

Genetic variability of *Passiflora* spp. against collar rot disease

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Abstract

Collar rot caused by the fungus *Fusarium solani*, is one of the main diseases in passion fruit orchards. Some disease resistance sources were previously identified in wild species of *Passiflora*; however, information is limited and contradictory concerning disease resistance within *Passiflora* species. The objective of this study was to evaluate intra and interspecific variability of ten *Passiflora* species regarding to their resistance characters against *Fusarium solani*. Cuttings (clones) from four different genotypes from the ten species studied were prepared, with a total of 40 genotypes. Resistance or susceptibility was evaluated by the plant survival period and by a disease assessment scale. Data was analyzed using variance analysis, test of means, graphical dispersion and the hierarchical clustering method UPGMA. Intra and interspecific variability of resistance against *F. solani* was confirmed, being *P. quadrangularis*, *P. nitida* and *P. foetida* evaluated as highly resistant. The most suitable species for plant breeding programs is *P. nitida*, since it showed a high resistance level without genetic variability within the characters studied.

Keywords: Genetic resistance. *Fusarium solani*. Passion fruit. Cutting. Plant Breeding.

Abbreviations: t_ tonelada; UNEMAT_ Universidade do Estado de Mato Grosso; UFV Universidade Federal de Viçosa; UENF_ Universidade Estadual do Norte Fluminense Darcy Ribeiro; km_ kilometer; BAG_ germplasm active bank; BDA_ potato, dextrose, agar; B.O.D_ biochemical oxygen demand; PVC_ polyvinyl chloride; mm_ millimeter; DAI_ days after inoculation; NS_ Note scale; PS_ Survival period; UPGMA_ unweighted pair group method with arithmetic mean; SQM_ mean square residue.

Introduction

Yellow passion fruit (*Passiflora edulis* Sims) belongs to the Genera *Passiflora*, the biggest and most important within the family Passifloraceae, it is original from the tropical and subtropical America (Vanderplank, 2000) and widely cultivated in countries like Brazil, Ecuador, Venezuela, Colombia and other countries outside America as South Africa, Kenya, Zimbabwe, India and New Zealand (Vanderplank, 1996; Manicom et al., 2003). Brazil has a vast diversity of passion fruit species with about 141 species already known (Bernacci et al., 2014). Brazil is considered one of the biggest world producing countries and the biggest producer of yellow passion fruit (FAO, 2014). However, in 2012 a reduction of approximately 16% production was verified when compared to 2011, with a production of 776.097 t in 2012 contrasting with a 923.035 t in 2011. The reduction in passion fruit production is related primarily with the incidence of diseases (Cavichioli et al., 2011). Amongst the main plant disease problems contributing for the low productivity of passion fruit orchards in Brazil, basal or collar rot has a leading role (Viana and Costa 2003; Fischer et al., 2005a), caused by the fungus *Fusarium solani*, the imperfect form of *Haematonectria haematococca* (Berkeley & Broome) Samuels & Nirenberg (Leslie & Summerell 2006). This is a polyphagous fungus affecting a wide number of cultivated plants as peanut (Casasnovas et al., 2013), tomato (Castaño et al., 2012), beans (Sasan et al., 2013), etc,

however, recent studies have demonstrated that *F. solani* f. sp. *passiflorae* is a specialized form that infects yellow passion fruit (Bueno et al., 2014). Symptoms of the disease consist of a light apical wilt followed by color alterations of leaves and a subsequently severe wilt, defoliation and death, as a result of a complete necrosis of the collar region. Necrosis extends 2-10 cm above the soil and in some cases it advances to the roots (Bueno et al., 2014). The pathogen develops resistance structures, the chlamydospores that remain in the soil for several years, even in the absence of the host plant (Fischer et al., 2005a). Due to these characteristics collar rot is a difficult disease to control. In passion fruit orchards the disease incidence results in a significant reduction of productivity, need of constant migration or scape to new areas free from the pathogen and reduction of the orchard's lifespan. The development of an effective strategy to control collar rot disease is still at preliminary stage (or experimental), as for example biological control using *Trichoderma* spp. and chemical products (Meza et al., 2008; Fischer et al., 2010b; Silva et al., 2014). Disease control using rootstocks has been also studied. Junqueira et al. (2006) verified that commercial passion fruit grafted with *P. nitida* shows good performance in areas previously affected by the disease. In the study performed by Fischer et al. (2010b), also evaluating rootstocks, they concluded that the species *P. maliformis*, *P. suberosa* and *P. alata* are resistant to collar

Table 1. Summary of analysis of variance of genetic resistance of 10 species of *Passiflora* about the *F. solani*.

Source of variation	G.L ¹	SQM ²	
		EN	PS
Blocks	3	0.74	62.58
Species	9	6.57 **	524.19**
Waste	27	0.82	100.65
Average		4.72	31.55
C.V (%)		19.21	31.80

(**) Significant at 1% by test F; ¹G.L_degree of freedom; ²SQM_mean square residue; EN_assessment scale and PS_survival period.

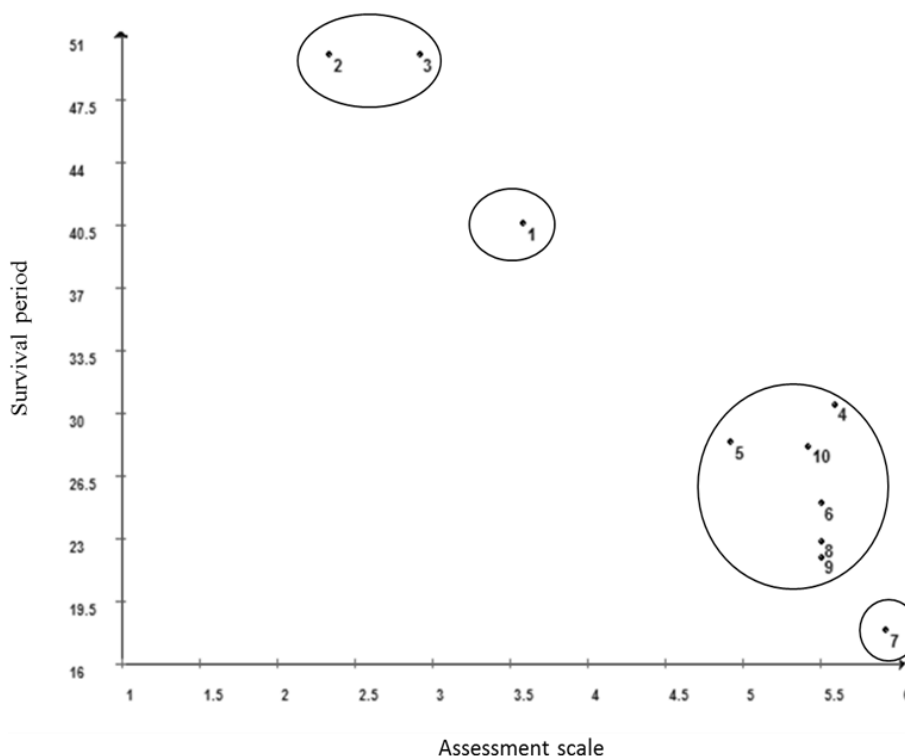


Fig 1. Graphical dispersion of measurements obtained in the assessments of rating scale and survival period (average of 4 plants) in relation to the behavior of 10 species of *Passiflora* to *Fusarium solani*. Species are represented by the numbers: 1- *P. quadrangularis*; 2- *P. nitida*; 3- *P. foetida*; 4- *P. eichleriana*; 5- *P. alata*; 6- *P. cincinnata*; 7- *P. mucronata*; 8- *P. suberosa*; 9- *P. morifolia* e 10- *P. edulis*.

rot. Considering the significance of the yellow passion fruit cultivation in Brazil, as well as the importance of the disease caused by *F. solani*, the lack of more efficient control strategies and the need for exploiting the genetic diversity of the *Passiflora* species that can reveal sources of resistance, this work was developed with the aim to: 1) find one resistant species against the fungus *F. solani* within the Genera *Passiflora*, 2) evaluate the existence of genetic variability concerning resistance against the pathogen within the species.

Results

Detection of resistant species (interspecific variability)

Significant differences were observed for all characteristics evaluated (Table 1), thus indicating the possibility of selection of resistant species against collar rot, either for developing plant breeding programs or for their use as rootstock plants. Plant death was observed five days after inoculation (DAI) in *P. mucronata*, *P. morifolia*, *P. suberosa*, *P. cincinnata*, *P. alata* and *P. eichleriana*. These species had the lowest mean values for the survival period and the highest

mean values in the disease assessment scale (Table 2). Thus, these were the most susceptible species against the pathogen. On the other hand, the species *P. nitida* and *P. foetida* had the higher survival period, all plants survived 50 evaluation days and had the lowest mean values or the disease assessment scale. The species were gathered according to the means observed for both resistance characters by the method of distance projection 2D (Fig. 1), these results confirm the previously ones showed above. It was possible to observe the formation of four clusters with different levels of resistance. *P. nitida* (2) and *P. foetida* (3), included within the same cluster, were the most resistant against the pathogen, followed by *P. quadrangularis* (1). In contrast, *P. mucronata* (7) was the most susceptible to the fungus. The majority of the species formed a cluster of plants considered susceptible, since the mean values for the period of survival character were low while the ones for the disease assessment scale were high. This cluster comprehends the species *P. alata*, *P. eichleriana*, *P. edulis*, *P. cincinnata*, *P. suberosa*, *P. morifolia*. In a study performed by Fischer et al., (2005b), they also verified susceptibility of the species *P. cincinnata*, *P. suberosa* and *P. edulis*.

Table 2. Assessment scale assigned to the symptoms of collar rot and period of survival of the species of *Passiflora* in 50 days of ratings.

Species	Resistance characteristics ¹	
	EN	PS
<i>P. quadrangularis</i>	3.58 bcd ²	40.58 ab
<i>P. nitida</i>	2.33 d	50.0 a
<i>P. foetida</i>	2.92 cd	50.0 a
<i>P. eichleriana</i>	5.58 ab	30.5 ab
<i>P. alata</i>	4.92 abc	28.42 ab
<i>P. cincinnata</i>	5.5 ab	25.0 b
<i>P. mucronata</i>	5.92 a	17.92 b
<i>P. suberosa</i>	5.5 ab	22.92 b
<i>P. morifolia</i>	5.5 ab	22.0 b
<i>P. edulis</i>	5.42 ab	28.17 ab

¹Averages of four plants (repetitions). ²Means followed by the same letter in the column do not differ (Tukey, p<0,05); EN_assessment scale and PS_survival period.

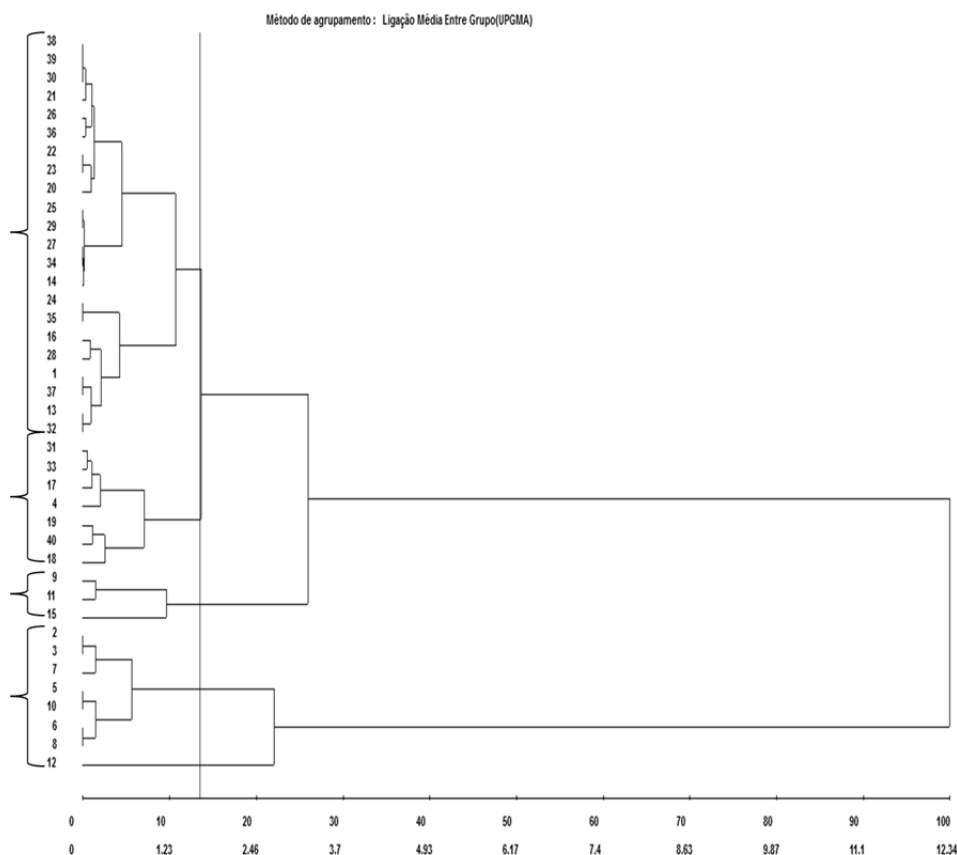


Fig 2. Dendrogram the resulting analysis of 40 genotypes of *Passiflora* based on two features of resistance to the fungus *F. solani* obtained by the UPGMA clustering method, using Mahalanobis distance as a measure of the genetic distance. Where the 1-4 are the species *P. quadrangularis*; 5 a 8 *P. nitida*; 9 a 12 *P. foetida*; 13 a 16 *P. eichleriana*; 17 a 20 *P. alata*; 21 a 24 *P. cincinnata*; 25 a 28 *P. mucronata*; 29 a 32 *P. suberosa*; 33 a 36 *P. morifolia* e 37 a 40 *P. edulis*.

Genetic diversity of 40 genotypes of *Passiflora* based in characteristics of resistance against *F. solani*.

From a total of 360 plants, 153 wilted and died. The pathogen was re-isolated from some of those plants to confirm the cause of death. The cophenetic correlation coefficient obtained between the generalized distance of Mahalanobis (D^2) matrix and the cophenetic distance (C) matrix was of 0.80, thus revealing a good correlation between the graphic representation of the distances and its original matrix (Rohlf, 2000), allowing to achieve inferences by means of a visual evaluation of the Fig. 2. Based on the arrangement in different clusters by the UPGM method (Fig.2) it is possible to confirm that there is genetic variability for resistance

against collar rot within the species studied. With a split-up at number 15 it is possible to observe the formation of four clusters with different degrees of resistance against the pathogen. The first group was formed with 52.5% of genotypes with three genotypes of species *P. edulis* (38, 39 and 37), one *P. alata* (20), four *P. mucronata* (26, 25, 27 and 28), three *P. suberosa* (30, 29 and 32), three *P. morifolia* (36, 34 and 35), three *P. eichleriana* (14, 16 and 13), four *P. cincinnata* (21, 22, 23 and 24), one *P. quadrangularis* (1), according to the order of numbers represented in Fig. 2. These genotypes showed high susceptibility degree, once the mean values for the maximum survival period was of 35 days and the lowest assessment scale value was 5.

Table 3. Representation of four distinct genotypes of each species, a total of 40 genotypes (treatment) evaluated.

Genotypes	Species	Genotypes	Species	Genotypes	Species	Genotypes	Species
1	<i>P. quadrangularis</i>	11	<i>P. foetida</i>	21	<i>P. cincinnata</i>	31	<i>P. suberosa</i>
2	<i>P. quadrangularis</i>	12	<i>P. foetida</i>	22	<i>P. cincinnata</i>	32	<i>P. suberosa</i>
3	<i>P. quadrangularis</i>	13	<i>P. eichleriana</i>	23	<i>P. cincinnata</i>	33	<i>P. morifolia</i>
4	<i>P. quadrangularis</i>	14	<i>P. eichleriana</i>	24	<i>P. cincinnata</i>	34	<i>P. morifolia</i>
5	<i>P. nitida</i>	15	<i>P. eichleriana</i>	25	<i>P. mucronata</i>	35	<i>P. morifolia</i>
6	<i>P. nitida</i>	16	<i>P. eichleriana</i>	26	<i>P. mucronata</i>	36	<i>P. morifolia</i>
7	<i>P. nitida</i>	17	<i>P. alata</i>	27	<i>P. mucronata</i>	37	<i>P. edulis</i>
8	<i>P. nitida</i>	18	<i>P. alata</i>	28	<i>P. mucronata</i>	38	<i>P. edulis</i>
9	<i>P. foetida</i>	19	<i>P. alata</i>	29	<i>P. suberosa</i>	39	<i>P. edulis</i>
10	<i>P. foetida</i>	20	<i>P. alata</i>	30	<i>P. suberosa</i>	40	<i>P. edulis</i>

The second cluster was constituted only 15% of the genotypes with one genotype of specie *P. suberosa* (31), one *P. morifolia* (33), three *P. alata* (17, 19 and 18), one *P. quadrangularis* (4) and one *P. edulis* (40). These genotypes showed a higher degree of resistance when compared with the previous cluster, once the mean value for the maximum period of survival was of 40 days. The constitution of these two clusters evidences the genetic diversity of the genotypes regarding resistance against *F. solani*, once there were genotypes of the same species gathered in both clusters, as for example *P. alata*. The third cluster was formed with the lowest number of genotypes, only 7.5%, constituted by the species *P. foetida* (genotypes 9 and 11) and *P. eichleriana* (genotype 15). Genotypes of this group showed moderated resistance against the pathogen due to the survival of all genotypes during 50 evaluation days, but having relatively high mean values for the disease assessment scale, of approximately 5. The last cluster was formed by 20% of the genotypes, constituted by the species *P. quadrangularis* (genotypes 2 and 3), *P. nitida* (genotypes 7, 5, 6 and 8) and *P. foetida* (genotypes 10 and 12). Plants within this group were considered highly resistant once the mean value for the survival period was of 50 days and the disease assessment scale value ranged from 1 to 2.6. The species showing the higher variation of resistance was *P. quadrangularis*. Genotypes of this species are present within three clusters, from the most susceptible to the most resistant. In contrast, genotypes form the species *P. nitida* had no variation in resistance degree, besides being the species with the best performance.

Discussion

In this work the species *P. mucronata* showed the highest susceptibility to the pathogen. Previous work by Fischer et al. (2010b) confirms this result, when *P. mucronata* used as a rootstock was evaluated as susceptible against the pathogen. In a study performed by Fischer et al. (2005b), evaluating different species within the Genera *Passiflora* against *F. solani*, the authors confirmed species *P. nitida* and *P. alata* as resistant and *P. cincinnata* as susceptible, matching results observed in the present study except for *P. alata*, evaluated as susceptible in the present study. Contradictory results to the present study were observed by Fischer et al. (2010b), where these authors observed found *P. alata* and *P. suberosa* species, as being resistant against collar rot disease when used as rootstocks. When comparing different studies, contrasting results are observed, this variation may be associated to a genetic diversity within the species resistance against the fungus. The species *P. quadrangularis*, *P. nitida* and *P. foetida* were evaluated as the most resistant. Some

studies had also demonstrated the resistance of *P. nitida* against soilborne pathogens (Fischer et al., 2005b; Junqueira et al., 2010). According to these authors, *P. nitida*, in addition to be rustic, possesses significant resistance against the disease and has potential to be used in breeding programs that will include interspecific hybridization. High levels of genetic variability were observed within the genotypes, even when belonging to the same species. Bueno et al. (2014), while characterizing a *F. solani* population, identified isolates with different levels of aggressivity on yellow passion fruit, the authors attributed such differences to the variability within the species or variability of the pathogen. In the present study, variability is derived only from the genotypes studied, with inocula being originated from a single spore (monosporic). This variation in *Passiflora* resistance, pathogen aggressivity and its significant interactions must be considered when developing breeding programs. In previous studies accomplished by Silva et al. (2013) to characterize resistant passifloras against *Fusarium oxysporum* f. sp. *Passiflorae*, resistance variability of genotypes of the same species was also observed. These results reveal that resistance probably depends of the genotype. The genotypes form the *P. edulis* species studied were considered susceptible and having variability in their resistance degree, as previously observed by Fisher et al. (2010a), when besides detecting susceptibility against an isolate of *F. solani* in the yellow passion fruit variety Afruvec, they detected high variability in the resistant reaction in different genotypes of yellow passion fruit. Further studies (Cavichioli et al., 2011; Meza et al., 2008) also detected that genotypes of these species had no resistance against collar rot disease. This results show the need to incorporate genes of resistance into the commercial species (*P. edulis*), found in wild species, by means of classic methods of plant breeding. Differences in resistance between and within each genotype may be associated with the high level of heterozygosity of the *Passiflora* species, therefore justifying the selection of intraspecific resistant species. This diversity must be considered in plant breeding programs aiming resistant cultivars against the collar rot disease, being of substantial importance to include an evaluation for intra and interspecific resistance in the germplasm characterization. The species *P. nitida* had no variability against collar rot disease, once all the genotypes of the species evaluated were grouped as resistant, as observed in previous studies (Fischer et al., 2005b; Roncetto et al, 2004). This species showed resistance to the pathogen, therefore is the most fitted to be used in breeding programs targeting the transference of resistance genes against *F. solani* to the commercial *P. edulis* species.

Materials and Methods

Experimental area

The experiment was performed at the experimental area of the Universidad of Estate de Mato Grosso (UNEMAT), Campus Cáceres, located in the Southwest region of the Mato Grosso state, Brazil, latitudes 15° 27' and 17° 37' South and longitudes 57° 00' and 58° 48' West, at an altitude of 118 m above the sea level. The county is part of the middle-region of the Center-South State of Mato Grosso and the Alto Pantanal micro-region, at a distance of 215 km from the capital, with a tropical hot and humid climate and a dry winter (Neves et al., 2011).

Implementation of the working collection and preparation of cuttings

The implementation of the *Passiflora* working collection at UNEMAT was accomplished by the planting of 10 species in field conditions: *P. nitida*, *P. alata*, *P. mucronata*, *P. cincinnata*, *P. morifolia*, *P. suberosa*, *P. foetida*, *P. eichleriana*, *P. quadrangularis*, and one genotype of the commercial species *P. edulis* derived from the breeding program from UFV/UNEMAT/UENF. All the seeds used were originated from the Active Germplasm Bank (BAG) from UNEMAT. After the establishment of the plants in the field, cuttings containing two or three buds were made from the median portion of vegetative branches. In order to evaluate the variability within the 10 species under study, four cuttings of different plants were prepared, with a total of 40 genotypes that were identified by a number, as shown in Table 3. Cuttings were rooted in trays of 72 cells distributed in random block design with 40 treatments (genotypes), three replicates and three plants per parcel, with a total of 360 plants evaluated.

Isolate preparation and inoculation of *Fusarium solani*

After rooting, cuttings were inoculated with the fungus *Fusarium solani*. To avoid interference of genetic variability of the fungus, the monosporic isolate FS04, from the mycology collection at the Laboratory of Genetic Breeding – UNEMAT, was used for inoculation. For the inoculum preparation, monosporic isolate FS04, preserved in filter paper, was replicated in Petri dishes containing BDA and incubated in growing chamber (B.O.D.) at 24 °C and light period of 12 h during 7 days. Inoculation was performed as described by Fischer et al. (2003). A discus (5 mm diameter) of culture media containing profuse mycelium growth was retired from the Petri dishes and fixed with PVC plastic tape over a small injury in the plant stem, at a distance of 2 cm from the soil. The plastic tape was removed five days after inoculation (DAI). The same procedure was applied to the control treatment, but without the media culture discus containing fungus.

Disease evaluation

The response of the inoculated plants were analyzed by means of: i- the survival period, considered as the number of days elapsed from the inoculation until the plant death or until 50 days of evaluation and ii- through a disease assessment scale, modified from Roy (1997). The disease assessment scale was applied in the last day of evaluation,

using a scale ranging from 1 to 6, being 1= absence of symptoms; 2= light symptoms: necrosis in just a part of the plant, less than 50% of the stem circumference; 3= moderate symptoms: necrosis in more than 50% of the stem circumference, with destruction of the cortex but without destruction of the plant medulla; 4= moderate symptoms: with a less extent of the cortex necrosis but with extended necrosis of the medulla; 5= severe symptoms: extensive colonization of the stem and necrosis of the medulla and cortex; 6= dead plant.

Statistical analysis

Initially, data of resistance characters (disease assessment scale and survival period) were submitted to variance analysis in order to verify genetic variability between the ten species studied, then, the mean values were compared by Tukey's test at 5% probability. Means were also grouped according the methodology of Graphical Dispersion Projection 2D. In order to reveal the intraspecific genetic variability of the resistance within *Passiflora* species, the 40 genotypes were distinguished by the UPGMA hierarchical clustering method, using as dissimilarity measure the average Mahalanobis distance (D^2) and the correction between distances' matrix and the dendrogram were estimated by the cophenetic correlation coefficient (ccc). All analysis was performed with the aid of the software Genes® (Cruz, 2013).

Conclusions

Intra and interspecific variability of resistance against *Fusarium solani* do exists within the studied *Passiflora* species. Species *P. nitida*, *P. foetida* and *P. quadrangularis* had the highest resistance level. The most adequate species for plant breeding programs of passion fruit aiming resistance against *F. solani* is *P. nitida*, once those genotypes showed no variation in resistance.

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