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Short Communication

Biolistic inoculation of cassava (*Manihot esculenta* Crantz) with *South African cassava mosaic virus*

M. Makwarela[1,2], N.J. Taylor[2], C.M. Faquet[2] and M.E.C. Rey[1]*

[1]School of Molecular and Cell Biology, University of the Witwatersrand, Private Bag 3, WITS 2050, Johannesburg, South Africa.
[2]International Laboratory for Tropical Agricultural Biotechnology (ILTAB), Danforth Plant Science Center, 975 Warson Road, St Louis, MO 63132, Missouri, U.S.A.

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Cassava *mosaic* disease (CMD) is undoubtedly the most widespread disease in cassava *(Manihot esculenta Crantz*) in sub-Saharan Africa. While African cassava mosaic virus-Kenya (ACMV-KE), African cassava mosaic virus-Uganda (ACMV-UG), African cassava mosaic virus-India (ACMV-IC), East African cassava mosaic virus-Cameroon (EACMV-CM) and a recombinant East African cassava mosaic virus-India/Cameroon (EACMV-CM/IC) can be transmitted easily and repeatedly to *Nicotiana benthamiana* plants, difficulty in mechanical transmission and multiplication of EACMV-UG and South African cassava mosaic virus (SACMV) in N. *benthamiana* appears to place them biologically in a different group. In vitro grown 3-week-old cassava plants were biolistically inoculated with SACMV DNA A and B *dimers* and infectivity measured using a system based on visual assessment. We report for the first time successful infection, induced by SACMV DNA A and B *dimer,* of cassava cultivar TMS60444 using biolistic inoculation. Typical mosaic symptoms started to show at 14 days post-inoculation (dpi) in infected cassava plants, and SACMV replication was confirmed using PCR. Potential applications of biolistic infection of SACMV are also discussed.

Key words: Cassava, biolistic, inoculation, SACMV.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is the primary food crop in sub-Saharan Africa. Cassava mosaic disease (CMD) is one of the most important diseases of this crop and it is caused by whitefly-borne viruses of the genus *Begomovirus* (family *Geminiviridae*) (Fondong et al., 2000). These include *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV)and the recently isolated virus, *South African cassava mosaic virus* (SACMV) (Berrie et al., 2001). SACMV has been fully sequenced (accession numbers: AF155806; AF155807).

The distinguishing feature of most begomoviruses is that they consist of two DNA components, DNA-A and DNA-B. DNA-A is autonomously replicating and encodes five to six genes (one or two on the virion and four on the complementary strand) (Pita et al., 2001) whereas the dependant DNA-B component encodes two genes (one on each strand) necessary for efficient systemic spread of the virus throughout the plant . The two genomic components share approximately 200 bp of sequence (encompassing the conserved TAATATTAC sequence) homology within the intergenic region (IR) which is then termed the “common region” (CR). Both DNA-A and B

*Corresponding authors E-mail:chrissie@gecko.biol.wits.ac.za. Tel: +27 11 717 6351.

Abbreviations: CMD, Cassava mosaic disease; ACMV, African cassava mosaic virus; EACMV, East African cassava mosaic virus; ACMV-KE, African cassava mosaic virus-Kenya; ACMV-UG, African cassava mosaic virus-Uganda; ACMV-IC, African cassava mosaic virus-India; EACMV-CM, East African cassava mosaic virus-Cameroon; EACMV-CM/IC, East African cassava mosaic virus-India/Cameroon; SACMV, South African cassava mosaic virus; dpi, days post-inoculation; PCR, polymerase chain reaction; IR, intergenic region; CR, common region.

Table 1. Primers used to amplify AC1 N-rep from both cassava and tobacco infected plants.

Name	Sequence	Virus	Target
PN-REPC2611	AATGAATTCCTCACGTATCCG	SACMV	AC1
PN-REPV1990	CGATGAGGATCCTACTCGG	SACMV	AC1

components are essential during infection (Chatterji et al., 1999).

Presently, several clones including ACMV-NG (Briddon et al., 1998), ACMV-CM, EACMV-CM and ACMV-CM (Fondong et al., 2000), pseudorecombinant EACMV-UG2 DNA-A + EACMV-UG3 DNA-B (Pita et al., 2001) were reported to be infectious to cassava by biolistic inoculation. However, some clones including ACMV-KE and ACMV-NG were shown to be infectious to various tobacco species but the original isolate of ACMV-KE was not infectious to cassava (Stanley, 1993). This lack of infectivity was attributed to a probable mutation in the DNA-B component. In this report, the results of infection by the SACMV begomovirus from South Africa, dimer A and B (using the particle inflow gun) of cassava is presented.

MATERIALS AND METHODS

SACMV dimer A and B from the newly isolated South African cassava begomovirus were used in both tobacco and cassava infectivity tests. Full-length head-to-tail dimers of DNA-A and DNA-B components of SACMV were constructed by digestion with *Sal*I or *Eco*RI, respectively (Berrie et al., 2001). Relevant fragments were then religated to form dimers in pBluescript KS^+. DNA-A and DNA-B dimers were then subcloned into pBIN19 (pBINSA-A and pBINSA-B, respectively).

To 30 mg of gold particles, 500 µl of 100% ethanol was added and the mixture was vortexed for 3 min and the gold particles pelleted by spinning at 12 000 rpm for 30 s. The supernatant was discarded and 500 µl of fresh 100% ethanol added followed by vortexing for 90 s. The gold particles were pelleted by spinning the mixture again at 12 000 rpm for 1 min and ethanol was discarded. A volume of 500 µl sterile distilled water was added to the gold particles pellet. The gold particles were resuspended by vortexing for 1 min and then pelleted with the supernatant discarded. This step was repeated followed by the addition of 500 µl of 100% ethanol per 30 mg of gold particles. While vortexing at low speed both components of pBINSA-A and pBINSA-B were added to give 100 ng of each component per shot. Then 20 µl spermidine (0.05 M), 50 µl $CaCl_2$ (1 M) were added successively as the mixture was vortexed. The mixture was incubated for 10 min at room temperature. The particles were pelleted by centrifuging for 10 sec at 10 000 rpm, then the supernatant was then discarded and 50 µl of cold 100% ethanol added. After resuspending the pellet, the mixture was added to microcarriers and left to dry at room temperature.

The DNA-coated particles were used to shoot 3-week-old cassava plantlets (cv. TMS60444) in magenta boxes at a pressure of 1500 psi using the Bio-Rad PDS-1000/He biolistic device. Thirty-day-old *Nicotiana benthamiana* seedlings were also inoculated in the same manner. In both cases, young tender uppermost leaves were targeted (five plants inoculated and another 5 as control). After bombardment, both cassava plantlets and tobacco seedlings were maintained at 28°C and 16 h photoperiod greenhouse for symptom development. The symptom severity on fully expanded leaves was scored based on a 0-5-point scale described earlier by Fauquet and Fargette (1990). Replication of SACMV was confirmed by PCR amplification (Table 1).

RESULTS AND DISCUSSION

Cassava plantlets and tobacco seedlings inoculated with SACMV dimers showed infection by visibility of distinct CMD symptoms, while control plantlets that were not inoculated were symptomless. Symptoms caused by these dimers are shown in Figures 1(A-B). Symptoms appeared 7 days post-inoculation (dpi) in tobacco whereas mosaic symptoms became visible 14 dpi in cassava. Infected cassava leaves displayed yellow chlorosis and at 14 dpi symptoms were assigned a severity score of 4. Cassava symptom severity scores never went up to 5 and remained constantly at 4 up until 30 dpi. In the case of *N. benthamina*, leaves were assigned a symptom severity score of 5 at 7 dpi, where severe stunting on the growth of the plant was observed. Although the severity of cassava symptoms was not comparable to field conditions, symptoms caused by the dimers were phenotypically the same as those in field conditions (Figure 1C). The results of the study also demonstrated that SACMV dimers inoculated by means of a biolistic device proved to be infectious. Previously reported studies by Briddon et al. (1998) using cloned ACMV-Nigeria with a hand held biolistic device at a pressure range between 200 to 300 psi proved infectious whereas the current study used pressures at 1500 psi.

Symptoms spread systemically from first inoculated leaves to young tender developing ones confirming the fact that both components are required for infection and movement from cell to cell. Plants displaying symptoms were screened for the presence of SACMV-DNA-A by PCR amplification using primers designed to amplify the N-Rep region of the SACMV AC1 gene (Table 1). Amplified N-Rep PCR products became visible in plants that displayed symptoms and where not obtained in plants that had no symptoms.

We report here successful biolistic infection of SACMV dimer A and B in cassava using the Bio-Rad PDS-1000/He biolistic device for the first time. SACMV symptoms are similar to EACMV, but no early recovery was observed in this study, after SACMV infection of cassava which is not the case with EACMV (unpublished observation). The results of this study are useful, as our laboratory is currently investigating various strategies that aim to engineer local South African cassava cultivars for

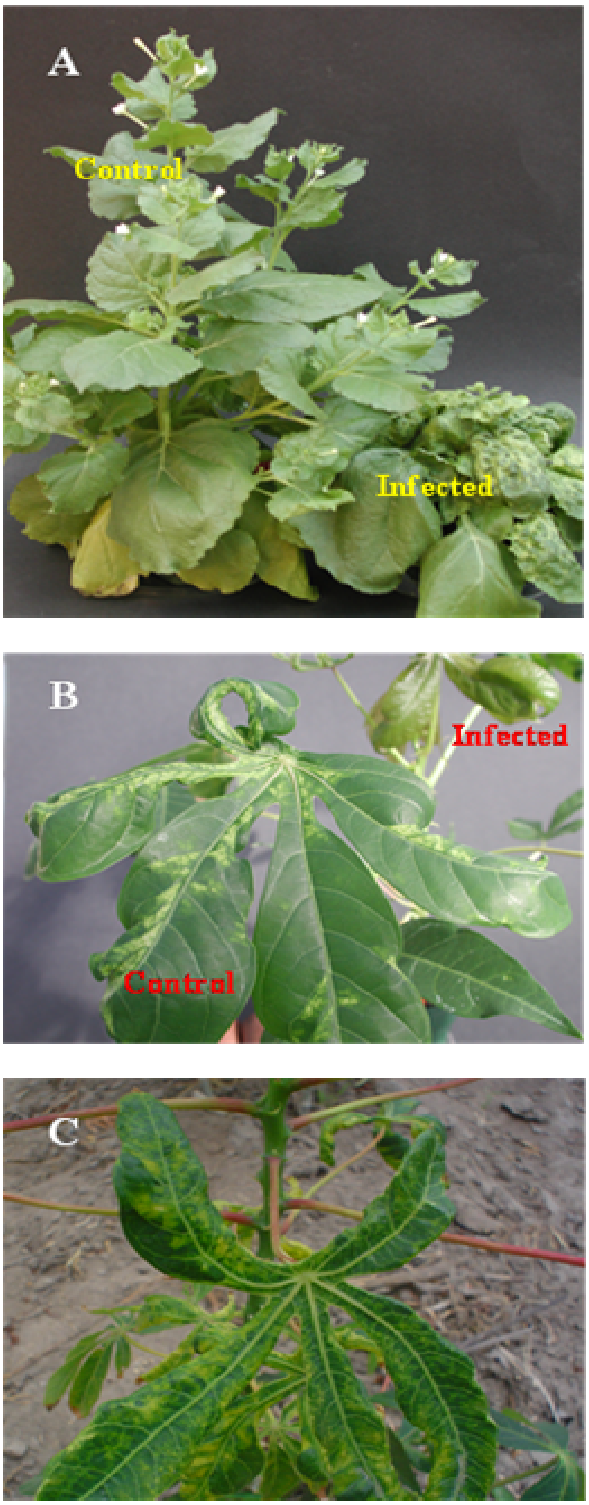

Figure 1. Infection with SACMV (A) Stunting and leaf distortion of tobacco leaves in infected plants (right) and normal looking healthy control (left). Plants were photographed at 28 days post-inoculation (dpi). (B) CMD symptoms on young systemically infected leaves of cassava (right) and young healthy leaves (left). Plants were photographed at 30 dpi. (C) Leaf showing CMD mosaic symptoms in a cassava field in Mpumalanga Province, South Africa.

resistance to SACMV. We believe that biolistic inoculation of SACMV dimer infectious to cassava will expedite screening for SACMV resistance without having to rely on whitefly inoculation, since SAMCV is not mechanically transmissable.

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