II SIMPÓSIO EM PROTEÇÃO DE PLANTAS

Campus de Engenharias e de Ciências Agrárias
Rio Largo, Alagoas
10 e 11 de março de 2020

CONSTRUCTION OF AN AGROINFECTIOUS CLONE OF BEGOMOVIRUS *Bean golden mosaic virus* USING GIBSON ASSEMBLY

Mayra Machado de Medeiros Ferro[1]; Roberto Ramos Sobrinho[2]; Sarah Jacqueline Cavalcanti[1]; Maria Helloá Costa de Oliveira[1]; Gaus Silvestre de Andrade Lima[1]; Tatsuya Nagata[3]; Iraildes Pereira Assunção[1]

[1]Setor de Fitossanidade/Centro de Ciências Agrárias, Universidade Federal de Alagoas, Rio Largo, AL, 57100-000, Brazil, mayra.ferro@hotmail.com; sarah.silva@ceca.ufal.br; helloacosta1@hotmail.com; gaus@ceca.ufal.br; iraildes.assuncao@ceca.ufal.br. [2]School of Plant Sciences, University of Arizona, Tucson, AZ 85721, USA, robertorsobrinho@gmail.com; [3]Departamento de Biologia Celular, Instituto de Ciências Biológicas, Universidade de Brasília, Brasília, DF, 70910-900, Brazil, tatsuya@unb.br.

RESUMO

Viruses belonging to the genus *Begomovirus* (family *Geminiviridae*) infect cultivated and wild plants in tropical and subtropical regions, causing serious economic losses. The begomoviruses have circular single-strand DNA genome encapsidated into quasi-icosahedral particles, being transmitted by whitefly cryptic species into the sibling group *Bemisia tabaci* (Homoptera: Aleyrodidae). Therefore, this study aimed to obtain an infectious clone of BGMV, using the PCR-Gibson Assembly method. For this study, DNA-A and DNA-B genomic components of the begomovirus BGMV isolate 173 AL (GenBank accession KJ939749 for DNA-A, MH925107 for DNA-B), previously cloned into pBluescript KS+plasmid vectors were used to construct infectious clones by PCR and Gibson Assembly (GA). Aliquots of the GA reactions were used to transform *Escherichia coli* DH10B electrocompetent cells, and binding of the viral fragments into pJL-89 binary vector was confirmed by enzymatic digestion and sequencing. Plasmid DNA from the confirmed constructs was used for transformation of *Agrobacterium tumefaciens* (strain GV3101), and the infectivity of the clones was tested by agroinoculation in *Phaseolus vulgaris* 'cv. Pérola'. Total DNA was extracted from leaf samples (systemically infected) collected at 15- and 30-days post agroinfiltration (dpa), and used as template for viral detection using specific primers. Common bean seedlings displayed severe yellow mosaic and stunt symptoms 15 dpa with DNA-A and DNA-B of BGMV and both genomic components were detected by PCR. The approach based on PCR-GA protocol is a fast and useful tool to obtain infectious clones of a circular DNA plant virus

PALAVRAS-CHAVE: *Geminiviridae*, Gibson Assembly, infectious clones.

APOIO: CAPES; FAPEAL; CNPq**.**