UNIVERSIDADE FEDERAL DE LAVRAS – UFLA
PRÓ-REITORIA DE PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO EM
BIOTECNOLOGIA VEGETAL

Caracterização de isolados virais associados ao endurecimento de frutos do maracujazeiro-amarelo (*Passiflora edulis* Sims f. *Flavicarpa* O. Deg) provenientes de Livramento de Nossa Senhora, Brasil

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2008

Ficha Catalográfica Preparada pela Divisão de Processos Técnicos da Biblioteca Central da UFLA

Moreira, Charles Neris.
Caracterização de isolados virais associados ao endurecimento de frutos do maracujazeiro 'amarelo' (*Passiflora edulis* Sims f. *flavicarpa* O. Deg) provenientes de Livramento de Nossa Senhora, Brasil. / Charles Neris Moreira. – Lavras : UFLA, 2008.
27 p. : il.

Dissertação (Mestrado) – Universidade Federal de Lavras, 2008.
Orientador: Antônia dos Reis Figueira
Bibliografia.

1.PWV. 2. Capa protéica. 3. Maracujá. 4. Vírus. 5. PCR. 6. CABMV. I. Universidade Federal de Lavras. II. Título.

CDD – 634.425

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Dissertação apresentada à Universidade Federal de Lavras como parte das exigências do Programa de Pós-Graduação em Biotecnologia Vegetal, para a obtenção do título de "Mestre".

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APROVADA em 31 de agosto de 2008

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LAVRAS
MINAS GERAIS - BRASIL

Aos meus pais, Gilton e Júlia pelo eterno amor
Aos meus irmãos, Júnior e Michelle pelo companheirismo e afeto
Aos meus familiares, em especial Tia Júlia e meu primo Thiago, pela
força nas horas difíceis e por acreditar sempre
também pela confiança
e pelo amor
À todos os meus amigos
Dedico

AGRADECIMENTOS

À Deus pelo dom da vida.

À Universidade Federal de Lavras, através do curso de Biotecnologia Vegetal, pela oportunidade de cursar o mestrado e realizar este trabalho, que muito contribuíram para minha formação pessoal e profissional.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior CAPES pela concessão da bolsa.

À orientadora Prof. Dra. Antônia dos Reis Figueira pela paciência, orientação, confiança, amizade e ensinamentos.

Ao meu co-orientador Prof. Dr. Antonio Carlos de Oliveira pela grande amizade, ensinamentos e constante disponibilidade em me auxiliar.

À Dra. Luciane Vilela Resende pela disponibilidade de participação na banca.

Ao Prof. Dr. Luciano Vilela Paiva pela amizade e pelo exemplo de determinação à frente do curso de Biotecnologia Vegetal.

À toda a equipe da Fitovirologia, Biotecnologia Vegetal e Laboratório Central de Biologia Molecular pela amizade e pelos ensinamentos.

Aos funcionários do Centro de Indexação de Vírus da UFLA pelos serviços prestados e amizade.

À José Romário F. Melo (IC/FAPESB) e a Natanael V. Nascimento (ICJr./FAPESB), colegas do grupo de Pesquisa GenPlanta (UESB/CNPq), pelo suporte na geração de material biológico vegetal e viral.

Aos meus pais Giltom e Júlia, minha irmã Michelle, meu irmão Júnior, minha Tia Júlia, meu primo Thiago e todos os meus familiares pelo amor e por sempre acreditar em mim.

A todos os meus amigos pela amizade e pelos agradáveis momentos de convivência.

SUMMARY

RESUMO

MOREIRA, Charles Neris. **Caracterização de isolados virais associados ao endurecimento de frutos do maracujazeiro-amarelo (*Passiflora edulis* Sims f. *flavicarpa* O. Deg) provenientes de Livramento de Nossa Senhora, Brasil.** 2008. 27 p. Dissertação (Mestrado em Biotecnologia Vegetal) – Universidade Federal de Lavras, Lavras.

No presente trabalho foram estudados 10 isolados virais, coletados em maracujazeiros 'amarelo' com sintomas de endurecimento dos frutos, em lavouras comerciais do município de Livramento de Nossa Senhora, no Sudoeste da Bahia. Esses isolados foram inoculados mecanicamente em plantas de cinco famílias botânicas e um fragmento genômico da região da capa protéica, amplificado com *primers* específicos para o *Cowpea aphid-borne mosaic virus* (CABMV) foi seqüenciado e analisado. Todos os isolados virais causaram infecção sistêmica em *N. benthamiana*, soja, feijão cv. 'Preto 153', caupi cvs 'Clay' e 'Pitiúba'. A cultivar 'Redl. Gre B' reagiu de modo diferente à maioria dos isolados, apresentando mosaico e lesões locais cloróticas e necróticas nos isolados LNS-3, LNS-9 e LNS-10, apenas mosaico aos isolados LNS-7 e LNS-8, lesões locais e necróticas e cloróticas ao isolado LNS-1, apenas lesões necróticas ao isolado LNS-2 e foi imune aos isolados LNS-4, LNS-5 e LNS-6. A identidade entre os isolados aqui estudados, quanto à seqüência de nucleotídeos, variou de 90% (entre o LNS-2 e o LNS-8) a 98% (entre LNS-9 e os isolados LNS-4, LNS-5 e LNS-6). A identidade mínima de aminoácidos entre os dez isolados estudados foi ainda menor do que a observada nos nucleotídeos, variando de 82% (entre o LNS-2 e o LNS-8) a 98% (entre o LNS-4 e o LNS-9), sendo considerado estirpes de CABMV por apresentarem identidade maior que 70% de nucleotídeos. Ao se comparar a seqüência de aminoácidos dos isolados brasileiros com a dos outros isolados de CABMV disponíveis no GenBank, observou-se que o LNS-2 apresentou baixas identidades, chegando ao mínimo de 74%, o que sugere a possibilidade de ser uma espécie diferente. Quanto comparado com isolados de PWV encontrou-se valores de porcentagem de 63% (entre o LNS-2 e o U67149). Entretanto, existe a necessidade de se fazer uma análise mais criteriosa, mediante o seqüenciamento e análise do genoma completo dos isolados descritos nesse trabalho para se validar essa possibilidade. Os 10 isolados estudados se distribuíram em quatro grupamentos distintos nas árvores filogenéticas. Tanto o LNS-2 como o DQ397525 apresentaram uma maior distância de seus parceiros nos respectivos clados. Os dados obtidos indicaram que os isolados virais que causam endurecimento do fruto do maracujazeiro, de um modo geral, apresentam uma alta variabilidade genética na região da proteína do capsídeo.

Palavras-chave: CABMV**,** capa protéica, maracujá, PCR, PWV, vírus

Viral characterization of isolates associated to yellow passion fruit woodiness disease (*Passiflora edulis* Sims f. *flavicarpa* O. Deg) from Livramento de Nossa Senhora, Brazil

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ABSTRACT

Ten viral isolates, collected from passion fruit plants with fruit woodiness symptoms, growing in commercial crops from Livramento de Nossa Senhora, in southeastern Bahia – Brazil, were studied. They were mechanically inoculated in plants of 5 botanical families and one genomic fragment of coat protein region, amplified with specific primers for *Cowpea aphid-borne mosaic virus* (CABMV), and then analyzed. Every virus isolate induced systemic symptoms in *N. benthamiana*, soybean, bean cv. Preto 153 and cowpea cvs. Clay and Pitiúba. The identity among the 10 isolates ranged between 90% (LNS-2 and LNS-8) and 98% (LNS-9 and LNS-4, LNS-5 and LNS-6) and the amino acid identity varied from 82% (LNS-2 and LNS-8) to 98% (LNS-4 and LNS-9). When compared with CABMV isolates from GenBank, the LNS-2 showed a low amino acid identity, with the lowest one reaching 74%, suggesting that it could be a different virus

species. However, a more accurate analysis of the entire genome of Brazilian CABMV isolates should be done before the final conclusion. The phylogenetic trees showed four distinct groups, with the LNS-2 and DQ397525 keeping some distance from other isolates in their respective clades. Data results showed that the viral isolates, which cause fruit woodiness in passion fruit plants, present a high genetic variability in the coat protein region.
Keywords: PWV, coat protein, passion fruit, virus, PCR

1. INTRODUCTION

Passiflora include many species of passion fruit, being *Passiflora edulis* Sims f. *flavicarpa* O. Deg, or yellow passion fruit, the most important one (Winks et al., cited by Menzel & Simpson, 1994). This specie was first described in 1818 by Sims, then in 1872 by Masters, and the term *flavicarpa* was finally proposed in 1932 by O. Degener. Passifloracea are largely distributed throughout the tropics (Oliveira, 1987), with the most important geographic distribution center in central and north regions of Brazil. *Passiflora* is the dominat species within the family, having a huge range of genetic variability that needs to be explored (Faleiro et al., 2005).

Since last year, an expressive reduction of productivity in passion fruit plants has been observed. It has mainly been caused by the occurrence of diseases in this culture. Within these, passion fruit woodiness disease is considered for many authors as one of the most important disease due its association with economic losses, wherever passion fruit is cultivated (Junqueira et al., 2003).

In Brazil, this disease is widespread in all regions where passion fruit is cultivated. It was first observed in the State of Bahia in the 1970's (Yamashiro & Chagas, 1979). Since then, an increasing evidence of high incidences of this disease has been reported in many states, with values of 71,8% and 73,1% in São Paulo and Ceará, respectively (Chagas et al, 1981; Loreto & Vital, 1983; Chagas et

al, 1992). Alternative methods of control for this virus is very difficult due to the abundance of inoculums and presence of sources near crops.

The characterization of the causal agent of passion fruit woodiness disease started with the pioneering studies conducted by Cobb (1901) in Australia (cited by Shukla & Ward, 1988), who named the virus as *Passion fruit woodiness virus* - PWV. However, in the last decades, many authors have reported that other viruses can cause woodiness disease, like *Cowpea aphid-borne mosaic virus* (CABMV) in Africa (McKern et al., 1994) and Brazil (Santana et al., 1999; Nascimento et al., 2004, Cerqueira-Silva et al., 2008), *South African passiflora virus* (SAPV) (McKern et al., 1994) and *East asian passiflora virus* (EAPV) in South Africa and Asia (Iwai et al., 2006).

Studies based on serological and biological characterization in Brazil had previously indicated passion fruit woodiness virus to be the main causal agent of this disease (Yamashiro & Chagas, 1979; Chagas et al., 1981; Inoue et al., 1995; Bezerra et al., 1995; Moraes et al., 2002; Gioria et al., 2004). However, classification based on simptomatology is not very reliable and although DAS-ELISA has shown to be successful in the identification of this virus. When indirect ELISA is used, differentiation between CABMV and PWV has proven not being effective, which may lead us to erroneous classification (Yamashiro & Chagas, 1979; Chagas et al., 1981; Inoue et al., 1995; Bezerra et al., 1995).

The recent popularization of molecular techniques has allowed the correct identification of causal agent of this disease based on genomic sequence. Pioneer studies by Nascimento et al. (2004; 2006) and subsequent studies of Cerqueira-Silva et al. (2008) using amino acids sequence analysis of coat protein from different viral isolates from symptomatic yellow passion fruit have found strong similarities with CABMV isolates in Brazil. Additionally, the causal agent turned out to be considered as a isolate of CABMV and not PWV. Others have proposed that passion fruit woodiness disease in Brazil is also caused by CABMV (Santana et al., 1999 and Gioria, 2003). However, experimental evidence is as scarce as genome sequencing studies themselves, for example.

CABMV and PWV belong to the same Potyvirus gender and Potyviridae family. Structurally, they have positive ssRNA, 9465 nucleotides in length coding at least eight proteins P1, HC-Pro, P3, CI, 6K2, NIa, NIb and CP , produced by enzymatic activity of three proteinases in the same poliprotein (P1, HC-Pro e NIa) (Shukkla et al., 1994).

Genome sequencing of etiological agents of passion fruit woodiness disease has improved our knowledge, as evidenced by the partial sequencing of genomes of SAPV (Brand et al., 1993 and Braz et al., 1998), EAPV (McKern et al., 1994), PWV (Shukla et al., 1998; Santana et al., 1999) and CABMV (Pio-Ribeiro et al., 2000; Gioria et al., 2004; Nascimento et al., 2004; 2006, Novaes & Rezende 2005 and Cerqueira-Silva et al., 2008) and the complete sequencing of CABMV made recently by Barros (2007). Even though we acknowledge the importance of previous studies about host range and molecular characterization of CABMV isolates from Brazil (i.e., Santana et al. 1999; Pio-Ribeiro et al., 2000; Gioria et al., 2004; Nascimento et al., 2004; 2006; Novaes & Rezende 2005; Barros 2007 and Cerqueira-Silva et al., 2008). It is important as well to consider the fact that new isolates from other cities in Brazil have already been sampled with plants of passion fruit associated with this disease, especially in the cities of Itaberaba and Jaquaquara in the State of Bahia, were a similar work had been made. Additionally, novel viral isolates from yellow passion fruit woodiness disease, from other regions in Brazil, as well new probable host plants such as CABMV need to be more thoroughly studied in order to better characterize the causal agent, because the precise identification of an etiological agent is very important to improving both the application of control methods and studies about genetic interaction between host-pathogen.

Thus, acknowledging the need for further research, , the present study presents 10 viral isolates causing passion fruit woodiness disease in passion fruit which were collected and analyzed. Such viral isolates were naturally found infecting crops in the village of Livramento de Nossa Senhora, the biggest producing area of passion fruit culture in Northeast Brazil, located in the Southeastern region of Bahia. Some serological and biological properties were

determined and a genomic fragment of coat protein gene with 860 nucleotides were amplified, sequenced and molecularly analyzed for each one of the isolates.

2. MATERIALS AND METHODS

Viral Isolates

Ten isolates of the yellow passion fruit woodiness disease virus were collected in field conditions, designated LNS1 to LSN10, and placed at three different areas in the village of Livramento de Nossa Senhora, Bahia State, Brazil. Leaf samples of yellow passion fruit infected with the woodiness virus were taken to the Molecular Biology Laboratory at the Universidade Estadual do Sudoeste da Bahia/UESB, in Vitória da Conquista, Bahia State, Brazil. Each isolate was individually inoculated in yellow passion fruit plants, which were kept under greenhouse then sent to the Molecular Virology Laboratory at the Universidade Federal de Lavras/UFLA, Minas Gerais State, Brazil. By mechanical inoculation, isolates were maintained in cowpea plants (*Vigna unguiculata* L.) subsp. *unguiculata* Walp. cv. Pitiúba as well as *Nicotiana benthamiana* L.. For inoculation, leaves previously infected were ground with Potassium Phosphate buffer (0.01M, pH 7.0) (1:20 w/v). Then, mechanical inoculation was performed using extract of infected plant tissues previously dusted with carborundum. The resulting inoculated leaves were then washed with running water and maintained in greenhouse for symptoms evaluation for at least 30 days. After symptoms appearing, leaves were selected based in symptomatology, dehydrated and kept in freezer (- 80 ºC).

Biological characterization of the isolates

For host range evaluation, isolates were mechanically inoculated using the same methodology in *Chenopodium amaranticolor* Coste & Reyn, *C. ambrosioides* L., *C. quinoa* Willd., *N. benthamiana* L., tobacco (*N. tabacum* L.), pumpkin (*Cucurbita pepo* L.), soybean (*Glycine max L.*), cowpea (*Vigna unguiculata* L. subsp. *unguiculata*), cvs 'Pitiúba' and 'Clay', common bean (*Phaseolus vulgaris* L.), cvs. 'IPA7419', 'Preto 153' and 'Preto Campinas', and in eleven cultivars of common bean, which were used to separate groups of isolates of the *Bean common mosaic virus* (BCMV) (BOARI, 1992): 'Amanda', 'GN-123', 'Imuna', 'Jubila', 'Pinto 114', 'Redl Gre-B', 'Redl Gre-C', 'Red Mexican', 'Semilac', 'Top Crop' and 'Widusa', not previously reported as used for host range experiments with CABMV. All species and cultivars used in the host range screening were kindly provided by Éderson Kido (Universidade Federal de Pernambuco, UFPE), Kaesel Damasceno (Embrapa Meio Norte), Genira Andrade (UFPE), Heloisa Torres (Embrapa Arroz e Feijão), Marília Lobo (Embrapa Recursos Genéticos e Biotecnologia) and Sérgio Carbonell (Instituto Agronômico de Campinas). All plants were sowed in 2-kg capacity pots containing a 3:1:1 substrate mixture (soil: sand: manure). Two plants per pot were used. Common bean plants were inoculated in the primary stage of two leaves whereas the other species were inoculated at the stage of four leaves. Twenty-three plant species and/or cultivars, with three replications of eight plants for each isolate were used. . The inoculated plants were evaluated in a daily basis regarding type and severity of symptoms in leaves, during a period of at least 30 days. To confirm infection, extracts of leaves

with or without symptoms were inoculated twice into healthy plants of *N. benthamiana*.

Detection and Identification

The serological identification was performed with indirect ELISA tests described by Almeida (2001), using polyclonal antisera against PWV and CABMV (kindly provided by A. J. Albérsio de Lima, Universidade Federal do Ceará, Brazil). The indirect ELISA method was used to detect the presence of the virus in yellow passion fruit plants and in the other plants inoculated in this work. Samples were considered positive once the absorbance values showed more than twice the value of the average sample.

Molecular characterization

The RNA was extracted following TRIzol protocol (AFGC Protocols, 2002) then analyzed by agarose gel electrophoresis (0.7%). Primers used in the amplification of the viral genome were designed from isolates of CABMV available at the GenBank (coat protein gene position: nucleotides 1 to 828). The sequences were: CAB-F (5´TCTGATGGAAAGGACAAAG3´ sense: nucleotides 1-19) and CAB-R (5'CGATAACTGTGGCGAGGGCG3' anti-sense: nucleotides 860-841). The PCR was performed as described by Krause-Sakate et al. (2001). The amplicons were purified directly from the agarose gel (1.2%), using the gel purification GFX PCR DNA and Gel Band Purification Kit (Biosciences) and sequenced, in both directions, in the ABI 3100 machine at the Center for Applied Biology from Embrapa Milho e Sorgo, Sete Lagoas, Minas Gerais State, Brazil.

Genetic analysis

The sequences from all isolates were then analyzed using the BLAST program (Altschul et al. 1990). The sequences of amino acids and nucleotides were compared with sequences of CABMV available at the "GenBank" (Table 1). The multiple sequence alignments were performed by CLUSTALW and phylogenetic trees obtained with the MEGA 4.0 program using the UPGMA method for nucleotides and *Neighbor-Joining* to aminoacids, with bootstrap and considering values higher than 2,000 repetitions, with Poisson correction.

3. RESULTS

Biological and serological characterization

All viral isolates caused systemic infection and mosaic in *N. benthamiana*, soybean, common bean ‘Preto 153’, cowpea cvs ‘Clay’ and ‘Pitiúba’ while mosaic and chlorotic local lesions followed by necrosis were found in common bean ‘Preto Campinas’ and yellowing of veins in pumpkin plants. Plants of *C. ambrosioides*, *N. tabacum* and common bean cv. ‘IPA 7419’ showed apparent immunity. Plants of *C. amaranticolor* and *C. quinoa* reacted with chlorotic local lesions for all isolates. All ten isolates induced chlorotic local lesions in *C. amaranticolor* and *C. quinoa*. Six of the eleven cultivars of common bean (‘Amanda’, ‘GN 123’, ‘Imuna’, ‘Jubila’, ‘Semilac’, and ‘Top Crop’) showed apparent immunity. Two cultivars of common bean (‘Pinto 114’ and ‘Widusa’) showed systemic mosaic and only one cultivar (Redl Gre-C), which responded with chlorotic local lesions and necrosis

for all isolates, did not show differences. The cultivar ‘Redl. Gre B’ responded in a different fashion for most of the isolates: presence of mosaic and chlorotic and necrotic local lesions (LNS3, LNS9 and LNS10 isolates), only mosaic (LNS7 and LNS8), local, chlorotic and necrotic lesions (LNS1), only necrotic lesions (LNS2),, and immunity (LNS4, LNS5, and LNS6). This cultivar should be investigated in future studies to uncover its ability to differentiate CABMV isolates. Boari (1992), in a study for classification of isolates of BCMV, found that the cultivar 'Redl. Gre B' was able to differentiate I, II, III, Va and Vb BCMV isolates. In the indirect ELISA test, polyclonal antisera to CABMV reacted strongly with all ten isolates whereas the antisera to PWV reacted weakly to most of them. The highest viral concentrations, estimated by absorbance of ELISA test, were detected in cowpea cv. ‘Pitiúba’.

Analysis of coat protein gene of the isolates

Although amplicons of 860 pairs of nucleotides in size containing all the nucleotides that encode the coat-protein (1-828) were obtained, the analysis was performed in only 786 nucleotides (262 amino acids). The identity of nucleotides among the isolates and between them and others available at the GenBank were used for comparison. The identity of the isolates studied in this work ranged from 90% (between the LNS2 and LNS8) and 98% (between the LNS9 and LNS4, LNS5 and LNS6 isolates). By comparison with the sequences of CABMV/PWV deposited at the GenBank, the magnitude of variation was greatly significative: 63% (between isolates LNS2 and U67149 from PWV) and 95% (between isolates LNS6 and DQ397532). Even among isolates of CABMV available at the GenBank,

the variability was very significative, showing identity values ranging from 68% to 99%.

The minimal identity value of aminoacid residues among the ten isolates studied was lower than that observed in nucleotides, ranging from 82% (LNS2 vs LNS8) and 98% (LNS4 vs LNS9) (Table 2). It means that the replacement of most nucleotides resulted in aminoacids substitution, indicating a non-synonymous replacement. For the other individual isolates employed for comparison, the identity between LNS2 and U67149 remained in 63%. The isolate DQ397532 showed the greatest identities values with the isolates studied in this work, however it also ranged from 83% (if compared with LNS2) and 96% (if compared with LNS4 and LNS6). Among the isolates of CABMV available at the GenBank, the identity was between 73% (DQ397531 vs DQ397525) and 100% (AY505342 vs AY433951).

The isolate DQ397525, which presents identities ranging from 73% to 88% with the other isolates studied in this work, showed an identity level even lower (57%) as compared to the PWV-Culness isolate, suggesting that it cannot be an isolate of this virus, which means there is a need to investigate these viral isolates related to woodiness passion fruit viruses.

The phylogenetic tree was constructed based on the sequence of nucleotides (Figure 1) and showed that the isolates from Livramento de Nossa Senhora were grouped separately of the others isolates of CABMV and PWV available at the GenBank, and the LNS2 isolate showed a greater distance if compared to the other isolates. The isolates from GenBank grouped into three

different clades and the isolate U67149 remaining alone, i.e., separated from the CABMV typical isolate. The same fashion was observed in the amino acids phylogenetic tree (Figure 2).

4. DISCUSSION

The symptom analysis evaluated in the present work did not show any differences in severity caused by all isolates in susceptible plants (*N. benthamiana*, soybean, common beans 'Preto 153', cowpea cvs 'Clay', 'Pitiúba' and 'Preto Campinas', pumpkin, *C. amaranticolor* and *C. quinoa*), occurrence of mosaic or in the quantity and/or severity of chlorotic lesions and/or induced necrosis, unlike it was observed for other Potyvirus (Truta et al., 2004).

In this work, studies of symptomatology induced by the isolates had the same results as those obtained by other authors, showing that Brazilian isolates of CABMV was capable of causing symptoms in species of *Solanaceae, Chenopodiaceae* and *Cucurbitaceae* families as previously reported (Braz et al., 1998; Santana et al., 1999; Nascimento et al., 2004, 2006).

All isolates caused systemic infection in cowpea cv. 'Pitiúba', as confirmed by Nascimento et al., (2004, 2006). However, previous studies of Inoue et al. (1995) and Costa (1996) did not detect symptoms in cowpea when inoculated with viral isolates causing passion fruit woodiness disease. For some authors, there are evidence of an unknown mechanism of resistance to CABMV in cultivars of cowpea, like *Vigna unguiculata* L. subsp. *unguiculata* Walp. cv 'Pitiúba' and

‘Clay’ (Nascimento et al. 2004, 2006). In this work, cowpea cultivars showed susceptibility to all isolates.

Studies conducted by McKern et al. (1994) and Sithole-Niang et al. (1996) showed that CABMV isolates could infect passion fruit, however other studies had already reported that typical isolates of CABMV could not infect this plant but, instead, other leguminosae like common beans and cowpea (Bock & Conti, 1974; Pio-Ribeiro et al., 2000).

Similar work performed in other countries identified isolates of CABMV causing woodiness disease in fruits, like in Morocco (McKern et al., 1994) and Zimbawe (Sithole-Niang et al., 1996). On another hand, PWV is described as the causal agent of this disease in many countries in Africa, Asia, and Europe (Patel e Kuwite, 1982; Mali et al, 1988; Hampton et al., 1992).

The sequence of the coat protein region of all the ten isolates associated with woodiness disease in passion fruit in Livramento de Nossa Senhora – BA and the detailed analysis of sequences available at the GenBank confirmed the results previously obtained (Nascimento et al., 2004, 2006 and Cerqueira-Silva et al., 2008), showing that this disease can be caused by isolates of CABMV, indicating an interesting result regarding to variability.

If the criteria normally used to discrimination of species and isolates of virus highly related to Potyviridae are taking into consideration (Adams et al., 2005), the viral isolates causing woodiness disease in fruits of passion fruit could belong to different isolates and even a wide range of species, caused probably by low identity of nucleotides and amino acids observed between some of them.

The variability observed between isolates of CABMV probably indicates that the causal agent belongs to more than one species of virus; additionally, when nucleotide and amino acids identities reach values lower than 80%, they are considered as a different species for most of virus, for example, the *East Asian Passiflora virus* (Iwai et al., 2006).

Adams et al. (2005), analyzing Potyvirus isolates available at the GenBank, proposed a criterium for species discrimination, considering as different species isolates with identity ranging lower than 76-77% to nucleotides and 80% to amino acids. Following this criterium, isolates studied remained in these limits of identity to be considered isolates of CABMV. However, to evaluate amino acids identity, it has been noted that LNS2 presented values of identity lower than 80% with other GenBank isolates. These lowest identity values indicate a great difference capable to classify LNS2 and DQ397525 in different species. This means that there is a need to do a more detailed analysis of these isolates to evaluate the link to passion fruit woodiness disease, and following for the next step to the complete genetic sequencing of Brazilian isolates such as LNS2. In fact, molecular characterization could identify isolates and maybe even novel species of virus associated to woodiness disease in passion fruit.

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6. ANEXOS

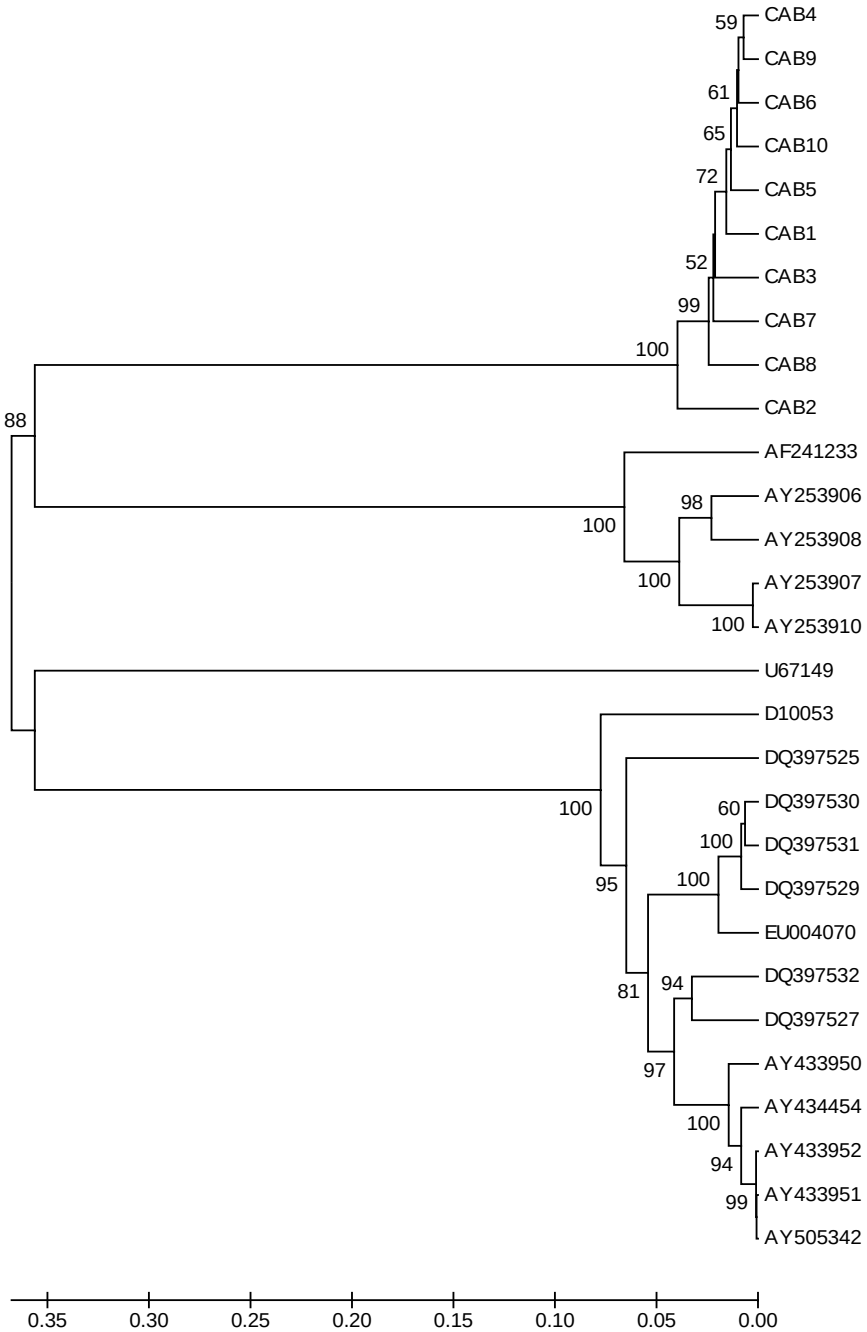

Figure 1 - Phylogenetic tree built based in nucleotide sequence of CABMV isolates. Bootstrap values were obtained by MEGA and UPGMA program, with 2.000 repetition.

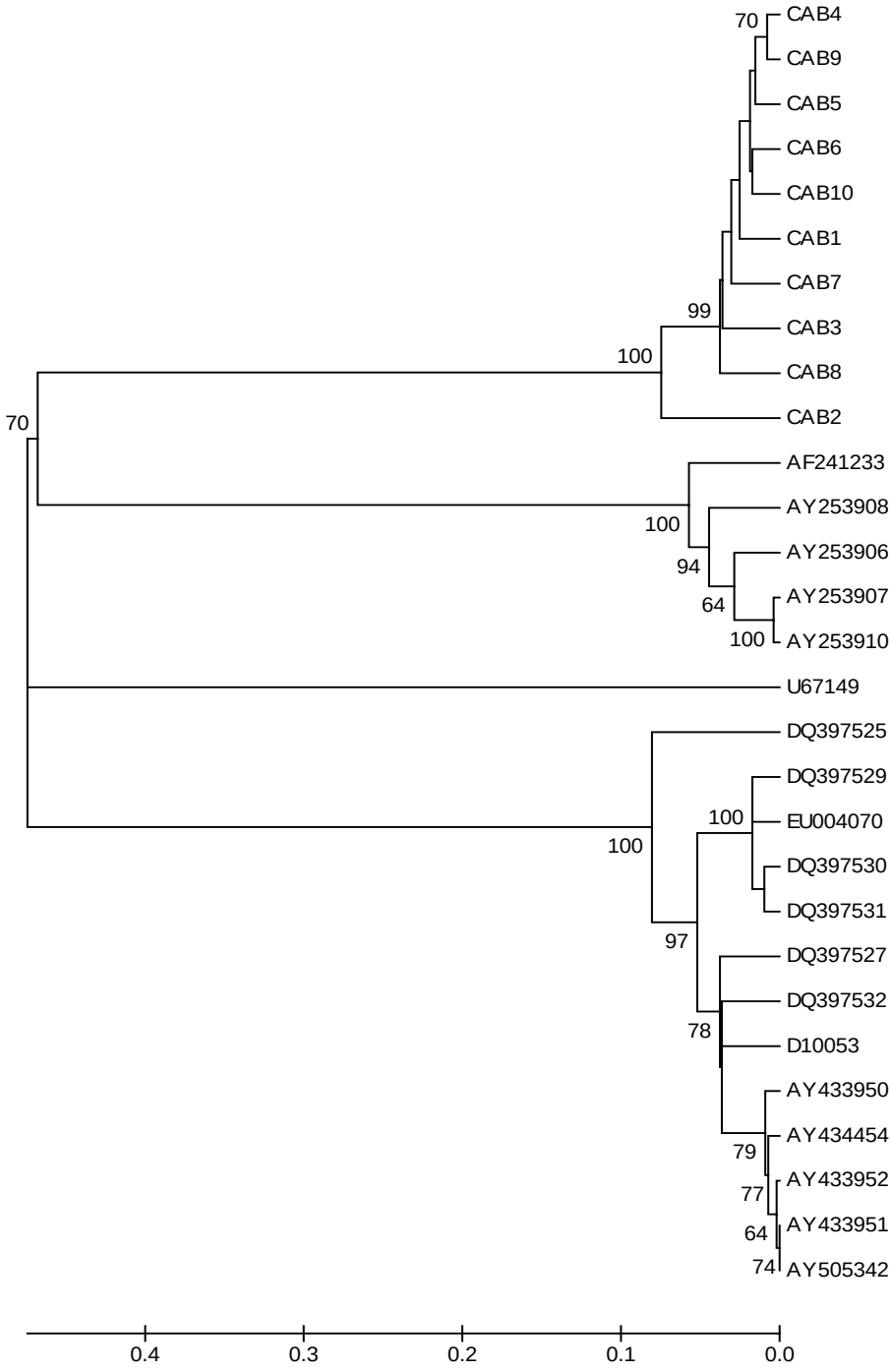

Figure 2 - Phylogenetic tree built based in aminoacid sequence of CABMV isolates. Boostrap values were obtained by MEGA and Neighbor-joining, with 2.000repetition.

Table 1: Isolates obtained from GenBank (NCBI, 2008) and used for comparison between isolates studied.

ACESSO	ORIGEM	ESTIRPE	AUTORES/PUBLICAÇÃO
AY433950	Brazil	CABMV-SP	Gioria et al. (2004)
AY433951	Brazil	CABMV-F101	Gioria et al. (2004)
AY433952	Brazil	CABMV-M2	Gioria et al. (2004)
AY434454	Brazil	CABMV-M3	Gioria et al. (2004)
AY505342	Brazil	CABMV-F144	Novaes e Rezende (2005)
AY253906	Brazil	CABMV-PE2	Nascimento et al. (2006)
AY253907	Brazil	CABMV-PB1	Nascimento et al. (2006)
AY253908	Brazil	CABMV-PE3	Nascimento et al. (2006)
AY253910	Brazil	CABMV-PB2	Nascimento et al. (2006)
DQ397528	Brazil	CABMV-ITB	Nascimento et al. (2006)
DQ397529	Brazil	CABMV-Vni	Nascimento et al. (2006)
DQ397531	Brazil	CABMV-Prp	Nascimento et al. (2006)
DQ397532	Brazil	CABMV-Brs	Nascimento et al. (2006)
DQ397525	Brazil	CABMV-MG	Nascimento et al. (2006)
DQ397527	Brazil	CABMV-Jgr	Nascimento et al. (2006)
AF241233	Brazil	CABMV	Pio-Ribeiro et al. (2000)
U67149	Australia	PWV-Culness	Sokhandan et al. (1997)
Y18634	Marrocos	CABMV-Mor	Van Boxtel et al. (2000)
D10053	Africa	SAPV	Brand et al. (1993)

Table 2 – Identity percent in amino acids sequence from coat protein between all the ten CABMV isolates from Livramento de Nossa Senhora (LNS) in comparison with 19 isolates sequence from "GenBank", obtained by multiple aligment using CLUSTAL W.

N	Isolados	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1	CAB1	84	92	95	95	94	94	91	95	93	91	94	94	91	93	93	93	93	93	93	89	89	89	88	95	85	93	89	69
2	CAB2	-	85	86	85	85	85	82	85	84	81	84	81	80	81	83	83	83	83	83	79	78	77	77	83	74	82	78	63
3	CAB3		-	93	93	92	91	89	93	92	87	91	89	87	89	89	90	89	89	90	85	84	85	83	91	81	89	84	68
4	CAB4			-	96	96	94	94	98	97	91	95	94	91	93	94	94	94	94	94	90	89	89	88	96	85	93	89	70
5	CAB5				-	94	94	92	96	95	90	94	93	90	93	92	92	92	92	92	88	88	88	87	95	85	91	88	69
6	CAB6					-	92	93	96	96	90	95	93	91	93	93	94	93	94	94	89	89	89	88	96	85	93	89	69
7	CAB7						-	90	93	93	88	92	90	88	90	91	91	91	91	91	87	87	87	86	92	82	90	87	68
8	CAB8							-	93	94	88	92	91	88	90	91	91	91	91	91	87	86	87	85	93	83	91	86	68
9	CAB9								-	96	91	95	93	91	93	93	94	93	93	94	89	88	89	88	95	85	93	88	70
10	CAB10									-	90	95	93	91	93	93	93	93	93	93	89	88	89	88	95	85	93	88	68
11	AF241233										-	89	89	86	89	92	91	91	91	91	89	88	88	88	89	81	88	88	69
12	AY253906											-	94	93	94	93	94	93	94	94	88	90	90	89	96	88	94	89	66
13	AY253907												-	90	99	92	93	93	93	93	87	90	90	89	94	86	93	89	65
14	AY253908													-	90	89	90	90	90	90	85	86	86	85	91	85	90	86	64
15	AY253910														-	92	93	93	93	93	87	90	89	89	93	85	93	89	67
16	AY433950															-	98	98	97	98	92	90	91	92	93	84	93	89	69
17	AY433951																-	99	98	100	91	90	91	92	94	85	93	89	69
18	AY433952																	-	98	99	91	90	90	91	93	85	93	89	69
19	AY434454																		-	98	90	90	91	92	94	85	93	89	69
20	AY505342																			-	91	90	91	92	94	85	93	89	69
21	D10053																				-	86	86	87	88	79	88	86	68
22	DQ397529																					-	97	98	90	84	90	96	65
23	DQ397530																						-	98	90	85	89	95	65
24	DQ397531																							-	89	73	89	96	65
25	DQ397532																								-	86	93	88	66
26	DQ397525																									-	86	92	57
27	DQ397527																										-	90	66
28	EU004070																											-	64
29	U67149																												-

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