

ABSTRACT

Cassava mosaic disease (CMD) is one of the major viral crop diseases in sub-Saharan Africa (SSA). In Kenya, CMD is caused by four cassava mosaic begomovirus (CMB) species namely *East African cassava mosaic virus* (EACMV), *African cassava mosaic virus* (ACMV), *East African cassava mosaic Kenya virus* (EACMKV), *East African cassava mosaic Zanzibar virus* (EACMZV), and several recombinant viruses notably the Ugandan variant of EACMV (EACMV-UG). Different plant species are playing an important epidemiological role as virus reservoirs for the whitefly vector *Bemisia tabaci* and as sources of virus diversity. So far, only *Jatropha curcas* has been reported as an alternative host for CMBs in Kenya. This study was done to determine the epidemiological role begomoviruses infecting non-cassava plants play in the CMB-cassava pathosystem in Kenya by (a) identifying alternative hosts for CMBs in coastal and western Kenya, (b) evaluating infectivity of begomoviruses infecting non-cassava in cassava, and (c) determining the evolutionary and molecular evolution of begomoviruses infecting cassava and non-cassava plants. This will help to elucidate the potential of begomoviruses infecting non-cassava plants to evolve and emerge as a threat to cassava production in Kenya.

During diagnostic surveys conducted in 2014 and 2017, a total of 500 leaf samples were collected from fourteen plant species belonging to nine plant families exhibiting begomovirus-like symptoms from cassava fields in coastal and western Kenya. Using species-specific primers, EACMV-like DNA was amplified from 4 out of 14 plant species namely *Deinbollia borbonica*, *Markhamia lutea*, *Cissampelos andromorpha*, and *Capsicum annum*. Infected *M. lutea*, *C. andromorpha*, and *C. annum* leaf samples tested positive for EACMV-UG infection. Next-Generation Sequencing (NGS), *de-novo*, and reference sequence assembly were able to reveal the bipartite genomes of viruses associated with *M. lutea* and *D. borbonica* only. DNA-A of the virus associated with *M. lutea* shared 99% sequence identity with an EACMV-UG isolate (AJ717522) previously identified in cassava in western Kenya. Infected *D. borbonica* samples were found to be infected by a novel begomovirus named *deinbollia mosaic virus* (DMV). DMV DNA-A was most closely related to DNA-A of *Tomato leaf curl Mayotte virus* (AM701764) and *South African cassava mosaic virus* (SACMV; KJ888019) at 75% sequence identity.

The infectivity of DMV was tested in a limited host range in plants belonging to the families *Solanaceae* and *Euphorbiaceae* using concatemeric DNAs and DNA partial dimers using sap and biolistic inoculation methods. *Nicotiana benthamiana* bombarded with concatemeric DNAs resulted in systemic infection associated with yellow mosaic symptoms, leaf deformation, and stunting, while DNA partial dimers caused asymptomatic systemic infection. “Koch’s postulate” was fulfilled with concatemeric DNAs after reintroduction of DMV in *N. benthamiana* through biolistics caused symptomatic infection. Hypersensitive and necrotic responses were observed on biolistically inoculated cassava leaves preventing further virus movement from the point of inoculation and subsequent disease establishment demonstrating a resistant or incompatible host-virus interaction. Sap inoculation of DMV failed to cause infection in the tested plants.

Adaptive mutation was identified as the evolutionary constraints acting on the DMV genome. Predominantly purifying or negative selection was the driving force supporting the adaptation of DMV in its known natural host, *D. borbonica*. The coat protein (CP) gene was found to be mutating rapidly at a mean rate of 1.25×10^{-3} nucleotide substitutions/site/year (subs/site/year). Evolutionary analysis of DMV and selected CMBs showed EACMV, ACMV, and DMV shared a common lineage, and divergence occurred about 200 years ago coinciding with the period when cassava was introduced into the East African coast from Latin America.

This study identified *M. lutea* as a potential alternative host for EACMV-UG revealing the adaptive potential for the virus and expanding our current knowledge of the host range of CMBs in Kenya. The discovery of DMV revealed additional diversity of begomoviruses at the coast. DMV is thought to have the potential to affect cassava production due to its close phylogenetic and evolutionary relationship with CMBs.