

# Characterization and selection of passion fruit (yellow and purple) accessions based on molecular markers and disease reactions for use in breeding programs

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**Abstract** *Passiflora edulis* Sims, which is native to South America, stands out as a passion fruit species with major potential for fruit production and marketing. This species is popularly known as yellow or purple passion fruit, depending on the color of the fruits produced. Brazil is the major worldwide producer of passion fruit; however, the average productivity of the country is low compared with its potential for culture. Fungal, bacterial and viral pathogens are among the factors limiting the productivity of passion fruit. Furthermore, no existing

cultivars exhibit both productivity and resistance to disease. To select genetic material that will be useful for core collections and for increasing the genetic resistance of passion fruit cultivars to pathogens, we characterized 36 accessions based on 23 microsatellite loci and six variables related to the reactions to three diseases (woodiness virus, scab and anthracnose). We identified 127 alleles (an average of 5.52 alleles per locus), 30 % of which were private for yellow or purple passion fruit accessions. Analysis of variance and mean comparison tests indicated differences in five of the six variables ( $p < 0.05$ , Scott-Knott test). Differences between the average reactions of the yellow and purple passion fruit accessions were also observed for the symptoms of woodiness virus and anthracnose ( $p < 0.05$ , Mann–Whitney test). Together with these results, molecular and phenotypic estimates allowed the identification of groups of preferential accessions for use in breeding programs, for example, accessions BGP029, 071, 168, 205 and 277.

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## Introduction

Passion fruit (*Passiflora* spp.) is grown in different parts of the world, primarily in tropical regions, with Brazil, Colombia, Peru and Ecuador representing the

major producers of these fruits (Borges et al. 2005). Although Brazilian production has varied, it has generally increased in recent decades, with an average production of 676,000 tons being recorded during the last 10 years (IBGE 2012). However, despite dominating global passion fruit production, Brazilian productivity has remained stable (approximately  $14 \text{ tha}^{-1} \text{ year}^{-1}$ ) and well below this crop's productivity potential, which is estimated at approximately  $50 \text{ tha}^{-1} \text{ year}^{-1}$  (Meletti et al. 2005).

Different factors contribute to the low productivity of passion fruit in Brazil, such as the occurrence of fungal, bacterial and viral pathogens. Records in the literature indicate the occurrence of numerous pathogens associated with passion fruit crops. Discussions of advances and perspectives related to the primary diseases that affect passion fruit cultivation (such as fusariosis, scab, anthracnose, bacteriosis, and woodiness virus) are also available in the literature (Cerqueira-Silva et al. 2014a; Silva et al. 2013; Faleiro et al. 2011; Meletti et al. 2005; Faleiro et al. 2005).

Recent and ongoing research has aimed to identify or to generate genotypes and hybrids with greater resistance to the diseases that affect passion fruit. To identify sources of resistance to diseases, we highlight studies addressing the characterization of passion fruit varieties and cultivars (Batistti et al. 2013; Fischer et al. 2010; Junqueira et al. 2003), as well as accessions maintained in collections and germplasm banks (Oliveira et al. 2013; Silva et al. 2013; Cerqueira-Silva et al. 2008; Junqueira et al. 2006). In general, the results presented in the published literature vary, indicating the differing susceptibility of the evaluated cultivars and the broad genetic variability of the specimens in germplasm banks, for both *P. edulis* and wild passion fruit species.

Various investigators have indicated a need for research that contributes to advancing genetic breeding programs for passion fruit and to releasing cultivars that combine resistance characteristics (Faleiro et al. 2011; Meletti et al. 2005; Faleiro et al. 2005; Junqueira et al. 2003). Promising results associated with identifying potential sources of resistance have been observed in some accessions of *P. edulis* for resistance to bacteriosis and anthracnose; in accessions of wild species such as *P. actinia*, *P. setacea* and *P. coccinea* for resistance to virosis; in *P. odontophylla*, *P. gibertii*, *P. caerulea*, *P. actinia*, and *P. mucronata* for resistance to bacteriosis; and in *P. serrato-digitata*, *P. gibertii*, *P.*

*coccinea*, *P. actinia*, *P. setacea*, *P. nitida*, and *P. caerulea* for resistance to anthracnose (Oliveira et al. 2013; Faleiro and Junqueira 2009; Faleiro et al. 2005). However, basic information associated with agronomic evaluations and the available estimates of genetic variability remain limited for many of these materials. Therefore, pre-breeding activities, such as the prospecting and characterization of accessions, are required to maintain the variability in collections, and germplasm banks could be effectively used as a genetic resource allowing expansion of the genetic basis of passion fruit breeding programs (Faleiro et al. 2011; Meletti et al. 2005; Faleiro et al. 2005).

Costs are generally high for research activities specifically associated with pre-breeding (such as performing agronomic characterizations, determining diversity estimates and identifying preferred crossings), and the time required to obtain results is considerable (Nass 2011; Nass and Paterniani 2000). The use of molecular markers is fundamentally important for enhancing the amount of information generated and for reducing the time required to obtain results (Ferreira and Rangel 2011). In this context, we note a considerable increase in research devoted to obtaining estimates of genetic variability using molecular markers in both commercial (Ortiz et al. 2012; Costa et al. 2012; Cerqueira-Silva et al. 2010a; Bellon et al. 2007; Viana et al. 2003) and wild passion fruit species (Cerqueira-silva et al. 2010b, c, 2012a; Junqueira et al. 2007). These studies have identified low variability among commercial accessions of *P. edulis* and generally wide variability among wild accessions of passion fruit, reinforcing the potential for the use of these materials as a resource in breeding programs.

Significant advances in research devoted to constructing genetic maps and identifying regions associated with resistance control, at least for *Xanthomonas axonopodis* pv. *passiflorae* yellow passion fruit, have occurred since 2002 (Lopes et al. 2006; Carneiro et al. 2002). In this context, with the addition of a new set of microsatellite markers, the first integrated map of passion fruit was published for *P. edulis* (Oliveira et al. 2008), providing the ability to use this information for identifying quantitative trait loci (QTLs). The construction of a genetic map using multiple markers, such as amplified fragment length polymorphisms (AFLPs), microsatellite-AFLPs (M-AFLPs), simple sequence repeats (SSRs), resistance gene analogs (RGAs) and

single nucleotide polymorphisms (SNPs), has also been reported for *P. alata* (Pereira et al. 2013). In addition, recent efforts associated with developing and characterizing bacterial artificial chromosomes (BACs) have led to the construction of physical maps for passion fruit (Santos 2013).

Although recent, the inclusion of molecular markers in breeding programs for passion fruit is already occurring at different stages (Faleiro et al. 2012). Molecular markers are currently being employed for purposes such as identifying ornamental hybrids and improving fruit production, using both dominant and co-dominant markers (Santos et al. 2012; Conceição et al. 2011; Junqueira et al. 2008). Molecular markers have also contributed to the monitoring of genetic variability in segregant populations subjected to cycle selection (Reis et al. 2012, 2011; Costa et al. 2012) and backcrossing (Fonseca et al. 2009). These referenced works, together with the growing number of results concerning the identification and characterization of microsatellite markers in passion fruit species (Cerqueira-Silva et al. 2014b; Penha et al. 2013; Cazé et al. 2012; Cerqueira-Silva et al. 2012b; Padua et al. 2005; Oliveira et al. 2005) should effectively contribute to advancing breeding programs and to proposing strategies for managing and conserving passion fruit.

Considering the need for research that contributes to the genetic improvement of passion fruit cultivars, thereby increasing resistance to major diseases affecting the fruits, in the present study, we characterized *P. edulis* (yellow and purple shell) accessions based on molecular markers and their reactions to diseases to select genetic material that will be useful for establishing core collections and working populations dedicated to understanding and increasing genetic resistance in passion fruit cultivars.

## Materials and methods

### Biological material

Thirty-six accessions were used in this study, one of which was a cultivated passion fruit variety (BRS Gigante Amarelo). Most of the accessions (34) originated from 18 cities distributed across five Brazilian states, and the remaining two accessions originated from Venezuela and Portugal (Fig. 1) (Supplementary Material—SM 1). The accessions encompassed

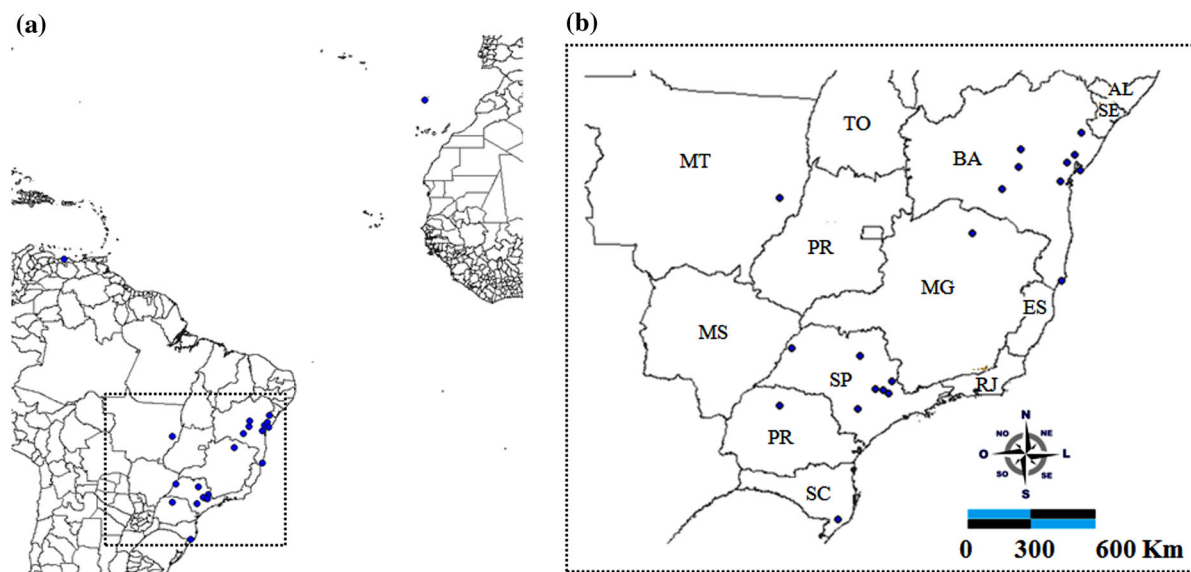
popularly known variations, such as purple and yellow passion fruits, both of which are found in *P. edulis* Sims (Bernacci et al. 2008). All the accessions belong to the Active Germplasm Bank for *Passiflora* of Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) Cassava and Fruits (CNPMP/EMBRAPA), Cruz das Almas, Bahia, Brazil (12°48'38''S and 39°06'26''W).

### Characterization of microsatellite locus polymorphisms

Genomic DNA was isolated from fresh leaves using the cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle 1990) and stored in duplicate samples in an ultra-cold freezer at the Laboratory of Applied Molecular Genetics of the Universidade Estadual do Sudoeste da Bahia (UESB), Itapetinga, Bahia, and at the Laboratory of Molecular Genetic Analysis of the Universidade Estadual de Campinas (UNICAMP), Campinas, São Paulo. Every accession was represented by three plants (grown from seeds of fruits obtained through controlled pollination). DNA sample quality was assessed via electrophoresis (90 V electric current for 90 min) in 1 % (m v<sup>-1</sup>) agarose gels and staining with ethidium bromide. DNA concentrations (ng μL<sup>-1</sup>) were estimated based on comparisons with known quantities of lambda phage DNA (undigested lambda DNA).

A set of 23 SSR loci was used, 11 of which came from the series *mPc-UNICAMP*, *mPe-UNICAMP* and *mPs-UNICAMP* (Cerqueira-Silva et al. 2012b, 2014b) and 12 from the series *USP* (Oliveira 2006). Polymerase chain reaction (PCR) assays to obtain diversity estimates were conducted in a final volume of 15 μL, containing 12 ng of template DNA, 0.4 mM each primer (reverse and forward) and 1.0 U of Taq DNA polymerase (Invitrogen Co, Carlsbad, CA, USA). For other reagents (dNTP concentrations, MgCl<sub>2</sub> and buffers) and amplification conditions (annealing temperatures and characteristics of PCR cycling), the exact procedures described in the original articles addressing the identification and characterization of the loci were used.

The products were initially visualized through horizontal electrophoresis in 3 % (m/v) agarose gels run in 0.5× TBE and stained with ethidium bromide to confirm amplification and subsequently through vertical electrophoresis in denaturing 6 % (m v<sup>-1</sup>) polyacrylamide gels run in 1× TBE and stained with silver



**Fig. 1** Geographical areas where passion fruit (*Passiflora edulis* Sims) accessions were collected (a), highlighting areas of the Brazilian territory where most of the collections were performed (b). The blue dots correspond to sampled areas. (Color figure online)

nitrate to characterize the amplification profiles. The sizes of the products were determined using a 10-bp DNA ladder (Invitrogen), and the repeatability of the electrophoretic profiles was evaluated by repeating the vertical electrophoresis runs for a minimum of 10 % of the samples considered.

#### Characterization of the disease reactions of the passion fruit accessions

Experimental field data were collected as part of the characterization activities (and subsequent stages of the pre-breeding program) conducted on the *Passiflora* Germplasm Bank of EMBRAPA Cassava and Fruits from April to July in 2009, which are the months with the highest natural incidence of disease. Artificial inoculations had not been previously performed, and a randomized block design was adopted, with four repeats and ten plants per parcel. The spacing between rows was 2.6 m, while that between plants was 3.7 m, and a system of vertical trellises was used for the conduction of branches.

The evaluation of symptoms was based on previously developed note scales that are routinely used to characterize the reactions of passion fruit to woodiness virus (Table 1) (Novaes and Rezende 1999), scab and anthracnose (Table 2) (Junqueira et al. 2003). Then,

**Table 1** Scale used to quantify symptoms of woodiness virus in the leaves and fruits of 36 passion fruit (*Passiflora edulis* Sims) accessions from the active germplasm bank of EMBRAPA Cassava and Fruits, Cruz das Almas, Bahia, Brazil

Note scale	Symptoms of woodiness disease in passion fruit		
	Leaves	Fruits	Plants
1	No symptoms	No symptoms	Few leaves with mild mosaic and wrinkling
2	Mild mosaic, no deformation of leaves	Mildly deformed	Moderate quantity of leaves with mild mosaic and wrinkling
3	Mild mosaic, blisters and deformation of leaves	Totally deformed, fruit woodiness, spots	Large quantity of leaves with mosaic and severe wrinkling
4	Severe mosaic, blisters and deformation of leaves	–	–

the observed data were employed to quantify the reactions of the accessions to each of the three diseases considered in this study.

To evaluate disease symptoms caused by woodiness virus, scab and anthracnose on fruits, 15 fruits

**Table 2** Scale used to quantify symptoms of scab on the fruits and branches and anthracnose on the fruits in 36 passion fruit (*Passiflora edulis* Sims) accessions from the active germplasm bank of EMBRAPA Cassava and Fruits, Cruz das Almas, Bahia, Brazil

Note	Symptoms of scab and anthracnose on passion fruit scale
1	No symptoms
2	Surface of the fruit/branch covered with lesions $\leq 10$ %
3	Surface of the fruit/branch covered with lesions between 11 and 30 %
4	Surface of the fruit/branch covered with lesions $> 30$ %

were randomly collected at the beginning of the maturation process. To assess the severity of scab on branches, we examined three young branches on each of five central plants in the plot. Finally, to assess the severity of woodiness virus symptoms in leaves, the fifth leaf of young branches and the overall distribution of woodiness virus symptoms were evaluated in five central plants in the plot.

#### Data analysis and diversity estimates

Initially, we performed a descriptive statistical analysis of the 23 SSR loci considering all 36 accessions (with each accession represented by three plants). The number of alleles per locus ( $N_a$ ), observed heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ) and fixation index ( $F$ ) were calculated using the GenAlEx v. 6.5.3 program (Peakall and Smouse 2012), and the polymorphic information content (PIC) of each locus was calculated using the macro MSTools application (Excel Microsatellite Toolkit v. 3.1) in Microsoft Excel 2007. The data obtained from the assessment of disease symptoms based on the note scales were standardized (each observed value was divided by the highest value in each scale) such that all observations ranged from 0 (resistant) to 1 (susceptible), regardless of the disease evaluated. The data were analyzed using descriptive statistics by estimating the mean and extreme values and the variance of the samples using BIostat 5.3 software (Ayres et al. 2007).

Diversity estimates were performed for the molecular data based on the modified Rogers' distance (Wright 1978), with each accession being examined as a group composed of three plants, and the agronomic

data concerning disease reactions were assessed based on the Mahalanobis distance; these analyses were conducted using the TFPGA (Miller 1997) and GENES (Cruz 2008, 2006) programs, respectively. Clusters were graphically represented as dendrograms using the unweighted pair-group method with arithmetic mean (UPGMA) clustering algorithm, which was implemented in DARwin software (Perrier and Jacquemoud-Collet 2006). The efficiencies of the cluster matrices were evaluated through bootstrap analysis (1,000 resampling values), and the correlation between the matrices was verified using the Mantel test (with 10,000 simulations), which was implemented in the GENES program (Cruz 2006).

Comparisons regarding the intensity of the symptoms presented by the yellow and purple passion fruit accessions for each of the evaluated diseases were performed based on the Mann–Whitney test using BIostat 5.3 software (Ayres et al. 2007). Diversity and genetic structure were also assessed between the yellow and purple passion fruit accessions. Both parameters were estimated using the GenAlEx v. 6.5.3 program (Peakall and Smouse 2012) based on the occurrence of private alleles, the value of Nei's  $G_{st}$  and the percentage of variation between groups through analysis of molecular variance (AMOVA).

#### Selection of accessions and establishment of core and working collections

The accessions were sorted based on the intensity of the observed symptoms for all six studied variables and subjected to analyses of variance (ANOVAs), and univariate grouping tests (Scott-Knott), which were implemented in the GENES program (Cruz 2006, 2008). Using the ANOVA results as a basis, the coefficient of variation (CV) and the broad-sense heritability (BSH) for each variable were also estimated. The same analysis procedures were applied jointly considering the variables related to each disease, where the values used in the analysis represented the average values presented by the accessions for the three variables related to woodiness virus (in leaves, fruits and plants) and the average values for the two variables related to scab (on fruits and branches).

Considering the ranks of the accessions, which were based on the severity of symptoms for the three diseases, groups were formed that encompassed 25, 50, 75 and 100 % of the accessions in ascending order

**Table 3** Characteristics of the microsatellite markers used in the genotyping of 36 passion fruit (*Passiflora edulis* Sims) accessions from the germplasm bank of EMBRAPA Cassava and Fruits, Cruz das Almas, Bahia, Brazil

Markers	Na	H <sub>0</sub>	H <sub>E</sub>	PIC	F
mPc-UNICAMP11	2	0.15	0.14	0.13	−0.08
mPs-Unicamp01	7	0.62	0.73	0.68	0.15
mPs-Unicamp02	7	0.58	0.73	0.68	0.2
mPs-UNICAMP05	3	0.21	0.29	0.27	0.3
mPs-Unicamp06	4	0.82	0.66	0.59	−0.25
mPs-UNICAMP09	2	0.01	0.01	0.01	0
mPs-Unicamp10	4	0.08	0.17	0.16	0.49
mPs-UNICAMP15	2	0.14	0.13	0.12	−0.08
mPs-UNICAMP16	3	0.45	0.53	0.45	0.15
mPe-UNICAMP01	2	0.03	0.05	0.04	0.39
mPe-Unicamp02	4	0.44	0.43	0.37	−0.02
USP 07	16	0.81	0.87	0.86	0.06
USP 08	6	0.36	0.57	0.52	0.36
USP 15	8	0.6	0.69	0.64	0.13
USP 16	9	0.62	0.77	0.75	0.19
USP 17	4	0.48	0.51	0.46	0.06
USP 18	9	0.69	0.68	0.64	−0.02
USP 19	9	0.52	0.8	0.77	0.34
USP20	3	0.41	0.39	0.32	−0.06
USP 23	6	0.35	0.75	0.72	0.54
USP 24	8	0.66	0.73	0.68	0.09
USP 42	3	0.07	0.08	0.08	0.19
USP 58	6	0.59	0.62	0.55	0.04
Average	5.52	0.42	0.49	0.46	0.14
Confidence interval <sup>a</sup>	4.3–6.7	0.32–0.51	0.38–0.58	0.34–0.54	0.06–0.21

Na number of alleles, H<sub>0</sub> observed heterozygosity, H<sub>E</sub> expected heterozygosity (Nei's genetic diversity), PIC polymorphic information content, F fixation index  
<sup>a</sup> Confidence interval estimated through bootstrapping (100,000 simulations)

of symptom intensity. The number of microsatellite alleles maintained in each group that was formed was estimated using the GenAEx v. 6.5.3 program (Peakall and Smouse 2012). Estimations for the core collections based only on molecular data were also performed with the COREFINDER program using 99 interactions (Cipriani et al. 2010). Statistical analyses were conducted considering the probability level of significance to be 5 % ( $p < 0.05$ ).

## Results

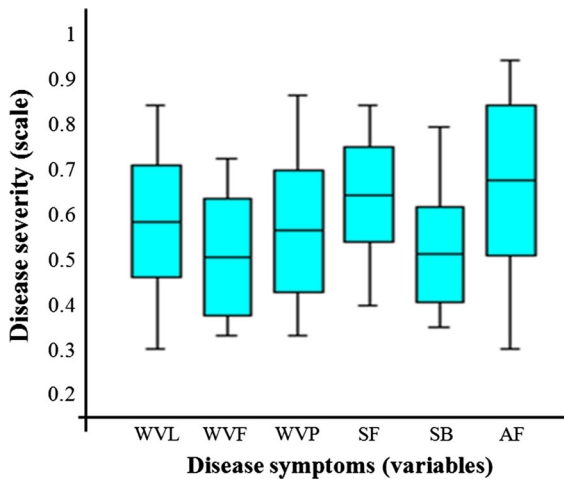
### Genetic diversity based on microsatellite markers

We identified 127 alleles among the 36 passion fruit accessions (with an average of 5.52 alleles per assessed locus), and an excess of homozygotes was detected based on the observed heterozygosities (H<sub>0</sub>; mean value = 0.42) and the expected heterozygosities

(H<sub>E</sub>, Nei's genetic diversity; mean value = 0.49) (Table 3). The PIC presented a mean value of 0.46 (ranging from 0.01 to 0.86), and the fixation index (F) values were positive for most of the SSR loci (70 %), with a mean value of 0.14 being obtained (ranging from −0.25 to 0.54). No genetic structure was observed between the purple and yellow passion fruit accessions (Nei's G<sub>st</sub> = 0.012), and only 1 % of the existing diversity occurred among the groups of yellow and purple passion fruit (AMOVA based on 999 permutations). Approximately 30 % of the alleles were identified as private, corresponding to 34 and five alleles restricted to accessions of yellow and purple passion fruit, respectively.

### Characterization of the disease reactions of the passion fruit accessions

The symptom-associated values observed for the six variables related to the reactions of the accessions to



**Fig. 2** Box-plot representing the average, standard deviation and extreme values observed for the symptoms of woodiness virus in leaves (WVVL), woodiness virus in fruits (WVVF), woodiness virus in plants (WVVP), scab on fruit (SF), scab on branches (SB), and anthracnose on fruits (AF) in 36 passion fruit (*P. edulis* Sims) accessions

the three analyzed diseases ranged from 0.30 to 0.95 (when the data were standardized to range from 0 to 1). The average values observed in the 36 accessions for each variable were as follows: 0.58 for woodiness virus in leaves, 0.50 for woodiness virus in fruits, 0.57 for woodiness virus in plants, 0.65 for scab on fruit, 0.51 for scab on branches, and 0.68 for anthracnose on fruits (Fig. 2).

The ANOVA identified significant differences for all six variables ( $p < 0.05$ ), and the univariate test for the comparison of means (Scott-Knott) allowed the accessions to be classified into two distinct groups in terms of the severity of symptoms (identified with the letters a and b) for four of the six variables considered (symptoms of woodiness virus in leaves, woodiness virus in fruits, woodiness virus in plants and scab on fruit) (Table 4). No significant difference was observed between the average values of the variables related to scab on branches and anthracnose. The correlations between the six variables were extremely low ( $-0.12 < r < 0.36$ ), with the only significant correlations being detected between the symptoms of scab on fruits and woodiness virus on fruits (Supplementary Material—SM 2). Finally, when the variables related to each disease were analyzed together, we identified a significant difference between the groups for the symptoms related to woodiness virus ( $p < 0.05$ , Scott-Knott test), but not for those related to scab (Table 4).

### Estimation of diversity, grouping and selection of accessions

The modified Rogers' distance (Wright 1978) presented a mean value of  $0.41 \pm 0.07$  among the pairs of accessions evaluated using SSR markers, with the values ranging from 0.21 to 0.62. Based on the disease reaction data, a mean value of  $5.06 \pm 3$  was observed for the Mahalanobis distance among the pairs of accessions, with a range of 0.38–22.9. Details regarding the distances observed between pairs of accessions for each of the estimations are presented in dendrograms (Fig. 3). The Mantel correlation test was significant for the two distance matrices ( $p < 0.001$ ); however, the value of the correlation coefficient was low ( $r = 0.16$  with 10,000 simulations).

A significant difference in the mean values of symptoms was observed between the groups formed based on different percentages of selection (25, 50 and 75 % of accessions per group) and based on the total set of accessions evaluated ( $p < 0.05$ ; Mann-Whitney test) (Table 5). The number of alleles present in each group ranged from 98 (in the group formed by the nine accessions showing the lowest intensity of symptoms for anthracnose) to 125 (in the group formed by the 27 accessions showing the lowest intensity of symptoms for scab), representing 77 and 98 %, respectively, of the total alleles identified in the 36 characterized accessions. For all groups, the values of Nei's genetic diversity were similar, ranging from 0.48 to 0.52 (Table 5). Additionally, five accessions were identified (BGP029, 071, 168, 205, 277) as being present in both of the groups displaying lower intensities of symptoms (groups 1 and 2) for the three evaluated diseases. The average values for the observed symptoms differed significantly between the accessions of yellow passion fruit (0.57 for woodiness virus, 0.59 for scab, and 0.66 for anthracnose) and purple passion fruit (0.51 for woodiness virus, 0.58 for scab, and 0.72 for anthracnose) for woodiness virus ( $p < 0.05$ , Mann-Whitney test), but not for scab and anthracnose ( $p < 0.1$ , Mann-Whitney test) (Table 6).

The estimates performed for the core collections with the COREFINDER program (Cipriani et al. 2010) indicated that four and 16 accessions represented 70 and 100 % of the genetic diversity of alleles, respectively (Fig. 4).

**Table 4** Average values observed in 36 passion fruit (*P. edulis* Sims) accessions for symptoms of woodiness virus in leaves (WVL), woodiness virus in fruits (WVF), woodiness virus in plants (WVP), scab on fruit (SF), scab on branches (SB), and anthracnose on fruits (AF) and the average symptoms observed for woodiness virus and scab

Accessions <sup>a</sup>	Woodiness virus				Scab			Anthracnose
	WVL	WVF	WVP	Average	SF	SB	Average	AF
BGP007	0.65a	0.60a	0.60b	0.62a	0.85a	0.35a	0.60a	0.70a
BGP009	0.65a	0.73a	0.53b	0.64a	0.60b	0.40a	0.50a	0.55a
BGP017	0.65a	0.67a	0.47b	0.59a	0.50b	0.55a	0.53a	0.85a
BGP022	0.75a	0.33b	0.53b	0.54b	0.45b	0.40a	0.43a	0.90a
BGP023	0.65a	0.40b	0.40b	0.48b	0.70a	0.55a	0.63a	0.75a
BGP028	0.70a	0.60a	0.60b	0.63a	0.60b	0.55a	0.58a	0.70a
BGP029	0.55b	0.40b	0.60b	0.52b	0.45b	0.60a	0.53a	0.60a
BGP033	0.55b	0.60a	0.53b	0.56a	0.75a	0.55a	0.65a	0.95a
BGP034	0.70a	0.60a	0.73a	0.68a	0.80a	0.60a	0.70a	0.70a
BGP041	0.80a	0.60a	0.53b	0.64a	0.65a	0.50a	0.58a	0.45a
BGP043	0.70a	0.40b	0.60b	0.57a	0.80a	0.55a	0.68a	0.85a
BGP044	0.40b	0.40b	0.67a	0.49b	0.60b	0.65a	0.63a	0.70a
BGP048	0.55b	0.67a	0.53b	0.58a	0.70a	0.60a	0.65a	0.50a
BGP051	0.60a	0.73a	0.80a	0.71a	0.70a	0.50a	0.60a	0.85a
BGP064	0.50b	0.60a	0.80a	0.63a	0.80a	0.80a	0.80a	0.30a
BGP071	0.50b	0.47b	0.47b	0.48b	0.65a	0.50a	0.58a	0.50a
BGP078	0.50b	0.73a	0.53b	0.59a	0.70a	0.40a	0.55a	0.65a
BGP079	0.65a	0.47b	0.47b	0.53b	0.80a	0.40a	0.60a	0.85a
BGP092	0.50b	0.33b	0.67a	0.50b	0.55b	0.50a	0.53a	0.85a
BGP094	0.85a	0.40b	0.80a	0.68a	0.70a	0.60a	0.65a	0.55a
BGP116	0.70a	0.33b	0.47b	0.50b	0.60b	0.55a	0.58a	0.80a
BGP121	0.55b	0.53a	0.47b	0.52b	0.55b	0.65a	0.60a	0.65a
BGP123	0.50b	0.47b	0.53b	0.50b	0.75a	0.50a	0.63a	0.70a
BGP158	0.30b	0.53a	0.33b	0.39b	0.65a	0.65a	0.65a	0.65a
BGP168	0.45b	0.33b	0.33b	0.37b	0.65a	0.40a	0.53a	0.35a
BGP181	0.75a	0.73a	0.53b	0.67a	0.75a	0.35a	0.55a	0.85a
BGP185	0.50b	0.60a	0.53b	0.54b	0.70a	0.50a	0.60a	0.70a
BGP186	0.45b	0.40b	0.67a	0.51b	0.65a	0.70a	0.68a	0.80a
BGP188	0.45b	0.40b	0.40b	0.42b	0.40b	0.50a	0.45a	0.95a
BGP205	0.45b	0.47b	0.47b	0.46b	0.65a	0.35a	0.50a	0.65a
BGP208	0.45b	0.33b	0.73a	0.51b	0.60b	0.35a	0.48a	0.90a
BGP222	0.65a	0.67a	0.53b	0.62a	0.65a	0.45a	0.55a	0.70a
BGP227	0.80a	0.40b	0.73a	0.64a	0.55b	0.60a	0.58a	0.45a
BGP277	0.60a	0.40b	0.33b	0.44b	0.55b	0.45a	0.50a	0.45a
BGP311	0.50b	0.47b	0.87a	0.61a	0.55b	0.45a	0.50a	0.65a
BRS Gigante Amarelo	0.65a	0.47b	0.60b	0.57a	0.70a	0.50a	0.60a	0.50a
CV	35 %	40 %	36 %	23 %	28 %	37 %	22 %	33 %
BSH	46 %	50 %	57 %	54 %	45 %	36 %	41 %	65 %

Averages followed by the same letter within a column do not significantly differ by the Scott-Knott test at a 5 % probability

CV coefficient of variation, BSH broad-sense heritability

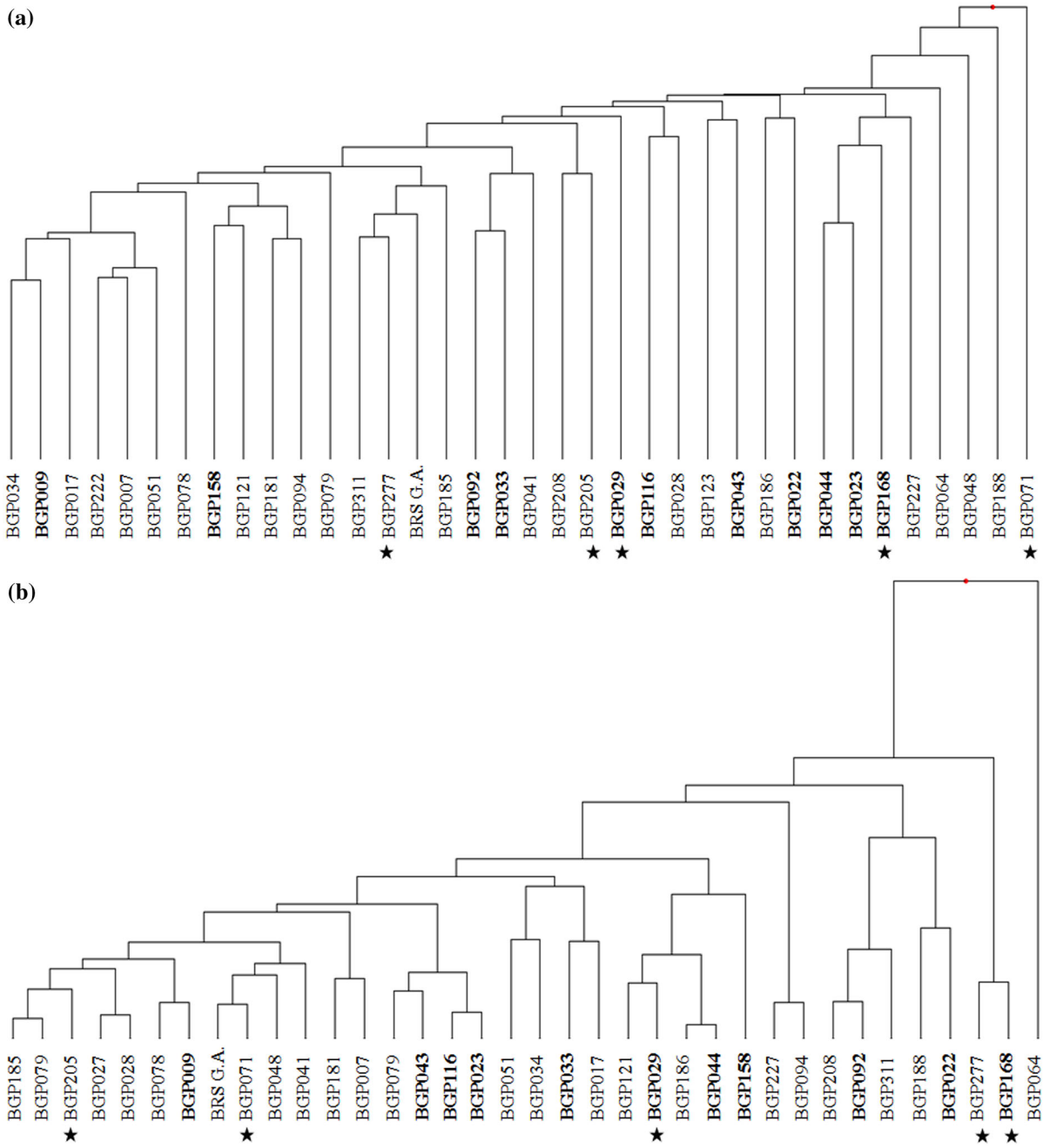
<sup>a</sup> Details of the accessions are available in the Supplementary Material - SM 1

## Discussion

Considering the need to release cultivars exhibiting combined resistance to the major diseases that affect

passion fruit, the results obtained in this study will contribute to passion fruit pre-breeding activities aimed at intensifying the use of the accessions available in the active germplasm bank of EMBRAPA. From this





**Fig. 3** Dendrograms obtained through UPGMA cluster construction based on the modified Rogers’ distance (Wright 1978) for microsatellite data (a) and for the Mahalanobis distance for disease reaction data (b), considering 36 passion fruit accessions

from the germplasm bank of EMBRAPA Cassava and Fruits, Cruz das Almas, Bahia, Brazil. *Note* Accessions that presented a lower intensity of symptoms for the three diseases evaluated in this study are identified with a *star*

perspective, the estimates of molecular genetic variation and of the gradient of disease resistance observed for the accessions enable the identification of preferred crossings to establish populations that are useful in both

recurrent selection cycles and for genetic map construction and QTL identification.

The average number of alleles (5.52 per locus) observed in the 36 accessions (Table 3) is consistent with

**Table 5** Descriptive presentation of the average intensity of symptoms, number of microsatellite alleles (Na) and Nei's genetic diversity ( $H_E$ ) for the different groups formed after classification of the severity of symptoms in 36 passion fruit accessions for the three evaluated diseases

Diseases	Percentage of accessions selected <sup>a</sup>	Symptoms	Na	$H_E$	Set of accessions <sup>b</sup>
Woodiness virus	Group 1 (25 %)	0.45	105	0.52	BGP023, 044, 071, 092, 158, 168, 188, 205, 277
	Group 2 (50 %)	0.48	119	0.51	BGP022, 029, 079, 116, 121, 123, 185, 186, 208
	Group 3 (75 %)	0.52	121	0.50	BGP017, 028, 033, 043, 048, 078, 222, 311, BRS-GA
	Group 4 (100 %)	0.55	127	0.49	BGP007, 009, 034, 041, 051, 064, 094, 181, 227
Scab	Group 1 (25 %)	0.49	99	0.48	BGP009, 022, 029, 092, 188, 205, 208, 277, 311
	Group 2 (50 %)	0.53	118	0.50	BGP017, 028, 041, 071, 078, 116, 168, 181, 222
	Group 3 (75 %)	0.55	125	0.50	BGP007, 023, 044, 051, 079, 121, 185, 227, BRS-GA
	Group 4 (100 %)	0.58	127	0.49	BGP033, 034, 043, 048, 064, 094, 123, 158, 186
Anthracnose	Group 1 (25 %)	0.45	98	0.48	BGP009, 041, 048, 064, 071, 168, 227, 277, BRS-GA
	Group 2 (50 %)	0.55	113	0.49	BGP007, 028, 029, 078, 094, 121, 158, 205, 311
	Group 3 (75 %)	0.61	120	0.49	BGP017, 023, 034, 044, 116, 123, 185, 186, 222
	Group 4 (100 %)	0.68	127	0.49	BGP022, 033, 043, 051, 079, 092, 181, 188, 208

<sup>a</sup> Accessions of group 1 are also part of the accession list of group 2, accessions of group 1 and 2 are part of the accession list of group 3, as well as accessions from group 1, 2 and 3 are part of accessions list of the group 4

BRS-GA BRS Gigante Amarelo

<sup>b</sup> Details of the accessions are available in the Supplementary Material - SM 1

**Table 6** Presentation of the symptoms of three diseases in 25 yellow passion fruit and 11 purple passion fruit accessions, including the average intensity, standard deviation and results of the Mann–Whitney test

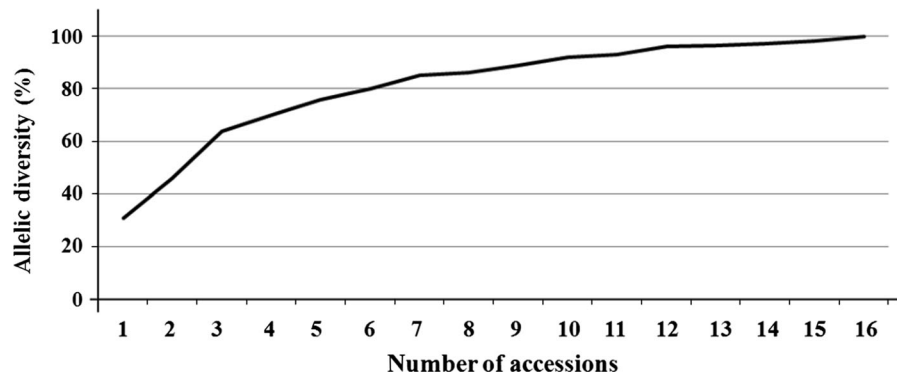
Disease	Average intensity of the symptoms		Mann–Whitney
	Yellow	Purple	
Woodiness virus	0.57 ( $\pm 0.08$ )	0.51 ( $\pm 0.07$ )	Z value = 2.18*
Scab	0.59 ( $\pm 0.07$ )	0.58 ( $\pm 0.08$ )	Z value = 0.11
Anthracnose	0.66 ( $\pm 0.17$ )	0.72 ( $\pm 0.18$ )	Z value = 1.04

\* Differ significantly by the Mann–Whitney test at a 5 % probability ( $p$  value observed = 0.014)

the values obtained in previous genetic characterizations performed in passion fruit using microsatellite markers (Cerqueira-Silva et al. 2014c). The few such studies conducted to date have indicated a low number of alleles at the loci characterized for the commercial species *P. edulis* (4.7) (Oliveira et al. 2005; Cerqueira-Silva et al. 2014b) and *P. alata* (3.1) (Padua et al. 2005).

For wild passion fruit species, such as *P. contracta*, *P. cincinnata* and *P. setacea*, the average number of alleles per locus has also been found to be low, ranging from 2.8 to 4.9 (Cerqueira-Silva et al. 2014b; Cazé et al. 2012). In addition, the number of alleles detected in passion fruit undergoing recurrent selection has been reported to be fewer than three alleles per locus (Reis et al. 2011). Considering the apparent pattern of low diversity at SSR loci observed in passion fruit (Cerqueira-Silva et al. 2014c), the number of alleles identified in the set of evaluated accessions is considered favorable for establishing strategies for selecting and forming base populations for breeding.

The obtained Nei's genetic diversity values (average of 0.49) were consistent with the expectation for outcrossing species and with the values observed in a previous study for *P. edulis* (Oliveira et al. 2005). The mean value of  $F$  (0.14) can be interpreted as indicating a loss of heterozygotes and corroborates the higher values obtained for  $H_E$  than for  $H_0$ . These results may be partly explained by the origins of the accessions



**Fig. 4** Allelic diversity as a function of the number of accessions included in the core collection of passion fruit (*Passiflora edulis* Sims). Note The order of the accessions included in the core collection was as follows: BGP007;

BGP208; BGP041; BGP023; BGP028; BGP123; BGP071; BGP116; BGP188; BGP185; BGP181; BGP051; BGP168; BGP064; BGP017; BGP079

found in germplasm banks; i.e., the transport of seeds among production regions and the use of seeds produced by a given plantation for its next generation of seedlings are common practices, particularly for commercial passion fruit species (Santos et al. 2011).

The amplitude of the observed values for the six measured variables (0.65, considering a scale from 0 to 1) (Fig. 2) and the significant results of ANOVA ( $p < 0.05$ ) for all six variables indicate the existence of variability that is potentially useful for breeding programs, particularly in the early stages of mass selection and the establishment of progenies for recurrent selection. The results obtained from the univariate test for the comparison of means (Scott-Knott) corroborate the existence of variability in the reaction of the passion fruit accessions to at least four of the six considered variables (Table 4).

Descriptions of the reactions of *P. edulis* (yellow and purple shell) to different pathogens are available in the literature and indicate the existence of both variability available for selection in collections and accessions maintained in germplasm banks (Oliveira et al. 2013; Silva et al. 2013; Cerqueira-Silva et al. 2008; Junqueira et al. 2006) and low disease resistance or tolerance in passion fruit cultivars (Batistti et al. 2013; Fischer et al. 2010; Junqueira et al. 2003).

The low correlations observed among the six variables considered in evaluating the reactions of the passion fruit accessions to the three studied diseases ( $-0.12 < r < 0.36$ ) indicate possible difficulties in obtaining balanced genetic gains using direct selection procedures, given the trend toward unwanted losses of

genetic gains due to the existence of uncorrelated features. Similar correlation results were observed by Freitas et al. (2012) when evaluating the same six variables among 43 accessions of yellow passion fruit. However, the obtained correlation values do not prevent the use of selection indices, particularly because no significant negative correlations were found. The application of different selection indices for agronomic production traits and disease resistance was recently evaluated in passion fruit accessions (Freitas et al. 2012), and an imbalance was observed in the genetic gains associated with production characteristics and with levels of disease resistance when using the strategy of direct selection. In this work, Freitas et al. (2012) also detected more balanced genetic gains using the Mulamba and Mock index.

The variability estimates obtained from both the modified Rogers' distance (Wright 1978) and the Mahalanobis distance allowed genetic differentiation of all the evaluated accessions (Fig. 3). Estimates of genetic distance between passion fruit accessions or within natural passion fruit populations based on microsatellite loci are in their incipient stages, with the results being restricted to recent diversity estimates, such as estimates from the SSR locus cross-amplification (Oliveira et al. 2013). The use of these markers has also contributed to the monitoring of the variability present in segregating populations undergoing recurrent selection (Reis et al. 2012, 2011) and to developing genetic maps for *P. edulis* (Oliveira et al. 2005) and for *P. alata* (Pereira et al. 2013).

Based on the obtained estimates of the modified Rogers' distance, it is possible to identify pairs of

convergent and divergent accessions, highlighting the pair BGP009 and BGP034 (or BGP051), with a distance value of 0.21, and the pair BGP071 and BGP186, with a distance value of 0.62. Characterizing the genetic distance within collections and germplasm banks based on molecular markers contributes to both the monitoring of variability and the definition of preferred crosses to be performed at different stages of a breeding program. Genetically divergent accessions, such as BGP071 and BGP186, may be useful in establishing segregating populations and in producing hybrids with a potential heterosis effect. In turn, identifying genetically similar accessions can reduce the time and number of crossings required in back-crossing cycles whose goal is to introgress specific traits into cultivars of interest.

In turn, extreme values were observed for the Mahalanobis distance between the accession pair BGP044 and BGP186 (0.38) and the accession pair BGP064 and BGP188 (22.9). Considering the low correlations found among the reactions of the accessions to the different diseases and the consequent difficulty of applying a direct selection strategy grounded in individual characteristics, we understand that identifying convergent crosses potentiates the average gain of resistance to diseases. In turn, the use of divergent accessions, based on the Mahalanobis distance, can be useful in generating mapping populations, which increases the possibility of identifying QTLs for resistance, at least for the studied diseases.

Variability data based on molecular markers and the reactions of accessions to diseases potentiate the establishment of populations showing high genetic variability (considering divergent values for the modified Rogers' distance) and displaying reduced variance in their reactions to the evaluated diseases (considering convergent values for the Mahalanobis distance). Freitas et al. (2012) indicated that maximizing the variability among the accessions used in the composition of base populations undergoing recurrent selection is a strategy employed to avoid unwanted narrowing of the available genetic base in the initial selection cycles.

The low correlations observed between the matrices of distance estimates obtained from the molecular data (such as microsatellite markers) and the agronomic data (such as the reactions of the accessions to disease) ( $r = 0.16$ ; Mantel test with 10,000 simulations) are expected due to the nature of these data.

Most of the observed variability in the molecular markers is, by definition, nonadaptive, unlike phenotypic characteristics, which are affected by selection and influenced by interactions with the environment. Reis et al. (2012) reported similar results when comparing data from the genotyping and phenotyping of passion fruit undergoing recurrent selection cycles.

Classification of the accessions into groups according to the percentages of selection (25, 50, 75 and 100 %) considering the rank of the accessions based on the ascending order of symptom intensity for the three diseases (Table 5), contributed to defining the accessions to be prioritized in breeding programs dedicated to resistance. As the accessions classified as belonging to groups 1 and 2 (Table 5) exhibited the lowest intensities of symptoms (average symptom values ranging from 0.48, for woodiness virus, to 0.55, for anthracnose) and retained, on average, 85 % of the alleles identified in the microsatellite loci, these accessions should be prioritized in the composition of breeding populations. These results hold greater relevance when compared with the set of accessions suggested for use in a core collection considering only genotyping data from microsatellite loci (Fig. 4). The average values of symptoms observed in this accession collection varied between 0.55, for woodiness virus, and 0.70, for anthracnose.

Considering that a core collection should retain from 5 to 20 % of the accessions available in a collection and should represent at least 70 % of the existing diversity (Brown et al. 1987; Nass 2011), both the set of accessions suggested by the COREFINDER program (Fig. 4) and the accessions from groups 1 and 2 (Table 5) are suitable for forming core and working collections. Identifying accessions with favorable characteristics for passion fruit genetic breeding is of great importance because studies conducted by Freitas et al. (2011) on *Passiflora* genetic resources demonstrated the need for genetic recombination among accessions to further explore and select superior progenies, as accessions of passion fruit combining the quantitative and qualitative characteristics favorable for production were not identified by these authors.

Notably, BGP029, 071, 168, 205 and 277 were ranked among the accessions displaying weaker symptoms for the three evaluated diseases when considering a selection percentage of 50 %. Assuming a more restrictive selection percentage (25 %),

accessions BGP029 and BGP205 were both classified into group 1, displaying a low intensity of symptoms for woodiness virus and scab. In turn, accession BGP168 was classified into group 1 based on its symptoms related to woodiness virus and anthracnose. Finally, accession BGP277 was the only accession classified into group 1 for all three evaluated diseases.

Although few studies have focused on the agronomic characterization of accessions maintained in collections and germplasm banks, important associations can be identified based on the available publications. Data related to the resistance of passion fruit to *Fusarium oxysporum* f. sp. *passiflorae* (Silva et al. 2013) indicate that the BGP208 and BGP029 accessions, which were classified into the groups showing weaker symptoms for woodiness virus and scab, are also resistant to *F. oxysporum*. In turn, agronomic characterizations performed by Reis et al. (2011) indicate that BGP208 presents high fruit production (80–98 fruit per plant) compared with other accessions in the germplasm bank.

The ‘BRS Gigante Amarelo’ cultivar was classified into group 3 due to its reactions to both woodiness virus and scab because this cultivar was more susceptible to these diseases than most of the accessions included in this study (Table 5). Considering anthracnose, however, the ‘BRS Gigante Amarelo’ cultivar was classified into group 1 because of being less susceptible to this disease. These results are consistent with the data available in the literature (Batistti et al. 2013; Fischer et al. 2010; Junqueira et al. 2003) indicating the susceptibility of passion fruit cultivars to the most important diseases related to fruit culture. In this context, different authors have indicated the need for research focused on increasing the resistance of existing cultivars or on developing new cultivars using both intraspecific and interspecific crosses (Faleiro et al. 2011; Meletti et al. 2005; Faleiro et al. 2005). The genetic variability and the gradient of resistance identified in this study reinforce the possibility, which was also presented by Oliveira et al. (2013), of employing intraspecific crossing as a strategy to reduce the susceptibility of passion fruit cultivars to the primary diseases that affect passion fruit culture.

Although significant genetic structure was not observed among the purple and yellow passion fruit accessions (Nei’s  $G_{st} = 0.012$ ) and although no clear evidence of subgroups (Fig. 3) was observed, the private alleles identified among these groups of

accessions could be useful in monitoring hybrids between these variants of *P. edulis*. In addition, the results of the comparison of averages (Table 6) suggest that the purple passion fruit accessions can be prioritized in the search for resistance to woodiness virus. Additionally, a tendency toward reduced anthracnose symptoms was observed in the yellow passion fruit accessions.

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