacific Island Group) that is polyploid. There are also known tetraploid *Acacia* species in the 'Asiatic and African Group' and the 'Cosmopolitan Group'. The 'American Group' of *Acacia*'s appears to be comprised solely of diploid species (Darlington and Wylie 1955).

The base chromosome number in *Acacia* is x = 13. Koa is a tetraploid (2n = 4x = 52), while other members of its group are diploid (2n = 2x = 26) (Darlington and Wylie 1955). However, it may be possible that samples used to assess ploidy through early chromosome counts only included part of the range of the species and didn't include the 'subspecies'. Knowing whether all koa populations are tetraploid would help in interpreting disease resistance data and planning any breeding efforts.

METHODS

Twenty-five seed from each of two sources were received at NFGEL on August 2, 2004: "Big Island, Kapa 5" and "Kauai, Kokee l, Lower Ditch" ('**A'**, below).

A. 

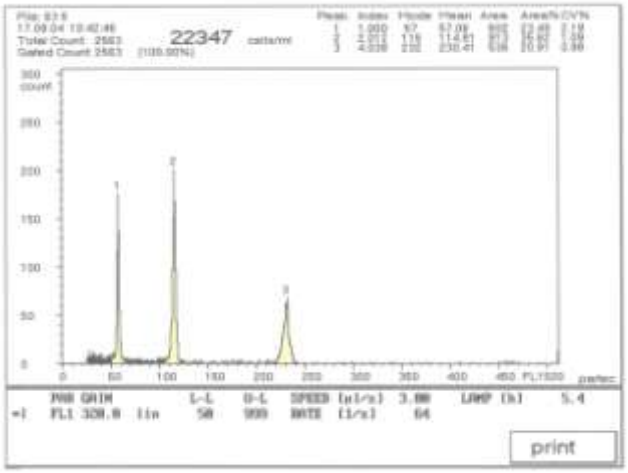
B. 

Six seed per source were scarified (a small cut was made with a scalpel through each seed coat) and soaked in H_2O at room temperature overnight. Three seed per source were prepared for ploidy analysis using the 2-step Partec protocol by extracting approximately ¼ of the seed including endosperm, embryo, and seed membrane. The remaining three seed per source were placed in petri dishes lined with 1% H_2O_2 soaked kimpack and placed in an incubator for germination. All six seeds germinated ('**B**', above), and after 10 days, the root tip from each germinant was prepared for ploidy analysis using the 2-step Partec protocol.

The 2-step Partec protocol consists of the following steps: (1) mince tissue in 0.5ml extraction buffer, (2) incubate at room temperature for five minutes, (3) filter slurry through a green Celltrics filter, (4) incubate at room temperature for fifteen minutes, (5) add 1.5ml stain solution, and (6) read sample on the PA-I using a gain of 320 and LL of 50.

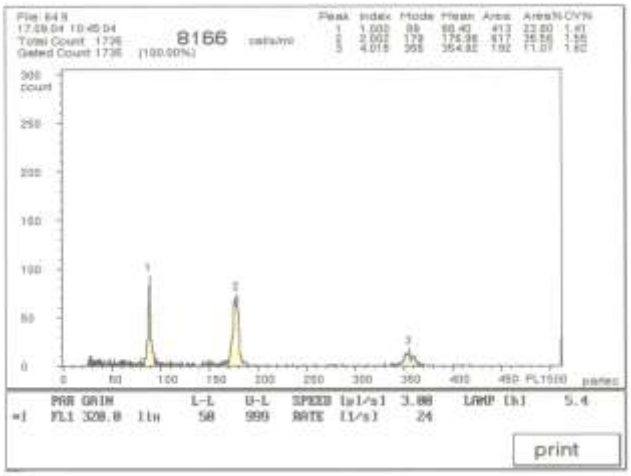
RESULTS

- Ploidy results using seed tissue were the same as those derived from using root tip tissue.
- All six samples analyzed from the Kauai source showed identical ploidy patterns. Without a known diploid or tetraploid control, we cannot definitively identify the ploidy level of these samples. However, we can say they all share the same ploidy level.



Peak position 57 = 2 C
Peak position 115 = 4 C
Peak position 232 = 8 C

- Five of the six samples analyzed from the Big Island source showed the same pattern as that observed in the Kauai source (see figure directly above).
- One of the six samples from the Big Island showed a unique pattern relative to the other 11 samples analyzed (see figure directly below). This sample differs from the other samples in that there appears to be variation in its chromosome number. Therefore, all 12 samples have the same ploidy level, with this one seedling having an apparent chromosome number shift. This sample was also run together with one from Kauai to verify peak positions (plot not shown).



Peak position 89 = 2 C
Peak position 179 = 4 C
Peak position 355 = 8 C

CONCLUSION

The twelve koa seed analyzed produced the same ploidy pattern indicating that all tested samples have the same ploidy level (likely either diploid or tetraploid). Since the species is thought to be a tetraploid (Darlington and Wylie 1955), it is likely these samples are all tetraploid. If tetraploid, the 2 C peak in the plots represents the tetraploid peak. The 4 C peak is the result of chromosome replication before mitosis. The 8 C peak (and in some samples a hint of a 16 C peak) is observed endopolyploidy. Endopolyploidy is more common in certain tissues, such as root tips. If further ploidy analysis is performed in koa, leaf tissue from seed germinates can be used to minimize the endopolyploid peaks.

One of the twelve seed (one sample from the Big Island source), though sharing the same ploidy level as the other 11 samples analyzed, appears to have a variable chromosome number. This can be the result of many things including chromosome imbalance, aneuploidy, chromosome fragmentation, chromosome fusions, and repeated ploidy events (Briggs and Walters 1997).

REFERENCES

Briggs, D. and S.M. Walters. 1997. Plant Evolution, 3rd Edition. Cambridge University Press.

Darlington, C.D., and A.P. Wylie. 1955. Chromosome Atlas of Flowering Plants. University Press, Aberdeen, Great Britain. 519 pp.