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Abstract

Construction of full-length infectious clones for Ugandan cassava brown streak virus (UCBSV).

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Abstract:

Cassava is one of the most important staple foods to most of the African populations with over 800 million people depending on cassava as the main source of starch. However, low average yields of cassava are caused by a number of factors including susceptibility to pests and diseases. Among the viral diseases, cassava brown streak disease (CBSD), caused by Cassava brown streak virus and (CBSV) and Ugandan cassava brown streak virus (UCBSV) family Potyviridae and genus Ipomovirus, is one of the most devastating. Disease spread has been controlled by planting clean and healthy cuttings. Breeding for resistant varieties has been undertaken, however, there is a need to screen the generated breeding lines for resistance to CSBD, and this has necessitated a need for infectious clones. The main aim of this work was to construct a full-length infectious clone of UCBSV Kikombe. Infectious clones of RNA viruses provide a homogenous and reproducible source of viral infection for effective screening of breeding lines. In order to construct the full-length infectious clone, initially, the complete genome of Kikombe isolate (UCBSV) was amplified in sections by reverse transcription polymerase chain reaction (RT-PCR) and Sanger sequenced. The complete sequence (GenBank accession number KX753356) was found to consist of 9070 nucleotides (excluding the poly-A tail) which translated into a polyprotein of 2902 amino acids. A full-length in-vitro UCBSV infectious clone (GenBank accession KX753357) was generated by cloning the complete genome of the virus in a backbone pYES2.1 vector. An SP6 promoter was introduced at the 5' end of the UCBSV genome to allow for in-vitro transcription. The constructed infectious clone was confirmed to infect tobacco and cassava plants as shown by the mosaic like symptoms observed in the infectious clone infected plants. RNA was extracted from the symptomatic plants and used in RT-PCR. PCR and sequencing results confirmed the infection. In conclusion, this work has for the first time reported construction of an intron-less, in-vitro infectious clone of the full length cDNA of the UCBSV.

Biography:

Research Consultant Department of Biotechnology, National Crops Resources Research Institute (NaCRRI) Developed quick diagnostic tools for viruses infecting yam plants, which are essential for early diagnosis to control disease spread. Developed excellent scientific data analysis and interpretation skills as revealed by gaining hands-on expertise in sequence analysis.

Publication of speakers:

1. Tomlinson, Katie & Pablo Rodriguez, Jose & Bunawan, Hamidun & Nanyiti, Sarah & Green, Patrick & Miller, Josie & Alicai, Titus & Seal, Susan & Bailey, Andy & Foster, Gary. (2019). Cassava brown streak virus Ham1 protein hydrolyses mutagenic nucleotides and is a necrosis determinant. Molecular Plant Pathology. 20. 10.1111/mpp.12813.
2. Duff, Celia & Mbanzibwa, Deusdedith & Nanyiti, Sarah & Bunawan, Hamidun & Pablo Rodriguez, Jose & Tomlinson, Katie & James, A. & Alicai, Titus & Seal, Susan & Bailey, A. & Foster, Gary. (2019). Supplementary Material
3. Nanyiti, Sarah. (2018). Duff-Farrier2018 Article StrategiesForTheConstructionOf.
4. Duff, Celia & Mbanzibwa, Deusdedith & Nanyiti, Sarah & Bunawan, Hamidun & Pablo Rodriguez, Jose & Tomlinson, Katie & James, A. & Alicai, Titus & Seal, Susan & Bailey, A. & Foster, Gary. (2018). Strategies for the Construction of Cassava Brown Streak Disease Viral Infectious Clones. Molecular Biotechnology. 61. 10.1007/s12033-018-0139-7.

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