Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Virus Research 119 (2006) 154–162
Virus Research
www.elsevier.com/locate/virusres

Studies on the activity of a bidirectional promoter of *Mungbean yellow mosaic India virus* by agroinfiltration

K.S. Usharani, M. Periasamy, V.G. Malathi *

Plant Virology Unit, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi 110 012, India

Received 24 March 2005; received in revised form 16 December 2005; accepted 16 December 2005
Available online 3 February 2006

Abstract

The AV promoter expressing AV1 and AV2 genes and AC1 promoter expressing AC1 gene are present in opposite orientation in the intergenic region of *Mungbean yellow mosaic India virus* (MYMIV). Transient *Agrobacterium*-mediated delivery of putative promoter constructs into *Nicotiana benthamiana* and different legumes, followed by reporter gene (β-D-glucuronidase, GUS) assay, identified the promoter region of both AC1 and AV genes that is necessary for transcriptional initiation. Transcription activator protein-independent activity of AV promoter and differential regulation of AC1 promoter are unique to MYMIV. The AV promoter is a composite core promoter having both TATA box and Initiator elements ($TATA^{+}Inr^{+}$). Many transcription factor binding sites were identified in the upstream promoter sequences of both virion and complementary sense genes, which might be used in the transcription regulation studies of the host plant as well as the virus.
© 2006 Elsevier B.V. All rights reserved.

Keywords: *Mungbean yellow mosaic India virus*; Geminivirus; Begomovirus; Promoter; Agroinfiltration

1. Introduction

Geminiviruses (Family: *Geminiviridae*) are plant viruses with circular, ss-DNA genome. *Mungbean yellow mosaic India virus* (MYMIV) is a whitefly transmitted geminivirus (genus-*Begomovirus*) infecting legumes in South Asia. Other genera of *Geminiviridae* are monocot infecting, leafhopper transmitted *Mastrevirus*, dicot infecting, leafhopper transmitted *Curtovirus* and dicot infecting, treehopper transmitted *Topocuvirus*.

Begomoviruses are either monopartite or bipartite in genome organisation. Bipartite begomoviruses have DNA A and DNA B components, each of ∼2.7 kb in length. In DNA A, ORFs AV1 (Coat protein, CP) and AV2 are present in the virion sense. ORFs AC1 (Replication initiator protein, Rep), AC2 (Transcription activator protein, TrAP), AC3 (Replication enhancer protein, REn), AC4 and AC5 are present in the complementary sense. CP encapsidates the viral genome and plays a vital role in vector transmission. Rep and REn are essential for viral DNA replication (Harrison and Robinson, 2002). TrAP, which transactivates virion sense genes in both DNA A and DNA B (Sunter and Bisaro, 1992) is a well-known post-transcriptional gene silencing (PTGS) suppressor (van Wezel et al., 2002). The function of ORFs AV2, AC4 and AC5 has not yet been clearly ascertained. In DNA B, ORF BV1 (Nuclear shuttle protein, NSP) is encoded in the virion sense and ORF BC1 (Movement protein, MP) in the complementary sense. Both the proteins direct virus movement in the host. There is an intergenic region (IR) in begomoviruses having the origin of replication and regulatory elements required for replication as well as transcription. The DNA A and DNA B components share a ∼200 bp common region (CR) with near-identical sequences that is present in the IR (Harrison and Robinson, 2002). MYMIV is exceptional in that the CR between DNA A and DNA B components has only 84% identity (Usharani et al., 2004).

The transcription of CP and Rep genes are governed by a bidirectional promoter that is present in the IR. Rep down regulates its own expression by binding to an iterative motif located between the TATA box and its transcription start site (Eagle and Hanley-Bowdoin, 1997). TrAP transactivates CP expression. For *Tomato golden mosaic virus* (TGMV), TrAP activated CP expression in mesophyll cells and derepressed the promoter in phloem cells (Sunter and Bisaro, 1997). Later, Sunter and Bisaro (2003) identified a bipartite sequence element in TGMV CP promoter that is involved in both activation and derepression

* Corresponding author. Tel.: +91 11 25842438; fax: +91 11 25843113.
E-mail address: vgmalathi@rediffmail.com (V.G. Malathi).

0168-1702/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.virusres.2005.12.013

of virion sense promoter by TrAP. In the case of *African cassava mosaic virus* (ACMV) (Zhan et al., 1991) and *Cotton leaf curl Multan virus* (CLCuMV) (Xie et al., 2003), the complementary sense promoter was found to have stronger activity than virion sense promoter. A conserved sequence element (CLE) had been identified to play a role in TrAP activation of virion sense expression though there are conflicting reports on its requirement. CLE is present in many Old World begomoviruses and in few New World begomoviruses. For *Pepper Huasteco virus* (PHV) from South America, CLE was found to be essential for transactivation (Ruiz-Medrano et al., 1999) whereas Sunter and Bisaro (2003) had shown that in TGMV, CLE is not necessary for TrAP transactivation.

In this paper, *Agrobacterium*-mediated delivery into plants was used as a transient assay system for qualitative studies on promoter activity. The 5′ start site of AV and AC1 transcripts of MYMIV were identified and the promoter sequences required for their expression were defined by using nested fragments of the upstream regions. It was found that AC1 promoter was differentially regulated in roots and leaves and also that transactivation by TrAP was not necessary for the basal expression of the virion sense promoter.

2. Materials and methods

2.1. Transcript mapping

From 100 mg leaves of French bean (*Phaseolus vulgaris*) agroinoculated with pBMA2 and pBMB2 (Mandal et al., 1997)—the tandem repeat (two copies) constructs of DNA A (AF126406) and DNA B (AF142440) components of MYMIV respectively in the binary vector pBIN19, total RNA was isolated using TRIzol™ reagent (InVitrogen™, life technologies). The RNA pellet was dissolved in DEPC-treated water. The 5′ RNA Ligase Mediated Rapid Amplification of cDNA Ends (5′ RLM-RACE) was performed using the isolated total RNA by following the manufacturer's protocol (FirstChoice™ RLM-RACE, Ambion). The PCR reactions were carried out using nested 5′ RACE outer and inner primers and virus specific primers. For mapping of viral sense transcripts, the primers AV2 Betra (5′ TGTAAGGTACGAGTAACCTA 3′) and RepC3 (5′ GAGAATTCGCAGGCAGCATGA 3′) were used. The complementary sense transcript of AC1 was mapped by using RepV1 primer (5′ TCAAGCTTTGCGTTTATAGCAAA 3′). The amplified products were cloned into pGEM™-T Easy Vector Systems (Promega) and sequences were determined using ABI Prism automated sequencing facility available at University of Delhi.

2.2. Construct preparation

The following primers along with RepV1 and RepC3 were used to amplify the upstream promoter region of virion sense and complementary genes of pMYAH13 [MYMIV DNA A in pUC18 vector] (Varma et al., 1991):

- RepC1: 5′ GAGAATTCACCGGTCGCGGGGCAC 3′;
- RepC2: 5′ GAGAATTCTTGCCAACATGCACCGGA 3′;
- CPV1: 5′ TCAAGCTTCCGGTGCATGTTGGCA 3′;
- CPV2: 5′ TCAAGCTTGTGCCCCGCGACCGGT 3′.

The position and orientation of the primers in the IR of MYMIV DNA A are depicted in Fig. 1A. The eighth nucleotide A of the conserved nonanucleotide sequence (TAATATTAC) is numbered as the first nucleotide of MYMIV genome as per

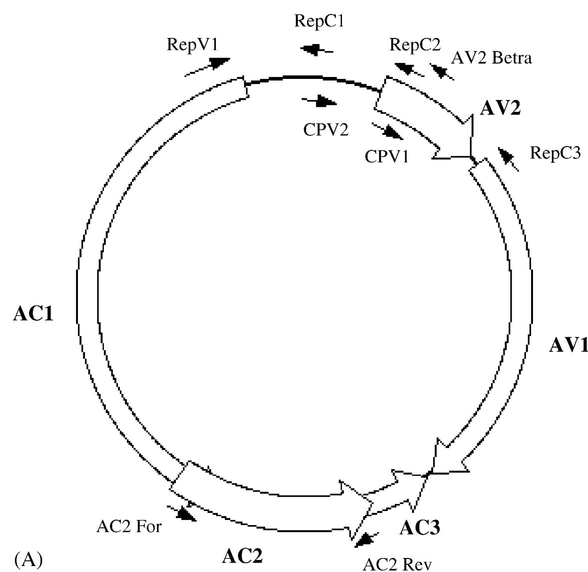

Fig. 1. (A) The location and orientation of the primers in the genome of MYMIV DNA A, that were used for transcript mapping and for amplifying the AV and AC1 promoters (AV2 Betra: nt 284–265; RepV1: nt 2591–2607; RepC1: nt 22–7; RepC2: nt 219–202; RepC3: 408–396; CPV1: 203–218; CPV2: 7–22) and ORF AC2 (AC2 For: 1640–1628; AC2 Rev: 1230–1242). The ORFs AC4 and AC5 are not shown in the figure. (B) Diagrammatic representation of the promoter constructs. The eighth nucleotide 'A' of the conserved nonanucleotide sequence in the origin of replication (shown as stem and loop) is numbered as the first nucleotide. The position of the transcript start sites (TSS) of AC1 and AV genes along with the start and stop codons of the genes, wherever they are present in the promoter constructs are highlighted: (i) AC1 promoter constructs; (ii) AV promoter constructs.

Download English Version:

https://daneshyari.com/en/article/3431257

Download Persian Version:

https://daneshyari.com/article/3431257

Daneshyari.com