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Evaluation of Different Strategies to Engineer Cassava Brown Streak Virus (Potyviridae) Resistance in Cassava (*Manihot esculenta*).

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Abstract

Cassava Brown Streak Virus (CBSV) has emerged as a factor causing important losses in cassava field in East Africa. Even though it was first identified in 1930's, molecular diversity and dissemination dynamics remain poorly studied. Classical symptoms are restricted to the storage roots and it renders early disease diagnostic complicated. CBSV is an RNA virus belonging to the Potyviridae family. The CBSV genome encodes for nine proteins, the coat protein (CP) being the most conserved sequence amongst the different CBSV isolates.

Degenerated primers were used to amplify the full CBSV CP sequence from CBSV-infected cassava collected in Tanzania. In order to evaluate the RNA interference mechanism in this system, different strategies were conducted: The characterised CBSV CP partial sequence was used to produce cassettes for expression of sense, antisense transcripts as well as their untranslatable versions. In addition, a cassette for exogenous expression of double-stranded hairpin homologous to CBSV CP sequence was produced to test the efficacy of conventional RNA interference against CBSV.

Transgenic cassava lines were produced for the complete set of binary vectors covering the above presented approaches. Transgenic cassava lines were selected based on transgene copy number and expression levels. Relative transcript quantification through qRT-PCR allowed the selection of transgenic lines containing high and low level of CP sense and antisense transcripts. The transgenic lines were also characterised for the load of short RNAs homologous to CBSV CP sequence through Northern blot procedures adapted for short RNAs detection. The selected transgenic cassava lines are being evaluated for CBSV resistance by grafting transgenic scions on wild-type cassava rootstock infected with a virulent CBSV species. Virus quantification methods were established for the CBSV - cassava host system and they are used to assess virus tolerance and/or resistance in the transgenic cassava lines.

Keywords: Cassava transformation, Plant resistance, RNAi