

# Genetic basis of the resistance of a passion fruit segregant population to *Cowpea aphid-borne mosaic virus* (CABMV)

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**Abstract** *Cowpea aphid-borne mosaic virus* (CABMV) infects many species of economic interest, accounting for up to 100 % reduction in productivity in passion fruit. The present work aimed to identify resistant genotypes in passion fruit populations from backcross and study resistance inheritance. The assessments were conducted in 369 genotypes, including the parents *P.edulis* (susceptible), *P.setacea* (resistant), the hybrid (*P.edulis* X *P.setacea*) and RC<sub>1</sub>. Evaluations were performed by AUDPC, obtained through a rating scale, and asymptomatic plants were subjected to ELISA test. ANOVA was significant ( $p < 0.05$ ) for AUDPC. The estimation of genetic parameters indicated heritability of 94 %; IV of 0.26 and CVe of 27.9 %. The percentage of resistant genotypes in the progenies was 14.87 and 22.64, according to ELISA, for progenies one and two, respectively. The quantities of resistant and susceptible individuals obtained in backcrosses were significantly different from the amounts of expected resistant and susceptible individuals ( $\chi^2 > 3.84$ ; G.L. 1;  $p > 0.05$ ), and thus the hypothesis of monogenic inheritance was rejected. The inheritance for resistance is polygenic, and there is gain with selection provided that large populations and more complex

methods are used, such as backcross breeding and recurrent selection.

**Keywords** *P.edulis* · *P.setacea* · Woodiness virus

## Introduction

Brazil is a major center of diversity of the genus *Passiflora* (Bernacci et al. 2014), and the world's largest producer of passion fruit (Oliveira et al. 2013). The areas harvested in 2010 and 2011 were, respectively, 62.019 and 61.631 ha, with annual production of 920.158 t in 2010, and 923.035 in 2011 (IBGE 2011, 2012). However, phytosanitary problems have led to decreased production and significant decrease in the duration of plantations. In some areas, they have even prevented cultivation (Paula et al. 2010).

Among the major diseases affecting passion fruit, we highlight fruit woodiness caused by *Cowpea aphid-borne mosaic virus* (CABMV; genus *Potyvirus*, family *Potyviridae*) (Monteiro-Hara et al. 2011; Oliveira et al. 2013). This virus is transmitted by aphids but their chemical control is inefficient, since aphids do not colonize passion fruit plants and the virus is transmitted in a non-persistent manner (Fischer et al. 2007; Maciel et al. 2009). The acquisition and transmission of the virus takes only 15 to 30 s during probing, and there is no latent period (Fischer et al. 2007; Yuki et al. 2006; Sampaio et al. 2008).

This viral infection causes mosaic with different intensity on the leaves, yellowish ring-shaped spots, mottling, roughness and blistering. Internodes become shorter, thus reducing the size of the infected plants. The fruits are often deformed, smaller, and present a hardened and thick pericarp (Anjos et al. 2001). The presence of CABMV in passion fruit fields poses a great challenge. Some management recommendations

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were adopted aiming to extend the economic viability of the crop and minimize the losses due to disease (Fischer et al. 2007; Sampaio et al. 2008). However, these procedures have not been enough to reduce losses in productivity. The use of resistant varieties thus emerged as an alternative (Anjos et al. 2001; Junqueira et al. 2003, 2005).

The search for CABMV-resistant passion fruit genotypes is an ongoing quest for breeders. The degree of resistance to CABMV among *P. edulis* cultivars has been assessed in the field with artificial inoculation, and the plants are selected based on the severity of symptoms of the viral disease on leaves and plant yield (Leão et al. 2006; Cerqueira-Silva et al. 2008; Pinto et al. 2008).

The presence of considerable genetic variability for resistance to fruit woodiness among commercial species and the negative and significant correlation between disease severity and fruit production was reported by Cerqueira-Silva et al. (2008), and a positive correlation between viral concentration and symptom severity was reported by Leão et al. (2006). However, studies on genetic diversity among genotypes of commercial passion fruit cultivars report the absence of significant genetic variability among these genotypes (Viana et al. 2003).

Many *Passiflora* wild species have been studied and assessed to identify genes for resistance to major diseases that affect passion fruit crops (Junqueira et al. 2005; Maciel et al. 2009; Oliveira et al. 2013). These works report wide genetic variability between these species, which make them promising for use in breeding programs for disease resistance, especially as hybrids (Viana et al. 2003).

The species *P. suberosa* and *P. setacea* have shown resistance to CABMV isolates (Maciel et al. 2009). The species *P. suberosa* is incongruent in interspecific cross with the cultivated species *P. edulis*, since they belong to distinct genetic groups; *P. suberosa*,  $2n=4x=24$ , belongs to subgenus *Plectostemma*; *P. edulis*,  $2n=2x=18$ , belongs to subgenus *Passiflora* (Vanderplank 1991). However, *P. edulis* belongs to the same subgenus of the species *P. setacea* and both present  $2n=2x=18$  (Vanderplank 1991). The chromosomal homology of parent plants can minimize incongruity or cross incompatibility problems and thus enable the use of the hybrid (Soares-Scott et al. 2003).

Studies aimed at obtaining resistant *Passiflora* genotypes through interspecific hybridizations and the determination of genetic parameters related to resistance are incipient. Therefore, the present study aimed to identify CABMV-resistant genotypes, to determine the genetic parameters and to study the inheritance of resistance in a segregating population obtained from a backcross between a hybrid from an interspecific cross between *P. setacea* and *P. edulis*, and their recurrent parent (*P. edulis*), thus providing the guidelines for the best strategy to carry out a breeding program.

## Material and methods

### Obtainment of the segregating population

The interspecific pollinations between *P. setacea* and *P. edulis* were performed at the campus of the Universidade Estadual de Santa Cruz, in the city of Ilhéus, Bahia, from July to August 2010, to obtain the hybrid progenies. The species *P. edulis* was used as a pollen grain donor and *P. setacea* as female parent.

The  $H_1$  progenies were conducted at the Escola Técnica Estadual Agrícola Antônio Sarlo, in the municipality of Goytacazes, Rio de Janeiro (RJ) state. They were characterized and evaluated for resistance to CABMV in the field. Backcrossings were performed at the same place, from October to December 2012. Backcrossings between the resistant  $H_1$  hybrid H5-14 and the recurrent parent were conducted. *P. edulis* was used as donor and recipient of pollen grain in 13 and 74 crossings, respectively. The hybrid genotype H5-14 was selected to be the donor parent due to its good horticultural traits and resistance to CABMV under natural field conditions.

The transfer of pollen to the stigma was performed with the aid of tweezers by gently rubbing the anther on the stigma of each flower in pre-anthesis, which were protected by a paper bag. The flowers were tagged, and five days after pollination, the rate of fruit setting [(number of fruits obtained / number of pollinations performed)  $\times$  100] was assessed. Flowers that initiated fruit development were considered fertilized.

To obtain the percentages of emergence, seeds of each progeny obtained were sown in styrofoam trays containing commercial substrate. The emergence was assessed from the eighth day after sowing, according to the percentage of emerged plants.

### Assessment of CABMV resistance

Plants of two progenies were used to evaluate resistance to the disease. These plants were obtained from the backcross between a hybrid genotype (*P. setacea*  $\times$  *P. edulis*) and two genotypes of *P. edulis*, provided by the recurrent selection program of the Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF). Interspecific hybridizations were performed using *P. edulis* as male genitor and the  $H_1$  hybrid H5-14 as female parent, in order to obtain the progenies. This backcross was conducted to recover the genome of the cultivated species. The progenies assessed were named  $T_1$  and  $T_2$ . The  $T_1$  progeny consisted of 289 genotypes and the  $T_2$  progeny, of 54 genotypes.

The seedlings from  $T_1$  and  $T_2$  progenies were transplanted to 0.5 L plastic bags filled with substrate and taken to a greenhouse. The experimental design was completely randomized with five treatments. Those treatments had different number of plants and  $T_1$  progeny,  $T_2$  progeny, H5-14 hybrid, *P. edulis*

and *P. setacea*, where the number of replications matched the number of genotypes of each progeny or species. Eighty-one days after transplanting, the plants were mechanically inoculated with CABMV using leaf extracts from infected *P. edulis* plants. The extract was prepared in a mortar, by grinding 1 g of infected leaf tissue with 10 ml of sodium phosphate buffer 0.1 M, pH 7.0, using carborundum (600 mesh) as an abrasive.

The CABMV isolate was collected from *P. edulis* leaves presenting mosaic symptoms, blisters and foliar deformation, grown in the experimental field of the Colégio Agrícola Antônio Sarlo, in Campos dos Goytacazes, RJ. This isolate was characterized by RT-PCR, with the primers designed to anneal at the potyvirus genome portion corresponding to the cytoplasmic inclusion protein (CI; Ha et al. 2008), and also by Plate Trapped Antigen–Enzyme Linked Immunosorbent Assay (PTA-ELISA), and kept in susceptible passion fruit plants in a growth chamber for constant inoculum production (Santos et al. 2015).

Forty-eight hours after the first inoculation, the plants were re-inoculated to prevent the incidence of escapes. In each inoculation, the fully expanded younger leaves was targeted. As a control, 11 genotypes of *P. edulis* and the H5-14 hybrid as well as five genotypes of *P. setacea* were inoculated with buffer solution alone as negative controls.

Observations of disease symptoms in plants started 1 day after the second inoculation and lasted for 30 days. The severity of foliar symptoms was visually assessed using a rating scale (Novaes and Rezende 1999): 1 = no symptoms; 2 = mild mosaic with no leaf deformation; 3 = severe mosaic with no leaf deformation; and 4 = severe mosaic, blisters and leaf deformation (Fig. 1).

At the end of the assessments, the data obtained by the rating scale were used to calculate the Area Under the Disease

Progress Curve (AUDPC) (Campbell and Madden 1990) for each genotype evaluated, according to the expression:

$$\text{AUDPC} = \sum_{i=1}^{n-1} \frac{Y_i + Y_{i+1}}{2} T_{i+1} - T_i$$

where:

- $Y_i$  proportion of disease in the  $i$ -th observation  
 $T_i$  time in days of the  $i$ -th observation  
 $n$  number of observations.

The values obtained by calculating the AUDPC were subjected to analysis of variance using the Genes software system (Cruz 2013), according to the following statistical model:

$$Y_{ij} = \mu + G_i + e_{ij}$$

where:

- $\mu$  overall constant  
 $G_i$  genotype effect (NID, 0,  $\sigma^2_G$ )  
 $e_{ij}$  random error (NID, 0,  $\sigma^2$ )

The analysis of variance of the AUDPC provided estimates of expected mean square. The following parameters were estimated:

- a) Environmental variance ( $\hat{\sigma}_e^2$ ):

$$\hat{\sigma}_e^2 = \frac{QME}{r}$$

- b) Phenotypic variance ( $\sigma_f^2$ ):

$$\sigma_f^2 = \frac{\sigma^2}{r} + \sigma_g^2$$

- c) Genotypic variance ( $\sigma_g^2$ ):

$$\sigma_g^2 = \frac{QMG + QME}{r}$$

- d) Phenotypic coefficient of variation ( $CV_g$ ):

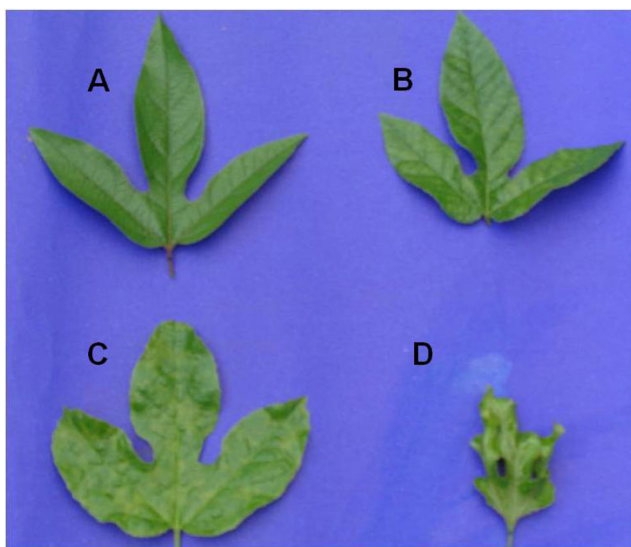
$$CV_g = \frac{100 \cdot \sqrt{\sigma_g^2}}{\bar{x}}$$

- e) Experimental coefficient of variation ( $CV_e$ ):

$$CV_e = \frac{100 \cdot \sqrt{\sigma_e^2}}{\bar{x}}$$

- f) Index of variation (IV):

$$IV = \frac{CV_g}{CV_e}$$



**Fig. 1** Leaves of the hybrids assessed and grades in the scale rating: **a** Grade 1; **b** Grade 2; **c** Grade 3; **d** Grade 4

g) Heritability ( $h^2$ ):

$$h^2 = \frac{\sigma_g^2}{\sigma_f^2}$$

### Study of the inheritance of the resistance

The study on inheritance was based on the evaluation of symptoms and ELISA, which resulted in two classes: resistant and susceptible. We used the chi-square test ( $\chi^2$ ), at 5 % probability, to adapt the proportions observed in the RC<sub>1</sub> generation, to those expected based on the hypothesis of monogenic inheritance (1:1) to control the character CABMV resistance. The calculated chi-square ( $\chi_c^2$ ) was estimated using the Genes software system (Cruz 2013), through the following statistics:

$$\chi_c^2 = \frac{(o_i - E_i)^2}{E_i}$$

where:

- $o_i$  observed number of individuals in the  $i$ -th phenotypic class
- $E_i$  expected number of individuals in the  $i$ -th phenotypic class
- $K$  number of phenotypic classes

## Results

### Obtainment of segregant population

Seventy-four crosses were conducted using the recurrent parent as pollen grain receptor. However, only one fruit was obtained, which was called T<sub>3</sub> progeny (Table 1). This fruit presented 44 seeds and an emergence percentage of 80 %, 22 days after sowing.

The highest percentage of fruit setting, 77 %, was obtained with the use of the recurrent parent (*P. edulis*) as a donor of pollen grain. These backcrosses provided ten fruits; eight of them from the T5-14×T10-10 progeny, which was called T<sub>1</sub> progeny, and two from the progeny T5-14×T10-3, which was called T<sub>2</sub> progeny (Table 2).

In the T<sub>1</sub> progeny, the highest emergence percentages at 22 days after sowing were 46.66; 40.81 and 34.84 %, found in fruits 3, 5 and 4, respectively. In the T<sub>2</sub> progeny, the highest percentage, 40 %, was found in fruit 2. The highest number of seeds in the T<sub>1</sub> progeny was 61, found in fruits 1 and 8. The lowest was 45, found in fruit 3.

**Table 1** Number of pollinated flowers, fruits obtained and rate of fruit setting of reciprocal backcrosses between the species *P. edulis* and the segregating population (SP) obtained from a *P. setacea* × *P. edulis* interspecific cross

Parents	Number of crosses	Number of fruit	Fruit setting (%)
<i>P. edulis</i> × SP	74	1	1.35
SP × <i>P. edulis</i>	13	10	77

### Genetic basis of CABMV resistance

The character “resistance to CABMV” presented a heritability of 0.94. The environmental variance found was 0.39, indicating that this character was little influenced by the environment. The genotypic variance observed was 6.61. This variance contributed most to the phenotypic variance, which was 7.00 (Table 3).

A significant difference ( $p < 0.05$ ) was found between genotypes by the F test for the character resistance (AUDPC) in the population studied, which indicates the existence of genetic variability.

Plants of the T<sub>1</sub> and T<sub>2</sub> progenies were inoculated with CABMV and evaluated for resistance. It was observed that, in the T<sub>1</sub> progeny, out of the 289 genotypes inoculated with the virus, 54 showed no symptoms of the disease (grade 1). An additional 18 genotypes, although showing symptoms of the disease, were given scores ranging from 1 to 2 during the observation period. These genotypes showed mild mosaic and no leaf deformation. In the T<sub>2</sub> progeny, 13 genotypes showed no symptoms, and four presented only mild mosaic and no leaf distortion.

Seven clones of the hybrid considered as resistant were submitted to the ELISA test after being mechanically inoculated with the virus and assessed by a rating scale. Three plants showed no symptoms of the disease and were regarded as immune by the ELISA test, while four plants showed

**Table 2** Progenies obtained from backcrosses using the segregating population as female parent, number of seeds per fruit, percentage (%) of seeds emerged 22 days after sowing

Progenies	Number of seeds per fruit	% Emergence
T1-1	61	26.23
T1-2	53	24.53
T1-3	45	46.66
T1-4	58	34.48
T1-5	49	40.81
T1-6	53	26.41
T1-7	56	23.21
T1-8	61	27.87
T2-1	73	35.61
T2-2	30	40.00
T3	44	80.00



**Table 3** Analysis of variance and estimation of genetic parameters of the area under the disease progress curve (AUDPC), in progenies obtained from backcross between the hybrid (S<sub>1</sub>) and the recurrent parent *P. edulis*

Source of variation	Mean squares
Genotypes	1973.20**
Errors	109.15
Parameters genetic	
$\hat{\sigma}_f^2$	7.00
$\hat{\sigma}_e^2$	0.39
$\hat{\sigma}_g^2$	6.61
$h^2$	0.94
$CV_g$	6.87
$CV_e$	27.9
IV	0.26

$\hat{\sigma}_f^2$  phenotypical variance,  $\hat{\sigma}_e^2$  environmental variance,  $\hat{\sigma}_g^2$  genetic variance,  $h^2$  heritability,  $CV_g$  coefficient of genetic variation,  $CV_e$  coefficient of experimental variation, IV variation index

\*\*Significant at 1% by F test

symptoms of the disease and were ELISA-positive. Importantly, the evaluations were conducted in three new, fully expanded leaves from the main branch, i.e., the plant was not evaluated as a whole.

Out of the 54 asymptomatic plants from T<sub>1</sub> progeny, 43 were immune (were not infected by CABMV) according to the ELISA test. In the T<sub>2</sub> progeny, out of the 13 asymptomatic plants, 12 were immune, according to the same test. Therefore, it was found that 14.87 % of the T<sub>1</sub> progeny genotypes were resistant to CABMV, while the T<sub>2</sub> progeny showed 22.64 % of resistant genotypes. The mortality rate due to virus infection was 1.62 % for progeny T<sub>1</sub> and 2.43 % for T<sub>2</sub> progeny (Table 4).

The hypothesis of monogenic inheritance for resistance to CABMV was not confirmed by the segregation of the H<sub>1</sub> backcross with the susceptible parent. The number of resistant and susceptible individuals obtained in backcrosses were significantly different from the expected number of resistant and susceptible individuals ( $\chi^2 > 3.84$ ; G.L. 1;  $p > 0.05$ ) (Table 5).

**Table 4** Percentage values of CABMV-resistant and -susceptible individuals, and the mortality rate in progenies obtained from backcross between the resistant hybrid and recurrent parent susceptible to the virus

Progeny	% Resistant	% Susceptible	% Mortality
T <sub>1</sub>	14.87	85.13	1.62
T <sub>2</sub>	22.64	77.36	2.43

**Table 5** Chi-square between the number of expected and observed resistant and susceptible individuals in the progeny from the hybrid (H<sub>1</sub>) X *P. edulis* (recurrent parent) backcross, assuming a monogenic model of inheritance (GL=1)

Progeny	Phenotypic class	EF	OF	$\chi^2$
T <sub>1</sub>	R	144.5	43	142.5916
	S	144.5	246	
T <sub>2</sub>	R	26.5	12	15.8679
	S	26.5	41	

EF expected frequency, OF observed frequency

## Discussion

### Obtainment of segregating population

The results obtained suggest that, in this work, a greater number of seeds do not necessarily assure the achievement of more seedlings. The low percentage of viable hybrid seeds may be due to incongruity caused by post-fertilization barriers, such as the death of the embryo from endosperm degeneration. Problems with seed viability were also observed in interspecific hybridization using wild species and cultivated *P. edulis* (Junqueira et al. 2006).

### Genetic basis of resistance to CABMV

Vivas et al. (2012) evaluated the response of papaya progenies to blight and also found that the genotypic variance was greater than the environmental variance, which conferred greater discriminating power.

Heritability values ( $h^2$ ) equal or close to one indicate that phenotypic variances among individuals are caused solely by genetic differences between them (Allard 1971). Therefore, the high value obtained for  $h^2$  (0.94) suggests that the observed phenotypic variation has more additive genetic origin. However, Fuzatto and Cia (2003) reported high broad-sense heritability values for low environmental effect. These authors found high heritability for ramulose, between 0.89 and 0.87, and low values for environmental variance. The scenario that might be favorable for breeding changed when the probability of occurrence of resistant plants was calculated.

The ANOVA for the area under the disease progress curve (AUDPC) lead us to infer whether there were differences among genotypes for this variable. The estimate of genetic parameters demonstrates whether this difference is due to genetic or environmental factors. It is known that variance is the sum of the squared differences obtained from the values of each measurement ( $x_i$ ) minus the mean of each measurement ( $\bar{x}$ ), divided by the number of original measurements minus one. Therefore, the calculation of variances included the average of susceptible and resistant plants, which probably explains the high value observed for the heritability of the character AUDPC.

The index of variation (IV) refers to the quotient between the coefficient of genetic variation ( $CV_g$ ) and the experimental coefficient of variation ( $CV_e$ ), which was not affected, therefore, by the average character (Gonçalves et al. 2007). According to Pereira et al. (2012),  $CV_e$  with values ranging from 6 to 26 % indicate good experimental precision. However, Borges et al. (2014) claim that  $CV_e$  varies according to the type of trait, experimental design and the species under study. According to Silva et al. (2012), high  $CV_e$  values may not indicate inaccuracy in the performance of the experiment or data measurement. Rather, they may reveal that the traits under study are controlled by multiple genes and, therefore, highly affected by the environment. Thus, the high  $CV_e$  value equal to 27.9 % found in this study results from the fact that the character “resistance to CABMV” is of polygenic inheritance.

According to Dias et al. (2011), when the descriptors evaluated present IV lower than 1 and an estimated broad-sense heritability of medium to high magnitude, the selection process does not favor such features in magnitude. Nevertheless, it is possible to achieve long-term gains as significant as, or even higher than, those of the other characteristics evaluated due to  $CV_g$ , since this is an important indicator of the relative magnitude of the possible changes that can be obtained for each descriptor by selection.

The results indicate that the greater the number of progenies obtained by backcrossing, the greater the success of the breeding program in obtaining resistant genotypes. Furthermore, the use of simple breeding methods will not provide significant gains during the selection process. Thus, breeding methods based on family performance are more suitable than those using selection based on the performance of individual plants.

Genotypes that obtained grade 2, which showed only mild mosaic and no leaf distortion, can be classified as moderately resistant according to Leão et al. (2006). The presence of symptoms restricted to very few leaves, compared to the total leaf volume presented by the accessions, may be a mechanism of disease resistance (Oliveira et al. 2013). A resistant genotype is not necessarily free from symptoms of the disease, but will develop mechanisms to prevent the development and reproduction of the pathogen. Accordingly, accessions of *P. setacea* free from symptoms of the disease in fruits and with low distribution in the plant, despite showing mild leaf mosaic and some deformities, were considered resistant to CABMV (Oliveira et al. 2013).

Thus, the hybrid H5-14 can be considered to be a resistant genotype, which is confirmed by the fact that this genotype originated CABMV-resistant individuals when back-crossed with the species susceptible to viral infection.

Studies have been published on the genetic variability among genotypes of *P. edulis* for resistance to CBMV in the field and the degree of resistance and susceptibility in *P. edulis* plants subjected to controlled greenhouse conditions and artificially inoculated with CABMV (Leão et al. 2006; Pinto et al.

2008; Oliveira et al. 2013). These research groups have also evaluated wild *Passiflora* species for resistance to CABMV and performed interspecific crosses aiming to obtain genotypes with resistance to the virus and desirable horticultural traits (Cerqueira-Silva et al. 2008). However, inheritance of resistance to the virus has not been reported in passion fruit plants.

In a work conducted in Brasília, where 60 RC<sub>5</sub> plants from base crosses between *P. edulis* and *P. setacea* were mechanically inoculated with CABMV, it was found that both genotypes of the recurrent parent (*P. edulis*) and the genotypes of the five generations of backcrossing (RC<sub>5</sub>) populations were susceptible to the virus (Faleiro et al. 2005; Fonseca et al. 2009). According to Faleiro et al. (2005), resistance to CABMV is lost as backcross generations advance, a process named “diluted resistance” by those authors. However, based on the results obtained in this study, we infer that in the absence of pre- or post-fertilization barriers which lead to incongruity, such resistance is not lost with the advance of generations. Since CABMV resistance is a character that does not follow the 1:1 segregation ratio, it is necessary to assess a very large number of individuals as backcross generations advance.

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