



AGRARIAN SCIENCES

Compatibility in pollen-pistil interaction of interspecific crossings with *Passiflora* spp.

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Abstract: Intraspecies or interspecies crossings transfer relevant alleles between plants. However, some interspecies crossings involving *Passiflora* species impede ovule fertilization and the viable development of seeds. Thus, the purpose of this study was to verify the viability of interspecific crossings and monitor pollen tube development. The experiment had six species of *Passiflora* in the reciprocal crossings. Histochemical tests aimed to evaluate the percentage of intraspecies or interspecies crossings that resulted in fruit development and pollen tube development. Ovule fertilization and fruit development occurred in determined directions of crossings when controlling the female or male genitor, but only one case of reciprocal crossing had success. In crossings with no fruit development, histological analysis showed that some callus developed in the stigma and style, confirming unilateral and interspecies incompatibility in the genus *Passiflora* to some species and some directions of crossings.

Key words: interspecific hybridization, incompatibility, plant crossings, genetic breedings.

INTRODUCTION

The contribution of the genus *Passiflora* for plant diversity outstands in the family Passifloraceae with an expressive number of species. Only in Brazil, there are more than 135 species, from which there are 81 species and eight taxonomic varieties that are endemic in tropical and subtropical areas (Cervi & Imig 2013, Bernacci et al. 2013). In Brazil, *Passiflora* species have an economical relevance to both food production and medical drugs as well as some have the potential to be ornamental plants (Cervi et al. 2013). The ornamental potential of plants is still in process to be explored (Santos et al. 2012).

In addition, it has a high potential for the production of new varieties of *Passiflora* by the formation of hybrid plants with abundant size and flowering varieties (Abreu et al. 2009). With the aim of producing new varieties, some

breeding programs can use wild *Passiflora* species in crossings, since they have genes that promote adaptation to several natural areas (Debouck & Libreros 1995, Bruckner 1997).

Breeding programs have the purpose to create a new variety using the germplasm of individual species from populations or genotypes. It is a challenge to generate a new variety of cultivar with the maximum possible number of favorable alleles in only one individual, with the alleles originating from different parental genotypes. In order to develop new cultivars, most plant breeding programs use a set of genotypes for developing favorable genetic combinations (Peel & Rasmusson 2000, Reddy & Comstock 1970).

The majority of results for interspecific crossings in a F_1 generation contains intermediate phenotypes for some traits such as: stipule size,

morphology and position of nectaries; variation on series number, structure and coloring of corona filaments on flowers and variation on lobules number and leaf formats (Ulmer & Macdougall 2004). When the purposes are to transfer favorable traits from native species to commercial species of *Passiflora edulis* Sims., such as genes of resistance to premature death and nematodes tolerance, more than 500 hybrids was obtained in *Passiflora* (Ataíde et al. 2012, Fischer 2004). However, interspecific crossings had other purposes such as coloring matches on flowers and cold tolerance between native species (Bugallo et al. 2011).

However, sometimes interspecific crossings can present incompatibility barriers that impede ovule fertilization and, therefore, it is impossible to a F_1 generation with hybrid plants to develop. There is a need to know about reproductive systems and chromosomal homology between species that will be used in crossings (Meletti et al. 2005, Santos et al. 2012). In the literature, there are some studies related to reproductive biology, nevertheless their focus are on morphology, flowering biology and agents of pollination. There is also a lack in study approaches about reproductive systems, pollination efficiency and pollen viability (Amorim et al. 2011, Shivanna 2012).

Researches on pollen viability and *in vivo* fertilization are fundamental to studies with the purpose of reproductive biology and development of hybrid plants, since they guide the researcher to plan the crossings, which can evidently favor the identification of promising cross-breeding. Strategies for plans and crossings improve the experiment with reduction in time for work execution. Therefore, the purpose of this study was to evaluate the viability of interspecific reproduction among six species of *Passiflora*, so that it is possible to collect information about the potential use

of species in crossings and their future use in breeding programs.

MATERIALS AND METHODS

The experiment was conducted from April 2014 to July 2015 in the experimental field from the Department of Plant Breeding of Universidade Federal de Viçosa (UFV), localized in the municipality of Viçosa, Minas Gerais, Brazil (20°45'52.3"S 42°51'09.5"W). The city has 680 meters of altitude, a climate predominantly of tropical altitude, dry and cold winter, hot and humid summer and an average of annual rain precipitation of 1200 mm.

The species used in this study were: *Passiflora cincinnata* Mast.; *Passiflora gibertii* N. E. Brown; *Passiflora mucronata* Lam.; *Passiflora alata* Curtis; and *Passiflora edulis* Sims. The plants were established in vineyards, drip irrigated and subjected to pruning, fertilization and spraying. The laboratory analyzes were performed at the Laboratory of Plant Anatomy of the Department of Plant Biology.

The crossings from the experiment had seven plant genotypes for each species that were randomly chosen in the field. Species phenology was observed to make viable crossings under the flowering coincidence. The flower-bud in pre-anthesis used in the breedings were filled into a paper bag on the day before anthesis, in order to avoid exogenous pollen contamination, with the exception of *P. edulis*, that had their flower-bud filled into paper bags in the morning of the breeding day.

Controlled crossings followed these steps: the female genitors were unsacked and emasculated, meanwhile the pollen was collected from the male genitor with the use of swabs and transferred to the stigma of the emasculated flower. Hybridizations under

a reciprocal mode in each pair of plants had five flowers being used in each combination of crosses.

The verification of crossing success occurred after seven days, by the evaluation of fruit formation. When harvesting fruits, they were bagged with a nylon net until their complete ripening. Then, seeds were extracted from fruits, dried at room temperature and maintained in paper bags in a refrigerator. The interpretation of data was performed by descriptive statistics, with the observation of the percentage of crossings between plants within each species and between species.

In order to verify the formation of the pollen tube, a histochemical test was performed, with the evaluation of ten flowers per plant that were recipients of pollen in reciprocal crossings. In a period of 24 hours after pollination, all flowers were collected and submitted to a fixation solution composed by Formaline at 37%, Acetic acid and Alcohol at 70% (FAA) in the proportion of 5:5:90 for 48 hours. Then, pollen tubes were maintained in Alcohol at 70% until slide preparation for analysis. Slide preparation consisted in softening biological material with sodium hydroxide (NaOH) at 10% and clearing with sodium hypochlorite at 1% and, afterwards, biological material on the slide was stained with Aniline Blue. Then, the laminule was positioned in the slide and a pressure was applied on the stigma making possible the subsequent examination of the biological material under Ultraviolet Light Microscope, in the blue hue. Images were captured in the Olympus Optical AX70TRF Microscope, with a digital camera connected to it (Spot Insightcolour 3.2.0, Diagnostic Instruments Inc., USA), in the Plant Anatomy Laboratory at UFV.

RESULTS

Breedings between *Passiflora gibertii* N. E. Brown and *Passiflora mucronata* Lam.

There were 5% of fruit production, when the female genitor was *P. gibertii* and the male genitor was *P. mucronata*. On the other hand, there was no fruit development in the reciprocal crossing (Table I). In some cases, the genotypes in the crossings influenced fruit development. Four out of seven genotypes of *P. gibertii* were female genotypes and had fruit production ranging from 3% to 17%. Seven genotypes of *P. mucronata* were pollen donors in the experiment, but only five genotypes fertilized the ovules of *P. gibertii*. At these crossings, with *P. mucronata* as the male genitor and *P. gibertii* as female genitor, there was no visualization of the pollen tube development by histochemical analysis. The average number of seeds per fruit from viable crossings was of 27.18 seeds per fruit, considering that *P. gibertii* plants produce small fruits (Table II).

Crossings between *Passiflora alata* Curtis and *Passiflora mucronata* Lam.

In the crossings between *Passiflora alata* as the female genitor and *Passiflora mucronata* as male genitor, the percentage of fruit production was of 1%. On the other hand, no fruit production occurred in the reciprocal crossing (Table III). Among the seven genotypes of *P. alata* used as female genitor in the crossings, only two cases of crossings had fruit development, which corresponds to 3%. On the other hand, from the genotypes of *P. mucronata* used as pollen donors, only one genotype fertilized the ovule of the female genitor *P. alata* and produced fruits (Table III). No cellular structures indicated pollen grains germination in the histochemical evaluation. Finally, the crossings with fruit production had an average of 28 seeds per fruit (Table II).

Table I. Percentage of fruit development in reciprocal crossings between *Passiflora gibertii* N. E. Brown and *Passiflora mucronata* Lam. UFV, Viçosa-MG, 2016.

<i>P. gibertii</i> (♀)	<i>P. mucronata</i> (♂)							Average		(%)
	22	23	24	25	26	27	28	NP	F	
7	20	0	0	60	0	0	0	35	4	11
8	0	0	0	0	0	0	0	35	0	0
9	0	0	20	60	0	0	40	35	6	17
10	0	0	0	0	0	0	20	35	1	3
61	0	0	0	0	0	0	0	35	0	0
65	0	0	0	0	0	0	0	35	0	0
69	0	0	0	0	14	0	20	35	2	5
Average	3%	0%	3%	17%	3%	0%	11%	245	13	5
	<i>P. gibertii</i> (♂)							Average		
<i>P. mucronata</i> (♀)	7	8	9	10	61	65	69	NP	F	(%)
22	0	0	0	0	0	0	0	35	0	0
23	0	0	0	0	0	0	0	35	0	0
24	0	0	0	0	0	0	0	35	0	0
25	0	0	0	0	0	0	0	35	0	0
26	0	0	0	0	0	0	0	35	0	0
27	0	0	0	0	0	0	0	35	0	0
28	0	0	0	0	0	0	0	35	0	0
Average	0%	0%	0%	0%	0%	0%	0%	245	0	0

NP: pollination number; F: fruit production; ♀: female genitor; ♂: male genitor.

Crossings between *Passiflora edulis* Sims. and *Passiflora mucronata* Lam.

The crossing with *P. edulis* as the female genitor and *P. mucronata* as male genitor had an average of 1% of fruit production. However, the crossing with *P. mucronata* as the female genitor had no fruit. In the crossings with fruit production, one out of seven genotypes of *P. edulis* used as female genitor was responsible for 6% of fruit development. Similarly, *P. mucronata* as the male genitor (pollen donor) had only one out of seven genotypes being responsible for 6% of fruit development (Table IV).

No cellular structures indicated pollen grains germination in the histochemical analysis. The crossings between the female genitor *P. edulis*

and the male genitor *P. mucronata* produced 44 seeds at total, which is a small value in relation to crossings with *P. edulis* that had an average of 330 seeds per fruit. It is worth to mention that most of the 44 seeds resulted from the crossings between the female genitor *P. edulis* and the male genitor *P. mucronata* (Table II).

Crossings between *Passiflora gibertii* N.E. Brown and *Passiflora alata* Curtis

In crossings with *P. gibertii* as the female genitor and *P. alata* as male genitor, there was 1% of fruit production and in the reciprocal crossing there was 0.41% of fruit production. Additionally, only one out of seven genotypes of *P. gibertii* showed to be responsive to crossings, when it was female

Table II. Average of seeds per fruit, obtained in open and controlled crosses with *Passiflora* spp. UFV, Viçosa-MG, 2016.

Random -pollination			Number of seeds	Standard deviation
<i>P. cincinnata</i>				
<i>P. mucronata</i>			89	7.26
<i>P. gibertii</i>			25	4.66
<i>P. alata</i>			191	9.86
<i>P. edulis</i>			330	16.08
Controlled crossings				
♀		♂	Number of seeds	Standard deviation
<i>P. gibertii</i>	X	<i>P. cincinnata</i>	17.10	4.86
<i>P. gibertii</i>	X	<i>P. mucronata</i>	27.18	2.98
<i>P. gibertii</i>	X	<i>P. alata</i>	25	6.86
<i>P. gibertii</i>	X	<i>P. edulis</i>	9	4.88
<i>P. alata</i>	X	<i>P. cincinnata</i>	122	5.61
<i>P. alata</i>	X	<i>P. mucronata</i>	28	4.36
<i>P. alata</i>	X	<i>P. edulis</i>	120	6.34
<i>P. edulis</i>	X	<i>P. cincinnata</i>	180.12	8.57
<i>P. edulis</i>	X	<i>P. mucronata</i>	44	7.06

Table III. Percentage of fruit production in reciprocal crossings between *Passiflora alata* Curtis and *Passiflora mucronata* Lam. UFV, Viçosa - MG, 2016.

	<i>P. mucronata</i> (♂)								Average		
<i>P. alata</i> (♀)	22	23	24	25	26	27	28	NP	F	(%)	
11	0	0	0	0	0	0	0	35	0	0	
12	0	0	0	0	0	0	0	35	0	0	
13	0	0	0	0	0	0	0	35	0	0	
16	0	0	0	0	0	0	0	35	0	0	
17	0	14	0	0	0	0	0	35	1	3	
89	0	0	0	0	0	0	0	35	0	0	
94	0	20	0	0	0	0	0	35	1	3	
Average	0%	5%	0%	0%	0%	0%	0%	245	2	1	
	<i>P. alata</i> (♂)								Average		
<i>P. mucronata</i> (♀)	11	12	13	16	17	89	94	NP	F	(%)	
22	0	0	0	0	0	0	0	35	0	0	
23	0	0	0	0	0	0	0	35	0	0	
24	0	0	0	0	0	0	0	35	0	0	
25	0	0	0	0	0	0	0	35	0	0	
26	0	0	0	0	0	0	0	35	0	0	
27	0	0	0	0	0	0	0	35	0	0	
28	0	0	0	0	0	0	0	35	0	0	
Average	0%	0%	0%	0%	0%	0%	0%	245	0	0	

NP: pollination number; F: fruit production; ♀: female genitor; ♂: male genitor.

and male genitors. A similar behavior occurred with *P. alata* (Table V). Additionally, these crossings had no visualization of the pollen tube by histochemical analysis. The average number of seeds per fruit was of nine seeds, which is a lower value than the expected value, since *P. gibertii* had an average of 25 seeds per fruit (Table II), as mentioned before.

Crossings between *Passiflora gibertii* N.E. Brown and *Passiflora cincinnata* Mast.

There were 9% of fruit production, when *P. gibertii* was the female genitor and *P. cincinnata* Mast. was the male genitor. The reciprocal crossing had no fruit. In the direction of the crossing with fruit production, six out of the seven genotypes of *P. gibertii* used as female genitor had fruit development varying from 3% to 20%. Among *P. cincinnata* genotypes used as the male genitor only four produced fruits, ranging from 6% to 20% of fruit development. In crossings with fruit production, *P. gibertii* had an average of 17.10 seeds per fruit (Table VI).

In the crossings with fruit development, histochemical analysis of stigmas can confirm pollen germination under the stigma surface and pollen tube formation. On top of the developed callus in the stigma occurred the development of pollen tube (Figure 1a). Fruit development occurs after egg fertilization, but histochemical analysis confirms that not all the pollen tubes formed can reach the ovary of the flower. In the reciprocal crossings, germination was evidenced in pollen grains (Figure 1b), with occurrence of the same phenomenon of callus deposition in the pollen tube, with no fruit development.

Crossings between *Passiflora alata* and *Passiflora cincinnata*

The crossings with the female genitor *P. alata* and the male genitor *P. cincinnata* had 0.41% of fruit production. The reciprocal crossing had

no fruit development (Table VII). Among the genotypes of *P. alata*, only one had 3% of fruit development. This result also occurred with the male genitor *P. cincinnata*. Crossings between *P. alata* as the female genitor and *P. cincinnata* as the male genitor had an average of 122 seeds per fruit, which is a median value, in relation to crossings with *P. alata* that presented an average of 191 seeds (Table II). Histochemical analysis of stigmas in the direction of fertilization showed a low percentage of fruit development due to the development of callus that blocked the development of the pollen tube (Figure 2).

Crossings between *Passiflora edulis* Sims. and *Passiflora cincinnata* Mast.

In crossings between the female genitor *Passiflora edulis* Sims. and male genitor *Passiflora cincinnata* Mast., there were 5% of fruit production. On the other hand, the reciprocal crossing had 0.41% of fruit development.

Six plants of *P. edulis* were used in the crossings, resulted in well-succeeded fruit production, ranging from 3% to 14% of fruit development. Five plants of the male genitor *P. cincinnata* had 3% to 20% of fruit production (Table VIII).

In the reciprocal crossing with the male genitor *P. edulis*, only one genotype had 3% of fruit production. On the other hand, the reciprocal crossing with the female genitor *P. cincinnata* had only one out of seven genotypes with 3% of fruit production. On these crossings, there was no visualization of the pollen tube by histochemical analysis. In crossings between the female genitor *P. edulis* and the male genitor *P. cincinnata*, the average number of seeds per fruit was of 180.12 seeds being an approximate value to the species that is 330 seeds (Table II).

Table IV. Percentage of fruit production in reciprocal crossings between *Passiflora edulis* Sims. and *Passiflora mucronata* Lam. UFV, Viçosa-MG, 2016.

	<i>P. mucronata</i> (♂)								Average	
<i>P. edulis</i> (♀)	22	23	24	25	26	27	28	NP	F	(%)
102	0	0	0	0	0	0	0	35	0	0
105	0	0	0	0	0	0	0	35	0	0
110	0	0	40	0	0	0	0	35	2	6
139	0	0	0	0	0	0	0	35	0	0
143	0	0	0	0	0	0	0	35	0	0
157	0	0	0	0	0	0	0	35	0	0
169	0	0	0	0	0	0	0	35	0	0
Average	0%	0%	6%	0%	0%	0%	0%	245	2	1
	<i>P. edulis</i> (♂)								Average	
<i>P. mucronata</i> (♀)	102	105	110	139	143	157	169	NP	F	(%)
22	0	0	0	0	0	0	0	35	0	0
23	0	0	0	0	0	0	0	35	0	0
24	0	0	0	0	0	0	0	35	0	0
25	0	0	0	0	0	0	0	35	0	0
26	0	0	0	0	0	0	0	35	0	0
27	0	0	0	0	0	0	0	35	0	0
28	0	0	0	0	0	0	0	35	0	0
Average	0%	0%	0%	0%	0%	0%	0%	245	0	0

NP: pollination number; F: fruit production; ♀: female genitor; ♂: male genitor.

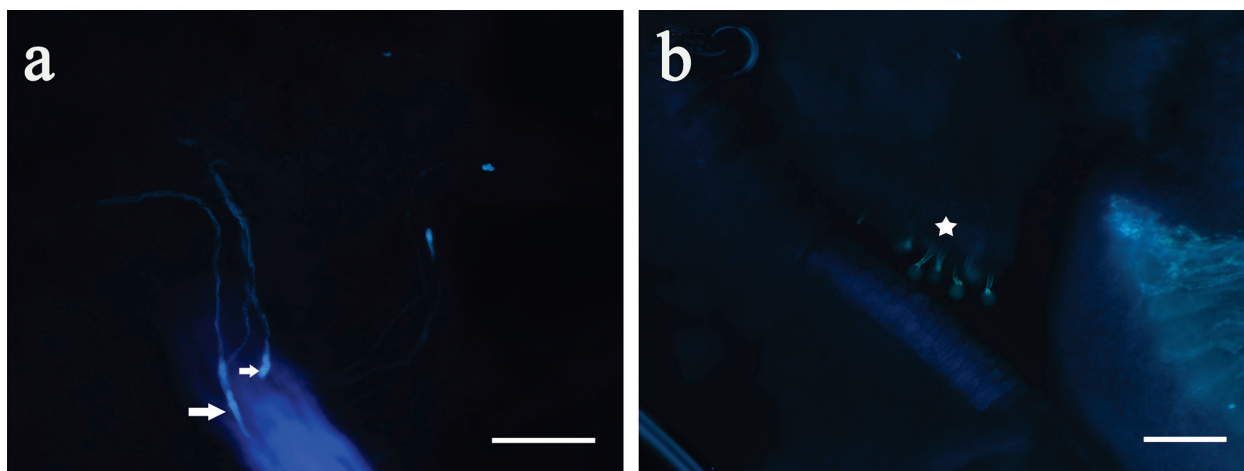


Figure 1. Fluorescence microscopy in pistil of *P. gibertii* pollination with incompatible pollen. The pistils were stained with Aniline Blue. a) *P. gibertii* and *P. cincinnata* crossing. Bar = 150 μ m; arrows indicate callus; b) *P. cincinnata* and *P. gibertii* crossing. Pollen grains germinating under the stigma and tubes with posterior callus development during pollen tube formation; star indicates pollen grain germination. Bar = 300 μ m.

Table V. Percentage of fruit production in reciprocal crossings between *Passiflora gibertii* N. E. Brown and *Passiflora alata* Curtis. UFV, Viçosa- MG, 2016.

	<i>P. alata</i> (♂)							Average		
<i>P. gibertii</i> (♀)	11	12	13	16	17	89	94	NP	F	(%)
7	0	0	0	0	0	0	0	35	0	0
8	0	0	0	0	0	0	0	35	0	0
9	0	0	0	0	0	0	0	35	0	0
10	0	0	0	0	40	0	0	35	2	6
61	0	0	0	0	0	0	0	35	0	0
65	0	0	0	0	0	0	0	35	0	0
69	0	0	0	0	0	0	0	35	0	0
Average	0	0	0	0	5	0	0	245	2	1
	<i>P. gibertii</i> (♂)							Average		
<i>P. alata</i> (♀)	7	8	9	10	61	65	69	NP	F	(%)
11	0	0	0	0	0	0	0	35	0	0
12	0	0	0	0	0	0	0	35	0	0
13	0	0	0	0	0	0	0	35	0	0
16	0	0	0	0	0	0	0	35	0	0
17	0	0	20	0	0	0	0	35	1	3
89	0	0	0	0	0	0	0	35	0	0
94	0	0	0	0	0	0	0	35	0	0
Average	0	0	3	0	0	0	0	245	1	0.41

NP: pollination number; F: fruit production; ♀: female genitor; ♂: male genitor.

Crossings between *Passiflora gibertii* N.E. Brown and *Passiflora edulis* Sims.

The crossings with the female genitor *P. gibertii* and the male genitor *P. edulis* had 0.41% of fruit development. The reciprocal crossing had no fruit development (Table IX). In the crossing with fruit development, the female genitor *P. gibertii* had one out of the seven genotypes with approximately 3% of fruit development. Similarly, the male genitor *P. edulis* had only one genotype being responsible for 3% of fruit production.

On these breedings, there was no visualization of the pollen tube by histochemical analysis. Crossings with the female genitor

P. gibertii and the male genitor *P. edulis* had a production of nine seeds per fruit, which is a relatively low value, when compared to the expected value of 25 seeds (Table II).

Crossings between *Passiflora alata* Curtis and *Passiflora edulis* Sims.

The crossing with the female genitor *P. alata* and the male genitor *P. edulis* had 3% of fruit development, but the reciprocal crossing had no fruit development (Table X). In crossings with fruit production, there are three genotypes of *P. alata* with fruit development, ranging from 6% to 11%. From *P. edulis* genotypes, only one genotype was a pollen donor and had 23% of fruit production (Table X). Histochemical analysis did

Table VI. Percentage of fruit production in reciprocal crossings between *Passiflora gibertii* N. E. Brown and *Passiflora cincinnata* Mast. UFV, Viçosa – MG, 2016.

	<i>P. cincinnata</i> (♂)							Average		
<i>P. gibertii</i> (♀)	2	3	4	5	18	19	50	NP	F	(%)
7	0	0	20	40	0	0	0	35	3	9
8	0	0	20	0	0	0	0	35	1	3
9	0	0	20	20	0	80	0	35	6	17
10	0	0	0	0	0	0	0	35	0	0
61	20	0	40	0	0	0	0	35	3	9
65	0	0	40	0	0	0	0	35	2	6
69	20	0	20	0	0	0	0	35	7	20
Average	6	0	37	9	0	11	0	245	22	9
	<i>P. gibertii</i> (♂)							Average		
<i>P. cincinnata</i> (♀)	7	8	9	10	61	65	69	NP	F	(%)
2	0	0	0	0	0	0	0	35	0	0
3	0	0	0	0	0	0	0	35	0	0
4	0	0	0	0	0	0	0	35	0	0
5	0	0	0	0	0	0	0	35	0	0
18	0	0	0	0	0	0	0	35	0	0
19	0	0	0	0	0	0	0	35	0	0
50	0	0	0	0	0	0	0	35	0	0
Average	0	0	0	0	0	0	0	245	0	0

NP: pollination number; F: fruit production; ♀: female genitor; ♂: male genitor.

not show any pollen tube formation. And, finally, the average number of seeds per fruit was of 120 seeds in crossings with fruit production (Table II).

DISCUSSION

It was observed a difference in the rate of crossings in hybridizations between plants within each species. It reinforces the idea that the success of hybridizations does not only depend on the species chosen, but also on the plants selected.

The compatibility in the crosses between the species evaluated in this work was only possible due to the phylogenetic proximity between the species, although in some cases compatibility occurred only in one direction of the crossings. The species are included in the same subgenus *Dysosmia* and have the same chromosome number ($2n=18$) in *P. cincinnata* (Guerra 1986), *P. alata* (Meletti et al. 2003), *P. mucronata* (Souza et al. 2003), *P. gibertii* (Mayeda & Vieira 1995) and *P. edulis* (Soares-Scott et al. 2003).

Similar results were found by Soares et al. (2015). The authors describe about the experiment success, when using the following

Table VII. Percentage of fruit production in reciprocal crossings between *Passiflora alata* and *Passiflora cincinnata* Mast. UFV, Viçosa – MG, 2016.

<i>P. alata</i> (♀)	<i>P. cincinnata</i> (♂)							Average		
	2	3	4	5	18	19	50	NP	F	(%)
11	0	0	20	0	0	0	0	35	1	3
12	0	0	0	0	0	0	0	35	0	0
13	0	0	0	0	0	0	0	35	0	0
16	0	0	0	0	0	0	0	35	0	0
17	0	0	0	0	0	0	0	35	0	0
89	0	0	0	0	0	0	0	35	0	0
94	0	0	0	0	0	0	0	35	0	0
Average	0	0	3	0	0	0	0	245	1	0.41
<i>P. cincinnata</i> (♀)	<i>P. alata</i> (♂)							Average		
	11	12	13	16	17	89	94	NP	F	(%)
2	0	0	0	0	0	0	0	35	0	0
3	0	0	0	0	0	0	0	35	0	0
4	0	0	0	0	0	0	0	35	0	0
5	0	0	0	0	0	0	0	35	0	0
18	0	0	0	0	0	0	0	35	0	0
19	0	0	0	0	0	0	0	35	0	0
50	0	0	0	0	0	0	0	35	0	0
Average	0	0	0	0	0	0	0	245	0	0

NP: pollination number; F: fruit production; ♀: female genitor; ♂: male genitor.

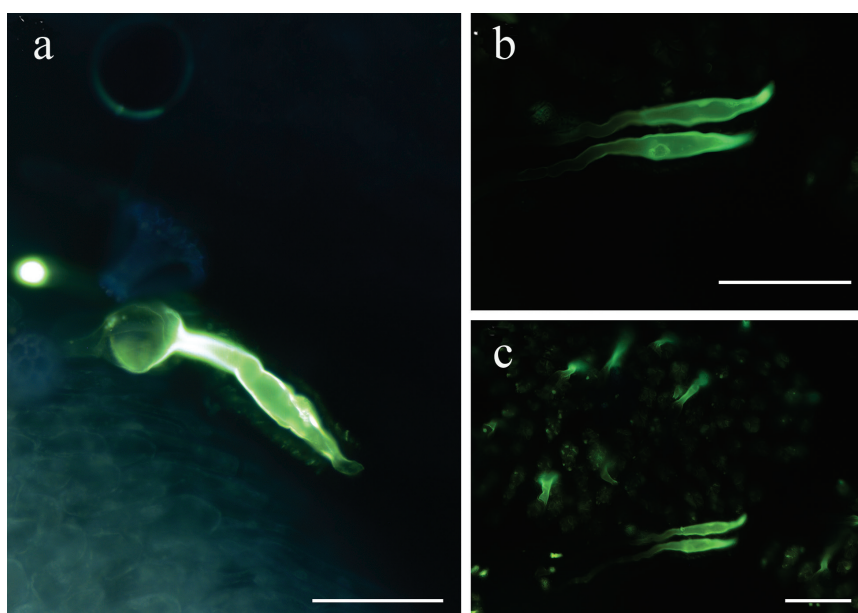


Figure 2. Fluorescence microscope of pistils in the crossing between *P. alata* and *P. cincinnata*, stained with Aniline Blue. a) Deformed pollen grain by callus excessive deposition is still inhibited on the stigma; b, c) Top of pollen tubes after going through the stigma, being blocked by callus synthesis after 24 hours of pollination. Bar = 150 μm respectively.

Table VIII. Percentage of pollinations with fruit production in reciprocal crossings between *Passiflora edulis* Sims. and *Passiflora cincinnata* Mast. UFV, Viçosa - MG, 2016.

	<i>P. cincinnata</i> (♂)								Average		
<i>P. edulis</i> (♀)	2	3	4	5	18	19	50	NP	F	(%)	
102	0	0	0	0	20	0	0	35	1	3	
105	0	0	20	0	0	20	0	35	2	6	
110	0	20	0	0	0	0	0	35	1	3	
139	0	0	0	0	40	0	0	35	2	6	
143	0	0	0	0	0	0	0	35	0	0	
157	0	0	0	0	0	0	100	35	5	14	
169	0	0	0	0	0	0	40	35	2	6	
Average	0	3	3	0	9	3	20	245	13	5	
	<i>P. edulis</i> (♂)								Average		
<i>P. cincinnata</i> (♀)	102	105	110	139	143	157	169	NP	F	(%)	
2	0	0	0	0	0	0	0	35	0	0	
3	0	0	0	0	0	0	0	35	0	0	
4	0	0	0	0	0	0	0	35	0	0	
5	0	0	20	0	0	0	0	35	1	3	
18	0	0	0	0	0	0	0	35	0	0	
19	0	0	0	0	0	0	0	35	0	0	
50	0	0	0	0	0	0	0	35	0	0	
Average	0	0	3	0	0	0	0	245	1	0.41	

NP: pollination number; F: fruit production; ♀: female genitor; ♂: male genitor.

species with the same chromosome number ($2n=18$) in the crossings: *P. racemosa*, *P. gibertii*, *P. edmundoi*, *P. mucronata*, *P. edulis f. flavicarpa*, *P. galbana* and *P. tenuiflora*. Souza et al. (2008) describe the positive results in interspecies crossings with the same chromosome number ($2n=18$) in the following crossings: between *P. edulis f. flavicarpa* Deg. and *P. setacea*; *P. coccinea* and *P. glandulosa* Cav. According to Pereira et al. (2005) the expected chromosomal homology between genetically similar species can induce viable interspecific crossings and, then, reduce the incongruity, allowing the development of hybrid plants.

It is interesting to note that in the present study some reciprocal crossings were not successful and had no fruits. This phenomenon is referred in the literature as incongruity or unilateral incompatibility. Although there are some exceptions of successful crossings such as the hybridizations between *Passiflora gibertii* and *Passiflora alata*, and also between *Passiflora edulis* and *Passiflora cincinnata*, which had the development of fruits in reciprocal crossings. In these cases, reciprocal crossings had differences in results with no fruit production in some of them.

Table IX. Percentage of fruit development in reciprocal crossings between *Passiflora gibertii* N. E. Brown and *Passiflora edulis* Sims. UFV, Viçosa – MG, 2016.

	<i>P. edulis</i> (♂)							Average		
<i>P. gibertii</i> (♀)	102	105	110	139	143	157	169	NP	F	(%)
7	0	0	20	0	0	0	0	35	1	3
8	0	0	0	0	0	0	0	35	0	0
9	0	0	0	0	0	0	0	35	0	0
10	0	0	0	0	0	0	0	35	0	0
61	0	0	0	0	0	0	0	35	0	0
65	0	0	0	0	0	0	0	35	0	0
69	0	0	0	0	0	0	0	35	0	0
Average	0	0	3	0	0	0	0	245	1	0.41
	<i>P. gibertii</i> (♂)							Average		
<i>P. edulis</i> (♀)	102	105	110	139	143	157	169	NP	F	(%)
102	0	0	0	0	0	0	0	35	0	0
105	0	0	0	0	0	0	0	35	0	0
110	0	0	0	0	0	0	0	35	0	0
139	0	0	0	0	0	0	0	35	0	0
143	0	0	0	0	0	0	0	35	0	0
157	0	0	0	0	0	0	0	35	0	0
169	0	0	0	0	0	0	0	35	0	0
Average	0	0	0	0	0	0	0	245	0	0

NP: pollination number; F: fruit production; ♀: female genitor; ♂: male genitor.

This unilateral incongruity occurs with a certain stability in the genus *Passiflora*, as already mentioned by Bugallo et al. (2011). These authors performed interspecific hybridizations with *P. alata*, *P. caerulea*, *P. amethystina*, *P. edulis* and *P. violacea*. They discovered that *P. alata* and *P. caerulea* had success in crosses in both directions, though the other interspecific combinations in crossings had one unilateral incongruity.

Soares et al. (2015) verified that in the reciprocal crossing between *P. racemosa* and *P. capsularis*, fruit development only occurred in the crossing with *P. racemosa* as the male genitor. Junqueira et al. (2005) verified that *P. setacea*, *P.*

coccinea and *P. glandulosa* are responsive in the crossings in which they were the female genitors; Also, Conceição et al. (2011) described that the reciprocal crossings between *P. watsoniana* x *P. gardneri* and between *P. gardneri* x *P. gibertii* had unilateral incompatibility. Additionally, Santos (2013) described that the percentage of fruit development doubled when *P. edulis* was used as the female genitor (100%) or as the male genitor (50%) in crossings with *P. setacea*. Finally, Ocampo et al. (2016) described fruit production ranging from 2.1% to 60% in crossings with *P. vitifolia*, *P. mucronata*, *P. edulis f. edulis* and *P. edulis f. flavicarpa* as female genitors. On the other hand, no fruit development occurred

Table X. Percentage of pollination with fruit production in reciprocal crossings between *Passiflora alata* Curtis and *Passiflora edulis* Sims. UFV, Viçosa - MG, 2016.

	<i>P. edulis</i> (♂)							Average		
<i>P. alata</i> (♀)	102	105	110	139	143	157	169	NP	F	(%)
11	0	0	0	0	0	0	0	35	0	0
12	0	0	0	0	0	0	0	35	0	0
13	0	40	0	0	0	0	0	35	2	6
16	0	0	0	0	0	0	0	35	0	0
17	0	0	0	0	0	0	0	35	0	0
89	0	40	0	0	0	0	0	35	2	6
94	0	80	0	0	0	0	0	35	4	11
Average	0	23	0	0	0	0	0	245	8	3
	<i>P. alata</i> (♂)							Average		
<i>P. edulis</i> (♀)	11	12	13	16	17	89	94	NP	F	(%)
102	0	0	0	0	0	0	0	35	0	0
105	0	0	0	0	0	0	0	35	0	0
110	0	0	0	0	0	0	0	35	0	0
139	0	0	0	0	0	0	0	35	0	0
143	0	0	0	0	0	0	0	35	0	0
157	0	0	0	0	0	0	0	35	0	0
169	0	0	0	0	0	0	0	35	0	0
Average	0	0	0	0	0	0	0	245	0	0

NP: pollination number; F: fruit production; ♀: female genitor; ♂: male genitor.

in the reciprocal crossing, which confirms the occurrence of unilateral and interspecific incompatibility.

Probably such restrictions to fruit development in some crossings can result in some degree of genetic incompatibility or any zygotic barrier in pre or post fertilization. It can have a rejection mechanism in pollen recognition process under the stigma, style or ovary, which proteins block the germination of pollen grains or inhibit/suspend the development and growth of the pollen tube, besides the ratio pollen:ovary, which can influence on the efficacy of the pollen grain to reach the compatible stigma (Bugallo et al. 2011, Jones et al. 2012, Ferreira et al. 2014).

Also the embryonic sac immaturity, ovule degeneration or lack of synchronism between male and female gametes can directly affect fertilization and, thus, fruit development (Guerra et al. 2011).

However, this mechanism of unilateral inconsistency, even well frequent in *Passiflora*, it is not exclusive of this genus, which can also be evidenced in other species of plants such as in the genus *Capsicum* spp. For example, Martins (2014) described differences in reciprocal crossings with a better fruit production in the crossing between *C. annum* var. *annuum* and *C. baccatum*, which had nine fruits, with two fruits in the reciprocal crossing. Nascimento et

al. (2012) had 50% of fruit development in the crossing between *C. chinense* and *C. baccatum*, and the reciprocal crossing showed to be unviable.

The histochemical analysis by fluorescence also evidenced the development and inhibition of pollen tubes due to the development of callus. Such response occurs in a fast manner in the stigma surface, limiting the growth of incompatible pollen tubes. Madureira et al. (2012) states that in cases in which crossings are compatible, callus were not visible due to their association to tube elongation.

Madureira et al. (2014) reinforces that besides callus development, in cases of incompatibility, there is an occurrence of cellular disorganization, which deforms the anatomy of the pollen tube. This is an effect of the actine reduction, which is related to the action of the cytoskeleton in the pollen tube making them very thick. This fact can be observed in the images 1 and 2 obtained in this study, with visualization of the pollen tubes with thicker or thinner areas.

According to Payán & Martín (1975), Bugallo et al. (2011) and Conceição et al. (2011), there is a second barrier to the occurrence of crossings in *Passiflora* species that is the abortion of seeds, even if it is possible some crossings between species. The degree of similarity between the number and size of parental chromosomes is associated to the maternal effects that normally promote the endosperm growth, meanwhile the parental effect suppress the endosperm development (Kinoshita et al. 2008). The reason is that fertilization can cause negative interactions between nucleus-cytoplasm, resulting in risks for embryo development, due to failure in endosperm development (Nimura et al. 2003). This is the explanation for non-germination of seeds obtained from the crosses between *P. alata* and *P. edulis*; *P. gibertii* and *P. edulis*; *P.*

edulis and *P. cincinnata*, since there is embryo death and a posterior barrier to hybridization.

Failure in the embryo development was described by Conceição et al. (2011) in the crossing between *P. gardneri* and *P. cincinnata*, which fruits had 100% of seeds with no endosperm. Furthermore, the crossings between *P. gibertii* and *P. kermesina* and between *P. gibertii* and *P. alata* had fruits with no seed production. Similarly, Junqueira et al. (2005) found fruits with a rare production of seeds in crossings between *P. caerulea* and *P. edulis f. flavicarpa*. However, when changing the direction of the crossings using the male genitor *P. edulis* Sims., fruits from F1 generation had viable seeds.

CONCLUSIONS

Thus, the experiment results described in this paper can be helpful to other researchers as an auxiliary tool during the plan of intra-crossings or inter-crossings, improving the establishment of breeding programs with the purpose to use interspecific hybrid plants. Although further studies are necessary in order to increase knowledge about this rejection mechanism.

The analysis of the crossings performed in the experiment showed the following crossings with the highest potential for reproduction: *P. gibertii* x *P. cincinnata*; *P. gibertii* x *P. mucronata*, *P. gibertii* x *P. alata*, *P. gibertii* x *P. edulis*, *P. alata* x *P. cincinnata*, *P. alata* x *P. mucronata*, *P. alata* x *P. edulis*, *P. edulis* x *P. cincinnata*, *P. edulis* x *P. mucronata*.

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