Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

Detection of a resistance gradient to *Passion fruit woodiness virus* and selection of 'yellow' passion fruit plants under field conditions

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Genet. Mol. Res. 7 (4): 1209-1216 (2008)
Received July 25, 2008
Accepted August 29, 2008
Published November 4, 2008

ABSTRACT. Productivity of 'yellow' passion fruit (*Passiflora edulis* Sims. f. *flavicarpa* O. Deg.) is reduced by infection with *Cowpea aphid-borne mosaic virus* (CABMV). We examined resistance in 72 yellow passion fruit plants grown from open-pollinated commercial seed. Plants were mechanically inoculated with CABMV virus and maintained in the field in order to select contrasting genotypes for resistance. Isolates were obtained from symptomatic leaves of yellow passion fruit plants from field production in Livramento de Nossa Senhora, Bahia State and were characterized by sequencing the viral coat protein gene. Severity of leaf symptoms of the disease, evaluated through a global leaf disease index, was measured during the eighth month of growth. Morpho-agronomic variables of fruit were evaluated from months 10 to 12. Significant linear regressions between the quantification of the leaf symptoms and the morpho-agronomic characteristics related to productivity were detected ($5.17\% \leq R^2 \leq 11\%$; $0.002 \leq P \leq 0.028$). Based on evaluations of fruit productivity, severity of leaf symptoms of the disease, and the application of a selection index of 10%, four contrasting groups of ‘yellow’ passion fruit plants considered as “resistant”, “mildly resistant”, “susceptible” and “extremely susceptible” in their reaction to CABMV (0.0001 < P < 0.024) were selected. These plants could be useful for genetic studies and for breeding yellow passion fruit plants resistant to this disease.

Key words: CABMV; Passion fruit; *Passion fruit woodiness virus*; Tolerance; Susceptibility; Virology

INTRODUCTION

Passion fruit (Passifloraceae; *Passiflora*) originates from tropical America (Viana et al., 2003); it possesses considerable genetic variability, which should be studied, preserved and used in breeding programs (Faleiro et al., 2005). The ‘yellow’ passion fruit (*Passiflora edulis* Sims. f. *flavicarpa* O. Deg.) is the most widely cultivated *Passiflora* species in the world and predominates in the Brazilian market (Bellon et al., 2007). The main producing countries are located in South America, Brazil accounts for 70% (491,619 metric tons) of the world’s production (IBGE, 2004; Ferreira, 2005); 95% (37,252 ha) of the commercial plantings in this country consist of yellow passion fruit (IBGE, 2004). The Brazilian northeast accounts for 43% of the production, and the main producing State is Bahia, with 21% of the national production (Viana-Silva, 2003).

Production and quality of passion fruit is affected by pests and pathogens (Lima et al., 1999); *Cowpea aphid-borne mosaic virus* (CABMV) (Kitajima et al., 1986; Nascimento et al., 2004), the cause of Passion Fruit Woodiness Disease (PWD) in Brazil, is one of the main problems. The first register of PWD occurrence, in Brazil, was in commercial ‘yellow’ passion fruit plants and in *P. alata* Ait (‘sweet’ passion fruit) in the State of Bahia, at the end of the 1970s (Yamashiro and Chagas, 1979); later it was found in the States of Pernambuco (Loreto and Vital, 1983), Sergipe, Ceará (Kitajima et al., 1986), São Paulo (Chagas et al., 1992) and Minas Gerais (São José et al., 1994).

Pre-immunization strategies, as used in Australia by Simmonds (1959), did not protect passion fruit plants in Brazil against CABMV (Novaes and Rezende, 2003). Currently, tests are being made with transformed plants resistant to CABMV (Alfenas et al., 2005; Trevisan, 2005). However, until now, these plants have not been developed efficient gene silencing when challenged with multiple CABMV isolates.

There is little information on germplasm characterization and genetic improvement of yellow passion fruit in Brazil (Oliveira, 1980; Meletti et al., 2000; Nascimento et al., 2003; Viana et al., 2003; Farias et al., 2005a,b). Research on resistance of yellow passion fruit to CABMV are still preliminary (Tempesta Jr. et al., 2004; Leão et al., 2006; Faleiro et al., 2007; Fonseca et al., 2007). Damage and production loss caused by CABMV in yellow passion fruit have been evaluated in greenhouse production (Gioria et al., 2000).

We mechanically inoculated yellow passion fruit plants in the field with CABMV to determine their degree of resistance.

MATERIAL AND METHODS

The experiment was conducted in an experimental field of the Universidade Estadual do Sudoeste da Bahia (UESB), located in Vitória da Conquista, Bahia State (south latitude 14°53' and west longitude 40°48', average altitude 900 m, annual average precipitation 700-800 mm, concentrated between November and March, annual average temperature 20-22°C) (Instituto Nacional de Meteorologia/Ministério da Agricultura e Abastecimento). Seventy-two yellow passion fruit plants (*P. edulis* f. *flavicarpa*) were tested; these were started from seeds purchased in the central market of Vitória da Conquista.

The leaves were infected with CABMV UESB-01 isolate, by light friction of foliar limbs with a vegetal extract obtained from the maceration of leaves of yellow passion fruit showing severe symptoms of CABMV infestation, including mosaic leaf deformations and blisters, diluted 1:20 (weight:volume) in 0.02 M potassium phosphate buffer, pH 7.0. This virus isolate was originally collected from yellow passion fruit plants in the producing region surrounding the city of Livramento de Nossa Senhora, Bahia. In order to minimize loss of infectivity of the viral particles, the leaves to be used as a source of inoculation were kept at 5°C from the time of collection until they were macerated. In order to minimize escape possibilities, inoculations were performed three times, with four- and five-month-old plant three. Three-month-old plants were transferred from plastic containers to a field plot with wire supports, with 2.5 x 2.0 m spacing and drip irrigation. The plants were cultivated according to cultural treatment normally recommended for this culture.

Molecular characterization was carried out by RT-PCR, using the pair of primers CABMV-F 5' tctgatggaaaggacaaag 3' and CABMV-R 3' cgataactgtggcgagggcg 5'. These primers were used for amplification of the fragment of the coat protein gene of CABMV according to Nascimento et al. (2006), with modifications (without bases referent to enzyme restriction site, once the fragments were not cloned). The PCR were conducted according to Krause-Sakate et al. (2001) and the genomic fragment generated was used to determine percentage of identity of this PWD causal agent with other sequences of CABMV available in GenBank (http://www.ncbi.nlm.nih.gov/Genbank/).

The leaf symptoms caused by PWD were quantified in plants eight months of age; a 'global leaf disease index' (varying from 0 to 1), calculated by ΣGS.L/TNL x HGS, where GS = grade of the scale determined for each leaf, L = number of leaves showing to each grade of the scale, TNL = total number of leaves, and HGS = highest grade of the scale. This phytophatometric variable is an adaptation of McKinney's (1923) disease index, which consists of evaluation of the absence or presence of different levels of leaf symptoms of the disease in all leaves of the plant. The scale of grades (0 = without symptoms; 1 = mild mosaic without leaf deformations; 2 = severe mosaic without leaf deformation, and 3 = severe mosaic, blisters and leaf deformation) employed in this study to calculate the global leaf disease index was described by Novaes and Rezende (1999).

The morpho-agronomical characteristics of the fruit from the 72 yellow passion fruit plants infected with CABMV were measured bi-weekly from months 10 to the 12, including total number of the fruits, total weight of the fruits, average weight of the fruits, and average diameter of the fruits. A digital pachymeter (STARRET - 727-2001; with a precision of 0.01 mm) was employed to measure fruit diameter, and a digital scale (BG 1000 with precision of 0.01 g) was used for fruit weight.

Analysis of the global leaf disease index and the fruit variables consisted of standard deviation calculation, normality test, linear regression, correlation via bootstrapping (10,000 resamplings) and a means comparison test (*t*-test bootstrap), using the Bioestat v4.0 software (Ayres et al., 2005). When necessary, data normalization was made through transformation, using the 'Box and Cox' test (Ayres et al., 2005).

RESULTS

The CABMV UESB-01 isolate that we used for inoculation of yellow passion fruit plants exhibited a high percentage of identity (>93%) with the amino acid sequences of coat protein of the strains of the CABMV available in GenBank. Considering that such molecular criteria are precise and such criteria are accepted by the International Committee of Virus Taxonomy for designation of species of *Potyvirus* (Van Regenmortel et al., 2000), we classified our UESB-01 sample, which caused PWD, as a strain of the CABMV.

The size and weight data are listed in Table 1. Assuming global leaf disease index as the variable predictor and the morpho-agronomic characteristics of the fruit as the dependent variables, a linear regression statistically significant was detected between the quantification of severity of the leaf symptoms of the disease with three of four characteristics evaluated [total weight of fruit, mean weight of fruit and mean diameter of fruit ($5.17\% \leq R^2 \leq 11\%$; $0.002 \leq P \leq 0.028$)], a significant R^2 was not identified ($P = 0.060$) for the regression of global leaf disease index versus total number of fruit (Table 2). However, significant correlations ($0.154 < r < 0.280$; $0.001 \leq P \leq 0.033$), by means bootstrap analysis, between the quantification of the leaf symptoms and all variables referring to the fruit productivity, including total number of fruit, were detected.

Table 1. Descriptive statistics of morpho-agronomic variables of fruits and severity of leaf symptoms measured in 72 yellow passion flower plants that were mechanically inoculated with *Cowpea aphid-borne mosaic virus* isolate UESB-01.

Statistical parameters	TNF	TWF (kg)	AWF (g)	ADF (mm)	GLDI
Mean	19.2	3.6	184.9	80.4	0.60
Median	13	3.6	191.3	82	0.56
Standard deviation	5.76	1.23	29.9	3.8	0.097
Coefficient of variation	30	34	16	5	16
Minimum value	10	1.4	127	71.0	0.35
	(UESB-E27)	(UESB-E27)	(UESB-D2)	(UESB-E27)	(UESB-A1)
Maximum value	30	6.3	260	88.6	0.84
	(UESB-B2)	(UESB-B5)	(UESB-C3)	(UESB-E31)	(UESB-A9)

TNF = total number of fruits; TWF = total weight of fruit per plant; AWF = average weight of the fruits; ADF = average diameter of the fruits; GLDI = global leaf disease index (0-1); UESB = identification of the genotype for each extreme value.

Table 2. Correlation and linear regression between the global leaf disease index (predictor variable) and the morpho-agronomic characteristics (dependent variables) measured in 72 yellow passion fruit plants mechanically inoculated with *Cowpea aphid-borne mosaic virus* isolate UESB-01.

Dependent variable	r (r^2)	P[1]	(R^2)	P[2]
Total number of fruits	0.154 (2.4%)	0.033	3.47%	0.060
Total weight of the fruits	0.280 (7.8%)	0.001	11%	0.002
Average weight of the fruits	0.259 (6.7%)	0.014	5.17%	0.028
Average diameter of fruits	0.171 (2.9%)	0.006	7%	0.013

[1]Estimated value of P for the correlation (r and r^2) based on 10,000 bootstrap resamplings. [2]Estimated value of P for R^2.

The plants were selected based on the severity of the leaf symptoms due to CABMV and productivity, adopting a selection index of 10%; 16 contrasting genotypes were designated as 'resistant' (UESB-A1, UESB-B2, UESB-A21, and UESB-E31), 'mildly resistant' (UESB-A22, UESB-A12, UESB-A23, and UESB-B5), 'susceptible' (UESB-E27, UESB-D10, UESB-E26, and UESB-D2), and 'extremely susceptible' (UESB-E32, UESB-E20, UESB-D8, and UESB-A9). Those genotypes, which presented lower global leaf disease index values (0.35-0.47) and higher total weight of fruit (5.57-6.30 kg) (Table 3), were considered to be resistant and mildly resistant. Similarly, those plants, which presented, concomitantly, higher values of global leaf disease index (0.72-0.84) and lower values of total weight of fruit (1.40-4.38 kg) (Table 4), were considered to be sensitive and extra-sensitive.

Table 3. Average results of morpho-agronomic characteristics of fruits and severity of leaf symptoms caused by *Cowpea aphid-borne mosaic virus* in eight yellow passion fruit plants selected as 'resistant' ('R') and 'moderately resistant' ('MR').

Genotype	Resistance level	TNF	TWF (kg)	AWF (g)	ADF (mm)	GLDI
UESB-A1	'R'	25	5.776	203.1	84.9	0.35
UESB-B2	'R'	30	5.121	170.1	78.8	0.39
UESB-A21	'R'	25	5.401	207.0	80.7	0.41
UESB-E31	'R'	18	4.292	238.4	88.6	0.44
UESB-A22	'MR'	14	2.814	187.0	79.0	0.45
UESB-A12	'MR'	28	4.609	158.9	80.3	0.45
UESB-A23	'MR'	25	5.571	195.0	85.6	0.46
UESB-B5	'MR'	27	6.308	233.3	8.41	0.47
Mean		24	4.990	199.2	8.27	0.43
Standard deviation		5	1.01	25.9	0.33	0.038
Coefficient of variation (%)		20.8%	20.3%	13%	4%	8.86%

See Table 1 for abbreviations.

Table 4. Average results *per se* of morpho-agronomic characteristics of fruits and severity of leaf symptoms of the *Cowpea aphid-borne mosaic virus* in eight "yellow" passion flower plants selected as 'susceptible' ('S') and 'extremely susceptible' ('ES') to the UESB-01 isolated.

Genotype	Resistance level	TNF	TWF (kg)	AWF (g)	ADF (mm)	GLDI
UESB-E27	'S'	10	1.400	139.9	71.0	0.72
UESB-D10	'S'	15	2.270	151.3	76.0	0.72
UESB-E26	'S'	12	1.964	163.0	77.0	0.73
UESB-D2	'S'	22	2.811	127.0	80.5	0.73
UESB-E32	'ES'	15	3.608	240.0	81.2	0.73
UESB-E20	'ES'	27	4.388	162.0	78.5	0.77
UESB-D8	'ES'	13	2.485	191.1	80.1	0.77
UESB-A9	'ES'	20	3.396	161.7	78.0	0.84
Mean		16.7	2.790	167.0	77.8	0.75
Standard deviation		5.37	0.09	32.7	0.305	0.04
Coefficient of variation (%)		32.11%	32.4%	19.5%	3.92%	5.3%

See Table 1 for abbreviations.

The group of eight yellow passion fruit plants classified as resistant and mildly resistant (Table 3) had significantly higher morpho-agronomic variables [total number of fruit (P = 0.016), total weight of fruit (P = 0.002), mean weight of fruit (P = 0.042), and mean diameter of fruit (P = 0.0001) bootstrap (10,000 resamplings) *t*-test] and significantly lower global leaf disease index values (P = 0.0001; bootstrap (10,000 resamplings) *t*-test) compared to the eight yellow passion fruit plants classified as sensitive and extrasensitive (Table 4).

DISCUSSION

The high percentage of amino acid identity (>93%) of the region of the coat protein (gene) of the CABMV UESB-01 isolate that we used with other identified strains of CABMV helps confirm that PWD in Brazil is caused by CABMV (Nascimento et al., 2004).

Among the four morpho-agronomic characteristics evaluated (Table 1), mean fruit diameter is very important, because consumers prefer fruit with an equatorial diameter greater than 65 mm (Silva and Rossi, 2005). Since mean fruit diameter ranged from 71 mm (genotype UESB-D10) to 88.6 mm (genotype UESB-E31), the germplasm that we worked with proved to be of good quality for this characteristic. Similarly, the ranges of values observed for mean fruit weight (127 g for genotype UESB-D2 and 238.4 g for genotype UESB-E31) are also close to published ranges for yellow passion fruit (Meletti et al., 2000; Nascimento et al., 2003).

The low linear regression values and the low correlation between gold leaf disease index and the morpho-agronomic variables of the fruit (Table 2) could be a result of high genetic variability between plants originated from commercial seeds (Meletti et al., 2000; Bellon et al., 2007) and the small and variable amount of pollen deposited on the stigma of the flowers by pollinating insects. Higher regression values for severity of leaf symptoms and fruit productivity parameters would be expected if full-sibling families from known parentals were used and/or if the flowers were manually pollinated, which would result in higher uniformity in fruit growth and development.

The significant differences found between the mean morpho-agronomic variables between the resistant/mildly resistant and sensitive/extra-sensitive passion fruit plants (0.0001 < P < 0.042; bootstrap *t*-test) could be due to the reduction of photosynthetic capacity because of disease (Chaves, 2002). Similar relationships between pathogen-affected leaf area and loss in the photo-assimilated production have been reported to other viral patho-systems, such as *Rupestris stem pitting associated virus* in wine grapes (*Vitis* spp; Fajardo et al., 2004) and *Melon yellowing-associated virus* in melons (*Cucumis melo*; Santos et al., 2004).

This is the first published report of inter-specific genetic variability of productivity among yellow passion fruit plants related to resistance to CABMV in production fields. Leão et al. (2006) reported on a gradient of resistance and susceptibility of yellow passion fruit plants to the CABMV under greenhouse conditions, evaluating leaf symptomatology, but they did not measure fruit production. There also have been evaluations of vegetative growth of passion fruit plants infected by CABMV under field conditions, again without evaluation of productivity (Tempesta Jr. et al., 2004). The relation between various other diseases of yellow passion fruit, including bacteriosis, anthracnosis, verrucosis and septoriosis, and fruit productivity has been evaluated previously (Junqueira et al., 2003).

We conclude that there is considerable inter-specific genetic variability in the resistance of yellow passion fruit to CABMV. We also found that severity of the disease correlates negatively and significantly with fruit production.

ACKNOWLEDGMENTS

We thank Maurício Robério Silva Soares for helping cultivate the plants and CNPq for a Master of Science fellowship for C.B.M. Cerqueira-Silva (#132590/2007-7).

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