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A quick evaluation method of AtNPR1 transgenic plants for resistance to HLB

Febres, V.J., Rezende-Muniz, F., and Moore, G.A.

We have produced a number of 'Carrizo' citrange (*Citrus sinensis x Poncirus trifoliata*) transformed with the Arabidopsis thaliana NPR1, a transcriptional co-activator that is key in the regulation of systemic acquired resistance (SAR) and the expression of pathogenesis related (PR) genes. Over-expression of this gene has been shown to induce broad spectrum disease resistance in several species. One of the limitations in obtaining genetically resistant citrus plants to HLB is how lengthy it is to propagate and evaluate the transgenic plants. Using grafting with infected budwood takes several months, is labor intensive and normally requires specialized greenhouse space which can be limited. We have developed a system to quickly screen AtNPR1 transgenic lines and determine if they exhibit an enhanced defense response to *Candidatus* Liberibacter asiaticus PAMPs. First, we used a synthetic peptide of L-flg22 (22 amino acid flagellin epitope derived from CLas) capable of triggering immunity in citrus. Second, using real time PCR, we determined changes in the expression levels of a battery of genes associated with defense in citrus in a time course of up to 72 hours after infiltration with L-flg22 and compared it with the expression in wild type plants. Certain lines consistently showed an enhanced defense response when exposed to L-flg22 thus identifying the ones with the most potential. The advantage of this method as a first step in the screening process is that is quick, controlled and does not require specialized greenhouse space. The selected lines are being further evaluated through graft inoculation for their tolerance to HLB.