CONSTRUCTION OF FULL-LENGTH IN-VITRO INFECTIOUS CLONE OF UGANDAN CASSAVA BROWN STREAK VIRUS.

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1. Introduction

a) About Cassava brown streak virus

- Causes Cassava brown streak disease (CBSD).
- The virus belongs to the family Potyviridae and genus Potomovirus.
- Two forms of the virus identified as Cassava brown streak virus (CBSV) and the Ugandan cassava brown streak virus (UCBSV).
- Infected cassava plants show root necrosis symptoms (Fig 1A) making the cassava root inedible.

b) About Infectious clones

- Infectious clones of full length cDNA are important tools for investigating the molecular biology of plant RNA viruses.
- Construction involves production of a double stranded DNA copy of the virus cloned inside a suitable vector.
- Placing the viral sequence under the control of a bacteriophage promoter such as SP6 allows infectious transcripts to be generated in-vitro. A range of genetic manipulations such as site direct mutagenesis, re-arrangement, insertions and deletions can be done.

2. Aims and Objectives

- Sequence the complete genome of Ugandan cassava brown streak virus (UCBSV), Kikombe isolate.
- Construction of infectious clones of Ugandan cassava brown streak virus (UCBSV), Kikombe isolate.

3. Methods

a) Genome Sequencing

- Degenerate primers were designed based on the conserved regions sequenced UCBSV isolates in NCBI database.
- RT-PCR was done to amplify approximately 1Kb sections which were cloned and sequenced.
- Genome was assembled using sequencher (5.0) software.

b) Construction of in-vitro infectious clone

- pYES2.1 vector backbone cloned with complete genome of UCBSV. Eight overlapping sections 1-8 were joined together by homologous yeast recombination to get the complete UCBSV genome cloned inside a pYES2.1 vector.
- The different sections (section 1 to section 8) comprising the complete genome of UCBSV(Fig 2) were joined by homologous yeast recombination technique.
- The technique involves yeast gap repair cloning which joins overlapping DNA sections.
- A yeast adapted plasmid pYES2.1 was used and the complete UCBSV genome was cloned inside pYES2.1 vector. An SP6 promoter was introduced and the plasmid used as template in in-vitro transcription to synthesis run off transcripts which were inoculated onto N. benthamiana and N. clevelandii plants.

4. Results

a) Genome sequencing

- Full genome of UCBSV Kikombe isolate was sequenced using sanger sequencing.
- Genome was assembled and found to consist of 9070 nucleotides which translated into an ORF of 2902 amino acids.
- All predicted ptoyvirus polyprotein cleavage sites were present in the UCBSV genome.
- MEGA phylogenetic analysis was done to compare the complete genome of the sequenced UCBSV isolate with the other isolates in the NCBI database (Fig 3).

b) In-vitro infectious clone

- Full length genome of UCBSV Kikombe isolate was cloned inside pYES2.1 vector.
- Integrity of the cDNA clone was confirmed by RT-PCR, Restriction analysis and sequencing.
- Clone was used in in-vitro transcription to generate run off transcripts which were inoculated onto N. benthamiana and N. clevelandii plants.
- The inoculated plants showed mosaic like symptoms (Fig 4) characteristic of the virus. RNA was extracted from the symptomatic plants and used in RT-PCR (Fig 5). PCR and sequencing results confirmed the infection.

5. Summary of Results

- Sequenced and assembled the complete genome of wild type Kikombe isolate (UCBSV).
- Constructed full length in-vitro clone of UCBSV (Kikombe isolate) RNA with an SP6 promoter.
- Sequenced and assembled Infectious clone complete genome.
- Testing the in-vitro transcripts in indicator plants to confirm infectivity of the Infectious clone.
- Confirmed wild type infection status of UCBSV wild type Kikombe isolate in indicator plants.
- Testing the infectious clones in cassava host plants.
- Now using Infectious clones to study UCBSV pathogenicity.

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7. References