11.3 Progress Using Transgenic Approaches and Biotechnology-Facilitated Conventional Breeding to Develop Genetic Resistance/Tolerance to HLB in Commercial Citrus

Grosser, J.W., Dutt, M., Shohael, A., Barthe, G.A. UF-IFAS Citrus Research and Education Center, Lake Alfred, FL, USA

A primary strategy of our program to produce HLB-resistant citrus is genetic engineering to incorporate bacterial resistance genes not found in citrus. Antimicrobial peptides (AMPs) are part of the innate immune response and can be found among all classes of life, including humans. These peptides are usually small proteins and have an ability to associate with membranes. Antimicrobial peptides are also characterized by their broad spectrum antibiotic property. Incorporation of one or more genes encoding for antimicrobial peptides into the citrus genome via genetic engineering could potentially result in development of cultivars resistant to HLB without otherwise altering varietal integrity. We have made significant progress moving several AMP genes into commercial citrus cultivars, mostly grapefruits and sweet oranges, using standard Agrobacterium-mediated citrus transformation and an alternative protoplast/GFP citrus transformation developed previously in our laboratory. A second strategy being employed is to incorporate genes that may turn on systemic acquired resistance (SAR). We have also tested and identified several phloem-limited promoters that function efficiently in citrus. Genetically modified plants containing antimicrobial genes or SAR-induction genes driven by a phloem-specific Arabidopsis sucrose synthase promoter have also been produced. The targeting of antimicrobial gene expression using phloem specific promoters is expected to minimize the expression of the foreign transproteins in subsequent fruit and juice products. Hundreds of transgenic plants from independent transformation events have already been regenerated and propagated, and greenhouse and field testing (at two sites in heavy HLB-pressure areas) of these transgenic plants is underway. Our goal is to identify the most effective yet safe transgene(s) against HLB and then transform it (them) into selected high-quality sweet orange cultivars with a sequential range of maturity dates. We are also testing complex hybrid rootstock candidates to determine their affect on HLB disease establishment and severity in grafted sweet orange scions. A preliminary experiment showed that complex ‘tetrazyg’ rootstocks infer variable tolerance when grafted with HLB-infected sweet orange scion. ‘Tetrazygs’ are allotetraploid hybrids obtained from crosses of somatic hybrids created by protoplast fusion. The working hypothesis is that the identification of a rootstock that can prevent HLB bacterium replication and has a higher efficiency of pumping specific nutrients affected by the HLB disease complex could mitigate the development and spread of the disease when challenged in the field.