Meiosis and male fertility in F1 interspecific hybrids (Passiflora vitifolia vs. Passiflora hatschbachii)

Analu Cruz Souza
Universidade Estadual de Santa Cruz
Margarete Magalhães Souza (souzamagg@yahoo.com.br)
Universidade Estadual de Santa Cruz https://orcid.org/0000-0003-0292-7988
Gonçalo Santos Silva
Universidade Estadual de Santa Cruz
Manuella Palmeira Lavinscky
Universidade Estadual de Santa Cruz
Viviane de Oliveira Souza
Universidade Estadual de Santa Cruz
Jôsie Cloviane de Oliveira Freitas
Universidade Estadual de Goiás: Universidade Estadual de Goias

Research Article

Keywords: Interspecific hybridization, Meiosis, GISH, Ornamental Passiflora

Posted Date: May 5th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-479829/v1

Abstract

Interspecific hybrids can be studied using methodologies in which the male gamete with high reproduction potential, viability, and fertility is prioritized. *Passiflora* species, with lush, showy, and exotic colors, have great potential for the ornamental plant market. In addition, artificial *Passiflora* hybrids were developed without many difficulties because of weak reproductive barriers. Thus, meiotic and post-meiotic behaviors were analyzed with 2% acetic carmine staining. Confirmation of interspecific hybridization was performed using SSR markers and GISH technique was used to detect genomic differentiation. The pollen viability of the parental and hybrids genotypes was tested using Alexander solution, fluorescein diacetate and *in vitro* germination tests were performed using culture medium. The meiotic behavior was regular and displayed haploid number $n = 9$ with nine bivalent (II) chromosomal, pairing in 90% of the cells in diakinesis. There was a significant difference ($p < 0.05$) in terminal and interstitial chiasma frequencies. Meiotic irregularities observed were as follows: early and/or delayed chromosomes, disorientation of spindle fibers, transverse spindles, tripolar spindles, and asynchrony; and consequently irregular post-meiotic products were observed: monads, dyads, triads, and polyads. GISH was used in the interspecific hybrids and pairing between homeologous chromosomes, and bivalent and tetravalent formation were observed. From this study, we could conclude that hybrid genotypes are fertile and pollen grains are viable and can be used in breeding programs. We also hypothesize that interspecific hybrid genotypes of *Passiflora* can be obtained with regular meiosis, which could be viable and fertile.

Introduction

The Passifloraceae family (A. L. de Jussieu ex. Kunth) belongs to the order Malpighiales encompassing approximately 17 genera and more than 700 species (Bernacci et al. 2013). The genus *Passiflora* L. is the largest in terms of number of species, that is, more than 525 species (Cervi and Imig 2013), distributed in the tropical and subtropical regions (Ulmer and MacDougal 2004). Brazil is regarded as the center of origin and geographical distribution of a large number of species of this genus (Meletti et al. 2000; Souza et al. 2003). These species present high levels of genetic diversity (Meletti et al. 2005; Silva et al. 2014) and are of economic interest; they can be used for pharmacological, nutritional and cosmetic purposes, and are of the ecological and ornamental interest (Abreu et al. 2009; Ocampo et al. 2016). In recent years, new species of the genus have been described (Cervi and Linsingen 2010; Bernacci and Souza 2012; Cervi and Imig 2013).

Passiflora species have great potential for the ornamental plant market because the flowers exhibit exuberant, showy, and exotic colors (Abreu et al. 2009). However, in Brazil, the use of these species for ornamental purpose is minimal, while in Europe these species are used for the decoration of pergolas, walls, and gardens (Junqueira et al. 2008; Abreu et al. 2009; Conceição et al. 2011). In addition, artificial *Passiflora* hybrids can be developed without many difficulties (Vanderplank 2000) because of weak reproductive barriers (Belo et al. 2018; Souza et al. 2020). The morphology of the species, flowers, and especially of the androgynophore which are composed of complex corona and consisting of filaments arranged in one or more concentric rows, are beautiful. Also there is interspecific variation in size, shape, and color, which hare striking features for the genus (Vanderplank 2002).

Interspecific hybrids of *Passiflora* with interesting features can be developed as a strategy to create varieties for the ornamental plant market. Interspecific hybrids are sources of variability, which may allow the selection of promising genotypes for plant breeding. Hybrid genotypes usually give a higher flower that yield and provide a wider variety of shapes and colors (Conceição et al. 2011). Ensuring the success of interspecific hybridization through regularities of meiotic events such as pairing, even partially, between the homeologous chromosomes, chromosomal recombination, regular meiosis, and viable pollen grains (PG) are important (Souza et al. 2003). Meiosis depends on the regularity of events such as chromosomal pairing of bivalents, formation of the synaptonemic complex, occurrence of crossover and chiasmata, recombination, and formation of regular post-meiotic products. Regular meiotic behavior enables the formation of viable gametes (Belo et al. 2018), and thus, the formation of fertile hybrids (Lavinscky et al. 2017).

Time and cost reduction are interesting factors for improvement programs. The costs of plant maintenance can be reduced by confirming the hybridization still in seedling phase, and molecular markers have been widely used for this purpose (Bellon et al. 2007; Junqueira et al. 2008; Conceição et al. 2011), in addition to genomic *in situ* hybridization (GISH) techniques (Silva and Souza 2013; Souza et al. 2020). Molecular markers are also used to verify the occurrence of DNA polymorphism (Fajardo et al. 1998) once the markers generate information on allelic diversity of the analyzed groups and phylogenetic relationships (Oliveira 2005). The simple sequence repeats (SSR) are short nucleotide sequences (1 to 10 bp), repeated *in tandem*, occurring in prokaryotes and eukaryotes (Pádua et al. 2005; Vieira et al. 2016). Sequences that flank the microsatellites are conserved even among species, which are used for drawing primers and amplification of SSR (Goetze et al. 2013). Studies have been already performed in which SSR were used to confirm interspecific hybridization between species of the genus *Passiflora* (Melo et al. 2016). In addition, successful assessment of the transferability rates of SSR primers in wild species of *Passiflora* has been made (Silva et al. 2014).

GISH can be used to confirm interspecific crossings (Souza et al. 2020); this technique can be explored by distinguishing the parental genomes in hybrid genotypes, differentiating the chromosomal lots from the supposed parents (Silva and Souza 2013; Younis et al. 2015; Ramzan et al. 2017). The use of GISH can be broadened, that is, the technique can be used to assess the phylogenetic inferences between species with genomic homology (Melo et al. 2017; Silva and Souza 2020). GISH technique also can be used to observe the occurrence of introgression of genes of interest in the genetic improvement of plants (Ramzan et al. 2017), beyond confirming hybridization produced by apomythic species and allopolyploid species (Younis et al. 2015). In reports based on the study of meiotic behavior, it has been revealed that the GISH technique assists in the analysis of chromosomal matching between homologous and homeologous chromosomes (Xie et al. 2010, 2014). It is possible, using GISH, to observe the formation of multivalent, bivalent, univalent chromosomes, and chiasmata frequency among the homeologous chromosomes (Younis et al. 2015). GISH can also be used to obtain important information about factors that cause meiotic irregularities and how these factors affect fertility in plants (Silva and Souza 2013). Thus, in this study, we aimed to (i) produce interspecific hybrids of *Passiflora* for ornamental use; (ii) confirm hybridization with the use of molecular markers and GISH; (iii) analyze meiotic behavior, pollen viability of the parental species, and hybrid genotypes.

Material And Methods

Plant material and Interspecific hybridizations

Passiflora species used for artificial interspecific crossing in the present study were maintained at the *Passiflora* work collection, located at the Universidade Estadual de Santa Cruz (UESC), in Ilhéus, Bahia, Brazil (39° 10" W, 14° 39" S, 78 m a.s.l.). Artificial crossings were performed between species *P. vitifolia* Kunth (accession 481) vs. *P. hatschbachii* Cervi (accession 486) from October 2015 to January 2016, during the early hours of the day, that is, between 7:00 and 8:00 a.m. in the anthesis flowers *P. vitifolia* and *P. hatschbachii* were used as pollen recipients and donors, respectively. On the previous day, pre-anthesis flower buds of the maternal parent *P. vitifolia* were emasculated and protected using paper bags. PG from the paternal parent (*P. hatschbachii*) were collected in bulk and the flowers with receptive stigma from the maternal parent, already curved, were pollinated manually, and protected again using paper bags. The fruits, because of crosses performed, were identified and protected with nylon nets until complete maturation.

Cultivation Conditions

The collected seeds were placed in Promalin® for 72 h to break dormancy. In all, 202 seeds were placed in plastic trays containing a commercial substrate. Sixty-seven hybrid seedlings were transplanted and kept in 1.5 L polyethylene bags, containing substrate, and in 50% shade in a green house. After 90 days, hybrid plants were brought to the field in and kept in sunlight. All plants obtained from the interspecific crosses were named as progeny HD26. The analyzes were carried out in the parents and eight hybrid progeny genotypes that were randomly chosen: HD26-104, HD26-105, HD26-118, HD26-136, HD26-137, HD26-143, HD26-146, and HD26-152.

Extraction of genomic DNA and SSR amplification

The total genomic DNA was extracted from fresh and young leaves from parents and putative hybrids using a CTAB-based protocol (Doyle and Doyle 1990) with some modifications (Viana et al. 2006). DNA quantification was done, and quality was assessed using a Nanodrop DN-1000 spectrophotometer. We performed marker SSR cross-amplification tests of primers originally developed for *P. alata* (Pádua et al. 2005) and *P. edulis* (Oliveira 2008) (Table 1) in the parents and putative hybrids. Amplification reactions for microsatellite markers were performed in a total volume of 20 µL containing 20 mM (pH 8.3) Tris–HCl, 50 mM KCl, 2.5 mM $MgCl_2$, 2.5 mM of each desoxinucleotides (dATP, dTTP, dGTP, and dCTP), 10 pmol of each primer (forward and reverse), one unit of Taq polymerase enzyme, and 20 ng/µL of DNA. The amplification conditions were as follows: initial denaturation at 95°C for 5 min, followed by 38 denaturation cycles at 94°C for 1 min, annealing temperature of 56°C for 40 s, extension at 72°C for 50 s, and a final extension at 72°C for 7 min. Agarose gel (1% stained SYBR®) was used to check amplification profiles, using TBE 1× and the molecular marker Ladder 100 bp to observe the size of the amplified DNA fragments, allowing confirmation of hybridization.

Table 1
Sequences of SSR primers used to confirm cross-fertilization in F_1 HD26 progeny hybrids (*P. vitifolia* vs. *P. hatschbachii*)

Primers	Sequence of primers		
	Primer forward	*Primer reverse*	Ta (°C)
Pa04	GGGCGGAAGAAAAGAGAAG	GAAACACACGATGCGAAAA	58°C
Pa07	CACATTTGCCGTCACTGG	CGGCATACGATAAATCTCCTG	56°C
Pe54	TGGTGTGTGTGGGTGATTAG	CATTCTCCTGCCACCTGAGT	58°C
Pe59	GAACACTTCGCATGGCTAGA	TTCCGAATCAAACCGTAACT	56°C
Pe64	ATCAATTACGCACCCCAAAC	GGAACGTCAATCAAGTGAGGA	56°C
Pe90	TCAGGAAGATTGCATGTTAGT	CTGGGTTTTGTTTATGTTGC	58°C
Pa, *P. alata*; Pe, *P. edulis*; At, Temperature of annealing			

Meiosis and post-meiosis

Flower buds at different stages of development were collected in the morning. The buttons were placed in Carnoy solution [ethanol: glacial acetic acid (3:1, v/v)] at room temperature (RT) (Johansen 1940) with exchange of Carnoy being carried out at 30 and 180 min and stored at -20°C for 24 hours. The buds were transferred to 70% ethanol and kept at ± 10°C until analysis. For the observation and counting of meiotic phases and post-meiotic products, one anther from each button was analyzed, and temporary slides were prepared using the squash technique and stained with 1% acetic carmine (Souza and Pereira 2011). Fifty cells of each genotype were observed for each meiosis phase. The frequencies of terminal, interstitial, and total chiasmata were verified by calculating the recombination index (RI = [Σn° total number of chiasmata ÷ n° of cells analyzed] + *n* value) (Darlington 1958). We considered the occurrence of one chiasma for the paired bivalents in baton (rod) and two chiasmata in ring-shaped pairing (ring) (Senda et al. 2005). The meiotic index (MI (%) = [n° of normal tetrads × 100] ÷ n°. of post-meiotic products counted) (Love 1951) was calculated from the number of post-meiotic products (monads, dyads, triads, tetrads, and polyads) and for analysis of variance (ANOVA), considering a completely randomized design, with replicates of four slides per genotype, the SISVAR open-source computer program was used.

In order to register meiotic irregularities, slides of meiotic phases were prepared in accordance with the report by Pierre et al. (2011), for the removal of the callose and staining with DAPI. Adaptations were made using this methodology: the anthers were washed twice using distilled water, for 5 min each time, by agitating, and macerated in the enzyme pectinase (Sigma); and the Eppendorf tubes with the macerated anthers were incubated at 37°C for 20 min and then centrifuged for 10 min to 2000 rpm to obtain suspension cells. The supernatant was discarded and centrifuged for 10 min at 2000 rpm with distilled water. Again, the supernatant was discarded and 20 µl of 45% acetic acid was added to obtain the cell suspension, which was dripped on the slides, covered with 20 × 20 mm coverslips, and then placed in liquid nitrogen for 15 min for the fixation of the material on the slides. Furthermore, the slides were dried at RT for

another 15 min and stained with 2 µL DAPI (2 µg/mL; Sigma) and detected with a U-MWU filter (330–385 nm excitation/emission > 420 nm) and photographed under an Olympus BX41 epifluorescence microscope equipped with a 5M Olympus DP25 digital camera and DP2-BSW software.

Preparation of probes for genomic in situ hybridization (GISH)

For genomic differentiation using the GISH technique, slides were made with the use of anthers in the diakinesis subphase (prophase I) and metaphase I phase, and the procedure for the preparation of slides was the same as that used for meiosis with irregularities, as described in the previous section. To make the probe, the genomic DNA of the parental species was fragmented using a sonicator (QSonica), through programming: 40% amplitude, 2 s on, and 2 s off in a total period of 5 min each (Jauhar and Peterson 2006), and the fragment size was verified by electrophoresis in 2% agarose gel (Pronadisa) using a 100 bp ladder as the marker (New England Biolabs) and stained with SYBR safe (Invitrogen), with the preference of using cleaved fragments between 200 and 1000 bp. The probes were marked by Nick Translation following the protocol proposed by the manufacturer, labeling the paternal parent probe (*P. hatschbachii*) with Biotin-16-dUTP (Roche Diagnostics) and maternal parent probe (*P. vitifolia*) with digoxigenin-11-dUTP (Roche Diagnostics), with a final concentration of 1 µg of cleaved DNA from each parent.

GISH in meiosis

The GISH technique followed the protocol proposed by Schwarzacher and Haslop-Harrison (2000) with modifications by Melo et al. (2015). Slides with cytological preparations were dried at 37°C for at least 1 h. Following treatment with 50 µL of a solution containing 100 µg/ml RNase (Sigma-Aldrich) in 2×SSC (saline-sodium citrate buffer; 0.3 M sodium chloride [Sigma] and 0.03 M sodium citrate [Sigma]), the slides were incubated in a humid chamber at 37°C for 1 h. The slides were washed twice in 2×SSC for 5 min each. HCl 50 µl 10 mM hydrochloric acid (HCl; Vetec) were added over the slides for 5 min. Following this, HCl was removed and replaced with 50 µl of pepsin (Sigma) [10 mg pepsin/ml, 10 mM HCl (1:100 v/v)] and the slides were incubated in a humidified chamber for 20 min at 37°C. Then, the slides were washed twice in 2×SSC for 5 min, immersed in 4% paraformaldehyde (Sigma) at RT for 10 min, and washed twice in 2×SSC for 5 min. The slides were dehydrated in 70% and 95% ethanol for 5 min each and dried at RT for 30 min. The hybridization mixture (50% formamide, 10% dextran sulfate, 2×SSC, and 0.13% sodium dodecyl sulfate) was added at a final volume of 15 µL. For the hybridization mixture, 33 ng of each DNA probe parent was added, which was heated at 75°C for 10 min in a thermocycler (Eppendorf, Mastercycler) and placed on ice for 5 min. The slides with the hybridization mixture were denatured in a thermocycler with a slide adapter (Techne, TC-412) at 75°C for 10 min and incubated overnight at 37°C in a humidified chamber. After hybridization, slides were immersed in 2×SSC for 5 min at RT to facilitate coverslip removal, moved to a Dubnoff bath (Marconi, MA093/1/E) set at 42°C, and immersed in 2×SSC for 5 min each, twice in 0.1×SSC for 5 min each, and twice again in 2×SSC for 5 min each. Finally, slides were dipped in 4×SSC containing 0.2% Tween 20 (Sigma) at RT for 5 min and then treated with 50 µl of 5% bovine serum albumin (BSA; Sigma). Biotin-labeled probe was detected using 0.7 µL of avidin-fluorescein isothiocyanate (FITC; Vector), and the digoxigenin-labeled probe was detected using 0.7 µL of anti-digoxigenin-rhodamine plus 18.6 µL of 5% BSA solution per slide. Slides containing the antibody for detection were incubated in a humid chamber

at 37°C for 1h, and three baths were performed in 4×SSC/0.2% Tween 20 in RT for 5 min each to remove excess antibodies. The slides were quickly immersed in 2×SSC, mounted, and counterstained with DAPI/Vectashield (Vector H-1200), and stored at 8–10°C until analysis.

Hybridizations were photodocumented using an epifluorescent Olympus BX41 microscope equipped with a 5 MP Olympus DP25 digital camera and DP2-BSW software. Hybridizations detected using avidin FITC were visualized with a U-MWB filter (excitation 450–480 nm/dichroic cutoff 500 nm/emission > 515 nm), while hybridizations detected using anti-digoxigenin rhodamine were visualized using a U-MWG filter (excitation 510–550 nm/dichroic cutoff 570 nm/emission > 590 nm). DAPI counterstaining was detected with a U-MWU filter (excitation 330–385 nm/dichroic cutoff 400 nm/emission > 420 nm). Slide images were processed using Photoshop SC5.

Pollen viability and in vitro germination

To perform pollen viability and *in vitro* germination tests, flowers were collected randomly at the beginning of the anthesis in the morning. PG corability was tested with (*i*) fluorescein diacetate (FDA) (Sigma) (Heslop-Harrison and Heslop-Harrison 1970), fluorochrome which indicates the presence of esterase enzyme activity related to the integrity of the plasmalemma, PG whose cytoplasm were stained fluorescent greenish yellow were considered viable, and (*ii*) Alexander solution (Alexander 1969), which used triple staining with Orange G (intensifier), basic Fuchsin (stains the cytoplasm red; Sigma) and Malachite green (stains the PG wall green) which provided the reactivity of the wall/cytoplasm. With the use of FDA dye, viable PG showed a yellow-green fluorescent color. We used 750 µL of the FDA solution [20 mg FDA diluted in 10 mL of acetone (Merck) + sucrose solution (Synth) 30% (3 g sucrose + 10 ml water)] and 250 µl glycerol (Dinamica) in the ratio 3:1 v/v. The slides were prepared by adding a drop of glycerol to the FDA and incubated for 10 min in a humid chamber at 37°C. The PG were counted and observed for colorability using the Olympus BX41 epifluorescence microscope with U-MWB filter (450–480 nm excitation/emission > 515 nm), equipped with an Olympus DP25 5M digital camera and DP2-BSW software. We used four different button anthers of each genotype (four repetitions), totaling 2,000 PG counted by genotype. Alexander solution was used to differentiate the PG into viable (with stained and intact cytoplasm) and unviable, which were classified as T1 (contracted cytoplasm); T2 (absence of cytoplasm - empty); T3 (micropollen), and T4 (giant). We used four different button anthers of each genotype (four repetitions), totaling 4,000 PG counted by genotype. The results were submitted to ANOVA with the aid of the open-source computer program SISVAR.

For *in vitro* germination of pollen, culture medium was used by following the protocol proposed by Bruckner et al. (2000), with modifications. The PG from each anther were put in a slide with a drop of autoclaved medium (0.10 g/L of boric acid (H_3BO_4) (Merck), 50 g/L of sucrose (Merck), and 0.3 g/L calcium nitrate tetrahydrate ($Ca(NO_3)_2$. $4H_2O$) (Merck); 0.2 g/L magnesium sulfate heptahydrate ($MgSO_4$. $7H_2O$) (Merck), and 0.1 g/L of potassium nitrate (KNO_3) (Merck)) and incubated in a humid chamber at 37°C for 24 h. Germinated GP were counted by genotypes totaling 500 GP per slide. The results were submitted to Skott–Knott test with SISVAR (Ferreira 2011).

Results

Interspecific crossing confirmation via SSR

Hybrid progeny derived from *P. vitifolia* vs. *P. hatschbachii* were named as HD26. Of the tested primers, Pa07 amplified the polymorphic locus in the parents, confirming the hybrid character of the F_1 hybrid progeny. The DNA amplification products showed an informative band of the maternal parent of approximately 280 bp, and another informative band of the paternal parent was about 257 bp. The heterozygote profile with bands inherited from the genitor species can be observed in hybrids (Fig. 1).

Meiotic behavior

The parents and hybrids progeny analyzed presented haploid number $n = 9$ and regular meiosis. In chromosomal pairing, nine bivalents (II) were observed in 90% of diakinesis cells. The species used in the interspecific hybridization in this study, *P. vitifolia* and *P. hatschbachii* have high cariotypical compatibility, with a chromosomal number of $2n = 18$; this made it possible to observe a high frequency of bivalent chromosomes in F_1 hybrids (above 90%). It were analyzed interspecific hybrids and observed regular meiotic behavior with chromosomal alignment in the metaphase plate, anaphase with regular segregation, and formation of four haploid nuclei at the end of telophase II (Fig. 2a-h) in 90% of the cells. Regarding the chromosomal pairing configuration, the formation of bivalents in diakinesis was greater than 90%, both in parents and in hybrids, but it was observed some irregular chromosomal pairing (Table 2; Fig. 3).

Table 2
Chromosomal pairing configuration in diakinesis in *Passiflora* parents and F_1 HD26 progeny hybrids (*P. vitifolia* vs. *P. hatschbachii*)

Genotype	Chromosomal Pairing Configuration (%)										
n = 9	9II	6II + 6I	5II + 8I	5II + 2I + 1III	5II + 2IV	4II + 10I	3II + 12I	2II + 10I + 1IV	2II + 14I	1II + 16I	18I
P. vitifolia	95.8	-	-	-	-	-	-	2.2	-	-	2.0
P. hatschbachii	90.5	1.8	-	-	-	2.2	-	2.0	1.5	-	2.0
HD26-104	94.2	-	-	-	-	-	1.8	-	-	2.0	2.0
HD26-105	93.0	-	-	-	-	2.0	-	-	1.8	1.5	1.7
HD26-118	93.0	-	2.0	-	-	-	-	-	1.5	1.0	2.5
HD26-136	92.4	-	-	-	-	0.8	-	-	2.8	1.5	2.5
HD26-137	92.2	-	-	-	1.8	2.0	-	-	2.0	-	2.0
HD26-143	91.8	0.8	-	-	0.6	1.0	1.8	-	1.8	0.8	1.4
HD26-146	90.8	1.6	-	-	1.0	1.3	1.5	-	-	1.8	2.0
HD26-152	94.0	-	-	1.8	-	-	-	-	-	2.0	2.2

I, Univalent; II, Bivalent; III, Trivalent; IV, Tetravalent

Terminal and interstitial chiasmata were observed in all genotypes, except in the HD26-152 hybrid that did not present terminal chiasmata and resulted in the highest mean of interstitial chiasmata. RI ranged between 9.3 (HD26-118) and 11.1 (HD26-146) (Table 3). The analysis of variance showed a significant difference ($P < 0.05$) in the frequencies of terminal and interstitial chiasmata between hybrid genotypes and parents (Table 4). In this study, there was occurrence of interstitial chiasmata in all genotypes examined. In the HD26-152 genotype, we could not visualize terminal chiasmata; however, recombination was not impaired, because it is the interstitial chiasmata that guarantees recombination between chromosomes.

Table 3
Number and location of chiasmata observed per cell in diakinesis and recombination index (RI) in *Passiflora* parents and F_1 HD26 progeny hybrids (*P. vitifolia* vs. *P. hatschbachii*)

Genotype (*n* = 9)	Terminal		Interstitial		TCh Mean	RI
	Mean	(Min – Max ± sd)	Mean	(Min – Max ± sd)		
P. vitifolia	6.50	(2–8 ± 3)	4.75	(4–5 ± 0.5)	11.25	9.9
P. hatschbachii	13.50	(10–20 ± 4.72)	8.25	(3–10 ± 3.5)	21.75	10.7
HD26-104	4.50	(3–6 ± 1.73)	3.50	(3–4 ± 0.57)	8.00	9.6
HD26-105	5.75	(4–8 ± 1.70)	6.75	(6–8 ± 0.95)	12.50	9.7
HD26-118	1.50	(0–3 ± 1.73)	2.50	(1–5 ± 1.73)	4.00	9.3
HD26-136	3.00	(3–3 ± 0)	3.00	(3–3 ± 0)	6.00	9.5
HD26-137	10.50	(10–12 ± 1)	5.00	(4–6 ± 0.81)	15.50	10.2
HD26-143	5.50	(4–6 ± 1.29)	7.50	(6–9 ± 1.29)	13.00	10.9
HD26-146	7.25	(6–8 ± 0.95)	5.75	(5–7 ± 0.95)	13.00	11.1
HD26-152	0.00	(0–0 ± 0)	12.50	(10–15 ± 2.38)	12.50	10.9

Min, minimum; Max, maximum; sd, standard deviation; TCh, total chiasmata.

Table 4
Summary of analysis of variance for chiasmata (C) types in *Passiflora* parents and F_1 HD26 progeny hybrids (*P. vitifolia* vs. *P. hatschbachii*)

SV	DF	MS	
		C Terminal	C Interstitial
Genotype	9	0.00*	0.00*
Error	30	1.03	0.80
CV (%)		35.82	26.93

SV, source of variation; DF, degree of freedom; CV, coefficient of variation; MS, mean square, *P < 0.05

Irregularities in meiotic behavior were also observed during meiosis I and II (Fig. 2i–k; Table 5), such as early and/or laggard chromosomes, spindle irregularities and asynchrony (Fig. 4). Some meiocytes presented chromosomal bridge formation during anaphase II segregation (Fig. 4f and 4h). Spindle irregularities were found in all genotypes in telophase II, and transverse spindles (chromosomal sets positioned on the equatorial plate in “T” shape) and tripolar spindles (“V” shape) were observed in meiocytes in metaphase and anaphase II (Fig. 4h and 4i). In meiosis II, asynchrony was the irregularity least found among genotypes (Table 5), with meiocytes presenting a chromosomal group in prophase II, while the other was in metaphase

II, or one chromosome group in metaphase II and the other initiating anaphase II and meiocytes with one chromosomal group in anaphase II and the other beginning and/or end of telophase II.

Table 5
Cells displaying irregularities in meiotic behavior in *Passiflora* parents and F_1 HD26 progeny hybrids (*P. vitifolia* vs. *P. hatschbachii*)

Genotype	Early/laggards Chromosome (MI - TII)						Spindle irregulaties (MII - TII)			Laggards/spindle irregulaties (MII - AII)		Asynchrony (MII - AII)	
	MI	AI	TI	MII	AII	TII	MII	AII	TII	MII	AII	MII	AII
P. vitifolia	-	-	-	-	-	-	-	-	58	-	-	-	-
P. hatschbachii	-	14	-	-	-	-	-	-	-	-	-	16	12
HD26-104	-	-	-	10	10	8	30	34	24	-	2	12	10
HD26-105	28	-	12	-	6	-	-	16	16	-	-	-	-
HD26-118	-	-	14	-	-	8	-	-	22	-	-	-	-
HD26-136	2	6	4	50	28	14	50	36	52	-	4	18	12
HD26-137	-	-	2	6	-	-	-	8	2	-	-	-	-
HD26-143	46	2	52	42	24	-	-	6	10	26	-	-	-
HD26-146	-	-	-	6	8	2	8	6	52	-	-	14	8
HD26-152	8	10	2	2	6	-	-	28	2	-	4	-	-
M, metaphase; A, anaphase; T, telophase; I, meiosis I; II, meiosis II													

The progeny hybrids and parental *Passiflora* evaluated presented a meiotic index (MI) above 90%, which can be considered to represent cytological stablity and the hybrids being potentially fertile. The analysis of post-meiotic products showed normal tetrad in more than 90% of genotypes (Table 6, Fig. 2l). However, irregular post-meiotic products such as monads, dyads, triads, and polyades were also found (Table 6, Fig. 2m – 2o). Triads were the most frequently found irregularities in all genotypes (Fig. 2m), while other irregularities did not reach 0.5% of the analyzed cells. The micronuclei observed in this study probably originated as a result of the metaphases with early chromosomes and laggard chromosomes in the anaphase (I and II).

Table 6 Mean values of post-meiotic products (normal tetrads and irregularities) and meiotic index (MI) in *Passiflora* parents and F_1 HD26 progeny hybrids (*P. vitifolia* vs. *P. hatschbachii*)

Genotype	Normal Tetrads	PMP Irregularities				Total PMP	MI (%)
		Monads	Dyads	Triads	Poliads		
P. vitifolia	1.250	-	-	0.28	-	1.278	97.8%
P. hatschbachii	1.735	-	-	0.32	-	1.767	98.2%
HD26-104	1.400	-	-	0.37	-	1.437	97.4%
HD26-105	1.800	0.01	-	0.31	0.06	1.838	97.9%
HD26-118	1.555	-	-	0.41	-	1.596	97.4%
HD26-136	1.890	-	-	0.23	-	1.913	98.7%
HD26-137	1.654	-	-	0.29	-	1.683	98.3%
HD26-143	1.598	-	-	0.32	0.04	1.634	97.7%
HD26-146	1.757	-	-	0.39	-	1.796	97.8%
HD26-152	1.457	-	0.01	0.34	-	1.492	97.6%

PMP, post-meiotic product; MI, meiotic index

GISH

Interspecific hybridization was confirmed with the use of the GISH technique, which was applied in the diakinesis phases of prophase I and at the beginning of metaphase I. It was possible for us to distinguish the genome of the parent species in the hybrid genotypes and visualize pairing between homeologous chromosomes in the HD26 progeny ($n = 9$), with the formation of bivalents (Fig. 5). In HD26-143, homeologous chromosomes paired on the metaphasic plate and connected to the spindle fiber were observed, showing regular meiosis (Fig. 5a). In HD26-146, tetravalent formation was observed among homeologous chromosomes (Fig. 5b).

Pollen viability and in vitro germination

The PG reacted positively to the histochemical test with the dyes used, Alexander solution, and fluorescein diacetate both in the parents and HD26 progeny hybrids (Fig. 6). Alexander test was useful in enabling the observation of viable PG with intact wall and cytoplasm, in addition to the four types of unviable PG. The percentage of viable PG was 65.5% and 96.5% in the *P. vitifolia* and *P. hatschbachii* parents, respectively. In hybrids, the highest percentage of viable PG was observed in the HD26-136 genotype with 84.1%, and the lowest percentage in the HD26-152 genotype with 52.8% (Table 7). Among the parents, a higher percentage of unviable PG was found in *P. vitifolia*, with predominance of unviable PG T4. For hybrids HD26-136 and HD26-143 genotypes presented lower percentages of unviable PG. The highest percentage of PG T1 was found in the hybrid HD26-152 (17.9%), PG T2 was found in the hybrid HD26-152 (15.2%), PG T3 was found

in HD26-136 (2.8%) and PG T4 was found in the hybrid HD26-118 (5.7%) (Table 7; Fig. 6). The analysis of variance showed a significant difference ($P < 0.05$) in pollen viability with Alexander solution and for the different types of unviability, T1 to T4 (Table 8). With regard to FDA staining, the analysis of variance showed no significant difference between analysed variables (viable and unviable pollen grains) (Table 8), however the use of this technique was efficient, revealing viable fluorescent yellow-green PG with percentage values from 34.3 (parental) to 95.6 (hybrid).

Table 7
Mean percentage values of viable and unviable PG using Alexander solution and fluorescein diacetate (FDA) in *Passiflora* parents and F_1 HD26 progeny hybrids (*P. vitifolia* vs. *P. hatschbachii*)

	Alexander solution					**FDA**
Genotype	% PG Viable	% PG T1	% PG T2	% PG T3	% PG T4	% PG Viable
P. vitifolia	66.5	10.4	9.4	1.6	12.0	34.3
P. hatschbachii	96.5	2.3	1.0	0.09	0.06	54.0
HD26-104	70.5	11.1	14.8	0.1	2.5	35.2
HD26-105	73.9	10.5	10.3	0.7	4.6	95.6
HD26-118	63.2	11.9	17.3	1.8	5.7	49.8
HD26-136	84.1	5.7	5.5	2.8	1.9	44.7
HD26-137	64.8	11.1	18.7	0.2	5.2	53.7
HD26-143	83.8	8.2	3.6	0.3	4.1	64.9
HD26-146	69.0	10.7	14.4	1.0	4.9	61.2
HD26-152	52.8	17.9	24.7	1.1	3.4	93.4
PG, pollen grains; T1-T4, unviable; T1, contracted, T2, empty; T3, micropollen; T4, giant						

Table 8
Summary of analysis of variance for total viable (V) and unviable (Unv) PG with fluorescein diacetate and Alexander solution in *Passiflora* parents and F_1 HD26 progeny hybrids (*P. vitifolia* vs. *P. hatschbachii*)

MS								
Fluorescein diacetate				Alexander solution				
SV	DF	PG V	PG Unv	PG V	PG Unv T1	PG Unv T2	PG Unv T3	PG Unv T4
Genotype	9	10.04^{NS}	10.04^{NS}	0.08*	0.00*	0.00*	0.03*	0.00*
Error	30	3064.3	3064.3	25481.1	1111.4	1659.0	123.2	493.9
CV (%)		53.44	75.90	26.30	39.16	39.30	117.18	56.80

MS, mean square; SV, source of variation; DF, degree of freedom; CV, coefficient of variation; PG, pollen grains; T1, contracted; T2, empty; T3, micropollen; T4, giant; *P < 0.05; NS, not significant

For *in vitro* germination, the paternal parent obtained the highest percentage of germinated PG (62.3%) (Fig. 5b) and among hybrid genotypes, HD26-137 showed the very low percentage (11.2%). In general, hybrid genotypes presented a low percentage of pollen germination, and there was no germination for some hybrid genotypes analyzed (Table 9). The floral anthesis of hybrid genotypes and maternal parent (*P. vitifolia*) occurs around 7.30 a.m. in the morning. For the reason that collection was done in the winter, during rainy days, collection was done in the morning between 9:00 a. m. and 10:00 a.m. Hence, the collection occurred after anthesis of the genotypes, because of which unviable PG were observed in histochemical tests, and which also have affected *in vitro* germination. The high percentage in the *in vitro* germination test found for parent *P. hatschbachii* corroborated with the high pollen viability shown with Alexander solution. We observed a significant difference between genotypes, evaluated by the Skott-Knott average test, with *in vitro* pollen germination.

Table 9
In vitro germination of pollen grains (PG) in *Passiflora* parents and F_1 HD26 progeny hybrids (*P. vitifolia* vs. *P. hatschbachii*)

Genotype	Number of germinated PG	% germinated PG
P. hatschbachii	311.50a	62.30a
P. vitifolia	15.00b	3.00b
HD26-104	3.25b	0.40b
HD26-105	0.00b	0.00b
HD26-118	19.50b	3.90b
HD26-136	0.00b	0.00b
HD26-137	56.00b	11.20b
HD26-143	0.00b	0.00b
HD26-146	33.50b	6.70b
HD26-152	6.25b	1.25b

Discussion

The molecular marker SSR confirmed interspecific crossing with the presence of specific band of each parent in hybrid genotypes. As a highly polymorphic, co-dominant marker, SSR allows differentiation between homozygous and heterozygous individuals (Zanella et al. 2017). This differentiation of polymorphic profiles through the SSR marker is possible because flanking regions are conserved within and between species as well as between genera or at higher taxonomic levels (Turchetto-Zolet al. 2013). Compared to other molecular markers, SSR is easily visualized and stable (Song et al. 2005). Furthermore, phylogenetically close species share conserved DNA sequences, and by homology, the transferability of primers in SSR regions in hybrid genotypes is facilitated (Kalia et al. 2011). SSR flanking regions can be found at many sites in the genome; however, these segments may be unique, and thus there may be a need for specific primers to be designed for SSR loci (Jones et al. 1997). In this study, we tested SSR primers drawn for *P. edulis* f. *flavicarpa* Deg. and *P. alata* Curtis. We obtained only amplification primers for *P. alata*, while other studies of *Passiflora* with primers of *P. edulis* f. *flavicarpa* were successful with high transferability (Belo et al. 2018). However, amplification of a few primers can confirm interspecific crossings. A specific locus of each genitor present in the hybrid progeny was sufficient to confirm hybridization (Conceição et al. 2011; Souza et al. 2012; Melo et al. 2016).

Chromosomal pairing between homologous characterizes meiosis I (Bozza and Pawlowski 2008; Anderson et al. 2015) and is based on a homologous sequence of DNA, which occurs along with recombination and synapses in prophase I (Bozza and Pawlowski 2008), marking one of the most important events of meiosis, which ends with the formation of crossover. In interspecific hybrids, the absence of pairing between the different parental genomes in the meiosis phases may compromise the reproductive potential of hybrids. In

addition, the genetic distance between the species involved in the crossing may lead to irregularities of linkage in meiosis, as was observed in the crossbreeding hybrids of Orchdaceae Juss., *Paphiopedilum delenatii* Guillaumin× *P. callosum* (Rchb.f.) Stein and *P. delenatii* × *P. glaucophyllum* J.J.Sm. (Lee et al. 2011). The pairing of homeologous chromosomes in hybrids increases the possibility of new allelic combinations and is directly related to the viability/fertility of hybrids (Abreu et al. 2009).

Different chromosomal association such as univalent, trivalent, and tetravalent (evidence for structural change) found in this study suggest similar genetic structure between the species involved in the crossing and are the most common for *Passiflora* species (Souza et al. 2012). Furthermore, these irregular chromosome pairing configurations did not interfere with regular chromosome segregation at the end of meiosis, because, there was the presence of at least one chiasmas per bivalent, confirming chromosomal pairing in hybrid genotypes. Univalent formation is directly related to low-frequency chiasmata, which can affect pollen viability (Pagliarini 2000; Souza and Pereira 2011). In addition, chromosomal configurations such as univalent and multivalent are frequent in *Passiflora* hybrids (Soares-Scott et al. 2003; Souza and Pereira 2011).

Terminal chiasmata ensures perfect disjunction as well as segregation of homologous chromosomes, not interfering with recombination index (Souza et al. 2012). Other studies on *Passiflora* species and hybrids corroborate high index of interstitial chiasmata (Souza et al. 2003; Souza and Pereira 2011). The average frequency of chiasmata per bivalent has relevance in meiosis stability (Pagliarini 2000), and the average in our study can be considered high for *Passiflora* species, which is an important aspect that can prevent premature separation of bivalents, resulting in univalent chromosomes (Souza and Pereira 2011). Chiasmata plays a key role in maintaining bivalents together during prophase I and is the result of crossing over.

The meiotic irregularities found here most commonly occur in *Passiflora* species (Pagliarini 2000; Souza et al. 2003; Souza et al. 2008; Pereira et al. 2017; Souza et al. 2020) such an asynchronism, chromosomal bridges and disorientation of spindle fibers that can be caused by the depolymerization of spindle fibers, leading simultaneously to two chromosomal sets being in different stages of meiosis (Souza et al. 2003). As well as, asynchronism occur because these chromosomes do not reach the telophase phase for regular chromosomal segregation (Soares-Scott et al. 2003; Corrêa et al. 2005; Damasceno et al. 2010; Kiihl et al. 2011; Souza and Pereira, 2011). In addition, paracentric translocations and inversions can cause the emergence of chromosomal bridges (Levin 2002; Paiva et al. 2012). In studies based on cereals, it was reported that sticky chromosomes may have caused the origin of chromosomal bridges in the phases of anaphases (Paiva et al. 2012).

The meiotic index between 90% and 100% give the plants a stable cytology confidence, and the lower limit is 82.77% and higher than 93.63%, which consists of the confidence interval for the meiotic indices (Love 1951). In our study we found meiotic indices of 90% which represent cytological stability. The main irregular post-meiotic products such as triads may be caused by the occurrence of irregularity spindle fibers (Souza and Pereira 2011) as well as errors in cytoplasmic division during meiosis I and II (Moreira et al. 2017). In addition, micronuclei can also be formed by cytomixis (transfer of material from one cell to another)

(Diegues et al. 2015), but this event was not visualized in this study. Post-meiotic products with irregularities can originate from gametes with an extra chromosomal set, non-degraded gametes, or generate aneuploid gametes (Souza et al. 2003), or unbalanced PG (Moreira et al. 2017) or sterile (Souza et al. 2008).

The GISH technique confirmed the interspecific crossing between the parental species *P. vitifolia* and *P. hatschbachii* applied to meiocytes in the diakinesis subphase (Prophase I) and in metaphase I as well as allowing the visualization of chromosomal pairing. Although the diakinesis subphase in prophase I is the ideal stage for viewing chromosomal pairing because of the difficulties encountered in eliminating or decreasing physical barriers in meiocyte (cell wall, calosis, and cytoplasm) cells were also used in metaphase I. In studies based on banana (*Musa* L.), the same difficulties were observed with regard to breaking of the cell wall and the cytoplasmic density (Capdeville et al. 2009; Jeridi et al. 2011); these physical barriers hinder the probe of chromosomes (Jeridi et al. 2011), requiring an adequate number of protocols.

In this study, using GISH, it was possible for us to observe the pairing between homeologous chromosomes as well as pairings between chromosomes of the same genome. Plant genomes share many repetitive DNA regions, and in *Passiflora*, this type of DNA is part of the genome (Silva et al. 2018). Chromosomes of the same genome are common repetitive DNA regions, which may explain the pairing between these chromosomes. Homeologous chromosome pairings were observed in all hybrid genotypes evaluated, confirming the existence of homology between genomes, which consequently allows recombination between chromosomes of different species. GISH on meiotic chromosomes was used to confirm polyploidy in *Dahlia* Cav. by interspecific crossing, which formed bivalents in cell pairing between parental genomes in the metaphase I subphase; homeologous and homologous chromosomes were observed in the formation of tetravalents, trivalents, and univalents (Gatt et al. 1999). In this study, the formation of tetravalents and multivalents was observed. Interspecific hybrids of *Musa acuminata* Colla (AA) and *M. balbisiana* Colla (BB) were also found to be univalent, bivalent, trivalent, and multivalent among homeologous chromosomes (Jeridi et al. 2011). The use of two probes simultaneously for the differentiation of parental genomes in hybrid genomes was satisfactory in this work; the application of this methodology has already been reported to be successful in other studies, which, in addition to differentiating parental genomes in interspecific hybrids in *Passiflora*, has also allowed the observation of recombinant chromosomes in backcrossed hybrids (Melo et al. 2015; Souza et al. 2020).

Pollen viability is directly related to regular meiosis. Plants with regular meiotic behavior present viable PG (Pereira et al. 2017), and it is important to measure the potentiality of fecundation and fertilization of the PG (Biondo and Battistin 2001; Soares et al. 2013). A high percentage of viable PG is related to the high index of normal tetrads, a result of regular meiotic behavior (Corrêa et al. 2005). In this study, the histochemical test with Alexander solution showed some hybrid genotypes with high pollen viability percentages, with 70% approximately. For *Passiflora* species, percentage value above 70% is considered high (Souza et al. 2002). Pollen viability can be favored by several factors, such as temperature variation, humidity, time, and storage conditions of the PG (Franzon et al. 2005). In addition, in *Passiflora*, high viability is favored because the morphology of the PG presents a substance known as pollenkitt that offers a sticky consistency to the PG, serving as protection, allied to physiological factors avoiding dehydration of the PG (Souza et al. 2002). In

some species, the correlation between the loss of pollen viability and water loss has been reported (Zanatto et al. 2009). Pollen viability with an average of less than 30% is considered low, while between 30% and 69% is considered moderate. In this study, the hybrid genotype with the highest percentage of pollen viability was 84% in HD26-136. In studies with interspecific pepper hybrids of the genus *Capsicum* L., the pollen viability of 72.5% was considered satisfactory (Moreira et al. 2017).

Over time, after flowering, pollen viability may be compromised, with the tendency to decrease. Variations throughout the day, such as temperatures, possibly cause the PG to become unviable (Belo et al. 2018). In addition to abiotic factors that may interfere (Abreu et al. 2009), pollen viability may vary among genotypes of the same species, cultivars, flowers of the same plant, anthers in the same flower, among other factors (Souza et al. 2002; Coelho et al. 2012). Studies about pollen feasibility that dynamically shows the changes that occur during flowering stages in a plant are important to assist in the characterization of reproductive barriers and in the selection of fertile PG (Deng et al. 2017). Floral development studies were performed by Soares et al. (2018), who evaluated *Passiflora* species, with regard to pollen viability, stigma receptivity, and *in vivo* and *in vitro* germination of PG, in the three stages of flowering: pre-anthesis, anthesis, and post-anthesis. The results show the ideal moment of PG fertilization potential and consequently the fructification is in the anthesis for the vast majority of the species analyzed. This moment of PG fertilization is fundamental to overcome pre-fertilization and post-fertilization barriers at crossings (Soares et al. 2018).

Contrary to what has been seen for the maternal parent (*P. vitifolia*) and some hybrid genotypes that had pollen viability and *in vitro* germination in high humidity, the humid environmental condition may have allowed water retention in the PG in *P. hatschbachii* and its viability may have been extended for a longer time after anthesis, maintaining its optimum temperature for germination (Souza et al. 2002). The anthesis of this species occurs early in the morning and the flowers remain open until dawn in optimum temperature conditions. Thus, environmental factors may influence pollen viability and consequently pollen germination, as was observed for the parent species in our study. Other tests of viability and germination of PG in *Passiflora* species are recommended, as there are few studies on pollen viability (Soares et al. 2013). Contracted PG are formed because of mutations in genes in the post-meiotic phase, which affect the androgametogenesis in *Passiflora* (Pagliarini 2000; Souza et al. 2003; Souza et al. 2008). The unviability of pollen can also be attributed to meiotic irregularities, such as univalent chromosomes and early and laggard chromosomes (Souza et al. 2002; Damasceno et al. 2010).

Pairing events, synapses, recombination that characterize regular meiosis and a post-meiosis with high pollen viability are likely interdependent and co-regulated (Hamant at al. 2006). Important multigene families that regulating the expression these genes have been identified as *DV* (Divergent spindles), *MS28* (Male sterility 28), *MS43* and *MPS1* (Multipolar Spindle 1) (Golubovskaya and Distanova, 1986; Jiang et al. 2009). The genes Synapsin 1 (*SYN1*), Differentiation-inducing factor 1 (*DIF1*), and the cohesin *AtREC8* in the plant pre-meiotic S-phase code for multiprotein cohesion complex (*REC8*/ *RAD2*/ *SMC1* and *SMC3*) which are required for replication sister chromatids, associated protein such as Aabsence of first division (*AFD1*) (Hamant et al. 2006, Lee and Orr-Weaver, 2001). Early prophase I stage the structures of meiotic chromosomes are established and the gene mutation *SWITCH1* (*SW11*) destabilizing the sister chromatid cohesion which compromises the formation of bivalents (Mercier et al. 2003).

Synapsis of homologous chromosomes during zygotene (prophase I) ensure pairing between bivalents with the installation of the synaptonemal complex (Hamant et al. 2006). The gene Meiotic asynaptic mutant 1 (*ASY1*) plays a significant role in the synapsis between homologous chromosome in *Arabidopsis* and the mutant *asy1* cause failure in the synapsis (Armstrong et al. 2002), as well as the mutants Meiotic recombination protein 1 (*atdmc1*) also led formation univalent chromosome (chromosomes remain unpaired) at late prophase I, it indicated that *AtDMC1* gene plays an important role in the formation of bivalents and chromosome segregation, in addition to restoring fertility in plants (Couteau et al. 1999). In studies with dicotyledonous plants from Indian (Kaur and Singhal, 2019) were found asynaptic mutants formation with chromosomes univalents and as consequences were observed high frequency of aberrant microspores, sterile gametes and heterogeneous sized PG. Another study with polyploidy wheat the 5B meiotic gene copy (*TaZIP4-B2* (gene *Ph1*); Rey et al. 2018) in addition homologous pairing, synapsis, crossover and suppressing homeologous crossover it preserves pollen viability and grain number fertile in polyploidy wheat (Alabdullah et al. 2021).

The culture medium used for the *in vitro* germination test simulates the conditions of receptivity of the stigma with the stimulus for pollen tube germination (Soares et al. 2013; Belo et al. 2015). The concentrations and the presence and absence of reagents determine the balance necessary for the success of the technique (Belo et al. 2015). Several compositions of the culture medium can be tested to arrive at the best specific protocol for each species (Vida et al. 2011). Some authors consider the addition of stimulating elements to the culture medium as a favorable factor for the germination of PG (Taylor and Hepler 1997). It has been reported in a study conducted on *Actinidia chinensis* var. *delicious* (A. Chev.) A. Chev. that when boric acid was added to the culture medium, the effect was significantly greater for the germination of the pollen in comparison to the condition in which boric acid was not added (Borghezan et al. 2011). The same result with the addition of boric acid was corroborated in a study with *Passiflora* species (Vida et al. 2011). Thus, the low percentage of germination of PG in the genotypes evaluated in this study may be moreover related to the late time of the collection and the environmental variables. The condition of the culture medium used in this study was also used in other studies for *P. suberosa* L. and *P. sublanceolata* (Killip) MacDougal, from which we could infer that the liquid culture medium was suitable for PG germination and also negatively pointed out the collection time hours after anthesis (Cruz et al. 2008; Belo et al. 2015).

Conclusions

The crossing of *P. vitifolia* versus *P. hatschbachii* resulted in progeny hybrid with behavior meiotic regular, high pollen viability and PG germination. The interspecific hybrids with high pollen viability, found in this study, may be useful in breeding programs as well as in the ornamental plant market because they may also be fertile and contribute with polymorphic loci in outcrossing for better use of genetic variability. The molecular tools such as SSR markers and GISH are effective to confirm the paternity of interspecific hybrids in *Passiflora*.

Declarations

Acknowledgements

The authors would like to thank UESC (Universidade Estadual de Santa Cruz), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and CAPES (Coordenação de Aperfeiçoamento de Pesssoal de Nível Superior) for the financial support and scholarships.

Funding:

The research was supported by Universidade Estadual de Santa Cruz with financial resources; by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) with the scholarship given for the first and third authors; CNPq for the research grant given for the second author.

Conflicts of interest/Competing interesting:

The authors are unaware of any conflict of interest or possible competition.

Availability of data and material:

Not applicable.

Code availability:

Not applicable.

Authors' contributions:

.Analu Cruz Souza: Conducting laboratory experiment, data analysis, article writing.

.Margarete Magalhães Souza: Assistance and collaboration in all stages of the research.

.Gonçalo Santos Silva: Assistance in writing the article.

.Manuella Palmeira Lavinscky: Assistance in laboratory

.Viviane de Oliveira Souza: Assistance in laboratory

.Jôsie Cloviane de Oliveira Freitas: Assistance in data analysis

Ethics approval:

Not applicable.

Consent to participate:

Attached emails.

Consent for publication:

Attached emails.

References

Abreu PP, Souza MM, Santos EA, Pires MV, Pires MM, Almeida AF (2009) Passion flower hybrids and their use in the ornamental plant market: perspectives for sustainable development with emphasis on Brazil. Euphytica 166:307-315

Alabdullah AK, Moore G, Martín AC (2021) A duplicated copy of the meiotic gene ZIP4 preserves up to 50% pollen viability and grain number in polyploidy wheat. Biology 10:290-310

Alexander MP (1969) Differential staining of aborted and non-aborted pollen. Stain Technology 44:117-122

Anderson CM, Oke A, Yamp P, Zhuge T, Fung JC (2015) Reduced crossover interference and increased ZMM-Independent recombination in the absence of Tel1/ATM. Plos Genetics 11:1-27

Armstrong S, Caryl AP, Jones GH, Franklin FCH (2002) Asy1, a protein required for meiotic chromosome synapsis, localizes to axis-associated chromatin in *Arabidopsis* and *Brassica*. Journal of Cell Science 115:3645-3655

Bellon G, Faleiro FG, Junqueira PK, Junqueira NTV, Santos EC, Braga MF, Guimarães CT (2007) Variabilidade genética de acessos silvestres e comerciais de *Passiflora edulis* Sims. com base em marcadores RAPD. Revista Brasileira de Fruticultura 1:124-127

Belo GO, Souza MM, Souza VO, Melo CAF (2015) Reproductive and cytogenetic characterization in *Passiflora sunlanceolata*. Biologia 70:1-11

Belo GO, Souza MM, Silva GS, Lavinscky MP (2018) Hybrids of *Passiflora*: *P. gardneri* versus *P. gibertii*, confirmation of paternity, morphological and cytogenetic characterization. Euphytica 214:1-13

Bernacci LC, Cervi AC, Giovanni R, Borges RAX, Hering RLO, Serrano T, Santos Filho LAF (2013) Passifloraceae. In: Martinelli G, Moraes MA (org). Livro vermelho da flora do Brasil. Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, Rio de Janeiro pp 830-834

Bernacci LC, Souza MM (2012) *Passiflora cacao* (Passifloraceae), a new species from southern Bahia, Brazil. Novon 22:1-7

Biondo E, Battistin A (2001) Comparação da eficiência de diferentes corantes na estimativa da viabilidade de grãos de pólen em espécies dos gêneros *Eriosema* (DC.) G. Don e *Rhynchosia* Lour (Leguminosae-Faboideae), nativas na região Sul do Brasil. Bioikos 1:39-44

Borghezan M, Clauman AD, Steinmacher DA, Guerra MP, Orth AI (2011) *In vitro* viability and preservation of pollen grain of kiwi (*Actinidia chinensis* var. *deliciosa* (A. Chev.) A. Chev). Crop Breeding and Applied Biotechnology 11:338-344

Bozza CG, Pawlowski WP (2008) The cytogenetics of homologous chromosome pairing in meiosis in plants. Cytogenet Genome Research 120:313-319

Bruckner CH, Silva MM, Falleiro TM, Andrade BB, Moreira AE (2000) Viabilidade do pólen de maracujazeiro sob diferentes condições de armazenamento. Revista Ceres 47:523–531

Capdeville G, Júnior MTS, Szinay D, Diniz LEC, Wijnker E, Swennen R, Kema GHJ, Jong HD (2009) The potential of high-resolution BAC-FISH in banana breeding. Euphytica 3:431-443

Cervi AC, Imig DC (2013) A new species of *Passiflora* (Passifloraceae) from Mato Grosso do Sul, Brazil. Phytotaxa 103:46-50

Cervi AC, Linsingen LV (2010) *Passiflora Kikiana*, a new species of Passifloraceae from the Brazilian Amazon. Acta Botanica Brasilica 24:1062-1064

Coelho APD, Morais KP, Laughinghouse IVHD, Giacomini SJ, Tedesco SB (2012) Pollen grain viability in accessions of *Crotalaria juncea*. (FABACEAE). Agrociencia 46:481-487

Conceição LDHCS, Souza MM, Belo GO, Santos SF, Freitas JCO (2011) Hybridization among wild passion flower species. Revista Brasileira de Botânica 34:237-240

Corrêa MGS, Viégas J, Silva JB, Ávila PFV, Busato GR, Lemes JS (2005) Meiose e viabilidade polínica na família Araceae. Acta bot. Bras. 19:295-303

Couteau F, Belzile F, Horlow C, Grandjean O, vezon D, Doutriaux MP (1999) random Chromosome Segregation without Meiotic arrest in both male and female meiocytes of a *dmc1* mutant of *Arabidopsis*. The Plant Cell 11:1623-1634

Cruz TV, Souza MM, Roza FA, Viana AJC, Belo GO, Fonseca JWS (2008) Germinação *In Vitro* de grãos de pólen em *Passiflora suberosa* L. para sua utilização em hibridação interespecífica. Revista Brasileira de Fruticultura 30:875-879

Damasceno JPC, Pereira TNS, Neto MF, Pereira MG (2010) Meiotic behavior of *Carica papaya* and *Vasconcellea monoica*. Caryologia 63:229-236

Darlington CD (1958) Evolution of genetic systems. London: Oliver and Boyd

Deng Y, Sun X, Gu C, Jia X, Lijian L, Su J (2017) Identification of pre-fertilization reproductive barriers and the underlying cytological mechanism in crosses among three petal-types of *Jasminum sambac* and their relevance to phylogenetic relationships. Plos One 12:1–19

Diegues IP, Damasceno JPC, Ribeiro NVS, Reis MVM, Abboud ACS (2015) Comportamento meiótico e viabilidade polínica na espécie *Jatropha curcas* L. Semina: Ciências Agrarias 36:141-150

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13-15

Fajardo D, Angel F, Grum M, Tohme J, Lobo M, Roca W. M, Sanchez I (1998) Genetic variation analysis of the genus *Passiflora* L. using RAPD markers. Euphytica 101:341-347

Ferreira, DF (2011) Sisvar: a computer statistical analysis system. Ciência e Agrotecnologia 35:1039-1042.

Franzon RC, Corrêa ER, Raseira MCB (2005) *In vitro* pollen germination of feijoa (*Accasellowiana* (Berg) Burret). Crop Breeding and Applied Biotechnology 5:229-233

Gatt M, Hammett K, Murray B (1999) Confirmation of ancient polyploidy in *Dahlia* (Asteraceae) species using Genomic *In Situ* Hybridization. Annals of Botany 84:39-48

Goetze M, Lozada RB, Wanderley MGL, Souza LM, Bered F, Palma-Silva C (2013) Development of microsatellite markes for genetic diversity analysis of *Aechmea caudata* (Bromeliaceae) and cross-species amplification in other bromeliads. Biochemical Systematics and Ecology 48:194-198

Golubovskaya IN, Distanova EE (1986) Mapping meigene ms43 by B-A translocation stocks. Genetica 22:1173–1180

Hamant O, Ma H, Cande WZ (2006) Genetic of meiotic prophase I in plants. Annual Review of Plant Biology 57:267-302

Heslop-Harrison J, Heslop-Harrison Y (1970) Evaluation of pollen viability by enzymatically induced fluorescence; intracellular hydrolysis of fluorescein diacetate. Stain Technology 45:115-120

Jauhar PP, Peterson TS (2006) Cytological analyses of hybrids and derivatives of hybrids between durum wheat and *Thinopyrum bessarabicum*, using multicolor fluorescent GISH. Plant Breeding 125:19-29

Jeridi M, Bakry F, Escoute J, Fondi ECF, Ferchichi A, D'hont A, Rodier-Goud M (2011) Homoeologous chromosome pairing between the A and B genomes of *Musa* spp. revealed by genomic *in situ* hybridization. Annals of Botany 108:975-981

Jiang H, Wang FF, Wu YT, Zhou X, Huang XY, Zhu J, Gao JF, Dong RB, Cao KM, Yang ZN (2009) MULTIPOLAR SPINDLE 1 (MPS1), a novel coiled-coil protein of *Arabidopsis thaliana*, is required for meiotic spindle organization. The plant journal 59: 1001-1010

Johansen DA (1940) Plant microtechnique. Mc Graw Hill, New York

Jones CJ, Edwards KJ, Castaglione S et al (1997) Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. Molecular Breeding 3:381-390

Junqueira KP, Faleiro FG, Junqueira NTV, Bellon G, Ramos JD, Braga MF, Souza LS (2008) Confirmação de híbridos interespecíficos artificiais no gênero *Passiflora* por meio de marcadores RAPD. Revista Brasileira de Fruticultura 30:191-196

Kalia RK, Rai MK, Kalia S, Singh R, Dhawana K (2011) Microsatellite markers: an overview of the recent progress in plants. Euphytica 177:309-334

Kaur D, Singhal VK (2019) Meiotic abnormalities affect genetic constitution and pollen viability in dicots from Indian cold deserts. BMC Plant Biology 19:1-11

Kiihl PRP, Pereira ARA, Godoy SM, Stenzel NMC, Risso-Pascotto C (2011) Chromosome stickiness during meiotic behavior analysis of *Passiflora serrato-diditata* L. (Passifloraceae). Ciência Rural 41:1018-1023

Lavinscky MP, Souza MM, Silva GS, Melo CAF (2017) Contributions of classical and molecular cytogenetic in meiotic analysis and pollen viability for plant breeding. Genetics and Molecular Research 16:1-15

Lee YI, Chang FC, Chung MC (2011) Chromosome pairing affinities in interspecific hybrids reflect phylogenetic distances among lady's slipper orchids (*Paphiopedilum*). Annals of Botany 108:113-121

Lee JY, Orr-Weaver TL (2001) The Molecular Basis of Sister-Chromatid Cohesion. Annual Review of Cell and Developmental Biology 17:753-777

Levin DA (2002) The role of chromosomal change in plant evolution. University Press, Oxford

Love RM (1951) Varietal diferences in meiotic chromosomes behavior of Brazilian wheats. Agronomy Journal 43:72-76

Mercier R, Armstrong SJ, Horlow C, Jackson NP, Makaroff CA, Vezon D, Pelletier G, Jones GH, Franklin FCH (2003) The meiotic protein SWI1 is required for axial element formation and recombination initiation in *Arabidopsis*. Development 130:3309-3318

Meletti LMM, Santos RR, Minami K (2000) Melhoramento do maracujeiro-amarelo: obtenção do cultivar 'composto IAC-27'. Scientia Agricola 57:491-498

Meletti LMM, Soares-Scott MD, Bernacci LC (2005) Caracterizacão fenotípica de três seleções de maracujazeiro-roxo (*Passiflora edulis* Sims). Revista Brasileira de Fruticultura 27:268-272

Melo CAF, Silva GS, Souza MM (2015) Establishment of genomic *in situ* hybridization (GISH) technique for analysis in interspecific hybrids of *Passiflora*. Genetics and Molecular Research 14:2176-2188

Melo CAF, Souza MM, Viana AP, Santos EA, Souza VO, Corrêa RX (2016) Morphological characterization and genetic parameter estimation in backcrossed progenies of *Passiflora* L. for ornamental use. Scientia Horticulturae 212:91–103

Melo CAF, Souza MM, Silva GS (2017) Karyotype analysis by FISH and GISH techniques on artificial backcrossed interspecific hybrids involving *Passiflora sublanceolata* (Killip) MacDougal (Passifloraceae). Euphytica 213:2-17

Moreira NF, Pereira TNS, Martins KC (2017) Meiotic analysis of interspecific hybrids between *Capsicum frutescens* and *Capsicum chinense*. Crop Breeding and Applied Biotechnology 17:159-163

Ocampo J, Arias JC, Urrea R (2016) Interspecific hybridization between cultivated and wild species of genus *Passiflora* L. Euphytica 209:395-408

Oliveira JE, Pádua JG, Zucchi MI, Camargo LEA, Fungaro MH, Vieira MLC (2005) Development and characterization of microsatellite markers from the yellow passion fruit (*Passiflora edulis* f. *flavicarpa*). Molecular Ecology Notes 2:331-333

Oliveira EJ, Vieira MLC, Garcia AAF, Munhoz CF, Margarido GRA, Consoli L, Matta FP, Moraes MC (2008) An integrated molecular map of yellow passion fruit based on simultaneous maximum-likelihood estimation of linkage and linkage phases. Journal of the American Society for Horticultural Science 133:35-41

Pádua JG, Oliveira EJ, Zucchi MI, Oliveira GCX, Camargo LEA, Vieira MLC (2005) Isolation and characterization of microsatellite markers from the sweet passion fruit (*Passiflora alata* Curtis: Passifloraceae). Molecular Ecology Note 5:863-865

Pagliarini MS (2000) Meiotic behavior of economically important plant species: the relationship between fertility and male sterility. Genetics and Molecular Biology 23:997-1002

Paiva EAA, Bustamante FO, Barbosa S, Pereira AV, Davi LC (2012) Meiotic behavior in early and recent duplicated hexaploid hybrids of napier grass (*Pennisetum purpureum*) and pearl millet (*Pennisetum glaucum*). Caryologia: International Journal of Cytology, Cytosystematics and Cytogenetics 65:114-120

Pereira TNS, Geronimo IGC, Rossi AAB, Pereira MG (2017) *Passiflora cristalina* and *Passiflora miniata*: meiotic characterization of two wild species for use in breeding. Crop Breeding and Applied Biotechnology 17:273-279

Pierre PMO, Souza SM, Davide LC, Machado MA, Viccini LF (2011) Karyotype analysis, DNA content and molecular screening in *Lippia alba* (Verbenaceae). Anais da Academia Brasileira de Ciências 83:994-1005

Ramzan F, Younis A, Lim (2017) Application of genomic *In Situ* hybridization in horticultural science. International Journal of Genomics 2017:1-12

Rey MD, Martin AC, Smedley M, Hayta S, Harwood W, Shaw P, Moore G (2018) Magnesium increases homoeologous crossover frequency during meiosis in *ZIP4* (*Ph1* Gene) Mutant Wheat-Wild Relative Hybrids. Frontiers in Plant Science 9:1-12

Schwarzacher T, Haslop-Harrison P (2000) Practical *in situ* hybridization. Bios Scientific Publishers, Oxford

Senda T, Hiraoka Y, Tominaga T (2005) Cytological affinities and interfertilities between *Lolium temulentum* and *L. persicum* (Poaceae) accessions. Hereditas 142:45-50

Silva GS, Souza MM (2013) Genomic *in situ* hybridization in plants. Genetics and Molecular Research 12:2953-2965

Silva MAA, Souza MM, Silva GS, Melo CAF, Corrêa RX, Araújo IS, Conceição LDHCS (2014) Analysis of transferability of microsatellite primers (SSR) in wild *Passiflora* species and intraspecific genetic diversity in *Passifloraalata*. Genetics and Molecular Research 13:5908-5918

Silva GS, Souza MM, Melo CAF, Urdampilleta JD, Forni-Martins ER (2018) Identification and characterization of karyotype in *Passiflorahybrids* using FISH and GISH. BMC Genetics 19:1-11

Silva GS, Souza MM (2020) Origin of the cultivated passion fruit *Passifloraedulis* f. *flavicarpa* and genomic relationships among species of the subgenera *Decaloba* and *Passiflora*. Plant Biology 22:533-540

Soares-Scott MD, MelettI LM, Recco-Pimentel S (2003) Meiotic behavior and pollen fertility in sexual and somatic hybrids of *Passiflora* species. Caryologia 56:129-138

Soares TL, Jesus ON, Santos-Serejo JA, Oliveira EJ (2013) *IN VITRO* pollen germination and pollen viability in passion fruit (*Passiflora* spp.). Revista Brasileira de Fruticultura 35:1116-1126

Soares TL, Jesus ON, Souza EH, Oliveira EJ (2018) Floral development stage and its implications for the reproductive success of *Passiflora* L. Scientia Horticulturae 238:333–342

Song QJ, Shi JR, Singh S, Fickus EW, Costa JM, Lewis J, Gill BS, Ward R, Cregan PB (2005) Development and mapping of microsatellite (SSR) markers in wheat. Theoretical and Applied Genetics 110:550-560

Souza MM, Pereira TNS, Martins ER (2002) Microsporogênese e microgametogênese associadas ao tamanho do botão floral e da antera e viabilidade polínica em maracujazeiro-amarelo (*Passiflora edulis* Sims f. *flavicarpa* Degener). Ciência e Agrotecnologia 26:1209-1217

Souza MM, Pereira TNS, Viana AP, Pereira MG, Bernacci LC, Sudré CP, Silva LC (2003) Meiotic irregularities pollen viability in *Passiflora edmundoi* Sacco (Passifloraceae). Caryologia: International Journal of Cytology, Cytosystematics and Cytogenetics 56:161-169

Souza MM, Pereira TNS, Vieira MLC (2008) Cytogenetic studies in some species of *Passiflora* L. (Passifloraceae): a review emphasizing Brazilian species. Brazilian Archives of Biology and Technology 51:247-258

Souza MM, Pereira TNS (2011) Meiotic behavior in wild and domesticated species of *Passiflora*. Revista Brasileira de Botânica 34:63-72

Souza MM, Pereira TNS, Sudré CP, Rodrigues R (2012) Meiotic irregularities in *Capsicum* L. species. Crop Breeding and Applied Biotechnology 12:138-144

Souza VO, Souza MM, Silva GS, Melo CAF, Freitas JCO, Souza AC (2020) Morphological and cytogenetic characterization of new ornamental *Passiflorahybrids* (*P*. 'Vivis' and *P*. 'Jhovi'). Crop Breeding and Applied Biotechnology 20:1-8

Taylor LP, Hepler PK (1997) Pollen germination and tube growth. Annual Review of Plant Physiology and Plant Molecular Biology 48:461-491

Turchetto-Zolet AC, Segatto ALA, Turchetto C, Palma-Silva C, Freitas LB (2013) Guia Prático para estudos Filogeográficos. Sociedade Brasileira de Genética, São Paulo

Vanderplank J (2000) Passion Flowers. The MIT Press, Cambridge

Vanderplank RJR (2002) More Wonderful Cultivars. Plassiflora 12:1-8

Viana AJC, Araújo IS, Souza MM, Ahnert D, Corrêa RX (2006) Otimização da extração de DNA de espécies de *Passiflora* L. visando obtenção de marcadores RAPD. Magistra 18:88

Vida AM, Soares TL, Oliveira EJ, Jesus ON, Souza FVD, Cerqueira TT, Santos-Serejo JA (2011) Germinação in vitro e viabilidade polínica em Passifloras silvestres. In: Congresso Brasileiro de Melhoramento de Plantas. Anais Búzios. https://www.alice.cnptia.embrapa.br/handle/doc/899005. Accessed 02 March 2021

Vieira MLC, Santini L, Diniz AL, Munhoz CF (2016) Microsatellite markers: what they mean and why they are so useful. Genetics and Molecular Biology 39:312-328

Xie S, Khan N, Ramanna MS, Niu L, Marasek-Ciolakowska A, Arenss P, Tuyl J M (2010) An assessment of chromosomal rearrangements in neopolyploids of *Lilium* hybrids. Genome 53:439-446

Xie S, Ramanna MS, Arens P, Tuyl JM (2014) GISH Investigation of crossover events during meiosis of interspecific hybrids of Lily. Acta Horticulturae 1027:143-148

Younis A, Ramzan F, Hwang Y-J, Lim K-B (2015) FISH and GISH: molecular cytogenetic tools and their applications in ornamental plants. Plant Cell Reports 34:1477-1488

Zanatto M, Brammer SP, Nascimento JA, Scagliusi SM (2009) Viabilidade polínica como seleção assistida no programa de melhoramento genético de Triticale. Ciência e Agrotecnologia 33:2078-2082

Zanella CM, Turchetto C, Palma-Silva C (2017) Microssatélites: Metodologias de identificação e análise. In: Turchetto-Zolet AC, Turchetto C, Zanella CM, Passaia G (ed) Marcadores Moleculares na Era Genômica: Metodologias e Aplicações. SBG, São Paulo, pp 94 – 113

Ulmer T, MacDougal JM (2004) Passiflora: Passionflowers of the world. Timber Press, Portland

Figures

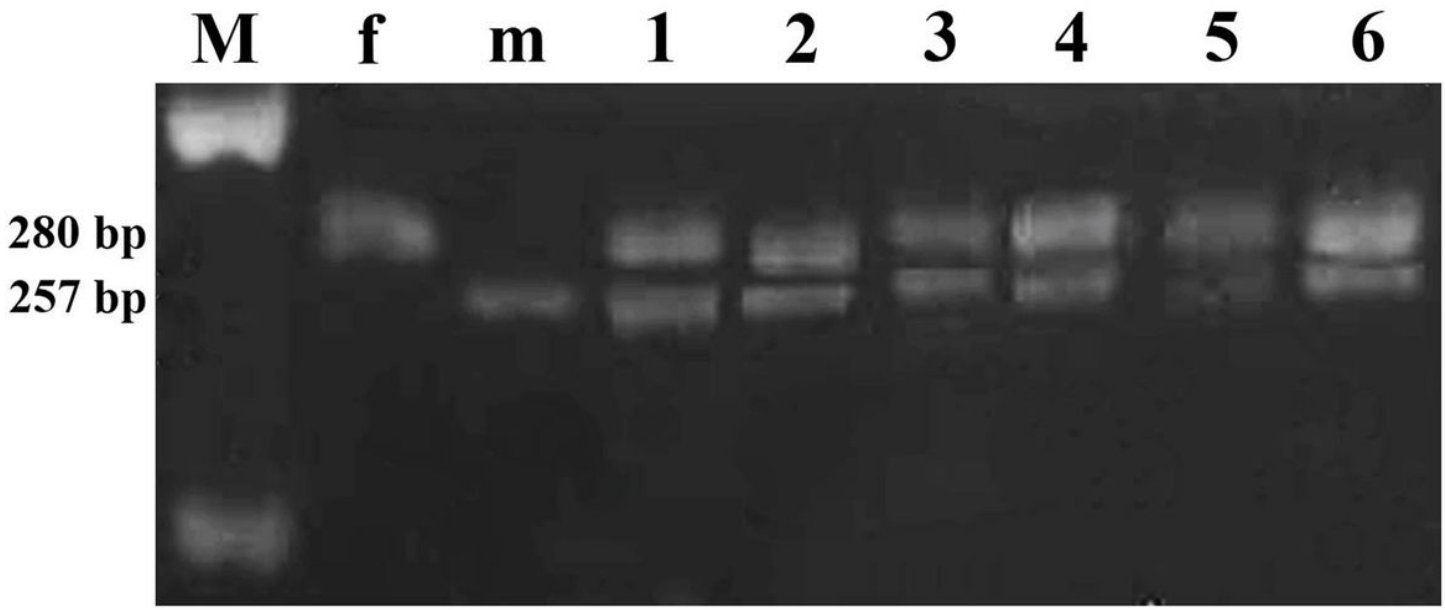

Figure 1

SSR profile generated by the primer Pa07 in Passiflora parents and F1 HD26 progeny hybrids (P. vitifolia vs. P. hatschbachii). M 100 bp Ladder molecular marker; f P. vitifolia (maternal parent); m P. hatschbachii (paternal parent); 1 HD26-104; 2 HD26-105; 3 HD26-118; 4 HD26-136; 5 HD26-146; 6 HD26-152.

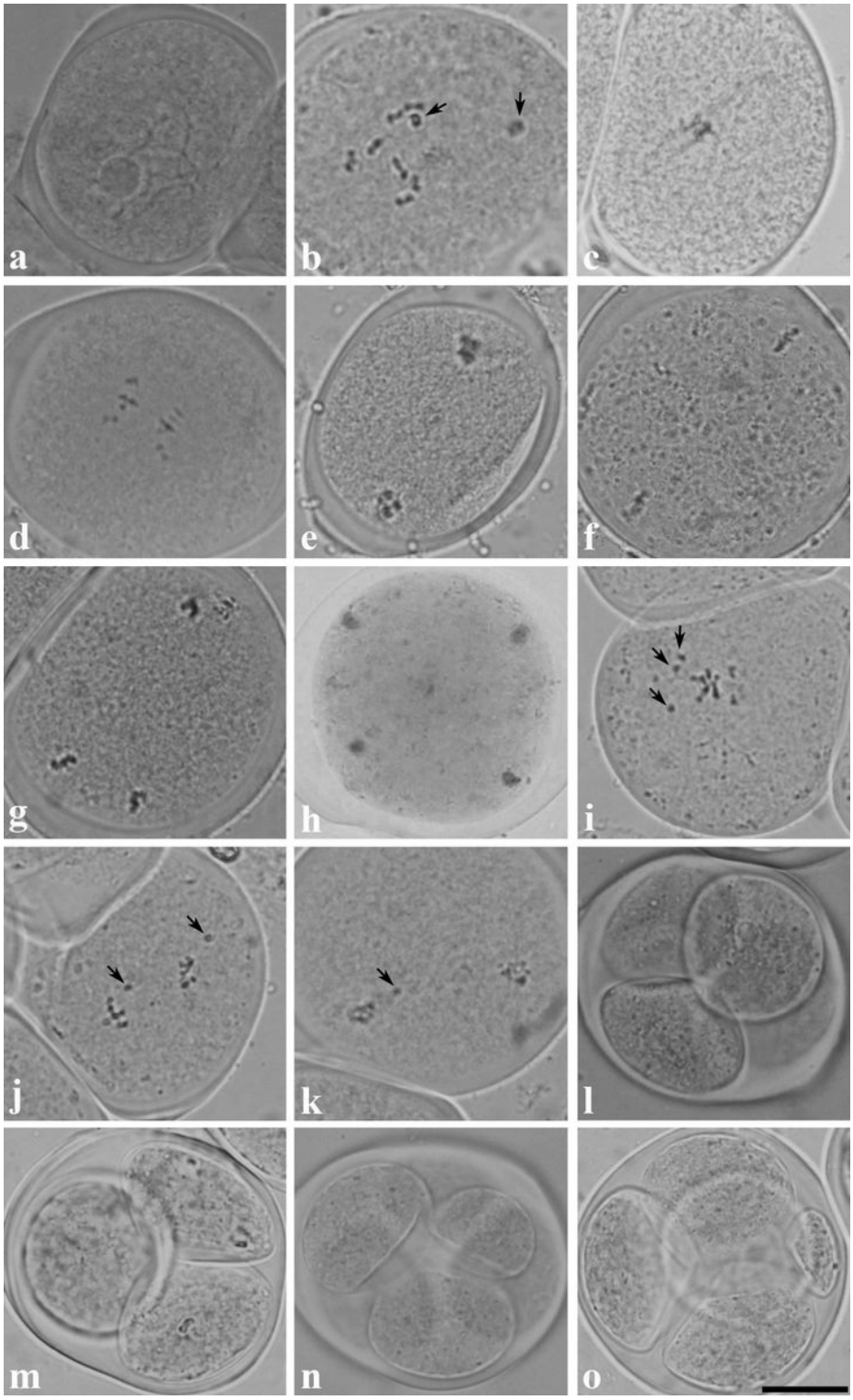

Figure 2

Meiotic behavior in Passiflora parents and F1 HD26 progeny hybrids (P. vitifolia vs. P. hatschbachii) (n = 9). a - h Regular meiotic behavior; i - o Irregularities and post-meiotic products, stained with acetic carmine. a HD26-137 Pachytene; b HD26-146 Diakinesis with 9II (Chiasmata terminals - arrows); c HD26-143 Metaphase I; d HD26-118 Anaphase I; e HD26-105 Telophase I; f Metaphase II of P. hatschbachii (paternal parent); g HD26-104 Anaphase II; h HD26-152 Telophase II; i HD26-143 metaphase I with lagging chromosomes (arrows); j HD26-136 Anaphase I with lagging chromosome (arrows); k HD26-118 Telophase I

with retarded chromosome (arrow); l Tetrad normal in P. vitifolia (maternal parent); m Triad in HD26-146; n Polyad in HD26-104; o Tetrad with micronucleus in HD26-105. Bar = 20 μm

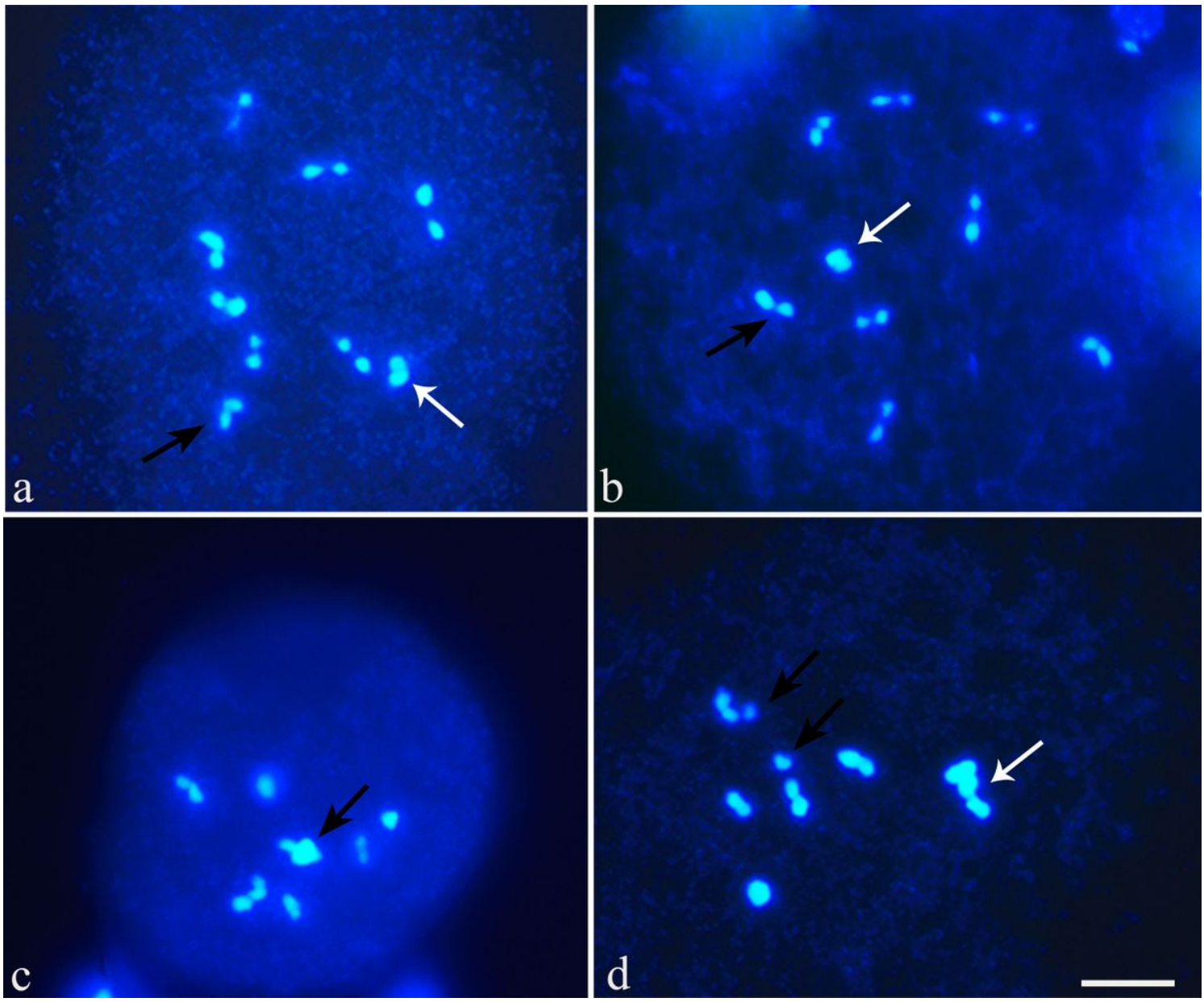

Figure 3

Chromosomal pairing configuration of HD26 hybrid progeny (P. vitifolia vs. P. hatschbachii) (n = 9) stained with DAPI. a HD26-105 with 9 bivalents with one (black arrow) and two (white arrow) chiasmata; b HD26-136 with 9 bivalents with one (black arrow) and two (white arrow) chiasmata; c HD26-105 with 7 bivalents and 1 tetravalent (arrow); d HD26-152 with 6 bivalents, 2 univalents and 1 multivalent. Bar = 10 μm

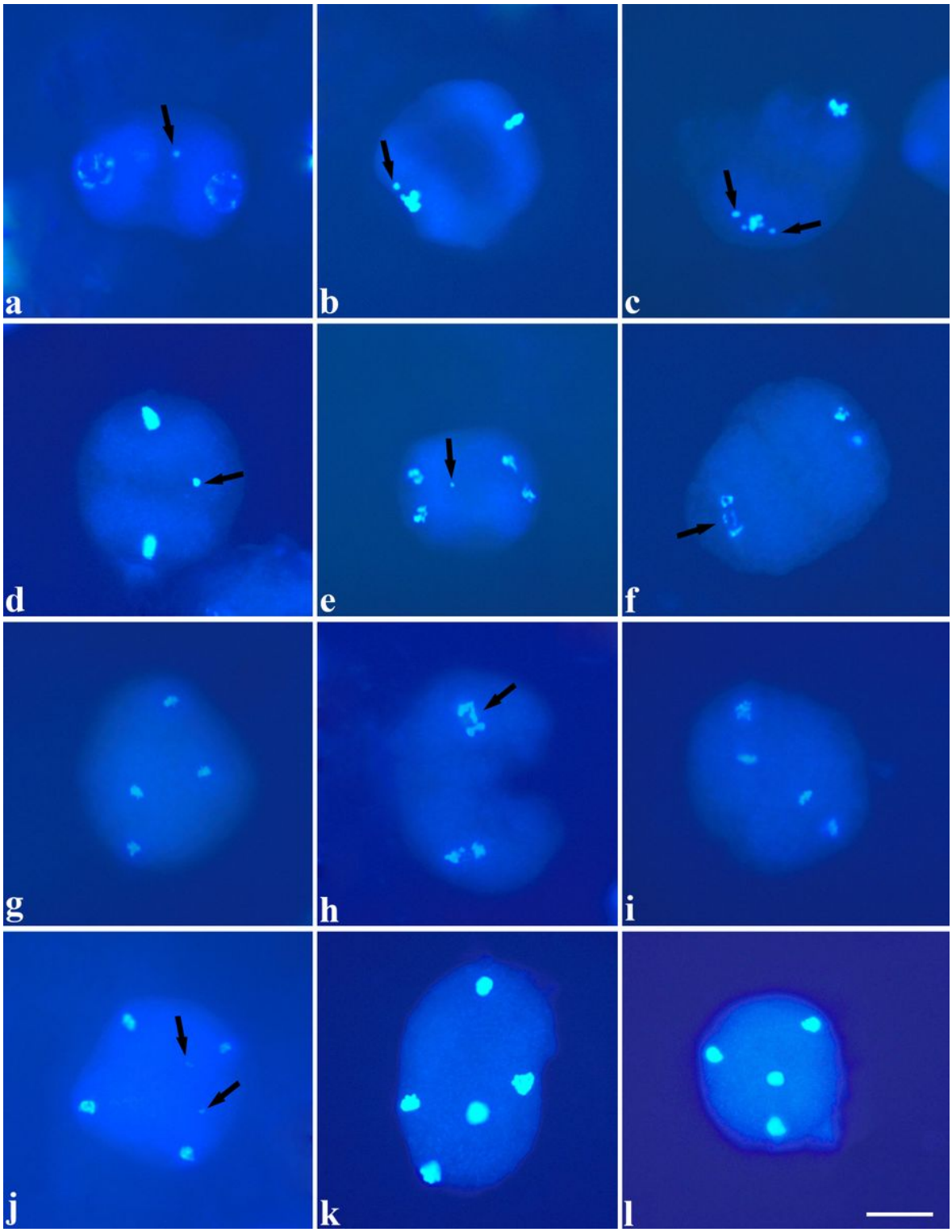

Figure 4

Irregular meiotic behavior in F1 HD26 progeny hybrids (P. vitifolia vs. P. hatschbachii) stained with DAPI (n = 9). a HD26-146 Prophase II with laggard chromosome (arrow); b HD26-152 Metaphase II with laggard chromosome (arrow); c HD26-143 Metaphase II with laggard chromosome (arrows); d HD26-104 Metaphase II with early chromosome (arrow); e HD26-136 Anaphase II with laggard chromosome (arrow); f HD26-118 Anaphase II with chromosomal bridge (arrow); g HD26-105 Anaphase II with spindle irregularity; h HD26-137 Anaphase II with chromosomal bridge (arrow) and tripolar spindle; i HD26-118 Anaphase II with transverse

spindle; j HD26-137 Telophase II with two laggard univalent (arrows); k HD26-105 Telophase II polynuclear (five nuclei); l HD26-104 Telophase II with spindle irregularity. Bar = 20 µm

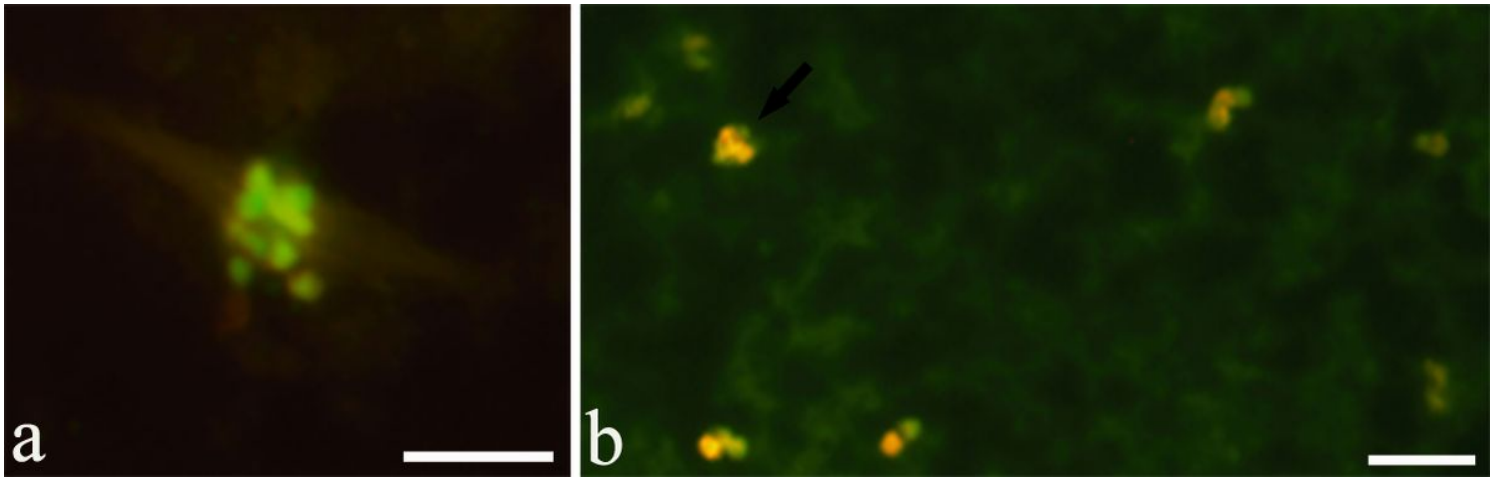

Figure 5

Genomic In Situ Hybridization (GISH) in F1 hybrids of the HD26 progeny (P. vitifolia vs. P. hatschbachii) (n = 9). a HD26-143 with homeologous chromosomes at metaphase I; b HD26-146 with homeologous chromosomes paired at diakinesis and one tetravalent (arrow). Bar = 10 µm

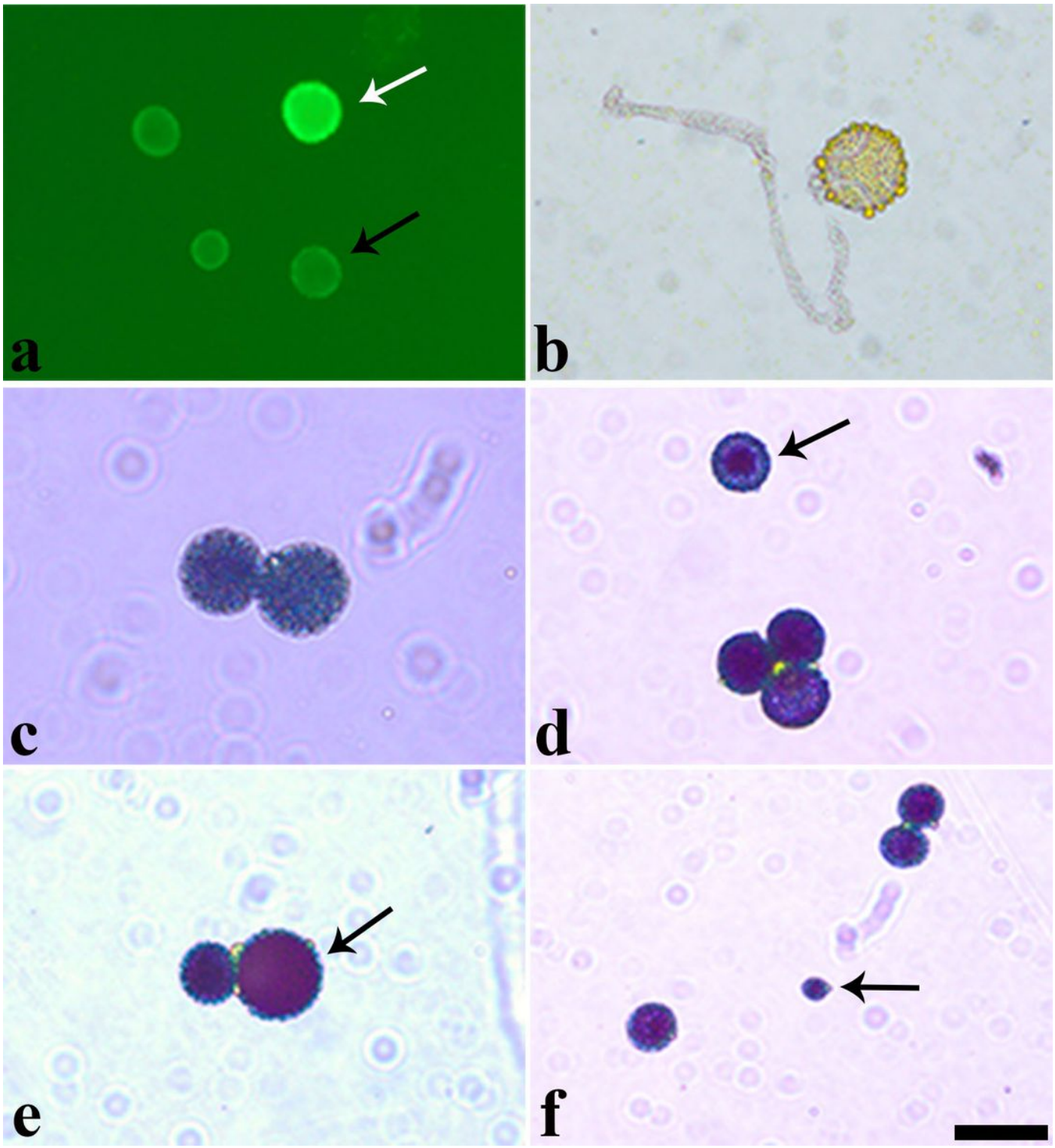

Figure 6

Pollen grains (GP) of Passiflora parents and F1 HD26 progeny hybrids (P. vitifolia vs. P. hatschbachii). a Fluorescein diacetate test demonstrating viable GP (white arrow) and non-viable GP (black arrow) in HD26-137; b Pollen tube germinated in the paternal genitor P. hatschbachii; c - f Test with Alexander solution; c Empty PG in HD26-136; d Contracted PG in HD26-104 (arrow); e Giant PG (arrow) and viable PG in HD26-152; f Micropollen in HD26-105 (arrow). Bar = 100 µm