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Clivia miniata Seedlings Germinated *in Vitro* under Different Sucrose Concentrations and Used as Explants for Callus Induction

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Abstract

Clivia miniata is an ornamental plant with pharmaceutical potential, but its cultivation is costly. The plant's slow growth has prompted the search for alternative cultivation methods aimed at producing phenolic compounds, such as the *in vitro* culture. This study investigated the effect of sucrose concentration and fruit maturity on the *in vitro* germination (IG) of *C. miniata* seeds and subsequent callus induction (CI). Sucrose was added to the MS medium: 0 - 40 g·L^{-1}. During IG, the concentration of sucrose affected the speed of germination, and at 0 and 10 g·L^{-1}, seeds from ripe fruit had a higher germination percentage (63% and 67%). Mature seeds showed significantly higher percentages than immature ones. When inducing callus, a high concentration of growth regulators (GR) resulted in significant mortality of the explants. Combinations such as 2.4-D mg·L^{-1} + 0.52 mg·L^{-1} PIC + 2 mg·L^{-1} BAP and 4 mg·L^{-1} BAP + 4 mg·L^{-1} ANA showed high survival rates and CI. The results demonstrate that sucrose concentrations > 5.1 g·L^{-1} reduce germination in mature seeds, likely due to osmotic stress, while lower doses (0 - 10 g·L^{-1}) optimize germination (up to 67%), indicating limited reliance on exogenous carbon sources. The cytokinin BAP proved to be essential for callogenesis, regardless of the auxin used.

Keywords

Callogenesis, *In Vitro* Germination, Osmotic Concentration, Plant Growth Regulator, Seed Germination

1. Introduction

The species *Clivia miniata* (Amaryllidaceae), native to South Africa [1], produces

unique alkaloids, particularly lycorine - a compound with proven antiviral activity (effective against herpes and poliomyelitis) and uterotonic properties (traditionally used to induce labor), though it shows toxicity at high doses. In African traditional medicine, its extracts are used to treat fevers, tuberculosis, HIV, arthritis, and snakebites, while computational studies suggest antidiabetic and antimicrobial potential. However, the lack of clinical and toxicological studies limits its pharmaceutical development [2]. Additionally, its field cultivation is labor-intensive, encouraging the search for alternative methods to produce its bioactive secondary metabolites.

The use of cell, tissue, and organ culture has emerged as a valuable approach for the production of plant secondary metabolites [3] [4]. Numerous studies have demonstrated the successful *in vitro* production of phenolic compounds [5]-[7], highlighting the efficiency of these systems as economical and scalable alternatives for obtaining natural compounds under controlled laboratory conditions [8]. Among these techniques, callus culture has garnered significant attention due to its versatility in both research and industrial applications. As a result, callus-based systems are increasingly recognized as an ideal platform for the sustainable production of commercially valuable metabolites [9] [10].

Cell culture systems offer several distinct advantages over conventional whole-plant cultivation for secondary metabolite production. First, they enable the consistent generation of target compounds independent of external variables such as soil composition or climatic conditions. Second, the controlled *in vitro* environment eliminates risks of microbial contamination or insect predation that commonly affect field-grown plants. Third, this approach facilitates the sustainable utilization of rare or endangered species by maintaining their cells indefinitely for metabolite production [11]. Notably, plants derived from *in vitro* germinated seeds develop aseptically, making them particularly valuable as source material for subsequent tissue culture applications.

In vitro germination success is influenced by multiple intrinsic and extrinsic factors. Intrinsic factors include seed characteristics such as collection time, habitat origin, developmental stage, dormancy status, and tegument composition [12]-[14]. Extrinsic parameters encompass physical growth conditions (light, temperature), nutritional components of the culture medium, and supplementation with phytoregulators, carbohydrates, vitamins, and organic additives [12]-[14].

Due to reduced photosynthetic capacity under *in vitro* conditions, cultured plants often require exogenous carbon supplementation. While glucose, fructose, and sucrose (or their mixtures) are commonly used [15], sucrose has emerged as the preferred carbon source in contemporary tissue culture systems [16]. Its superiority stems from multiple roles: 1) As a phloem-mobile carbohydrate, it serves as both carbon and energy source; 2) It participates in developmental regulation [17]; 3) It directly modulates secondary metabolic pathways [18] [19]. The hexoses derived from sucrose metabolism feed directly into fundamental biochemical

pathways including glycolysis and the pentose phosphate pathway [20], ultimately promoting enhanced growth, leaf expansion, and biomass accumulation [21] [22].

The culture medium composition during *in vitro* germination plays a multifaceted role beyond simple nutrition. The ionic and osmotic properties of salt and sugar solutions critically influence water potential regulation, mitigating cellular water stress while simultaneously affecting germination efficiency and embryonic development [23]. This osmotic regulation demonstrates species-specific requirements, as evidenced by Zeng *et al.* [24], who observed that while some species can germinate in sugar-free media, others necessitate 2% - 3% sugar supplementation for successful germination.

This study examined the influence of sucrose concentration and seed maturity on *in vitro* germination efficiency in *Clivia miniata*. The resulting seedlings were subsequently employed as explant sources for callus induction experiments.

2. Material and Methods

2.1. Experiment I—*In Vitro* Germination

Fruits of *Clivia miniata* were randomly collected from adult plants located in the municipality of Porto Amazonas, Paraná (25˚32'42''S, 49˚53'24''W).

To determine the color of the skin, the fruit was analyzed using a Konica Minolta Colorimeter, model CR-400. This equipment provides, for each fruit analyzed, the luminosity value (L), which ranges from 0 to 100 (0 being equivalent to black and 100 to white), the a-axis (a), representing the variation between the opposite colors green and red—with negative values closer to green and positive values closer to red—and finally, the b-axis (b), representing the variation between the opposite colors blue and yellow—with negative values closer to blue and positive values closer to yellow. The following equation is used to determine color: Hue = $\tan^{-1}(b/a)$. For the *in vitro* germination tests, fruits with entirely bright red skin were classified as mature ($a = 32.45$, $b = 19.82$, $h = 31.43˚$), while fruits with shades varying from green to yellow along their length were designated as immature ($a = -6.73$, $b = 27.26$, $h = 166.12˚$) (**Figure 1**).

2.1.1. Seed Viability Test

Seed viability of Clivia was evaluated using the tetrazolium test. For each maturity stage, four replicates of 25 seeds (100 seeds total per maturity level) were analyzed. The seeds underwent hydration in distilled water for 24 hours at 25˚C ± 1˚C, followed by longitudinal bisection to expose embryonic tissues for staining.

The prepared seeds were then immersed in a 1% (w/v) 2,3,5-triphenyltetrazolium bromide (TTC) solution and subjected to a two-phase incubation: first for 3 hours at 30˚C, followed by 21 hours at room temperature (25˚C) under complete darkness [25]. Post-incubation, samples were rinsed under running water and blotted dry on absorbent paper for visual assessment and photographic documentation.

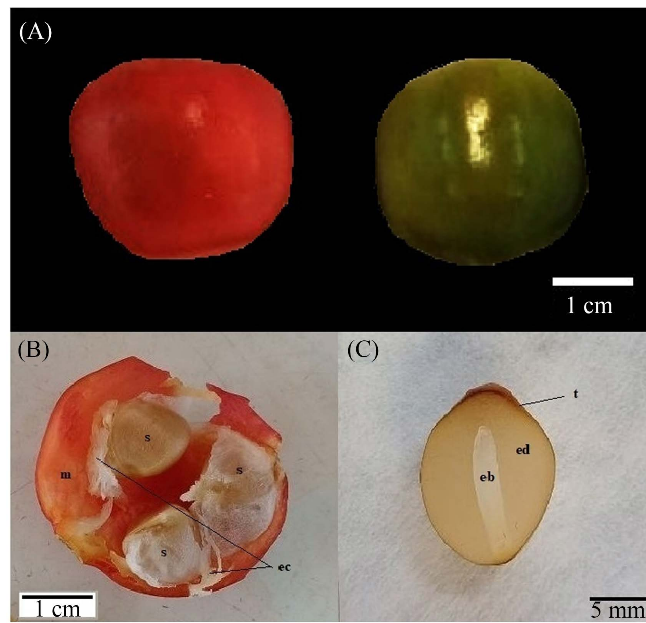

Figure 1. (A) Fruits of *Clivia miniata*. On the left: mature fruit; on the right: immature fruit; (B) Opened mature fruit of *C. miniata*; (C) Seed of *C. miniata* sectioned longitudinally exposing the embryo. m—mesocarp, s—seed, ec—endocarp, eb—embryo, ed—endosperm, t—tegument.

The test principle relies on the reduction of colorless TTC to red formazan by dehydrogenase enzymes in metabolically active tissues. Since this redox reaction occurs exclusively in viable cells and the resulting formazan pigment is non-diffusible, it provides clear visual discrimination between living (stained) and non-viable (unstained) tissues (**Figure 2**).

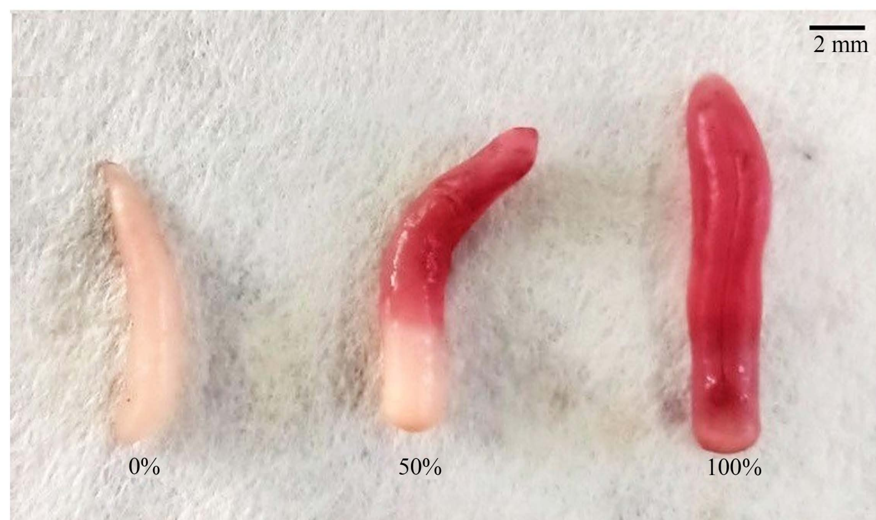

Figure 2. *C. miniata* embryos stained with tetrazolium chloride.

Viability scoring was based on the percentage of embryo staining (0%, 50%, or 100%), with only fully stained (100%) embryos being classified as viable.

2.1.2. *In Vitro* Introduction

The collected seeds were washed in running water and disinfected with 70% (v/v) alcohol for 60 seconds, followed by immersion in a 2% - 2.5% (v/v) sodium hypochlorite solution for 20 minutes under agitation. After asepsis, the seeds were transferred to a laminar flow hood, where they were rinsed three times with autoclaved distilled water (sterilized at 120˚C and 1 atm pressure) for 20 minutes each. Subsequently, the seeds were placed in test tubes containing 10 mL of MS basal medium [26] supplemented with increasing concentrations of sucrose (0, 10, 20, 30, and 40 $g \cdot L^{-1}$).

The sucrose concentration range (0 - 40 g/L) was selected based on previous studies with ornamental monocots, such as orchids and other slow-growing species, which demonstrate contrasting effects: low concentrations (0 - 20 g/L) promote germination, while concentrations above 30 g/L may induce osmotic stress and inhibit embryonic development. Furthermore, concentrations approaching 40 g/L were included to evaluate critical tolerance thresholds in both mature and immature seeds.

2.1.3. Statistical Analysis

The data on seed viability assessed by tetrazolium testing were not subjected to statistical analysis. To evaluate the *in vitro* physiological quality of the seeds, the experiment was conducted in a completely randomized design with twenty-five replications, where each replication consisted of one seed per test tube. The treatments were arranged in a 2 × 5 factorial scheme, representing two maturity stages (ripe and unripe) and five sucrose concentrations (0, 10, 20, 30, and 40 $g \cdot L^{-1}$).

Data on seed maturity were analyzed using the F-test, with mean comparisons performed using Tukey's test at a 5% significance level. For sucrose concentration effects, polynomial regression analysis was applied, testing both linear and quadratic models. The most significant equations ($p \leq 0.05$) with the highest coefficient of determination (R^2) were selected. When significant interactions between factors were detected, further breakdown analyses were conducted.

2.2. Experiment II—Callus Induction

2.2.1. Obtaining the Explants

After 90 days of *in vitro* germination, roots and leaves were removed from the seedlings, and the stems containing the apical meristem were longitudinally sectioned along the leaf bases, dividing them into two equal halves (**Figure 3**). The resulting explants were further divided into smaller segments measuring 0.5 ± 0.2 cm in length and inoculated into flasks containing 30 mL of culture medium for callus induction.

2.2.2. Cultivation Medium

All culture media consisted of MS basal salts supplemented with growth regulators, 10 $mg \cdot L^{-1}$ myo-inositol, and 40 $g \cdot L^{-1}$ sucrose, solidified with 6 $g \cdot L^{-1}$ agar. The pH was adjusted to 5.8 prior to agar addition, followed by autoclaving at 120˚C and 1 atm pressure for 20 minutes.

Figure 3. Sectioned stem segment and leaf bases of *C. miniata* germinated *in vitro* used as a source of explants for callus induction.

The flasks were maintained in a growth chamber at 25˚C ± 2˚C in complete darkness for 120 days.

2.2.3. Histological Evaluation

Calli obtained after 120 days of *in vitro* culture were sectioned and fixed in FAA70 (formalin: glacial acetic acid: 50% ethyl alcohol, in a 1:1:18 ratio) for 24 hours. Subsequently, the samples were dehydrated in an increasing ethanol series up to 70% alcohol, pre-infiltrated in resin (hydroxyethyl methacrylate) and 70% alcohol in a 1:1 v/v ratio, and embedded in pure resin with the addition of an activator, following the manufacturer's instructions (Leica Microsystems, Wetzlar, Germany). The entire process was performed under vacuum.

Sections of 7 μm were cut using a rotary microtome (LEICA RM2145, Leica Microsystems, Wetzlar, Germany) and stained with toluidine blue (0.5% in distilled water) for 8 minutes, then dried at room temperature.

The sections were photographed using a Nikon® optical photomicroscope, model Eclipse E200, equipped with an attached The Imaging Source® camera, model DFK 23UX236.

2.2.4. Statistical Analysis

The experiment was conducted using a completely randomized design. Each of the 10 treatments consisted of five replicates, with each replicate containing five fragments. Explant survival and callus induction percentage were analyzed using the F-test, and mean values were compared by Tukey's test at a 5% significance level.

3. Results

3.1. Experiment I—*In Vitro* Seed Germination

The tetrazolium viability test revealed that for both degrees of ripeness, the percentage of viable seeds did not exceed 70% (Table 1).

Following experimental setup, daily monitoring of seed germination was conducted for 90 days (Figure 4). Initial germination events were sparse during the first 7 days. The shortest mean germination time (27.5 days) occurred in mature

seeds cultured on 30 g·L^{-1} sucrose medium, while the longest (41.4 days) was observed in immature seeds on sucrose-free medium (0 g·L^{-1}), representing a 13.9-day difference (Table 2). However, Tukey's test ($\alpha = 0.05$) indicated no statistically significant differences in germination time across the sucrose concentrations tested.

Table 1. Viability of zygotic embryos of *Clivia miniata* stained with 2, 3, 5 triphenyl tetrazolium bromide. 0% correspond to dead embryos; 100% stained embryos were considered viable.

Embryo staining (%)	Fruit ripeness (%)	
	Unripe	Ripe
0	18	12
50	26	23
100	55	65
Total	100	100

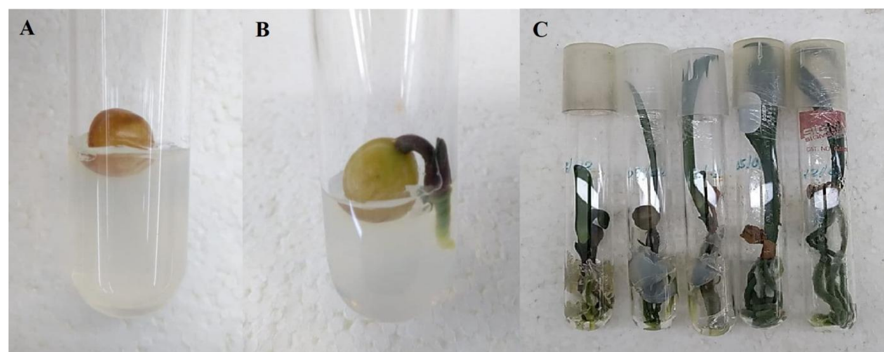

Figure 4. Germination of *C. miniata in vitro* in semi-solid medium; (A) Seed immediately after sowing; (B) Germinated seed with seedling showing primary root and leaf; (C) Clivia seedlings 90 days after germination.

Table 2. Time elapsed in days until germination of *Clivia miniata* seeds *in vitro* as a function of sucrose concentration and degree of maturation.

Sucrose (g·L^{-1})	Days to germination	
	Unripe	Ripe
0	41.40^{a}	30.93^{b}
10	35.83^{a}	31.86^{a}
20	37.37^{a}	30.36^{a}
30	35.87^{a}	27.55^{a}
40	35.81^{a}	33.70^{a}

Equal letters do not differ according to Tukey's test at the 5% probability level.

Across all maturity stages, no treatment achieved germination rates exceeding 70%. The highest mean germination percentage (66.6%) was observed in mature

seeds cultured on sucrose-free medium (0 g·L^{-1}), while the lowest rate (33.3%) occurred in immature seeds grown on media containing 20 or 30 g·L^{-1} sucrose (Table 3). Statistical analysis revealed significantly higher germination percentages in seeds derived from mature fruits compared to their immature counterparts.

Table 3. Percentage germination of *Clivia miniata* seeds as a function of sucrose concentration and fruit ripeness after 90 days of *in vitro* cultivation.

Sucrose (g·L^{-1})	Ripeness (%)	
	Unripe	Ripe
0	42^{ABb}	67^{Aa}
10	50^{Ab}	63^{Aa}
20	33^{Bb}	46^{Ba}
30	33^{Ba}	38^{Ba}
40	46^{ABa}	42^{Ba}

Equal uppercase letters in the columns and lowercase letters in the rows do not differ by Tukey's test at the 5% probability level.

Figure 5 shows that at low sucrose concentrations, the highest Germination Percentage was obtained in seeds from ripe fruit, which decreased from 5.1 g·L^{-1} onwards. For immature fruit, the concentration of sugar was not significant.

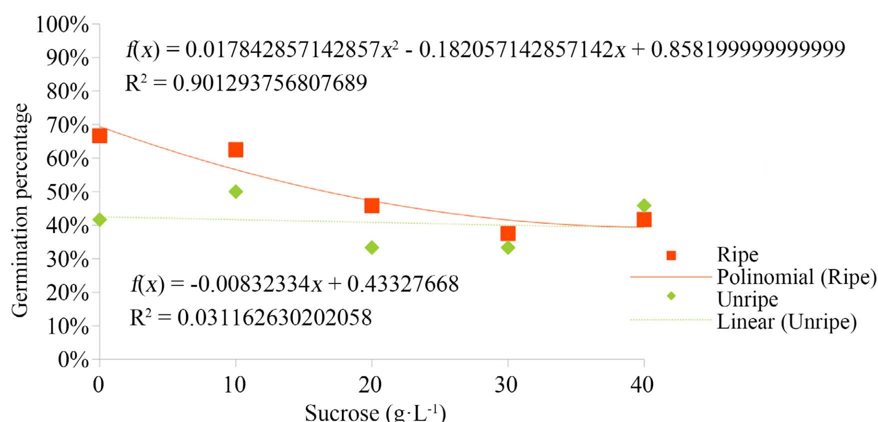

Figure 5. *In vitro* germination of *Clivia miniata* seeds from mature and immature fruit as a function of sucrose concentration in the culture medium.

3.2. Experiment II—Callus Induction

Following four months of *in vitro* cultivation on callus induction medium, the explants exhibited highly variable responses, with survival rates ranging from 0% to 90% across treatments (Table 4). Callus induction percentages showed particularly marked variation, with up to 80% difference observed between the most contrasting treatments.

Table 4. Survival and percentage of callus induction from stems and leaf bases of 90-day-old *Clivia miniata* germinated *in vitro* after 4 months in culture medium with growth regulators.

Growth regulators ($mg \cdot L^{-1}$)	Variables	
	Survival (%)	Callus induction (%)
Control (no regulator)	25^{bc}	33^{ns}
2 2,4-D + 0.52 PIC	75^{ab}	35
4 2,4-D + 1.05 PIC	70^{ab}	31
8 2,4-D + 2.1 PIC	5^{c}	0
2 2,4-D + 0.52 PIC + 2 BAP	80^{ab}	75
4 2,4-D + 1.05 PIC + 2 BAP	95^{a}	38
8 2,4-D + 2.1 PIC + 2 BAP	0^{c}	-
2 2,4-D + 2 BAP + 2 NAA	90^{a}	43
4 2,4-D + 4 BAP + 4 NAA	45^{abc}	62
4 BAP + 4 NAA	60^{abc}	80

Equal letters do not differ by Tukey's test at 5% probability. ns = not significant.

In control treatments lacking growth regulators, explants showed limited survival (25%) and callus induction (33%), consistent with expected baseline performance under these culture conditions. Supplementation with 2 $mg \cdot L^{-1}$ 2,4-D + 0.52 $mg \cdot L^{-1}$ PIC significantly improved explant survival to 75%, while maintaining comparable callus induction rates (35%). Doubling these regulator concentrations (4 $mg \cdot L^{-1}$ 2,4-D + 1.05 $mg \cdot L^{-1}$ PIC) yielded similar results (70% survival, 31% callus induction). However, the highest tested concentration (8 $mg \cdot L^{-1}$ 2,4-D + 2.1 $mg \cdot L^{-1}$ PIC) proved phytotoxic, reducing survival to 5% and completely inhibiting callus formation.

The inclusion of cytokinin (2 $mg \cdot L^{-1}$ BAP) to the optimal auxin combination (2 $mg \cdot L^{-1}$ 2,4-D + 0.52 $mg \cdot L^{-1}$ PIC) synergistically enhanced both explant survival (80%) and callus induction (75%). While increasing auxin concentrations (4 $mg \cdot L^{-1}$ 2,4-D + 1.05 $mg \cdot L^{-1}$ PIC) with constant BAP maintained high survival (95%), callogenic capacity declined substantially (38%). The previously observed toxicity of 8 $mg \cdot L^{-1}$ 2,4-D + 2.1 $mg \cdot L^{-1}$ PIC was exacerbated by BAP addition, resulting in 100% explant mortality.

Interestingly, the ternary regulator system (2,4-D + BAP + NAA) exhibited concentration-dependent inhibition, with survival rates declining from 90% to 45% as PGR concentrations increased, despite moderate improvement in callus induction (43% to 62%). Notably, the simplest combination – 4 $mg \cdot L^{-1}$ BAP + 4 $mg \cdot L^{-1}$ NAA without auxin-produced optimal callogenic response (80% induction) with acceptable survival (60%).

Callus response varied significantly across media formulations (**Figure 6(A)** and **Figure 6(B)**). While BAP + 2,4-D + PIC suppressed differentiation, NAA-

promoted rhizogenesis, and BAP + 2,4-D induced shoot organogenesis. Globular structures, initially hypothesized as somatic embryos, lacked protodermal organization and displayed isolated tracheary differentiation, ruling out embryonic identity (**Figure 6(C)**).

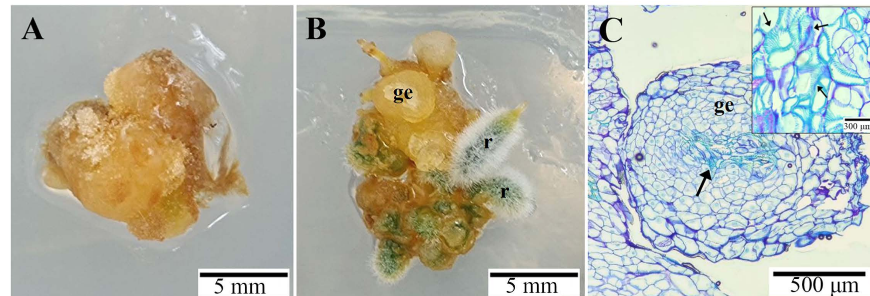

Figure 6. *Clivia miniata* callus after 120 days under distinct growth regulator treatments. (A) Compact callus; (B) Organogenic callus with adventitious roots (r) and emerging globular structures (ge); (C) Histology of globular structure (arrow: developing vascular tissue).

4. Discussion

Udomdee *et al.* [27] investigated the effects of sucrose concentration and seed maturity on *in vitro* germination of *Dendrobium nobile* hybrids (another monocot species). In contrast to our findings with clivia, they observed no significant effect of sucrose concentration on seed germination. However, they reported an inverse relationship between sucrose concentration and seedling development, with fewer embryos progressing to the seedling stage as sucrose levels increased.

Soluble sugars, typically glucose, fructose, or sucrose, are commonly added to culture media at concentrations ranging from 10 to 30 $g \cdot L^{-1}$ [28]. However, elevated sucrose levels may retard cellular development, potentially inducing cell cycle arrest under nutrient-limited conditions [29] [30]. In *Cymbidium aloifolium*, Deb and Pogen [31] demonstrated sucrose concentration-dependent effects on immature seed germination. Several studies have further shown that high sucrose concentrations can inhibit nutrient uptake by reducing the medium's water potential [32] and inducing osmotic stress [33]. Johnson *et al.* [34] proposed that the observed suppression of germination and growth in *Bletia purpurea* (Orchidaceae) at high sucrose concentrations could result from either osmotic stress at elevated molarities or inhibitory effects of sucrose hydrolysis byproducts generated during autoclaving.

Seeds of *Campomanesia adamantium* and *Campomanesia pubescens* exhibited high initial *in vitro* germination rates (94%) in sucrose-free medium. Germination percentages progressively declined with increasing sucrose concentrations. At 20 $g \cdot L^{-1}$ sucrose, C. pubescens showed 64% germination, while 40 $g \cdot L^{-1}$ sucrose reduced emergence by 44% (*C. adamantium*) and 65% (*C. pubescens*). Both species demonstrated germination rates below 10% at 80 $g \cdot L^{-1}$ sucrose. These results indicate that sucrose significantly alters the medium's osmotic potential, affecting water availability. Such osmotic stress may impair seed physiological quality, re-

ducing both vigor and germination capacity [35].

The highest germination percentages at 0 and 10 g/L (67% and 63%, respectively) confirm that *C. miniata* does not require exogenous sucrose for germination, similar to Campomanesia [35]. This suggests that the endosperm of mature seeds provides sufficient carbohydrates for embryo development, making sucrose supplementation unnecessary or even detrimental at higher concentrations. The observed germination decline above 20 g/L (33% - 46%) aligns with studies in orchids [31] and *B. purpurea* [34], where high sucrose concentrations induce osmotic stress or inhibition from sucrose hydrolysis byproducts during autoclaving.

Lee *et al.* [36] reported that immature *Cypripedium formosanum* seeds germinated faster and more successfully than mature seeds. The low germination of mature seeds in some studies may be related to the physical restriction imposed by the seed coat (testa) and the presence of germination inhibitors such as abscisic acid [27] [36]. Variations in abscisic acid (ABA) levels have been shown to restrict germination during embryo development [37]-[39]. In contrast, clivia seeds from ripe fruits germinated at higher percentages, suggesting that the seed's protein and lipid composition, as well as potential hormonal influences, may not significantly affect their germination.

The results observed for *Clivia miniata*, where mature seeds showed higher germinative capacity compared to immature ones and where elevated sucrose concentrations significantly reduced germination, can be interpreted in light of recent evolutionary models on seed behavior. Barbedo *et al.* [40] proposed a dynamic model in which different species exhibit variations in the timing of acquiring physiological traits such as dry matter accumulation, desiccation tolerance, and germinability, influenced by genotypic and environmental factors. In this context, immature clivia seeds may represent a developmental stage in which they have not yet reached the peak of these traits, which would explain their lower *in vitro* germination efficiency. The reduction in germination in media with high sucrose concentrations may reflect the osmotic sensitivity typical of seeds still undergoing maturation, or a partially recalcitrant behavior, as already observed in other species. Thus, interpreting seed maturation as a continuous process rather than a fixed event may offer broader insight into the response of *C. miniata* seeds to the *in vitro* environment.

Embryos from immature fruits of *Acrocomia aculeata*, without fully developed endosperm, are capable of producing seedlings with fully formed roots and leaf sheaths, indicating early embryogenesis in this species. It is therefore possible to use immature fruits as sources of embryos for *in vitro* cultivation [39], as has also been observed in the palm species *Astrocaryum ulei* Mart. [41] and *B. capitata* [42]. The results observed for *C. miniata* in this study also showed that seeds from immature fruits can be used for *in vitro* germination or zygotic embryo cultivation, although they exhibit lower germination rates when compared to seeds from mature fruits.

For future studies, a deeper investigation of the immature stage of *Clivia miniata* seeds, with an emphasis on their physiological and biochemical characteriza-

tion during development, would be of great value. Assessments such as dry matter accumulation, variations in hormonal content (especially ABA), and the progressive acquisition of desiccation tolerance may help elucidate the factors limiting the *in vitro* germination of immature seeds. Furthermore, understanding the point in development at which the seed reaches its maximum germinative capacity could contribute to optimizing *in vitro* cultivation protocols, enabling the efficient use of immature fruits as a source of viable embryos—an approach that may be advantageous for accelerating propagation cycles and supporting the conservation of the species.

The second phase of this study, focused on inducing callus formation from plants obtained through *in vitro* germination, yielded somewhat conflicting results. In both treatments containing 8 mg·L^{-1} of 2,4-D combined with 2.1 mg·L^{-1} of picloram (PIC), more than 90% of the explants died, likely due to the high concentration of plant growth regulators (PGRs). This hypothesis aligns with findings by Pan *et al.* [43], who reported that at concentrations above 6 mg·L^{-1}, 2,4-D may inhibit somatic embryogenesis (SE), possibly due to membrane depolarization.

The response to exogenous hormones, particularly auxins and cytokinins (CK), is critical in *in vitro* regeneration, which occurs in three distinct stages: 1) hormonal perception and cellular dedifferentiation, 2) cell fate determination mediated by hormonal balance, and 3) morphogenesis independent of exogenous hormones [44]. The synthetic auxin 2,4-D is widely used in SE induction, with optimal concentrations varying significantly among species—typically 2 mg·L^{-1} for Poaceae and 5 - 10 μM for medicinal plants. While essential for embryogenic callus induction, 2,4-D often becomes dispensable in later developmental stages. Conversely, cytokinins play a pivotal role in adventitious shoot induction and SE, acting synergistically with auxins. High CK/auxin ratios promote shoot formation, whereas low ratios favor rhizogenesis [44].

Interestingly, SE has been successfully induced at much higher auxin concentrations—over 100 mg·L^{-1} of 2,4-D in *Serenoa repens* [45] and 72.42 mg·L^{-1} of picloram in *Bactris gasipaes* [46]. Additionally, in Dactylis glomerata L., 8 mg·L^{-1} of 2,4-D effectively induced callus formation [47], underscoring the species-specific variability in PGR responses.

The composition and relative concentration of PGRs are decisive factors in explant responsiveness and the type of morphogenic response induced [48]. Auxins and cytokinins, whether applied alone or in combination, can produce highly divergent effects on cell proliferation in plant tissue culture. These responses are complex and often unpredictable, as the mechanisms by which PGRs modulate cell metabolism remain incompletely understood. Therefore, empirical determination of optimal PGR types and concentrations for each species and culture condition is essential [49].

5. Conclusion

In summary, this study demonstrates that reducing or eliminating sugar from the

culture medium significantly enhances the germination of mature *Clivia miniata* seeds, reaching 67% germination in sugar-free conditions, while germination potential declines sharply above 5.1 g·L^{-1} sucrose. In contrast, sucrose concentration had no observable effect on seed germination in immature fruits. Germination speed remained unaffected by sucrose levels or seed maturity. Furthermore, the cytokinin 6-benzylaminopurine (BAP) was critical for callus induction, independent of auxin type, whereas high concentrations of 2,4-D and picloram (PIC) negatively impacted explant survival and cell proliferation.

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Authors' Contributions

Formal analysis, methodology, visualization, writing—original draft, dos Reis, C. A.; Conceptualization, funding acquisition, investigation, methodology, project administration, resources, supervision, writing—review, AYUB, R. A.

Conflicts of Interest

The authors declare no conflict of interest.

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