

Micropropagation and phytopathology of calla lily (*Zantedeschia* spp.) *in vitro*Micropropagación y fitopatología de la cala (*Zantedeschia* spp.) *in vitro*AMPARO MARTÍNEZ-HERNÁNDEZ¹JOSÉ LUIS RODRÍGUEZ DE LA O¹JUAN GUILLERMO CRUZ-CASTILLO^{2,3}JOSÉ OSCAR MASCORRO-GALLARDO¹MA. DE JESÚS JUÁREZ-HERNÁNDEZ¹LUIS ÁNGEL BARRERA-GUZMÁN²HÉCTOR TECUMSHÉ MÓJICA-ZÁRATE²

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White calla lily (*Zantedeschia aethiopica* L. Spreng) seedling obtained from embryo isolation and *in vitro* culture.

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ABSTRACT

Calla lily (*Zantedeschia* spp.) is an herbaceous flowering plant that belongs to Araceae family with worldwide distribution. Native to the swampy or mountainous regions of South Africa. It's valued as ornamental plant due to its extraordinary shape and decorative leaves. However, its production has decreased due to the presence of diseases. *In vitro* culture of plant cells and tissues has been successfully applied to precisely diagnose and control diseases to produce disease-free plants. This study analyzes information from *in vitro* techniques applied to this crop, highlighting phytopathological aspects. The description of the different stages of *in vitro* culture, obtention and propagation of healthy or pathogen-free plants was considered. *In vitro* culture has proven to be an effective tool for rapid clonal propagation and multiplication of *Zantedeschia* spp. A sanitary management before the *in vitro* culture is recommended.

Additional key words: tissue culture; embryogenesis; organogenesis; totipotency; *Pectobacterium*.

RESUMEN

La cala lily (*Zantedeschia* spp.) es una planta herbácea con flores que pertenece a la familia Araceae de amplia distribución mundial. Originaria de las regiones pantanosas o montañosas de Sudáfrica. Es valorada como planta ornamental por su extraordinaria espata y hojas decorativas. Sin embargo, su producción ha disminuido por la presencia de enfermedades. El cultivo *in vitro* de células y tejidos vegetales se ha aplicado exitosamente tanto para el diagnóstico preciso de enfermedades y su control como para la producción masiva de plantas libres de enfermedades. Esta revisión analiza información de las técnicas *in vitro* aplicadas a este cultivo, poniendo de relieve aspectos fitopatológicos. Se consideró la descripción de las diferentes etapas que refieren al cultivo *in vitro*, la obtención y propagación de plantas sanas. El cultivo *in vitro* ha demostrado ser una herramienta efectiva para la propagación y multiplicación clonal rápida de *Zantedeschia* spp. Un manejo sanitario previo a su cultivo *in vitro* es recomendado.

Palabras clave adicionales: cultivo de tejidos; embriogénesis; organogénesis; totipotencia; *Pectobacterium*.

INTRODUCTION

In Mexico, calla lily production is concentrated in the provinces of Veracruz, Puebla, Jalisco, Chiapas, Oaxaca, Colima, and the State of Mexico (Cruz-Castillo and Hernández-Montes, 2022). Its production is limited by *Pectobacterium carotovorum* or *Erwinia carotovora* subsp. *carotovora* (Trejo-Téllez *et al.*, 2013; Cruz-Castillo and Torres-Lima, 2017) and other unknown pathogens (Delgado-Paredes *et al.*, 2021). Calla lilies are popular in Mexico and are used to decorate weddings and other celebrations (Cuellar-Mandujano *et al.*, 2022). Most calla lilies produced in Mexico are perennial with white spathes. In Latin America, this calla lily grows in Argentina, Bolivia, Brazil, Colombia (Casierra-Posada *et al.*, 2014), Ecuador, and Guatemala (Cruz-Castillo, 2022).

The calla lily (*Zantedeschia aethiopica* L. Spreng) is the most well-known species (Fig. 1), but there are other species with flowers with different colours from pink to brown. The genus *Zantedeschia* is divided into two sections or botanical groups: the *Zantedeschia* section and the

Aestivae section (He *et al.*, 2020). The first comprises the species *Z. aethiopica* and *Z. odorata*, while the *Aestivae* section includes *Z. albomaculata*, *Z. rehamannii*, *Z. elliottiana*, *Z. jucunda*, *Z. valida*, and *Z. petlandii* (Wei *et al.*, 2017). It has been demonstrated that the *Aestivae* section is more susceptible to soft rot caused by bacteria of the genus *Pectobacterium* due in part to the ecological adaptation of each section (Guttman *et al.*, 2021). This issue has reduced the production of calla lilies, and *in vitro* cultivation techniques were introduced to obtain pathogen-free plants (Nery *et al.*, 2018).

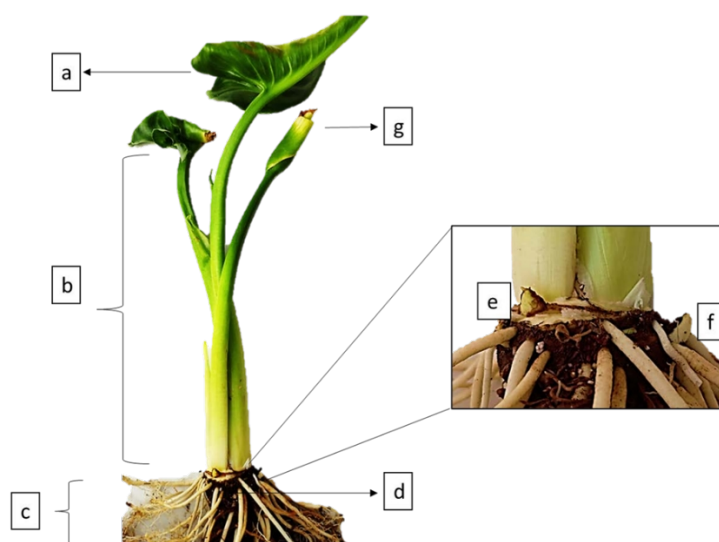


Figure 1. Calla lily (*Zantedeschia aethiopica* L. Spreng) var. 'Utopia'. (a) leaf; (b) petiole; (c) roots; (d) rhizome; (e) apical bud; (f) adventitious bud; (g) immature spathe.

A review regarding tissue culture of *Zantedeschia* was published (Sun *et al.*, 2023) but rot causal agents of explants were not considered. Frequently, the micropropagation of *Z. aethiopica* has been difficult because explant contamination of cultured rhizome tissue (Martínez-Hernández, 2022). Therefore, *in vitro* techniques in *Zantedeschia*, highlighting phytopathological aspects to reduce the contamination of the explants were reviewed.

Phytopathology of the *Zantedeschia* genus

The main factor limiting the production of calla lilies is the diseases. Soft rot, caused by *Pectobacterium carotovorum* subsp. *carotovorum* has bring on a significant worldwide threat to *Zantedeschia* crop (Githeng'u *et al.*, 2016), others may be associated such as: *Pseudomonas marginalis* (Krejzar *et al.*, 2008; Yan *et al.*, 2014), *Pseudomonas veronii*, *Chryseobacterium*

indologenes (Mikiciński *et al.*, 2010), *Cellvibrio zantedeschia* sp. Nov. (Sheu *et al.*, 2017), *Pectobacterium zantedeschiae* (Waleron *et al.*, 2019), and *Pectobacterium aroidearum* (Chen *et al.*, 2020). The main symptoms of this disease include dwarfing, and chlorosis along the leaf margin progressing in a "V" shape that may turn brown, the stem may bend, and eventually desiccate (Ciampi *et al.*, 2009).

Viruses that often attack *Zantedeschia* include dasheen mosaic virus (DsMV) classified as the most prevalent among Aroids (Cafrune *et al.*, 2011), and its distribution was recorded in Bosnia and Herzegovina, the crops presented the following symptomatology; yellowing and distortion of leaves as well as mosaic and chlorotic ring symptoms (Grausgruber-Gröger *et al.*, 2016).

For the konjac mosaic virus (KoMV), *Mizus persicae* and *Aphis gossypii* were identified as main vectors, as well as vegetative propagation and seed transmission, its symptoms appear as mosaics, green islands, interveinal chlorosis, leaf deformity, short peduncles, and discolored pigmented spots on the spathe (Liao *et al.*, 2020).

The calla lily latent virus (or zantedeschia mild mosaic virus [ZaMMV]) is a potyvirus isolated from the Black Magic cultivar, and manifests yellow spots and stripes, green islands, and an unusually mild mosaic (Rizzo *et al.*, 2015). For the detection of viruses, *Zantedeschia* has been cultured *in vitro* (Purmale *et al.*, 2023) and with this type of propagation the viruses were not eliminated, therefore, studies on thermotherapy, chemotherapy, cryotherapy or electrotherapy (Szabó *et al.*, 2024) to eliminate virus in *Zantedeschia* could be evaluated.

Pathological control and eradication methods

Endogenous contamination is a recurring issue in *in vitro* establishment, particularly in rhizome tissue. Sanitary management before *in vitro* cultivation is recommended. Species of the *Pectobacterium* family propagate easily through decaying plant material and there are no efficient control agents for the disease they cause (van der Wolf *et al.*, 2021; Niyokuri and Nyalala, 2023). The soil fumigation with metam sodium and the application of antagonistic bacteria CAE 01 of the Enterobacteriaceae family reduced the percentage of infection in rhizomes (García-López, 2010). As a biological control for *Pectobacterium carotovorum* subsp. *carotovorum*, the high specificity of the bacteriophage virus PP1 of the Podoviridae family was studied (Lim *et al.*, 2013). The use of *Bacillus*, showed that *Bacillus amyloliquefaciens* subsp. *plantarum* reduced the growth of the phytopathogen in both *in vitro* and field conditions (He *et*

al., 2021). Protein profiles from *in vitro* and *in vivo* conditions for *Pectobacterium carotovorum* following interaction with *Zantedeschia elliotiana* 'Black Magic' were also generated. Using two-dimensional electrophoresis, 53 essential proteins for their virulence were identified, showing differential expression *in vitro*, *in vivo*, or both compared to the control (Wang *et al.*, 2018).

Methods of incorporation to *in vitro* conditions

The eradication of pathogens previously to calla lily *in vitro* establishment has been a challenge. The use of mercury chloride (HgCl₂) at 0.1-0.2 % (w/v) for 15 min (Xiao-Chun, 2010) or for 30 min for leaf and rhizome at doses of 0.1 and 0.2 % (w/v) respectively with prior treatment with benomyl at 1 % (w/v) in shaking for 30 min (Hlophe *et al.*, 2015) yielded good results, even doses of 0.5 % of HgCl₂ (w/v) for 15 min were effective at this stage in *Z. rehmannii* with pre-treatment of immersion in 70 % ethanol (v/v) for 30 s (Kulpa, 2016). Despite the effectiveness of these treatments, a concentration of 0.25 mg L⁻¹ has negative environmental consequences, on amphibian development (Muñoz-Escobar and Palacio-Baena, 2010).

Hernández-Villaseñor *et al.* (2018) disinfected with a 15 % (w/w) chlorine solution followed by 500 mg L⁻¹ of streptomycin, 500 mg L⁻¹ of oxytetracycline, and 500 mg L⁻¹ of carboxamide for 60 min. Finally, the explants were placed into a 70 % (v/v) ethanol solution for 30 s. Similarly, the application of chlorine dioxide (ClO₂) at a concentration of 60 to 180 mg L⁻¹ (v/v) for 5 min followed by hydrogen peroxide (H₂O₂) at 5 % (v/v) for 15 min provided a survival rate >75 % for *Z. aethiopica* (Chen *et al.*, 2017).

Due to the need to improve the disinfestation protocols that have less environmental impact, studies based on antibacterial activity of *Coptis chinensis* extract has been developed against the soft rot, 100% *C. chinensis* made an inhibition effect comparable to streptomycin sulphate (Githeng'u *et al.*, 2016) an antibiotic widely used in the 90 s. This protocol has not been tested on *Zantedeschia* explants. Hashemidehkordi *et al.* (2021) evaluated the effect of hot water in the disinfection of rhizome explants and achieved a survival rate of 90% at 45°C for 35 min followed by immersion in 70% ethanol for 30 s and 1% (v/v) sodium hypochlorite (NaOCl) for 10 min. This procedure represents a good alternative to reduce the use of previously mentioned products and their impact in terms of environmental, economic impact, and antibiotic resistance. Table 1 shows the control methods for the *in vitro* culture of calla lilies.

Table 1. Methods for controlling contaminants in *Zantedeschia* spp. cultured *in vitro*.

Plant material	Origin of explant	Control method	Survival rate (%)	Reference
<i>Z. hybrida</i>	Rhizome	HgCl ₂ at 0.1-0.2% for 15 min for rhizome segments	Not reported	Xiao-Chun, 2010
<i>Z. aethiopica</i>	Leaf and rhizome	HgCl ₂ at 0.2% for rhizome segments and 0.1% for leaves for 30 min	28% in rhizome and 78.3% in leaf	Hlophe <i>et al.</i> , 2015
<i>Z. rehmannii</i>	Rhizome	Immersion in 0.5% HgCl ₂ for 15 min	73%	Kulpa, 2016
<i>Z. aethiopica</i> L. Spreng	Rhizome	15 % chlorine + 500 mg L ⁻¹ streptomycin, 500 mg L ⁻¹ oxytetracycline, and 500 mg L ⁻¹ captan for 60 min + 70 % ethanol (v/v) for 30 s	Not reported	Hernández-Villaseñor <i>et al.</i> , 2018
<i>Z. aethiopica</i> L. Spreng	Rhizome-buds	ClO ₂ at 60 to 180 mg L ⁻¹ (v/v) for 5 min + 5% H ₂ O ₂ (v/v) for 15 min	>75 %	Chen <i>et al.</i> , 2017
<i>Zantedeschia</i> spp ‘Orania’ and ‘Sunclub’	Rhizome-buds	Hot water bath at 45°C for 35 min + immersion in 70 % ethanol for 30 s and 1 % sodium hypochlorite (NaOCl) (v/v) for 10 min	>90 %	Hashemidehkordi <i>et al.</i> , 2021
<i>Z. aethiopica</i> ‘Déjà vu’ and ‘Utopia’	Rhizome	Agrimicyn® (2 g L ⁻¹) added to the fungicide Benomyl® (2 g L ⁻¹)	‘Utopía’: 60%; ‘Déjà vu’; 80 %	Martínez-Hernández, 2022
<i>Z. aethiopica</i>	Seed	70 % ethanol for 1 min + 10% NaOCl for 15 min, and 10 min of 30% H ₂ O ₂	100 %	Martínez-Hernández, 2022

In vitro propagation of *Z. aethiopica* cultivars in Mexico has not been easy due to contamination of the explants. The antibiotic Agrimicyn® (2 g L⁻¹) added to the fungicide Benomyl® (2 g L⁻¹) reduced the endophytic contamination by fungi and bacteria in explants from rhizomes of the cultivars Deja Vú and Utopia. For seed germination *in vitro* contamination was not observed using 70% ethanol for 1 min followed by 10% sodium hypochlorite for 15 min, and 10 min of 30% hydrogen peroxide (Martínez-Hernández, 2022).

Direct organogenesis

The process of organogenesis provides the basis for asexual plant propagation largely from nonmeristematic somatic tissues (Schwarz and Beaty, 2000). Through this method, pathogen-free plants have been obtained via meristem culture (Tab. 2). In *Z. aethiopica*, the addition of

benzylaminopurine (BAP) at 22.19 μM produced 2.6 shoots per explant (Ribeiro *et al.*, 2014). Rhizomes and tubers have been the main organs targeted for obtaining explants despite the likelihood of presenting endogenous contamination. Singh *et al.* (2009) obtained 5 to 6 shoots of *Z. aethiopica* 10.0 mg L⁻¹ of BAP supplemented with 50 mg L⁻¹ of ascorbic acid. In *Z. rehmannii*, culture was obtained using a concentration of 3 mg L⁻¹ BAP, and a rate of 4.3 shoots per explant was obtained with 2.5 mg L⁻¹ BAP (Kulpa, 2016).

Table 2. Research relating to the direct organogenesis of *Zantedeschia* spp.

Plant material	Origin of the explant	Medium	Growth regulators	Multiplication rate (shoots per explant)	Reference
<i>Z. aethiopica</i>	Rhizome	MS	10.0 mg L ⁻¹ BAP, 50 mg L ⁻¹ ascorbic acid, 25 mg L ⁻¹ adenine sulfate, L-arginine and citric acid	5-6	Singh <i>et al.</i> , 2009
<i>Z. rehmannii</i>	Rhizome-buds	MS	3 mg L ⁻¹ and 2.5 mg L ⁻¹ BAP + 100 mg L ⁻¹ ascorbic acid	4.3	Kulpa, 2016
<i>Z. aethiopica</i>	Shoot tip	MS	22.19 μM BAP	2.6	Ribeiro <i>et al.</i> , 2014

Indirect organogenesis

Shin *et al.* (2020), reconstructed the process of regeneration of new shoots from callus and differentiated four stages: acquisition of pluripotency (in a medium rich in auxins), formation of pro-meristems, establishment of shoot progenitor, and shoot growth. Some species from the genus *Zantedeschia* have been propagated through this pathway (Tab. 3).

Table 3. Research related to indirect organogenesis of *Zantedeschia* spp.

Plant material	Origin of the explant	Medium	Growth regulators	Reference
<i>Z. hybrida</i> 'Feng Yang'	Rhizome	MS	BA (2.0 mg L ⁻¹) + NAA (0.1 ~ 0.2 mg L ⁻¹)	Xiao-Chun, 2010
<i>Zantedeschia</i> spp. 'Sunlight', 'Chiante', 'Pink Persuasion'	Apices derived from callus shoots and adventitious shoots	MS + 70-90 g L ⁻¹ sucrose	BA (2-3 mg L ⁻¹)	Lee and Ko, 2005

<i>Zantedeschia</i> sp. 'Pink Giant'	Leaf	MS	2,4-D (2.0 mg L ⁻¹) and BA (2.0 mg L ⁻¹) or NAA (2.0 mg L ⁻¹) and BA (2.0 mg L ⁻¹)	Gong <i>et al.</i> , 2008
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MS, Murashige and Skoog.

The *in vitro* dedifferentiation in *Z. aethiopica* and *Z. elliottiana* was observed in petiole, leaf, and spathe segments, achieving over 80% callus formation on Murashige and Skoog (MS) medium supplemented with indoleacetic acid (IAA) at 0.5 mg L⁻¹ + BAP 2.0 mg L⁻¹ for leaves; IAA (2.0 mg L⁻¹) + BAP (2.0 mg L⁻¹) for spathes; and IAA (1.0 mg L⁻¹) + BAP (2.0 mg L⁻¹) for petioles. The petioles showed the greatest dedifferentiation capacity among the organs mentioned. Furthermore, *Z. elliottianna* demonstrated a higher differentiation capacity than *Z. aethiopica*, as well as the ability to generate callus at high doses of BAP (4.0 mg L⁻¹) combined with NAA (0.1 mg L⁻¹) (Jonytienè *et al.*, 2017). In *Zantedeschia* spp., callus formation was induced by culturing leaf segments on MS medium supplemented with 2,4-D (2.0 mg L⁻¹) and BA (2.0 mg L⁻¹) or with naphthaleneacetic acid (NAA) (2.0 mg L⁻¹) and BA (2.0 mg L⁻¹) (Gong *et al.*, 2008).

The hormonal balance between auxins and cytokinin is crucial in the indirect organogenesis of *Zantedeschia in vitro* (Cheng *et al.*, 2013). Lee and Ko (2005), achieved up to 56.7% callus formation on a medium containing BA (2.0 mg L⁻¹) and a shoot regeneration rate of 70% at 3.0 mg L⁻¹ of BA. Cytokinin induced the formation of multiple shoots; 2ip (1.0 mg L⁻¹), BA (5.0 mg L⁻¹), and BA (1.0 mg L⁻¹) brought on 16, 14, and 12 multiple shoots in the cultivars Sunlight, Chiante, and Pink Persuasion, respectively. Xiao-Chun (2010) promoted callus formation on an MS medium + 2.0 mg L⁻¹ BA + 0.1-0.2 mg L⁻¹ NAA and achieved 100% rooting with an MS medium + indole butyric acid (IBA) at a concentration of 0.3 mg L⁻¹.

Somatic embryogenesis

Few studies on induced somatic embryogenesis in *Zantedeschia* exist (Tab. 4). The first reported research on somatic embryogenesis in calla lilies was conducted on 3 hybrids with explants derived from rhizomes and anthers. However, the formation of somatic embryos was only achieved from rhizome segments. The best results were obtained in media supplemented with plant growth regulators (Nic-Can *et al.*, 2016), as NAA (2.0 mg L⁻¹) and BA (0.6 to 2.0 mg

L⁻¹). Conversion to normal seedlings was achieved in MS medium supplemented with vitamins, micro and macronutrients, 1.0 mg L⁻¹ 2iP, 3% sucrose, and 0.7% agar (Duquenne *et al.*, 2006).

Stem segments containing apical meristems derived from the cultivar Gag-si were cultured on MS medium, a 25% induction rate of yellow embryogenic calli was obtained with MS + 0.5 mg L⁻¹ NAA and 1.5 mg L⁻¹ BA. In the regeneration experiments from embryogenic calli, the MS medium supplemented with 0.5 mg L⁻¹ IAA and 2.0 mg L⁻¹ BA showed the highest rates, approximately 85 ~ 90%. Table 4 summarizes research related to somatic embryogenesis of *Zantedeschia* species (Han and Kim, 2019).

Table 4. Research related to somatic embryogenesis of *Zantedeschia* spp

Species	Origin of explant	Medium	Growth regulators	Reference
<i>Zantedeschia</i> hybrids	Rhizomes and anthers	MS	BA (0.22 mM)	Duquenne <i>et al.</i> , 2006
<i>Zantedeschia hybrida</i>	Petiole	MS	0.5 mg L ⁻¹ NAA, 1.5 mg L ⁻¹ BA, 0.5 mg L ⁻¹ IAA, 2.0 mg L ⁻¹ BA	Han and Kim (2019)

Plant mass propagation through bioreactor

Research with *Zantedeschia* grown in liquid media has focused on determining the optimal frequency of immersion, comparing temporary immersion systems (TIS) with other culture systems such as semisolid and agitated liquid, and the effect of growth regulators. A multiplication coefficient of 9.7 shoots per explant was obtained using active buds from *Zantedeschia* spp rhizomes in a modified MS medium with 100 mg L⁻¹ myo-inositol, 1 g thiamine, 4 mg L⁻¹ BAP, and the use of paclobutrazol at 0.3 mg L⁻¹ with an immersion frequency of 4 h every 4 min (Sánchez *et al.*, 2009). Sánchez *et al.* (2010), achieved multiplication rates of 7.93, 10.66, and 11.30 shoots per explant in an MS medium + 1, 2, and 4 mg L⁻¹ BA and an immersion frequency of 4 h every 3 min.

In vitro zygotic embryos germination

Mature embryos from *in vitro* seeds have also been used to generate transgenic plants of *Zantedeschia* (Sun *et al.*, 2022). Studies on the *in vitro* germination of embryos (seeds) of species in the genus *Zantedeschia* determined that temperatures of 14-20°C, high concentrations of sucrose and agar (Ngamau, 2001), fruit maturity, and Ga₃ can influence the germination rate

(Nery *et al.*, 2015). *In vitro* germination of *Zantedeschia* studies focused on early flowering, and salt and/or temperature tolerance. Seedlings from seeds established at low temperatures had greater growth under lower night temperatures than those germinated at high temperatures, and clones from seeds exposed to high levels of sodium chloride (NaCl) achieved greater growth. Also, early seeds showed better growth, early flowering, and a higher number of spathes (Ngamau, 2008). The germination of *Z. aethiopica* seeds *in vitro* was enhanced in a medium supplemented with 3.0 mg L⁻¹ of GA₃ and 0.3 mg L⁻¹ of BAP, and germination occurred 35 d after establishment (Martínez-Hernández, 2022) (Fig. 2).

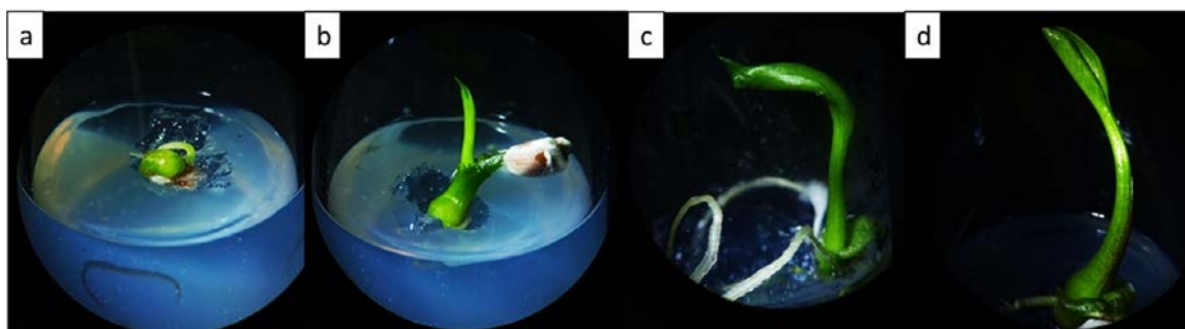


Figure 2. Growth *in vitro* of white calla lily (*Zantedeschia aethiopica* L. Spreng) seed cultured. A. Development of the radicle and emergence of the plumule. B and C. Elongation of the root and plumule. D. First leaf.

Martínez-Hernández *et al.* (2022) isolated zygotic embryos from mature and immature white calla lily fruits, the seeds were pretreated with sterile water and 30% (v/v) H₂O₂. The isolated embryos were cultured in a medium with MS salts (100%) added BAP (0, 0.3 and 3 mg L⁻¹) and AG₃ (0, 0.3 and 3.0 mg L⁻¹). The embryos development was not affected by the composition of culture media, the seed pretreatments or the fruit ripening (Fig. 3).



Figure 3. White calla lily (*Zantedeschia aethiopica* L. Spreng) seedling obtained from embryo isolation and *in vitro* culture.

***In vitro* flowering**

Naor *et al.* (2004a) investigated the hormonal control of floral induction and the development of the inflorescence *in vitro* in day-neutral plants of *Zantedeschia* spp., as well as the effects of GA and BA on the development of inflorescences in regenerated tissue culture seedlings. The seedlings were immersed in GA₃ and BA solutions before replanting in new media. GA₃ was mandatory for the transition from the vegetative to the reproductive stage. Inflorescence development in the apical bud was observed after 30-50 d in seedlings treated with GA₃ grown *in vitro*. However, they observed inflorescences with incomplete male and female flowers. BA did not affect flower induction but in the presence of GA₃, BA at 4.4 M enhanced inflorescence differentiation. They concluded that the floral initiation was not influenced by any environmental stimuli, and that the inflorescence development in the apical bud resembled that which occurs under natural conditions (Naor *et al.*, 2004a). GA₃ had a dual action in the flowering process, leading to the induction of inflorescence differentiation and promotion of floral stem elongation. They inferred that the flowering pattern might be a gradient in the distribution of GA and that this gradient along the axes of the tuber and the shoot is likely controlled by the apical dominance of the primary bud (Naor *et al.*, 2004b).

Future studies

Genotype influences the growth and development of the explants *in vitro*. Thus, the propagation of new varieties will need specific protocols. The evaluation of artificial light

sources for illumination to improve the quality of the explants may be explored. The use of nanomaterials in modulating *in vitro* *Zantedeschia* tissue growth and development could be evaluated. Organic additives on the micropropagation to enhance the growth and health protection of the explants could be tested.

CONCLUSION

This review provided a historical overview of the applications of *in vitro* cultivation of *Zantedeschia*, and phytopathological issues that limit the propagation of this ornamental crop. The establishment of *Zantedeschia* in the field free from pathogens is ensured using healthy *in vitro* propagated explants. *In vitro* culture has proven to be an effective tool for rapid clonal propagation and multiplication of *Zantedeschia* spp. However, the propagation *in vitro* of *Z. aethiopica* has not been easy in some cultivars due to the contamination of the explants.

Conflict of interests: The manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

BIBLIOGRAPHIC REFERENCES

Cafrune, E.E., F. Asinari, C.F. Nome, C.F. Perotto, M.C. Quiroga, and V.C. Conci. 2011. Identificación y caracterización del Dasheen mosaic virus en calla (*Zantedeschia aethiopica*) en Córdoba, Argentina. *Fitopatol. Colomb.* 35(1), 63-67.

Casierra-Posada, F., M.M. Blanke, and J.C. Guerrero-Guío. 2014. Iron tolerance in calla lilies (*Zantedeschia aethiopica*). *Gesunde Pflanzen* 66, 63-68. Doi: <https://doi.org/10.1007/s10343-014-0316-y>

Chen, L.-R., T.-C. Hsiung, K.-H. Lin, T.-B. Huang, M.-Y. Huang, and A. Wakana. 2017. Supplementary effect of hydrogen peroxide as a pre-disinfectant for sterilizing rhizome bud explants of *Zantedeschia aethiopica* L. with chlorine dioxide. *J. Fac. Agric. Kyushu Univ.* 62(1), 81-86. Doi: <https://doi.org/10.5109/1799306>

Chen, L.R., P.R. Lin, and C.J. Huang. 2020. First report of *Pectobacterium aroidearum* causing soft rot disease of white calla lily in Taiwan. *Plant Dis.* 104(2), 563. Doi: <https://doi.org/10.1094/PDIS-07-19-1462-PDN>

Cheng, Z.J., L. Wang, W. Sun, Y. Zhang, C. Zhou, Y.H. Su, W. Li, T.T. Sun, X.Y. Zhao, X.G. Li, Y. Cheng, Y. Zhao, Q. Xie, and X.S. Zhang. 2013. Pattern of auxin and cytokinin responses for shoot meristem induction results from the regulation of cytokinin biosynthesis by AUXIN RESPONSE FACTOR3. *Plant Physiol.* 161(1), 240-251. Doi: <https://doi.org/10.1104/pp.112.203166>

Ciampi, L., J. Nissen, E. Venegas, R. Fuentes, M. Costa, R. Schöbitz, E. Alvarez, and P. Alvarado. 2009. Identification of two species of *Fusarium* link that cause wilting of colored callas (*Zantedeschia aethiopica* (L.) Spreng.) cultivated under greenhouse conditions in Chile. *Chilean J. Agr. Res.* 69(4), 516-525. Doi: <https://doi.org/10.4067/S0718-58392009000400006>

Cruz-Castillo, J.G. 2022. ‘Utopia’: a new *Zantedeschia aethiopica*. *HortScience* 57(3), 443. Doi: <https://doi.org/10.21273/HORTSCI16321-21>

Cruz-Castillo, J.G., and A. Hernández-Montes. 2022. ‘Sublime’, una nueva variedad de alcatraz (*Zantedeschia aethiopica* (L.) Spreng). *Agro-Divulgación* 2(4), 57-58.

Cruz-Castillo, J.G. and P.A. Torres-Lima. 2017. ‘Deja Vu’: a new calla lily (*Zantedeschia aethiopica*) cultivar. *Rev. Chapingo Ser. Hortic.* 23(2), 97-101. Doi: <https://doi.org/10.5154/r.rchsh.2017.01.005>

Cuellar-Mandujano, A.K., A. Hernández-Montes, and J.G. Cruz-Castillo. 2022. Atributos intangibles en alcatraz blanco (*Zantedeschia aethiopica* (L.) K. Spreng): significados psicológicos y estructura de valores humanos para el consumidor. *Nova Sci.* 14(29). Doi: <https://doi.org/10.21640/ns.v14i29.3129>

Delgado-Paredes, G.E., C. Vásquez-Díaz, B. Esquerre-Ibañez, P. Bazán-Sernaqué, and C. Rojas-Idrogo. 2021. *In vitro* tissue culture in plants propagation and germplasm conservation of economically important species in Peru. *Sci. Agropec.* 12(3), 337-349. Doi: <https://doi.org/10.17268/sci.agropecu.2021.037>

Duquenne, B., T. Eeckhaut, S. Werbrouck, and J. Van Huylenbroeck. 2006. *In vitro* somatic embryogenesis and plant regeneration in *Zantedeschia* hybrids. *Plant Cell Tiss. Organ Cult.* 87(3), 329-331. Doi: <https://doi.org/10.1007/s11240-006-9161-8>

García-López, F. 2010. Efecto de la cepa bacteriana CAE-01 y fumigación al suelo sobre la pudrición blanda en el cultivo de alcatraz en La Perla, Veracruz. Msc thesis. Universidad Autónoma Chapingo, Chapingo, Mexico.

Githeng'u, S.K., S. Nyalala, and L. Gaoqiong. 2016. Antibacterial activity of *Coptis chinensis* extract against *Pectobacterium carotovorum* subsp. *carotovorum*. *Int. J. Phytopathol.* 5(2), 61-66. Doi: <https://doi.org/10.33687/phytopath.005.02.1159>

Gong, X., F. Qu, C. You, W. Sun, and M. Wang. 2008. Establishment of callus induction system from leaves in *Zantedeschia*. *J. Yantai Univ. (Nat. Sci. Engi. Ed.)*. 3, 221-225.

Grausgruber-Gröger, S., S. Richter, J.M. Salapura, D.K. Jošić, V. Trkulja, and H. Reisenzein. 2016. First report of *Dasheen mosaic virus* in *Zantedeschia* in Bosnia and Herzegovina. *New Dis. Rep.* 33(1), 13-13. Doi: <https://doi.org/10.5197/j.2044-0588.2016.033.013>

Guttman, Y., J.R. Joshi, N. Chriker, N. Khadka, M. Kleiman, N. Reznik, Z. Wei, Z. Kerem, and I. Yedidia. 2021. Ecological adaptations influence the susceptibility of plants in the genus *Zantedeschia* to soft rot *Pectobacterium* spp. *Hort. Res.* 8(1), 13. Doi: <https://doi.org/10.1038/s41438-020-00446-2>

Han, I.-S. and J.B. Kim. 2019. Establishment of a regeneration system for the production of calla plants (*Zantedeschia* spp.) via embryogenic callus culture. J. Plant Biotechnol. 46(1), 32-36. Doi: <https://doi.org/10.5010/JPB.2019.46.1.032>

Hashemidehkordi, E., S.N. Mortazavi, and P. Azadi. 2021. An efficient *in vitro* propagation protocol of pot calla lily (*Zantedeschia* spp cv. Orania and Sunclub) via tuber production. Int. J. Hort. Sci. Technol. 8(4), 343-351.

He, P., W. Cui, P. He, S. Munir, X. Li, Y. Wu, Y. Li, S. Asad, P. He, and Y. He. 2021. *Bacillus amyloliquefaciens* subsp. *plantarum* KC-1 inhibits *Zantedeschia hybrida* soft rot and promote plant growth. Biol. Control 154, 104500. Doi: <https://doi.org/10.1016/j.biocontrol.2020.104500>

He, S., Y. Yang, Z. Li, X. Wang, Y. Guo, and H. Wu. 2020. Comparative analysis of four *Zantedeschia* chloroplast genomes: expansion and contraction of the IR region, phylogenetic analyses and SSR genetic diversity assessment. PeerJ, 8, e9132. Doi: <https://doi.org/10.7717/peerj.9132>

Hernández-Villaseñor, L.A., M.Z. Reyna-Villela, S.M. Aceves-Villarruel, J. Salazar-flores, E.D. Torres-Sánchez, J.H. Torres-Jasso, and D. Rojas-Bravo. 2018. Effect of carbamazepine on the *in vitro* propagation of *Zantedeschia aethiopica*. Int. J. Environ. Sci. Toxicol. Res. 6(2), 12-17.

Hlophe, N.P., M. Moyo, J. Van Staden, and J.F. Finnie. 2015. Micropropagation of *Zantedeschia aethiopica* (L.) Spreng.: towards its commercial use in the cut flower industry. Propag. Ornam. Plants 15(2), 73-78.

Jonytienè, V., R. Masienè, N. Burbulis, and A. Blinstrubienè. 2017. Factors affecting *Zantedeschia* Spreng. dedifferentiation *in vitro*. Biologija 63(4), 334-340. Doi: <https://doi.org/10.6001/biologija.v63i4.3608>

Krejzar, V., J. Mertelík, I. Pánková, K. Kloudová, and V. Kúdela. 2008. *Pseudomonas marginalis* associated with soft rot of *Zantedeschia* spp. Plant Protect. Sci. 44(3), 85-90. Doi: <https://doi.org/10.17221/16/2008-PPS>

Kulpa, D. 2016. Micropropagation of calla lily (*Zantedeschia rehmannii*). Folia Hort. 28(2), 181-186. Doi: <https://doi.org/10.1515/fhort-2016-0021>

Lee, Y.S. and J.A. Ko. 2005. Effect of plant growth regulators on *in vitro* micropropagation of colored calla lily (*Zantedeschia* spp.). Korean J. Plant Res. 18(1), 154-160.

Liao, M.-T., D.-F. Mou, Y.-C. Chang, and C.-W. Tsai. 2020. Vector transmission of konjac mosaic virus to calla lily (*Zantedeschia* spp.) by aphids. Ann. App. Biol. 177(3), 367-373. Doi: <https://doi.org/10.1111/aab.12628>

Lim, J.-A., S. Jee, D.H. Lee, E. Roh, K. Jung, C. Oh, and S. Heu. 2013. Biocontrol of *Pectobacterium carotovorum* subsp. *carotovorum* using bacteriophage PP1. J. Microbiol. Biotechnol. 23(8), 1147-1153. Doi: <https://doi.org/10.4014/jmb.1304.04001>

Martínez-Hernández, A. 2022. Propagación *in vitro* de nuevas variedades de alcatraz (*Zantedeschia aethiopica* L. Spreng). MSc thesis. Universidad Autónoma Chapingo, Chapingo, Mexico.

Mikiciński, A., P. Sobiczewski, M. Sulikowska, J. Puławska, and J. Treder. 2010. Pectolytic bacteria associated with soft rot of calla lily (*Zantedeschia* spp.) tubers. J. Phytopathol. 158(4), 201-209. Doi: <https://doi.org/10.1111/j.1439-0434.2009.01597.x>

Muñoz-Escobar, E.M., and J.A. Palacio-Baena. 2010. Efectos del cloruro de mercurio (HgCl₂) sobre la sobrevivencia y crecimiento de renacuajos de *Dendrosophus bogerti*. Acta. Biol. 32(93), 189-197. Doi: <https://doi.org/10.17533/udea.acbi.13814>

Naor, V., J. Kigel, and M. Ziv. 2004a. Hormonal control of inflorescence development in plantlets of calla lily (*Zantedeschia* spp.) grown *in vitro*. *Plant Growth Regul.* 42(1), 7-14. Doi: <https://doi.org/10.1023/B:GROW.0000014889.16196.f7>

Naor, V., J. Kigel, M. Ziv, and M. Flaishman. 2004b. A developmental pattern of flowering in colored *Zantedeschia* spp: effects of bud position and gibberellin. *J. Plant Growth Regul.* 23(4), 269-279. Doi: <https://doi.org/10.1007/BF02637250>

Nery, F.C., V.L.A. Goulart, M.A. Viol, P.D.O. Paiva, R. Paiva, D.O. Prudente and M.C. Nery. 2015. *In vitro* germination and chemical composition of *Zantedeschia aethiopica* callus. *Acta Hortic.* 1083, 189-196. Doi: <https://doi.org/10.17660/ActaHortic.2015.1083.22>

Nery, F.C., D.O. Prudente, R. Paiva, M.C. Nery, P.D.O. Paiva, and D. Domiciano. 2018. Micropropagation and histological analysis of calla lily. *Acta Hortic.* 1224, 183-190. Doi: <https://doi.org/10.17660/ActaHortic.2018.1224.24>

Ngamau, K. 2001. Development of an *in vitro* culture procedure using seeds from *Zantedeschia aethiopica* 'Green Goddess' as explants. *Gartenbauwissenschaft* 66(3), 133-139.

Ngamau, K. 2008. Selection for early flowering, temperature and salt tolerance of *Zantedeschia aethiopica* 'Green Goddess'. *Acta Hortic.* 766, 155-162. Doi: <https://doi.org/10.17660/ActaHortic.2008.766.19>

Nic-Can, G.I., J.R. Avilez-Montalvo, R.N. Aviles-Montalvo, R.E. Márquez-López, E. Mellado-Mojica, R.M. Galaz-Ávalos, and V.M. Loyola-Vargas. 2016. The relationship between stress and somatic embryogenesis. pp. 151-170. In: Loyola-Vargas, V. And N. Ochoa-Alejo (eds.). *Somatic embryogenesis: fundamental aspects and applications*. Springer, Cham, Switzerland. Doi: https://doi.org/10.1007/978-3-319-33705-0_9

Niyokuri, A.N. and S. Nyalala. 2023. Calla lily soft rot causal agents, symptoms, virulence and management: a review. *Int. J. Hort. Sci.* 29(1), 60-68. Doi: <https://doi.org/10.31421/ijhs/29/2023/11380>

Purmale, L., A. Korica, and R. Joffe. 2023. First accounts of *Zantedeschia* sp *Dasheen* and *Konjac mosaic virus* detection in Latvia. *Acta Hortic.* 1359, 173-178. Doi: <https://doi.org/10.17660/ActaHortic.2023.1359.21>

Ribeiro, M.N.O., M. Pasqual, A.B. Silva, and V.A. Rodrigues. 2014. Propagação *in vitro* de copo-de-leite: sulfato de adenina e 6-benzilaminopurina. *Ornam. Hortic.* 20(1), 21-26. Doi: <https://doi.org/10.14295/rbho.v20i1.660>

Rizzo, D., A. Panattoni, L. Stefani, M. Paoli, B. Nesi, S. Lazzereschi, S. Vanarelli, P. Farina, M. Della Bartola, A. Materazzi, and A. Luvisi. 2015. First report of *Zantedeschia* mild mosaic virus on *Zantedeschia aethiopica* in Italy. *J. Plant Pathol.* 97(2), 399.

Sánchez, J., I. Capote, and M. Daquinta. 2010. Multiplicación *in vitro* de brotes de tres variedades de callas (*Zantedeschia* sp.) empleando sistema de inmersión temporal. *Cienc. Tecnol.* 3(1), 1-5. Doi: <https://doi.org/10.18779/cyt.v3i1.90>

Sánchez, J., M. Daquinta, and I. Capote. 2009. Multiplicación *in vitro* de *Zantedeschia* spp. variedad Treasure en sistemas de inmersión temporal. *Biotechnol. Veg.* 9(4), 211-215.

Schwarz, O.J. and R.M. Beaty. 2000. Organogenesis. pp. 125-138. In: Trigiano, R.N (ed.). *Plant tissue culture concepts and laboratory exercises*. 2nd ed. Routledge. Doi: <https://doi.org/10.1201/9780203743133>

Sheu, S.-Y., C.-W. Huang, M.-Y. Hsu, C. Sheu, and W.-M. Chen. 2017. *Cellvibrio Zantedeschiae* sp. nov., isolated from the roots of *Zantedeschia aethiopica*. *Int. J. Syst. Evol. Microbiol.* 67(9), 3615-3621. Doi: <https://doi.org/10.1099/ijsem.0.002178>

Shin, J., S. Bae, and P.J. Seo. 2020. *De novo* shoot organogenesis during plant regeneration. J. Exp. Bot. 71(1), 63-72. Doi: <https://doi.org/10.1093/jxb/erz395>

Singh, M., M.S. Rathore, K. Coudhary, and N.S. Shekhawat. 2009. Direct shoot bud formation and tuberization from aseptically cultured root tubers of calla lily (*Zantedeschia aethiopica* L.). J. Plant Biochem. Biotechnol. 18(2), 203-207. Doi: <https://doi.org/10.1007/BF03263320>

Sun, X., X. Wang, B.S. Subedi, Y. Jiang, D. Wang, R. Gou, G. Zhang, W. Xu, and Z. Wei. 2023. Tissue culture of calla lily (*Zantedeschia* Spreng.): an updated review on the present scenario and future prospects. Phyton Int. J. Exp. Bot. 92(8), 2413-2428. Doi: <https://doi.org/10.32604/phyton.2023.029667>

Sun, X., Y. Wang, T. Yang, X. Wang, H. Wang, D. Wang, H. Liu, X. Wang, G. Zhang, and Z. Wei. 2022. Establishment of an efficient regeneration and *Agrobacterium* transformation system in mature embryos of calla lily (*Zantedeschia* spp.). Front. Gen. 13, 1085694. Doi: <https://doi.org/10.3389/fgene.2022.1085694>

Szabó, L.K., F. Desiderio, Z. Kirilla, A. Hegedűs, E. Várallyay, and A. Preininger. 2024. A mini-review on in vitro methods for virus elimination from *Prunus* sp. fruit trees. Plant Cell Tiss. Organ Cult. 156(42). Doi: <https://doi.org/10.1007/s11240-023-02670-9>

Trejo-Téllez, B.I., N.I. Torres-Flores, L.I. Trejo-Téllez, and V.M. Cisneros-Solano. 2013. El alcatraz blanco (*Zantedeschia aethiopica* (L.) K. Spreng) en el municipio de La Perla, Veracruz. Agro Productividad 6(3). <https://revista-agroproductividad.org/index.php/agroproductividad/article/view/457>. Consulta: mayo de 2021.

van der Wolf, J.M., S.H. De Boer, R. Czajkowski, G. Cahill, F. van Gijsegem, T. Davey, B. Dupuis, J. Ellicott, S. Jafra, M. Kooman, I.K. Toth, L. Tsrer, I. Yedidia, and J.E. van der Waals. 2021. Management of diseases caused by *Pectobacterium* and *Dickeya* species. pp. 175-214. In: van Gijsegem, F., J.M. van der Wolf, and I.K. Toth (eds.). Plant diseases caused by *Dickeya* and

Pectobacterium species. Springer, Cham, Switzerland. Doi: https://doi.org/10.1007/978-3-030-61459-1_6

Wang, H., Z. Yang, S. Du, L. Ma, Y. Liao, Y. Wang, I. Toth, and J. Fan. 2018. Characterization of *Pectobacterium carotovorum* proteins differentially expressed during infection of *Zantedeschia elliotiana* *in vivo* and *in vitro* which are essential for virulence. Mol. Plant Pathol. 19(1), 35-48. Doi: <https://doi.org/10.1111/mpp.12493>

Waleron, M., A. Misztak, M. Waleron, M. Franczuk, J. Jońca, B. Wielgomas, A. Mikicinski, T. Popović, and K. Waleron. 2019. *Pectobacterium zantedeschiae* sp. nov. a new species of a soft rot pathogen isolated from calla lily (*Zantedeschia* spp.). Syst. Appl. Microbiol. 42(3), 275-283. Doi: <https://doi.org/10.1016/j.syapm.2018.08.004>

Wei, Z., H. Zhang, Y. Wang, Y. Li, M. Xiong, X. Wang, and D. Zhou. 2017. Assessing genetic diversity and population differentiation of colored calla lily (*Zantedeschia* Hybrid) for an efficient breeding program. Genes 8(6), 168. Doi: <https://doi.org/10.3390/genes8060168>

Xiao-Chun, S.H.U. 2010. Study on the tissue culture and rapid propagation of *Zantedeschia hybrida* 'Feng Yan'. J. Anhui Agr. Sci. 38(26), 14268-14269.

Yan, L.I., X.T. Wei, Q.H. Gan, J.I. Ying, X.L. Shao, and Y.C. Wang. 2014. Isolation and identification of soft rot bacteria on imported *Zantedeschia*. J. Food Saf. Qual. 5(12), 3944-3946.