Colegio de Postgraduados

# Effects of foliar-applied homobrassinolide on the *ex vitro* acclimatization of *Guarianthe skinneri* (Bateman) Dressler & W.E.Higgins

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**ABSTRACT**

**Objective**: To identify the influence of foliar application of homobrassinolide at various doses and frequencies on the growth and *ex vitro* acclimatization of *Guarianthe skinneri* (Bateman) Dressler & W.E.Higgins.

**Design/methodology/approach**: *G. skinneri* seeds were germinated *in vitro* on Yasuda medium. Once the seedlings developed roots, leaves and reached a height of 2 cm, they were transferred to the nursery for *ex vitro* acclimatization. Coconut fiber was used as a substrate in 10 oz beakers. Hbr-based treatments were generated with three doses of Hbr (2, 4 and 6 $mgL^{-1}$) and three application frequencies (every 7, 14 and 21 days), plus a control with 10 replicates in a completely randomized design. he following variables were evaluated at 28, 56, 70, 77, and 91 days: plant height, number of green and dry leaves, and number of dead plants.

**Results**: Foliar application frequencies and concentrations of Hbr induce differential growth and survival of *G. skinneri*. Applying Hbr more frequently at higher concentrations improves *G. skinneri* survival in the nursery.

**Limitations on study/implications**: The results may vary under different environmental conditions during the acclimatization of *G. skinneri*.

**Findings/conclusions**: Foliar application of Hbr induced greater growth in *G. skinneri*, particularly in terms of plant height. More frequent applications and higher concentrations of Hbr improved the acclimatization and survival of *G. skinneri* in nursery conditions. Different concentrations and application intervals of foliar Hbr resulted in distinct effects on the growth and survival of *G. skinneri*.

**Keywords**: Orchids, Homobrasinolide, Survival.

**Citation**: Gálvez-López, A. L., Aguirre-Medina, J.F., Chilel-Pérez, E. H., De León-Roblero, J.M., Aguirre-Cadena, J.F., & Reyes-Reyes, J. (2025). Effects of foliar-applied homobrassinolide on the *ex vitro* acclimatization of *Guarianthe skinneri* (Bateman) Dressler & W.E.Higgins. *Agro Productividad*. https://doi.org/10.32854/ye703g68

**Academic Editor**: Jorge Cadena Iñiguez
**Associate Editor**: Dra. Lucero del Mar Ruiz Posadas
**Guest Editor**: Daniel Alejandro Cadena Zamudio

**Received**: June 17, 2025.
**Accepted**: September 13, 2025.
**Published on-line**: September XX, 2025.

*Agro Productividad, 18*(9) supplement. September. 2025. pp: 117-125.



## INTRODUCTION

The Orchidaceae family comprises approximately 800 genera and nearly 30,000 species, with a wide geographical distribution worldwide (Arditti & Ghani, 2000; Chase *et al*., 2003), ranging from sea level to elevations of up to 4,000 meters (Rollke, 2007; Téllez & Flores, 2007). Orchids are highly valued and admired for the beauty of their flowers, and they are particularly popular due to their diversity of colors, sizes, shapes, and fragrances. Currently, 188 orchid species are listed under a risk category in Mexico's NOM-059 regulation (Cabrera, 2006).

Orchid populations have declined in their natural habitats due to anthropogenic factors such as habitat fragmentation (Hágsater *et al.*, 2005; Newmarch *et al.*, 2024), climate change, dependence on specific pollinators and mycorrhizal fungi (Tsiftsis & Djordjević, 2020), and their limited seed germination capacity due to the absence of endosperm (Damon *et al.*, 2004). This condition hinders natural repopulation, as germination relies on minimal carbohydrate reserves.

Currently, there are various efficient protocols for the *in vitro* regeneration of orchids (Orbovic *et al.*, 2008), and as a result, propagation protocols are now available that can support the multiplication of endangered species. During the *in vitro* stage, several plant growth regulators have been used, such as gibberellic acid in *G. skinneri* (Coello *et al.*, 2010). In *Epidendrum elongatum* Jacq., growth was also enhanced by supplementing the culture medium with activated charcoal, indole-3-acetic acid (IAA), and benzylaminopurine (BAP) (Pedroza, 2009).

In general, *in vitro* micropropagation is a useful technique for cultivating *G. skinneri* (Vázquez *et al.*, 2014; Park *et al.*, 2018); however, its effectiveness requires validation under *ex vitro* conditions (Gil Rivero *et al.*, 2017).

At this stage, it is recommended to consider nutritional, chemical, biological, and environmental factors that may influence plant growth. Growth regulators such as brassinosteroids, which are widely distributed throughout the plant kingdom, have been shown to induce pleiotropic effects in plants, among which their growth-promoting effect stands out (Yang, 2011). This is particularly significant considering that, during the early stages of acclimatization, seedlings exhibit low photosynthetic performance (Kadlecek *et al.*, 2001), as demonstrated in *in vitro* propagated *G. bowringiana*. However, once transferred to *ex vitro* conditions, a rapid increase in photosynthetic pigments (chlorophyll a, b, and carotenoids) has been observed (Buyun *et al.*, 2021). In addition, brassinosteroids have been shown to reduce the effects of abiotic stress factors such as water and heat stress (Bajguz & Tretyn, 2003), mainly by increasing cell membrane permeability (Hernández *et al.*, 2010; Bao-Fundora *et al.*, 2013).

Based on this background, the objective of this study was to identify the influence of foliar application of homobrassinolide at different doses and frequencies on the growth and *ex vitro* acclimatization of *Guarianthe skinneri* (Bateman) Dressler & W.E. Higgins.

## MATERIALS AND METHODS

The research was conducted at the Biotechnology Laboratory and the nursery of the Faculty of Agricultural Sciences, Campus IV of the Universidad Autónoma de Chiapas (15° 00′25.02" N and 92° 23′59.06" W, at an altitude of 32 meters above sea level). The study was carried out in two stages: the first involved seed germination and *in vitro* multiplication, and the second consisted of *ex vitro* acclimatization. The climate in which the plants were grown *ex vitro* is classified as Am(w′′)ig, corresponding to a tropical sub-humid climate (2,200 mm annual precipitation) with two dry periods —one occurring during the rainy season (mid-summer drought or canícula), and the other beginning in November. The average temperature is 28.5 °C, with a minimum of 15 °C and a maximum of 38 °C (García, 2004).

**Seeds and Propagation Medium**

Seeds were obtained from mature plants in the Soconusco region, Chiapas, and were sown eight days after capsule collection. Capsules were first washed with a soapy solution containing Tween 80 and treated with a fungicidal solution of 1.5 g $L^{-1}$ azoxystrobin for 20 min. Subsequently, they were disinfected with sodium hypochlorite (NaClO 3%) for 15 min and then kept in an antioxidant solution until sowing. Sterile capsules were cut longitudinally to extract the seeds, which were germinated *in vitro* using Yasuda *et al.* (1985) medium, supplemented with the vitamins described by Gamborg (2002) and 30 g $L^{-1}$ sucrose. To the medium, the following were added separately: indole-3-acetic acid (IAA) (1.5 $mgL^{-1}$), activated charcoal (AC) (1 g $L^{-1}$), and homobrassinolide (Hbr) (4 $mgL^{-1}$). One treatment was prepared using only the base Yasuda medium as a control. The medium was adjusted to pH 6.3 and solidified with Phytagel (5 g $L^{-1}$). It was sterilized in an autoclave for 15 min at 1.0 kg $cm^2$ pressure and 250 °C. Culture medium (20 mL) was dispensed into Gerber® jars, where seed sowing was carried out.

**Incubation Conditions**

The experimental units for the *in vitro* growth stage were maintained in a controlled-environment growth chamber under cool white fluorescent light, with photosynthetically active radiation of 50 $ME^2$ $s^{-1}$. The photoperiod was 16/8 h (light/dark), with an average temperature of 26±1.0 °C and 50% relative humidity. Once the seeds had germinated, protocorms were selected and transferred to individual jars, which served as experimental units. The culture media used in the multiplication stage contained activated charcoal, with the Yasuda basal medium serving as the control. For the *ex vitro* stage, *Guarianthe skinneri* (Bateman) Dressler & W.E. Higgins plantlets with developed roots and leaves were selected. These were transplanted into 10 oz Styrofoam cups containing ground coconut fiber substrate (Thomas Willey-Medal, Philadelphia, USA).

**Homobrassinolide**

The homobrassinolide (Hbr) CIDEF-4 (Natura del Desierto, S.A.) contains 80% steroidal content, of which 10% is active. It is presented as a non-toxic, water-soluble formulation that is compatible with fertilizers, insecticides, and fungicides (according to label information).

**Experimental Management and Irrigation**

Foliar application of Hbr was carried out at 7, 14, and 21 days after transplanting (DAT) at concentrations of 2, 4, and 6 $mgL^{-1}$.

**Treatments, Experimental Design, and Number of Replications**

The treatments were generated by combining three doses of Hbr (2, 4, and 6 $mgL^{-1}$) with three application frequencies (7, 14, and 21 days), plus a control. All treatments, with 10 replications each, were distributed in a completely randomized design on the nursery benches.

### Variables

Morphological variables and plant survival were recorded at 28, 56, 70, 77, and 91 days after transplanting (dat). Plant height was measured using a graduated ruler from the base to the apex. In addition, the number of green leaves, dry leaves, and dead plants were recorded.

### Statistical Analysis

Plant height was statistically analyzed using SAS software version 9.0 for Windows (SAS Institute Inc., Cary, NC 27513, USA), and means were compared using Tukey's test ($p \leq 0.05$). The other variables were graphed using Sigma Plot software (version 11.0) from Jandel Scientific.

## RESULTS AND DISCUSSION

The applications of Hbr increased the height of *G. skinneri* (Bateman) Dressler & W.E. Higgins compared to the control, but the effect depended on the concentration and the frequency of application to the plant (Figure 1).

The treatment that induced the greatest plant height was when 6 mg $L^{-1}$ of Hbr was applied every 21 days, and this was statistically different from the other treatments ($P \leq 0.05$). Plant height also increased when 4 mg $L^{-1}$ of Hbr was applied at 7 and 14 days after transplanting (dat), but these values were not statistically different.

The increase in plant height with the application of Hbr at different frequencies has been previously reported by Joaquín-Torres *et al.* (2006) and Torres-Ruíz *et al.* (2007) in other species such as Guinea grass (*Panicum maximum* Jacq.) and maize. This effect is attributed to one of the known processes induced by Hbr application, namely cell elongation through its influence on gene expression and/or enzyme activity (Khripach *et al.*, 2000). Furthermore,

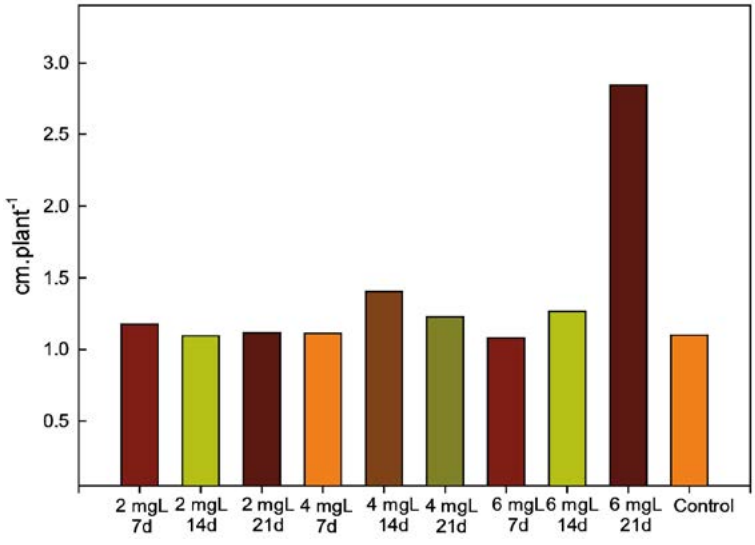


**Figure 1**. Height of *Guarianthe skinneri* (Bateman) Dressler & W.E. Higgins) with Hbr application under nursery conditions at 91 days after transplanting (dat). Means with the same letter are not significantly different (Tukey, $p \leq 0.05$). CV=20.5%.

brassinosteroids play an important role in tolerance to abiotic stress such as drought and temperature fluctuations (Bajguz & Hayat, 2009; Gomes, 2011; Vriet *et al.*, 2012).

**Number of Green and Dry Leaves**

The number of green and dry leaves recorded at different evaluation dates is presented in Table 1.

The initial average number of green leaves with the Hbr application was 12% higher compared to the control. At the end of the evaluation, at 91 dat, the lowest average was 12 for the treatments with 2 mg $L^{-1}$ applied at 7 and 14 days. In contrast, when applying 6 mg $L^{-1}$ at 14 and 21 dat, the highest number of green leaves was induced during the evaluation.

Regarding the interaction between application frequencies of Hbr and the concentrations of the brassinosteroid, it was found that at 21 dat, the highest number of green leaves occurred at the concentrations of 2 and 6 mg $L^{-1}$, while at 14 dat, the highest number was found when the 4 mg $L^{-1}$ concentration was applied.

Capote *et al.* (2009) mentions that the application of Hbr MH5 stimulated the number of leaves and root formation in the Bromeliaceae *Vriesea* sp., and notes that it suggests a synergistic or additive effect with auxins in this process.

The number of dry leaves increased in all treatments with Hbr at 28 dat, but decreased in the control. The most notable effect was observed when Hbr was applied every 7 days. At the end of the evaluation, at 91 dat, the highest number of dry leaves was observed when the 2 and 4 mg $L^{-1}$ doses were applied every 7 days. In contrast, when applying Hbr at a concentration of 6 mg $L^{-1}$, the lowest number of dry leaves was observed.

In this same context, the lowest number of dry leaves at the end of the evaluation, that is, at 91 dat, was recorded when 2, 4, and 6 mg $L^{-1}$ of Hbr were applied with a frequency of 14 days. In this regard, Izquierdo *et al.* (2012) report an increase in leaf area, dry biomass, and total soluble protein content in *Musa* spp.

**Table 1**. Number of green leaves and dry leaves of *G. skinneri* (Bateman) Dressler & W. E. Higgins under *ex vitro* conditions when different frequencies and concentrations of Hbr were applied in the nursery.

| Treatment | Green leaves (Time days) | | | | | | Dry leaves (Time days) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Initials | 28* | 56 | 70 | 77 | 91 | Initials | 28 | 56 | 70 | 77 | 91 |
| 2 mgL (7d) | 42 | 31 | 25 | 31 | 21 | 12 | 5 | 18 | 6 | 1 | 10 | 5 |
| 2 mgL (14d) | 35 | 29 | 32 | 37 | 23 | 12 | 3 | 9 | 12 | 2 | 11 | 0 |
| 2 mgL (21d) | 41 | 22 | 25 | 32 | 23 | 18 | 7 | 16 | 11 | 8 | 4 | 2 |
| 4 mgL (7d) | 35 | 20 | 26 | 30 | 31 | 16 | 0 | 20 | 4 | 0 | 2 | 6 |
| 4 mgL (14d) | 37 | 22 | 26 | 28 | 24 | 17 | 6 | 9 | 2 | 1 | 0 | 1 |
| 4 mgL (21d) | 40 | 22 | 29 | 29 | 25 | 17 | 10 | 9 | 2 | 3 | 3 | 3 |
| 6 mgL (7d) | 51 | 30 | 28 | 29 | 22 | 14 | 4 | 14 | 10 | 4 | 0 | 0 |
| 6 mgL (14d) | 38 | 24 | 31 | 34 | 29 | 22 | 10 | 7 | 4 | 3 | 4 | 0 |
| 6 mgL (21d) | 37 | 32 | 33 | 33 | 34 | 27 | 0 | 9 | 2 | 0 | 0 | 3 |
| Control | 34 | 22 | 27 | 29 | 29 | 17 | 11 | 5 | 3 | 2 | 1 | 3 |

***** Days after being established in the greenhouse.

**Dead plants by treatment and sampling**

The number of dead plants shows a certain relationship with the concentrations and frequencies of the applications. The highest concentration of 6 mg $L^{-1}$ and the most frequent application every seven days resulted in the lowest number of dead plants (Figure 2).

However, the lowest concentration of 2 mg $L^{-1}$, regardless of the application frequency, and the control treatment, showed the highest number of dead plants. A similar result was reported by Francisco *et al.* (2011) at 90 days, who found that the highest value for the start of acclimatization with *Laelia eyermaniana* occurred between 60 and 90 days, primarily in

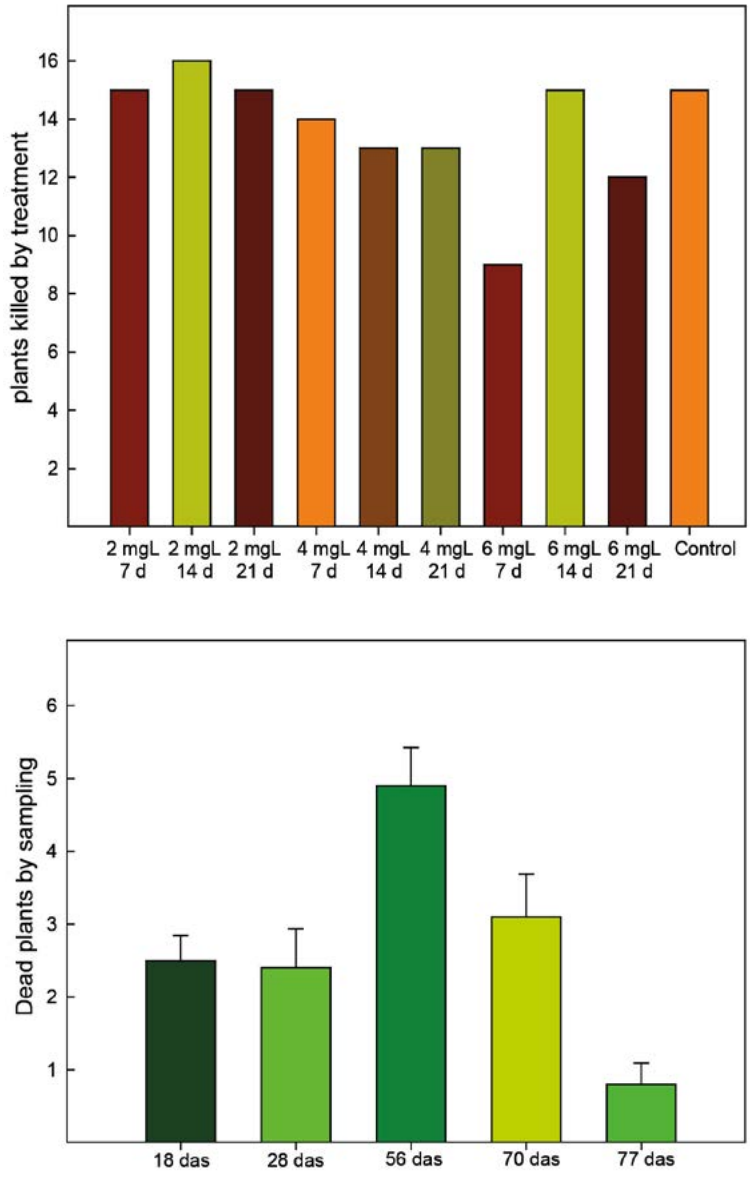


**Figure 2**. Number of dead *G. skinneri* (Bateman) Dressler & W. E. Higgins plants with different frequencies and applications of Hbr during *ex vitro* acclimatization. a) Dead plants by treatment b) Dead plants by sampling. The vertical line indicates ± the standard error.

small (21.87) and medium-sized (31.25) seedlings. They have also reported good results when using brassinosteroids (Bajguz and Hayat, 2009; Kagale *et al.*, 2007), as they mitigate both biotic and abiotic stress.

The previous behavior suggests that the concentration of Hbr applied influences plant acclimatization, but with a lesser effect from the frequency of application. Posadas *et al.* (2016) report an 80-82% increase in survival during the acclimatization of *Carica papaya* cv. Maradol Roja with the application of Hbr Pectimorf in Cuba. In *Tuberaria major* (Willk.), a plant found near the coasts of Portugal, 97% survival was achieved six weeks after transplanting (Goncalves *et al.*, 2010).

The samples taken at 18 and 28 days after transplanting show the lowest values of plant mortality, with an average of 2.5 dead plants in each sampling. After 56 days of establishment, the number of dead plants significantly increased across treatments. The average reached 4.9, representing almost 100% in relation to the first two samplings. After this increase, plant mortality gradually decreased to 3 plants at 70 ddt, and by 77 ddt, it was less than one.

Preece and Sutter (1991) mention that *ex vitro* acclimatization presents dehydration problems due to the loss of leaf water and restricted uptake, as the roots are incapable of absorbing water in the early stages. This is one of the main causes of plant mortality when they are transferred to *ex vitro* conditions. In the first weeks after transfer to the *ex vitro* environment, plants must adapt to new growing conditions and develop a normal and functional root system physiology (Debergh *et al.*, 2000). Capote *et al.* (2009) indicate that at 28 and 49 days, significant differences were found in the survival of plants treated with MH5 compared to the control group with no application of this regulator. The lowest percentages (66%) were observed in the plants that did not receive the application of the brassinosteroid analog at the end of this phase.

## CONCLUSIONS

The foliar application of the Brassinosteroid CIDEF-4 induced greater growth of *G. skinneri* (Bateman) Dressler & W.E. Higgins, which was expressed in height. The more frequent application of Br at higher concentrations improves the acclimatization and survival of *G. skinneri* (Bateman) Dressler & W.E. Higgins in the nursery. Brassinosteroid concentrations with foliar application at different frequencies induce differences in the growth and survival of *G. skinneri* (Bateman) Dressler & W.E. Higgins.

## ACKNOWLEDGMENTS

To the Faculty of Agricultural Scences-Campos IV. Autonomous Univernity of Chiapas, Huehuetán, México, for the facilities provided in the nursery and Laboratory.

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