CAPÍTULO 1

Cowpea aphid-borne mosaic virus (CABMV) IS WIDESPREAD IN PASSIONFRUIT IN BRAZIL, AND CAUSES PASSIONFRUIT WOODINESS DISEASE

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***Cowpea aphid-borne mosaic virus* (CABMV) is widespread in passionfruit in Brazil, and causes passionfruit woodiness disease**

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Running title: CABMV causing passionfruit woodiness in Brazil

SUMMARY

Leaf samples of yellow passionfruit (*Passiflora edulis* f. *flavicarpa*) displaying fruit woodiness symptoms were collected in seven Brazilian states and the Federal District. Viral infection was confirmed by host range and ELISA, and fourteen viral isolates were obtained. All isolates were capable of infecting several leguminous host species, although differences in symptom severity were noticeable. Woodiness symptoms were reproduced in yellow passionfruit, and mosaic symptoms were induced in common bean. All isolates infected cowpea, reported as a non-host of *Passionfruit woodiness virus* (PWV). Indirect ELISA demonstrated that all isolates were serologically related among each other and also to *Cowpea aphid-borne mosaic virus* (CABMV). The complete sequence of the capsid protein was determined for all isolates. Comparison of these sequences with those of other potyviruses indicated a highest identity with CABMV isolates (85 to 94%). Identity with PWV isolates ranged from 54 to 70%. Phylogenetic analysis grouped all Brazilian isolates in a monophyletic cluster with CABMV isolates, clearly distinct from PWV isolates. Furthermore, this analysis demonstrated that a group of previously characterized isolates from Brazil and designated as PWV should be reclassified as CABMV. Together, these results provide unequivocal evidence that, in Brazil, passionfruit woodiness disease is primarily caused by CABMV. The presence of PWV in Brazil has yet to be confirmed.

INTRODUCTION

Passionfruit woodiness disease (PWD) is the most important viral disease of yellow passionfruit (*Passiflora edulis* f. *flavicarpa*) worldwide. The disease is characterized by mosaic and distortion of the leaves and by "woodiness" of the fruit, which become dwarfed and lose much of the pericarp, therefore becoming unmarketable. Until recently, it was thought that only a single potyvirus species, *Passionfruit woodiness virus* (PWV), was capable of causing PWD. However, the molecular characterization of viral isolates causing the disease in the African continent identified a second species, *Cowpea aphid-borne mosaic virus* (CABMV), as an etiological agent of the disease [18, 24]. Both PWV and CABMV are typical potyviruses, transmitted in a non-circulative manner by several species of aphids [5, 10, 26]. Their particles are flexuous rods, 690-760 nm long and 11-16 nm wide, and their genomes are composed of a single molecule of single-stranded, positive-sense RNA of approximately 10,000 nucleotides [10]. The genome of a cowpea-infecting CABMV isolate from Africa has been completely sequenced [19].

In Brazil, the center of origin and the world's largest producer of passionfruit, PWD has been reported in all the major producing states and is a yield limiting factor for the crop [6, 7, 11, 17]. The etiological agent has always been designated as PWV based on biological and serological properties. However, the analysis of the amino acid sequence of the capsid protein of a number of Brazilian potyvirus isolates capable of inducing PWD indicated a higher sequence similarity with CABMV instead [20].

The precise identification of the etiological agent of PWD is of fundamental importance, considering that host resistance, either natural or engineered, is the only viable control measure for the disease [2, 25]. Therefore, this work was carried out in order to identify and characterize potyvirus isolates causing PWD in Brazil, using a combination of biological, serological and molecular techniques.

MATERIALS AND METHODS

Viral isolates. Plants of yellow passionfruit (*Passiflora edulis* f. *flavicarpa*) displaying symptoms of yellow mosaic and leaf distortion, as well as woodiness of the fruit, were identified in commercial fields in the states of Bahia, Espírito Santo, Minas Gerais, Paraíba, Pernambuco and Sergipe (Table 1). Sap was extracted from symptomatic leaves from these plants and used to mechanically inoculate plants of common bean (*Phaseolus vulgaris* 'Preto 153'), using 0.05 M potassium phosphate pH 7.2, 0.1% sodium sulphite, and carborundum (600 mesh) as an abrasive. Additional viral isolates from the state of São Paulo and from the Federal District, obtained in the same manner and previously identified as PWV [7, 11] were provided by Dr. Jorge A. Rezende and Dr. Elliot W. Kitajima (ESALQ-USP, Piracicaba, São Paulo, Brazil), respectively (Table 1). A CABMV isolate obtained from peanut in the state of Paraíba (CABMV-Br) [21] was also used as a control in all experiments. All viral isolates were propagated by successive sap-inoculation in plants of yellow passionfruit, bean or *Nicotiana benthamiana*. The isolates were also stored at -20^{o}C in the form of dried foliar material.

Biological and serological characterization. The partial host range of the viral isolates was determined by sap-inoculation of *Chenopodium amaranticolor*, *C. quinoa*, *C. murale*, *N. benthamiana*, *N. clevelandii*, bean cvs. Preto 153 and IPA7419, and cowpea (*Vigna unguiculata*) cvs. Pitiúba and Clay. Plants were inoculated at the two to four leaf stage, as described earlier, except that a second inoculation was performed three days after the first one. Three plants of each species were inoculated with each isolate, and one plant was mock-inoculated as a negative control. Results were evaluated by visual observation of local and systemic symptoms, for up to 30 days after inoculation. A serological analysis was carried out by indirect ELISA [8], using polyclonal antisera prepared against isolate MG-Avr [9], against a PWD-inducing potyvirus previously identified as PWV (isolate DF-Brs) [11], against a CABMV isolate from cowpea (provided by Dr. José Albérsio A. Lima, UFC,

Fortaleza, CE, Brazil) and against a CMV isolate from sweetpepper [4]. The assay was used in the preliminary identification of viral isolates obtained from the field and for viral detection during the host range experiment.

Sequencing of the capsid protein gene. Viral RNA was extracted as previously described [14] from a concentrated viral preparation [16] obtained from infected cowpea or *N. benthamiana* plants. The RNA was used as a template for RT-PCR, using the SuperScript Preamplification System for First Strand cDNA Synthesis (Invitrogen), an oligo-dT primer containing a *Sst* I restriction site, and a specific primer designed based on the sequence of isolate MG-Avr [2], containing a *Bam*H I site (poty-5: 5'GCG GGA TCC ATG TCT GAT GGA AAG GAC AAA GA-3', *Bam*H I site underlined). Amplification products were either digested with *Bam*H I and *Sst* I and cloned into pBLUESCRIPT KS+ using standard procedures [22] or directly cloned into the pGEM-T-Easy plasmid vector (Promega), according to the manufacturer's instructions. Cloned fragments were sequenced in both orientations using the BigDye Terminator Cycle Sequencing Ready Reaction kit and an ABI 310 automatic sequencer (Applied Biosystems).

Phylogenetic analysis. Deduced amino acid sequences of the capsid protein gene were compared to sequences available from GenBank (Table 1). Multiple sequence alignments were obtained with Clustal W [27]. Phylogenetic trees were obtained with MEGA version 3.1 [15], using the neighbour-joining method with Poisson correction. Tree branches were bootstraped with 1.000 replications.

RESULTS

Biological and serological characterization. All fourteen isolates obtained from yellow passionfruit plants were able to systemically infect yellow passionfruit, *N. benthamiana*, *N. clevelandii*, bean cv. Preto 153 and cowpea cvs. Pitiúba and Clay (Figure 1). Woodiness symptoms were reproduced in plants of yellow passionfruit (Figure 1B),

accompanied by severe mosaic and leaf distortion. The isolates induced chlorotic local lesions in *C. amaranticolor* and *C. quinoa*. No symptoms were observed in *C. murale* plants. Both cowpea cultivars displayed mild mosaic symptoms when infected by isolates PB-Alh, PB-Cnd, PE-Bcs1 and PE-Bcs2, and severe mosaic symptoms when infected by the remaining isolates. The bean cultivar Preto 153 reacted in a similar way, except that isolates PE-Ptr and SE-Nps also induced mild symptoms. Interestingly, the bean cultivar IPA7419 was not infected. The control isolate CABMV-Br induced severe mosaic symptoms in both cowpea cultivars and in bean 'Preto 153', but did not infect bean 'IPA7419' or passionfruit.

Results of indirect ELISA using a polyclonal antisera raised against isolate MG-Avr indicated the existence of a strong serological relationship among all isolates (Table 2). Also, all isolates reacted positively with a CABMV-specific polyclonal antiserum and with a polyclonal antiserum raised against isolate DF-Brs, a PWD-inducing potyvirus previously identified as an isolate of PWV (Table 2). None of the isolates reacted with a CMV-specific polyclonal antiserum (Table 2).

Sequencing of the capsid protein gene and phylogenetic analysis. The electrophoretic patterns of the capsid protein and viral RNAs extracted from concentrated viral preparations (using a method designed to concentrate most mesophyll-infecting viruses) confirmed infection by a potyvirus and the absence of mixed infection with non-potyviruses such as CMV or rhabdoviruses, for all samples (data not shown). RT-PCR amplification using the poty-5 and oligo-dT primers yielded a 1.3 kbp fragment. From this fragment, the complete nucleotide (nt) sequence of the capsid protein (CP) coding region and of the 3'-untranslated region (3'-UTR), and the deduced amino acid (aa) sequence of the CP, were determined for all isolates.

The CPs of all isolates are 275 aa long, except the one from the PE-Bnt isolate which is 276 aa long. All sequences contain the D/N-A-G triplet involved in aphid transmission, and conform to the well established pattern of potyvirus capsid proteins with a variable N-

terminus, a highly conserved core region and a conserved C-terminus (data not shown). Pairwise comparisons among the fourteen sequences indicated aa identity levels between 85 and 98% (Table 3), well above the 76-77% threshold level for potyvirus species [1] and thus indicating that all isolates should be classified within the same species. When compared to other potyviruses, the highest aa identity levels were observed with CABMV isolates (85 to 94%) (Table 3). Identity with PWV isolates ranged from 54 to 70% (Table 3), except for a group of previously characterized isolates from São Paulo state, which displayed 84 to 97% identity to the fourteen isolates. However, these isolates displayed >86% identity to CABMV isolates from GenBank, and <71% identity to Australian PWV isolates. Equivalent results were obtained for 3'-UTRs, which ranged from 226 to 230 nt in length among the fourteen isolates (data not shown).

The phylogenetic tree based on the complete CP aa sequences clearly indicates the close relationship between the Brazilian isolates and CABMV (Figure 2). All isolates characterized in this work, and also the five aforementioned Brazilian "PWV" isolates from São Paulo state, were grouped in a monophyletic cluster with a 100% bootstrap value which also included all CABMV isolates from different geographical regions. The most closely related CABMV isolates are CABMV-Br (obtained from a peanut plant in the state of Paraíba, Brazil) and CABMV-SAP (obtained from passionfruit in South Africa, and previously referred to as South African passiflora virus, SAPV) [18]. Australian PWV isolates were grouped in a separate cluster with a 99% bootstrap value (Figure 2). Two isolates from Japan and one isolate from Taiwan reported to induce PWD in yellow passionfruit [12] were grouped in a third, distinct monophyletic cluster with a 99% bootstrap value (Figure 2), in agreement with recent results indicating that a third potyvirus species is capable of inducing the disease [13].

DISCUSSION

Until the mid-1990's, PWV was the only potyvirus species reported as capable of inducing PWD. However, molecular studies demonstrated that CABMV strains can also cause the disease [18, 24]. Typical CABMV strains can infect cowpea, common bean and other leguminous hosts, but do not infect passionfruit [5, 21]. In Brazil, potyvirus isolates obtained from passionfruit plants showing symptoms of PWD have always been identified as PWV based on biological and serological properties [9, 11]. Here we report on the biological, serological and molecular properties of a group of fourteen potyvirus isolates from Brazil obtained from yellow passionfruit and capable of inducing PWD.

Results of the host range and indirect ELISA assays confirmed infection by a potyvirus in the field-collected plants. All fourteen isolates caused systemic infection in both cowpea cultivars tested (Pitiúba and Clay), although differences in symptom severity were observed among the isolates. Previous studies carried out in Brazil reported that PWD-inducing isolates were incapable of infecting cowpea, which contributed to their classification as PWV [3, 6, 7, 11]. However, the cowpea cultivars used in these studies were different from the ones used here. Considering the differences observed between the two common bean cultivars included in the host range assay ('Preto 153' being susceptible, and 'IPA7419' being resistant), it is quite possible that cowpea cultivars can also react differentially to infection by the virus. Whether cowpea cultivars Pitiúba and Clay are susceptible to true PWV isolates remains to be demonstrated.

Although serology has often been used to characterize Brazilian PWD-inducing isolates [3, 11], tests were always carried out using antisera raised against Brazilian isolates. Nevertheless, a serological relationship was observed between a PWD-inducing isolate from the state of Ceará (designated as PWV based on biological properties) and CABMV, *Clitoria mosaic virus* (ClMV) and *Sirato mosaic virus* (SrMV), based on the positive reaction observed when these two viruses were tested with a polyclonal antiserum raised againt the

"PWV" isolate [3]. There are no reports of serological relationships among PWD-inducing isolates from Brazil and from Australia (the only country in which PWV isolates have been characterized at the molecular level) [23, 25]. Our results show that all fourteen isolates from passionfruit displayed a positive ELISA reaction to a polyclonal antiserum raised against an isolate collected in the Federal District and designated as PWV at the time [11], but shown here to be a CABMV (isolate DF-Brs). All isolates also reacted with an antiserum raised from a Brazilian CABMV isolate from peanut (CABMV-Br). Together, these results demonstrate the existence of a serological relatioship among our fourteen isolates and CABMV. However, the existence of a serological relationship between PWD-inducing CABMV isolates from Brazil and true PWV isolates remains to be demonstrated.

Molecular characterization (analysis of the capsid protein coding region) demonstrated unequivocally that all fourteen isolates should be classified as CABMV. Nonetheless, a considerable degree of variation was observed among the fourteen isolates, with pairwise identity levels as low as 85% in several cases. Although such identity levels are perfectly within the threshold for species demarcation, this degree of genetic diversity could complicate disease management strategies based on host resistance. In particular, engineered resistance based on gene silencing should be targeted to more conserved regions of the viral genome, to increase the probability of obtaining broad spectrum field resistance.

Out of the six CABMV sequences used in the analysis, the one with the highest identity to the passionfruit isolates (88 to 94%) was CABMV-Br. CABMV-Br is a typical CABMV isolate obtained in Brazil (state of Paraíba) from a peanut plant, and does not infect passionfruit. The other five sequences are from CABMV isolates from Africa. One of them, CABMV-SAP, was obtained from passionfruit plants (originally designated as South African Passiflora virus, SAPV), and the remaining four were obtained from cowpea. Furthermore, isolates PB-Alh and PB-Cnd, obtained from plants collected at the state of Paraíba, clustered in the same branch of the phylogenetic tree, as well as two isolates from the state of

Pernambuco collected at the same city (PE-Bcs1 and PE-Bcs2). Therefore, a correlation can be observed between identity levels for the CP aa sequence and the geographical origin of the isolates, but not between the ability of a given isolate to infect passionfruit.

Incidentally, our analysis reinforced that a group of five PWD-inducing isolates obtained at the state of São Paulo (isolates F101, F144, M2, M3 and SP) and designated as PWV should be reclassified as CABMV, as previously noted [1]. These isolates display >86% identity to CABMV isolates (including the fourteen PWD-inducing isolates characterized here) and <71% identity to Australian PWVs (Table 3). Likewise, these five isolates cluster with CABMV isolates in the phylogenetic tree (Figure 2). Furthermore, two PWD-inducing isolates from Japan (AO and IB) and one isolate from Taiwan comprise a third, distinct species based on CP aa sequence and phylogenetic analysis [13]. Clearly, definitive taxonomical conclusions regarding PWD-inducing potyvirus isolates can only be reached once the complete sequence of the CP coding region (at least) has been determined.

Brazil is the center of origin of passionfruit, with more than 150 native species of *Passiflora*. Interestingly, PWD was first described in Australia, where it is caused by PWV. The potyvirus with the closest relationship to PWV is BCMV, and in fact PWV is capable of infecting beans. Therefore, it is reasonable to assume that PWV was originally an Australian bean-infecting virus, which acquired the ability to infect passionfruit. To this date, PWV has not been reported in any other country. In Africa and Brazil, PWD is caused by CABMV, which is also closely related to BCMV, but even more so to BCMNV. It is tempting to assume that two independent "species-jumping" events occurred in Africa and Brazil, since CABMV-Br (from Brazil, does not infect passionfruit) is closer to the PWD-inducing Brazilian isolates than CABMV-SAP (from Africa, infects passionfruit). In any event, it is noteworthy that all three potyvirus species capable of inducing PWD belong to the BCMV subgroup.

Our results indicate that CABMV is the primary causal agent of PWD disease in Brazil. All isolates characterized to date from Brazil should be classified as CABMV, and those designated as PWV in GenBank should have their taxonomical position updated. Whether PWV isolates are also present remains to be demonstrated. Passionfruit woodiness disease remains as a limiting factor to this crop in Brazil, and breeding programs, either by conventional or engineered approaches [2], should be targed at establishing resistance to CABMV.

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Table 1. Viral isolates and amino acid sequences analyzed in this study.

Potyvirus isolates obtained from yellow passionfruit (*Passiflora edulis* f. *flavicarpa*) in Brazil		
Isolate	**Origin (City, State)**	**Accession number**
BA-Itb	Itaberaba, BA	DQ397528
BA-Jgr	Jaguaquara, BA	DQ397527
DF-Brs	Brasília, DF	DQ397532
ES-Vni	Venda Nova do Imigrante, ES	DQ397529
MG-Avr	Areias Vermelhas, MG	DQ397525
PB-Alh	Alhandra, PB	AY253907
PB-Cnd	Conde, PB	AY253910
PE-Bnt	Bonito, PE	DQ393726
PE-Ptr	Petrolina, PE	AY253906
PE-Bcs1	Bom Conselho, PE	AY253908
PE-Bcs2	Bom Conselho, PE	AY253909
SE-Nps	Neopólis, SE	AY253911
SP-Vcz	Vera Cruz, SP	DQ397530
SP-Prp	Parapuá, SP	DQ397531

Capsid protein amino acid sequences obtained from GenBank*			
Species-isolate	**Host**	**Origin**	**Accession number**
CABMV-Br	Peanut	Brazil	AF241233
CABMV-SAP	Passionfruit	South Africa	D10053
CABMV-Z	Cowpea	Zimbabwe	NC_004013
CABMV-Ib	Cowpea	Ivory Coast	AJ132414
CABMV-Mor	Cowpea	Morrocco	Y18634
CABMV-Mon	Cowpea	Zimbabwe	Y17822
PWV-K	Passionfruit	Australia	1906186A
PWV-M	Passionfruit	Australia	P32574
PWV-S	Passionfruit	Australia	P32575
PWV-TB	Passionfruit	Australia	P32576
PWV-299	Passionfruit	Australia	AJ430527
PWV-CL1	Passionfruit	Australia	U67149
PWV-SD1	Passionfruit	Australia	U67150
PWV-AO	Passionfruit	Japan	D85849
PWV-IB	Passionfruit	Japan	AB185021
PWV-Taiwan	Passionfruit	Taiwan	AF208662
PWV-F101	Passionfruit	Brazil	AY433951
PWV-F144	Passionfruit	Brazil	AY505342
PWV-M2	Passionfruit	Brazil	AY433952
PWV-M3	Passionfruit	Brazil	AY434454
PWV-SP	Passionfruit	Brazil	AY433950

* Sequences of BCMV, BCMNV, BYMV, JGMV, JYMV, PeMoV, PRSV, PVY, SMV, SPFMV, TEV, TuMV and ZYMV are "reference" sequences retrieved from GenBank's Viral Genomes Resource (www.ncbi.nlm.nih.gov/genomes/VIRUSES/Viruses.html).

Table 2. Results of indirect ELISA (absorbance values at 405 nm) with passionfruit woodiness-inducing potyvirus isolates from Brazil using four different polyclonal antisera. Each value represents the average of four replications.

	Antiserum			
Isolate	**MG-Avr**	**DF-Brs**	**CABMV-Br**	**CMV**
BA-Itb	0.893	0.821	0.881	0.221
BA-Jgr	0.979	0.896	0.919	0.205
DF-Brs	0.998	0.893	0.945	0.207
ES-Vni	0.999	0.897	0.985	0.213
MG-Avr	1.390	0.987	0.923	0.205
PB-Alh	0.895	0.718	0.728	0.207
PB-Cnd	0.924	0.642	0.757	0.217
PE-Bnt	0.925	0.720	0.810	0.230
PE-Ptr	0.899	0.660	0.719	0.229
PE-Bcs1	0.945	0.671	0.676	0.180
PE-Bcs2	0.973	0.511	0.645	0.222
SE-Nps	0.965	0.760	0.815	0.216
SP-Vcz	0.934	0.820	0.887	0.230
SP-Prp	0.978	0.892	0.924	0.203
Mock	0.204	0.088	0.109	0.119

Table 3. Percent amino acid identity[a] for the complete capsid protein sequences of PWD-inducing potyvirus isolates from Brazil and some closely related potyviruses.

Species-isolate[b]	BA-Itb	BA-Jgr	DF-Brs	ES-Vni	MG-Avr	PB-Alh	PB-Cnd	PE-Bnt	PE-Ptr	PE-Bcs1	PE-Bcs2	SE-Nps	SP-Vcz	SP-Prp
BA-Itb	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BA-Jgr	94	-	-	-	-	-	-	-	-	-	-	-	-	-
DF-Brs	94	93	-	-	-	-	-	-	-	-	-	-	-	-
ES-Vni	90	90	90	-	-	-	-	-	-	-	-	-	-	-
MG-Avr	86	86	85	85	-	-	-	-	-	-	-	-	-	-
PB-Alh	98	93	94	90	86	-	-	-	-	-	-	-	-	-
PB-Cnd	98	93	93	90	85	98	-	-	-	-	-	-	-	-
PE-Bnt	86	85	85	85	92	85	86	-	-	-	-	-	-	-
PE-Ptr	95	94	96	90	86	94	94	87	-	-	-	-	-	--
PE-Bcs1	91	91	92	87	85	92	92	86	94	-	-	-	-	-
PE-Bcs2	92	90	92	86	85	92	92	86	94	98	-	-	-	-
SE-Nps	92	93	93	91	85	93	92	86	93	92	92	-	-	-
SP-Vcz	89	88	90	97	85	90	89	85	90	87	87	91	-	-
SP-Prp	89	88	88	98	85	89	89	85	89	86	86	93	98	-
PWV-F101	93	93	94	90	85	93	93	86	94	91	90	97	91	92
PWV-F144	93	93	94	90	85	93	93	86	94	91	90	97	91	92
PWV-M2	92	93	93	90	85	93	93	86	93	90	90	96	90	91
PWV-M3	93	93	94	90	85	93	93	86	94	91	90	97	91	92
PWV-SP	92	93	93	90	84	92	92	86	93	90	89	97	91	92
CABMV-Br	92	90	91	90	88	92	92	88	91	90	90	94	90	90
CABMV-SAP	89	89	89	87	87	89	88	87	89	86	86	88	88	88
CABMV-Z	88	88	88	86	87	87	87	87	88	87	86	90	87	87
CABMV-Ib	88	87	88	87	85	88	88	85	88	86	86	88	86	87
CABMV-Mor	87	86	88	85	85	87	87	85	87	86	86	89	86	87
CABMV-Mon	88	86	87	86	85	88	88	85	87	86	86	87	85	85
PWV-K	69	68	69	67	60	69	69	59	68	66	66	70	66	67
PWV-M	58	58	57	58	55	58	59	54	58	59	59	59	57	59
PWV-S	59	59	57	58	56	59	58	54	59	60	60	59	58	59
BCMV	70	68	69	67	61	70	70	61	65	65	65	77	67	74
BCMNV	75	77	76	75	68	75	79	65	76	72	72	78	74	75
SMV	71	70	72	68	61	71	72	60	71	67	67	71	71	68
ZYMV	67	70	67	69	61	72	70	60	70	67	67	72	65	68
SPFMV	54	52	53	51	47	54	54	46	54	52	52	56	51	51

[a] Pairwise comparisons made with DNAMan version 4.0, using the Fast Alignment option with the following parameters: BLOSUM matrix, K-tuple = 1, Gap penalty = 4, Gap open = 10, Gap extension = 0.1.
[b] As in Table 1.

Figure 1. Symptoms induced by isolate MG-Avr in (A) yellow passionfruit leaves and (B) fruit (left, infected; right, healthy), (C) common bean (*Phaselous vulgaris* 'Preto 153') and (D) cowpea (*Vigna unguiculata* 'Pitiúba')

Figure 2. Neighbour-joining tree based on the complete amino acid sequences of the capsid protein gene from PWD-inducing potyvirus isolates from Brazil and some closely related potyviruses. Horizontal distances are proportional to the genetic distances among the isolates. Vertical distances are arbitrary. The numbers on each branch represent bootstrap values (1,000 replications). The fourteen isolates characterized in this study are underlined. Isolates indicated by the asterisk should have their taxonomical position updated to CABMV in the databases.

Figure 1, Nascimento *et al.*

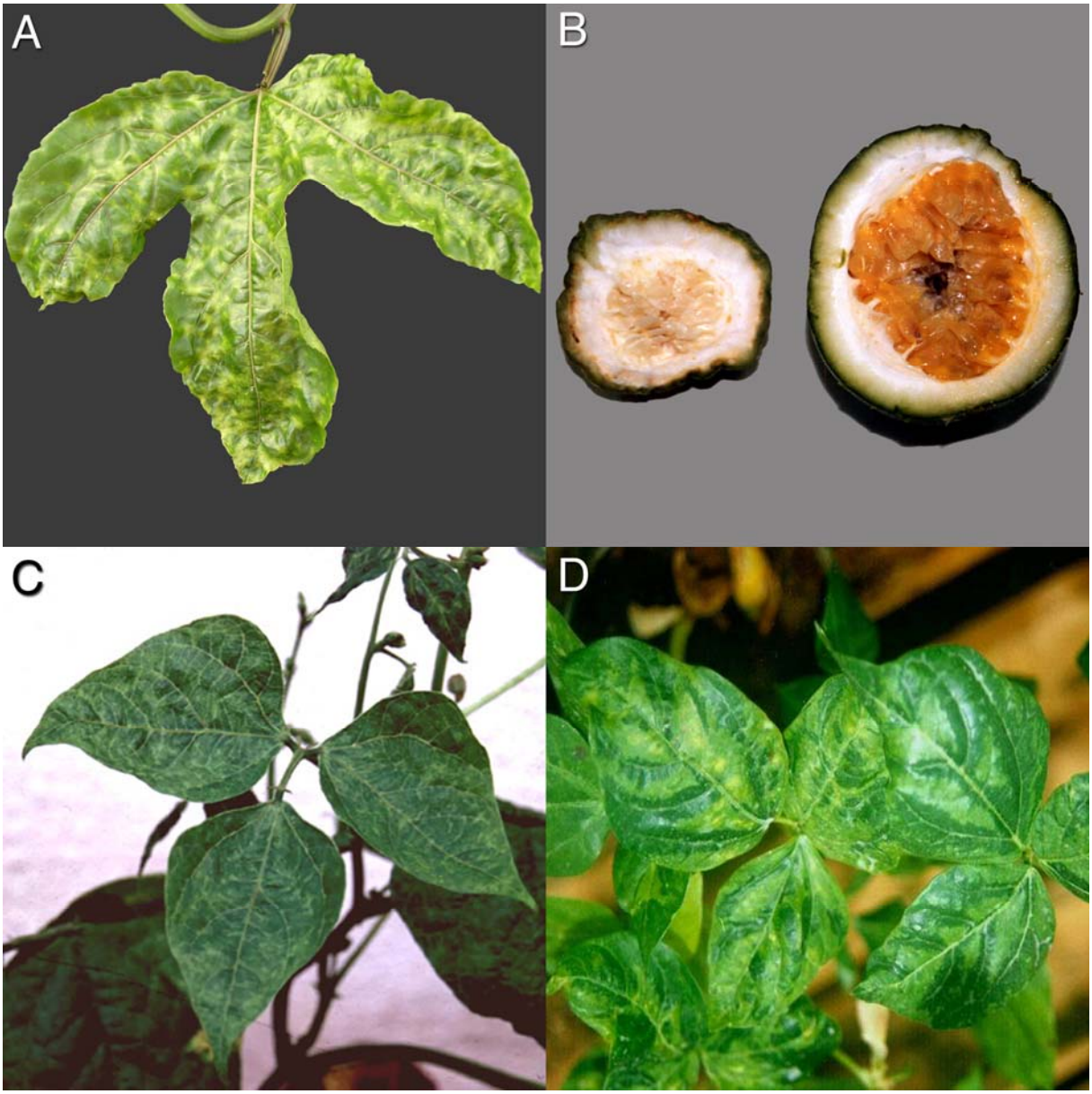

Figure 2, Nascimento *et al.*