13.3 Developing Transgenic Solutions for HLB Resistant Citrus at the US Horticulture Research Laboratory

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No HLB-resistance has been identified within cultivated citrus, making transgenic solutions the priority to develop plant material which will permit economic citrus production where HLB is endemic. Little is known about the HLB pathosystem and thus antimicrobial peptides (AMPs) have been the focus for current work. Related efforts are underway at Texas A&M University, the University of Florida, and the Sylvio Moreira Citrus Center in Brazil, and ongoing discussions ensure that these efforts are complementary rather than duplicative. In the USHRL program, priority AMPs are those which are from plants or synthetic rather than animal origin, are already reported to be effective against gram-negative bacteria, and have negligible potential for human health problems. There are numerous bottlenecks in efficiently mobilizing AMPs to confer HLB-resistance. Due to the urgency for identifying solutions we are moving full speed with best available information, while also attempting to improve transformation efficiency and screen AMPs for important traits. D4E1, a 17 amino acid synthetic AMP which forms a beta sheet (Lucca et al., 1998), is active against Agrobacterium tumefaciens in poplar (Mentag et al., 2003) and has been utilized extensively in our initial efforts. More than a 1000 putative transformants have been developed with D4E1 driven by D35S, representing a broad range of scion and rootstock genotypes. In vitro assessment of minimum inhibitory concentration (MIC) has been conducted using Sinorhizobium and Agrobacterium as surrogates for Liberibacter as they are closely related alpha proteobacters (Bastianel et al., 2005). The causal agent of citrus canker (Xanthomonas smithii pv. citri) is also included in these analyses, in the hope that HLB and canker resistance can be achieved with the same AMP transgene. Thus far D4E1 is the most active AMP tested, with an MIC less than 1 µM. More than 100 transformants are verified and established in the greenhouse. Subpropagations of each independent transformant will be available in early 2009 for graft inoculation with HLB. Procedures have been developed that provide ~90% infection with symptoms and PCR positive response in around six weeks. Several other AMPs targeting Liberibacter and lectins targeting the psyllid are also being utilized. Phloem-specific promoters are being investigated with AMPs. Additional transgenes will be used as opportunities are identified to target Liberibacter gene products and virulence mechanisms.