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Geminivirus Induced Gene Silencing for Functional Characterisation of Plant Genes and to Induce Virus Resistance by Rna Interference in Cassava

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Abstract

Virus induced gene silencing (VIGS) is a powerful tool to study gene function of unknown plant genes and to induce co-suppression by RNA interference with replicating RNA viruses. The expression of genes or gene fragments via VIGS permits gene function analysis and the study of candidate genes in a simple, fast and robust method prior or as an alternative to the transgenic approach. For crops, like cassava, recalcitrant to plant transformation and regeneration, VIGS can be an attractive alternative. For this purpose we have constructed a geminivirus VIGS vector from the genomic DNA A and DNA B components of an East African cassava mosaic virus, cloned within the left and right boarders of a binary vector. The gene to be studied is introduced into the recombinant DNA A genome replacing the viral coat protein which is dispensable for movement in planta. Upon plant inoculation and during infection, the recombinant virus expresses the foreign gene "fragment" and induces the degradation of a homo-/orthologous gene by RNAi. If an endogenous gene is targeted, an altered mutant phenotype resulting from 'gene knockouts' allows assignment of function to unknown genes. The expression an endogenous cassava magnesium chelatase gene fragment with the replicating virus lead to silencing of the nuclearly encoded sulphur gene significant for chlorophly formation resulting in inhibition of chlorophyll biosynthesis and total bleaching of the plants, hence validating the use of the VIGS system. By using a similar approach, sequences of a destructive cassava infecting RNA virus, Cassava brown streak Ipomovirus (CBSV), were expressed. For this virus not much information is available and there exists no basis of natural resistance in cassava. Hence overexpression of CBSV sequences that results in RNA interference and sequence specific degradation and elimination of infecting CBSV can be an interesting avenue to induce virus resistance. The resistance phenotypes resulting from this approach in different cassava breeding lines infected with CBSV or mixed infected with CBSV and geminiviruses will be presented as examples to discuss the scope of the method.

Keywords: Cassava brown streak virus, Magnesium chelatase Cassava geminiviruses

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