

Project Concluding: Summary Report

Breeding a Red-Fleshed Mandarin (Candidate Gene Analysis)

Project Leaders:

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The title of this project is “Breeding a Red Fleshed Mandarin,” subtitled “Candidate Gene Analysis.” The project has been a collaboration between Jose Chaparro at the University of Florida, Andrew Breksa at the USDA-ARS facility in Albany, California, and Greg McCollum at the USDA-ARS-HRL at Ft. Pierce, Florida.

The primary objective of the project is to gain an understanding of the genetic control of color development in citrus fruit. Peel and flesh color is an important quality trait in citrus. External appearance is the most important factor affecting consumer choice in an impulse (first) purchase of fruit.

The primary pigments of citrus fruit are xanthophylls (yellows and orange pigments) and lycopene (red). The markers and tools developed in this project will benefit the citrus industry by providing breeders with tools for the early selection of seedlings with deep orange peel and flesh color, provide an understanding of the genetic control of flesh and peel color, and identify candidate genes for the modification of existing cultivars using transformation. The project has the potential to develop a novel citrus fruit type in the form of a red fleshed mandarin with increased nutraceutical value resulting from increased levels of lycopene and provitamin A.

The project covers three areas: (1) Identification and mapping of genes controlling flesh and peel color in citrus; (2) Identification of gene(s) responsible for lycopene accumulation in the Cara Cara navel orange; (3) Identification of gene(s) responsible for lycopene accumulation in pummelos. Progress through 2004-2005:

Identification and mapping of genes controlling flesh and peel color in citrus: PCR primers were designed to genes of the carotenoid biosynthetic pathway to allow the amplification of the genes from citrus genomic DNA. Primers for each gene were tested on genomic DNA of a panel of 8 genotypes each, representing mandarin, sweet orange, pummelo and grapefruit. Direct sequencing of the PCR products was performed to search for gene sequence variation and markers designed for 8 of the genes in pathway. Colorimeter readings were obtained from the (pummelo x trifoliolate) x sweet orange juice samples collected in the fall of 2004. DNA was extracted from leaf samples collected in August 2005. The available progeny will be genotyped using the tilling based markers in the spring of 2006.

Identification of gene(s) responsible for lycopene accumulation in the Cara Cara navel orange: The partial sequences of the carotenoid biosynthetic genes in Cara Cara were compared to that of other sweet oranges. Comparison of the partial gene sequences did not reveal any polymorphisms. Cara Cara navel was used to generate segregating progeny. In 2005, 200 open pollinated seedlings of Cara Cara sweet orange were planted in half-gallon pots. Most seedlings appear to be of nucellar origin. The seedlings will be fingerprinted in 2006 using molecular markers to identify the zygotic progeny prior to planting out.

Identification of gene(s) responsible for lycopene accumulation in pummelos: In 2005, pummelo fruit samples from 2004 were analyzed for flesh color. Colorimeter measurements and Lycopene content values were obtained from 72 fruiting pummelos. Vesicle extracts from 21 of the pummelos were further characterized by HPLC. Leaf samples were collected in August 2005 from trees that fruited in 2004, and DNA has been extracted from all the leaf samples. The trees will be genotyped in spring 2006.