A Sobemovirus Hindering the Utilization of *Calopogonium mucunoides* as a Forage Legume in the Lowland Tropics

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ABSTRACT

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The cultivation of calopo, *Calopogonium mucunoides*, as a promising forage legume for the lowland tropics, was hindered by its susceptibility to a severe yellow mosaic disease observed in the Eastern Plains of Colombia. An isometric virus ca. 28 nm in diameter was observed by electron microscopy in leaf extracts and purified preparations, and in phloem cells of systemically infected calopo plants. The virus was transmitted by mechanical means and by the chrysomelid beetle *Diabrotica balteata*. The host range of the virus was restricted to the legumes *Phaseolus vulgaris, Vigna unguiculata, V. radiata, Centrosema* spp., and *Senna occidentalis*. The physical and chemical properties of the calopo virus were similar to those reported for the sobemovirus group, and the virus was antigenically related to the bean and cowpea strains of southern bean mosaic virus (SBMV). However, the host range of the calopo virus differed from the pathogenicity spectra of the bean, cowpea, Ghanaian, and Mexican strains of SBMV. Nucleotide sequence analysis of a 609 bp fragment amplified from the coat protein region of the calopo sobemovirus revealed similarities of 81.8 and 66.1% with the corresponding regions of the bean and cowpea strains, respectively, of SBMV. The respective homologies increased to 83.7 and 67.8% when the deduced amino acid sequences of these viruses were compared. It is concluded that the mosaic disease of calopo is caused by a previously undescribed sobemovirus for which the name calopo yellow mosaic virus is suggested.

The neotropical legume *Calopogonium mucunoides* Desv. is used as a cover crop throughout the tropics (20), and it is currently under evaluation as a promising forage species for acid, infertile soils in tropical America (2,15). The main biotic problem of *C. mucunoides,* observed in agronomic trials conducted in the Eastern Plains (Meta) of Colombia, has been a striking variegation characterized by an intense yellowing and foliar malformation (Fig. 1). As the disease progresses, bleaching of the leaf lamina may occur.

Calopogonium mucunoides has been cited as a natural host of Centrosema mosaic potexvirus in Papua New Guinea (23,24), passionfruit woodiness potyvirus in Australia (8), cowpea severe mosaic comovirus in Brazil (11), and Clitoria yellow vein mosaic virus in Malaysia (1). This investigation was conducted to identify the causal agent of the intense yellowing of calopo in Colombia.

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MATERIALS AND METHODS

Isolation and maintenance of the pathogen. Variegated leaves of *C. mucunoides* collected in an experimental field near Villavicencio (Meta) were used as source of the isolate selected for this investigation. For the mechanical inoculation tests, symptomatic leaves were ground and diluted (1:10, wt/vol) in tap water. The inoculum was applied onto test plants using a cheesecloth pad. The causal agent was mechanically transmitted to and maintained in bean (*Phaseolus vulgaris* L.) cvs. Bountiful and Michelite 62, under glasshouse conditions with a maximum light intensity of 1,100 μE s^{-1} m^{-2}, an average temperature of 27°C, and 75% relative humidity.

Pathogenicity tests. The following species were mechanically inoculated: *Arachis hypogaea* PI 259747; *Calopogonium mucunoides; Canavalia* sp.; *Centrosema acutifolium* CIAT 5568; *C. brasilianum* CIAT 5234; *C. macrocarpum* CIAT 5252; *C. pascuorum* CIAT 5195; *C. pubescens* CIAT 438; *Glycine max* cvs. Buffalo, Davis, ICA L-21, Improved Pelican, Kwanggyo, Marshall, Ogden, Williams, and York; *Lablab purpureus* CIAT 17192; *Macroptilium lathyroides*; *Phaseolus acutifolius* var. *latifolius* CIAT 40163; *P. lunatus* cv. Henderson; *P. vulgaris* cvs. Amanda, Black Turtle Soup, Bountiful, Dubbele Witte, Great Northern 31, Great Northern 123, Improved Tendergreen, Jubila, Kentucky Wonder, Imuna, Monroe, Pinto 114, Red Mexican 34, Red Mexican 35, Redlands Greenleaf B, Redlands Greenleaf C, Sanilac, Stringless Green Refugee, Topcrop, and Widusa; *Pisum sativum* cv. Alaska; *Senna occidentalis* CIAT 15538; *Vigna aconitifolia* cv. AC-10; *V. radiata* cv. R-5; *V. umbellata* cv. U-7; and *V. unguiculata* cvs. Blackeye, S-2, S-5, S-10, and S-18, in the Leguminosae. Additional plant species tested in other families included: *Capsicum frutescens; Chenopodium album, C. amaranticolor, C. murale*, and *C. quinoa; Cucumis sativus; Datura stramonium; Hibiscus esculentus; Ipomoea* sp.; *Lycopersicon esculentum* cv. Marglobe; *Nicandra physalodes; Nicotiana benthamiana, N. glutinosa,* and *N. tabacum* cv. White Burley. All test plants were serologically assayed for systemic infection after inoculation using foliar tissue.

Virus purification. Approximately 150 g of leaves were harvested from systemically infected bean cv. Stringless Green Refugee plants inoculated 12 days earlier. Infected tissue was homogenized in a blender for 3 min in 300 ml of cold 0.05 M KPO_4 buffer, pH 7.2, containing 0.1% 2-mercapto-ethanol and 0.15% thioglycollic acid. The homogenized mixture was filtered through cheesecloth and centrifuged at 10,400 × *g* for 10 min. The supernatant was blended with 8% polyethylene glycol 6000 and 1% NaCl, by stirring for 3 h at 4°C and centrifuged at 16,300 × *g* for 10 min. The pellet was resuspended in 30 ml of 0.02M KPO_4 buffer, pH 7.2, and then clarified at 3,000 × *g* for 5 min.

Fig. 1. Mosaic and leaf malformation in *Calopogonium mucunoides* induced by the calopo sobemovirus.

The suspension was treated with chloroform (4:1, vol/vol, respectively) and then centrifuged at 3,000 × *g* for 5 min. The resulting supernatant was subjected to centrifugation at 102,900 × *g* for 90 min. The pellet was resuspended in 2 ml of 0.02M KPO_4 buffer, pH 7.2, and the suspension was centrifuged at 1,075 × *g* for 3 min. The virus was further purified by equilibrium density gradient centrifugation (95,770 × *g* for 210 min) in a preformed 26 to 40% (wt/wt) suspension of CsCl prepared in 0.02 M KPO_4 buffer, pH 7.2. The viruslike band was diluted with 0.02 M KPO_4 buffer, pH 7.2, in 10-ml tubes to be concentrated by centrifugation at 102,900 × *g* for 90 min. The resulting pellet was resuspended in 200 µl of 0.02M KPO_4 buffer, pH 7.2, before a final clarification at 1,075 × *g* for 3 min.

Electron microscopy. Leaf extracts and purified preparations from infected test plants were negatively stained in 2% uranyl acetate, pH 3.7, and examined for the presence of virus particles using a JEOL SX-100 electron microscope. Leaf tissue of symptomatic *C. mucunoides* plants was prepared for cytology as described earlier (13). Thin sections were cut with a diamond knife using a MT 6000 Sorval ultramicrotome.

Spectrophotometry. Absorption spectra of purified virus preparations were obtained with a Beckman DU 50 spectrophotometer in the range of 360 to 240 nm. Since a relationship to the sobemoviruses was assumed, an extinction coefficient of 5.8 was used (21) to determine the concentration of the purified calopo virus.

Electrophoresis. For coat protein analysis, purified virus preparations were analyzed in 10% polyacrylamide gels containing sodium dodecyl sulfate (SDS) as described by Weber and Osborn (25). Samples were dissociated by adding two volumes of a solution containing 0.1 ml of the $NaPO_4$ buffer used for electrophoresis, 0.25 ml of 10% SDS, 25 µl of 2-mercaptoethanol, and 0.25 ml of 60% sucrose, and boiling the mixture for 1 min. Bovine serum albumin (M_r 66,000), ovalbumin (M_r 45,000), and carbonic anhydrase (M_r 31,000) were used as markers for molecular weight determinations.

For the analysis of viral dsRNA, virus-free and virus-infected bean cv. Bountiful leaf tissue was used as control and source of viral RNA, respectively, according to the procedure described by Dodds and Bar-Joseph (6). Approximate dsRNA weights were estimated using a 1-kb ladder (Bethesda Research Laboratory [BRL], Bethesda, MD).

Serology. An antiserum was prepared by injecting New Zealand white rabbits with purified virus preparations standardized to a concentration of 1 mg/ml. A series of four intramuscular injections was given at weekly intervals. Each injection consisted of 0.5 ml of the virus emulsified with an equal volume of complete (first injection) or incomplete (subsequent injections) Freund's adjuvant. The antiserum was collected at weekly intervals for a month after the last injection.

The antiserum to the bean strain of southern bean mosaic virus (SBMV) had been previously prepared at CIAT (9), and the antiserum to the cowpea strain of SBMV was kindly provided by George Thottappilly, virologist, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

The Ouchterlony, double antibody sandwich–enzyme-linked immunosorbent assay (DAS-ELISA), and serologically specific electron microscopy (SSEM) tests were conducted as described by Purcifull and Batchelor (16), Clark and Adams (4), and Derrick (5), respectively.

Seed transmission tests. Mature and green pods were harvested from systemically infected *C. mucunoides* plants. Green pods and mature and immature seeds were serologically assayed (ELISA) for the presence of virus, using the homologous antiserum prepared in this investigation. Approximately 150 mature seeds were grown out and the resulting seedlings were tested by ELISA for viral infection 15 days after sowing.

Insect transmission tests. Individuals of *Diabrotica balteata* LeConte reared on *Phaseolus vulgaris* L. were tested as vectors. The chrysomelids were starved for 4 h before being transferred to bean cv. Bountiful plants systemically infected with the calopo virus. After a 24-h acquisition access period, the chrysomelids were transferred in groups of four to three virus-free Bountiful plants and allowed a 24-h inoculation access period. Following the inoculation period, the insects were eliminated with an insecticide.

Extraction of total nucleic acid. Approximately 40 mg of *C. mucunoides* tissue obtained from plants infected by the calopo virus, was ground into a powder under liquid nitrogen, and then vortexed with 800 µl of 4 M guanidinium isothiocyanate for 2 min (19). The RNA was precipitated with 0.7 volumes of 100% ethanol and concentrated by centrifugation for 5 min at 13.000 *g*. The pellet was dissolved in 450 µl of 6 M NaI, and 40 µl of 3 M sodium acetate, pH 5.2, and then fractionated in a Glass-Max (Gibco BRL, Bethesda, MD) column according to supplier's directions for RNA. The total nucleic acid was eluted by adding 45 µl of diethyl-pyrocarbonate (DEPC)–treated water, pre-heated to 65°C, and centrifugation for 20 s at 13.000 × *g*. The concentration of the total nucleic acid isolated was determined spectrophotometrically (A_{260}) at a 1:50 dilution.

cDNA synthesis and PCR amplification. Double-stranded cDNA was synthesized using 200 U of Superscript Reverse Transcriptase (Gibco BRL) and following the protocol suggested by the supplier. The oligonucleotides SBMV-BFOR (5′ TGA GGT ATC CAT GGC CCC 3′) and SBMV-BREV (5′ TAT AGG CGT CCA GTA CTC ACA GC 3′), derived from the capsid regions of the bean and cowpea strains of SBMV, were used as upstream and downstream primers, respectively, at a final concentration of 125 nM.

The cDNA was amplified in a 25 µl polymerase chain reaction (PCR) reaction, using 1.0 unit of *Taq* DNA polymerase (Promega, Madison, WI), 0.2 mM of each dNTP, 0.1 µM of each oligonucleotide and 2 mM $MgCl_2$. The reaction was performed in a thermal controller (MJ Research, Watertown, MA) running 29 cycles of the following profile: 94°C/30 s; 56.5°C/45 s; 72°C/1 min.

Cloning and sequencing of PCR products. PCR products were purified by the Wizard PCR system (Promega) and subjected to a blunt-end reaction using the Klenow fragment. The DNA was purified and ligated into the *Srf*I site of pCR-Script SK(+) vector (Stratagene, La Jolla, CA) according to the supplier directions. Ten microliters of the ligation reaction was used to transform the DH5α strain of *Escherichia coli*. Sequencing was done by the dideoxynucleotide termination chain method (18) using the Sequenase version 2.0 (United States Biochemical, Cleveland, OH) with the primers T3 and T7. To obtain the complete sequence, a subclone was produced by restriction enzyme digestion and autoligation. Sequence analyses and comparisons were done using DNASIS (Pharmacia LKB, Uppsala, Sweden) and Seqaid II (D. D. Rhoads and D. J. Roufa, 1989, Kansas State University, Manhattan).

RESULTS

As determined by symptom expression and ELISA, the causal agent was mechanically transmitted to the following legumes: *C. mucunoides*; the five *Centrosema* species tested; the nine soybean cultivars inoculated; the bean cvs. Amanda, Bountiful, Improved Tendergreen, Jubila, Redlands Green Leaf B and C, Stringless Greenleaf Refugee and Topcrop; *V. radiata*; and *V. unguiculata* cvs. S-2 and S-5. *Senna occidentalis* gave a positive reaction in ELISA despite the absence of visible symptoms. *Phaseolus acutifolius* and the bean cvs. Great Northern 31 and 123, Imuna, Kentucky Wonder, Monroe, Pinto 114, Sanilac, Red Mexican 34 and 35 ,and Widusa reacted with local lesions on the inoculated primary leaves. The remaining legumes tested were symptomless and negative in ELISA. None of the non-leguminous test plants inoculated showed symptoms or was shown to be systemically infected as determined by ELISA.

The purification procedure yielded approximately 25 mg of a nucleoprotein with an $A_{260/280}$ ratio of 1.8 (uncorrected for

light scattering) per kg of infected tissue. The nucleoprotein band recovered from the CsCl gradients, was shown by electron microscopy to contain isometric viruslike particles with a diameter of ca. 28 nm. This fraction was infectious when manually inoculated onto Bountiful bean seedlings. Similar particles were observed in thin sections of mosaic-affected foliar tissue of *C. mucunoides*, mostly in phloem cells. The viruslike particles were often associated with viroplasms and fibrils (Fig. 2A), and were sometimes observed inside vesicles (Fig. 2B).

A single protein species of M_r 31,000 was detected by SDS–polyacrylamide gel electrophoresis (PAGE) of purified virus preparations. A predominant band of ca. 4,000 bp was observed in 5% acrylamide gels of ds-RNA extracts from infected bean plants. This ds-RNA band had an equivalent mass of ca. 2.77×10^6 Da, or 1.38×10^6 Da for an ss-RNA molecule. These protein and nucleic acid bands were not observed in extracts prepared from healthy calopo plants.

Due to quarantine restrictions preventing the introduction of the cowpea strain of SBMV, only the bean strain of SBMV and calopo sobemovirus could be antigenically compared in reciprocal double immunodiffusion tests. The results obtained (Fig. 3) showed the formation of weak but clear spurs with the antisera to the calopo sobemovirus and the bean strain of SBMV, respectively, over the heterologous antigens. The antiserum to the cowpea strain of SBMV also precipitated the bean strain of SBMV and the calopo sobemovirus.

In SSEM tests, the use of the homologous antiserum to the calopo virus resulted in a 35-fold increase in the number of virus particles trapped with respect to the number of virus particles observed without treating the grid with antiserum. Similar results (25- to 30-fold increase in the number of calopo virus particles trapped) were obtained with the antisera to the bean and cowpea strains of SBMV.

In DAS-ELISA tests, positive reactions (optical density [OD] A_{405} of 0.40 to 0.45 after a 15 min substrate incubation period) were obtained with the calopo virus antiserum, using the homologous antigen and the bean strain of SBMV. The antiserum to the latter strain, recognized its homologous antigen (OD A_{405} of 0.75, 15 min after addition of substrate) but it did not react with the calopo virus (OD A_{405} of 0.02) under the same experimental conditions and substrate incubation time. However, a weak, positive reaction of OD A_{405} of 0.2 was obtained following a 4-h substrate incubation period. Virus-free bean and calopo plant extracts had OD A_{405} values that ranged from 0.02 to 0.03 following substrate incubation periods of 4 h.

None of the 150 *C. mucunoides* seedlings grown out from seed harvested from systemically infected plants reacted positively in ELISA tests. However, positive ELISA reactions were observed for extracts of immature (5/17) and mature (3/17) *C. mucunoides* seeds and green pods (3/6).

The chrysomelid *D. balteata* transmitted the causal agent of calopo yellow mosaic to Bountiful bean seedlings, which reacted with a striking mosaic and were positive in SSEM tests using the calopo virus antiserum.

The PCR reaction resulted in the amplification of a ca. 0.6-kb DNA product. The resulting cDNA clone was 609 nucleotides long and contained an open reading frame of 203 amino acid residues (Fig. 4). In optimal alignments of this coat protein gene fragment and the corresponding regions of the bean and cowpea strains of SBMV, there were nucleotide sequence similarities of 81.8 and 66.1% between the calopo virus and the bean and cowpea strains of SBMV, respectively. A comparative analysis of the deduced amino acid sequence of the calopo virus coat protein fragment revealed slightly higher similarities (83.7 and 67.8%) between this virus and the bean (14) and cowpea (26) strains of SBMV, respectively.

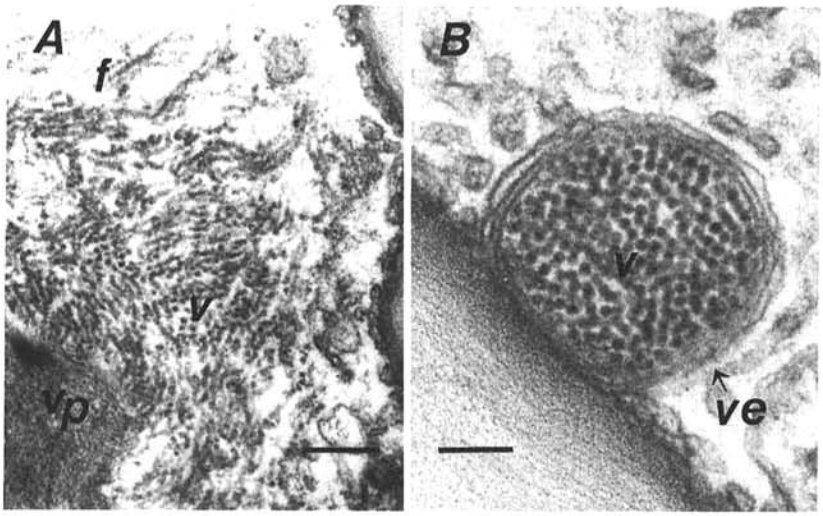

Fig. 2. Thin sections of *Calopogonium mucunoides* leaves infected by the calopo sobemovirus. **(A)** viroplasm (vp), virus particles (v), and fibrils (f) in the cytoplasm of a phloem cell. Scale bars= 0.44 µm. **(B)** virus particles (v) contained in a vesicle (ve) formed inside a phloem cell. Scale bars = 0.2 µm.

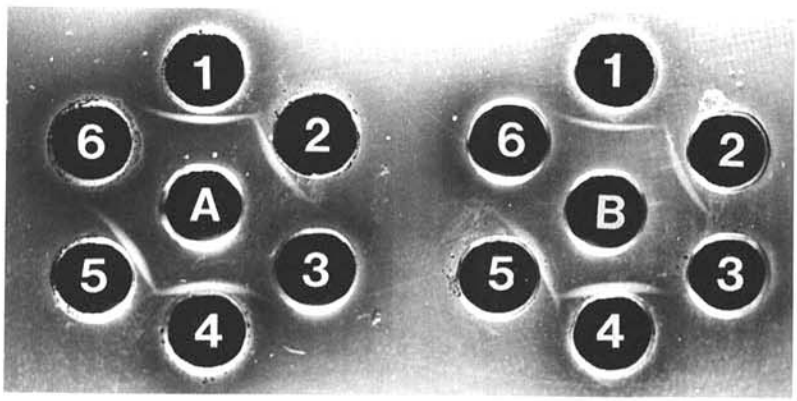

Fig. 3. Reciprocal double immunodiffusion tests with the bean strain of southern bean mosaic virus and the calopo sobemovirus. The center wells contain antisera to **(A)** the calopo sobemovirus and **(B)** the bean strain of SBMV. The peripheral wells contain the following antigens: 1 and 4, calopo sobemovirus; 2 and 5, SBMV-bean strain; 3 and 6 healthy bean extracts. Both antigens and the virus-free control were obtained from bean cv. Bountiful plants.

DISCUSSION

The morphology, nucleic acid and coat protein molecular weights, biological and mechanical transmission properties, antigenic relationships, cytopathology (7,21), and sequence homology of the causal agent of calopo yellow mosaic, demonstrate that the pathogen is a sobemovirus related to SBMV.

According to the literature (21), there are four major SBMV variants. First, the bean strain of SBMV, which, unlike the calopo virus, does not infect *V. unguiculata* or *V. radiata*. Second, the cowpea strain of SBMV, which infects cowpea but not common bean genotypes, except cv. Pinto (local lesions and symptomless infection). In contrast, the calopo virus sys-

temically infected various common bean genotypes in this investigation. The third and fourth major SBMV variants, the Ghanaian strain and the Mexican (or severe) strain, infect both common bean and cowpea genotypes. Reportedly, the Ghanaian strain does not induce systemic symptoms in most bean cultivars tested, including Bountiful (10), whereas the calopo sobemovirus induced a noticeable mosaic in the majority of the common bean cultivars inoculated in this investigation, and particularly in Bountiful, which reacts with severe mosaic. The Mexican strain of SBMV, on the contrary, is highly pathogenic on *P. vulgaris*, infecting cultivars, such as Great Northern 31, Great Northern 123, Red Mexican 34, Red Mexican 35, and Sanilac (27), that were not infected by the calopo sobemovirus.

Considering the lack of antigenic relatedness among different members of the sobemovirus group (21), and the close antigenic relationship observed in this study between the bean or cowpea strains of SBMV and the calopo sobemovirus, this virus could be tentatively considered as a strain of SBMV. However, there is only a 67.6% similarity between the nucleotide sequences of the coat proteins of the bean and cowpea strains of SBMV, and there is little sequence homology in their 3′ noncoding regions (12). The complete sequence of the bean strain of SBMV was determined (14) and the overall nucleotide sequence homology between the bean and calopo sobemoviruses was 55%. The open reading frame 1 only had a 24% amino acid identity between the two strains. Othman and Hull suggested that consideration be given to classifying the cowpea and bean strains as distinct viruses (14). Thus, it seems that the bean and cowpea strains of SBMV are in fact different viruses, despite their antigenic relatedness. A similar case is that of the whitefly-transmitted geminiviruses, which are distinct but serologically related viruses (17).

The partial coat protein sequence data obtained in this investigation shows that the calopo virus is more closely related to the bean than to the cowpea strain of SBMV. Nevertheless there is only 81% amino acid identity between the bean strain of SBMV and the calopo sobemovirus. Since the coat protein has the highest level of homology between the cowpea and bean strains of SBMV (14), the overall homology between the strains of SBMV and the calopo sobemovirus is expected to be considerable less than 81%, therefore the calopo sobemovirus should be considered a distinct species of the SBMV subgroup of sobemoviruses.

The environmentally determined perennial or annual growth habit of *C. mucunoides,* and its known susceptibility to leaf-eating beetles (20), greatly hinder the implementation of economically feasible control practices other than genetic improvement. Also, since SBMV has been shown to be transmitted by seed contamination in bean and cowpea genotypes (3,10), this mode of dissemination may also occur in *C. mucunoides*, as suggested by the results obtained in this investigation. Therefore, the collection of seeds from virus-infected calopo plants should be avoided to prevent further dissemination of the calopo sobemovirus.

From the above results and previous investigations on the pathogenic variability of SBMV (21,22), it is concluded here that calopo yellow mosaic is caused by a previously undescribed sobemovirus, for which we suggest the name calopo yellow mosaic virus.

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TGACCTATCCATGGCCCCTATTGCTCAGGGGTCTATGGTTCGCCTTCGTGAACCAGCGTTGCGCACGGCTCGCGGAGTGACTGTCCTAAC CaYMV
 D  L  S  M  A  P  I  A  Q  G  S  M  V  R  L  R  E  P  A  L  R  T  A  R  G  V  T  V  L  T  SBMV-Ca
 Q  V                        T                          S                 G                    SBMV-B
 G  V                        T           K              P     M           S  S  M  D        I     S  SBMV-C

GCACTCTGAGCTCTCAGCAGAGCTCGCTGTGACGAATGCGACGGTTGTTACCTCAGAGCTAGTTATGCCCTTCACAATGGGCACTTGGCT CaYMV
 H  S  E  L  S  A  E  L  A  V  T  N  A  T  V  V  T  S  E  L  V  M  P  F  T  M  G  T  W  L  SBMV-Ca
                  T           S                 I     I                          Y                    SBMV-B
    C             T                       D  T  I                                      V              SBMV-C

TAGAGGCGTTGCGTCCAATTGGTCTAAGTATAGCTTGGAGTCGGTGAGATATACCTATCTCCCCTCGTGTCATCCAACGACACCTGGGTC CaYMV
 R  G  V  A  S  N  W  S  K  Y  S  L  E  S  V  R  Y  T  Y  L  P  S  C  H  P  T  T  P  G  S  SBMV-Ca
               A                          L           T                          P  S        S        SBMV-B
               Q                    A  N  V  A  I                                P  T        S     A  SBMV-C

CATTCATATGGGTTTCCAATATGATATGGCTGACACCTTGCCCGTATCCGTTAACCAGTTATCCAACCTTAGAGGCTATGTGTCGGGACA CaYMV
 I  H  M  G  F  Q  Y  D  M  A  D  T  L  P  V  S  V  N  Q  L  S  N  L  R  G  Y  V  S  G  Q  SBMV-Ca
                                                                                              SBMV-B
                                                                        K           T     P  SBMV-C

GGTTTGGTCGGGTTCTTCTGGCCTCTGCTACGTCAATGGCACCAAATGCTTAGATGTGGCGGCTGCTATCTCCACCACCTTGGATGTGAG CaYMV
 V  W  S  G  S  S  G  L  C  Y  V  N  G  T  K  C  L  D  V  A  A  A  I  S  T  T  L  D  V  S  SBMV-Ca
                           I           R           T     N           T                    G  SBMV-B
       E     Q                 F           N           P     T  S  R           T  I  A           T  N  SBMV-C

TAAGCTAGGTAAGAAATGGTACCCGTACAAGACTAGTGCTGACTATACGACTGCCGTTGGCGTGAATGCCGACGTTGCGACGCCCCTTGT CaYMV
 K  L  G  K  K  W  Y  P  Y  K  T  S  A  D  Y  T  T  A  V  G  V  N  A  D  V  A  T  P  L  V  SBMV-Ca
 Q                        F           T     F                          V  N  I                 SBMV-B
 E  V  S  E     R           F           A  T        A                    N  I  G  N  I        SBMV-C

TCCGGCGAGGCTAGGGATAGCCATGCTAGACGGGGCGGGAACTACGGCTGTGAGTACTGGACGCCTATA                        CaYMV
 P  A  R  L  G  I  A  M  L  D  G  A  G  T  T  A  V  S  T  G  R  L                           SBMV-Ca
               I                       S  S  S                                             SBMB-B
               V           E  G        S  S  K           N                                 SBMV-C
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Fig. 4. The nucleotide and predicted amino acid sequences of a coat protein fragment of a sobemovirus infecting *Calopogonium mucunoides* in Colombia. The amino acid differences with the bean (SBMV-B) and cowpea (SBMV-C) strains of southern bean mosaic virus are shown below the calopo (CaYMV) sobemovirus sequences.

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