

Molecular Characterization of Bean Calico Mosaic Geminivirus

A.O. Loniello¹, R.T. Martinez¹, M.R. Rojas¹, R.L. Gilbertson², J.K. Brown³, and D.P. Maxwell¹

¹Dept. of Plant Pathology, University of Wisconsin-Madison, 53706; ²Dept. of Plant Pathology, University of California-Davis, 95616; and ³Dept. of Plant Pathology, University of Arizona, Tucson, 85721

Whitefly-transmitted geminiviruses have a ssDNA genome generally divided into two circular components, DNA-A and DNA-B (Howarth *et al.*, 1985). The two components have different nucleotide sequences with the exception of a 200 nucleotide region which is nearly identical between both components of each geminivirus and different among different geminiviruses. This region is designated the Common Region. Both components are necessary for infection for geminiviruses from the Western Hemisphere (Gilbertson *et al.*, 1991). Geminiviruses are found throughout the Caribbean Basin, Central America, South America, Africa, the Middle East, and Asia. They are a serious constraint on vegetable production in those areas (Bock, 1982; Gálvez and Morales, 1989).

Bean calico mosaic geminivirus (BCMoV) was described by Brown *et al.* (1990) as a new whitefly-transmitted geminivirus from Sonora, Mexico. Brown observed a 60% occurrence of BCMoV in infected bean fields (*Phaseolus vulgaris*). Characteristic particles of the geminivirus were observed in infected bean plants (Brown *et al.*, 1990).

The symptoms of BCMoV include bright yellow calico and mosaic patterns on the leaves and stunting of the beans. These symptoms are similar to those caused by bean golden mosaic geminivirus (BGMV). Thus, it is necessary to determine if BCMoV is a new virus, or if it is a strain of a previously discovered geminivirus, e.g., BGMV from Guatemala (Gilbertson *et al.*, 1991).

The double-stranded replicative form of BCMoV was extracted from infected beans (Gilbertson *et al.*, 1991) and used for cloning. Three putative full-length DNA-B clones (pBCB1, pBCB2 and pBCB3) were obtained. Endonuclease restriction maps showed that these BCMoV DNA-B clones were different than those for other bean-infecting geminiviruses characterized in our laboratory (unpublished); i.e., BGMV from Brazil, Guatemala and the Dominican Republic and bean dwarf mosaic geminivirus (BDMV). The nucleotide sequence of the presumed Common Region of pBCB1 has the characteristically conserved stem-loop (Fig. 1).

Nucleotide sequence identities of the Common Region of BCMoV with those of DNA-Bs' for BGMV from Guatemala, BGMV from Brazil, and BDMV were 45%, 60% and 61%, respectively. The Common Region of BCMoV had the greatest nucleotide sequence identity (86%) to the restricted host range isolate of squash leaf curl geminivirus (SqLCV) from California (Fig. 1; Lazarowitz, 1991).

The polymerase chain reaction technique (Rojas, 1992) was used to amplify a portion of the BCMoV DNA-A containing a portion of the AL1 gene (the putative replicative protein gene), the Common Region (the geminivirus origin of replication), an intergenic region and part of the AR1 gene (the coat protein gene). Part of the AL1 gene and the AR1 gene were sequenced and compared to sequences from other geminiviruses. The nucleotide sequence of the AL1 region of BCMoV was compared with respective regions for other bean-infecting geminiviruses and identities were

always less than 80%. Identities of greater than 90% are indicative of strains of the same geminivirus (unpublished data).

In conclusion, BCMoV is a distinct bean-infecting geminivirus and is not closely related to BGMV isolates from Central America, the Caribbean Basin or Brazil or BDMV; but appears to be more closely associated with the squash leaf curl geminivirus types (Rojas, 1992).

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	1				50
BCMoV	ATTAGGG.TT	CAGTGGCAT.	TGTCGTAAAT	AAGACCCGGG	ACACCAGGGG
SqLCR	GTTAGGGTTT	CAGTGGCATA	TTTGGTAAAT	ATGAACCCGGG	ACACCAGGGG
CONS	.TTAGGG.TT	CAGTGGCAT.	T.T.GTAAAT	A.GA.CCGGG	ACACCAGGGG
	51				100
BCMoV	GGAGCTCCTC	TCAAAAACCC	TATTGTTGTT	GGTGCCTGG	TGTCCCATAT
SqLCR	.GAGCTC.TC	TCTAAAACC.	TATTATTGCT	GGTGCCTGG	TGTCCCATTT
CONS	.GAGCTC.TC	TC.AAAACC.	TATT.TTG.T	GGTGCCTGG	TGTCCCAT.T
	101				150
BCMoV	ATACTACAAG	TCTCTTTAGG	CCCTTTTAGG	ACACCATGGG	CAAAATCGGC
SqLCV	A...TACAAC	TCTCTGGGG.AGG	ACACCAGGGG	CAAAATCGGC
CONS	A...TACAA.	TCTCT...G.AGG	ACACCA.GGG	<u>CAAAATCGGC</u>
	151				194
BCMoV	CATCCGAAAT	AATATTACCG	GATGGCCG.A	AATTTTTTGG	TGTCC
SqLCV	CATCCGCAAT	AATATTACCG	GATGGCCGCA	AATTTTTTGG	TGTCC
CONS	<u>CATCCG.AAT</u>	<u>AATATTACCG</u>	<u>GATGGCCG.A</u>	AATTTTTTGG	TGTCC

Fig. 1. Common Region of BCMoV DNA-B compared to the Common Region of the restricted host range isolate of SqLCV DNA-B. The stem-loop region, which is conserved in all geminiviruses, is underlined. CONS = consensus sequence. A period (.) indicates no consensus.