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Molecular Genetics of Avocado

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Our primary goal is to obtain a large number of DNA clones from avocado that can be used as genetic markers. To begin work towards these objectives, we have cloned random DNA fragments into a plasmid vector; and we have selected 404 clones for characterization. From this large set, we have obtained 21 single-copy clones that are polymorphic when tested against material representing the three major avocado varieties and are thus good RFLP markers. In addition, we have characterized seven multiple-copy sequences that appear to be highly polymorphic between cultivars within varieties. These "hypervariable" probes may be especially useful in cultivar identification.

Total cellular DNA has been isolated from 42 avocado cultivars. These DNAs are being screened using cloned avocado DNA probes to establish a genetic "fingerprint" for each cultivar. DNA samples have also been prepared from 85 self-pollinated progeny of a single Hass tree and these can be regarded as F_2 progeny with respect to loci heterozygous in the Hass parent. This material will be used to test for genetic linkage among single-copy probes.

We have begun to use our DNA probes to screen 14 cultivars. (The cultivars screened are Hass, H670, H287, Reed, Gwen, Thille, Nabal, Whitsell, Esther, Pinkerton, HX48, Fuerte, Bacon, and Zutano.) To date this panel has been screened with 10 probes (8 single-copy and 2 multi-copy). We have been particularly interested in determining the potential pollen parent of Gwen and in asking whether Pinkerton could have arisen as a Hass x Fuerte cross.

We have also invested considerable effort in exploring the use of the polymerase chain reaction technique (PCR) for the production of DNA based markers in avocado. This method permits the direct amplification of specific DNA fragments from a total DNA preparation. If successful, the method can be used to screen DNA markers in avocado without the very time consuming steps of molecular cloning and probe hybridization. The PCR based methods would therefore greatly increase the number of plants that could be screened per unit time. This large increase in efficiency has obvious utility for our research effort. Of particular interest is a new PCR application called the RAPD method that allows the generation of random DNA markers based on small random primer molecules. Our initial efforts with this method have been unsuccessful; however, we believe these technical difficulties can be overcome.

Genetic markers will have substantial utility in avocado improvement because (a) they will permit the retrospective identification of lineages that have contributed to successful

cultivars; (b) they will facilitate the genetic dissection of complex traits like drought or salt tolerance, disease resistance, and fruit quality; and (c) once the genetic basis of such traits is understood, markers can be used to monitor their genetic transfer in sexual crosses.