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Resistance to *Cowpea aphid-borne mosaic virus* in species and hybrids of *Passiflora*: advances for the control of the passion fruit woodiness disease in Brazil

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Abstract The potyvirus-induced passion fruit woodiness disease (PWD) is considered the most important limiting factor for passion fruit production in several countries. In Brazil, PWD is caused by the *Cowpea aphid-borne mosaic virus* (CABMV), and to date there are no reports on the existence of *P. edulis* genotypes resistant to this virus. Thus, resistance gene introgression from wild *Passiflora* species for a commercial species, via interspecific hybridization, is one of the strategies adopted in order to control the disease. The current study's goals were to: confirm CABMV occurrence under field conditions; assess the resistance to CABMV in 178 *Passiflora* genotypes constituted by interspecific hybrids and their parents (*P. edulis and P. setacea*), as well as to estimate genetic parameters for the area under the disease progress curve (AUDPC), in order to obtain cultivars of sour passion fruit resistant to CABMV in future. The experimental design was set according to unbalanced randomized blocks with two repetitions. Data referring to the AUDPC were analyzed by means of the mixed models methodology (REMI/BLUP). CABMV infections were confirmed in sour passion fruit plants and in interspecific hybrids by observing foliar mosaic symptoms and by PTA-ELISA with specific antiserum against CABMV. There was a difference on the intensity of symptoms induced by CABMV for the 178 *Passiflora* genotypes assessed under natural occurrence conditions. The higher AUDPC values were obtained for 41 hybrids and for all *P. edulis* genotypes. In turn, lower values were estimated for 115 hybrid genotypes and for all *P. setacea* individuals. Of the 31 genotypes assessed by PTA-ELISA, 28 were considered resistant, out of those three *P. setacea* genotypes and 25 hybrids. Estimated AUDPC heritability values (0.99) and accuracy (0.99) enable inferring that resistance to CABMV within the assessed population was highly inheritable, allowing high selective efficiency. Resistant hybrid plants will be able to be selected and recombined with *P. edulis* genotypes and, again, assessed in order to corroborate the resistance to the virus, providing means of following up with the breeding genetic program on CABMV resistance.

Keywords *P. edulis* · *P. setacea* · Interspecific hybrids · CABMV · AUDPC · Mixed models

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Introduction

The *Passiflora* genus contains more than 400 species, and about 130 of them are native to Brazil (Bernacci et al. 2013). Currently, Brazil is the world's largest producer of passion fruit, and sour passion fruit (*Passiflora edulis*

Sims) represents more than 95 % of the country's commercial cultivation (Meletti 2011). The Brazilian production of sour passion fruit was 923,035 t in 2011, and 776,097 t in 2012, corresponding to 146,938 t reduction (IBGE 2014). The occurrence of diseases in the passion fruit orchards is one of the main factors associated with this significant reducing of yield and quality (Cerqueira-Silva et al. 2014).

Passion fruit woodiness disease (PWD) is considered the most economically important and a limiting factor for passion fruit production in Brazil (Cerqueira-Silva et al. 2014). Infected plants exhibit mosaic, blistering and distortion on the leaves, reduction of plant size, woodiness, deformation and pulp reduction of the fruits, and a reduction of the lifespan of the orchards (Nascimento et al. 2006; Oliveira et al. 2013).

In Brazil, the first occurrence of this disease was recorded in commercial orchards of sweet and sour passion fruit in the state of Bahia, in the late 1970s (Yamashiro and Chagas 1979; Chagas et al. 1981) and, afterwards, in the states of Pernambuco, Sergipe, Ceará (Kitajima et al. 1986), São Paulo (Chagas et al. 1992), Distrito Federal (Inoue et al. 1995), Pará (Trindade et al. 1999) and Rio de Janeiro (Maciel et al. 2009). Currently, PWD has been observed in all Brazilian passion fruit field productions (Cerqueira-Silva et al. 2014).

Initially, studies based on serological and biological properties considered *Passion fruit woodiness virus* (PWV) as the etiological agent of PWD in Brazil (Yamashiro and Chagas 1979; Chagas et al. 1981; Kitajima et al. 1986). However, subsequent molecular analyses discovered that woodiness disease in passion fruit, in Brazil, was actually associated with *Cowpea aphid-borne mosaic virus* (CABMV) (Nascimento et al. 2004; Nascimento et al. 2006; Cerqueira-Silva et al. 2008; Nicolini et al. 2012). Apart from CABMV in Brazil, other potyviruses may cause PWD, like PWV itself, described in Australia (Wylie and Jones 2011), *East Asian Passiflora virus* (EAPV) in Japan (IWAI et al. 2006), and Ugandan Passiflora virus (UPV) in Uganda (Ochwo-Ssemakula et al. 2012).

Losses caused by CABMV in Brazilian passion fruit cultivations are severe and lead to reductions in production, cultivated area and in the lifespan of the orchards. The high incidence of PWD has prompted producers to adopt remediating measures, like renewal of orchards on a yearly basis. However, such initiatives increase production costs, compelling producers to transfer plantations to other regions or even give up this agricultural activity. Several other recommendations should also be adopted in order to reduce the damage potentially of PWD, like using virus-free seedlings, elimination of infected and old plants in the field and avoiding mechanical transmission during horticultural practices (Fischer and Rezende 2008; Cerqueira-Silva et al. 2014). However, these measures were not effective to control or eradicate the disease (Novaes and Rezende 2003; Trevisan and Mendes 2006; Sampaio et al. 2008). Thus, identifying genetic resistance sources is the main goal of the sour passion fruit enhancement program in Brazil.

The genetic basis of the cultivars of sour passion fruit is relatively narrow. Hence, genetic breeding programs have been using wild species capable of increase such cultivars resistance levels. The wild species *P. setacea*, which is considered resistant to CABMV, presents other physical and chemical properties of fruits desirable to genetic breeding (Junqueira et al. 2005). Thus, another alternative should be the development of resistant cultivars obtained by means of gene introgressions of a resistant species for susceptible cultivars through interspecific hybridization. Accordingly, crosses between *P. edulis* and *P. setacea* should be excellent strategy to obtain promising recombinants for genetic breeding.

The identification of promising CABMV resistance sources is fundamental for the genetic breeding programs, in order to reduce productivity losses in passion fruit crops. Nevertheless, research on the reaction of passion fruit to CABMV infection are limited to commercial (Junqueira et al. 2003; Leão et al. 2006; Pinto et al. 2008; Cerqueira-Silva et al. 2008; Cerqueira-Silva et al. 2012) and wild species (Maciel et al. 2009; Oliveira et al. 2013). There are no reports associating symptom evaluations using a sensitive and specific method for CABMV detection in *Passiflora* interspecific hybrids to prove resistance to this virus. Besides, there is also no information about genetic parameters regarding features related to CABMV resistance in passion fruit.

By taking into account the importance of such information to the genetic breeding programs, this work aims to: (i) confirm CABMV occurrence in field; (ii) assess CABMV resistance in *Passiflora* interspecific hybrids and their genitors (*P. edulis* and *P. setacea)*, associating phenotypic evaluations and the presence of the virus; and (iii) estimate genetic parameters for characteristics related to CABMV resistance by using the REML/BLUP methodology, in order to identify resistant

hybrids to generation advance within the sour passion fruit genetic breeding program aiming to obtain CABMV resistant cultivars.

Material and methods

Genetic material

One hundred seventy-eight (178) individuals from the passion fruit genetic breeding program of the State University of North Fluminense Darcy Ribeiro (UENF) were assessed and described as follows: (i) 10 *P. edulis* genotypes, obtained from the UENF's recurrent selection program, and used as positive controls for CABMV susceptibility reaction; (ii) 12 *P. setacea* genotypes, species described as CABMV resistant (Junqueira et al. 2005), from the Active Germplasm Bank of the State University of Santa Cruz, UESC (BAG-Passifloras), municipality of Ilhéus, State of Bahia, Brazil; and (iii) nine full-sib progenies (UENFH-1, UENFH-2, UENFH-3, UENFH-4, UENFH-5, UENFH-6, UENFH-7, UENFH-8 and UENFH-9) from a crossing between *P. edulis* x *P. setacea* with 20, 21, 15, 20, 22, 3, 14, 22 and 19 individuals, respectively, totaling 156 interspecific hybrids.

Interspecific hybridizations were performed between July 14th and August 8th 2010 at UESC within a greenhouse by using *P. edulis* as female and male genitor, in order to obtain the progenies. Temperature inside the greenhouse ranged from 28 down to 22 °C, and relative humidity was between 60 and 98 %. Artificial crossings were performed in different times, from 13:30 till 14:30 for *P. edulis* genotypes and from 19:00 till 19:30 for the *P. setacea* genotypes. Flower buds from the genitors were protected with paper bags in the morning. Anthers from the donor species were collected, deposited in Petri dishes with silica gel and stored in a refrigerator (10 °C) till receptor flowers anthesis stage (12:30 pm anthesis in *P. edulis* plants and from 18:00 till 19:00 in *P. setacea* plants). During pollination, anthers from donor species were carefully rubbed on the receptor species stigma with tweezers. After the artificial hybridization, crossings were identified and flowers were once more protected for 24 h. Fruits resulting from the hybridizations were protected with a nylon mesh till their complete ripening.

Cultivation conditions

Genotypes were seeded in 128 cells Styrofoam trays containing *Basaplant*® organic substrate and, after the emergence of two definite pairs of leaves, seedlings were individually transferred to 1-l black polyethylene plastic bags, containing humus and cattle manure in the ratio of 2:1. Ninety five days after, seedlings were sent to an experimental area at Antônio Sarlo Agricultural School, in the municipality of Campos dos Goytacazes, North of the state of Rio de Janeiro, 21°45′ S, 41°20′ W, 11 m altitude. The experimental design was performed in unbalanced randomized blocks for plants within progenies with two repetitions. The system used for plant trellising was the vertical cordon with 2.5 m high fence posts, set 4 m apart from each other with 12 metal wires from 1.80 m down to the ground. The distance between the planting rows was 3.5 m. Cultivation treatments were performed according to recommendations for the passion fruit cultivation.

Identification of the viral isolate occurring in the region

In order to confirm CABMV identity, leaves of *P. edulis* [*Pe*(7)] and an interspecific hybrid [H9(29)] with foliar deformation and mosaic symptoms were collected in the field, in October 2012, and assessed by means of PTA-ELISA (Plate Trapped Antigen – Enzyme Linked Immunosorbent Assay) using a specific polyclonal antiserum against CABMV.

Healthy *P. edulis* foliar samples (1 g) were used as negative control (−) and were ground in a coating buffer (1.59 g of $Na_2CO_3$and 2,93 g of $NaHCO_3$for 500 mL of H_2O, 0.05 M, pH 9.6) in the ratio of 1/5 (g/ml). Leaves of CABMV-infected *P. edulis* showing mosaic and foliar deformation were used as positive control (+). They were also ground in a coating buffer in the ratio of 1/5 (g/ml). Foliar samples from sour passion fruit were ground in a solution (0.1126 g of DIECA, 0.0372 of EDTA, 0.057 g of sodium thioglycolate for 0.1 l of water) in the ratio of 1/5 (g/ml) and diluted in PBSTPo (1.200 ml of PBS-T, 2 % of polyvinilpirrolidone, PVP) in the ratio of 1/1 (ml/ml). Extracts from the positive and negative controls and the samples were added to 96-well polystyrene plates in triplicate and incubated at 37 °C for 2 h. The plates were washed three times in a phosphate buffer with Tween (PBST) (0.05 % of Tween-20® in PBS 0.1 M,

pH 7.4), blocked with 1 % skimmed powder milk, diluted in PBSTPo, and incubated at 37 °C for 2 h. Plates were washed with PBST and the specific antiserum against CABMV (kindly provided by Dr. J.A.M. Rezende, ESALQ, USP) was added. The antiserum was previously cross-absorbed with healthy plant extract and diluted in the ratio of 1:2000 in presence of PBSTPo. Plates were incubated once more at 37 °C for 2 h, and washed (three times using PBST) and, as a next step, the conjugated anti-rabbit+alkaline phosphatase (Sigma) diluted in the ratio of 1:30000 in PBSTPo was applied. Plates were incubated at 37 °C for 2 h. Substrate application was performed after the last wash in PBST (p-nitrophenylphosphate). After approximately 30 min the plates were read with a Microplate reader 3550-UV (Bio-Rad), at a wavelength of 405 nm. Samples were considered positive when the average of the absorbance readings was three times higher than that obtained for the negative control.

In order to confirm the PTA-ELISA results, mechanical inoculations were also performed on leaves of *Chenopodium amaranticolor*, which react with local symptoms to CABMV infection (Silva 2012).

A RT-PCR (Reverse Transcription – Polymerase Chain Reaction) was also performed using primers to the cylindrical cytoplasmic inclusion (CI) protein sequence (Ha et al. 2008). Total RNA was extracted from a 0.2 g of infected leaves of passion fruit using the commercial product *TRizol® Reagent* (Invitrogen), according to manufacturer's recommendations. Complementary DNA (cDNA) synthesis was done with M-MLV reverse transcriptase (Invitrogen) enzyme, according to manufacturer recommendations, using the primer CIRev. About 100 ng of total RNA were subjected to a reverse transcription reaction. For the PCR, 5 μl from the cDNA and 10 pmoles/μl from each primer were used, CIRev and CIFor. It enabled amplification of the approximately 600 bp DNA fragments. The PCR conditions were: 5 min at 92 °C, followed by 40 cycles of 50 s at 92 °C, 1 min at 54 °C and 30 s at 72 °C, with final extension of 5 min at 72 °C. Samples were amplified in PTC100 (MJ Research) thermocycler and the DNA fragments were visualized in 1 % agarose gel in the presence of ethidium bromide, under ultraviolet light (Sambrook et al. 1989).

RT-PCR products [Pe(7) and H9(29)] were eluted from the agarose gel using the kit Concert™ Rapid Gel (Gibco BRL™), following the manufacturer's instructions. The purified DNA products were submitted to direct sequencing using the kit Big Dye (Applied Biosystems) and sequenced in an automatic sequencer ABI 377, ABI Prism (Applied Biosystems), following the manufacturer's instructions. CI sequencing was performed with the same primers used in PCR (Rodrigues et al. 2015). The DNA sequences were compared to other sequences deposited in GenBank using the software Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI), and percentage of nucleotide identity was calculated using software Bio Edit 7.2.0.

Symptom evaluation

Symptoms, induced by CABMV, were assessed by visual analysis after the natural in-field occurrence of the disease in 12-month-old plants. The evaluation was performed on a weekly basis from August till December 2012, totalling 16 evaluations. The incidence of the disease in the genotypes was assessed taking the percentage of plants presenting characteristic symptoms of the disease under consideration. The severity of the foliar symptoms was assessed by means of a scoring scale (varying from 1 to 4), as proposed by Novaes and Rezende (1999): 1=no symptoms; 2=light mosaic without foliar deformation; 3=severe mosaic without foliar deformation; and 4=severe mosaic, blisters and foliar deformations. Data obtained from the scoring scale were used to calculate the area under the disease progress curve (AUDPC), according to the equation of Campbell and Madden (1990).

Estimating genetic parameters by Restricted Maximum Likelihood (REML)

Values obtained by the AUDPC were used in order to estimate genetic parameters by means of the software Selegen-REML/BLUP (Resende et al. 2013). The analysis followed the statistical model $y=Xr+Zg+Wp+e$ in which y is the data vector, r is the vector of repetition effects (assumed as fix) added to the general mean, g is the vector of individual genotypic effects (assumed as random), p is the vector of portion effects (random) and e is the vector of errors or residues (random). Capital letters represent the incidence matrix for the

mentioned effects. The following variance components were estimated (Individual REML):

$\widehat{\sigma}_g^2$ genotypic variance among full sib progenies, equivalent to ½ of the additive genetic variance plus ¼ of the prevalent genetic variance, ignoring the epistasis

$\widehat{\sigma}_f^2$ individual phenotypic variance

$\hat{h}^2 mp$ progenies heritability average, assuming complete survival

Acprog accuracy of the progeny selection, assuming complete survival.

Assessing the resistance to CABMV

After visual evaluations and identification of *Passiflora* asymptomatic genotypes, PTA-ELISA was performed, as described above, aiming to confirm plants presenting resistance to CABMV. Thirty one (31) individuals corresponding to three *P. setacea: Ps(4), Ps(365)* and *Ps(367)* genotypes and 28 interspecific hybrids were evaluated. Twenty one (21) individuals from the progeny UENFH-1 [H1(1), H1(8) and H1(20)], UENFH-2 [H2(2), H2(18) and H2(20)], UENFH-3 [H3(9), H3(10) and H3(11)], UENFH-4 [H4(2), H4(3) and H4(9)], UENFH-7 [H7(3), H7(11) and H7(12)], UENFH-8 [H8(13), H8(47) and H8(55)] and UENFH-9 [H9(11), H9(56) and H9(57)], two individuals from the progeny UENF-6 [H6(6) and H6(27)] and five individuals from the progeny UENFH-5 [H5(1), H5(12), H5(14), H5(19) and H5(21)] were evaluated. Due to the large number of asymptomatic plants, only those with higher productivity were selected.

Results and discussions

Identification of the viral isolate occurring in the region

P. edulis Pe(7) samples and a sample from a H9(29) interspecific hybrid with mosaic symptoms, blisters and foliar deformation, analyzed by PTA-ELISA, reacted positively with the anti-CABMV polyclonal antiserum (As-CABMV) (Fig. 1).

These samples were also subjected to total RNA extraction and RT-PCR with primers for *Potyvirus* species (Ha et al. 2008), and the amplicons (Fig. 2) sequenced. The sequences showed 94 to 100 % nucleotide identity with other CABMV sequences deposited in GenBank. Mechanical inoculations on *C. amaranticolor* resulted in local lesions (data not shown). These results allowed us to confirm that the passion fruit virus isolates associated to PWD from Campos dos Goytacazes (RJ) belong to the species CABMV, confirming the reports by Nascimento et al. (2006), Pinto et al. (2008), Cerqueira-Silva et al. (2008), Barros et al. (2011), Nicolini et al. (2012) and Rodrigues et al.

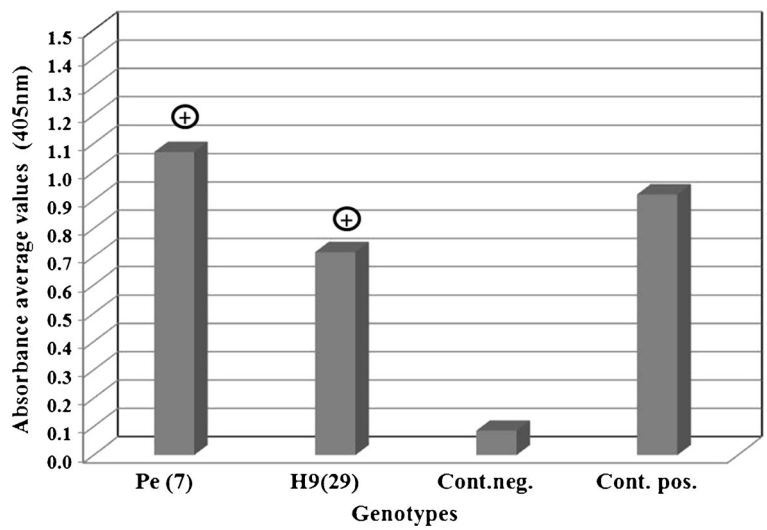

Fig. 1 Absorbance values (405 nm) obtained by PTA-ELISA with specific antiserum to *Cowpea aphid-borne mosaic virus* (CABMV) in *Passiflora* genotypes analyzed for CABMV resistance. (+) values obtained for plants which reacted positively. Each value represents an average of three repetitions. *Cont. pos.* positive control, *Cont. neg.* negative control, *Pe*(7) *P. edulis* genotype, *H9(29)* genotype (29) from progeny H9

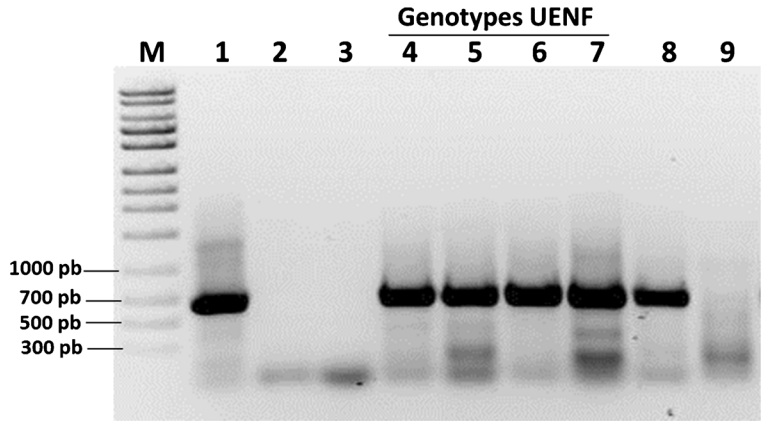

Fig. 2 Agarose gel (1.2 %) analysis of the RT-PCR amplified DNA products with ca. 600 base pairs obtained with CIRev/CIF primers (Ha et al. 2008) (M)=1 kb DNA ladder (Norgen); (*1*) total RNA extracted from CABMV infected sour passion fruit (positive control); (*2*) total RNA from healthy sour passion fruit; (*3*) RT reaction negative control; (*4* and *5*) total RNA from sour passion fruit [*P. edulis* isolate UENF (Pe (7)]; (*6* and *7*) total RNA from sour passion fruit [hybrid isolate UENF (H9(29)]; (*8*) positive control – Caserta infected with *Zucchini yellow mosaic virus* (*Potyvirus*); (*9*) PCR negative control

(2015), which state that, in Brazil, CABMV is the only species associated to PWD.

Assessment of symptoms induced by CABMV in P. edulis, P. setacea and interspecific hybrids

During the period of the field experiment, temperatures varied from 12 to 40 °C, and average monthly rainfall was 76.27 mm, varying from 6.1 to 203 mm (Fig. 3). Temperature condition during planting time and the time that the experiment was being done were good for the cultivation of *P. edulis* and *P. setacea* species as well as for interspecific hybrids and for CABMV replication in the assessed plants. After the ninth monitoring month (August 2012), 40 % of the *P. edulis* genotypes and 26 % of the assessed hybrids presented typical CABMV-induced symptoms. Cavichioli et al. (2011) have estimated that the first symptoms of the disease occurred at the 90th day after the seedlings were planted in the field and reached, at the 180th day, 100 % of the *P. alata* and *P. gibertti* plants and 97.5 % of the *P. edulis* genotypes.

The amount of deviance (1278.69) obtained for AUDPC, by means of mixed models analyses indicates that there was a difference in reaction of CABMV-induced symptoms for 178 *Passiflora* genotypes assessed under natural occurrence conditions. A wide variation of symptoms was observed in different individuals, from asymptomatic plants to plants with severe symptoms of mosaic with blisters and foliar deformation.

All the hybrid plants from the UENH-8 and UENF-9 progenies and the *P. edulis* genotypes showed typical symptoms of CABMV infection. The H8(64), H9(1) hybrids with 329 and 343, respectively, and the *P. edulis Pe*(7) and *Pe*(10) genotypes (both with 315) presented the higher AUDPC values and, consequently, exhibited the most severe symptoms of the disease, thus being considered as highly susceptible genotypes. On the other hand, the lower values within the progenies (UENFH-8 and UENFH-9) and between the *P. edulis* genotypes (CABMV susceptible) were estimated for the H8(68), H9(5) and *Pe*(2) individuals, with 217, 220 and 157, respectively (Fig. 4).

In turn, all individuals from progenies UENFH-1, UENFH-2, UENFH-3, UENFH-4, UENFH-5, UENFH-6 and UENFH-7, as well as the *P. setacea* genotypes, species seen as resistant to the virus, presented the lower AUDPC values (105). These individuals did not present characteristic symptoms of CABMV infection and they scored 1 in all assessments, thus appearing to be resistant (Fig. 4). Similar results were obtained by Junqueira et al. (2005). The authors have verified that hybrids from the *P. edulis* x *P. setacea* and the species *P. setacea* were resistant to the virus.

The differences in responses from the assessed progenies regarding the CABMV induced symptoms may be attributed to the genetic variability of segregating populations, resulting from interspecific crossings. The other factors might have also contributed such as viral inoculum, low concentration in plants, and/or inoculum

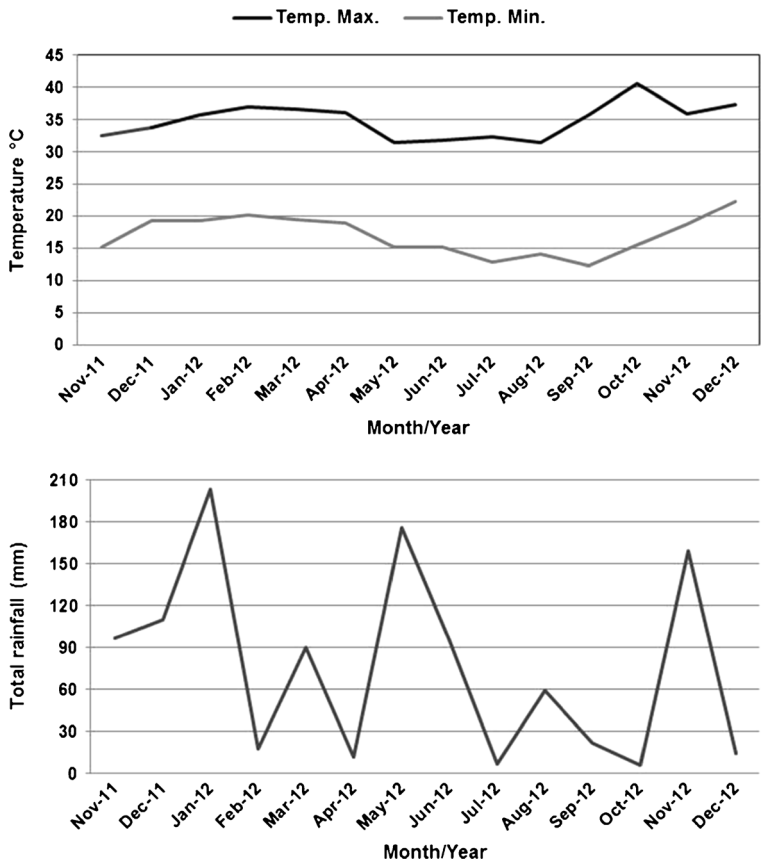

Fig. 3 Climate data regarding in field experiment: maximum and minimum temperatures (Temp. Max and Temp. Min), in °C, and total rainfall, in mm, provided by Instituto Nacional de Meteorologia (INMET). They are monthly recorded at the EstaçãoAutomática de Campos-RJ, between November 2011 and December 2012

levels found in the field (Pinto et al. 2008). Changes in certain environmental factors, mainly in climate variables, such as temperature and relative humidity, seedling's nutritional status and age difference among plants can influence the pathogen's virulence and, consequently, the expression of disease symptoms (Novaes and Rezende 1999; Vida et al. 2004). However, these factors might not be the cause of absence of symptoms in some plants, since all genotypes were the same age and were subjected to the same environmental and nutritional conditions.

Regarding the *P. edulis* genotypes, some plants presented less severe symptoms than others. Such variability can be also associated with species genetic heterogeneity, since they are crossed pollination plants. Cerqueira-Silva et al. (2008) and Oliveira et al. (2013) have described the reaction of many sour passion fruit genotypes according to CABMV induced symptoms, they also verified great variability within the species, although, all assessed genotypes presented some level of susceptibility to the disease.

The evaluation of CABMV resistance has being done in sour passion fruit cultivars. However, there was no detection of resistance levels able to offer satisfactory virus control. In most of the cases, plants are classified as moderately resistant and susceptible (Junqueira et al. 2003; Leão et al. 2006; Pinto et al. 2008). Variability on resistance, within the studied cultivars, is too low. It highlights the importance of genetic breeding work focused on the introgression of resistance genes in sour passion fruit cultivars.

Identifying sources of resistance to CABMV is a priority activity in the genetic breeding programs, since this virus induces a generalized disease in the main producing regions in Brazil. Maciel et al. (2009) verified the reaction of 16 *Passiflora* species to infection by four Brazilian CABMV isolates and also observed that *P. setacea* was only resistant to the CABMV-RJ isolate.

Oliveira et al. (2013) assessed the reaction of four *P. setacea* genotypes to CABMV under in-field natural infection and verified that only one (BGM237) was considered resistant to the three isolates of the virus

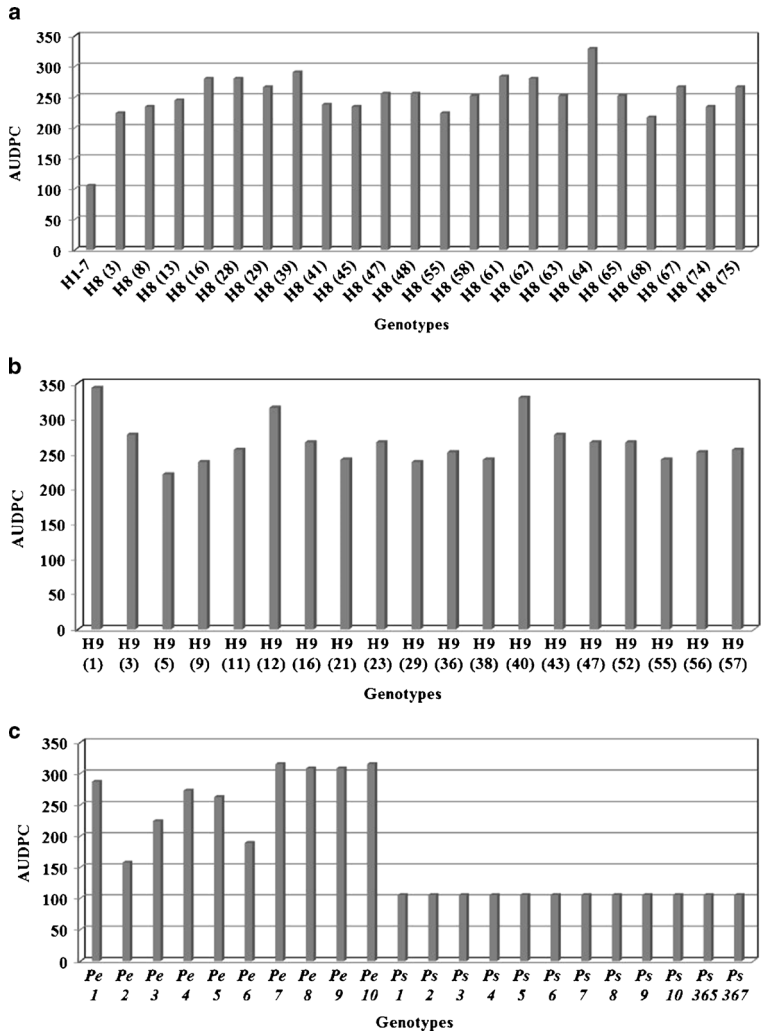

Fig. 4 AUDPC throughout 112 days in *Passiflora* hybrid genitors. **a** H1-7- include all genotypes from the progenies UENFH1, UENFH2, UENFH3, UENFH4, UENFH5, UENFH-6 and UENFH-7 with 20, 21, 15, 20, 22, 3, 14 individuals and the genotypes from progeny UENFH-8. **b** individuals from progeny UENFH-9 and **c** *Pe*1 to 10=*P. edulis* genotypes; *Ps*1 to 367=*P. setacea* genotypes

used. In the current study no *P. setacea* genotype presented characteristic symptoms of CABMV infection. It was verified, thus, that there is variability for CABMV reaction within *P. setacea*, and that evaluation for CABMV reaction must be done before using the genotypes in breeding programs focused on resistance introgression.

Corroborating CABMV resistance

Of the 31 genotypes analyzed, 28 were resistant to CABMV: H1 (1), H1 (8), H1 (20), H2 (2), H2 (18), H2 (20), H3 (9), H3 (10), H3 (11), H4 (2), H4 (9), H5 (1), H5 (12), H5 (14), H5 (21), H6 (6), H6 (27), H7 (11), H7 (12), H8 (13), H8 (47), H8 (55), H9 (11), H9 (56), H9 (57), Ps (4), Ps (365) and Ps (367) the CABMV. Hybrids' levels of absorbance were similar to those from genitors *Ps*(365) and *Ps*(367), a species reported as resistant to CABMV (Junqueira et al. 2005) (Table 1).

In turn, genotypes H4 (3), H5 (19) and H7 (3) reacted with antiserum to CABMV and thus were considered susceptible. Even with positive results in ELISA, hybrids, absorbance values were three times lower than those from the positive control (Table 1).

The presence or absence of symptoms in plants under natural occurrence conditions depends on the aggressiveness of the virus lineage found in the field, on the tested genotype and on the environmental conditions in which the plants are grown (Novaes and Rezende 1999; Nascimento et al. 2006).

In order to confirm ELISA results, H4(3), H5(19), and H7(3) samples were mechanically inoculated to plants of *C. amaranticolor*, which reacted with local

Table 1 Genotypes, PTA-ELISA absorbance values and final evaluation of three *Passiflora setacea* genotypes and 28 interspecific hybrids from a *P. edulis* x *P. setacea* crossing evaluated for CABMV resistance. Campos dos Goytacazes, UENF, 2013

Genotypes	Absorbance	[a]ELISA	[b]Final evaluation
H1(8)	0,065	−	R
H1(20)	0,066	−	R
H2(2)	0,069	−	R
H2(18)	0,067	−	R
H2(20)	0,067	−	R
H3(9)	0,067	−	R
H3(10)	0,096	−	R
H3(11)	0,062	−	R
H4(2)	0,093	−	R
H4(9)	0,110	−	R
H4(3)	0,273	+	S
H5(1)	0,084	−	R
H5(12)	0,065	−	R
H5(14)	0,081	−	R
H5(19)	0,192	+	S
H5(21)	0,107	−	R
H6(6)	0,095	−	R
H6(27)	0,079	−	R
H7(3)	0,168	+	S
H7(11)	0,097	−	R
H7(12)	0,119	−	R
H8(13)	0,102	−	R
H8(47)	0,145	−	R
H8(55)	0,117	−	R
H9(11)	0,127	−	R
H9(56)	0,070	−	R
H9(57)	0,078	−	R
P. setacea (4)	0,081	−	R
P. setacea (367)	0,068	−	R
P. setacea (365)	0,072	−	R
P. edulis healthy (negative control)	0,062	−	−
P. edulis infected by CABMV (positive control)	1,012	+	S

[a](+)=positive reaction to the presence of CABMV (−)=negative reaction to the presence of CABMV

[b]R=resistance; S=susceptibility

chlorotic lesions, indicating that passion fruit plants which reacted with the anti-serum were infected by CABMV. Nascimento et al. (2006) collected sour passion fruit samples with woodiness virus symptoms from seven Brazilian states and from Distrito Federal. They verified, by means of biological characterization, that all isolates were capable of systematically infecting sour passion fruit, *N. benthamiana*, *N. clevelandii*, as well as black bean (*Phaseolus vulgaris*) cultivars, cowpea (*Vigna unguiculata*) cultivars 'Pitiuba' and 'Clay' and *C. amaranticolor* and *C. quinoa.* Besides, all isolates reacted with the polyclonal anti-serum specific to CABMV (Nascimento et al. 2006).

Phenotypic evaluation x PTA-ELISA

By comparing the results from visual evaluation of the symptoms and the serological analysis, it was verified that hybrid plants considered as susceptible by the PTA-ELISA [H4(3), H5(19), H7(3)] did not present CABMV infection symptoms. The low absorbance values found for these genotypes, when compared with the positive

control, might indicate low resistance levels in hybrid plants. Possibly, such plants would need a longer time in order to express the symptoms, so they would become an infield CABMV reservoir, a situation that is undesirable. However, in order to corroborate this hypothesis, it is worth visually evaluating symptoms for longer and repeating the serological test. Similar outcomes were obtained by Sacoman (2013). The author verified that a *P. setacea* (*Ps*RJ 4) genotype considered resistant by the visual analysis was categorized as susceptible by the PTA-ELISA.

Novaes and Rezende (1999), by using scoring scales associated with PTA-ELISA analysis verified a high and positive (99 %) correlation between absorbance values and the severity of symptoms in three *Passiflora* species, suggesting the use of these variables for selecting CABMV resistant genotypes. However, data obtained in our work indicated that it is not possible to select resistant plants based only on disease symptom expressions.

On the other hand, the genotypes H8(13), H8(47), H8(55), H9(11), H9(56) and H9(57), described as susceptible, by means of symptom visual evaluation, were considered resistant (negative results in PTA-ELISA). For such genotypes, initial CABMV symptoms (mild mosaic on the leaves) had appeared in the beginning of August and scored maximum grades in the beginning of October, presenting more severe symptoms (blistery mosaic and foliar deformations). However, from the second fortnight of November, disease symptoms regressed and the genotypes were given as asymptomatic by the end of the evaluation process (Figs. 5 and 6).

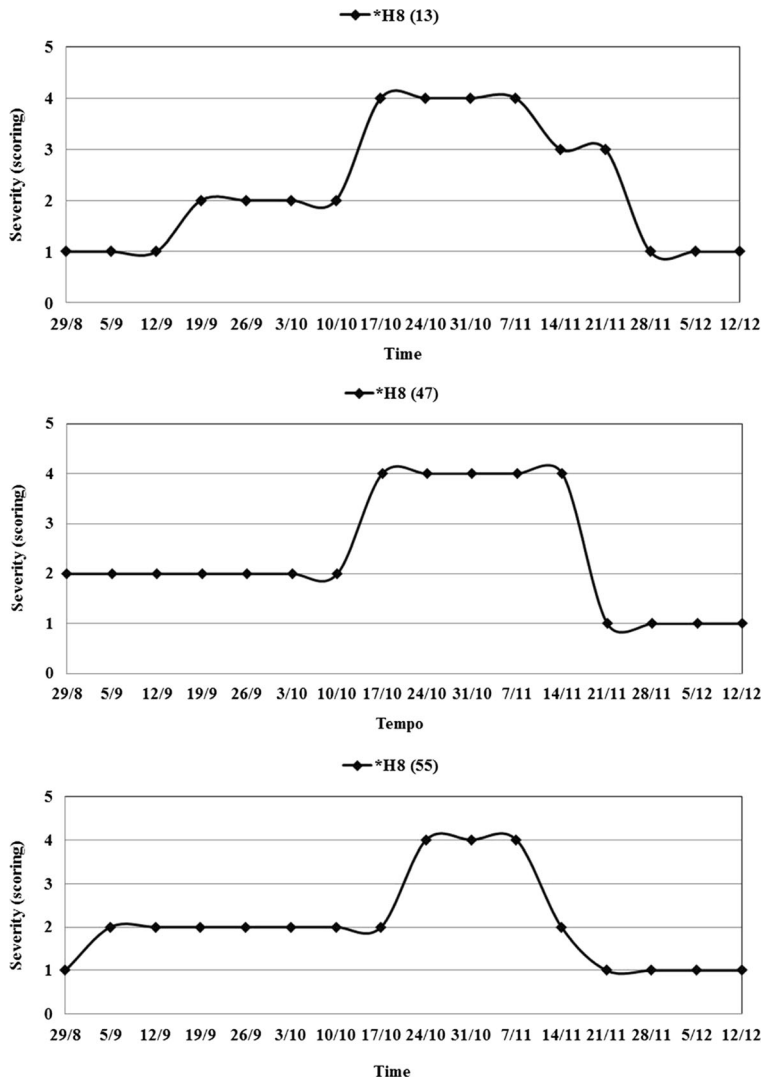

Fig. 5 Ratings of the severity of symptoms caused by CABMV in three UENFH-8 progeny genotypes based on scores weekly recorded between August and December 2012

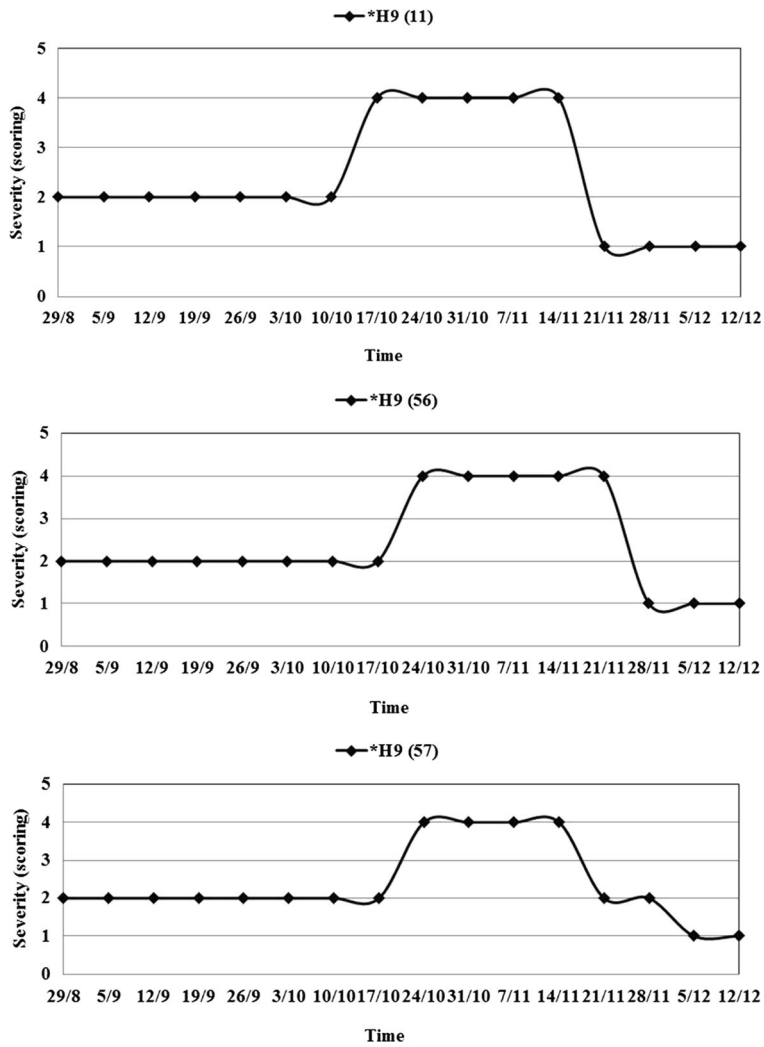

Fig. 6 Ratings of the severity of symptoms incited by CABMV in three UENFH-9 progeny genotypes based on scores weekly recorded between August and December 2012

Throughout the evaluation time, mainly in October, when most of the susceptible genotypes were presenting virus severe symptoms, there was an abrupt temperature change. The maximum mean temperature in October was 40 °C. It characterized the month as the warmest in the evaluation (Fig. 3). It is known that environmental factors might influence on host resistance mechanisms and, consequently, the symptom expression. Novaes and Rezende (1999) found that passion fruit inoculated in winter had presented lower virus concentration in comparison to those from plants inoculated in summer. The authors suggested that effects from temperature changes might have influenced virus concentration and symptom expression. In many studies on sour passion fruit, some evaluated genotypes were diagnosed sometimes as susceptible some as moderately susceptible, at different times of the year (Pinto et al. 2008; Leão et al. 2006).

Temperature and rainfall conditions might also influence aphid flights which disseminate CABMV. Garcêz et al. (2011) verified aphid population density decrease in summer, a time when rain is frequent. However, the drier months were favorable for aphid population growth. In the current study, the increase of CABMV symptoms occurred in August, a time when rain was sparse. Such a fact could be associated with aphid population growth (Garcêz et al. 2011).

The age of the plants is also an important variable related to the disease, since some genotypes present different responses (susceptibility or resistance) when they are inoculated in seedling stages or when they are adult plants (Leão et al. 2006; Pinto et al. 2008).

The posttranscriptional gene silencing (PTGS) mechanism or RNA silencing is another factor that may explain the disease symptom regression on hybrid genotypes over time (Prins et al. 2008). The observation that plants which have recovered from a first viral infection become resistant to reinfection with the same virus due to silencing activation and conservation, led to the hypothesis that RNA silencing would be an adaptive defense response to the virus (Al-Kaff et al. 1998). In a study on *Brassica napus* (canola) the same authors verified that the plants were capable of responding to pathogen attacks in order to stop the development of systemic infections. As this type of resistance is highly influenced by the environment, severity variations of the disease, due to the time of the year and geographic locations, are frequent. Thus, it is worth evaluating these progenies under different environmental conditions, favorable or unfavorable to the development of the disease in order to check whether the resistance is consistently expressed.

So far, published works about the sour passion fruit pathosystem x CABMV just report the virus' symptom progressions over time (Coimbra 2010; Cavichioli et al. 2011). Outcomes from the current study can be attributed to different causes resulting from genetic and/or environmental origins. However, the different hypotheses mentioned still need to be investigated.

Estimations on genetic parameters by mixed models

Genetic variation ($\widehat{\sigma}_g^2$) estimated values and phenotypic variance for AUDPC were 5366.86 and 5743.12, respectively. A low phenotypic variance was observed when compared to the genotypic one. It indicates small environmental effect in the expression of features, a fact that helps increasing h^2mp (99 %) estimations and selective accuracy (99 %). Estimated values inferred that resistance to CABMV in the evaluated population was highly heritable. It provides high selective efficiency. According to Beserra Júnior et al. (2006), high heritability values might indicate that the feature under investigation is under the control of a few genes. However, inheritance studies are demanding and they must use proper genetic designs in order to estimate the number and the effect of the genes which control CABMV resistance in sour passion fruit. Alves et al. (2009) used the mixed model methodology in *Theobroma grandiflorum* (cupuaçu tree) full sib progenies and found high values for progeny mean heritability (90 %) and selective accuracy (95 %) for features related to plants resistance to "witches-broom disease", similar to values found in this work.

Juhász et al. (2008) evaluated the genetic basis of resistance of *Solanum habrochaites* (syn *Lycopersicon hirsutum*) to *Pepper yellow mosaic virus* (PepYMV) and verified, by means of a quantitative analysis based on viral concentrations in each plant that PepYMV heritage was set by more than one gene with 99.22 % heritability. Beserra Júnior et al. (2006) assessed inheritance of resistance to *Watermelon mosaic virus* in watermelon segregating populations and found heritability estimations, in a broader sense, above 80 %. According to the authors, regardless of the fact that genetic control of resistance is oligo- or polygenic, there is no difficulty in obtaining resistant cultivars if the feature's heritability is high. In both cases, authors evaluated populations structured according to the genetic design called generation average analysis. They used variance analysis to estimate genetic parameters and found values different from those estimated via mixed models.

The results obtained in this manuscript advance prospects for possible control CABMV (by genetic resistance) in the passion fruit crop in Brazil. These results suggest that resistant hybrid plants can be selected and recombined with the *P. edulis* genotypes and, again, assessed in order to corroborate their resistance to CABMV, followed by an enhancement breeding program to find CABMV resistance for the Brazilian growing areas.

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