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Phenotypic characterization of the intergeneric hybrid *Miltonia flavescens* x *Brassia verrucosa* (Orchidaceae)

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ABSTRACT

Hybridizations between orchid species are often studied with the aim of combining distinct ornamental qualities and integrating aspects inherent to their cultivation and phenotype. *Miltonia* orchids are known for their large, showy flowers, often with striking patterns and vibrant colors. On the other hand, the genus *Brassia* is notable for its flowers that resemble spiders, with long, thin petals giving them an exotic and unique appearance. This study aimed to elucidate the main features of hybrids from the crossing between *Miltonia flavescens* Lindl. and *Brassia verrucosa* Bateman ex Lindl. The pollinia collected from the flowers of *B. verrucosa* was used to manually polinize the flowers of *M. flavescens*; the obtained capsules were harversted, and the seeding was carried out using the asymbiotic *in vitro* germination. After *in vitro* growth and cultivation, the seedlings were acclimatized and cultivated in a greenhouse until the flowering stage. The progeny from this crossing exhibited light-yellow petals and sepals, and a white labellum with wavy edges. Among the 15 plants evaluated, the blooming was observed between October and November, and the flowers present shelf life of 20 to 25 days. Studies like this contribute to a better understanding of feature segregation in intergeneric hybrids, exploring aesthetic beauty, biological robustness, and variability, with the goal of developing innovations in floriculture and promoting the use of wild orchid species in hybridization within breeding programs.

Keywords: Crossing, floriculture, improvement, orchid.

RESUMO

Caracterização fenotípica do híbrido intergenérico *Miltonia flavescens* x *Brassia verrucosa* (Orchidaceae)

As hibridações entre espécies de orquídeas são frequentemente estudadas com o objetivo de combinar qualidades ornamentais distintas e integrar aspectos inerentes ao seu cultivo e fenótipo. As orquídeas *Miltonia* são conhecidas por suas flores grandes e vistosas, frequentemente com padrões marcantes e cores vibrantes. Por outro lado, o gênero *Brassia* é notável por suas flores que se assemelham a aranhas, com pétalas longas e finas, conferindo-lhes uma aparência exótica e única. Este estudo teve como objetivo elucidar as principais características dos híbridos provenientes do cruzamento entre *Miltonia flavescens* Lindl. e *Brassia verrucosa* Bateman ex Lindl. As políneas coletadas das flores de *B. verrucosa* foram utilizados para polinizar manualmente as flores de *M. flavescens*; as cápsulas obtidas foram colhidas e a semeadura foi realizada utilizando a germinação *in vitro*. Após o crescimento e cultivo *in vitro*, as mudas foram aclimatadas e cultivadas em estufa até o estágio de floração. A progênie desse cruzamento apresentou pétalas e sépalas amarelo-claro, e um lábio branco com bordas onduladas. Entre as 15 plantas avaliadas, a floração ocorreu entre outubro e novembro, e as flores apresentaram uma durabilidade de 20 a 25 dias. Estudos como este contribuem para um melhor entendimento da segregação de características em híbridos intergenéricos, explorando a beleza estética, a robustez biológica e a variabilidade, com o objetivo de desenvolver inovações na floricultura e promover o uso de espécies de orquídeas selvagens para hibridizaçôes dentro dos programas de melhoramento.

Palavras-chave: Cruzamento, floricultura, melhoramento, orquídea.

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The flower and ornamental plant market is an important agricultural sector due to the high demand, seasonal nature, and rapid turnover of perishable floricultural products in supermarkets, fairs, and events. Data from the Brazilian Institute of Floriculture show the growth of this market, which earned R$ 17.8 billion in 2023, representing a 70% increase compared to 2017, and with excellent future growth expectations (Ibraflor, 2024). One of the factors driving the growth of this sector is the increasing interest in new cultivars on the market, with breeding programs being essential for increasing Brazil's autonomy in this technology (Neves & Pinto, 2015).

Among the commercialized plants grown in pots, orchids stand out because of their high ornamental value which can be attributed to the diversity of the shapes, colors, and aromas of their flowers. With respect to the total number of species, the family Orchidaceae is one of the largest plant families, with 29,485 species worldwide (WFO, 2024). A total of 2,643 species of orchids distributed across 247 genera are found in Brazil (Reflora, 2024).

Each year, in search of new varieties and hybrids, the commercialization of floricultural products induces the producers to search for innovations. In this context, genetic improvement is an essential strategy in the production of new plant varieties, in order to meet consumer demands for novelties (Devi *et al*., 2023).

According to Cardoso (2013), considering the vast genetic diversity of flowers in Brazil, a clever strategy for improving the country's flower market and make flower production self-sufficient is to invest in genetic improvement programs of flowers and ornamental plants.

Furthermore, the use of native species in genetic improvement programs is a strategy to ensure higher adaptability of plants to climatic and farming conditions in Brazil. However, to select the plants of superior quality, ornamental characteristics such as color, size, and proportionality of flowers should be considered, as well as horticultural characteristics such as fast growth, early flowering, flower durability, and the innovative aspect of the cultivar that might make it commercially attractive (Alves *et al*., 2018).

There are few studies on the genetic improvement of orchids due to their long-life cycle, which typically takes an average of three to four years until blooming, although this duration varies among different genera and species. This delay in flowering presents a significant challenge for breeding programs, as the prolonged cycle limits the evaluation and selection of new hybrids with desirable traits. Consequently, the enhancement of traits such as flower color, shape, and size remains a key focus for breeders aiming to meet market demands for more visually appealing and commercially viable varieties (Vichiato *et al*., 2014).

Faria *et al*. (2015) developed a new *Oncidium* hybrid resulting from the crossing of *Oncidium sarcodes* and *Oncidium "Aloha Iwanaga"*. This cultivar exhibits desirable agronomic traits, including a well-defined floral morphology characterized by symmetrical flowers that enhance its aesthetic appeal, an important factor for ornamental commercialization. Additionally, its vibrant yellow color and extended flower durability further contribute to its market potential, making it an attractive choice for consumers. Colombo *et al*. (2017) developed a new hybrid of *Cattleya* from the crossing between *Cattleya forbesii* and *Cattleya bowringiana*. Stulzer *et al*. (2019) developed a new hybrid by crossing *Cattleya forbesii* and *Cattleya loddigesii*, with the newly developed cultivar displaying early blooming and having the possibility of more than one blooming per year.

Among the orchid species cultivated in Brazil, *Miltonia flavescens* Lindl. and *Brassia verrucosa* Bateman ex Lindl. are frequently studied due to their high ornamental potential and common presence in Paraná (Paula *et al*., 2020; Hoshino *et al*., 2023). *M. flavescens* is an epiphytic orchid native to the southern region of Brazil and Paraguay, with high floristic value and rusticity, whose floral stems bear 7-10 flowers. Its graceful, arching flower stems and dark green, elongated leaves further enhance its ornamental appeal (Pomini, 2023).

The orchid *Brassia verrucosa* Bateman ex Lindl., commonly known as the spider orchid, is an epiphytic plant species distributed in Guatemala, Honduras, Venezuela, and Mexico. It grows in oak and pine forests, as well as in shade trees in coffee plantations, at altitudes ranging from 900 to 1,600 meters, and has high ornamental potential (Flores-Escobar *et al*., 2011). Its a medium-sized orchid with inflorescences of more than 12 fragrant flowers, featuring a bright white lip with green spots, which typically blooms from June to August (Baltazar-Bernal, 2024). Species of the genus *Brassia* and their hybrids are characterized by long sepals, which is where the common name "spider orchid" originates. The combination of the shape of the sepals and petals makes the flower resemble a spider, attracting a specific group of pollinators. The flowers have light green petals with brown spots at the base, and the lip is whitish with red spots and green warts, a feature that distinguishes it from other species (Schiff, 2018; Hoshino *et al*., 2023). Its petals are small, and the velamen is thick (Staebner *et al*., 2015; Tremblay *et al*., 2022).

Aiming at the goal of advancing the ornamental sector, this study sought to explore the potential of intergeneric hybrids between *M. flavescens* and *B. verrucosa*. It focused on characterizing the progeny of these hybrids to better understand their aesthetic and biological traits, and to identify promising characteristics for the development of new cultivars in floriculture.

Genetic origin and development

Healthy and vigorous *M. flavescens* and *B. verrucosa* plants without phytosanitary problems were selected in the nursery of the Center of Agriculture Sciences of the Department of Agronomy, State University of Londrina, Londrina, Paraná, Brazil, to be used as progenitors. The light intensity inside the greenhouse was assessed with a digital light meter (model LX1010B, Polyterm), which recorded a reading of 11×10^5 lux under full sun at noon. The orchids were cultivated in polypropylene pots (12.5×9.3×9.1 cm, volume: 725 cm^3) filled with a mixture of *Pinus* sp. bark (Imperial®) and charcoal (1:1), and the pots were positioned on a cement bench (Monteiro *et al.*, 2024). The pollinia of *B. verrucosa* flowers was collected 48 h after anthesis and immediately transferred to the stigma of recently opened *M. flavescens* flowers (48 h after anthesis) by manual pollination using a wooden stick. Self-pollination was avoided by enclosing the greenhouse with insect-proof nets, preventing any unintended pollinators from accessing the flowers. Additionally, right after anthesis, the pollinia were manually removed from the flowers using fine-tipped tweezers. The pollinia in *M. flavescens* are located inside the flower, in the column, where they are held by the viscidium, a sticky structure that attaches the pollinia to the pollinator or to the manual tools during the pollination process. By removing the pollinia promptly, we ensured that the pollen transfer occurred only through controlled pollination.

Capsules with mature seeds that look full, collected from different *M. flavescens* plants manually pollinated, were collected seven months after pollination (Dawa *et al.*, 2019). The seeds were germinated *in vitro* in vials of 10 cm in height, 5.5 cm in diameter, and 265 mL in volume. In each vial, 50 mL of MS culture medium (Murashige & Skoog, 1962) with half the usual concentration of macronutrients, 1.0 g/L activated carbon, 30.0 g/L sucrose, and 7.0 g/L agar were added, and the pH was adjusted to 6.0±0.2. After germination, the seedlings were subcultivated *in vitro* in the same culture medium for twelve months under a 16 h light photoperiod, with an irradiance of 52 $\mu mol/m^2/s$, and at a temperature of 25±2ºC until reaching a height of about 8.0 cm.

Subsequently, the seedlings were removed from the vials and allocated to styrofoam trays containing 200 cells, using sphagnum moss as substrate, and were grown in these trays for approximately two months. After this acclimatization, the plants were separately transplanted in black polypropylene pots (10.2 cm in diameter, 7.8 cm in height, and 415 mL in volume) using *Pinus* sp. bark and coconut fiber in the ratio of 1:1 (v/v) as substrate. After 24 months of initial growth, the plants were transferred to larger pots (13 cm in diameter, 9.8 cm in height, and 1,000 mL in volume), filled with the same substrate, and they remained in these pots until the beginning of the first bloom.

During the acclimatization period (about three months) and the cultivation period, the plants were kept in a protected environment, utilizing, in addition to insect-proof nets, a 75% shade net. Irrigation was performed manually three times a week, filling the pots with water and allowing it to percolate naturally. Fertilization was performed fortnightly after acclimatization. Forth© NPK mineral fertilizer composed of urea, mono ammonium phosphate, and potassium chloride (1:1:1 g/L) was diluted at a concentration of 3g/L and applied in the amount of 50 mL per pot.

After 48 months of sowing, fifteen hybridized plants were selected for the evaluations of morphological parameters, particularly precociousness, vigor, and health, due to their better phytosanitary conditions. The following phytometric characteristics of vegetative and floral parts were evaluated: plant height (HP); number (NPs), length (LPs), and diameter (DPs) of the pseudobulb; width (WLe) and length (LLe) of the longest leaf; width (WFl) and length (LFl) of the flower; width (WLa) and length (LLa) of the labellum; width (WPe) and length (LPe) of the petal; and width (WSe) and length (LSe) of the sepal. The height, length, width, and diameter were measured in cm.

The dimensions of floral whorls were determined by measuring two flowers from the basal, two flowers from the median, and two flowers from the apical region of the inflorescence, totaling six flowers per stem. The means of the variables phytometric were determined based on fifteen progenies, as well as based on the amplitude and standard error in each analyzed variable.

Performance and description

Vigorous hybrids with oval and elongated pseudobulbs were obtained, with a mean length of 8.1±0.6 cm and mean diameter of 1.4±0.2 cm; the plants usually had two coriaceous and dark green leaves per pseudobulb, with a mean size of 22.3±1.2 cm (Table 1, Figure 1). Plants obtained from the hybridization presented size similar to the male progenitor *B. verrucosa* (Table 2), with an average height of 32.5±3.3 cm, a characteristic that is important for commercialization in pots, as plants of this size are desirable for pot cultivation (Megersa *et al*., 2018).

The inflorescence of the obtained plants was racemose, containing on average 8.0±1.0 flowers per stem, and the flowers were separated along the stem. The obtained plants bloomed for the first time four years after *in vitro* sowing, with the flower shelf life of approximately 20 to 25 days after anthesis.

Figure 1. Plants of the intergeneric hybrid between *Miltonia flavescens* (♀) and *Brassia verrucosa* (♂). It is possible to notice that the flower stems are compact in relation to the plant. Londrina, State University of Londrina, 2019.

Table 1. Vegetative morphological characters of the intergeneric hybrid resulting from the crossing between *Miltonia flavescens* (♀) and *Brassia verrucosa* (♂): plant height (HP), number (NPs), length (LPs), and diameter (DPs) of pseudobulbs, and length (LLe) and width (WLe) of the longest leaf after 48 months of cultivation. Londrina, State University of Londrina, 2019.

Variable	Plant height (cm)	Pseudobulbs			Longest leaf (length, cm)
		Number	Length (cm)	Diameter (cm)	
Range	29.2-35.8	3.0-5.0	7.5-8.7	1.2-1.6	21.1-23.5
Mean	32.5±3.3	4.0±1.0	8.1±0.6	1.4±0.2	22.3±1.2
Standard error	1.88	0.58	0.35	0.12	0.70

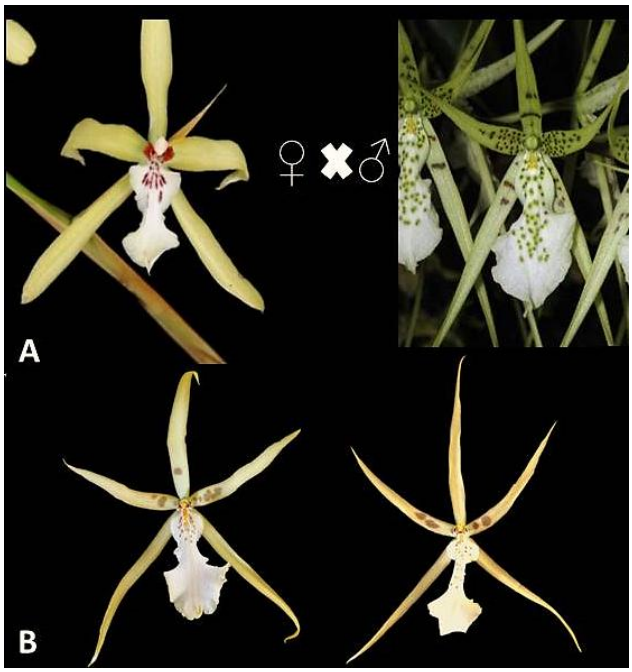

Figure 2. Flower morphology of (A) the progenitors *Miltonia flavescens* (♀) and *Brassia verrucosa (*♂) and (B) the selected hybrid. Londrina, State University of Londrina, 2019.

Table 2. Vegetative morphological characters of *Miltonia flavescens* (♀) and *Brassia verrucosa* (♂): plant height (HP), number (NPs), length (LPs), and diameter (DPs) of pseudobulbs, and length (LLe) and width (WLe) of the longest leaf after 48 months of cultivation. Londrina, State University of Londrina, 2019.

Variable	Plant height (cm)	*M. flavescens* (pseudobulbs)			Longest leaf	
		Number	Length (cm)	Diameter (cm)	Length (cm)	width (cm)
Range	23.1-33.5	8.0-11.0	6.5-8.7	1.3-2.0	14.9-32.3	1.5-2.8
Mean	27.3±3.2	9.8±1.0	7.6±0.6	1.7±0.2	20.7±5.4	2.1±0.4
Standard error	0.82	0.26	0.16	0.05	1.40	0.12
		B. verrucosa (pseudobulbs)			Longest leaf	
Range	25.7-40.2	3.0-9.0	4.0-9.3	1.5-5.0	22.5-30.2	2.2-4.4
Mean	33.8±4.5	5.1±1.9	6.8±1.4	2.9±1.0	26.7±2.3	2.9±0.6
Standard error	1.17	0.49	0.36	0.26	0.59	0.15

Table 3. Floral morphological characters of the intergeneric hybrid resulting from the crossing between *Miltonia flavescens* (♀) and *Brassia verrucosa* (♂): length (LFl) and width (WFl) of the flower, length (LLa) and width (WLa) of the labellum, length (LPe) and width (WPe) of the petal, and length (LSe) and width (WSe) of the sepal after 48 months of culture. Londrina, State University of Londrina, 2019.

Variable	LFl (cm)	WFl (cm)	LLa (cm)	WLa (cm)	LPe (cm)	WPe (cm)	LSe (cm)	WSe (cm)
Range	8.5-8.9	7.7-9.5	3.5-3.7	1.6-2.4	4.5-5.9	0.4-0.6	5.3-7.5	0.5-0.6
Mean*	8.7±0.2	8.6±0.9	3.6±0.1	2.0±0.4	5.2±0.7	0.5±0.1	6.4±1.1	0.5±0.1
Standard error	0.12	0.54	0.06	0.23	0.38	0.03	0.64	0.03

*Mean obtained from 90 flowers observed in 15 progenies.

The flower petals had a mean length of 5.2±0.7 cm, and the sepals measured 6.4±1.1 cm (Table 3). On the other hand, the shape and light-yellow color of the flowers was a characteristic presumably inherited from the female progenitor *M. flavescens.* The labellum was white, with undulations and a mean length size of 3.6±0.1 cm (Figure 2).

Miltonia flavescens and *Brassia verrucosa* typically bloom peaks between spring and summer, and the hybrid generated begins its flowering specifically from September onwards. No further evaluation was made to verify the possibility of more than one flowering per year. Regarding the differences in flower morphology, the hybrid exhibits spotting on the petals and occasionally on the dorsal sepal (as illustrated by the flower on the left in Figure 2-B). The labellum of the hybrid shows more prominent spotting compared to the *M. flavescens* parent. The described spots and the shape of the petals and sepals visually resemble the floral characteristics of the *B. verrucosa* parent.

During the cultivation period, no symptoms of the main insect pests and diseases associated with orchid cultivation were observed in the plants available. Among the desirable commercial characteristics of this new intergeneric hybrid, particular attention is drawn to its size, comparable to that of *B. verrucosa*, as opposed to *M. flavescens*, which is a more compact plant. Its floral stem proportional to plant size, flowers with spotted sepals and petals, white labellum with small spots, ease of cultivation, and the possibility of propagation by formation of clumps (Figure 1).

The hybrid generated in this breeding program can provide ecological benefits, such as increased adaptability to the environment, greater genetic diversity, and attracting pollinators at different times. This can enhance disease resistance, promote pollination, and contribute to ecological balance. Future studies to investigate these aspects will be crucial for scientifically validating the advantages of the hybrid, as well as biochemical or molecular analyses to confirm the relationship between the hybrid and the parental genotypes, and assess the degree of proximity to each parent and the traits derived from each.

Seed maintenance and distribution

Since 2019, the plants resulting from the crossing have been micropropagated and kept in a greenhouse at the Center of Agriculture Sciences, Department of Agronomy, State University of Londrina, Celso Garcia Cid Highway, Pr 445, km 380, University Campus, PO Box 10.011, Zip Code 86057-970, Londrina, Paraná, Brazil.

Author contribution: JCBP: Project conception, development, and experimental methodology; **KAM**: Drafting and editing of the manuscript; **GDS**: Data interpretation and statistical analysis; **HRG**: Experimental setup and data collection; **RTF**: Experimental design, guidance on execution, and support in data collection. All authors reviewed and approved the final version of the manuscript for publication in Horticultura Brasileira.

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