Genetic Maps of Sweet Orange and Trifoliate Orange

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This project is a collaboration involving the Roose and Close labs at UCR and the Fred Gmitter and Jose Chaparro labs at the University of Florida. The main goal is to develop genetic linkage maps of sweet orange and trifoliate orange. Genetic linkage maps are used to improve the efficiency of breeding and to identify genes that, if altered using biotechnology, would confer desired traits on the plant. The maps developed under this project will be the most detailed and accurate available for citrus. CRB funding enables us to obtain matching funds from the UC Discovery program (about $1.35 for each $1 of CRB funds).

Maps are developed by determining which form of a gene or DNA sequence (a “marker”) hybrid offspring inherit from each parent. A map reflects the linear order in which the markers occur in the hereditary material (chromosomes) of citrus and is very similar or identical for all individuals in a species.

Our maps include two types of markers: (1) SSR markers that will be highly informative in many different crosses and therefore make the reference map valuable to many researchers (this is the main type of marker used in human forensic investigation), and (2) a larger number of gene-specific markers detected using the GeneChip developed under this project and project 5200-121 (EST Libraries and Bioinformatics). Progress on developing and mapping these types of markers is summarized below.

SSR markers: In April we analyzed our database of 31,046 citrus genes to identify genes that contain the special repeated sequences that form the basis for developing SSR markers. We identified 3,438 SSRs in 2,945 different genes and designed PCR primers that allow us to study most SSRs. About 50% of these can be mapped in sweet orange and a smaller number in trifoliate orange. This analysis and previous work has allowed us to score the progeny for 107 informative SSR markers. Dr. Gmitter's laboratory has genotyped the progeny for 45 additional markers (their target number), and Dr. Chaparro's laboratory is currently working on mapping another 45 markers. We expect to reach the total project target of 250 markers by March 2006. The SSR markers form the “anchors” for the map, like large cities on a road map.

Gene Chip markers: Affymetrix Gene Chips® can be designed to detect markers when the variable base in the DNA is known. For example, two alternative forms of a gene may contain the sequences ACA and AGA. This variation, called a "single nucleotide polymorphism" or SNP, can be detected if the 25-base sequence deposited on the chip (the "probe") covers this variable region.

Detection occurs when DNA of a plant to be tested is labeled with a dye and hybridized to the chip. Only probes with an exact sequence match to the labeled DNA have high signal intensity. Therefore if the DNA comes from a progeny plant that has the AGA sequence, but not ACA, the corresponding probes on the chip will have high and low signal, respectively. We analyzed the database of expressed gene sequences (ESTs) to identify such variable positions and designed the Gene Chip® to contain 5,023 probe sets that serve as genetic markers for 3,219 genes. DNA variation can also be detected on Gene Chips when there is no knowledge of the variation that can be used for chip design. This applies to the portion of the chip which is designed to measure gene expression and we expect to be able to map at least 1000 more genes using such information.

We will begin mapping experiments in December 2005 or as soon as we obtain sufficient Gene Chips. We expect to complete the proposed mapping by January 2007.