

Hybridization among wild passionflower species

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ABSTRACT – (Hybridization among wild passionflower species). Passion fruits are appreciated for their ornamental value, since their flowers are showy and display a wide variety of colors. In addition, many hybrids have been produced and used in other countries. The genotypes used in selection of plants with ornamental characteristics are hybrid progenies which are used in various crossing strategies. Thus, the aim of this work was to obtain interspecific hybrids, perform backcrossing and obtain progenies from crossings between hybrids, and to determine the reproductive compatibility between the progenitors involved. The percentage of fertilized flowers, germination, and the number of fruits, seeds and plants obtained through crossing were recorded. A series of 374 crossings involved seven species and two hybrids. Crossings such as *Passiflora gibertii* N. E. Brown vs. *P. kermesina* Link & Otto and *P. gibertii* vs. *P. alata* Curtis did not produce seeds. The largest percentage of fertilized flowers (86%) was recorded for the crossing *P. gardneri* Mast. vs. *P. cincinnata* Mast.; yet, the seeds produced did not show endosperm. Interspecific hybrids were obtained from the crossings *P. gardneri* vs. *P. alata*, *P. watsoniana* Mast. vs. *P. alata*, *P. watsoniana* vs. *P. gardneri* and *P. gardneri* vs. *P. gibertii*. Seeds generated from backcrossings involving the hybrids *P. sublancoolata* (sin. *P. palmeri* var. *sublancoolata* (Killip) J. M. MacDougal) vs. *P. foetida* var. *foetida* L. (HD13-133 and HD13-141) and F₂ reached high germination percentages.

Key words - interspecific crossing, ornamental plants, *Passiflora*, seed germination

RESUMO – (Hibridação entre espécies silvestres de passifloras). As passifloras são apreciadas por seu valor ornamental, pois suas flores são vistosas e com enorme diversidade de cores, e muitos híbridos têm sido produzidos e utilizados em outros países. As fontes para seleção de plantas com características ornamentais são progênies híbridas utilizadas em variadas estratégias de cruzamentos. Assim, os objetivos do trabalho foram obter híbridos interespecíficos, realizar retrocruzamentos e obter progênies de cruzamentos entre híbridos e, desta forma, verificar a compatibilidade reprodutiva entre os genitores envolvidos. Foram registrados o percentual de flores fertilizadas e de germinação, além do número de frutos, de sementes e de plantas obtidos por cruzamento. Realizaram-se 374 hibridações envolvendo sete espécies e dois híbridos. Cruzamentos como *Passiflora gibertii* N. E. Brown vs. *P. kermesina* Link & Otto e *P. gibertii* vs. *P. alata* Curtis produziram frutos que não formaram sementes. Para o cruzamento entre *P. gardneri* Mast. vs. *P. cincinnata* Mast. registrou-se o maior percentual de flores fertilizadas (86%), porém as sementes produzidas não apresentaram endosperma. Foram obtidos híbridos interespecíficos dos cruzamentos *P. gardneri* vs. *P. alata*, *P. watsoniana* Mast. vs. *P. alata*, *P. watsoniana* vs. *P. gardneri* e *P. gardneri* vs. *P. gibertii*. Sementes geradas de retrocruzamentos envolvendo os híbridos *P. sublancoolata* (sin. *P. palmeri* var. *sublancoolata* (Killip) J. M. MacDougal) vs. *P. foetida* var. *foetida* (HD13-133 e HD13-141) e F₂ atingiram elevados valores percentuais de germinação.

Palavras-chave - cruzamentos interespecíficos, germinação de sementes, *Passiflora*, plantas ornamentais

Introduction

Interspecific hybridization in the genus *Passiflora* L. has been used as a strategy intended to obtain genotypes with interesting characteristics to the ornamental plant

market. Some hybrids are already known, such as *P. 'Sunburt'*, with orange flowers originated from the crossing between *P. gilbertiana* J.M. MacDougal and *P. jorullensis* H. B. K (Vanderplank 2000). Species with ornamental attributes, such as *P. alata* Curtis and *P. caerulea* L., among others, have been used in other countries for the creation of several hybrids. *Passiflora* 'Albo-nigra', *P. 'Amethyst'*, *P. 'Star of Bristol'*, *P. 'Star of Kingston'*, and other hybrids are commercialized and used in the decoration of American and European glasshouses (Vanderplank 2000, Ulmer & MacDougal 2004).

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In Brazil, the center of origin for at least one third of the *Passiflora* species (Ganga *et al.* 2004), the potential for ornamental plants is almost unexplored (Abreu *et al.* 2009). The wild species are considered proper for use in breeding programs aiming to produce ornamental *Passiflora* because of their exuberant foliage, attractive, colorful and exotic flowers, and a high number of flowers (Abreu *et al.* 2009). The sources of variability for selection of genotypes are progenies obtained from interspecific hybridization and backcrossing. Thus, the aim of this work was to obtain interspecific F₁ hybrids, perform backcrossings and obtain progenies from crossings between hybrids in order to determine the reproductive compatibility between involved progenitors; in addition, it aims at a future selection of plants with characteristics of interest to the ornamental plant market.

Material and methods

Crossings were performed from November 2006 to August 2008 and comprised accessions of the Germplasm Active Bank of Passionflowers from the Universidade Estadual de Santa Cruz, where seven species of the subgenus *Passiflora* L. were obtained: *P. alata* Curtis, *P. cincinnata* Mast., *P. gardneri* Mast., *P. gibertii* N. E. Brown, *P. kermesina* Link & Otto, *P. sublancoolata* (Killip) J. M. MacDougal (sin. *P. palmeri* var. *sublancoolata* Killip), *P. watsoniana* Mast., and the hybrids HD13 = 133 and HD13-141 (*Passiflora sublancoolata* vs. *P. foetida* var. *foetida* L.). A total of 374 crossings were carried out, among interspecific hybridizations, backcrossings, and the obtention of F₂ progenies (table 1). Controlled pollination was conducted according to the following procedure. Flower buds were protected using corn paper bags (10 cm × 20 cm) in pollen donor and receptor plants at pre-anthesis. On the following day, the recipient flowers were emasculated before flower opening. Pollination was held one hour after flowers

Table 1. Analysis of controlled crossings involving *Passiflora* L. (Set = fruit set percentage; Ger = germination; Pol = number of pollinations performed; F = number of fruits; S = seeds; P = plants obtained).

| Crossings | Pol | Set (%) | χ^2 Set | F | S | Ger (%) | χ^2 Ger | P |
|--|-----|---------|--------------|----|-----|---------|--------------|----|
| <i>P. alata</i> vs. <i>P. watsoniana</i> | 18 | 28 | 53.3 * | 4 | 568 | 0 | 495.2 * | – |
| <i>P. watsoniana</i> vs. <i>P. alata</i> | 13 | 31 | 44.6 * | 4 | 264 | 18 | 304.1 * | 37 |
| <i>P. gardneri</i> vs. <i>P. alata</i> | 30 | 83 | 16.9 *† | 9 | 839 | 3 | 460.2 * | 22 |
| <i>P. alata</i> vs. <i>P. gardneri</i> | 10 | 0 | 171.7 * | – | – | – | – | – |
| <i>P. gardneri</i> vs. <i>P. cincinnata</i> | 21 | 86 | 22.4 *† | 7 | 479 | 0 | 495.2 * | – |
| <i>P. cincinnata</i> vs. <i>P. gardneri</i> | 21 | 0 | 100.0 * | – | – | – | – | – |
| <i>P. gardneri</i> vs. <i>P. gibertii</i> | 14 | 57 | 1.7 ns† | 8 | 468 | 12 | 362.7 * | 31 |
| <i>P. gibertii</i> vs. <i>P. gardneri</i> | 3 | 0 | 100.0 * | – | – | – | – | – |
| <i>P. gibertii</i> vs. <i>P. alata</i> | 32 | 50 | 7.5 * | 12 | 0 | – | – | – |
| <i>P. alata</i> vs. <i>P. gibertii</i> | 10 | 0 | 171.7 * | – | – | – | – | – |
| <i>P. gibertii</i> vs. <i>P. kermesina</i> | 7 | 71 | 2.6 ns† | 5 | 0 | – | – | – |
| <i>P. kermesina</i> vs. <i>P. gibertii</i> | 1 | 0 | 100.0 * | – | – | – | – | – |
| <i>P. gibertii</i> vs. <i>P. watsoniana</i> | 42 | 40 | 23.1 * | 8 | 15 | 0 | 495.2 * | – |
| <i>P. watsoniana</i> vs. <i>P. gibertii</i> | 9 | 0 | 100.0 * | – | – | – | – | – |
| <i>P. sublancoolata</i> vs. <i>P. cincinnata</i> | 17 | 24 | 66.1 * | 4 | 0 | – | – | – |
| <i>P. cincinnata</i> vs. <i>P. sublancoolata</i> | 2 | 0 | 100.0 * | – | – | – | – | – |
| <i>P. watsoniana</i> vs. <i>P. cincinnata</i> | 67 | 16 | 95.8 * | 6 | 109 | 0 | 495.2 * | – |
| <i>P. cincinnata</i> vs. <i>P. watsoniana</i> | 9 | 0 | 171.7 * | – | – | – | – | – |
| <i>P. watsoniana</i> vs. <i>P. gardneri</i> | 5 | 60 | 0.4 ns† | 3 | 284 | 32 | 187.5 * | 51 |
| <i>P. gardneri</i> vs. <i>P. watsoniana</i> | 5 | 0 | 100.0 * | – | – | – | – | – |
| <i>P. sublancoolata</i> vs. HD13-133 | 7 | 43 | 17.5 * | 3 | 26 | 68 | 16.5 * | 13 |
| <i>P. sublancoolata</i> vs. HD13-141 | 14 | 21 | 76.6 * | 3 | 83 | 83 | 0.0 ns‡ | 30 |
| HD13-133 vs. HD13-141 | 8 | 38 | 27.3 * | 3 | 42 | 58 | 45.4 * | 14 |
| HD13-141 vs. HD13-133 | 9 | 44 | 15.9 * | 4 | 65 | 78 | 1.9 ns‡ | 25 |

*: Significance level $P < 0.05$; ns: not significant $P > 0.05$; †: set $\geq 63.2\%$ (Aular *et al.* 2005); ‡: germination $\geq 83.2\%$ (Ferreira *et al.* 2005); -: not survived.

opened, so that flowers with non-deflective styles and not receptive to pollen were avoided. The flowers were protected after the completion of manual pollination. After five days, abortion of the flower bud or start of fruit development were observed. The fruits were collected between 40-60 days after pollination.

The seeds were washed and dried at room temperature and stored at 5 °C. For germination, seeds were treated in sodium hypochlorite (2.5% active chlorine). Part of the seed coat was removed and then the seeds were placed on filter paper and daily moistened with a gibberellic acid solution (100 mg L⁻¹). Data on the percentage of fruit set (developing fruits five days after pollination), number of fruits, seeds, germination percentage and the number of surviving plants were obtained. Differences in flowering time and the need to use the seeds soon after collection, in order to prevent losses in germination capacity, precluded establishing an appropriate experimental design. Thus the χ^2 test ($P < 0.05$) was used for statistical analysis. Fruit set percentage of 63% (expected value) was therefore used as a parameter, as reported by Aular *et al.* (2004), for interspecific artificial hybridization in *P. cincinnata*. An average value of 83.2% was found by Ferreira *et al.* (2005) in a pre-germination treatment with gibberellic acid, and the removal of aril in *P. alata* was performed for germination.

Results

Fruit set in interspecific hybridizations ranged between 0% and 86% (table 1). All hybridizations involving backcrossings and H13-133 and HD13-141 generated progenies, and the values found for fruit set ranged between 21% and 44%. Interspecific hybrids were obtained from the crossings *P. gardneri* vs. *P. alata*, *P. watsoniana* vs. *P. alata*, *P. watsoniana* vs. *P. gardneri* and *P. gardneri* vs. *P. gibertii*, generating 22, 37, 51 and 31 plants, respectively. The crossings *P. sublancoolata* vs. *P. cincinnata*, *P. gibertii* vs. *P. kermesina* and *P. gibertii* vs. *P. alata* produced fruit, but not seeds. The seeds produced through the crossings *P. alata* vs. *P. watsoniana*, *P. gardneri* vs. *P. cincinnata*, *P. gibertii* vs. *P. watsoniana* and *P. watsoniana* vs. *P. cincinnata* did not germinate (table 1). Only five crosses had fruit set similar to or higher than expected, according to the reference value used in the chi-square test. In only two crosses the germination percentage observed reached the expected value.

Discussion

Despite the large number of ornamental hybrids produced, recorded and commercialized in many countries (Vanderplank 2000, Ulmer & MacDougal

2004), there are pre- and post-zygotic barriers in interspecific crosses involving the genus *Passiflora*. For most species of the subgenus *Passiflora* L. the chromosome number is $2n = 18$ (Souza *et al.* 2008). In the present study, the interspecific crosses involving the species *P. sublancoolata* ($2n = 22$; Abreu 2008) and *P. cincinnata* ($2n = 18$; Guerra 1986) failed probably due to this chromosome number difference. The progenitor species that originated the hybrids UESC HD13, *P. sublancoolata* and *P. foetida* var. *foetida* have the same chromosome number ($2n = 22$; Abreu 2008) and belong to the same section, *Dysosmia* DC., (MacDougal & Feuillet 2004). Promising results were obtained in crossings between *P. edulis* f. *flavicarpa* O. Deg. and *P. setacea*, *P. coccinea* and *P. glandulosa* Cav., all with $2n = 18$ (Souza *et al.* 2008), and reciprocal crossings (Junqueira *et al.* 2005).

Self-incompatibility has been studied in species of the genus *Passiflora* L., such as *P. quadrangularis* L., *P. cincinnata* (Howell 1976), *P. edulis* f. *flavicarpa* (Ruggiero 1976) and *P. edulis* Sims (Bruckner *et al.* 1995, Suassuna *et al.* 2003). Self-incompatibility reactions may be responsible for failure in interspecific crossings. Kimura *et al.* (2002) demonstrated the existence of *S* gene alleles common to two species of the genus *Brassica*, which have identical self-incompatibility reaction, except for interspecific hybridization. Incompatible species (IS) often show unilateral interspecific incompatibility to self-compatible species (SS): the pollen tube of IS grows into the style of SS, but the opposite does not occur. There are also reports of unilateral interspecific incompatibility in the genus *Lycopersicon* C. H. Müll., *Nicotiana* L., *Capsicum* L. and *Petunia* Juss. (Onus & Pickrsgill 2004). The crossings *P. watsoniana* vs. *P. gardneri* and *P. gardneri* vs. *P. gibertii* and their reciprocals showed results that suggest the occurrence of that phenomenon. More detailed studies on individual species and their crossings are therefore needed to confirm this unilateral incompatibility hypothesis.

Another possible barrier regarding interspecific hybridization is related to the combination nucleus-cytoplasm (Nimura *et al.* 2003). Negative interactions may endanger the embryo or cause failure in the development of the endosperm. In this study, the crossing *P. gardneri* vs. *P. cincinnata* with higher fruit set percentage (86%) produced hundreds of seeds; however 100% of the seeds did not contain endosperm. The hybridizations *P. gibertii* vs. *P. kermesina* and *P. gibertii* vs. *P. alata* produced fruits, however no seeds were formed (table 1). Similar results were found in *P. caerulea* vs. *P. edulis* f. *flavicarpa*, in which fruits containing seeds were rarely

generated. When *P. edulis* f. *flavicarpa* was used as the male progenitor, fruits with many fertile F_1 seeds were produced (Junqueira *et al.* 2005).

Except for *P. watsoniana* vs. *P. alata* (31%), three in five hybridizations with fruit set percentage equal to or above 63.2%, according to the chi-square test, were interspecific crosses capable of generating plants. All interspecific hybrids obtained in this study were confirmed using the molecular technique RAPD (Conceição *et al.* 2011). The high percentage of germination was decisive in the production of plants on backcrossings and crossings among hybrids. Although the crossings *P. sub lanceolata* vs. HD13-133 and HD13-133 vs. HD13-141 remained below the expected value of 83.2% (Ferreira *et al.* 2005), they were able to produce progeny.

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