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Cell Penetrating Peptides as an Alternative Transformation Method in Citrus

Jensen, S.P., Febres, V.J., and Moore, G.A.

Huanlongbing (HLB) has caused the loss of thousands of trees in Florida's multi-billion dollar citrus industry. An effective, long-term strategy to controlling this disease will be by the incorporation of genetic resistance into commercial genotypes. Because conventional breeding is limited by the lack of natural resistance in citrus to HLB, genetic engineering is now considered a significant alternative to incorporating such characteristics. In fact, despite general concerns from the public against genetically modified organisms (GMOs), one National Academy report[1] stated that genetic engineering will be the way to fully exterminate HLB, while growers' support of a transgenic approach for disease resistant traits also continues to rise. The primary transformation method of citrus typically uses *Agrobacterium*, in which explants are suspended with the bacterium and subsequently placed on selection media. After treatment, the explants produce shoots that can ultimately lead to stable transgenic plants. Due to the slow growth and lengthy maturation, this process takes several years to produce reproductive trees and must be optimized for each cultivar. Consequently, transformation efficiency is substantially less than other model systems. The commercialization of transgenic disease resistant cultivars is even slower due to regulations limiting GMOs worldwide. In order to decrease the dependence upon bacterial vectors and increase transformation efficiency, we have researched an alternative method for introducing nucleic acids into plants that does not involve *Agrobacterium* and instead uses cell penetrating peptides (CPPs). CPPs are short, positively charged amino acid sequences that bind to negatively charged molecules and subsequently translocate across cellular membranes. Most surprisingly plant cell walls can also be bypassed, as CPPs are currently used in plants in transient expression and gene silencing assays. Until now, CPPs have not been examined in citrus or other woody crops for stable transformation protocols. We have developed a method for the transient expression of reporter genes (GUS and GFP) using plasmid DNA and CPPs. Our data indicate that up to 50% of treated explants express GUS when CPPs are used alone. Several optimization steps have been tested and the expression efficiency can be increased up to 100% when CPPs are used in conjunction with a lipid transfection reagent. We have also produced hypocotyl segments which survived kanamycin selection. Some produced shoots that rooted and were planted in soil and have been maintained in a growth chamber. PCR and reporter gene analysis will confirm if stable integration has occurred. Our novel protocol could have far reaching effects for the successful integration of disease resistant GMOs in global markets by limiting the perceived negative effect of bacterial vectors.