Occurrence of African cassava mosaic virus (ACMV) and East African cassava mosaic virus – Uganda (EACMV-UG) in Jatropha curcas

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Background
Jatropha curcas is a drought resistant shrub native in tropical America, now widely grown in many tropical and subtropical regions for biodiesel production [1]. First reports on virus infections in Jatropha indicated the occurrence of viruses closely related to Cassava mosaic virus in India, reaching a disease incidence from 25 to 47%. This might represent a major constrain to the production of Jatropha in large scale [2]. The genome of Cassava mosaic geminiviruses (CMG) consist of two components termed DNA A and DNA B (~ 2.7 – 3.0 kb) [3]. Furthermore, Jatropha has been described as host of Cucumber mosaic virus (CMV) [2].

Methods
In this study we attempted to detect and molecularly characterize viruses infecting Jatropha in Eastern Africa (Kenya and Ethiopia). Detection methods will be valuable tools for early screening of plant viruses in order to make appropriate decisions and selection of planting material.

A total of 127 Jatropha samples from Ethiopia and Kenya (districts: Kakamega, Siaya, Busia and Nakuru showing typical virus symptoms and symptomless plants were used in this study. ELISA was performed to detect the presence of three RNA viruses: CMV, Cassava common mosaic virus (CsCMV) and Cassava brown streak virus (CBSV). PCR was performed using newly designed primers based on multiple alignments of full length DNA A sequences of geminiviruses available in the NCBI Genbank, reported to infect either Jatropha or cassava. This allowed to amplify the variable regions of full length (2800 bp) and shorter sequences (380-1085 bp). PCR products were sequenced. A phylogenetic tree was constructed from multiple alignments by performing a heuristic search. Multiple alignments were analyzed by maximum parsimony with full-length DNA A using Phylogenetic Analysis Using Parsimony (PAUP) and a bootstrap analysis with 1000 replicates.

Results and conclusions
None of the Jatropha samples analysed was infected with the RNA viruses CBSV, CMV and CsCMV. PCR primers amplifying a 380 bp fragment of AC1, AC2 and AC3 yielded positive results with 75% of the symptomatic samples from Kenya and further detected 20% of asymptomatic samples as positive. Furthermore, 61% of symptomatic Jatropha samples from Ethiopia were positive. Full length primers were able to detect 69% symptomatic Jatropha samples from Kenya, and also in 67% of asymptomatic samples. PCR analyses of sample K1J5 amplified the expected 2.8 kb of a near full length DNA A component of the Begomovirus sequence and an additional shorter fragment.

Complete nucleotide sequences of 34 DNA A components typical of Begomoviruses were determined in the Kenyan samples. Thirty three sequences ranged from 2770 bp to 2816 bp while one (K1J5) consisted only of 1416 bp and termed as a defective (Def) DNA. Phylogenetic analyses indicated that the defective molecule belongs to geminiviruses involved in CMG, representing a Def from DNA A of the bipartite Begomovirus ACMV. All viruses characterized in this study grouped with two previously identified Begomoviruses found in cassava in Western Kenya, namely EACMV – UG and ACMV. The Def DNA showed 96.6% sequence identity.
with the ACMV reference sequence [GenBank NC001467.1].

In this study, we report for the first time the detection of Begomovirus: ACMV and EACMV – UG in Jatropha from Kenya. From an evolutionary perspective, the phylogenetic data indicate that the virus isolates from the study were closely related to those isolated previously in Western Kenya from cassava [4,5]. Recombination and synergism that have long occurred in cassava [6,7] could have led to the recent spread of the virus in the field to infect Jatropha. Presence of EACMV – UG and ACMV on different Jatropha plants in the same field indicates the opportunity for mixed infections, hence offering good opportunities for more recombination to occur. EACMV – UG and ACMV are associated with severe synergistic epidemics on cassava. Synergism lead to a 10 - 50 fold increase in viral DNA accumulation which substantially increases the potential for a higher efficiency of vector transmission to even infect non cassava host plants [6,7]. This explain why EACMV - UG is the predominant virus in Jatropha. The deletions occurring in the DE DNA found in the study might affect the replication of the molecule and it might depend entirely on its helper virus for replication.

There is a possibility of Cassava mosaic virus in Jatropha being more wide spread than anticipated, since we have detected it also in Jatropha samples from Ethiopia.

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