

# *Ionocidium* ‘Cerrado 101’: intergeneric orchid hybrid with high quality of blooming<sup>(1)</sup>

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## ABSTRACT

Orchids are considered one of the most important potted-flowering in the world. *Oncidium* genus, as well their hybrids group (OHGs) has used for the flower market as pot or cut flower. However, some horticultural characteristics require improvements, e.g. the production of precocious cultivars, independent-season blooming or easy flowering induction, high quality and durability of flowers and variations in color of flowers, to compete with *Phalaenopsis* and *Dendrobium* orchids, as other flower groups. Aiming this purpose there were used hand-crossing pollination between the *Oncidium* ‘Sweet Sugar’ and *Ionopsis utricularioides*, a Brazilian wild species, for evaluate the capacity of crossing and to select progenies with interest of use in floriculture as new cultivar. The seeds obtained from this cross were seeded on *in vitro* conditions, followed by acclimatization and cultivation on greenhouse conditions until de flowering time. One of the plants obtained presented interesting characteristics, as good and rapid vegetative development and high quality of blooming. This hybrid obtained from an intergeneric crossing resulted in a plant with vegetative and flower type characteristics and color similar of *Oncidium* female parent, but with larger number of ramifications in inflorescence and number of flowers (60% and 219,4%, respectively) than *Oncidium* parent (♀), and with more similarity with *Ionopsis* (♂). This hybrid cultivar, called *Ionocidium* ‘Cerrado 101’ is one more option of OHGs in this competitive market and can be used either for pot and also for cut orchid flowers production, main because it longer inflorescence (83 cm).

**Keywords:** *Oncidium*, *Ionopsis utricularioides*, breeding, progenies selection, new hybrid.

## RESUMO

### *Ionocidium* ‘Cerrado 101’: híbrido intergenérico de orquídea com alta qualidade de floração.

As orquídeas estão entre as mais importantes flores de vaso do mundo. O gênero *Oncidium*, assim como seus híbridos (OHGs), são plantas com grande potencial para o mercado de flores de corte ou vasos. Entretanto, esse grupo ainda requer a melhoria de algumas características hortícolas, como florescimento precoce, produção de inflorescências independente das estações do ano ou com facilidade na indução e controle do florescimento, além de alta qualidade e durabilidade das flores e produção de cultivares com colorações diferentes daquelas existentes. Essas condições são necessárias para que os *Oncidium* possam competir no mercado com orquídeas de outro gênero como *Phalaenopsis* e *Dendrobium*, bem como outras espécies de vaso e corte. Para tanto, foram realizados cruzamentos entre *Oncidium* ‘Sweet Sugar’ e uma espécie nativa do Brasil, *Ionopsis utricularioides* com o objetivo de determinar a capacidade de cruzamento por polinização manual controlada, e selecionar progênies de interesse para propagação clonal e uso na floricultura como nova cultivar. As sementes obtidas do cruzamento foram colocadas para germinação e desenvolvimento das plântulas *in vitro*, seguido do cultivo em casa de vegetação até o momento do florescimento. Uma das plantas obtidas apresentou características de interesse hortícola como rápido e vigoroso desenvolvimento vegetativo, floração precoce e de alta qualidade. Esse híbrido obtido resultou numa planta com características vegetativas, tipo e coloração das flores similares ao híbrido de *Oncidium* (♀), porém com número de ramificações na inflorescência e número de flores superiores a *Oncidium* (60% e 219,4%, respectivamente), e similares a *Ionopsis* (♂). O híbrido intergenético obtido, chamado *Ionocidium* ‘Cerrado 101’ pode representar mais uma opção para o mercado de OHGs, tanto para cultivo em vaso como para produção de flores de corte, por possuir inflorescências com bom comprimento (83 cm).

**Palavras-chave:** *Oncidium*, *Ionopsis utricularioides*, melhoramento genético, seleção de progênies, novo híbrido.

## 1. INTRODUCTION

Orchidaceae family is the largest family in number of species of flowering plants, with more than 26,500 species (KEW, 2011) with one of the most geographical distribution in the world with species in all continents, with most part concentrated in tropical regions (CHASE et al., 2003).

*Oncidium* is one of the most important genus of orchids for flower market, together with *Dendrobium*, *Phalaenopsis* and *Cattleya* hybrid groups, and represents one of the genus with most ornamental applications, with several number of species and a large number of interespecific hybrids registered in Royal Horticultural Society and used in floriculture for pot and cut flowers production (FARIA

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et al., 2015; RHS, 2017). Two *Oncidium* Hybrid Groups (OHGs) deserve to be highlighted in the Brazilian flower market, the OHGs called 'Golden Rain Orchids' (including *Oncidium* Aloha 'Ywanaga', *O.* 'Aloha Case', *O.* 'Sweet Sugar') with large inflorescences with yellow canary flower color and the 'Chocolate Orchid' (*O.* 'Sharry Baby'), with red-brown flowers distributed in inflorescences and with a sweet smell that remember chocolate. *Ionopsis* is another genus from subtribe Oncidiinae with occurrence in Cerrado vegetation areas and riparian forests, and their ornamental potential and its use for OHGs breeding was previously reported (CARDOSO and ISRAEL, 2005; CARDOSO, 2014).

Breeding of orchids, including *Oncidium* hybridization are large explored in horticulture, but few papers described the methods of breeding and the segregation of morphological characteristics, as showed for *Cattleya* Hybrid Group (Cardoso et al. 2016; Cardoso, 2010), for *Dendrobium* (CARDOSO, 2012; FARIA et al., 2009, 2011, 2013) and for *Oncidium* (FARIA et al., 2015).

Among interesting characteristics for developing new *Oncidium* varieties are the resistance to pests and diseases, compact size of plants, season-independently flowering induction or easy control of blooming induction, high quality of blooming and flowers, e.g. size, number and new colors. Among the characteristics that difficult the breeding programs and selection of new cultivars by specific methods of breeding for orchids are the long period for blooming, ranging from 2 to 4 years after seeding, and the limited knowledge about heritability of characteristics (PAN et al., 2012). The absence of this type of information for orchids difficult the prediction of morphologic characteristic transmission to the progenies, in breeding programs which uses hybridization.

The high genetic variability and the capacity of crossings between species makes orchid breeding very exciting. The use of controlled crossings followed by the selection of superior progenies is the wide used method to obtain new cultivars in orchid industry (CARDOSO et al., 2016). In addition, the establishment of breeding programs of orchids and other species used in floriculture associated with technologies of propagation are required for the sustainability and self-sufficiency of the floriculture in developing countries, which is now, in most part of these countries, are extremely dependent of international breeders located in developed countries (CARDOSO, 2013), which cover royalties for use in protected cultivars.

*Oncidium* has different commercial hybrids used in floriculture (FARIA et al., 2015) and *Ionopsis* is a correlated genus wild from Cerrado and Atlantic Forest from southeast region of Brazil. Some interesting characteristics of *Ionopsis utricularioides* is the presence of flowers with rose color, associated with the production of more than one inflorescence/blooming/plant and inflorescences with very large number of flowers (CARDOSO and ISRAEL, 2005; CARDOSO, 2014), characteristics that partially are not common in *Oncidium* species and hybrids, requiring intergeneric crossings for this purpose.

Although *Oncidium* and *Ionopsis* are near botanically and morphologically (Subtribe Oncidiinae) and some hybrids were registered in Royal Horticultural Society (RHS, 2017), the inheritance of horticultural and ornamental characteristics from this intergeneric hybrids were not well reported. Also, breeding methods using commercial orchid hybrids (OHGs) in crossing with wild species could be resulted in hybrids which combine commercial characteristics with more adapted plants to local climatic conditions.

The aim of this experiment is to determine the capacity of crossing between these two species by hand crossing pollination, follow by the cultivation, evaluation and selection of superior progenies for breeding and use in floriculture as new cultivar.

## 2. MATERIAL AND METHODS

This program of breeding of *Oncidium* and correlated genera have been realized since the year of 2003, with works started at the Fundação Shunji Nishimura de Tecnologia, located at Pompeia City, Sao Paulo State, and after 2013 at Centro de Ciências Agrárias of University Federal of Sao Carlos, in Araras City, SP.

The actual breeding program consisted basically of selection of parents with characteristics of interest, followed by hand cross pollination and selection of superior progenies after *in vitro* and greenhouse *ex vitro* cultivation. Within this purpose there were selected as progenitors: the commercial hybrid of *Oncidium*, called *O.* 'Sweet Sugar', with general compact size and flowers with big size canary-yellow color, and; a selected plant of *Ionopsis utricularioides* obtained from a particular collection from author, with good number of inflorescences (3-4/blooming) with typical rose flowers. Both plants were used as female and male parent to improve the chances of successful to obtain fruit set and viable hybrid seeds.

The hand cross pollination was realized after emasculation of flowers used as female parent, in whom the pollineas were previously collected from the female flowers and bagged in an Eppendorf® of 1.5 ml. The pollineas from the male parent were immediately used for pollinate the stigma of female flowers parent, using a scalpel for pollination. Ten pollination of each crossing were realized. All these procedures were realized between 8 and 10:00 a.m and the pollinated flowers have between 7 to 14 days from anthesis.

Fruits obtained were collected approximately 120-140 days after pollination, when the fruits shown yellow color (instead of green) in its distal portion, signaling the begin of the fruit dehiscence. In laboratory conditions, the fruits were completely opened and the seeds were collected in a Petri dish and immediately were transferred to a flask for asepsis and *in vitro* germination. Asepsis were realized using sodium hypochlorite at 0.3% of active chlorine for 15 minutes, followed by three washings in distilled autoclaved water (unpublished, tested by author). For all stages of *in vitro* growth there was used the Murashige and Skoog

(1962) culture medium with macronutrients reduced by half concentration ( $MS\frac{1}{2}$ ), adding 30 g L<sup>-1</sup> of sucrose, 1 g L<sup>-1</sup> of activated charcoal, and solidified with 6 g L<sup>-1</sup> of Agar-Agar. The culture medium was autoclaved at 121 °C for 20 min.

The selection of progenies was based in a similar methodology described by Cardoso et al. (2016) for *Cattleya* breeding. After *in vitro* phases, which have duration of 9-months, only the seedlings with at least 5-cm length were used for acclimatization procedures, which were realized under greenhouse conditions protected with agricultural plastic and net shade of 80% of sunlight break. There was used 98 cells plastic tray and organic substrate consisted of coconut fiber for acclimatization. After three months of growth, survival plants were transplanted to definitive pots with 12 cm of diameter and 1,0 liter capacity, containing as organic substrate coconut chips and pinus bark 1:1 (v/v) (Vida Verde®, Mogi Mirim, Brazil).

Along the period of cultivation, the mean of temperature ranged from 12 to 28°C, depending of the period of the year. Irrigation was realized using micro-sprinklers applying 4 mm of water, three to four times a weekly, depending on climatic conditions. Fertigation was realized weekly, using ultra-soluble fertilizer containing 20% N, 20% K<sub>2</sub>O, 20% P<sub>2</sub>O<sub>5</sub>, 0,05% Mg, 0,25% Ca, 0,10% (SO<sub>4</sub>)<sup>-2</sup>, 0,0125% B, 0,0125% Cu-quelato, 0,05% Fe-quelato, 0,025% Mn-quelato, 0,005% Mo e 0,025% de Zn-quelato (Cardoso, 2010).

The selection of progenies as new cultivar was based on visual good and vigorous vegetative development with absence of pests and diseases, rapid growth. Reproductive development was observed mainly by the early blooming and high quality of flowers and inflorescences, based on: architecture and length of inflorescences, and flower size and color. There were characterized the first and second blooming of the selected progeny as new cultivar and a minimum of ten flowers for progeny or progenitor was measured, and the data were presented by the mean with standard deviation.

### 3. RESULTS AND DISCUSSION

The viability of the intergeneric crossing was possible only when *Oncidium* was used as female and *Ionopsis* as male parent, which resulted in fruits and seeds. The presence of embryos in seeds was observed using light microscope (data not showed). Using *Oncidium* 'Sweet Sugar' as female parent, the fruit set was 70% (7/10), instead of no fruit production when *Ionopsis utricularioides* was used as female. We observed that due to the high difference in flowers size (Table 2) and reproductive parts between these two genotypes (Table 1), the procedure of pollination in *Oncidium* stigma was not a problem, but the reverse hand-pollination maintained only one of the two pollineas of *Oncidium* in stigma of the small flower of *Ionopsis*, being a possible cause of unsuccessful fertilization when *Ionopsis* was used as female parent.

**Table 1.** Characteristics of inflorescences from male and female parent and the intergeneric progeny obtained as new cultivar *Ionocidium* 'Cerrado 101'.

Characteristic	Parents		New cultivar	
	<i>Oncidium</i> 'Sweet Sugar'	<i>I. utricularioides</i>	<i>Ionocidium</i>	Gains relative to <i>Oncidium</i> (%)
Pseudobulb length/diameter (cm)	9.4/2.9	1.5/0.3	9.1/3.8	-3.2/31.0
Leaf length/diameter (cm)	24.3/3.2	9.5/1.1	26.8/3.8	10.3/18.8
Number of Leaves/pseudobulb	2-3	2-3	2-3	0
Number of flowers	36	158	115	219.4
Number of inflorescences	1	3-4	1	0
Number of ramifications	5	13	8	60
Inflorescence length (cm)	51.0	46.5	83.0	62.0
Flower Diameter (cm)	4.1	1.2	4.0	-2.4

Values are the result of the second flowering time, considered as the commercial blooming for *Oncidium* 'Sweet Sugar' (♀) and also for the new cultivar *Ionocidium* 'Cerrado 101'

**Table 2.** Size of flower parts (Mean±SD) from male and female parents and the intergeneric progenie obtained as new cultivar *Ionocidium* 'Cerrado 101'

Characteristics	<i>Oncidium</i> 'Sweet Sugar'		<i>Ionopsis utricularioides</i>		<i>Ionocidium</i> 'Cerrado 101'	
	width (cm)	length (cm)	width (cm)	length (cm)	width (cm)	length (cm)
Flower	3.83 ± 0.29	4.09 ± 0.17	1.25 ± 0.04	1.15 ± 0.05	3.30 ± 0.22	4.01 ± 0.19
Petals	0.55 ± 0.09	1.08 ± 0.08	0.20 ± 0.00	0.50 ± 0.00	0.46 ± 0.04	1.33 ± 0.06
Sepals	0.50 ± 0.03	0.88 ± 0.08	0.18 ± 0.03	0.40 ± 0.00	0.41 ± 0.04	1.31 ± 0.05
Lip	3.83 ± 0.29	2.85 ± 0.18	1.25 ± 0.04	1.19 ± 0.78	3.30 ± 0.22	2.70 ± 0.14

Values are the result of mean ± standard deviation obtained from the measures of 10 flowers of each genotype

The fruits obtained produced a very small quantity of seeds that was rescued from the fruits and immediately seeded on culture medium on *in vitro* condition. In the Royal Horticultural Society (RHS, 2017), only five registered intergeneric hybrids between *Oncidium* and *Ionopsis* were founded, called: *Ionocidium* 'Blue Mist' (*Ionopsis utricularioides* x *Oncidium incurvum*) and *Ionocidium* 'Hinazakura' (*I. utricularioides* x *Oncidium* Twinkle), were both used *Ionopsis* as seed parent and; *Ionocidium* 'Chian-Tzy Catherin' (*O. Twinkle* x *I. paniculata*), *Ionocidium* 'Chian-TzyHoyen' (*Oncidium sotoanum* x *Ionopsis utricularioides*) and *Ionocidium* Seewatloppen (*O. bauerii* x *I. utricularioides*), which used *Oncidium* as seed parent. Other unregistered crossings were founded as *Ionocidium* 'Popcorn Haruri'. In Brazil, the only registered hybridization with OHGs is obtained from the crossing between *Oncidium sarcodes* x *O. Aloha* 'Iwanaga' (FARIA et al., 2015).

On *in vitro* conditions, after seeding, only 50-100 seedlings were obtained and after 9-months of *in vitro* cultivation, only 30 seedlings with more than 5-cm in length were acclimatized. At greenhouse conditions, 25 progenies were obtained and evaluated for obtaining new cultivar.

The selection of seedlings was started on *in vitro* conditions, and was realized observing the good vegetative development of progenies. The selection of progenies used a similar methodology described by Cardoso et al. (2016) for *Cattleya* breeding. On *in vitro* conditions, there was selected to the next phase only the part of seedlings that effectively differentiated into roots, pseudobulbs and leaves after germination, instead of the part that were maintained as protocorm for a long time since after transplantation, which were not used. In this method, only the progenies that differentiated into plantlets were maintained for rooting and acclimatization stages of the micropropagation. The differences among these progenies also was continued observed on greenhouse conditions, selecting for transplantation only the plants with rapid vegetative development, free of pests and diseases and that presented reduced time required for blooming compared

among progenies, which range from one to two and half years after acclimatization.

As characteristics, the seedlings obtained and acclimatized in greenhouse conditions showed rapid rooting, vigorous and quickly vegetative development, compared with another common *Oncidium* cultivars, showing characteristics of high hybrid vigor from this inter-generic crossing.

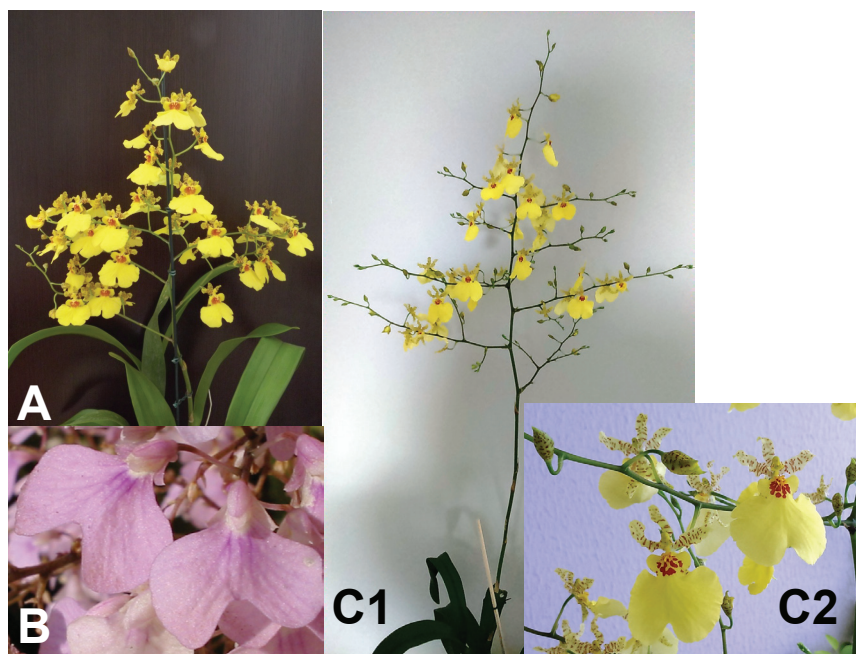
One selected progeny start it blooming early, approximately one year after acclimatization. However, commercial blooming in this hybrid, with higher number of flowers and longer inflorescences were obtained in the second blooming (plants with approximately 1.5 to 2 years after acclimatization), compared with the first ones. This characteristic of rapid flowering has importance for commercial production of orchids because allow the production of flowers in competitive period compared with other commercial genera as *Dendrobium* and *Phalaenopsis* orchids (CARDOSO, 2012; CARDOSO et al., 2016).

*Phalaenopsis* genus is actually the most commercialized pot flower in the world market and only in Brazil were commercialized 5 million pot plants in 2015, instead of 350 thousand of pot plants of *Oncidium* (TOGA, 2017). One explanation for these differences in the market is the largest quantity of varieties of *Phalaenopsis* compared with *Oncidium*. In *Oncidium* most of the species have yellow with brown spots or yellow or brown flower colors, including it hybrids (FARIA et al., 2015). Only the crossings among other correlated genera, as *Miltonia* (*Miltonidium*), *Odontoglossum* (*Odontocidium*) and others were capable to produce new cultivars with more diverse flower colors (LAKSHMAN et al., 2014) for the floriculture market.

The main aims of the actual crossing of yellow-canary predominant flowers from *Oncidium* 'Sweet Sugar' (♀), and *Ionopsis* (♂) with rose flowers (Figure 1) were select: 1) progenies with intermediary size between their parents (Table 1); 2) higher number of inflorescences per blooming than *Oncidium*, commonly with only one (from *Ionopsis*, Table 1); 3) inflorescences with more number of flowers than *Oncidium* (from *Ionopsis*, Table 1) and; 4) progenies with rose flowers (from *Ionopsis*, Figure 1). From these

four characteristics selected as targets for actual breeding program, only the characteristic of higher number of flowers/inflorescence were obtained in the selected progeny (Table 1). The work with the other targets (1, 2 and 4) will require new crossings using this progeny with another

species or back-crossings with *Ionopsis*. Interestingly, one not aimed characteristic, the longer inflorescence of the hybrid (Table 1), is a characteristic of interest for cut flower production, promoting the double purpose for the selected progeny as new cultivar, called *Ionocidium* ‘Cerrado 101’.



**Figure 1.** Intergeneric hybridization of orchids from Oncidiniinae subtribe (Orchidaceae): A) Female parent *Oncidium* ‘Sweet Sugar’; B) Male parent *Ionopsis utricularioides*; C) Type of inflorescence (1) and details of shape of cream-yellow flowers (2) in the moment of commercialization of the selected progeny called *Ionocidium* ‘Cerrado 101’

This intergeneric hybrid selected, as well as the progenies cultivated from this crossing, maintained most of the vegetative morphology, as size of plants, pseudobulbs and leaves similar to *Oncidium*-type plant (Table 1; Figure 1). About color, all progenies obtained from this crossing resulted in variations of yellow color flowers, ranging from yellow soft cream, e.g. the new cultivar selected (Figure 1), to yellow predominant color flowers (another progenies), showing that this characteristic is dominant in the crossing with *Ionopsis*. Also, the format and size of flowers is very similar to *Oncidium*-type flowers (Table 2; Figure 1).

The type of inflorescence has intermediary morphological characteristics between *Oncidium* and *Ionopsis*, with architecture similar to *Oncidium* with good distribution of flowers in inflorescence, but with number of ramifications and flowers more similar to *Ionopsis* (Table 1). Among the main superior characteristics of the progeny selected *Ionocidium* ‘Cerrado 101’ is the high quantity of ramifications (8 inflorescence<sup>-1</sup>) and number of flowers (115 flowers plant<sup>-1</sup>), more than three times the number of flowers of *Oncidium* ‘Sweet Sugar’ used as female parent (Table 1; Figure 1). These characteristics are of interest for the flower market, due to the greater filling of inflorescence with yellow-cream flowers.

Other characteristics of the *Ionocidium* ‘Cerrado 101’ and which still to be improved are the long length of

inflorescences (83.0 cm) compared with another OHGs used for pot culture (Table 1) and the maintenance of yellow-cream derived color, similar to another cultivars disposable in the market (with few novelties). In the Brazilian market there is no specific classification of characteristics for OHGs until the moment and that describe the size of plants and inflorescences suitable for the orchid market. In *Phalaenopsis*, one important pot flower orchid in the market, the plants in pots were classified in four classes according the length of inflorescences, being above from 30 cm (class I) to > 75 cm (class IV) and the presence of long inflorescences (Class IV) is not considered a defect for commercialization (VEILING HOLAMBRA, 2017). Similar classification was used for *Phalaenopsis* cut flowers, with inflorescence length above or below 60 cm in length.

According this classification for *Phalaenopsis*, the actual cultivar developed for OHGs could be considered appropriated for commercial production, both for pot flower, as well as for cut flower, maintaining characteristics of inflorescences similar to other disposable cultivars in the market, e.g. the *Phalaenopsis* hybrids.

The tentative of self-pollination of *Ionocidium* ‘Cerrado 101’ not resulted in fruits until the moment. Interestingly, the pollineas of this cultivar is white soft cream colored, instead of common yellow observed in both

*Oncidium* and *Ionopsis* parents, suggesting the possibility of male-sterility in this hybrid. Other tentative of crossings, as example the backcrossing with *Ionopsis*, using this last as male parent, aiming to obtain rose flowers are in progress by our breeding program.

*In vitro* micropropagated plantlets of *Ionocidium* 'Cerrado 101' are maintained in the Laboratory of Plant Tissue Culture and Physiology at Centro de Ciências Agrárias (UFSCar), located in Araras, SP.

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