Euphytica (2016) 208:493–507
DOI 10.1007/s10681-015-1588-8

Resistance to *Fusarium solani* and characterization of hybrids from the cross between *P. mucronata* and *P. edulis*

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Received: 22 June 2015 / Accepted: 26 October 2015 / Published online: 31 October 2015

Abstract The development of resistant cultivars is an alternative to control phytosanitary problems affecting passion fruit crops. This study was conducted to obtain progenies through interspecific crosses between *Passiflora edulis* × *Passiflora mucronata*, perform the genetic assessment of the progenies obtained, and evaluate and select genotypes resistant to *Fusarium solani*. When *P. mucronata* was used as female parent, 516 seeds were obtained, with 20 % germination and survival of nine hybrid genotypes. On the other hand, in the reciprocal cross, 9 seeds were obtained and only one genotype survived. Due to the small number of genotypes obtained, 10 hybrid genotypes and their parents *P. edulis* (susceptible) and *P. mucronata* (resistant) was propagated by cuttings. Nine plantlets of each genotype were taken to the field in a randomized block design, with three replications, aiming at morphological characterization. Twenty quantitative and 7 qualitative descriptors were assessed. Nine clones of each individual were kept in a greenhouse, inoculated with *F. solani*, and assessed 76 days after inoculation. Later, the fungus was re-isolated. The offspring genotypes of the cross in which *P. edulis* was the female parent did not flourish. For most quantitative and qualitative traits, hybrids were similar to *P. mucronata*. The genotypes studied formed six groups. Resistance to the fungus was detected in the genotypes of *P. mucronata*, from Bahia, and two hybrid genotypes. The resistant hybrid can be backcrossed with *P. edulis* and/or used as rootstock for sour passion fruit.

Keywords *Fusarium* · Genetic breeding · Interspecific hybrids · Sour passion fruit

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Introduction

Some authors describe Brazil as the center of origin of the sour passion fruit and other species of the genus *Passiflora* (Lima and Cunha 2004; Pacheco et al. 2014). They believe that one-third of the species of this genus are from Brazil (Ganga et al. 2004). However, Muschner et al. (2012) reported that the ancestors of *Passiflora* originated on the African continent, and reached the American continent through dispersion.

Brazil is one of the largest centers of diversity of the genus *Passiflora* (Bernacci et al. 2014), and the largest world producer of passion fruit. In 2013, it produced around 838,244 tons (IBGE 2014). It is cultivated in

almost all Brazilian states, and Bahia stands out as the largest national producer of sour passion fruit (IBGE 2014).

The expansion of commercial cultivation of passion fruit faces phytosanitary problems, which decrease production and significantly reduce the duration of plantations. These problems can even prevent orchard cultivation in certain areas (Paula et al. 2010). The main diseases affecting passion fruit plants include anthracnose, peanut scab or chladosporiosis, brown spot, a septoria disease, cowpea aphid-borne mosaic virus (CABMV), premature death, foot-rot and fusarium (Junqueira et al. 2003; Leão et al. 2006; Fischer et al. 2007, 2010). Thus, alternative control measures, such as the development of resistant cultivars, must be adopted.

Several authors have addressed genetic diversity among *Passiflora edulis* accessions targeting the achievement of superior genotypes in passion fruit breeding programs (Bellon et al. 2007; Reis et al. 2012; Lima et al. 2012). However, although the selection of more vigorous and resistant plants among genotypes of *P. edulis* has proved feasible by some authors (Ganga et al. 2004; Bellon et al. 2007; Negreiros et al. 2008; Reis et al. 2012), genetic variability for resistance among *P. edulis* cultivars is very low. Besides, they do not present satisfactory levels of resistance to bacterial, fungal or viral diseases (Cerqueira-Silva et al. 2012; Preisigke et al. 2015).

Several works have been conducted to assess the resistance of wild species to certain diseases affecting the passion fruit, aiming to use them in breeding programs to obtain interspecific hybrids (Crochemore et al. 2003; Viana et al. 2003; Roncatto et al. 2004; Fischer et al. 2005; Junqueira et al. 2006; Araújo et al. 2008; Junqueira et al. 2010; Fischer et al. 2010; Paula et al. 2010; Amorim et al. 2011; Conceição et al. 2011).

The successful use of wild species in breeding programs depends on the knowledge about their diversity, genetic compatibility, phenology, resistance to pests and diseases, as well as the variability of the pathogens that affect these species. In addition, it is necessary to investigate the potential of interspecific crosses between *P. edulis* and wild species (Junqueira et al. 2005).

New hybrids involving wild species have been obtained using direct and indirect crosses with sour passion fruit, but little information is available on wild species, mainly with regard to the compatibility of crosses between these species and *P. edulis*, the fertility of these hybrids and resistance of wild species and progenies obtained from interspecific pollinations to diseases affecting passion fruit crops.

Thus, this study aimed to obtain segregating progenies through interspecific crosses between *P. edulis* and *Passiflora mucronata*; assess segregating and parental populations for morpho-horticultural traits and resistance to *Fusarium solani* aiming at using them in breeding programs, for the achievement of genotypes resistant to fusarium.

Materials and methods

Population collection

The studied germplasm consisted of two genotypes of the species *P. mucronata* (accession Bahia) and two genotypes of the species *P. edulis*, grown in a greenhouse on the UENF campus. The genotypes of *P. edulis* used were obtained from the UENF recurrent selection program (Silva et al. 2009).

Interspecific crosses were performed between genotypes of *P. mucronata* and *P. edulis.* The crosses were reciprocal for the study of genetic compatibility between species and the achievement of hybrids. The flowers of the plants were protected with paper sack one day before anthesis.

The anthers of *P. mucronata* flowers were collected from 2 to 2:30 am in the morning and stored in petri dish containing silica gel and filter paper in a refrigerator at 20 °C until 12, the period of the anthesis of *P. edulis*. The anthers of *P. edulis* were collected at 12:30 pm and stored the same way as the anthers of *P. mucronata*, until 2:30 am. In this interval, all *P. mucronata* flowers opened. Forty-four hybridizations were performed using *P. mucronata* as female parent, and 46, as male parent.

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, which was previously protected with paper bag. The flowers were labeled, and, 5 days after pollination, the fruit set rate was determined (number of fruits obtained $\times$ 100 $\div$ number of pollinations conducted). Flowers that started the development of the fruit were considered fertilized.

To obtain the germination percentage, seeds of each progeny obtained were sown in Styrofoam trays containing commercial substrate. Germination was

assessed from the 8th day after sowing, according to the percentage of emerged plants.

Morphological characterization

On May 29, 2013, nine plantlets of each of the 10 hybrid genotypes and their parents (Table 1) were taken to the field. The experiment was conducted in a randomized block design with three replications, at the Escola Técnica Estadual Agrícola Antônio Sarlo in Rio de Janeiro state, with south latitude 21°45′, longitude 41° 20′W and 11 m of altitude. Vertical trellis was the conduction system used, with 2.5 m high poles, spaced at 4 m, with a wire number 12, at 1.80 m from the ground. The distance between the planting rows is 3.5 m. The experiment complied with the cultural practices recommended for passion fruit crops (Costa et al. 2008).

Descriptors assessed

The genotypes were characterized by the qualitative and quantitative descriptors (Table 2) present in the list of the Ministério da Agricultura Pecuária e Abastecimento (MAPA) (Ministry of Agriculture Livestock and Supply), established by the Serviço Nacional de Proteção de Cultivares (National Service for Cultivar Protection) for registration and protection of passion fruit cultivars.

Table 1 Identification of parental genotypes, progenies and hybrids assessed

Parents		
Species	Field identification	Genotype
P. edulis	89 (11)	2
P. edulis	139 (21)	12
P. mucronata	127	4
P. mucronata	PS	5
Hybrids		
Progenies		Genotype
127 × 139 (21)		13
127 × 139 (21)		6
127 × 139 (21)		10
127 × 139 (21)		8
127 × 139 (21)		9
127 × 139 (21)		7
127 × 139 (21)		3
127 × 139 (21)		11
89 (11) × 127		14
PS × 139 (21)		1

Pathogenicity test

The fungus *Fusarium solani* (CF/UENF 311) from the Northern region of the state of Rio de Janeiro was provided by the UENF plant disease clinic. It has been stored in sterile distilled water and cultivated by successive transfers in petri dishes containing "BDA" culture medium.

The fungus was inoculated by scraping the mycelium and conidia from a disc with 0.8 cm in diameter from the surface of the colony. Then, the inoculum mass was placed in a wound performed with stylus in the stem of the plantlet, with the aid of an autoclaved wooden toothpick. The inoculation site was protected with a moistened cotton sponge and wrapped by parafilm in order to maintain a moist chamber in the location. Next, to ensure inoculation, about 2 ml of the spore suspension was poured on the substrate around the stem of the plantlet (Fig. 1).

The seedlings were kept in a greenhouse, and 76 days after inoculation, the plants were taken to the UENF Plant Clinic, where they were assessed for fusarium incidence. Shoots taller than 20 cm of height were then cut and discarded. The symptoms of disease progression were assessed in the stem and root, both external (cicatricial callus, necrosis, tumors and the presence of perithecia) and internal (vascular staining). Fragments of the inoculation site were also removed and analyzed. Parts of the collar and root were observed under a microscope (stereoscopic and light microscope) and sown into culture medium for the reisolation of the pathogen. Thus, it was possible to assign descriptive grades from 1 to 5 to assess the symptoms in each individual, where 1 = healthy plant; 2 = Plant with no external symptoms, with internal symptoms; 3 = plant with external and internal symptoms; 4 = Dead and dried plant; and 5 = plant with presence of perithecia in the dry stem. Then, it was calculated the percentage of dead plants without the presence of the fungus, due to other causes (PLM); plants killed by the fungus (PLMF); live plants with external symptoms (PLVSE); live plants with internal symptoms (PLVSI); live plants with external and internal symptoms (PLVSEI) and asymptomatic live plants (PLVA).

Table 2 Qualitative and quantitative morphological descriptors observed in nine clones from each of the ten hybrid genotypes, two *P. mucronata* genotypes and two *P. edulis* genotypes. Campos dos Goytacazes, RJ, 2014

Classes observed according to descriptors for *Passiflora* spp. (MAPA)	
Qualitative descriptors	
Branch color	3: Purplish-green
Leaf shape	1: heart-shaped; 2: split
Division of the leaf blade	1: simple; 3: tri-lobed
Sinus	1: present; 2: absent
Depth of the Sinus	1: shallow; 2: average; 3: deep
Hairiness	1: absent
Nectary	1: adjacent to the leaf blade; 2: near the middle
Quantitative descriptors	
Stem diameter in mm (SD)	At the height of the main node of the main axis
Leaf length in mm (LL)	Longitudinal measurement of the largest extremity
Leaf width in mm (LW)	Cross measurement of the largest dimension
Length of petiole in mm (LP)	From insertion into the stem to insertion into the leaf
Flower diameter in mm (FD)	From extreme points of the flower
Corona diameter in mm (CD)	From extreme points of the corona filaments
Petal length in mm (PL)	From insertion into the flower to the apex
Sepal length in mm (SL)	From insertion into the flower to the apex
Petal width (PW)	Size of the largest dimension
Sepal width (SW)	Size of the largest dimension
Flower peduncle length in mm (LFS)	From insertion into flower receptacle to insertion into the stem
Length of androgynophore in mm (LA)	Throughout the area supporting the sexual organs
Ovarian length in mm (OL)	
Bract length in mm (BL)	From the insertion into the peduncle to the apex
Bract width (LB)	Size of the largest dimension
Mean mass of the fruits (FM)	Obtained with semi-analytical digital scale, and all ripe fruits were collected in the period assessed
Fruit longitudinal diameter in mm (FLD)	Determined in the longitudinal region of the fruits, with the aid of a digital caliper
Fruit cross diameter in mm (FCD)	Determined in the equatorial region of the fruits with the use of a digital caliper
Shell thickness in mm (ST)	Determined by the arithmetic means of the measurements of four spots of the external shell in the median portion of the fruits (transected, in the direction of the largest diameter), with the aid of a digital caliper
Mean mass of the pulp (PM)	Obtained by weighing the pulp (seeds with aryl), with the aid of a semi-analytical scale
Content of total soluble solids (SS)	Obtained by refractometry, using an ATAGO N1 portable digital refractometer reading within a line from 0° to 32° brix
Number of seeds (NS)	Manual counting (average of 10 fruits)

Analysis of variance for quantitative traits

The analysis of variance was conducted based on a randomized block design with three replications. The variables were analyzed using the Genes software system (Cruz 2013). The analysis followed the statistical model

$$Y_{ij} = \mu + g_i + B_j + e_{ij}$$

Fig. 1 Inoculation of fungus *Fusarium solani* in hybrid genotypes, their parents, *P. edulis* and *P. mucronata*—BA, and *P. mucronata*—RJ. **a** and **b** cutting made with blade in the plant collar; **c** mycelium and conidia scraped from a disk, 0.8 cm from the surface of the culture medium; **d** and **e** Inoculation of the pathogen with the aid of a wooden stick and; **f** Application of 2 ml of spore suspension on substrate around the stem

where is the Y_{ij} is the where i is the i-the genotype of the jth replication; μ is the overall mean of the assay; g_i: effect of the genotype i ($i = 1,2,\ldots g$), (NID, 0, σ_g^2); b_j is the effect of the block j ($j = 1, 2\ldots.r$), (NID, 0, σ_b^2); ε_{ij} is the experimental error residue, (NID, 0, σ^2).

The means of quantitative traits of the genotypes were compared by Scott-Knott grouping at 5 % probability.

Estimates of genetic parameters

The analysis of variance of the traits provided the mean square expectancy estimates. The following parameters were estimated:

(a) Environmental Variance $\hat{\sigma}_a^2$:

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

(b) Phenotypic variance $\hat{\sigma}_f^2$:

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

(c) Genotypic variance $\hat{\sigma}_g^2$:

$$\hat{\sigma}_g^2 = \frac{QMG - QME}{r}$$

(d) Coefficient of genetic variation CV_g:

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

(e) Coefficient of experimental variation CV_e

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

(f) Variation index (IV):

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

(g) Heritability (h^2):

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

The Mahalanobis distance was calculated for the analysis of the divergence of quantitative traits, and

the genotypes were grouped by the UPGMA method, using the Genes software software system (Cruz, 2013).

Results and discussion

Population obtaining

The flowers of the species *P. mucronata* open from 2 to 2:30 am, and close between 5:30 and 6:00 am. On cloudy days, with mild temperatures, the flowers of some genotypes remain open until about 10. Sazima and Sazima (1978) conducted a similar research and reported that the anthesis of *P. mucronata* flowers occurs at dawn and take from 1:00 to 2:00 am, extending to 7:00 or 10:00 am, depending on weather conditions. On the other hand, in the region of Monte Alegre do Sul, in the state of São Paulo, the anthesis of this species occurs from 18:00 pm until early the next morning (Meletti et al. 2011), which disagrees with the findings of this study for the Northern Rio de Janeiro state.

A fruit set rate of 11.3 % was observed when *P. mucronata* was the recipient of pollen. However, when it was pollen grain donor, the fruit set rate was 2.17 %. In crosses where *P mucronata* was used as female parent, five fruits and 516 seeds were obtained. However, only 20 % of these seeds germinated and only 103 were viable. Out of the 103 plants obtained, only nine genotypes survived and developed for later morphological characterization. In the reciprocal cross, nine seeds were obtained and only one genotype survived. The seed germination rate was, 55.5 %, when *P. edulis* was used as female parent, but only one fruit was obtained.

The greater number of fruits and seeds from the crossing *P. mucronata* × *P. edulis* compared to the reciprocal cross, might be due to difference in the size and shape of the reproductive system structures, such as the shorter length of the pollen tube *P. mucronata* compared to *P. edulis*. Although this study did not assessed the pollen viability of the parents, studies indicate that *P. edulis* pollen remains viable (above 75 %) until 24 h after the opening of the flower (Souza et al. 2002) and the pollen viability of *P. mucronata* is still high (75.8 %) for more than 12 h after anthesis (Meletti et al. 2011). Thus, pollen unfeasibility of the parents involved in the crossings may probably not be the cause of low fruit set, smaller number of fruits and seeds, at the crossing in which *P. edulis* was used as female parent.

The fruits obtained from crosses where the species *P. mucronata* was used as female parent presented a number of seeds that ranged from 52 to 230. Meletti et al. (2011) observed an average of 136 seeds per fruit (with a maximum of 321 seeds) in intraspecific crossings *P. mucronata*, higher than those observed in this study for the interspecific cross. Of the total obtained seeds, 80 % not germinate. The low percentage of viable hybrid seeds may probably be due to incongruity caused by post-fertilization barriers, such as the death of the embryo from endosperm degeneration. Conceição et al. (2011) also observed low percentage of germination, 3, 12 and 18 % in seeds obtained from interspecific crosses between *P. gardineri* × *P. alata*; *P. gardineri* × *P. gibertti*; and *P. watsoniana* × *P. alata*, respectively. Problems relating to seed production, number of seeds produced and seed viability have been observed in studies focusing on the crossability index between different wild species and between such species and *P. edulis* (Junqueira et al. 2005).

Vegetative and floral morphological characterization and genetic parameter estimate

Variability was observed for all traits related to the floral anatomy and for the traits length (LL) and width (LW) of leaves (Table 3).

In the analysis of variance, no significant differences were observed among the genotypes assessed for the variables SD and LP. Only the environmental effect affected these traits, so that the genetic variance for these variables was non-existent, and its value was zero. In turn, Santos et al. (2012) observed significant differences for all variables related to floral morphology and length and width of the leaves in progenies obtained from interspecific crosses between wild species *P. sublanceolata* and *P. foetida.*

The variables LL and LW recorded, respectively: genetic variance of 42.07 and 191.02; phenotypic variance of 49.47 and 199.20; environmental variance of 7.40 and 24.53, which contributed to the high heritability values of 85.03 and 95 %, respectively. IV was greater than unity, which agrees with the high values obtained for h^2 (Table 3). The values of the

Table 3 Analysis of variance and estimation of the genetic parameters of the traits flower diameter (FD mm); peduncle length (LFS mm); length of the petal (PL mm); petal width (PW mm); sepal length (SL mm); sepal width (SW mm); length of bract (BL mm); bract width (LB mm); diameter of the corona (CD mm); androgynophore height (LA mm) and length of the ovary (OL mm); collar diameter (SD mm); leaf length (LL mm); leaf width (LW mm) and length of the petiole (LP mm) in interspecific hybrids between *P. mucronata* and *P. edulis* and their parents

FV	GL	FD	LFS	PL	PW	SL	SW	BL	LB	CD	LA	OL	SD	LL	LW	LP
		Mean squares														
Block	2	6.07	330.42	1.44	0.06	1.61	2.52	0.02	1.13	67.60	3.12	0.07	2.34	21.91	117.74	1.40
Genotype	12[a];13[b]	23.91**	2149.91*	6.63*	3.26**	2.04**	30.38**	48.00**	45.90**	1207.47*	44.30*	3.06**	0.84[ns]	148.42**	646.69**	3.03[ns]
Residue	24	3.55	192.20	2.93	0.21	0.52	1.97	1.10	1.87	80.47	2.99	0.36	0.85	22.20	73.61	3.78
Total	38															
		Estimate of parameters														
σ_f		7.97	716.63	2.21	1.08	0.68	10.12	16.00	15.30	402.49	14.7	1.02	0.28	49.47	199.20	1.01
σ_e		1.18	64.06	0.97	0.07	0.17	0.65	0.36	0.62	26.82	0.99	0.12	0.28	7.40	24.53	1.26
σ_g		6.78	652.56	1.23	1.01	0.50	9.46	15.63	14.67	375.66	13.77	0.90	0.00	42.07	191.02	0.00
h^2		85.14	91.05	55.70	93.40	74.35	93.50	97.69	95.91	93.33	93.24	88.04	0.00	85.03	95.00	0.00
CV_g		3.16	28.24	3.02	12.38	1.88	36.40	20.82	38.76	54.16	11.93	12.30	0.00	8.45	16.34	0.00
CV_e		1.32	8.85	2.68	3.47	1.12	9.54	3.16	7.96	14.48	3.20	4.49	6.07	3.54	5.84	4.88
IV		2.39	3.19	1.12	3.56	1.67	3.81	6.58	4.86	3.74	3.72	2.73	0.00	1.37	2.79	0.00

FV source of variation, *GL* degree of freedom, σ_f^2 phenotypical variance, σ_e^2 environmental variance, σ_g^2 genetic variance, h^2 heritability, CV_g coefficient of genetic variation, CV_e coefficient of experimental variation, *IV* variation index

* Significant at 5 % by the F test; ** Significant at 1 %

[a] Degree of freedom of the flower traits

[b] Degree of freedom of vegetative traits

experimental variation coefficients, for the traits SD, LL, LW and LP ranged between 3.54 and 6.07 %, which provides good experimental accuracy (Table 3).

Except for the variable PL, which showed h^2 equal to 55.70 % (Table 3), all the other traits of floral morphology presented h^2 above 74 %. The highest value for h^2 was observed for BL (97.69), which is little affected by environmental variation (0.36) (Table 3). The floral traits are the descriptors that contribute most to divergence between individuals.

The IV values for the flower descriptors ranged from 1.12 to 6.58 % (Table 3), thus exceeding unity. It indicates a favorable situation for the selection of these traits (Vencovsky and Barriga, 1992). The CVg values ranged from 1.88 to 54.16 %. The highest values for CVg were 54.16, 38.76 and 36.40, observed for CD, LB and SW, respectively (Table 3). The genetic variation coefficient is a parameter whose estimate is directly proportional to the genetic variance (CV_g), which allows breeders to obtain a sense of the relative magnitude of the changes that can be achieved by selection throughout a breeding program (Silva et al. 2012).

The experimental variation coefficients for the morphological variables related to flower ranged from 1.10 to 14.48 %, which indicates accuracy and precision in data collection (Table 3). Santos et al. (2012) found CV_e values ranging from 2.12 to 11.78 %, in hybrid progenies from interspecific crosses between *P. sublanceolata* and *P. foetida*.

The determination of genetic parameters related to leaf and flower morphology was of great value, due to the absence of a published paper reporting and describing progenies or individuals from crosses using the species *P. edulis* and *P. mucronata*.

Genotype 14, the only hybrid derived from the cross that used *P. edulis* as female parent, presented a good vegetative growth, but did not flourish. Moreover, all genotypes from the crosses that used the species *P mucronata* as female parent flourished, and flower color was similar to that of *P. mucronata*. King et al. (2007), who crossed *P. mucronata* and *P. racemosa*, also observed that the progenies exhibited leaves and flower color similar to the species *P. mucronata*, and good flowering rate.

The flowers of the hybrids showed nocturnal anthesis and closed between 6:00 and 7:00 pm, like *P. mucronata*, which is pollinated by flitermouse. These flowers do not have attractive colors and do not show the characteristics of flowers pollinated by insects. However, some carpenter bees visited the flowers during floral anatomy data collection. However it was possible to observe the presence of carpenter bees during the morning.

Regarding the flower morphological traits, genotypes 2 and 12, which refer to the accessions of *P. edulis* used as parents in interspecific crosses, were placed in the same group by the Scott-Knott test and differed from all others for the variables PW, SW, BL, LB, CD, LA and OL. The hybrid genotypes were similar to those of the parents *P. mucronata* (genotypes 4 and 5) for these variables (Table 4). The lowest average values for the peduncle length (LSF) were 46.54 mm and 63.58 mm, found in genotypes 2 and 12, respectively. However, these genotypes showed the highest values for PW (9.26 and 9.42 mm), SW (11.96 and 12.34 mm) and CD (56.6 and 57.55 mm) (Table 4). The lowest LA values were observed for genotypes 2 and 12, 18.17 and 17.40 mm, respectively (Table 4).

Hybrids 3, 6, 9 and 13 and genotype 5 (*P. mucronata*) presented the lowest FD, and were classified in the same group by the Scott–Knott test. The following FD values were found for these genotypes: 77.5, 79.9, 80.6, 77.2 and 78.9 mm, respectively (Table 4). Hybrids 3, 6, 9 and 13 and the parents 5 and 12 and were allocated in the same group for the variable SL, since they presented the lowest values for this variable (Table 4). In the hybrid genotype 7, the mean value found for SL was significantly higher than the values observed for the genotypes of the species used as their parents (4, 5, 2 and 12) (Table 4).

Genotypes 2 and 12 showed the highest bract length and bract width (Table 4).

The hybrid genotype 8 had an average intermediate value between the parents for the trait PW and obtained the value of 8.3 mm, thus differing from all other genotypes (Table 4).

Wide variability was observed among the genotypes for the variable LL, with mean values ranging from 93.82 to 68.76 mm (Table 5). The hybrid genotype 14 presented the highest value (93.820 mm), while hybrids 1 and 3 obtained the lowest values, 68.76 and 68.98 mm, respectively. Significantly similar values were observed for the variable LL among genotypes 1 and 3; 4, 5, 9 and 10; 6, 7 and 13; 8 and 12 (Table 5).

Table 4 Averages and standard deviation of flower morphological variables in two genotypes of *P. edulis* (2 e 12), two genotypes of *P. mucronata* (4 and 5), and nine hybrids from the crosses between genotypes of *P. mucronata* and genotypes of *P. edulis*. UENF, Campos dos Goytacazes, RJ, 2014

Gen.	FD (mm)	LFS (mm)	PL (mm)	PW (mm)	SL (mm)
1	83.8 ± 3.8a	124.3 ± 31.5a	39.4 ± 15.3a	7.6 ± 0.5c	38.2 ± 2.1b
2	83.0 ± 5.1a	46.5 ± 22.2c	36.0 ± 2.5a	9.3 ± 1.1a	38.2 ± 2.1b
3	77.5 ± 3.2b	99.7 ± 23.7b	37.7 ± 16.3a	7.6 ± 0.5c	36.5 ± 1.7c
4	82.5 ± 7.2a	120.2 ± 27.1a	36.7 ± 2.3a	7.7 ± 0.6c	38.8 ± 2.2b
5	79.9 ± 3.4b	94.6 ± 18.6b	35.2 ± 2.1a	7.8 ± 0.4c	37.1 ± 2.1c
6	80.6 ± 3.8b	83.4 ± 19.1b	34.7 ± 3.4a	7.1 ± 0.7c	37.4 ± 3.0c
7	86.7 ± 5.4a	98.6 ± 25.1b	38.2 ± 3.8a	6.2 ± 0.4c	41.2 ± 1.8a
8	84.2 ± 4.3a	90.5 ± 25.2b	37.5 ± 2.2a	8.3 ± 0.4b	38.8 ± 1.8b
9	77.2 ± 5.1b	118.9 ± 23.0a	34.2 ± 2.4a	7.7 ± 0.6c	36.1 ± 2.4c
10	82.1 ± 2.9a	110.5 ± 33.2a	36.2 ± 2.6a	7.5 ± 0.5c	38.2 ± 1.6b
11	82.2 ± 4.9a	102.2 ± 24.2b	36.6 ± 2.7a	7.3 ± 0.7c	38.2 ± 2.6b
12	83.1 ± 3.7a	63.58 ± 10.5c	36.6 ± 2.7a	9.4 ± 1.3a	37.1 ± 2.3c
13	78.87 ± 4.5b	88.25 ± 25.1b	34.4 ± 2.2a	7.1 ± 0.5c	36.7 ± 1.7c

Gen.	SW (mm)	BL (mm)	LB (mm)	CD (mm)	LA (mm)	CO (mm)
1	6.9 ± 0.93b	15.4 ± 2.0b	6.4 ± 0.8b	25.5 ± 2.5b	27.5 ± 2.7a	7.2 ± 1.1b
2	12.0 ± 3.74a	23 ± 2.9a	13.9 ± 3.6a	56.6 ± 27.4a	18.2 ± 4.5b	9.1 ± 1.5a
3	6.3 ± 0.51b	15.8 ± 1.8b	7.7 ± 0.8b	24.9 ± 2.4b	23.1 ± 2.0a	7.2 ± 1.0b
4	6.8 ± 0.55b	15.1 ± 2.4b	7.3 ± 0.8b	24.4 ± 7.3b	27.3 ± 3.0a	7.1 ± 1.0b
5	6.4 ± 0.40b	18.3 ± 2.2b	8.3 ± 5.3b	23.1 ± 2.2b	25.3 ± 1.7a	7.0 ± 0.7b
6	6.5 ± 0.55b	16.1 ± 1.4b	7.6 ± 0.8b	24.6 ± 2.6b	25.5 ± 2.5a	7.0 ± 1.1b
7	6.3 ± 0.55b	15.9 ± 1.6b	7.3 ± 0.9b	29.0 ± 2.8b	25.6 ± 1.8a	7.2 ± 0.9b
8	7.4 ± 0.52b	17 ± 1.7b	8.0 ± 1.1b	26.5 ± 2.8b	26.6 ± 3.0a	7.4 ± 0.7b
9	6.9 ± 0.64b	18.7 ± 2.4b	8.4 ± 1.1b	25.6 ± 3.0b	25.5 ± 2.2a	6.9 ± 1.2b
10	6.8 ± 0.42b	17.1 ± 2.4b	7.84 ± 1.0b	24.1 ± 2.5b	26.5 ± 2.9a	7.4 ± 0.8b
11	6.5 ± 0.56b	18.2 ± 2.4b	8.5 ± 1.1b	26.3 ± 2.8b	26.4 ± 2.4a	7.4 ± 1.0b
12	12.3 ± 1.36a	23.0 ± 3.0a	14.9 ± 2.4a	57.5 ± 10.2a	17.4 ± 2.4b	8.8 ± 1.0a
13	6.39 ± 0.63b	17.0 ± 1.8b	7.46 ± 0.9b	26.2 ± 2.1b	24.1 ± 2.3a	6.9 ± 0.9b

Mean values followed by the same letter in the columns do not differ statistically by Scott–Knott grouping at 5 % probability

FD flower diameter, *LFS* peduncle length, *PL* petal length, *LP* petal width, *PW* sepal length, *SW* sepal width, *BL* bract length, *LB* bract width, *CD* corona diameter, *LA* androgynophore height, *OL* ovarian length in flowers

Gen. genotypes; Gen. 1 and 2 (*P. edulis*); gen. 5 and 6 (*P. mucronata*) and gen. 1, 3, 4, 6, 7, 8, 9, 10, 11 and 13 (hybrid genotypes)

For the trait LW, no significant difference was observed between the hybrid genotypes and the genotypes of *P. mucronata*. Both formed a single group. The genotypes of *P. edulis* (2 and 12) presented the highest average values for this trait (126.68 and 103.90, respectively), differing among themselves and from the other genotypes (Table 5). Santos et al. (2014) conducted a study similar to this and evaluated hybrids and parents coming from interspecific cross between *P. edulis* × *P. setacea* and found the highest values for LL and LW in the genotypes of *P. edulis*.

Morphological characterization of fruits and genetic parameter estimate

With the exception of genotype 14 (*P. edulis* × *P. mucronata*), which presented no flower or fruit, all genotypes assessed bore fruit, but not all fruits had seeds and/or, when they did, the seeds were not always viable. The hybrid genotypes 10, 11, and 13 obtained fruit, but no seeds were observed and the fruits were hollow.

Aborted seeds were observed in the hybrid genotypes 8 and 9. Infertility in these two genotypes may be related to genetic factors. All fruits produced by these genotypes hybrids during the observation period presented aborted seeds, while only a few fruits of the parent *P. mucronata* showed that kind of seed. Similar to this study Meletti et al. (2011) also observed empty fruit without seed in *P. mucronata*. The absence of seeds and/or the occurrence of aborted seeds in the fruits of hybrid plants can be explained by post-fertilization barriers, which cause partial or total sterility in hybrid plants.

Table 5 Averages and standard deviation of the leaf variables, in two genotypes of *P. edulis* (2 and 12), two genotypes of *P. mucronata* (4 and 5), and ten hybrids from the crosses between the genotypes of *P. mucronata* and the genotypes of *P. edulis*. UENF, Campos dos Goytacazes, RJ, 2014

Genotypes	LL (mm)	LW (mm)
1	68.98 ± 7.4g	74.69 ± 10.7c
2	86.9 ± 13.7b	126.68 ± 23.14a
3	68.76 ± 8.6g	73.74 ± 11.52c
4	76.15 ± 8.1d	81.87 ± 11.26c
5	77.49 ± 9.1d	86.57 ± 11.61c
6	71.47 ± 7.7f	75.77 ± 10.28c
7	71.91 ± 10.8f	74.07 ± 11.46c
8	75.15 ± 9.5e	77.79 ± 12.59c
9	76.81 ± 9.0d	81.73 ± 12.58c
10	75.83 ± 11.4d	90.07 ± 18.00c
11	83.49 ± 9.6c	76.53 ± 9.76c
12	73.62 ± 13.0e	103.90 ± 19.07b
13	73.02 ± 8.3f	74.96 ± 10.95c
14	93.82 ± 16.3a	85.21 ± 14.33c

Average values followed by the same letter in the columns do not differ statistically by Scott–Knott grouping at 5 % probability

LL leaf length, *LW* leaf width

Normal fruits with seed and pulp were found in hybrid genotypes 1, 3, 6 and 7. Hybrids with viable seeds were also observed by King et al. (2007) in progenies from interspecific cross between the species *P. mucronata* and *P. racemosa*.

Overall, the fruits of all hybrids showed morphological characteristics similar to the species *P. mucronata*. The analysis of variance of the traits of fruits from the four fertile genotypes and their parents showed significant difference for all variables of the fruits accessed (Table 6).

For all descriptors relating to the fruit, h^2 ranged from 89.23 to 99.71 %. The highest and lowest value for this variable was observed for the traits FM and SS, respectively (Table 6). Santos et al. (2015) conducted a similar research work using mixed model methodology (REML/BLUP) and found 89 % heritability for SS in a population of interspecific hybrids between *P. edulis* and *P. setacea*. In turn, Silva et al. (2012) evaluated progenies of full-sibs of sour passion fruit from the second recurrent selection cycle and verified for most traits related to fruit, heritability estimates below 50 %, which is lower than the values obtained in this work. The high h^2 estimates found in this study indicate that successful selection can be achieved for all traits related to fruit. However, the difference between the h^2 estimates found in this work and in those mentioned above is due to the fact that this parameter is a property not only of a character, but also of the population and environmental circumstances to which the individuals are subjected. Its value can be affected by changes in any of the components of genetic and phenotypic variance (Falconer 1987). Therefore, estimates should not be extrapolated to other populations.

The CV_e showed values between 2.94 and 17.20, except for PM, whose CV_e value was 39.87, which may be due to the greater effect of the environment on the expression of this trait (Table 6).

The Scott-Knott grouping showed no difference between hybrids for the variables ST, FM, PM, NS and SS (Table 7). For these traits, the fruits of the hybrids were similar to the fruits of the species used as female parent, *P. mucronata*. The highest values for the traits related to the fruit were observed for both genotypes of *P. edulis* used as male parents, which formed separate groups between the hybrids and *P. mucronata*. Similar results were observed for hybrids *P. edulis* and *P. setacea* and their parents (Santos et al. 2014).

For the variable FLD, it was observed, in the hybrid genotype 3, an average value (33.06 mm) lower than those obtained by their parents. The other hybrid genotypes showed values similar to those of the species *P. mucronata* (genotype 4) (Table 7).

Hybrid genotypes 3 and 7 obtained the lowest values for FCD, 17.4 and 15.1 mm, respectively, thus significantly differing from all other genotypes. However, the hybrids 1 and 6 showed average values for FCD similar to those of *P. mucronata* (genotype 4). The highest values for FCD (58.9 and 63.7) were observed in the cultivated species (genotypes 2 and 12) (Table 7).

The hybrids showed SS values ranging from 15.4° to 16.8° Brix (Table 7). According to Meletti et al. (2000), these values are higher than those accepted by juice industries, which range from 13 to 14°Brix. However, the fruits are small and do not meet the commercial standard.

Cerqueira-Silva et al. (2009) found high genetic dissimilarity between the accessions *P. edulis* and *P. setacea* through the physical–chemical traits of the fruits.

Table 6 Analysis of variance and estimate of genetic parameters of fruit traits in interspecific hybrids from crosses between *P. mucronata* and *P. edulis*, and their parents. UENF, Campos dos Goytacazes, RJ, 2014

FV	GL	FLD (mm)	FCD (mm)	ST (mm)	FM (g)	PM (g)	NS	SS (°Brix)
Mean squares								
Block	2	12.70	0.70	0.38	28.46	129.79	89.81	331
Genotype	6	848.02**	1330.31**	38.45**	6793.11**	1636.13**	12825.74**	1037**
Residue	12	5.91	3.82	0.82	57.64	154.23	858.36	111
Total	20							
Estimate of parameters								
σ_f^2		282.67	443.43	12.81	2264.36	545.37	4275.25	3.45
σ_e^2		1.97	1.27	0.27	19.21	51.41	286.12	0.38
σ_g^2		280.70	442.16	12.54	2245.15	493.97	3989.12	3.08
h^2		99.30	99.71	97.86	99.15	90.57	93.30	89.23
CV_g		35.16	68.45	116.93	139.85	123.67	61.98	11.71
CV_e		2.94	3.67	17.20	12.93	39.87	16.59	4.11
CV_g/Cv_e		6.89	10.76	3.91	6.25	1.79	2.16	1.66

** Significant at 1 % by the F test

FV source of variation, *GL* degrees of freedom, *FLD* fruit length, FCD fruit width, *ST* thickness of the fruit, *FM* fruit mass, *PM* pulp mass, NS number of seeds, *SS* content of total soluble solids

Table 7 Averages and standard deviation of the variables of fruits in two genotypes of *P. edulis* (2 and 12), one genotype of *P. mucronata* (4) and four hybrids from crosses between the genotypes of *P. mucronata* and the genotypes of *P. edulis*. UENF, Campos dos Goytacazes, RJ, 2014

Gen.	FLD (mm)	FCD (mm)	ST (mm)	FM (g)	PM (g)	SS (°Brix)	
2	68.9 ± 7.1b	58.9 ± 6.4b	8.3 ± 1.1a	87.8 ± 29.6b	54.2 ± 41.9a	11.3 ± 2.4c	154.0 ± 70.7b
12	74.8 ± 9.3a	63.7 ± 8.3a	8.3 ± 9.9a	117.0 ± 43.1a	49.9 ± 24.3a	13.9 ± 1.4b	220.2 ± 117.9a
1	39.2 ± 5.5c	20.5 ± 2.2c	0.9 ± 0.4b	7.9 ± 2.6c	5.4 ± 1.8b	15.4 ± 2.9a	93.8 ± 26.4c
3	33.1 ± 3.9d	17.4 ± 2.3d	0.8 ± 0.4b	5.0 ± 1.6c	3.3 ± 1.1b	15.6 ± 2.5a	56.3 ± 15.7c
6	40.7 ± 6.7c	18.6 ± 2.7c	0.9 ± 0.4b	6.7 ± 2.7c	4.2 ± 1.8b	16.3 ± 2.6a	57.2 ± 24.0c
7	33.4 ± 11.8c	15.1 ± 2.4d	0.9 ± 0.3b	4.2 ± 1.7c	2.6 ± 1.2b	16.8 ± 1.9a	32.7 ± 14.6c
4	38.4 ± 6.3c	20.8 ± 3.5c	1.1 ± 0.4b	8.7 ± 3.7c	6.0 ± 2.5b	15.6 ± 1.8a	99.0 ± 43.8c

Mean values followed by the same letter in the columns do not differ statistically by the Scott–Knott grouping at 5 % probability

Gen genotypes, FLD fruit length, *FCD* fruit width, *ST* thickness of the fruit shell, *FM* fruit mass, *PM* pulp mass, *SS* content of total soluble solids, *NS* number of seeds

Quantification of genetic diversity of hybrids and parents

The shortest distance (17.16) was observed between genotypes 5 and 10; and 5 is the female parent of the hybrid genotype 10, which explains the high similarity between these two genotypes. The genotypes 2 and 7 were the most distant (340.6) (Fig. 2). The cophenetic correlation coefficient (CCC) with estimated value of 83 % indicates good adjustment between the graphical representation and the original genetic distance matrix, which ensures the dendrogram inferences.

Genotype 14 was not included in the cluster analysis, because its inclusion would change the results, since it does not bloom. Therefore, the measurements of the flower morphological traits cannot be performed.

The genotypes were grouped into six groups according to their similarities (Fig. 2). The group I was formed by the hybrid 7; Group II, by genotype 2

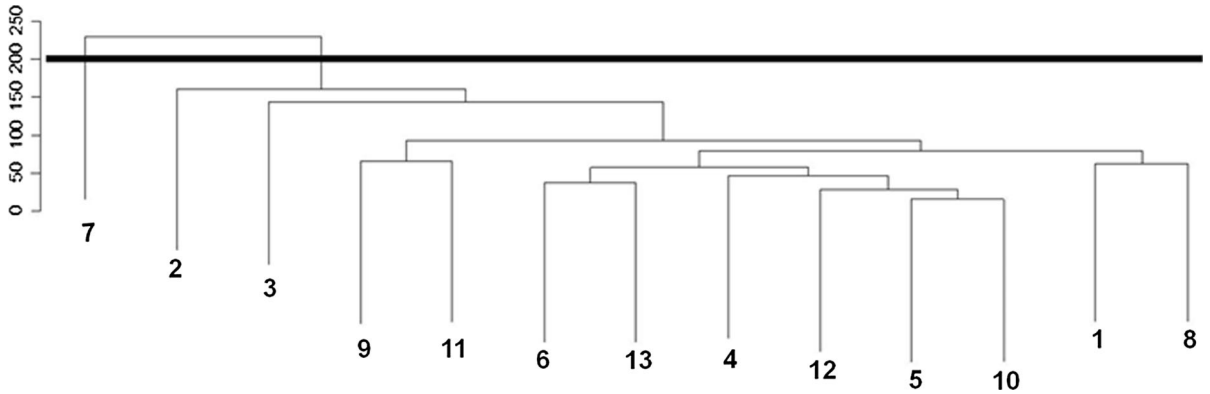

Fig. 2 Dendrogram of genetic diversity between interspecific hybrids (1, 3, 4, 6, 7, 8, 9, 10, 11, 13 and 14) and their parents (4 and 5 (*P. mucronata*); 2:12 (*P. edulis*)) obtained by the hierarchical method UPGMA, based on mean values for traits, using the Mahalanobis distance as the dissimilarity measurement

(*P. edulis*) and hybrid 3; Group III, by hybrids 9 and 11; group IV, by hybrids 6, and 13; Group V, by hybrid 10, parents and 4, 5 (*P. mucronata*) and 12 (*P. edulis*) and group VI was composed of hybrids 1 and 8 (Fig. 2).

The grouping of interspecific hybrids into various groups resulted from the implicit heterozygosity of passion fruit, which allows different interspecific hybrids obtained from crosses between *P. mucronata* and *P. edulis* were grouped alone or close to the parents. It is also observed that the genotype 12 (*P. edulis*) is close to the genotypes 4 and 5 *P. mucronata*. Santos et al. (2011) found that hybrids derived from interspecific cross between the species *P. sublanceolata* and *P. foetida* obtained intermediate average values for the morphological traits, compared to those of the parents or values close to one of their parents, similar from the findings of this study.

The trait that contributed most to the genetic divergence among genotypes was PW (25.10 %), followed by LW, SL, FD, LL, LB and CD, while LP and LA (Table 8) provided the least contribution. Tangarife et al. (2009) affirm that *Passiflora* species can be grouped according to the similarity corresponding to the variable flower size. These authors reported that the floral morphology was important for infrageneric discrimination of *Passiflora*. In this study, the variables PW, LW, SL, FD, LL, LB and CD contribute with 83.64 % of the total variance (Table 8). In other words, the descriptors related to flower (PW, SL, FD, LB and CD) would be enough to differentiate genotypes. Lorenz-Lemke et al. (2005) also found that the traits flower size and leaf shape were enough to distinguish the species *P. actinia* and *P. elegans*.

Table 8 Relative contribution of the traits for divergence between the genotypes. UENF, Campos dos Goytacazes, RJ, 2014

Variables	%	% Cumulative
PW	25.1033	25.10
LW	13.6411	38.74
SL	10.1847	48.92
FD	9.6468	58.57
LL	9.1763	67.75
LB	8.8057	76.56
CD	7.0747	83.64
LFS	5.5992	89.24
BL	2.863	92.10
PL	2.0089	94.11
OL	1.8727	95.97
SD	1.5211	97.49
SW	1.4054	98.90
LA	0.6515	99.55
LP	0.450	100.0

LA androgynophore height, *OL* length of the ovary, *FD* flower diameter, *LB* bract width, *CD* corona diameter, *PL* petal length, *SW* sepal width, *LP* petal width, *PW* sepal length, *LFS* peduncle length, *LL* leaf length, *BL* bract length, *LW* leaf width, *SD* collar diameter, *LP* petiole length

Pathogenicity test

The hybrid 13 obtained 100 % of living and healthy plants, free from the fungus after re-isolation. This genotype can be considered resistant to *Fusarium solani*, no infection or re-isolation of the pathogen was found in any of the inoculated plants. The absence of infection was also observed in both genotypes of *P. mucronata* accession Bahia (4 and 5) used as resistant parents in interspecific crosses to obtain the hybrids studied. However, the accession of *P. mucronata* from Rio de Janeiro was susceptible to the fungus, and 66.67 % of the plants were killed by *F. solani*, while 33.33 % of live plants showed external and internal symptoms (Table 9).

Hybrid 11 showed 100 % of its plants with external and internal symptoms. In turn, hybrid 14 showed 66.66 % of live plants free from symptoms of the disease and from the fungus, after re-isolation (Table 9). The plants from this genotype died due to the presence of the stem borer, also known as passion fruit borer (*Philonis* ssp.).

Hybrids 8 and 6 presented the highest percentage of plants killed by *F. solani*, 83.33 and 66.66 %, respectively. Genotype 12 is susceptible to the fungus and was used as male parent in interspecific crosses aiming to obtain these two hybrids. It showed 83.33 % of dead plants with symptoms of the disease. Thus, it can be said that hybrids 8 and 6 were the most susceptible to the pathogen, similarly to the male parent (Table 9).

The hybrid 10 had 16.67 % of its plants killed by *Fusarium solani*, and 83.33 % of live plants with internal and external symptoms. The hybrid 9 had 33.34 % of its plants killed by the fungus and 66.66 % with internal and external symptoms. Genotype 2 (*P. edulis*) had 100 % of live plants with internal and external symptoms. Hybrid 7 also showed 16.67 % of plants killed by *F. solani*, but 83.33 % of live plants showed only internal symptoms. Genotype 3 showed 66.66 of its plants killed by the fungus and 33.33 of living plants free from symptoms (Table 9).

The use of resistant (*P. mucronata*) and susceptible (*P. edulis*) genotypes in the crosses as donors or receptors of pollen grains did not affect the expression of resistance, since in hybrid genotype 13, evaluated as resistant, *P. mucronata* (genotype 2) was used as the female parent, but in hybrid genotype 14, also resistant, *P. mucronata* (genotype 2) was the male parent. Therefore, the results showed that the inheritance of resistance to *F. solani* in this population is probably not of cytoplasmic origin.

Table 9 Percentage of dead passion fruit plants (PLM), killed by the fungus (PLMF), alive with external symptoms (PLVSE), alive with internal symptoms (PLVSI), alive with external and internal symptoms (PLVSEI) and living asymptomatic (PLVA); and result of re-isolation after inoculation with *Fusarium solani*. UENF, Campos dos Goytacazes, RJ, 2014

Genotype	Inoculated Plants	PLM (%)	PLMF (%)	PLVSE (%)	PLVSI (%)	PLVSEI (%)	PLVA (%)	Re-isolation
11	6	–	–	–	–	100	–	+
14	6	33.34	–	–	–	–	66.66	–
8	6	–	83.33	–	16.67	–	–	+
10	6	–	16.67	–	–	83.33	–	+
13	6	–	–	–	–	–	100	–
6	6	–	66.66	–	–	33.33	–	+
9	6	–	33.34	–	–	66.66	–	+
3	6	–	66.66	–	–	–	33.33	+
7	6	–	16.67	–	83.33	–	–	+
4	6	–	–	–	–	–	100	–
5	6	–	–	–	–	–	100	–
MSF*	6	–	–	–	–	33.33	–	+
12	6	–	83.33	16.67	–	–	–	+
2	6	–	–	–	100	–	–	+

+ Presence of the fungus after re-isolation, − absence of the fungus after re-isolation * MSF. Genotype of *P. mucronata* from São Francisco do Itabapoana—RJ, included in the resistance test

The gene of resistance to *F. solani* is not probably present in all accessions of the species *P. mucronata*, since the accessions from Bahia were resistant, while the accession from São Francisco do Itabapoana—RJ was susceptible (Table 9).

Resistance to *Fusarium* depends on genetic processes, and since Passifloras are cross-pollinated plants (outcrossing), there is extensive segregation. Thus, within the same species, there may be variability for resistance among accessions or between genotypes. Furthermore, the genetic variability of the pathogenic agent (*F. solani*), and prolonged incubation may also affect the response to resistance to the fungus in accessions or genotypes of *Passiflora* (Silva et al. 2013). These authors reported that the incubation of the fungus lasted on average 36 days for all accessions of *Passiflora* with symptoms of the fungus.

Resistance to *F. solani* (in its asexual form, *Nectria haematococca*) was also found by Fischer et al. (2005) in genotypes of *P. mucronata*. In contrast, Preisigke et al. (2015) assessed the genetic variability for resistance to *F. solani*, and found that the UFV accession of *P. mucronata* was susceptible to the fungus. These authors report low survival rate for this accession, which remained alive for only 17 days after inoculation.

These results suggest that hybrid resistant to *F. solani* should be backcrossed with the species *P. edulis*, to verify the feasibility to obtain fruits with seeds and, subsequently, genotypes with promising horticultural traits and resistance to the fungus.

Acknowledgments The authors are thankful to the Graduate Program in Genetics and Plant Breeding; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES (Higher Education Personnel Improvement Coordination); Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro – FAPERJ (Foundation for Research Support of the State of Rio de Janeiro); Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq (National Council for Scientific and Technological Development) and Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF) for the financial support for this research.

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