

**EVALUATION OF DIFFERENT SUBSTRATES ON THE ACCLIMATIZATION OF  
*Epidendrum ibaguense* KUNTH PLANTLETS  
AVALIAÇÃO DE DIFERENTES SUBSTRATOS NA ACLIMATIZAÇÃO DE  
PLÂNTULAS DE *Epidendrum ibaguense* KUNTH**

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**ABSTRACT:** The objective of this work was to evaluate the effectiveness of different substrates for the acclimatization of micropropagated plantlets of *Epidendrum ibaguense* orchid species. Plantlets cultivated in Knudson culture medium for two months were used, after development of leaves and rooting. The substrates were mature coconut fiber, pine bark, termite-house, sphagnum and charcoal, distributed in trays with 50 cells in randomized blocks, with three replications and 10 plantlets per plot. After 60 days, plant height and number of leaves were evaluated. The highest growth and increase in leaf number were observed using charcoal and pine bark. Termite-house and sphagnum resulted in low plantlet development probably due to high water retention. The use of charcoal and pine bark for acclimatization of plantlets of *E. ibaguense* is recommended.

**Keywords:** Tissue culture, Orchidaceae, organic matter, Micropropagation.

**RESUMO:** O objetivo deste trabalho foi avaliar diferentes substratos na aclimatização de plântulas micropropagadas da orquídea *Epidendrum ibaguense*. Foram utilizadas plântulas cultivadas em meio Knudson por dois meses, após desenvolvimento de folhas e enraizamento. Os substratos utilizados foram: fibra de coco, casca de pinus, casa de térmita, esfagno e carvão, distribuídos em bandejas com 50 células em blocos ao acaso, com três repetições de dez plântulas. Após 60 dias, foram avaliados a altura das plântulas e o número de folhas. O maior crescimento e aumento em número de folhas foram observados nos tratamentos com carvão e casca de pinus. A casa de térmita e o esfagno resultaram em baixo desenvolvimento das plântulas provavelmente devido à alta retenção de água. Recomenda-se a utilização de carvão e casca de pinus para a aclimatização de plântulas de *E. ibaguense*.

**Palavras-chave:** Cultura de tecidos, Orchidaceae, Matéria orgânica, Micropropagação.

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## 1. INTRODUCTION

The Orchidaceae botanical family has more than 1,800 genera and near to 35,000 species, distributed around the entire world (RAVEN et al., 2001). These species are classified according to their natural habitats, most of them are epiphytes orchids and live on tree barks, where they fix and can absorb water from atmosphere and nutrients, some of those plants can also be terrestrial or lithophytic (LONE et al., 2008a).

*Epidendrum sp.*, known as the star, was originated from the Americas and is distributed from Mexico to Brazil. This group of plants has near to 1.100 species, which have good resistance to high and low temperatures, and high solar irradiance, independent of being terrestrial or epiphytic (FELIX, 2007).

Due to the predatory exploitation of orchids in their natural habitats, most of them are endangered. The destruction of the natural habitats is another problem because it causes the death of plants and insects pollinators (MULLER et al., 2007).

*In vitro* germination of orchid seeds is a rapid method for obtaining the maximum number of plantlets from their capsules - typical orchid fruits. Nowadays, the available refined methods used on the *in vitro* propagation of orchids can be an alternative to avoid the disappearance of many species. However, despite the relative success of these *in vitro* techniques, the acclimatization of plantlets remains a limiting factor, when the plant must adapt to new temperature and humidity conditions, presence of microorganisms and the absence of sugar from the culture medium (SORACE et al., 2007; SOARES et al., 2008).

Thus, it is essential to determine appropriate substrates for orchid acclimatization, which should ensure relatively thick texture and free drainage, providing access to air and light for the roots, as it occurs in nature. For a long time, the tree fern (*Dicksonia sellowiana* Hook) fiber was used for orchid acclimatization, but nowadays this species is considered endangered and its collection is forbidden by authorities (ASSIS et al., 2005). Sphagnum is a moss removed from the river banks and it is also one of the substrates used by orchid growers and producers in Brazil for acclimatization. Currently, fiber and powder of coconut are considered

promising as alternative substrates for the cultivation of orchids, materials that have already been used as substrates in the production of agricultural seedlings of vegetables (LONE et al., 2008b; SILVEIRA et al., 2002).

According to Meurer et al. (2008), the substrate is considered the basis of orchid cultivation. It must provide support for the plants, and must be characterized by optimum consistency to support the plants, good aeration for the roots, high water absorption and retention, high durability, and adequate pH levels (KAMPF, 2000). Therefore, the objective of this work was to evaluate the efficiency of alternative substrates for the development of *E. ibaguense* micropropagated plantlets during the acclimatization stage.

## 2. MATERIAL AND METHODS

The experiments were carried out at Embrapa Rondonia, in Porto Velho, Brazil (8°45'43"S; 63°54'14"W). Capsules from *E. ibaguense* plants were collected at Embrapa's experimental field. They were surface sterilized with 70% ethanol and 2.5% sodium hypochlorite and their seeds were inoculated in 250 mL glass flasks containing Knudson medium salts, 30.gL<sup>-1</sup> sucrose and 3.6 mg.L<sup>-1</sup> 6-benzylaminopurine, pH 4.8, during a 30-days period for shoot development. Shoots were rooted in the same conditions, with 1.6 mg.L<sup>-1</sup> indolbutiric acid for 30 days. These subcultures were kept in a growth room at 25±2°C, 35 mmol.m<sup>-2</sup>s<sup>-1</sup> of irradiance and 16 hours photoperiod (KNUDSON, 1946). Rooted plantlets were removed from flasks, washed in water and planted in five pre-autoclaved substrates: mature coconut fiber, pine bark, termite-house, sphagnum, and charcoal, distributed in three trays of 50 cells (4 cm height x 4 cm width x 9 cm depth) in randomized blocks, with 10 plantlets per plot and three replications. These plantlets were kept in a protected environment, with 80% shading and sprinkler irrigation for 3 minutes twice a day (8 a.m. and 5 p.m.). On the first day of cultivation and after 60 days, plant height and the number of leaves were evaluated. The differences between the first and the 60<sup>th</sup> day of cultivation in vertical length and number of leaves were expressed in percentage of increase. The averages were compared by Tukey test at a 5% level of significance using Genes program.

The chemical characteristics of the substrates were determined at Embrapa's Laboratory of Soils (Table 1).

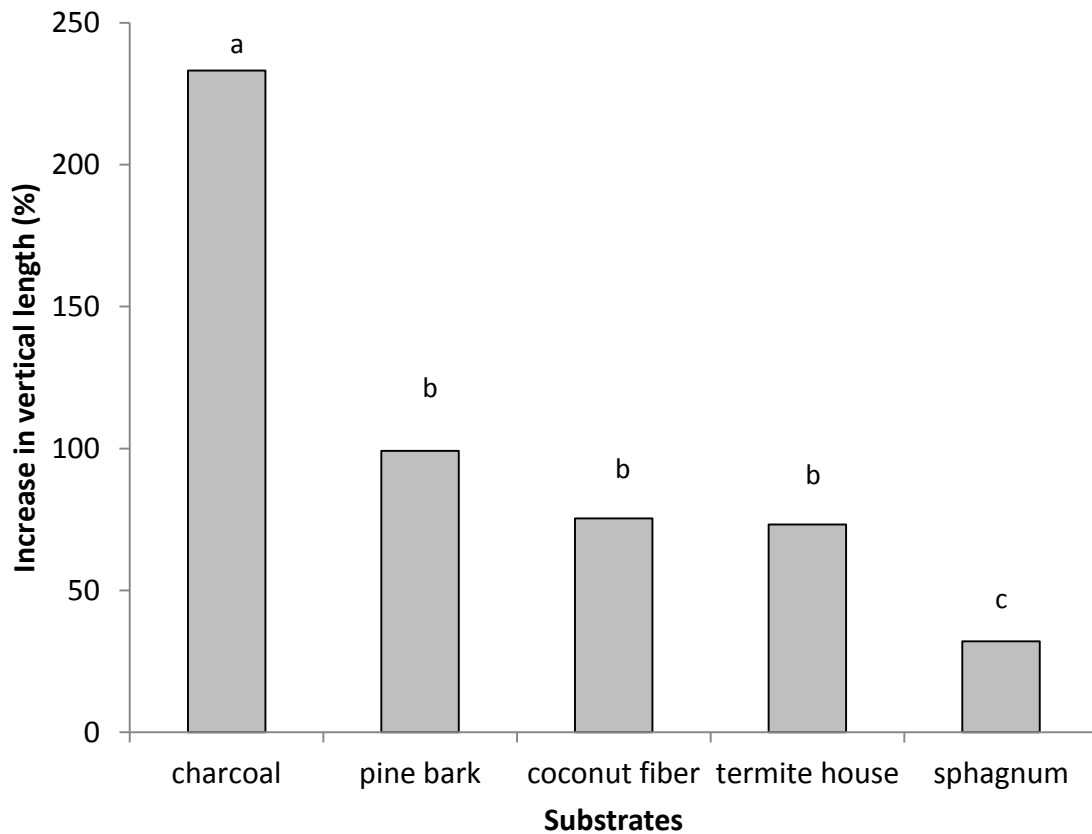
**Table 1.** Chemical characteristics of substrates used on the acclimatization of *Epidendrum ibaguense* plantlets.

Substrates	g.kg <sup>-1</sup>					
	N	P	K	Ca	Mg	S
Charcoal	4.18	0.16	1.2	1.9	0.5	1.71
Pine bark	3.52	0.23	1.8	2.2	1.0	0.49
Coconut fiber	3.50	0.40	3.9	5.0	4.9	1.44
Termite house	10.46	0.51	1.5	2.9	1.7	3.33
Sphagnum	11.72	1.34	5.1	7.8	3.0	2.83

### 3. RESULTS

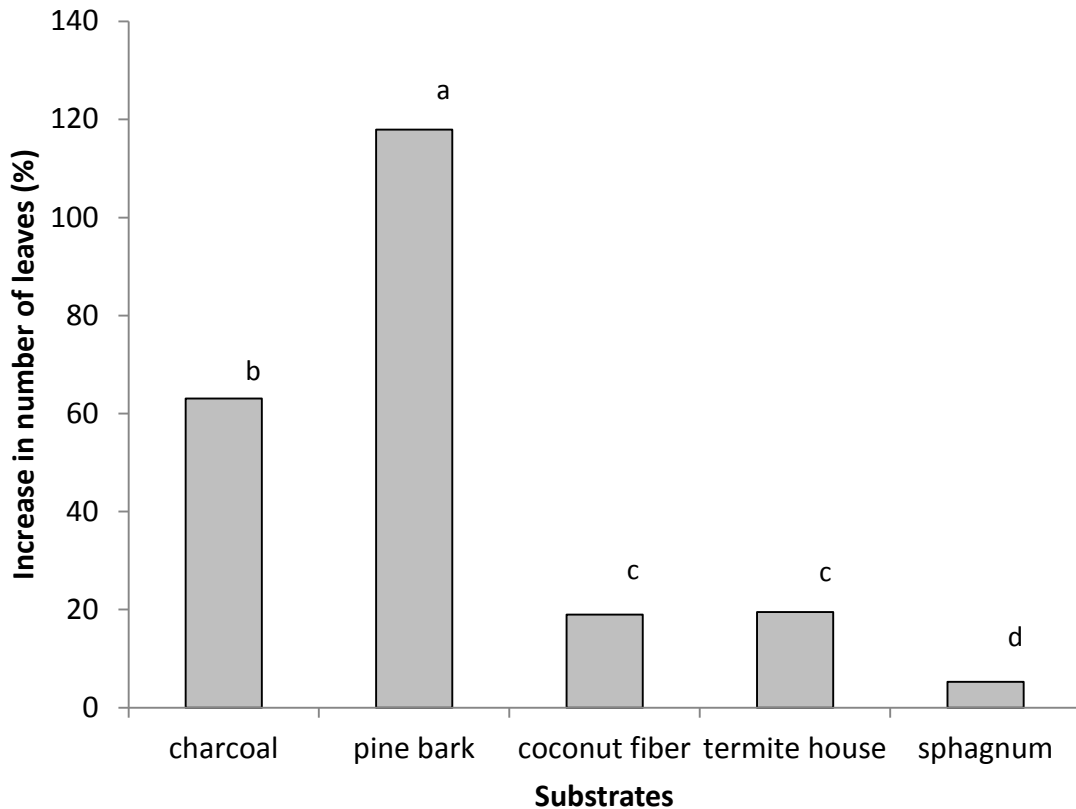
The evaluation of the seedling development of *E. ibaguense* during acclimatization showed significant differences in relation to the substrates to which they were subjected. Seedling survival was 100% in all treatments.

Charcoal was considered the most efficient substrate in relation to plantlet height; resulted in a 233.2% increase in vertical length and differed from the other treatments. Pine bark, coconut fiber and termite house substrate were not significantly different and resulted in increases in vertical length of 99.2, 75.4, and 73.2%, respectively. The lowest growth was observed with sphagnum that resulted in increases of 32.1% of the vertical length (Figure 1).



**Figure 1.** Percentages of increase in vertical length in *Epidendrum ibaguense* plantlets after 60 days of acclimatization in different substrates. Letters indicate difference by Tukey test at a 5% level.

In relation to the number of leaves, pine bark resulted an 117.9% increase in the number of leaves and was significantly superior to the other substrates. Charcoal was the second best substrate; resulted in a 63.1% increase and differed from the other substrates. Coconut fiber and termite house did not differ from each other and resulted in increases of 19.0 and 19.5%, respectively, very low in relation to charcoal and coconut fiber. The lowest number of leaves was observed with sphagnum, which resulted in an increase of 5.3% (Figure 2).



**Figure 2.** Percentages of increase in number of leaves in *Epidendrum ibaguense* plantlets after 60 days of acclimatization in different substrates. Letters indicate difference by Tukey test at a 5% level.

#### 4. DISCUSSION

In this study, charcoal was identified as the best substrate to provide the increase in vertical length to *E. ibaguense* plantlets. This is due to the fact that charcoal provides a highly porous structure which, if pure or mixed with soil or other types of substrates, can increase porosity, water-holding capacity and facilitate the proliferation of microorganisms beneficial to plant growth (YAMAKAMI, 2006). Moraes et al. (2002) studied substrates on the acclimatization of *Dendrobium nobile* Lindl. plantlets and observed the highest values of plant length with the use of a combination of charcoal, Plantmax (commercial substrate), and polystyrene (1:1:1). The authors indicate this substrate to *D. nobile* cultivation as an alternative to the commonly used tree fern, *Dicksonia sellowiana*, an endangered species. Several authors have obtained good results of vegetative development during orchid acclimatization and/or cultivation by using pure charcoal or charcoal mixed with

another plant, mineral, or synthetic substrates. Charcoal is a low-cost substrate and does not retain too much water, absorbing just the necessary amount for orchid species, with good aeration (FARIA et al., 2001; REGO et al., 2000; DEMATTÊ & DEMATTÊ, 1996).

Pine bark stood out from the other substrates in relation to the increase in a number of leaves and was the second best substrate, after charcoal, in relation to the increase in vertical length. Similar results were obtained by Sorace (2008), who evaluated alternative substrates during the acclimatization of *Cattleya skinneri* and concluded that pine bark resulted in the tallest plants. However, Yamakami et al. (2006) did not obtain efficient results in the cultivation of *Cattleya* hybrid plantlets when cultivated in this substrate.

Coconut fiber is a material resulting from the industrial processing of coconut husks (*Cocos nucifera*), which contain important nutrients for plant growth and development (Sorace, 2008). This substrate resulted in a relatively low increase in vertical length and number of leaves. This inefficient result can be explained because this substrate retains much humidity and has a lack of nitrogen (KAMPF, 2000). This is in accordance with Sorace (2008) who observed the low growth of plants of the genus *Cattleya* during acclimatization with coconut fiber. Several authors reported the good development of *Dendrobium nobile* Lindl. with pure coconut fiber and in mixture with other substrates (DEMATTÊ & GRAZIANO, 2000; ASSIS et al., 2005). Yamakami et al. (2006) concluded that coconut fiber can be considered an alternative to tree fern in the cultivation of the hybrid *Cattleya labiata* X *Cattleya forbesii*. This was confirmed by Lone et al. (2008b), who observed that coconut fiber and a mixture of coconut fiber with pine bark (1:1 v/v) were efficient as an alternative to tree fern for the acclimatization of *C. intermedia*.

Termite house resulted in relatively low growth in this study. This substrate was evaluated because many orchid growers in the Northern region of Brazil, more specifically in Rondonia state, use it for growing orchids because they believe that it is rich in nutrients.

The lowest growth was observed with sphagnum. This substrate is characterized by high moisture retention and is not ideal for orchid cultivation in the North region of Brazil where humidity is high. This is in agreement with Colombo et

al. (2005), who submitted a *Cattleya* (*Cattleya* “chocolate drop”) hybrid to different plant substrates and observed that sphagnum resulted in the lowest survival of plants.

## CONCLUSIONS

This study indicates that charcoal and pine bark substrates are more efficient for the acclimatization of *Epidendrum ibaguense* orchid species, resulting in highest growth in height and number of leaves, followed by coconut fiber and termite house. Sphagnum moss is not a suitable substrate for this species.

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