MOLECULAR GENETICS OF AVOCADO

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The primary goal of our research is to construct a genetic map of the avocado genome. To achieve this objective we are using two approaches, restriction fragment length polymorphisms (RFLP's) and random amplified polymorphic DNA markers (RAPD's). The RFLP technique uses clones of random fragments of avocado DNA to detect genetic variation among avocado varieties and cultivars. The RAPD method uses a process called polymerase chain reaction (PCR) to produce genetic markers that also detect these variations. These two types of markers can be used to address many aspects of avocado genetics, such as origins and relationships of cultivars, parentage and outcrossing, as well as to create a map which would enable us to identify markers corresponding to important traits like disease resistance.

The cloned DNA fragments (referred to as probes) used in the RFLP method often recognize many sites in the avocado genome. Those that recognize a single unique region (single-copy) are the most useful for the mapping project. Of the 500 probes that we have screened, we have isolated and characterized 35 single-copy probes and expect to have a final total of approximately 40 to use for constructing a map. To produce this map we have prepared DNA from 85 HASS avocado seedlings that we believe to be the products of self-fertilization. We have been using these to test our single-copy probes for genetic linkage. By establishing linkage groups using these probes we will then be able to identify the positions of genetic markers on the chromosomes and trace their transfer to subsequent progeny.

The RFLP techniques have many useful applications. Previously we have used 14 single-copy probes to screen 45 avocado cultivars, hybrids, and related Persea species from which we have DNA prepared. Based on these results we are able to assign a unique genotype or "fingerprint" to each which allows us to genetically identify these cultivars. These results were also used to illustrate the genetic relationships of the three avocado races and cultivars within each race that were included in this screening. . Another use of RFLP's has been in the detection of outcrossing. Our early attempts to test for linkage among the single-copy probes led to the discovery that our original HASS seedlings that were thought to be self-pollinated were in fact 40% outcrossed with the BACON cultivar. From this discovery we were able to identify several probes that enable us to detect outcrossing with BACON, FUERTE, and ZUTANO cultivars, and we are currently pursuing a more detailed study on the effects of outcrossing on yields.

The RFLP's have, unfortunately, proven to be costly and very labor intensive. The construction of a genetic map requires greater numbers of markers than we have

generated using the RFLP techniques. A more recent technology utilizing RAPD's has made it possible to generate markers at a much higher rate. Using small synthetic nucleotide chains called primers we can generate RAPD markers with a PCR tempcycler, a machine that incubates the primers with avocado DNA under extremely sensitive conditions. This method produces visual results in 1-2 days, whereas the RFLP's can take up to two weeks. These synthetic primers are commercially available in virtually unlimited numbers. To date we have screened approximately 100 primers and have found 40-50% to have useful markers for our mapping work, and we expect to have sufficient numbers to supplement the RFLP's and construct a genetic map before the year's end, thereby meeting our original projection of time required to complete this project.