

Molecular Genetics of Avocado

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Genetic markers provide an important tool to address a wide variety of practical problems in avocado improvement. Until recently, isozymes have provided the only source of genetic markers in avocado. It is now possible to develop an almost unlimited number of genetic markers using molecular techniques, and these can be used to address a number of significant problems.

Some of the problems which we are currently working on include: (1) A study of the genetic relationships between the major varieties of avocado. The origins and genetic relationships among the major botanical varieties are poorly understood and genetic markers are a major aid in unraveling these relationships. (2) The specific parentage of important cultivars is obscure in a number of cases, and detailed genetic markers will allow us to identify pollen parents through the use of DNA fingerprints. (3) A genetic map of the avocado genome can be constructed using molecular markers and, once such a map is constructed, important practical breeding problems can be addressed. Almost any character which has a strong genetic basis, ranging from fruit quality, flowering times, root-rot resistance, salt tolerance, drought tolerance, etc., could be analyzed and transferred if sufficient linked markers were available.

In the last several years, the methods of molecular genetics have provided genetic markers for mapping plant genomes. Molecular methods have significant advantages over traditional approaches such as the use of isozymes. Isozymes provide a limited number of markers (typically a maximum of 20 to 30 polymorphic gene loci), whereas an unlimited supply of restriction fragment length polymorphisms (RFLP's) can routinely be obtained from most plant species. Second, isozyme techniques have limited genetic resolution. Frequently, these methods are not informative at or above the interspecific level. In contrast, molecular markers can be selected that resolve genetic relationships at virtually any taxonomic level, ranging from individuals within a family through to subgeneric or higher level comparisons. Finally, the use of molecular markers provides a foundation for further biotechnological work on avocado improvement.

Project Objective

The primary objective of this project is to develop a large number of molecular markers to address a number of practical problems in avocado improvement.

Practical applications of this project include the use of DNA markers to (1) identify the genetic relationships among the major varieties of avocado; (2) to provide a precise genetic identification of various avocado cultivars within varieties; (3) to identify the

parentage of specific cultivars; and (4) to begin a program to use RFLP's to map the avocado genome.

Our current and preliminary work firmly establishes the feasibility of molecular methods as applied to avocado genetics.

Progress Report

We have used cloned DNA probes for a cellulase gene, for the ribosomal RNA genes, and for chloroplast genes to study the genetic relationships among avocado varieties. Our previous work, which is now in press (Furnier *et al*, Journal of Heredity, 1990), suggests that *Persea americana* var. *guatemalensis* has genetic markers from both *P. styermarkii* and *P. nubigena* which may indicate a hybrid origin for var. *guatemalensis*. Similarly, the rootstock cultivar G755 appears to be a hybrid of *P. americana* and *P. schiedeana* based on chloroplast DNA and ribosomal gene markers. Finally, our molecular genetic analyses indicate a pattern of relationships among the various *Persea* that is illustrated in Figure 1.

DNA has been prepared from 46 cultivars representing all three major varieties of avocado. Twenty of these samples have previously been screened using the cellulase probe. We found that some, but not all, cultivars within varieties were identifiable based on RFLP patterns. We are currently using anonymous single-copy clones (described below) for further evaluation of cultivars.

A large number of DNA clones are required to provide genetic markers for parental identification and for genome mapping. To begin work towards these two objectives, we have cloned random avocado DNA fragments into a plasmid vector and are characterizing these for their usefulness as genetic markers. To date, 15 clones have been characterized. Eleven of these clones represent single-copy sequences that are polymorphic when tested against cultivars representing three major avocado varieties and are thus good RFLP markers. Three of the remaining probes are multiple-copy sequences that appear to be highly polymorphic between cultivars within varieties. These "hypervariable" probes may be especially useful in cultivar identification. We are now characterizing 17 more clones and expect to increase the number of single-copy probes to 20-25. In addition, there are approximately 50 more clones yet to be screened and characterized.

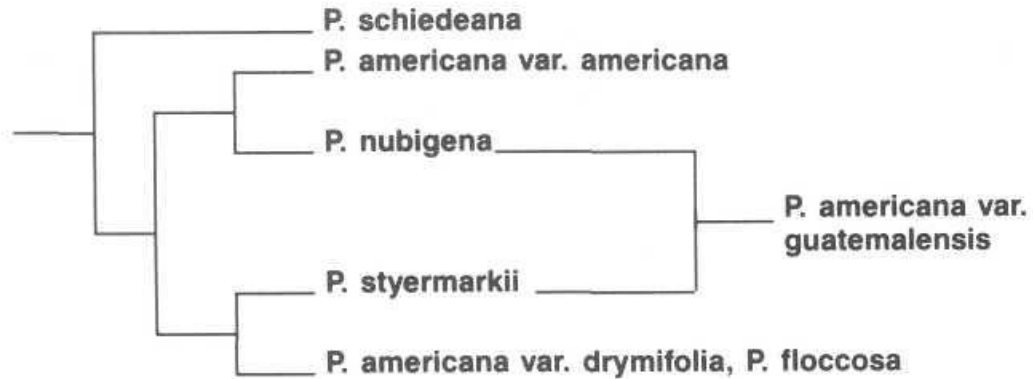


Fig. 1. Relationships among *Persea*.

We have now begun using our probes to screen a panel of cultivars representing several commercial and experimental selections in order to address certain questions of parentage and cultivar identification which might aid the breeding program and the industry. DNA has also been prepared from approximately 85 Hass seedlings that can be regarded as F2 selfed progeny with respect to loci heterozygous in the parent. This material is now being used to test for genetic linkage among single copy probes.

1989-90 Objectives and Expected Accomplishments

Our objectives during the current project year are; (1) To identify and characterize a large number of genomic clones from avocado. Ultimately, we hope to characterize more than 100 clones in order to establish a large number of RFLP markers. The genetic characterization requires that we hybridize each cloned fragment to a panel of avocado material in order to identify so-called "single copy" genes and to further identify those genes which are polymorphic; (2) We will also search among our cloned fragments for potential hypervariable sequences which will prove especially useful in determining genetic fingerprints of cultivars; (3) We plan to continue making DNA preparations from avocado cultivars and related *Persea* species; and (4) we will continue to screen panels of various cultivars to address certain problems in avocado breeding, as well as our F2 progeny to be used in constructing a genomic map of the avocado.

The data obtained in our work will be analyzed to (1) establish the genetic basis of each molecular clone (*e.g.*, single copy, multiple copy); (2) search for linkage relationships among RFLP's based upon the analysis of segregating F2 progenies; and (3) establish unique genetic identifications of all avocado cultivars.

In future years we intend to assemble a detailed RFLP map of the avocado genome where individual RFLP's will mark segments of all 12 avocado chromosomes. This technology has proved very powerful in tomato, maize, soybean, and other crop breeding efforts, and it should prove even more powerful when applied to the genetic characterization of long-lived crops such as avocado.