Design and Construction of a Tri-hybrid Hairpin Transformation Vector for Multiple Cassava Mosaic Begomovirus Resistant

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Background

Over 800 million people living in the tropical developing countries in Africa, Latin America and Asia depend on cassava for food and/or nutrition. Cassava is drought tolerant and produces higher carbohydrate yields per unit of land than all other crops. However, cassava production is severely threatened by several pests and diseases. Cassava mosaic disease (CMD), caused by ten distinct *Cassava mosaic begomoviruses* (CMB), is a severe viral disease of cassava and a major constraint for cassava production (Legg and Fauquet, 2004). Three of the ten CMB, *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV), and *South African cassava mosaic* (SACMV), have been reported in South Africa. One approach to achieving virus resistance is to engineer expressed RNA hairpins or inverted repeats (IR) into plants, which when processed generate small interfering (si) RNAs targeting homologous virus sequences. Devastating synergistic interactions which result during multiple begomovirus infection necessitate genetic modification of cassava for multiple virus resistance for broad spectrum resistance.

Methods

Based on superior begomovirus resistance results from a previous study and in silico predicted *begomovirus* siRNA hotspots, PCR primers were designed to amplify specific gene fragments from ACMV, EACMV and SACMV. The individual gene fragments were amplified using Phusion Hot Start II High-Fidelity DNA polymerase and joined using overhanging primers. The resulting ACMV/EACMV/SACMV amplicon was cloned, sequenced for sequence confirmation and sub-cloned into pHellsgate-8 in an inverted repeat format. Following further sequencing for confirmation of insert orientation, the transgene was excised from the pHellsgate-8 ACMV/EACMV/SACMV vector and sub-cloned into pCambia 1305.1.

Results

The designed primers yielded amplicons of the expected band sizes of 200 bp, 195 bp and 198 bp and an ACMV/EACMV/SACMV recombinant gene was successfully made using the overhang primers. The EACMV/SACMV sequences of all six clones were 100% identical however differences were observed in the ACMV sequences particularly within the last ten nucleotides of the 5ˈend. Two of the six clones sequenced had 100% nucleotide identity across the 586bp and were identical to the originally selected sequence but with a seven nucleotide deletion of the last seven nucleotide of the 5ˈ end of the ACMV gene fragment. One of these two identical clones was successfully ligated into two different points within the multiple cloning site of pHellsgate-8. Sequencing results confirmed the inverted repeat orientation. The transgene comprising the ACMV/EACMV/SACMV inverted repeat and the pdk intron was successfully cloned in to pCambia 1305.1 plant transformation vector.

Prospective Research

The pCambia 1305.1 ACMV/EACMV/SACMV vector will be used for for *Agrobacterium*-mediated transformation of friable embryogenic callus (FEC) of different varieties of cassava for subsequent evaluation.

References

Legg, J.P. and Fauquet, C.M. (2004). Cassava mosaic geminiviruses in Africa. *Plant Molecular Biology*. **56**: 585–599.