

Development of Molecular Markers, Microsatellite

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Benefit to the Industry

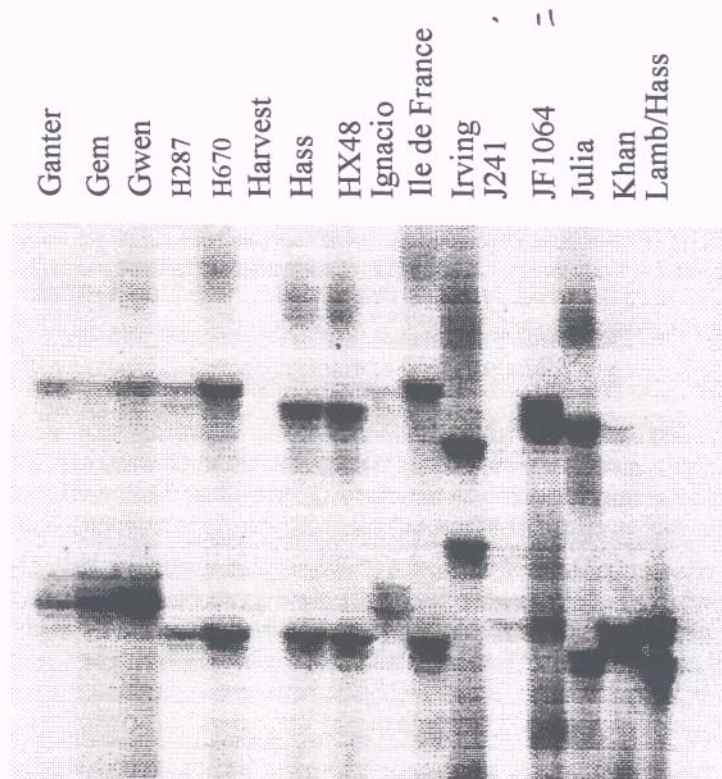
The development of microsatellites as molecular markers for avocado will permit a dissection of the inheritance of economically useful traits such as fruit characters, salt tolerance or disease resistance. Microsatellite markers will also allow the determination of the parentage of important crosses, they will facilitate a unique genetic identification of important breeding and commercial materials and they will assist in the identification of the pollen source (outcrossing) in field and experimental trials.

Objective

The main objective of this project is the development of 100 microsatellite markers. Comparison of microsatellite banding patterns among avocado varieties will provide unique varietal markers (Figure 1) which can be used to determine the pollen parent of seedling and fruit materials from two breeding programs. The first breeding program is the phytophthora-resistant rootstock breeding program being conducted by Dr. John Menge. The second is the B-type Hass-like breeding program supervised by Dr. Mary Lu Arpaia. By determining the pollen parent, we will assess the amount of outcrossing occurring among the 13 rootstock varieties and five varieties in each respective breeding program.

A second objective is the development of an avocado genetic map. The mapping of microsatellite markers and quantitative traits, such as fruit characters and disease resistance will provide a tool for marker-assisted breeding. At the seedling stage, we can assay for the presence of a microsatellite marker linked to a trait of interest. This early screening would enhance the efficiency of avocado breeding and improvement.

Figure 1. Microsatellite profiles of avocado varieties, using primer set, AVO102.



Discussion and Summary

Microsatellites are composed of repeated motifs of di-, tri- and tetranucleotides (for example, ATATAT..., GCAGCAGCA..., or CATGCATGCATG....) and are distributed in high numbers throughout the genome of plants and animals. The codominant inheritance of microsatellite loci allows for the discrimination of homozygous and heterozygous individuals. This provides more genetic information on the contribution of each parental gamete.

Using Hass avocado genomic DNA provided by our laboratory, a southern California company, Genetic Information systems, used a selection procedure to isolate the DNA fragments containing microsatellites, producing an avocado microsatellite-enhanced library. Once DNA sequences of these fragments are obtained, short spans of DNA, called primers, are designed to the two flanking regions surrounding the microsatellite locus. With the primers, we will compare microsatellite banding patterns of avocado varieties to obtain unique genetic identifier for a particular variety.

Our laboratory is currently developing primer pairs for 100 microsatellite loci. Immediate goal is the development of a unique microsatellite banding profile for each avocado variety for the 13 varieties of the phytophthora resistant rootstock breeding program and the five varieties of the B-type Hass-like breeding program. These unique profiles will be

used to determine the pollen parent for each fruit and seedling collected in the two breeding programs. Long term goals for the microsatellite marker is the development of unique profiles for avocado varieties that are important breeding and commercial materials.

Our laboratory has extracted embryo DNA from 720 Hass fruit collected from the B-type Hass-like plot in Ventura County. For the phytophthora resistant rootstock breeding program, extraction of leaf DNA from 374 seedlings is near completion. Once the appropriate microsatellite markers have been identified, we will analyze the DNA samples to determine the parentage of the fruit/seedling. The data on parentage will serve as a guide for outcrossing in the B-type Hass-like breeding program and the phytophthora resistant rootstock breeding program.