Ornamental Horticulture
ISSN 2447-536X | WWW.ORNAMENTALHORTICULTURE.COM.BR
SBFPO Sociedade Brasileira de Floricultura e Plantas Ornamentais

ARTICLE

Cytokinin and gibberellin influence growth and development of *Cattleya guttata* Lindl. (Orchidaceae) *in vitro* seedlings

Citocinina e giberelina influenciam o crescimento e o desenvolvimento de plântulas de *Cattleya guttata* Lindl. (Orchidaceae) *in vitro*

Marco Aurelio Ribeiro Schuffner[1], Otalício Damásio da Costa Junior[1], Luís Henrique Bueno[2], Josimar dos Santos Ladeira[1], Lucilene Silva de Oliveira[1], Maurecilne Lemes da Silva[3], and Diego Ismael Rocha[1,*]

[1]Universidade Federal de Viçosa, Departamento de Agronomia, Viçosa-MG, Brasil.
[2]Universidade Federal de Viçosa, Departamento de Biologia Vegetal, Viçosa-MG, Brasil.
[3]Universidade do Estado de Mato Grosso, Departamento de Biologia, Tangará da Serra-MT, Brasil.

Abstract
Few studies have investigated the effects of plant growth regulators (PGRs) on the development of native Brazilian orchids threatened with extinction and with high ornamental potential, such as *Cattleya guttata* Lindl. The present study aimed to investigate the effects of cytokinin and gibberellin on the early development of *Cattleya guttata* seedlings grown *in vitro*. Seedlings 120-days-old were cultured in MS medium supplemented with either 6-benzyladenine (BA; 4.44 µmol L^{-1}), gibberellic acid 3 (GA; 2.89 µmol L^{-1}), paclobutrazol (PBZ; 0.85 µmol L^{-1}), and BA+GA (BA: GA), or BA+PBZ (BA: PBZ) at the same concentrations. The control treatment consisted of MS medium without PGRs. After 140 days of *in vitro* growth, seedling development and leaf size and shape were assessed. BA supplementation significantly increased the formation of axillary shoots, resulting in modified seedling architecture. However, the simultaneous application of GA attenuated the effects of BA. Both BA and GA, whether applied individually or together, suppressed root development. When PBZ was added to the medium alone or in combination with BA, the root parameters were like the control. PBZ in the medium affected leaf morphology. BA and PBZ together produced a negative correlation between leaf width and length-width ratio. This study clarifies how cytokinin-gibberellin interactions affects *in vitro* growth and early development in this ornamental plant species.
Keywords: leaf development, plant growth regulators, phytohormones, orchids.

Resumo
Poucos estudos investigaram os efeitos de reguladores de crescimento vegetal (RCVs) no desenvolvimento de orquídeas brasileiras, nativas, ameaçadas de extinção e que possuem alto potencial ornamental de mercado, como é o caso da *Cattleya guttata* Lindl. O presente estudo teve como objetivo investigar o efeito da citocinina e giberelina no desenvolvimento inicial de mudas de *Cattleya guttata* cultivadas *in vitro*. Mudas de 120 dias de idade foram cultivadas em meio MS suplementado com 6-benziladenina (BA; 4,44 µmol L^{-1}), ácido giberélico 3 (GA; 2,89 µmol L^{-1}), paclobutrazol (PBZ; 0,85 µmol L^{-1}) e BA + GA (BA: GA) ou BA + PBZ (BA: GA) nas mesmas concentrações. O tratamento controle consistiu em meio MS sem RCVs. Após 140 dias de crescimento *in vitro*, o desenvolvimento das plântulas e o tamanho e a forma das folhas foram avaliados. A suplementação com BA aumentou significativamente a formação de brotos axilares, resultando em arquitetura modificada das plântulas. No entanto, a aplicação simultânea de GA atenuou os efeitos do BA. Tanto o BA quanto o GA, aplicados individualmente ou em conjunto, suprimiram o desenvolvimento radicular. Quando o PBZ foi adicionado ao meio de cultura, isoladamente ou em combinação com o BA, os parâmetros radiculares foram semelhantes aos do controle. O PBZ, no meio de cultura, afetou a morfologia foliar. A combinação de BA e PBZ resultou em correlação negativa entre a largura da folha e a relação comprimento/largura. Este estudo esclarece como as interações entre citocinina e giberelina afetam o crescimento *in vitro* e o desenvolvimento inicial desta espécie ornamental.
Palavras-chave: desenvolvimento foliar, fitohormônios, orquídeas, reguladores de crescimento vegetal.

Introduction

The Orchidaceae family comprises approximately 28,000 species, divided into five subfamilies, making it one of the largest and most diverse families of flowering plants. It represents about 8% of the total diversity of angiosperms cataloged worldwide (Chase et al., 2015). The remarkable diversity of orchids is attributed to their ability to thrive in diverse habitats, ranging from temperate climates to extensive tropical forests (Wraith and Pickering, 2019). Their adaptability is associated with their capacity to grow in soil, on phorophytes, and on rocks. Despite their adaptive success, these plants belong to one of the most threatened taxonomic groups worldwide (Wraith and Pickering, 2019).

In Brazil, several species of the genus *Cattleya* are listed as endangered species, including *Cattleya guttata* (Barros et al., 2015). This species belongs to the subgenus *Intermediae* and is found from Santa Catarina to Bahia State, predominantly in coastal plains. However, its distribution also extends to the interior of Paraná State and the eastern region of Minas Gerais State (Barros et al., 2015). *C guttata* has a peculiar morphology and a high ornamental market potential. It has a rhizomatous stem with two internodes on the rhizome (Barros et al., 2015). The longest pseudobulb can exceed 30 cm in length, varying from three to five internodes, with a cylindrical or thickened shape (Barros et al., 2015). The leaves are elliptical or oblong, and each pseudobulb can bear two to three leaves (Barros et al., 2015).

Despite the peculiar and appreciated morphology of orchids, information regarding the role of hormonal interactions in determining the identity and growth of plant orchid organs is incipient. Cytokinin and gibberellin are primary regulators of plant development, playing a pivotal role in the spatial and temporal development of plant tissues and organs (Petřík et al., 2024). Gibberellin is involved in several physiological processes in plants and has been linked to the regulation of plant height, leaf expansion, stem elongation, and floral initiation (Wang and Wang, 2022). In addition to gibberellin, paclobutrazol (PBZ), a gibberellin biosynthesis inhibitor, directly influences plant height, thereby reducing plant growth and development (Desta and Amare 2021). Cytokinins, in turn, induce multiple shoot formations under *in vitro* conditions (Nowakowska et al., 2022). They also cooperate in breaking axillary bud dormancy, mobilizing nutrients, chloroplast differentiation, responding to biotic and abiotic stresses, and inhibiting the senescence (Wu et al. 2021).

Several studies have investigated the use of plant growth regulators (PGRs) in the *in vitro* propagation of orchid species, including members

* **Corresponding author:** diego.rocha@ufv.br | https://doi.org/10.1590/2447-536X.v32.e322941 | Editor: Margherita Irene Beruto, International Society for Horticultural Science, Italy | Received: Apr 10, 2025 | Accepted: Nov 22, 2025 | Available online: Jan 19, 2026 |

of the genus *Cattleya*, such as *C. gaskelliana* (Hussien et al., 2024), *C. nobilior* (Nunes et al., 2025), *C. tigrina* (Menezes-Sá et al., 2021), *C. warneri* (Navarro et al., 2023), and *C. walkeriana* (Ramos et al., 2024). In most of these reports, 6-benziladenine (BA) has been used alone or in combination with other PGRs to induce shoot proliferation (Nongdam and Tikendra, 2014; Navarro et al., 2023; Hussien et al., 2024). However, only a limited number of these studies have examined in detail the morphophysiological effects of PGRs and their interactions on plant organ development. Nongdam and Tikendra (2014) highlighted that the application of PGRs, whether individually, in combination, or at varying concentrations, plays a critical role in inducing new shoots and leaf formation. Similarly, Silva et al. (2020) emphasized the importance of establishing a vigorous root system under *in vitro* conditions before acclimatization. Given that orchids typically exhibit slow growth, and the supplementation with exogenous PGRs can significantly enhance their development (Varfolomeeva et al., 2021), elucidating how PGRs influence the morphological patterns of *C. guttata*, particularly its architecture, leaf morphology, and root development, may be helpful for the successful establishment of *in vitro* culture systems for this species.

In the present study, we have evaluated the effects of BA, gibberellic acid 3 (GA), and PBZ supplementation in the culture media and their interactions on the development and architecture of *C. guttata in vitro* seedlings to understand how the balance between cytokinin and gibberellin modulates the peculiar morphological pattern observed in *C. guttata* and in other orchid species.

Materials and methods

Plant material

Fruits of *C. guttata* were obtained through crosses performed on mother plants of the same species. Before sowing, in a laminar flow chamber, the capsules were immersed in 92% alcohol and, before opening, were flamed. Using tweezers and a scalpel, the seeds were removed from the capsule and transferred to a previously autoclaved empty glass vial (125 x 55 mm). Then, sodium hypochlorite (NaClO) (0.5%) was added to disinfect the seeds, which were then stirred for 15 minutes. The seeds were then strained using a paper strainer into another vial, both of which were autoclaved. After this procedure, the seeds were not washed. Subsequently, using autoclaved tweezers and a spatula, the filter paper containing the seeds was placed on a sterile Petri dish. Then, the seeds were inoculated in flasks (125 x 55 mm) containing 40 mL of ½MS medium (Murashige and Skoog 1962), 30 g L^{-1} of sucrose, and 1.5% of activated charcoal. The pH was adjusted to 5.7 ± 0.1, and the mixture was solidified with 8 g L^{-1} bacteriological agar (SIGMA®) before autoclaving at 121 °C and 1.1 atm for 20 minutes. The flasks were stored in a growth room at 25 ± 2 °C with a 16:8-hour photoperiod (light-dark), provided by LED lamps with an irradiance of 50 µmol m^{-2} s^{-1}.

In vitro growth conditions

After germination, 120-day-old seedlings were selected according to their size (1 cm height) and transferred to MS medium, with the same composition mentioned above, but supplemented with 4.44 µmol L^{-1} BA (BA), 2.89 µmol L^{-1} GA (GA), 0.85 µmol L^{-1} PBZ (PBZ), 4.44 µmol L^{-1} BA + 2.89 µmol L^{-1} GA (BA: GA), and 4.44 µmol L^{-1} BA + 0.85 µmol L^{-1} PBZ (BA: PBZ). The control treatment consisted of culture medium without PGRs. The culture conditions were the same as described in paragraph Plant material.

Growth parameters

After 180 days of *in vitro* culture, the following parameters were measured: number of buds per explant over 0.5 cm, number of roots and leaves; longest root length (cm), fresh weight (g), dry weight (g); length (cm), width (cm), area (cm^2) of leaf, and ratio leaf length-to-width. When the explant consisted of more than one shoot and/or seedling, the number of roots, leaves, and root length were evaluated in the greatest shoot/seedling. Leaf parameters were measured in the second pair of leaves of the largest shoot/seedling of the explant. The morphometric parameters, including length, width, and thickness, were obtained through image analysis using ImageJ software, version 1.32j (Wayne Rasband, National Institutes of Health, USA). To determine the total dry matter, the plant material was placed in identified paper bags and dried in a forced-air circulation oven at 60 °C until a constant weight was achieved. Subsequently, they were weighed on an analytical balance to determine their dry matter content.

Statistical Analyses

The experiment was conducted in a completely randomized design, with six treatments (PGRs) and four replicates. Each replicate consisted of three test tubes (150 × 20 mm), with one explant per tube. The experimental data were subjected to the Shapiro-Wilk test to assess the normality assumption. Subsequently, they were subjected to analysis of variance (ANOVA), and the means when the F-test detected significant differences were compared using Tukey's test at a 5% probability level. Statistical analyses were conducted using R Studio, version 4.3.2 (R Core Team, 2024). All evaluated variables from *C. guttata* plants were subjected to Pearson correlation matrix analysis at a significance level ($p < 0.05$). Data correlation analyses were also performed using the RStudio statistical program.

Results

The PGR-treatments promoted significant changes in the morphology and architecture of *C. guttata* seedlings (Fig. 1A). However, no significant differences were observed in seedling height (data not shown). BA, alone or combined with PBZ, increased the number of axillary buds to a similar extent (Fig. 1B). Axillary buds in BA and BA: PBZ treatments were 70.19% and 78.17% higher than controls, respectively.

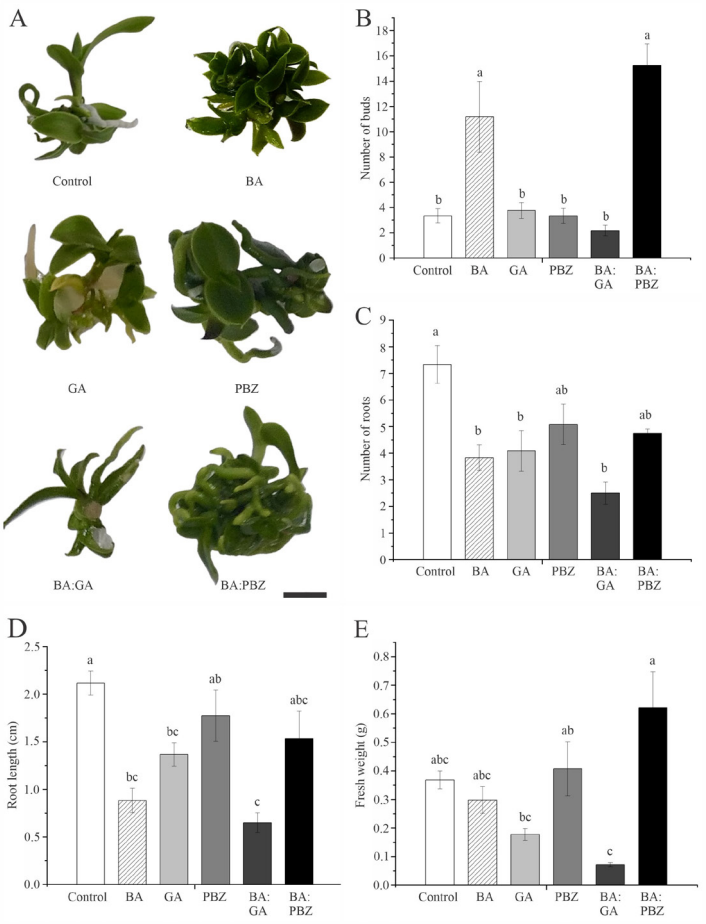

Fig 1. Morphological and morphometric analysis of *Cattleya guttata* seedlings grown in MS medium supplemented with different plant growth regulator treatments. Seedling morphology (A). Number of axillary buds (B). Number of roots (C). Root length (cm) (D). Fresh weight (E). Means represented in each graph, followed by equal letters, do not differ at a 5% probability level using the Tukey test. Abbreviations: BA, 6-benzyladenine; GA, gibberellic acid 3; PBZ, paclobutrazol. Bar = 1 cm.

The higher number of roots per shoot was scored when seedlings were grown on media with no PGRs. When media were supplemented with BA and GA (GA and BA: GA), shoots showed a significant reduction in the number of roots compared to the control (Fig. 1C). In contrast, shoots grown on media supplemented with the GA biosynthesis inhibitors (PBZ and BA: PBZ) showed a higher number of roots, somewhat reduced compared to the control but not significantly different from it (Fig. 1C). This trend was more or less reflected in the length of the root and shoots grown on media added with BA and PBZ (PBZ and BA: PBZ) provided greater root length, not statistically different from the control (Fig. 1D). The PBZ: BA treatment produced the highest fresh mass; the lowest fresh weight was recorded for GA and BA: GA treatments (Fig. 1E). Dry mass did not differ among treatments. The number, shape, and size of *C. guttata* leaves were also affected by the PGR-treatments (Fig. 2A). Treatments supplemented with GA (GA and BA: GA) showed a slight reduction in the number of leaves compared to the control (Fig. 2B). PBZ supplementation provided an increase in leaf width (Fig. 2C), and all PGR-treatments promoted a reduction in leaf length, in comparison to the control (Fig. 2D). However, only PBZ treatments (PBZ and BA: PBZ) showed changes in the length-width relationship of the leaves (Fig. 2E), presenting leaves with a more rounded shape (Fig. 2A). Leaf area was reduced in treatments supplemented with PGRs (Fig. 2F).

The Pearson correlation matrix enabled us to examine the relationships among morphometric variables in *C. guttata* seedlings. PBZ strongly influenced seedling morphology. Treatments supplemented with BA and PBZ resulted in a negative correlation between leaf width (LW) and the length-width ratio (LWR) (Fig. 3A), indicating that the wider the leaves, the smaller the LWR. BA and PBZ also significantly affected the number of leaves (NL) and the length-to-width ratio (Fig. 3A). The lower the length-to-width ratio, the higher the number of leaves in the seedlings. Conversely, treatments supplemented with BA and BA: GA exhibited a positive correlation between the number of buds (NB) and fresh weight (FW) (Fig. 3B).

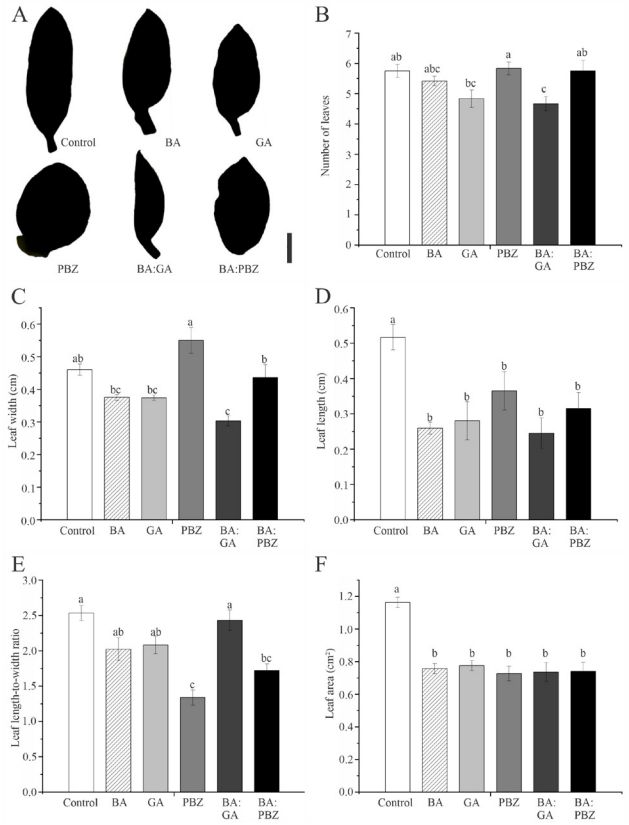

Fig 2. Leaf parameters of *Cattleya guttata* seedlings grown in MS medium supplemented with different plant growth regulator treatments. Leaf morphology (A). Number of leaves (B). Leaf width (cm) (C). Leaf length (cm) (D). Leaf length-width ratio (E) and leaf area (F). Means represented in each graph, followed by equal letters, do not differ at a 5% probability level using the Tukey test. Abbreviations: BA, 6-benzyladenine; GA, gibberellic acid 3; PBZ, paclobutrazol. Bar = 0.5 cm.

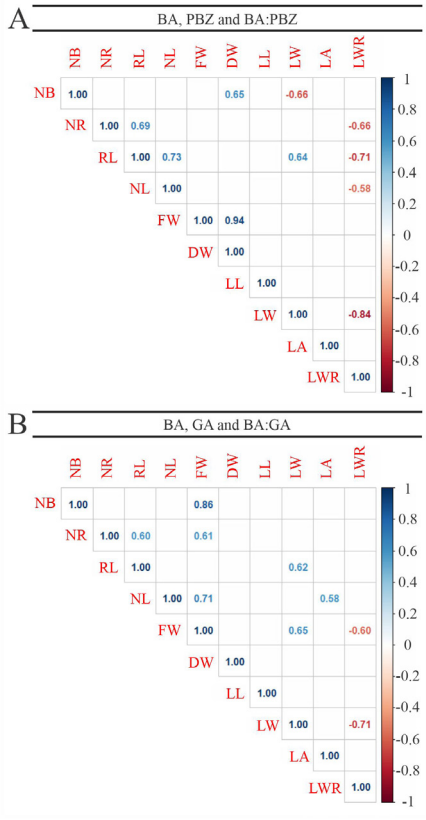

Fig 3. Pearson correlation analysis. Correlation among the growth parameters of the BA, PBZ, and BA: PBZ treatments (A) and BA, GA, and BA: GA (B). Growth parameters: number of buds (NB), number of roots (NR), root length (RL), number of leaves (NL), fresh weight (FW), dry weight (DW), leaf length (LL), leaf width (LW), leaf area (LA), and leaf length-to-width ratio (LWR). Abbreviations: BA, 6-benzyladenine; GA, gibberellic acid 3; PBZ, paclobutrazol.

Discussion

The supplementation of the culture medium with BA, GA, and PBZ promoted significant morphological changes in *Cattleya guttata* seedlings. Treatments supplemented with BA promoted a substantial increase in axillary buds, altering the branching and architecture of the seedlings. Cytokinins are essential for the formation and/or activation of buds and, through a negative interaction with auxins, coordinate the development of axillary buds in plants. Auxins inhibit cytokinin biosynthesis and suppress axillary bud growth (Nordstrom et al., 2004). The BA-supplementation altered the balance between auxins and cytokinins in aerial organs, due to an increase in the concentration of cytokinins in these organs, promoting the breakdown of apical dominance and, consequently, the activation of axillary buds (Wu et al., 2021). Interestingly, the association between BA and PBZ (BA: PBZ) significantly enhanced axillary bud proliferation. PBZ is a PGR that directly influences the hormonal content of plants, significantly increasing cytokinin levels while reducing gibberellin content (Castro-Camba et al., 2022).

On the other hand, the interaction between BA and GA (BA: GA) did not promote an increase in the number of axillary buds. Gibberellin is a negative regulator of cytokinin responses in many plants developmental processes (Fleishon et al., 2011; Rocha et al., 2025). Gibberellin significantly affects cytokinin metabolism and can control some of its actions. Additionally, gibberellin can stimulate auxin synthesis, inhibiting the role of cytokinins in axillary bud activation (Nordstrom et al., 2004). In *Bulbophyllum leopardinum*, gibberellin combined with cytokinins also inhibited the proliferation of new shoots. The use of cytokinin alone was the most suitable for bud induction in this species (Thapa et al., 2024).

BA inhibited root proliferation and elongation in *C. guttata*. Cytokinins are known to be a negative regulator of root development. Although it is biosynthesized in this organ and is essential in the early stages of root formation (Svolacchia and Sabatini, 2023; Zhao et al., 2024), cytokinins can inhibit the development of this organ (Ivanov and Filin, 2018), as observed here. Similarly, gibberellin also negatively regulated root growth in *C. guttata*, as evidenced by the GA treatments (GA; BA:GA), which showed a reduction in the number and length of roots. In contrast, the application of the gibberellin biosynthesis inhibitor (PBZ) did not alter these parameters compared to the control. In the literature, gibberellin plays a controversial role in root system development. While some authors have reported the benefit of gibberellin to promote root formation (Rizza et al., 2017), GAs have also been associated with root inhibition (Silva et al., 2020; Rocha et al., 2025), including in *Cattleya loddigesii* (Araújo et al., 2015), another orchid species. These contrasting results suggest that the effects of gibberellin on root system architecture are species-dependent (Silva et al., 2020) and that, in orchids, gibberellin may negatively regulate root development. However, further studies in other species within this family are necessary to confirm this hypothesis.

In the present study, PBZ significantly affected plant architecture for most leaf variables. Its use increased the number and width of leaves and reduced the length-width ratio, resulting in more rounded leaves. This can be explained by the inhibitory role of PBZ in gibberellin biosynthesis. Increased leaf width and reduction in the length-width ratio are conserved and commonly observed responses after PBZ application (Medeiros et al., 2024; Rocha et al., 2025). According to Sprangers et al. (2020), gibberellin positively affects proximal-distal, lateral, and dorsoventral leaf growth. However, gibberellin also stimulates longitudinal growth rates, increasing cell expansion anisotropy in the growing zone during leaf formation and consequently suppressing lateral and dorsoventral expansion of this organ. Our findings suggest that blocking GA biosynthesis through PBZ

application reduces cell expansion anisotropy, favoring leaf roundness. Indeed, leaf width and the length-width ratio exhibited a significant negative correlation in treatments supplemented with PBZ, indicating that plants treated with this growth regulator tend to develop more rounded leaves. This pattern has also been reported in ornamental pineapple seedlings (Téllez et al., 2020), demonstrating its influence in modulating leaf morphology.

In summary, we demonstrate that gibberellin functions as a central regulator of leaf morphology, modulating both size and shape. In contrast, cytokinin promotes the release of axillary buds, thereby altering the architectural pattern of *C. guttata* seedlings. Nonetheless, this morphogenic effect of cytokinin is antagonistically regulated by gibberellin. Furthermore, both cytokinin and gibberellin exert inhibitory effects on root initiation and elongation, highlighting their dual roles in shoot proliferation and root development. Our results can contribute to understanding how hormonal interactions may control the morphogenesis of *C. guttata* seedlings grown *in vitro*.

Conclusions

- Cytokinin supplementation, in the absence of gibberellin, induces the activation of axillary buds.

- Gibberellin negatively regulates cytokinin in the induction of multiple shoots.

- Cytokinin and gibberellin negatively regulate rooting and root elongation in *C. guttata*.

- Gibberellin plays a fundamental role in determining the size and shape of *C. guttata* leaves.

Acknowledgments

The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (Brasília, Brazil; Grant No. 308217/2025-0), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Brasília, Brazil; Grant No. 001), the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (Belo Horizonte, Brazil; Grant No. RED-00225-23), and the Rede Mineira de Biotecnologia em Multiplicação e Clonagem de Plantas.

Author Contribution

MARS: Conceptualization, Data Curation, Investigation, Methodology, Project Administration, Writing – Original Draft, Writing – Review & Editing. **ODCJ**: Data Curation, Formal Analysis, Methodology, Project Administration, Resources, Writing – Original Draft, Writing – Review & Editing. **LHB**: Methodology, Visualization, Writing – Original Draft, Writing – Review & Editing. **JSL**: Methodology, Writing – Original Draft, Writing – Review & Editing. **LSO**: Methodology, Writing – Original Draft, Writing – Review & Editing. **MLS**: Methodology, Writing – Original Draft, Writing – Review & Editing. **DIR**: Conceptualization, Data Curation, Funding Acquisition, Methodology, Resources, Writing – Original Draft, Writing – Review & Editing.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

Data will be made available upon request to the authors.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that the use of AI and AI-assisted technologies was not applied in the writing process.

References

ARAÚJO, A.G.de; PASQUAL, M.; RODRIGUES, F.A.; RODRIGUES, J.D.; DE CASTRO, E.M.; SANTOS, A.M. *In vitro* growth of *Cattleya loddigesii* Lindl. under different light spectra and gibberellic acid doses. **Revista Ceres**, v.56, n.5, p.542-546, 2015. https://ojs.ceres.ufv.br/ceres/article/view/3464

BARROS, F. de; VINHOS, F.; RODRIGUES, V.T.; BARBERENA, F.F.V.A.; FRAGA, C.N.; PESSOA, E.M.; FORSTER, W.; MENINI NETO, L.; FURTADO, S.G.; NARDY, C.; AZEVEDO, C.O.; GUIMARÃES, L.R.S. **Orchidaceae in Lista de Espécies da Flora do Brasil.** Jardim Botânico do Rio de Janeiro, 2015. Available at: http://floradobrasil2015.jbrj.gov.br/FB11336. Accessed at: 02/17/2025.

CASTRO-CAMBA, R.; SÁNCHEZ, C.; VIDAL, N.; VIELEBA, J.M. Plant development and crop yield: The role of gibberellins. **Plants**, v.11, n.19, p.2650, 2022. https://doi.org/10.3390/plants11192650

CHASE, M.W.; CAMERON, K.M.; FREUDENSTEIN, J.V.; PRIDGEON, A.M.; SALAZAR, G.; VAN DEN BERG, C.; SCHUITEMAN, A. An updated classification of Orchidaceae. **Botanical Journal of the Linnean Society**, v.177, n.2, p.151-174, 2015. https://doi.org/10.1111/boj.12234

DESTA, B., AMARE, G. Paclobutrazol as a plant growth regulator. **Chemical and Biological Technologies in Agriculture,** v.8, p.1, 2021. https://doi.org/10.1186/s40538-020-00199-z

FLEISHON, S.; SHANI, E.; ORI, N.; WEISS, D. Negative reciprocal interactions between gibberellin and cytokinin in tomato. **New Phytologist**, v.190, n.3, p.609-617, 2011. https://doi.org/10.1111/j.1469-8137.2010.03616.x

HUSSIEN, M.; MOLKANOVA, O.I.; RAEVA-BOGOSLOVSKAYA, E.N.; SERGEEVICH, M.S. Plant growth regulators and organic additives on the proliferation of protocorm-like bodies and plantlet regeneration of *Cattleya gaskelliana* (N.E.Br.) B.S.Williams. **Ciência e Agrotecnologia**, v.48, e018223, 2024. https://doi.org/10.1590/1413-7054202448018223

IVANOV, V.B.; FILIN, A.N. Cytokinins regulate root growth through its action on meristematic cell proliferation but not on the transition to differentiation. **Functional Plant Biology**, v.45, n.2, p.215-221, 2018. https://doi.org/10.1071/FP16340

MEDEIROS, B.O.; SILVA, L.A.S.; SARMENTO, S.N.; ROSA, D.A.; DE SOUZA BARBOSA, L.C.; MACHADO, M.; GIOPPATO, H.; DORNELAS, M.C.; KUSTER, V.C.; ROCHA, D.I. Antagonistic interactions between cytokinin and gibberellin during initial stem growth and leaf structure of royal poinciana [*Delonix regia* (Bojer ex. Hook.) Raf.]. **Trees**, v.38, p.415–1427, 2024. https://doi.org/10.1007/s00468-024-02562-1

MENEZES-SÁ, T. S. A.; COSTA, A. S. D.; ARRIGONI-BLANK, M. D. F.; BLANK, A. F.; MOURA, G. M. S.; SOARES, C. A. *In vitro* propagation and conservation of *Cattleya tigrina* A. Rich. **Ciência Rural**, v.52, n.5, e20200517, 2021. https://doi.org/10.1590/0103-8478cr20200517

MURASHIGE, T.; SKOOG, F. A revised medium for rapid growth and bioassays with tobacco tissue culture. **Physiologia Plantarum**, v.15, n.3, p.473-497, 1962. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

NAVARRO, Q. R.; DE OLIVEIRA CORRÊA, D.; BEHLING, A.; NOSEDA, M. D.; RIBAS, L. L. F. Effect of microalga *Desmodesmus subspicatus* and plant growth regulators on the *in vitro* propagation of *Cattleya warneri*. **Plant Cell, Tissue and Organ Culture**, v.153, n1, p.77-89, 2023. https://doi.org/10.1007/s11240-022-02442-x

NONGDAM, P.; TIKENDRA, L. Establishment of an efficient *in vitro* regeneration protocol for rapid and mass propagation of *Dendrobium chrysotoxum* Lindl. using seed culture. **The Scientific World Journal**, v.2014, n.1, p.740150, 2014. https://doi.org/10.1155/2014/740150

NORDSTROM, A.; TARKOWSKI, P.; TARKOWSKA, D.; NORBAEK, R.; ASTOT, C.; DOLEZAL, K.; SANDBERG, G. Auxin regulation of cytokinin biosynthesis in *Arabidopsis thaliana*: a factor of potential importance for auxin-cytokinin-regulated development. **Proceedings of the National Academy of Sciences**, v.101, n.21, p.8039–8044, 2004. https://doi.org/10.1073/pnas.0402504101

NOWAKOWSKA, K.; PIŃKOWSKA, A.; SIEDLECKA, E.; PACHOLCZAK, A. The effect of cytokinins on shoot proliferation, biochemical changes and genetic stability of *Rhododendron* 'Kazimierz Odnowiciel' in the *in vitro* cultures. **Plant Cell, Tissue and Organ Culture**, p.1–10, 2022. https://doi.org/10.1007/s11240-021-02206-z

NUNES, G. P.; SORGATO, J. C.; RAMOS, J. C. M.; SOARES, J. S.; REZENDE, R. K. S.; PEINADO, R. B. Innovations in micropropagation of the orchid *Cattleya nobilior*: the effect of bioreactor and enriched culture media. **Ciência Rural**, v.55, n.6, e20240271, 2025. https://doi.org/10.1590/0103-8478cr20240271

PETŘÍK, I.; HLADÍK, P.; ZHANG, C.; PĚNČÍK, A.; NOVÁK, O. Spatio-temporal plant hormonomics: from tissue to subcellular resolution. **Journal of Experimental Botany**, v.75, n.17, p.5295-5311, 2024. https://doi.org/10.1093/jxb/erae267

R CORE TEAM. R: **A Language and Environment for Statistical Computing**. Vienna: R Foundation for Statistical Computing, 2024. Available at: https://www.R-project.org/

RAMOS, J.C.M.; RIBEIRO, L.M.; NUNES, G.P.; SOARES, J.S.; FRANCISCO, P.M.S.; SORGATO, J.C. *Cattleya walkeriana* Gardner (Orchidaceae) propagation: culture medium, sealing system and irradiance. **Brazilian Journal of Biology**, v.84, e279803, 2024. https://doi.org/10.1590/1519-6984.279803

RIZZA, A.; WALIA, A.; LANQUAR, V.; FROMMER, W.B.; JONES, A. M. *In vivo* gibberellin gradients visualized in rapidly elongating tissues. **Nature Plants**, v.3, n.10, p.803-813, 2017. https://doi.org/10.1038/s41477-017-0021-9

ROCHA, A.C.F.; ROSA, D.A.; SILVA, L.A.S; Dias, L.L.L.; MOSQUEIRA, J.G.A.; MACHADO, M.; DIAS, L.L.C.; SILVA, M.L.; KUSTER, V.C.; ROCHA, D.I. Cytokinin and gibberellin interactions control organ size and shape in *Adenium obesum* (Forssk.) Roem. & Schult. **Tropical Plant Biology** 18, 55, 2025. https://doi.org/10.1007/s12042-025-09424-1.

SILVA, L.A.S.; COSTA, A.D.O.; BATISTA, D.S.; SILVA, M.L.D.; COSTA, A.P.D.; ROCHA, D.I. Exogenous gibberellin and cytokinin in a novel system for *in vitro* germination and development of African iris (*Dietes bicolor*). **Revista Ceres**, v.67, n.5, p.402-409, 2020. https://doi.org/10.1590/0034-737X202067050008

SPRANGERS, K.; THYS, S.; VAN DUSSCHOTEN, D.; BEEMSTER, G.T.S. Gibberellin enhances the anisotropy of cell expansion in the growth zone of the maize leaf. **Frontiers in Plant Science**, v.11, p.1163, 2020. https://doi.org/10.3389/fpls.2020.01163

SVOLACCHIA, N.; SABATINI, S. Cytokinins. **Current Biology**, v.33, n.1, p.R10-R13, 2023. https://doi.org/10.1016/j.cub.2022.11.022

TÉLLEZ, H.O.; DO BOMFIM, G.V.; DE CARVALHO, A.C.P.P.; DE AZEVEDO, B.M.; LOZANO, C.H.G. Paclobutrazol in the development of seedlings of ornamental pineapple plants. **Agrarian and Biological Sciences**, v.9, n.10, p.e2349108478, 2020. https://doi.org/10.33448/rsd-v9i10.8478

THAPA, B.B.; CHAND, K.; THAKURI, L.S.; BANIYA, M.K.; PANT, B. Ex-situ conservation of *Bulbophyllum leopardinum*, A threatened medicinal orchid of nepal. **Journal of Nepal Biotechnology Association**, v.5, n.1, p.1-7, 2024. https://doi.org/10.3126/jnba.v5i1.63739

VARFOLOMEEVA, N.I.; KAZAKOVA, V.V.; DINKOVA, V.S.; MANILOVA, O.Y. The influence of bioregulating adaptogens on the growth processes, development and decorative qualities of an orchid. **In IOP Conference Series: Earth and Environmental Science**, v.845, n.1, p.012068, 2021. https://doi.org/10.1088/1755-1315/845/1/012068

WANG, S.; WANG, Y. Harnessing hormone gibberellin knowledge for plant height regulation. **Plant Cell Reports** 41, 1945–1953, 2022. https://doi.org/10.1007/s00299-022-02904-8

WRAITH, J.; PICKERING, C. A continental scale analysis of threats to orchids. **Biological Conservation**, v.234, p.7-17, 2019. https://doi.org/10.1016/j.biocon.2019.03.015

WU, W.; DU, K.; KANG, X.; WEI, H. The diverse roles of cytokinins in regulating leaf development. **Horticulture Research**, v. 8, p. 118, 2021. https://doi.org/10.1038/s41438-021-00558-3

ZHAO, J.; WANG, J.; LIU, J.; ZHANG, P.; KUDOYAROVA, G.; LIU, C. J.; ZHANG, K. Spatially distributed cytokinins: Metabolism, signaling, and transport. **Plant Communications**, 2024. https://doi.org/10.1016/j.xplc.2024.100936