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Use of *Zingiber officinale* Roscoe (ginger) in the *in vitro* culture of *Oncidium cebolleta* (Jacq.) Sw.

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Abstract

In vitro cultivation of orchids has been widely employed as an efficient alternative for the propagation of species with difficult natural reproduction. In this context, the use of alternative plant-based supplements has proven to be a promising strategy to optimize the growth and acclimatization of micropropagated plants. This study aimed to evaluate the growth of *Oncidium cebolleta* protocorms in an alternative culture medium with different concentrations of *Zingiber officinale* Roscoe, in order to establish the best concentration for the propagation of the species. The experiment was conducted in a completely randomized design, with five treatments, five replications, and three protocorms per replication, using an alternative culture medium containing sucrose, agar, activated charcoal, B&G® fertilizer, and coconut water, supplemented with ginger extract at concentrations of 0, 0.5, 1.5, and 2 g L^{-1}. After 130 days of cultivation, morphological and biometric variables were evaluated. The concentration of 1.5 g L^{-1} showed the best results for the length of the largest leaf, number and length of roots, and total plant length. The results demonstrate that *Z. officinale* extract positively influences the in vitro growth of *O. cebolleta* and can be considered a promising natural supplement for optimizing micropropagation and acclimatization protocols of tropical orchids.

Keywords: Orchids; Micropropagation; Plant extracts; Alternative supplementation; Plant biotechnology.

INTRODUÇÃO

Orchids belong to one of the largest families of angiosperms and exhibit high diversity in tropical regions, with terrestrial, rupicolous, and epiphytic growth habits (Pereira, 2010; Colombo et al., 2004). In Brazil, approximately 235 genera and more than 2,400 species occur, of which 66 genera and 1,632 species are endemic (Barros et al., 2015).

Due to their great diversity in color, shape, and size, orchids are among the most widely marketed ornamental plants worldwide. In addition to their ornamental value, some species are used in traditional medicine and in the food industry, such as species of the genus *Vanilla* (Barros, 2015; Santos, 2023; Kayalvizhi et al., 2020; Yadav et al., 2024).

The species *O. cebolleta* has an epiphytic growth habit and belongs to a group of plants considered specialized for xeric environments due to their morphological and functional adaptations. The presence of pseudobulbs, root velamen, a thick mesophyll, and CAM metabolism represents highly efficient structural and physiological adaptations that enable this species to survive under conditions of limited water and nutrient availability (Queiroz, 2015).

Under natural conditions, sexual propagation through seeds results in a relatively small number of individuals. In contrast, plant tissue culture enables the production of a larger number of plants free from bacterial contamination, pests,

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and other sources of contamination, allowing to produce high-quality plant material on a large scale (Thakur et al., 2024).

In vitro tissue culture, the composition of the culture medium is essential for plant growth and development. Various compounds can be added to the culture medium to enhance plant growth, including fruits, peels, rhizomes, seeds, oils, and other natural products. Alternative supplements, such as activated charcoal and pyrolytic wood vinegar, have also been investigated for this purpose (Caramelo et al., 2022).

Some compounds added to culture media, such as ginger essential oil (*Zingiber officinale* Roscoe), have demonstrated inhibitory effects against *Fusarium graminearum* (Celestino, 2016) and reduced the germination of *Colletotrichum gloeosporioides* spores by 90% (Celoto, 2008). Similarly, Kalhoro et al. (2022) observed that ginger reduced the germination and growth of *Phytophthora* sp. These studies demonstrate the fungicidal potential of ginger rhizomes. Therefore, this study aimed to evaluate the growth of *Oncidium cebolleta* protocorms in an alternative culture medium supplemented with different concentrations of *Zingiber officinale* Roscoe, in order to determine the concentration most suitable for the propagation of this species.

MATERIAL AND METHODS

Experimental site

The experiment was conducted at the Laboratory of Cytogenetics and Plant Tissue Culture, located on Campus II of the State University of Mato Grosso (UNEMAT), Alta Floresta, Mato Grosso, Brazil.

Plant Material Origin

The mother plants from which the seeds were obtained were maintained at the Altaflorestense Orchidarium and originated from a rescue program associated with the Colíder Hydroelectric Power Plant, in Mato Grosso, Brazil. *Oncidium cebolleta* protocorms were obtained through *in vitro* seed germination on an alternative culture medium containing 30 g L^{-1} sucrose, 4 g L^{-1} agar, 2 g L^{-1} activated charcoal, 2 g L^{-1} B&G® fertilizer, and 100 mL L^{-1} coconut water, according to Rodrigues et al. (2012).

Inoculation and Culture Conditions

The experiment consisted of five treatments, with five replicates per treatment and three protocorms per replicate, totaling 25 culture vessels with a capacity of 300 mL, each containing 10 mL of the alternative culture medium. Before autoclaving, the medium was supplemented with different concentrations of freshly harvested and ground *Zingiber officinale* rhizome: 0, 0.5, 1.5, and 2.0 g L^{-1} (T1, T2, T3, and T4, respectively). A total of 20 protocorms were evaluated per treatment. The culture medium pH was adjusted to 5.5 and subsequently autoclaved at 121 °C and 1 kg cm^{-2} for 20 min.

After sterilization, the culture vessels were transferred to a laminar-flow cabinet and exposed to ultraviolet (UV) light for 15 min. The protocorms were then transferred to the culture medium and maintained under a 16-h photoperiod, with a light intensity of 2,000 lux, at a temperature of 22 ± 1 °C.

Evaluation

At 130 days after protocorm transplantation, the following growth parameters were evaluated: longest leaf length, determined as the distance from the plant collar to the leaf apex (cm); longest root length, determined as the distance from the plant collar to the root tip (cm); total plant length, determined as the distance from the apex of the longest root to the apex of the longest leaf (cm); number of leaves, determined by counting the leaves per plant; number of roots, determined by counting the roots per plant; and total fresh biomass (g).

Data Analysis

The experiment was conducted in a completely randomized design (CRD). The data were first subjected to tests of normality and subsequently to mean comparison tests and regression analysis, using a 5% significance level. Statistical analyses were performed in R software (R Core Team, 2016), with the ExpDes package (Ferreira, 2013).

RESULTS AND DISCUSSION

Only fresh mass (MF) and number of leaves (NF) showed no significant differences among the ginger concentrations evaluated (Table 1). Longest

leaf length (CMF) and total length (CT) showed significant responses at the 5% probability level, whereas longest root length (CMR) was significant at the 1% probability level and number of roots (NR) at the 0.1% probability level. Mean comparisons were performed using Tukey's test.

For the number of leaves (NF), no significant differences were observed among the ginger concentrations evaluated. The treatment without ginger supplementation showed the highest mean value, with 6.1 leaves per plant. Similarly, *Paphiopedilum glaucophyllum* showed significant differences in leaf number following the addition of banana to the culture medium, with the highest value observed for this variable. In contrast, *P. primulinum* showed no significant differences in leaf number among treatments (Safitri et al., 2024).

The longest leaf length (CMF) differed significantly among treatments, with T4 (60.70 mm) showing the highest value, followed by T5 (49.64 mm). Overall, leaf length increased with increasing ginger concentration, indicating a positive response of leaf elongation to ginger supplementation. Similar results were reported by Silva et al. (2017) for *Oeceoclades maculata* (Lindl.) Lindl., in which the addition of pyrolytic wood vinegar at low concentrations promoted plant growth under both *in vitro* and *ex vitro* conditions. However, increasing concentration inhibited plant growth, indicating that the response to natural supplements may depend on their concentration.

The increase in leaf size may contribute to plant development, as a greater leaf area may enhance photoassimilate production under adequate nutritional conditions (Schneider et al., 2024). Similarly, Freitas (2019) reported that the addition of banana pulp and peel to the culture medium promoted shoot growth in *Cattleya nobilior*. In *Dendrobium welirang*, shoot growth was also enhanced by increasing the concentration of activated charcoal in the culture medium (Warisman et al., 2024).

For the number of roots (NR), the treatment without ginger supplementation (T1) did not result in root formation, with plants developing only aerial parts. The highest mean values were observed in T4 and T5 (1.5 and 2.0 g L^{-1}, respectively), with 4.2 and 4.3 roots per plant, respectively, indicating that increasing ginger concentration favored root formation. This response may potentially enhance nutrient uptake from the culture medium. Similarly, Aquino et al. (2021) reported that the addition of banana pulp significantly affected this variable, increasing the number of roots in *Cattleya* sp., with no significant difference between the use of apple banana and plantain.

Table 1: Summary of the analysis of variance for the number of leaves (NF), longest leaf length (CMF, mm), number of roots (NR), longest root length (CMR, mm), total length (CT, mm), and fresh mass (MF, g) as a function of *Zingiber officinale* Roscoe concentration (g L^{-1}).

FV	GL	NF		CMF (mm)		**NR**	
		Q.M	P	Q.M	P	Q.M	P
Treatments	4	7.985	0.0566^{ns}	1867.96	0.017*	17.3628	0.00003***
Residuals	20	2.9		480.01		1.3828	
CV_{exp} (%)		35.33		62.47		50.11	
Overall mean		4.82		35.07		2.35	

FV	GL	CMR (mm)		CT (mm)		MF (g)	
		Q.M	P	Q.M	P	Q.M	P
Treatments	4	1951.63	0.0017**	3049	0.0438*	0.05612	0.1363^{ns}
Residuals	20	303.66		1020.8		0.02832	
CV_{exp} (%)		54.33		44.20		59.14	
Overall mean		32.08		72.28		0.28	

^{ns}, *, ** and ***: not significant, significant at 0.05, 0.01, and 0.001 probability (P), respectively. FV: Source of variation. GL: Degrees of freedom. CVexp: Experimental coefficient of variation (%).

Klaocheed et al. (2021) reported an increase in root number when *Dendrobium crumenatum* Sw. was cultured on a medium supplemented with 15% coconut water (v/v) and 0.2% activated charcoal (w/v). Similarly, in experiments with *Brassiocattleya* 'Pastoral' × *Laeliocattleya* 'Amber Glow', increasing the vitamin concentration in the culture medium promoted root formation, potentially enhancing nutrient uptake and fresh biomass production (Silva, 2003). In this context, banana has been used as a vitamin supplement and rooting stimulant due to its high potassium and mineral salt content, while its pulp is also a source of phytohormones, including cytokinins, auxins, and gibberellins (Dolce et al., 2020).

The longest root length (CMR) showed a significant difference at the 1% probability level, with T3 (1.0 g L^{-1} ginger) showing the highest response. The quadratic response indicated that relatively low ginger concentrations promoted root elongation, whereas higher concentrations resulted in a reduction in root length. Similar results were reported by Hwang et al. (2024), who observed increased root length in *Dendrobium moniliforme* (L.) Swartz when banana was added to the culture medium.

Total length (CT) reflects the combined response of leaf and root growth, with T4 showing the highest value. For fresh mass (MF), no significant differences were observed among treatments; however, T4 and T5 showed higher mean values than the other treatments.

Based on Figure 1, the ginger concentrations produced distinct responses in the *in vitro* growth characteristics of *O. cebolleta*. For NR, a positive linear response was observed as ginger concentration increased, with the highest predicted value at 2.0 g L^{-1}, corresponding to the upper limit of the concentrations evaluated. For CMR, the quadratic response allowed the estimation of an optimal concentration of approximately 1.25 g L^{-1}, corresponding to the maximum response predicted by the model. Overall, the highest observed mean values for CMF, NR, and CT were associated with ginger concentrations of 1.5 to 2.0 g L^{-1}, whereas the highest CMR value was observed at 1.0 g L^{-1}. For MF, although T4 and T5 showed higher mean values, no significant differences were observed among treatments.

For mean root length (CMR), the quadratic response indicated an estimated optimum ginger concentration of approximately 1.25 g L^{-1}, which is between 1.0 and 1.5 g L^{-1}. Above this concentration, a reduction in root growth was observed. Similar responses in root growth have been reported using different approaches in micropropagated plants. In *Brassiocattleya* 'Pastoral' × *Laeliocattleya* 'Amber Glow', root elongation was associated with the use of half-strength Knudson medium, in which reduced nutrient availability may stimulate plants to explore the culture medium for nutrients. Another approach involves increasing the vitamin concentration in Murashige and Skoog (1962) medium, which promoted root growth and had a beneficial effect on plant development (Silva, 2003).

For fresh mass (MF) and number of leaves (NF), no statistically significant differences were observed among the ginger concentrations evaluated. Among the treatments, T3 showed the highest mean value for NF, whereas T4 showed the highest mean value for MF.

For longest leaf length (CMF), number of roots (NR), and total length (CT), positive linear, rather than exponential, responses were observed with increasing ginger concentration. Therefore, an optimum concentration cannot be estimated for these variables within the evaluated range based on the linear models. The highest observed values were associated with T4 (1.5 g L^{-1}) or T5 (2.0 g L^{-1}), depending on the variable. Total length reflects the combined development of leaves and roots. An increase in total plant size was observed with increasing ginger concentration, which may contribute to greater plant suitability for acclimatization and facilitate the transition to *ex vitro* conditions. Amatuzzi et al. (2020) reported significant differences for this variable in *Epidendrum secundum*, in which supplementation with an aqueous extract of the seaweed *Kappaphycus alvarezii* at 50 mg L^{-1} resulted in increased fresh mass and greater plantlet development.

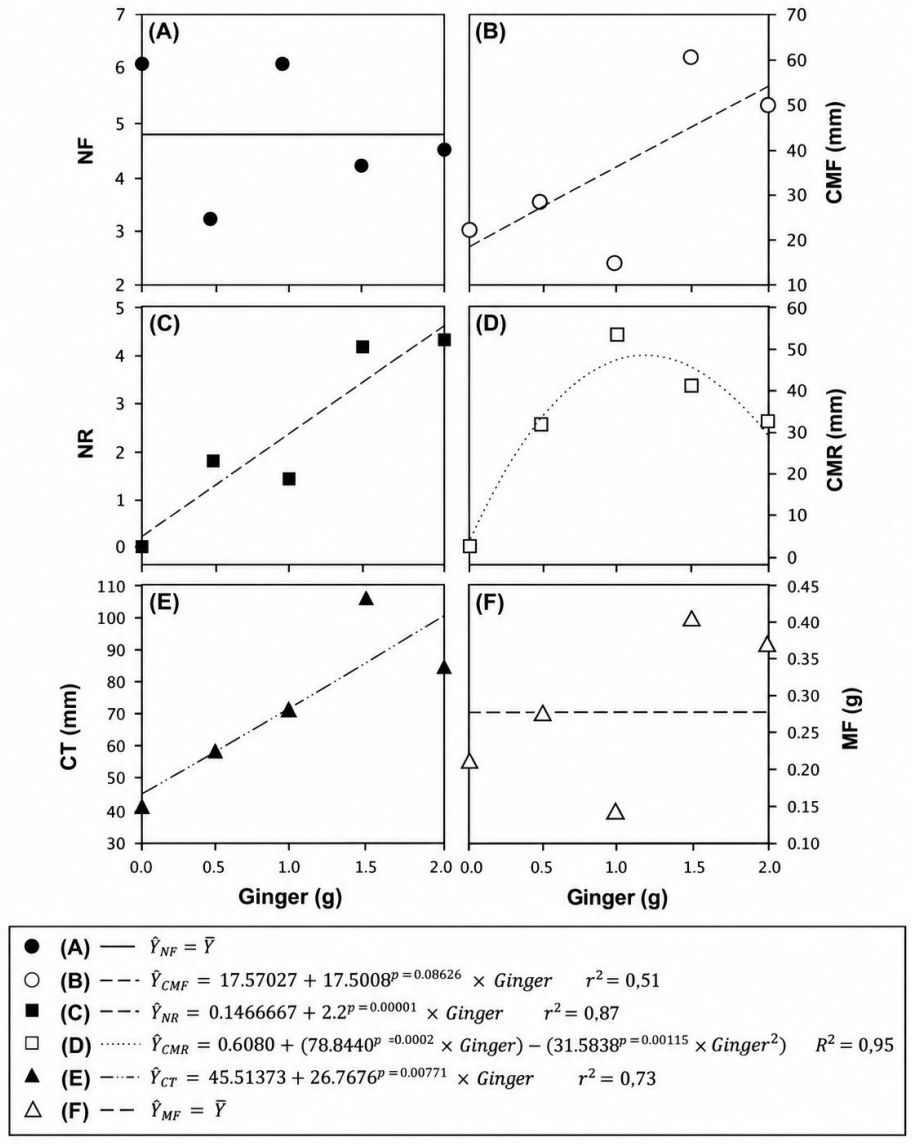


Figura 1: Número de folhas (NF), comprimento da maior folha (CMF, mm), número de raízes (NR), comprimento da maior raiz (CMR, mm), comprimento total (CT, mm) e massa fresca (MF, g) em função da concentração de Gengibre (g L^{-1}) (*Zingiber officinale Roscoe*). Source: The authors.

CONCLUSIONS

Based on the results obtained, it can be concluded that ginger extract influenced the growth of *O. cebolleta* protocorms, with generally higher growth responses at increasing ginger concentrations. Among the concentrations evaluated, 1.5 g L^{-1} resulted in the highest values for several growth-related traits. In addition, the quadratic response observed for mean root length allowed the estimation of an optimum ginger concentration of approximately 1.25 g L^{-1}, corresponding to the maximum root length predicted by the model. These results demonstrate the potential of ginger extract to promote protocorm development and the production of plants with characteristics favorable for acclimatization. However, further studies are needed to determine the concentration that provides the best balance between individual plant growth and plant production, thereby improving the efficiency of *O. cebolleta* propagation.

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