Canna paniculata as natural host of canna yellow streak virus in Brazil

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Abstract Canna yellow streak virus (CaYSV) was identified on *Canna paniculata* with foliar mosaic symptoms from Piracicaba municipality (São Paulo state, Brazil) by transmission electron microscopy, biological, serological and molecular assays. Pairwise analyses of nucleotide and amino acid sequences (potyvirus cylindrical inclusion region) with homologous sequences revealed the highest identity (88.6 and 97.8%) with CaYSV. This is the first detection of CaYSV in Brazil.

Keywords Potyvirus · Canna lily · Cannaceae

Cannaceae is of neotropical origin, with native species from the Southern United States to Northern Argentina (Maas-Van de Kamer and Maas 2008). Of the 217 scientific plant names cited by the Plant List (2013), only 12 are accepted as species names. In Brazil, the occurrence of four native *Canna* species (*C. glauca*, *C. indica*, *C. paniculata* and *C. pedunculata*) has been reported, all concentrated in the South and Southeast (Souza and Lorenzi 2012).

Few viruses have been reported in canna plants worldwide; these include Canna yellow mottle virus (CaYMV), a badnavirus, cucumber mosaic virus (CMV) and tomato

aspermy virus (TAV), cucumovirus, as well as bean yellow mosaic virus (BYMV) and sugarcane mosaic virus (SCMV), both potyviruses (Monger et al. 2007; Marino et al. 2008; Rajakaruna et al. 2014; Tang et al. 2016). Another potyvirus, Canna yellow streak virus (CaYSV), was reported in the United Kingdom in 2007, and other countries (Monger et al. 2007; Rajakaruna et al. 2014; Chauhan et al. 2015). Although the canna lily is commonly found in nature as well as in public and private gardens in Brazil, to date there has been no reported occurrence of viral diseases in these plants. As such, the aim of this study was to identify and characterize virus(es) associated with mosaic symptoms in *Canna paniculata* (sin. *C. brasiliensis*, *C. denudata*, *Distemon kunzei*, *D. miniatus*) leaf samples (Fig. 1) from a public garden in the municipality of Piracicaba, São Paulo state.

Elongated flexuous particles were observed by electron microscopy in extracts from negatively stained symptomatic canna plants (Canna Br01). Ultra-thin sections showed the presence of type II lamellar inclusions, according to Edwardson and Christie's (1996) classification, suggesting the sample was infected with a potyvirus.

Indirect ELISA with antisera against BYMV and CMV, and DAS-ELISA with Potyvirus group and banana bunchy top virus (BBTV, *Babuvirus*) – commercial antisera (AGDIA), exhibited negative results. BBTV was included because it is considered a quarantine pest in Brazil, and *C. indica* may be an alternative host of this virus even though it is closely associated with *Musa* sp. (Pinili et al. 2013).

A 684 bp fragment corresponding to the potyviral CI region was produced from Canna-Br01 after total RNA extraction with Trizol (Life Technologies Ambion) and RT-PCR using gene-specific primers for the cylindrical inclusion region (CI) of the potyvirus (Ha et al. 2008). Forward and reverse sequencing of the amplicon was performed in an automated DNA sequencer, followed by BLAST analysis and alignment with those

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Fig. 1 *Canna paniculata* with mosaic symptoms along leaf veins

retrieved from Genbank. Maximum likelihood (ML) analysis using PAUP v. 4.0b10 for Macintosh was performed after estimation of nucleotide substitution and gamma distribution (Modeltest v.3.06). The homologous sequence of the rymovirus ryegrass mosaic virus (RgMV) (Y09854) was used as outgroup, and bootstrap values were calculated (1000 resamplings). Identity percentage was determined using the PAUP program. BLAST analysis revealed that the Canna-Br01 (GenBank accession number KX897955) potyvirus sequence was 89% identical to CaYSV from the UK (GQ421689) in genomic RNA positions 3754 to 4433. Sequence analysis showed an identity percentage ranging between 94.6 and 97.8 at amino acid level (Table 1). Furthermore, the CaYSV-Br01 virus (KX897955) shares 71% identity with JGMV at the CI amino acid level. Monger et al. (2010) reported 76% identity for the same genome region with JGMV.

A monophyletic group with 100% bootstrap support formed by CaYSV-Br01 with CaYSV isolates available in GenBank was observed (Fig. 2). In addition, the group formed by CaYSV isolates, including a Brazilian isolate, shared a common ancestor with JGMV and the cocksfoot streak virus (CSV). Chauhan et al. (2015) showed that CaYSV belongs to the SCMV subgroup and is closely related to JGMV. On the other hand, in our phylogenetic analyses CaYSV and JGMV did not form a monophyletic group with SCMV.

Chauhan et al. (2015) compared the sequence corresponding to the CI of several American isolates of CaYSV (GenBank accession: KM882640-KM882648) and found 100% identity of nucleotides and amino acids, with the exception of the UK isolate (NC013261), which showed 94% sequence identity. In our study, CaYSV-Br01 shares more identity with the UK isolate, corroborating biological data.

To evaluate probable infections by a badnavirus, total DNA was isolated (Noris et al. 1994) and two degenerate primers (BadnaFP and BadnaRP) corresponding to the consensus sequences of the Reverse Transcriptase (RT) and RNaseH-coding regions of *Badnavirus* (Yang et al. 2003) were used. DNA from the *Bougainvillea spectabilis* chlorotic vein banding virus (BCVBV, *Badnavirus*) (KF600735) was used as positive control. PCR performed to detect the badnavirus did not amplify any fragment.

Attempts to infect seedlings of *Petunia x hybrida*, *Nicotiana benthamiana*, *Chenopodium amaranticolor*, *Catharantus roseus*, *Phasaelus vulgaris* cv. Preto, Carioca, Rajado and *Canna* sp., obtained from healthy plant seeds, with the virus from Canna-Br01 leaf extract failed. This isolate infected only 20% of the inoculated canna plants. The results of mechanical transmission attempts in this study were essentially similar to those reported by Rajakaruna et al. (2014),

Table 1 Comparison of identity percentage in nucleotides (NT) and amino acids (AA) of the potyvirus *Canna paniculata* (Canna-Br01) isolate, corresponding to the partial sequence of the cylindrical inclusion region, with potyvirus sequences retrieved from GenBank

Potyvirus isolations/accession number	NT	AA
Canna yellow streak virus (CaYSV)/GQ421689/NC013261	88.6	97.8
CaYSV isolate/KM882641 – KM882647	85.7	94.6
Johnsongrass mosaic virus (JGMV)/Z26920	67.1	71.0
JGMV/KT289893	65.1	71.1
Panax virus Y/NC014252	58.4	49.8
Clover yellow vein virus/AB732962	57.2	50.7
Pennisetum mosaic virus/ JX070156	56.9	52.9
Moroccan watermelon mosaic virus/EF579955	55.5	48.4
Sorgum mosaic virus/KM025048	55.5	48.4
Maize dwarf mosaic virus/JX185302	55.4	49.5
Potato virus Y/KR046206	55.1	46.2
Sugarcane mosaic virus/EU091075	54.7	48.0
Bean yellow mosaic virus/NC003492	54.4	48.0
Cocksfoot streak virus/NC003742	54.2	46.2
Turnip mosaic virus/KJ936093	53.9	51.1
Bidens mottle virus/EU250214	53.5	48.0
Yam mosaic virus/NC004752	53.0	43.5
Watermelon mosaic virus/DQ399708	52.0	44.9
Zucchini yellow mosaic virus/NC003224	49.6	44.9

Fig. 2 A maximum likelihood tree constructed using partial cylindrical inclusion sequences available in GenBank for *Potyvirus* species, with the ryegrass mosaic virus (*Rymovirus*) as outgroup. (TrN nucleotide

substitution model + G, G= 0.4402). Bootstrap values are indicated near the branches. The highlighted sequence corresponds to the Brazilian potyvirus isolate (Canna-Br01)

which state that CaYSV is poorly transmitted by mechanical inoculation using sap from canna leaves.

The occurrence of CaYSV has been reported in various parts of the world (Monger et al. 2010); however, this is the

first report in Brazil. The increased incidence of viral diseases has been exacerbated since cannas are predominantly propagated via rhizome cuttings and through the frequent international exchange of untested material (Monger et al. 2007). However, *C. paniculata* is a species whose center of origin is in Brazil, but is also found in Argentina, Paraguay, Peru, Bolivia, Ecuador, Venezuela and Southern Mexico (Maas-van de Kamer and Maas 2008). In contrast to what occurs with *C. indica*, which was introduced in different countries in Africa, Europe, the Middle East, Asia and Oceania, there are no reports of *C. paniculata* dispersion to other continents. Moreover, CaYSV dispersion may be associated with aphid transmission, since several polyphagous species have been reported associated with *C. indica* and *C. coccinea* species (Blackman and Eastop 2007).

In conclusion, electron microscopy, mechanical transmission, serological tests, RNA extraction and RT-PCR assays indicate that the symptoms observed in *Canna* from Piracicaba (SP), Brazil, are caused by CaYSV, with no other virus present in this plant.

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