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SCIENTIFIC ARTICLE

Grape-based residue as a substrate in *Oncidium baueri* Lindl. acclimatization

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Abstract

The use of agricultural residues as a substrate is a promising option for orchid acclimatization, which is a critical stage of micropropagation. Thus, this study aimed to evaluate the use of grape residue S-10 Beifort®, isolated or mixed with other agricultural residues, in the acclimatization of *Oncidium baueri* Lindl. Five treatments were studied (S-10 Beifort®; S-10 Beifort® + carbonized rice husk (CRH); S-10 Beifort® + coconut fiber (CF); S-10 Beifort® + CRH + CF; CRH + CF), with four replications containing 10 seedlings each. After 120 days, the highest survival rate occurred with the mixture CRH + CF (62.5%). The mixture of S-10 Beifort® with CRH provided a greater shoot length (3.7 cm), not differing from the mixture of the three substrates (2.9 cm). S-10 Beifort® + CRH and the mixture of the three substrates ensured greater root length (3.8 cm). The mixture of the three residues provided the highest average of fresh and dry mass accumulation. The pH variation between the substrates was from 5.2 to 6.3, while S-10 Beifort® presented the highest electrical conductivity (2030 $\mu S\ cm^{-1}$) and the mixture of this material with carbonized rice husk presented the highest water retention capacity. The use of a mixture of S-10 Beifort® with carbonized rice husk or with carbonized rice husk and coconut fiber (1:1:1) is indicated for the acclimatization of *Oncidium baueri* Lindl.

Keywords: agricultural residues, epiphytic orchids, micropropagation, substrates.

Resumo

Resíduo a base de uva como substrato para aclimatização de *Oncidium baueri* Lindl.

Na aclimatização os substratos podem contribuem para a adaptação vegetal após o cultivo *in vitro*. Este trabalho teve como objetivo avaliar o uso de S-10 Beifort®, um resíduo agrícola à base de uva, isolado ou em misturas com outros resíduos agrícolas na aclimatização de *Oncidium baueri* Lindl. Os substratos foram S-10 Beifort®, e as misturas de S-10 Beifort® com Casca de Arroz Carbonizada, Fibra de Coco, e Casca de Arroz Carbonizada + Fibra de Coco. Foi utilizado delineamento experimental inteiramente casualizado, com cinco tratamentos e quatro repetições, contendo 10 plântulas cada. Após 120 dias a maior taxa de sobrevivência ocorreu com a mistura de Casca de Arroz Carbonizada e Fibra de Coco (62,5%). A mistura de S-10 Beifort® com a Casca de Arroz Carbonizada propiciou maior comprimento de parte aérea das plantas (3,7 cm), não diferindo apenas da mistura dos três substratos juntos (2,9 cm). Estas misturas garantiram maior comprimento de raiz (3,8 cm). A mistura dos três resíduos propiciou maior acúmulo de massa fresca e seca para plantas. A variação de pH entre os substratos foi de 5,2 a 6,3. O S-10 Beifort® apresentou a maior condutividade elétrica (2030 $\mu S\ cm^{-1}$). Além disso, o substrato S-10 Beifort® em mistura com Casca de Arroz Carbonizada, apresentou a maior taxa de retenção de água. Podemos concluir que o uso da mistura de S-10 Beifort® com Casca de Arroz Carbonizada e com a Fibra de Coco (1:1:1) é indicado para a aclimatização de *Oncidium baueri* Lindl.

Palavras-chave: micropropagação, orquídeas epífitas, resíduos agrícolas, substratos.

Introduction

The species *Oncidium baueri* Lindl. is an orchid native to Brazil that occurs in several states of the country, especially in the Amazon and Atlantic Forest domains. It is known for its long floral stems, with numerous flowers showing yellow lips and greenish petals and sepals with brown spots (Reflora, 2020). It has great ornamental potential, being one of the most economically important species in floriculture (Faria and Colombo, 2015; Rodrigues et al., 2017).

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The seedling production of this orchid occurs both by vegetative propagation, through clump division, and by sexual propagation, through *in vitro* sowing. Furthermore, *in vitro* culture is a promising propagation tool for orchid conservation and has been successfully applied to many species (Kunakhonnuruk et al., 2018). From a commercial point of view, this alternative guarantees the production of a high number of seedlings in a short time, in addition to the formation of vigorous and healthy seedlings (Schuch et al., 2008; Bridgen et al., 2018).

In the micropropagation process, acclimatization is considered a critical step, as it is the moment when plants change from a heterotrophic condition to an autotrophic condition. In this phase, plant losses occur and factors such as humidity, temperature and luminosity must be strictly observed. Another significant factor in this process is the type of substrate used. In orchids, a great influence of substrates is observed on acclimatization and plant ability to develop in pots (Faria et al., 2018).

For orchid acclimatization, materials such as sphagnum have a relatively high cost, in addition to being extracted from nature in some places. As an alternative to this material, the use of agricultural residues is among the substrate options most reported in the literature once (Banitalebi et al., 2019; Rahman et al., 2019; Munna et al. 2021). In addition to reducing production costs, they avoid the accumulation of materials in the environment (Faria et al., 2018). In this context, it is important to select materials that have high water retention capacity, good aeration, pH, and electrical conductivity suitable for plant development. Furthermore, it is important as well as observe the local availability of these products, the cost, and the low environmental impact (Takane et al., 2013). Other factors can influence the plants micropropagated acclimatization, among which we can highlight the maintenance of the relative humidity of the air, containers, and temperature.

Among the agricultural residues commonly studied are coconut fiber, carbonized rice husk and pine bark. These substrates were used for the acclimatization of *Cattleya* and *Arundina graminifolia* "alba" hybrids (Lone et al., 2008; Zandoná et al., 2014). Other agricultural residues such as coffee husk, almond seeds, and sugarcane bagasse, isolated or mixed with other residues, were also tested in the pot development of *Cattleya*, *Dendrobiun*, *Miltonia* and *Oncidiun* (Faria et al., 2018). In addition, they were evaluated as structuring medium for orchids (Nadal et al., 2018).

Furthermore, to these materials, the use of commercial substrates, such as S-10 Beifort®, can be an option for plant acclimatization. This substrate has grapevine residues (*Vitis* sp) in its composition and was tested for the germination of papaya (*Carica papaya*) seeds (Almeida et al., 2020), pearl millet (*Pennisetum glaucum*) seeds (Suñe et al. 2019), and micropropagation of blueberry (*Vaccinium corymbosum*) (Nadal et al., 2020). However, considering the scarcity of information about its use in the acclimatization of orchids, this study aimed to evaluate the use of S-10 Beifort®, alone or in mixture with other agricultural residues, in the acclimatization of *Oncidium baueri* Lindl.

Material and Methods

The experiment was carried out in a greenhouse with a controlled temperature of 25 ± 2ºC. A completely randomized design was used, with five treatments and five replications containing 10 seedlings each.

Micropropagated *Oncidium baueri* seedlings were used, from *in vitro* sowing, carried out at the Laboratory of Tissue and Plant Cell Culture of Universidade Estadual de Londrina – UEL (*Oncidium baueri* – Figure 1). The plants had, on average, a shoot length of 1.0 ± 0.5 cm, and the roots were standardized at 1.0 cm before being transplanted to the substrates. The standardization of the roots was performed by cutting with scissors. Scissors were previously cleaned with 70% alcohol.

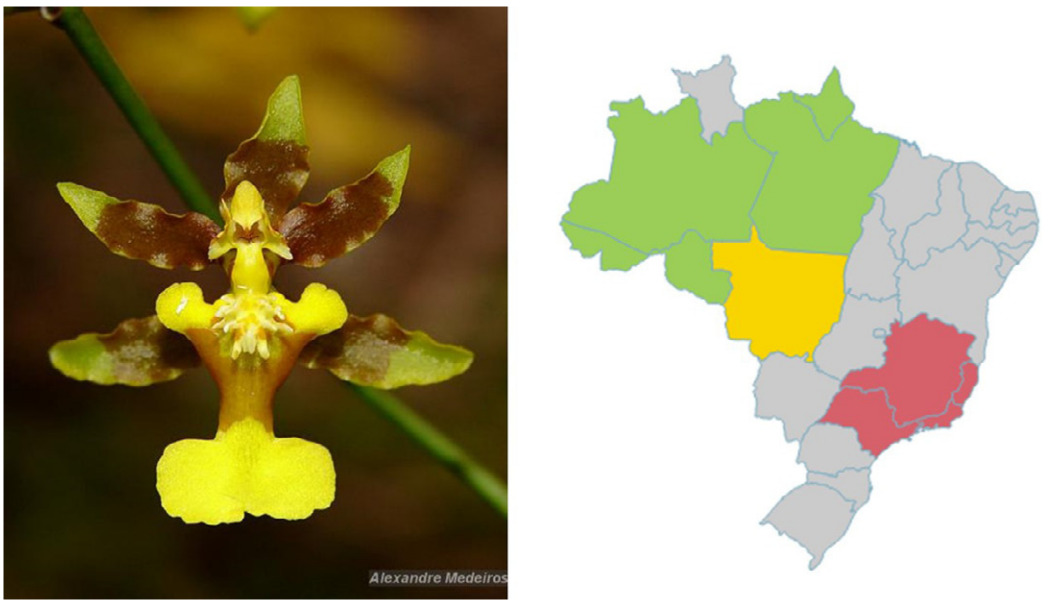

Figure 1. *Ocidium baueri* and the occurrence regions in Brazil. Source: Reflora (2020), Alexandre Medeiros

The treatments consisted of the substrates S-10 Beifort®; S-10 Beifort® + carbonized rice husk; S-10 Beifort® + coconut fiber; S-10 Beifort® + carbonized rice husk + coconut fiber; carbonized rice husk + coconut fiber. In the treatments with substrate mixtures, the proportions used were 1:1 (v:v) or 1:1:1 (v:v:v). S-10 Beifort® is a compound of Class A agro-industry organic waste (seed, bagasse, and stalk), ash, peat, and charcoal.

After being removed from the pots, they were washed in running water to remove the culture medium adhered to the roots, and they were then transplanted into transparent and articulated Sanpack® plastic packages (10 x 13 x 20 cm), containing 1 L of substrate, according to the treatment.

The plants were kept on a bench measuring 1.84 x 0.83 x 0.87 m. Manual watering was performed with the aid of a wash bottle in the morning every 48 hours, by supplying about 30 mL of water in each package. As a preventive measure, the fungicide Orthocide® 500 was applied fortnightly, at a concentration of 3 g L^{-1}.

Four months after the beginning of the experiment, the following parameters were evaluated: percentage of survival; shoot number; number of leaves; number of roots; shoot length and largest root (cm); total fresh and dry matter (g).

Shoot length and the largest root were measured with the aid of a graduated ruler. For fresh and total dry matter, a precision balance was used, and the dry matter mass was evaluated after drying in a forced ventilation oven at a temperature of 65 ºC for 24 hours.

Regarding the substrates, the values of pH, electrical conductivity (EC in dS m^{-1}) and water retention capacity (WRC in mL L^{-1}) were analyzed (Table 1), according to the methodology described by Kämpf (2006).

Table 1. Values of pH, electrical conductivity (EC - µS cm^{-1}) and water retention capacity (WRC - mL L^{-1}) for the substrates used for acclimatization of micropropagated *Oncidium baueri* Lindl. plants.

Substrates	pH µS cm^{-1}	EC mL L^{-1}	WRC
S-10 Beifort®	5.37	2030	317.55
Carbonized rice husk	8.58	450	400.30
Coconut fiber	5.61	1560	357.53
S-10 Beifort® + Carbonized rice husk	6.00	900	389.62
S-10 Beifort® + Coconut fiber	5.32	1610	261.88
Carbonized rice husk + Coconut fiber	6.38	1350	208.06
S-10 Beifort® + Carbonized rice husk + Coconut fiber	5.67	1160	335.53
CV %	6.94	27.48	20.63

Results and Discussion

In general, the use of S-10 Beifort® was more favorable when in mixture with the other tested agricultural residues. The highest percentage of plant survival was obtained with the mixture of carbonized rice husk and coconut fiber (62.5%), differing statistically only from the use of S-10 Beifort® mixed with coconut fiber (24%), where there was a lower survival rate (Table 2).

Table 2. Percentage of survival (PS), number of leaves (NL), shoot number (SN), number of roots (NR) of *Oncidium baueri* plants, 120 days after acclimatization, as a function of the substrate used.

Analyzed variables		Substrates										
		S -10*		S-10 + CRH		S-10 + CF		CRH + CF		S-10 + CRH + CF		CV (%)
PS	µ	32.0	ab**	48.0	ab	24.0	b	62.5	a	48.0	ab	46.5
CV (%)		57.3		24.3		56.5		28.6		20.4		
NL	µ	14.8	a	22.6	a	12.4	a	19.3	a	21.8	a	41.9
CV (%)		55.6		26.3		61.1		19.6		23.8		
SN	µ	0.2	a	0.8	a	0.6	a	0.3	a	1.2	a	101.3
CV (%)		200.0		50.0		133.3		173.2		33.3		
NR	µ	9.0	a	10.4	a	7.4	a	14.8	a	13.8	a	39.1
CV (%)		46.1		54.3		78.2		44.8		25.7		

*S-10 - Commercial Substrate S-10Beifort®; CRH – Carbonized rice husk; CF – Coconut fiber; **Same letters do not differ statistically by the Tukey test at 5%.

In the commercial multiplication of orchids through *in vitro* sowing, it is essential that there is success in the acclimatization phase (Shah et al., 2019). Thus, by adopting micropropagation, survival rates close to 100% are sought; it is essential to apply adequate measures for the adaptation of many species after the transfer from *in vitro* culture to the *ex vitro* environment. According to Bridgen et al. (2018), *in vitro* plants grow in an artificial environment with high humidity and this fact causes them to have a reduced layer of epicuticular wax, leaving them prone to desiccation when placed in lower humidity environments. Therefore, during the acclimatization period, wax development and stomatal regulation must occur.

Due to the small dimensions of the package and the management of constant irrigation, the microclimate inside the packages tends to be similar between treatments, becoming independent of the substrate used. Thus, from the point of view of relative air humidity, the difference between treatments tends to be minimized.

In this sense, observing the data obtained in this study, it is not possible to determine any relationship between the WRC and survival. But plant survival showed a positive relationship with the pH of the substrate, that is, for the data presented, the higher pH values represent the highest survival rates. Contrariwise, EC presents a negative relationship with the survival rates, in other words, with increasing EC values, survival rates decrease (Table 1).

S-10 Beifort® residue, has when compared to the other residues tested in this study, presents higher electrical conductivity (EC = 2030 µS cm^{-1}) (Table 1). When S-10 Beifort® is mixed with the other residues tested, EC values are reduced (900 µS cm^{-1} ≤ EC ≤ 2030 µS cm^{-1}). This value range is ideal for salinity sensitive plants, such orchids (Takane et al., 2013). Thus, it can be inferred that all tested substrates have adequate EC for *O. baueri*. However, it is noteworthy that substrates with lower EC values have higher survival rates.

However, in addition to the influence of the substrate, the articulated plastic container used helps maintain the humidity inside the package, since it is kept closed most of the time and only during the irrigation process these containers are opened more frequently. Moreover, these containers were kept in a temperature-controlled greenhouse. Under these conditions, the relationship between the environment and the change in the substrate is possibly lower when compared to greenhouse conditions. Especially because greenhouses have irrigation systems and greater temperature variation, which accelerates the structural changes in the substrates (Kämpf et al., 1999).

The survival rate varies between genera and species, Shah et al. (2019) obtained 100% survival in the acclimatization of *Cymbidium aloifolium* with a mixture of coconut fiber, gravel and charcoal (2:2:1) inoculated with beneficial microorganisms. None of the substrates studied in the present work guaranteed 100% survival rate for *O. baueri*. These results confirm the existence of survival variations between orchid species, highlighting the importance of carrying out experiments to increase the efficiency of the process for different species.

Evaluating alternative substrates to sphagnum in the acclimatization of *Arundina graminifolia* “alba”, Zandoná et al. (2014) obtained the highest survival percentage (80%), using the mixture of carbonized rice husk and coconut fiber. It is worth mentioning that Oncidiuns are epiphytic orchids and Arundinas are terrestrial orchids, developing under different conditions.

In addition to survival, adequate shoot and root development is essential for successful plant multiplication. In this context, the substrate as a means of mechanical support for seedlings also acts as a reservoir of nutrients, air and water for the roots (Mirani et al., 2017), which provides conditions for physiological plant development processes.

Regarding the number of leaves, shoots and roots, there were no statistical differences between the tested substrates (Table 2). However, the mixture of S-10 Beifort® with carbonized rice husk provided a greater shoot length (3.7 cm), not differing only from the mixture of the three substrates (2.9 cm). In these two treatments, greater root length was also recorded (3.8 cm), not differing from the use of S-10 Beifort® mixed with coconut fiber and the mixture of carbonized rice husk and coconut fiber, 2.6 and 2.1 cm, respectively (Table 3). For the number of shoots and roots, it was not observed the existence of a positive or negative relationship with pH, EC, and water retention capacity. However, for the number of leaves, there is a negative relationship to EC, that is, the higher EC-values, the lower number of leaves. Higher values of electrical conductivity represent higher concentrations of salts in the medium. This may reflect on the plant's ability to absorb water and nutrients.

Table 3. Mean shoot length (MSL - cm); Mean root length (MRL - cm); Total fresh matter (TFM - g); Total dry matter (TDM - g) of *Oncidiun baueri*, 120 days after acclimatization, as a function of the substrate used.

Analyzed variables		Substrates										
		S-10		S-10 + CRH		S-10 + CF		CRH + CF		S-10 + CRH + CF		CV (%)
MSL	μ (cm)	2.3	bc	3.7	a	2.2	bc	1.2	c	2.9	ab	53.93
CV (%)		28.8		8.0		17.3		20.2		32.1		
MRL	μ (cm)	1.5	bc	3.8	a	2.6	ab	2.1	ab	3.8	a	46
CV (%)		8.4		37.0		29.3		13.7		27.2		
TFM	μ (g)	0.91	ab	2.0	ab	0.79	b	0.90	ab	2.23	a	63.9
CV (%)		61.3		29.8		63.4		61.3		38.4		
TDM	μ (g)	0.04	b	0.12	ab	0.07	ab	0.10	ab	0.24	a	94.4
CV (%)		63.7		45.2		39.4		27.1		72.6		

*S-10 - Commercial Substrate S-10Beifort®; CRH – Carbonized rice husk; CF – Coconut fiber; **Same letters do not differ statistically by the Tukey test at 5%.

In the acclimatization of *Dendrobium nobile*, the use of coconut fiber mixed with sand and perlite (1:1:0.2) provided greater survival and better development of leaves and roots (Mirani et al., 2017). In another experiment, studying the pot development of a *Cattleya* hybrid, Assis et al. (2011) observed the highest formation of shoots in a mixture of coffee husk with coconut powder. For *Oncidium baueri*, mixtures of pine husk with coconut fiber or coffee bark provided the highest means of shoot development (Mora et al., 2015).

It is worth mentioning that except for *D. nobile*, the other studies presented were carried out in the pot development phase, and in different environmental conditions of this experiment. The substrates used, especially in the study with *O. baueri*, had as common characteristics, wide variation in WRC, pH values in the range of 5.2 to 6.1, and EC in the range of 252.0 and 339.6. These chemical substrates' characteristics are similar to those found in the mixtures used in the acclimatization phase carried out in this experiment for the same species. Possibly the differences in relation to the results are due to the physiological conditions of the plants, the stage of development, and the conditions of environmental control.

The formation of leaves and shoots is essential for the photosynthetic process and, consequently, for plant maintenance and development (Taiz et al., 2017). After the formation of these structures, orchids begin to accumulate reserves in the bulbs formed from the shoots. Commercially in Oncidiuns, this characteristic is very interesting, since greater the number of shoots, the greater number of flower stems.

Regarding the total fresh matter of the plants, the use of a mixture of the three substrates, S-10Beifort® + Carbonized rice husk + Coconut fiber, provided greater accumulation (2.23 g). However, there were no differences in this treatment in relation to the mixtures of S-10 Beifort® with rice husk; of rice husk with coconut fiber and the use of S-10 Beifort® as a unique substrate. On the other hand, although the greatest dry matter accumulation also occurred with the mixture of the three substrates, it differed only from the treatment with S-10 Beifort® (Table 3).

The greater dry matter accumulation provided by the mixture of the three substrates corroborates the result observed for the greater root length, and the non-statistical differentiation of the shoot length between this treatment and the treatment with S-10 Beifort® in mixture with carbonized rice husk.

There is a negative relationship between EC and total fresh and dry matters, in other words, the highest EC-values signify small values of total fresh and dry matters (Table 1). This indicates a negative relationship between carbon accumulation and EC, possibly related to lower water and nutrient absorption. The total fresh mass has a positive relationship with the WRC, that is, the higher WRC values, the greater the fresh mass. As WRC influenced only total fresh matter, this possibly indicates that the substrates with higher WRC have higher amounts of water available for plants to absorb.

Carbonized rice husk and coconut fiber are residues used as substrates mainly due to their physical properties.

In the case of carbonized rice husk, it has high drainage capacity and good aeration, and coconut fiber, high porosity. When mixed, they are a good substrate for the cultivation of several species of orchids (Faria et al., 2018). On the other hand, S-10 Beifort® is a substrate with high water retention capacity, density, and high infiltration speed. As observed in this study, it is as a possibility in the cultivation of orchids when mixed with other residues.

Analyzing the results obtained and the physical and chemical properties of the materials used in this study (Table 1), there was a pH variation from 5.2 to 6.3. For orchids the ideal pH is in the range of 5.5 to 6.5 (Takane et al., 2013). Thus, the substrate S-10 Beifort® used alone and the mixture of this material with coconut fiber do not fit the recommended pH range. This fact may justify the results verified in these treatments, since inadequate pH values affect nutrient availability in the medium, which may cause physiological disorders in the plants.

As for electrical conductivity, the values ranged between 900 and 2030 $\mu S\ cm^{-1}$, with S-10 Beifort® showing the highest value compared to the other treatments, 2030 $\mu S\ cm^{-1}$, and the mixture of S-10 Beifort® with carbonized rice husk at the lowest conductivity, 600 $\mu S\ cm^{-1}$. The mixture of the three substrates, which provided good results for the acclimatization of the species, presented an electrical conductivity of 1160 $\mu S\ cm^{-1}$.

Different genotypes of epiphytic orchids respond differently to electrical conductivity. There is little information about the characteristics of the substrates regarding the acclimatization period. For acclimatization of *Arundina graminifolia* "Alba", the mixture of agricultural residues presents values of 1413.0 $\mu S\ cm^{-1}$ (Zandoná et al., 2014). When it comes to pot development for the genus *Cattleya*, electrical conductivity values between 253.0 and 292.0 $\mu S\ cm^{-1}$ provided satisfactory growth (Assis et al., 2011). For *Miltonia regnelli* Rchb.f. x *Oncidium concolor* Hook., values of 206.5 $\mu S\ cm^{-1}$ were observed (Yamamoto et al., 2009).

It is important to observe that, in pot cultivation, leaching irrigations are carried out, which tends to reduce the amount of salts retained in the substrate. When acclimatizing in trays, this leaching is significantly lower due to the characteristics of the container, which provides favorable environmental conditions for acclimatization.

Considering the results obtained, the isolated use of the grape-based substrate is not indicated for the acclimatization of *O. baueri*. However, when S-10 Beifort® was mixed with carbonized rice husk, as well as with this substrate and coconut fiber, in general, it provided the best development in the acclimatization phase of that species. Therefore, the producer will be able to choose the most appropriate mixture for their region, as a function of the availability, cost of materials and the time required for labor to carry out such mixtures, among others.

Finally, once it is agricultural residues, the reduction in these materials in different ways, whether in agriculture or industry, will help reduce their volume in the environment. Such materials can assume the advantageous function of substrates for plants (Rodrigues et al., 2018).

Conclusions

The use of a mixture of S-10 Beifort® with carbonized rice husk (1:1) or with carbonized rice husk and coconut fiber (1:1:1) is indicated for the acclimatization of *Oncidium baueri* Lindl.

Author contribution

MCN: conducting and evaluating the experiment, statistical analysis, contextualization and writing the manuscript; **AMA:** experiment design, manuscript review, financial resources; **MWS:** manuscript review, financial resources; **RTF:** manuscript review, financial resources.

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