

## In vitro Micropropagation of Calla lily: An Overview

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Received: 2.03.2020 | Revised: 7.04.2020 | Accepted: 11.04.2020

### ABSTRACT

*Calla lily belonging to the Araceae family is used as a cut flower and in the landscaping of gardens. Plant parts such as leaves, rhizomes, roots, stems, and the whole plant are used as herbal medicine. Conventional methods used for propagation of calla lily do not produce adequate planting material as there is various fungal, bacterial and virus attack on the plant during plant propagation which leads to poor yield in flower and rhizome or some time it causes the death of the plant. Therefore, plant tissue culture is the only technique that allows fast clonal propagation also provides healthy and uniform plants. In most cases, growth regulators like BAP, IBA, and NAA were found to be essential for growth, multiplication of shoot and root formation. Some species of the calla lily are also used as herbal medicine having activity such as antibacterial, antifungal, antioxidant, antihistaminic, antialgal, antithrombotic, and anticoagulant activities. Therefore results show that there is a need for improvement in further research and development that need to be carried out to improve this crop, to developing improved varieties with commercially significant yield. In the present review, micropropagation methods and protocols are combined and gather the information to researchers for further investigation and to produce quality as well as quantity planting material.*

**Keywords:** Rhizome, Antifungal, Anticoagulant, Plant tissue culture, BAP

### INTRODUCTION

Calla lily, *Zantedeschia* spp. 'Southern Light' is a bulbous plant belonging to the genus *Zantedeschia*. It is native to Southern Africa and mainly distributed at an altitude range from 1,000 to 2,000 meters in temperate regions of South Africa, Zimbabwe, and Nigeria (Letty, 1973). It has been widely used as an ornamental plant because it has a unique leaf shape like flowers. Particularly, the

coloured calla has several different colours such as yellow, red, and purple which makes it as a cut flower, a potted flower, and a garden plant. In the early 1990s, the calla industry grew rapidly, with New Zealand's exports of cut flower increasing by 75% in five years, and an effort made to produce bulbs throughout the year (Clemens & Eddie Welsh, 1993).

**Cite this article:** Kumar, A., & Dogra, I. (2020). In vitro Micropropagation of Calla lily: An Overview, *Ind. J. Pure App. Biosci.* 8(2), 144-153. doi: <http://dx.doi.org/10.18782/2582-2845.7974>

Small rhizomes that are overwintered in pots under cover can be cut up into sections, and each of them has a visible bud. Large overwintered clumps in the garden can be split by lifting the plant before there is much top growth, and cutting through the roots with a spade and isolating into smaller sections. Nevertheless, large-scale production of the calla lily plants is narrow by tuber propagation, which is the traditional reproduction method used which result in low propagation coefficient. Specifically, because the plants will hurt during division propagation and created the wound can be easily infected by several fungal and bacterial infections during the cultivation practice. Growing of the calla lily has been studied broadly (Plummer et al., 1990; Corr & Widmer, 1991), the major efforts has been made to focused on the control of flowering and tuber storage (Corr, Widmer, 1987, 1988). Commercially, multiplication of calla lily through branches and tubers is applied to increase the size and the number of tubers. However, there is serious crop losses are caused by field-grown tubers infected by *Erwinia* soft rot (Chen et al., 2000). Because of high infection in individuals, Cohen (1981) and Ruiz (1996) with colleagues introduced plant propagation through an in vitro method. The in vitro technique for long has been elaborated commercially for the propagation of plant material for agricultural, horticultural, pharmaceutical, or research purposes, and for building up disease-free plant materials (Thorpe, 2007). With the induction of dedifferentiation is regulated by endogenous and exogenous growth regulators in the plant nutrient medium (MS) (Murashige & Skoog, 1962). An important role in dedifferentiation stimulation is played by the type and concentration of plant growth regulators and their contact (Khanam et al., 2000). It has been found that various combinations of auxins and cytokinins in the medium affected the strength of callus formation (Makunga et al., 2005). For

the induction dedifferentiation in *Zantedeschia spreng*, cytokinins are commonly used for callus formation (D'Arth et al., 2002; Naor et al., 2004; Yip et al., 2006). Indirect organogenesis offers strategy for dedifferentiating plant tissue into callus permitting transformation, selection procedures, and regenerating processes for development of in vitro whole plants from transformed callus (Robinson and Firozabadi, 1993). A variety of type of explants, such as rhizomes and buds (Chang et al., 2003; Ebrahim, 2004; Yip et al., 2006), shoots (Cohen, 1981) and leaves (Gong Xue Qin et al., 2008; Zheng, 2010) have been used for dedifferentiation of the calla lily. At the present time demand will need to be support by high biomass yield varieties with improved agronomical traits such as higher quality and quantity plant material production. Therefore this will generate the need for calla lily development through in vitro methods. The purpose of this overview is to summarize the existing literature for the in vitro culture of calla lily that may help to be acquainted from beginning to end tissue culture technology in calla lily and provide a baseline for further improvement in the calla lily propagation through in vitro techniques.

#### **Conventional methods used for calla propagation and diseases**

Calla lilies are conventionally multiplied through rhizomes commercially, annual multiplication of calla lily through offsets and tuber division is practiced to increase the number of tubers of flowering size. Several forms of rot can affect calla lilies which resulting in poor growth, wilting and possible plant death. However, differentiation of field grown tubers is subject to infection by *erwinia* soft rot, which causes serious losses in calla lily crop (Chen et al., 2000). Crown rot, root rot, and *pythium* rot are caused by separate different pathogens in poorly drained soil. Calla lilies are also affected by powdery mildew, *armillaria* rot, gray mold, blight, and

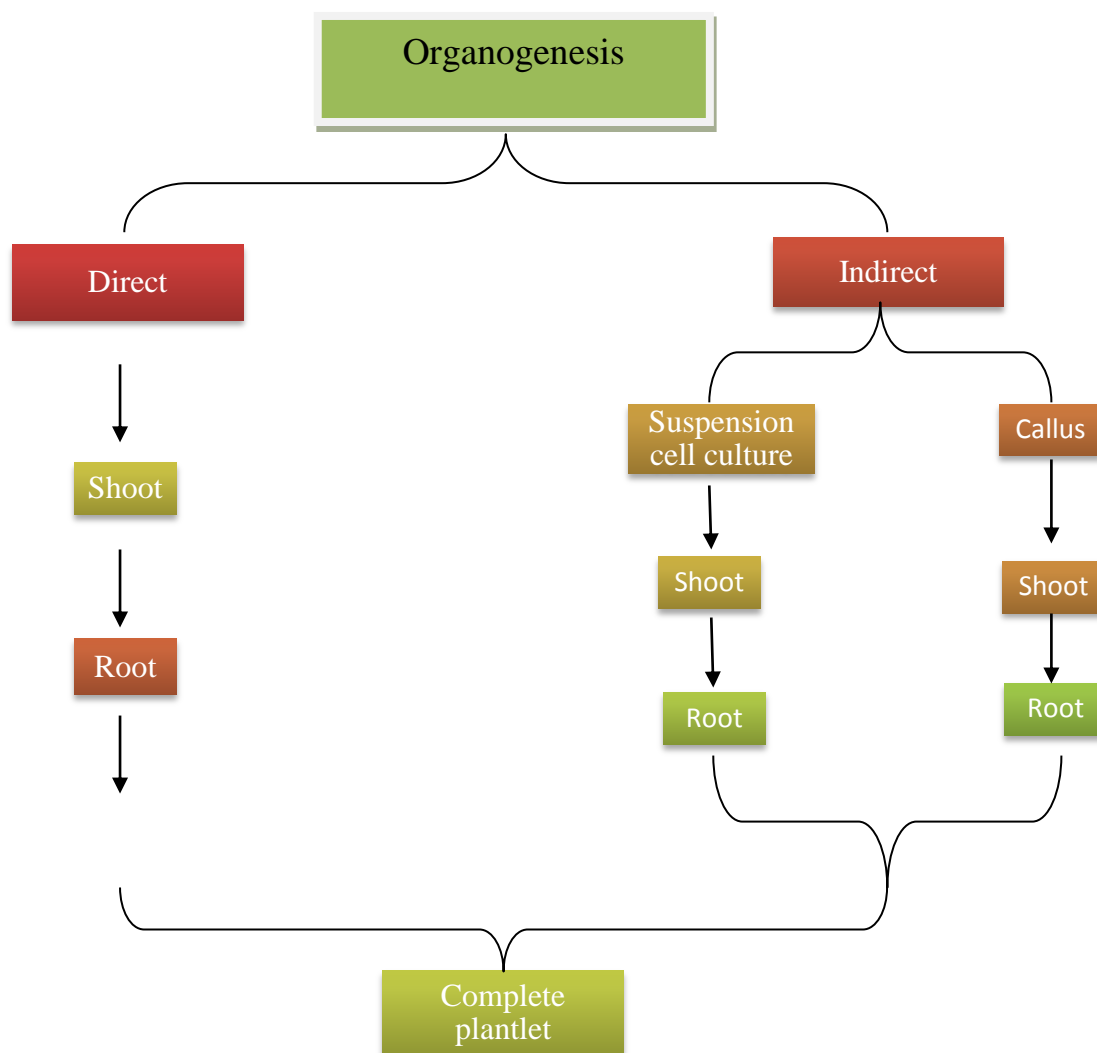
leaf spots. Spotted wilt and dasheen mosaic are two viruses that can attack calla lilies shows their effect on the leaf, flower stalks, and petioles.

**Plant tissue culture**

Plant tissue culture is a technique used to develop complete plantlets from a cell, tissue or any other part of the plant when cultured on a suitable medium under aseptic conditions.

**In vitro tissue culture of calla lily**

Plant regeneration from in vitro culture can be achieved by either organogenesis or embryogenesis. Tissue culture is used to introduce new cultivars and provide plant materials free from known viruses (Cohen, 1981; Yao et al., 1995). Tissue culture-derived plant material is proposed to be an alternative source of planting material for tuber production in calla lily (Clemens & Welsh, 1993; Cohen, 1981).



**Fig. 1: Micropropagation of plants by organogenesis**

**In vitro establishment of calla lily through tubers**

Danuta Kulpa., 2016 observed that the Culture initiation of calla lily on MS media supplemented with 3 mg dm<sup>-3</sup> BAP. During multiplication stage, adventitious shoots was

cultured on MS media supplemented with concentrations of BAP (0.5 to 5 mg dm<sup>-3</sup>), KIN (0.5 to 5 mg dm<sup>-3</sup>), TDZ (0.1 to 1 mg dm<sup>-3</sup>) and 2iP (2.5 to 15 mg dm<sup>-3</sup>) or BAP (0.5 to 7.5 mg dm<sup>-3</sup>) with IAA (0.5 to 2 mg dm<sup>-3</sup>). The highest multiplication rate for *Zantedeschia*

was obtained on MS medium supplemented with the concentration of BAP  $2.5 \text{ mg dm}^{-3}$ . Also MS medium with BAP of concentration  $2.5 \text{ mg dm}^{-3}$  shows the highest shoot length (3.41 cm) and the number of adventitious shoots (4.13). The highest rooting percentage was observed in MS medium fortified with  $0.1 \text{ mg dm}^{-3}$  IBA as compared to other rooting hormones used. Effects of different sucrose concentrations in the culture medium on the growth of colored *Zantedeschia* in vitro under  $\text{CO}_2$  enrichment conditions the plantlets in vitro of colored *Zantedeschia* had the largest root number, root weight, and root vigor under  $0\text{g/l}$  (sugar-free culture) treatment. And they had the largest plant height, leaf length, and leaf chlorophyll content, but poor root vigor under  $3.0 \text{ g/l}$  sugar (Wang et al., 2016). Ribeiro et al. (2009) observed that in vitro development of calla lily effect of sucrose and gibberellic acid concentrations. Plant tissue culture allows fast large-scale clonal propagation and provides healthy uniform plant material. During the in vitro micropropagation, the type of explant and concentrations of growth regulator could influence the growth of seedlings. It was found that sucrose and  $\text{GA}_3$  concentrations in MS medium increase the efficiency of the in vitro multiplication of calla lily. It was also found that the addition of  $60.5 \text{ g/L}$  sucrose and  $5 \text{ mg/L}$   $\text{GA}_3$  in MS medium shows the highest sprouts number. Whereas the higher length of the above-ground part the addition of  $45.3 \text{ g/L}$  sucrose and  $10 \text{ mg/L}$   $\text{GA}_3$  was found most effective. MS medium fortified with sucrose in the range of  $51.13 - 56.5 \text{ g/L}$  showed the highest root length and number of roots. Kozak et al., 2009 found that the effect of benzyladenine on shoot regeneration in vitro of *Zantedeschia aethiopica* 'Green Goddess'. Shoots of *Zantedeschia aethiopica* 'Green Goddess' obtained from aseptically grown shoot clusters were used as the source of explant cultured in vitro on MS medium supplemented with BAP of different

concentrations i.e; 5, 10, 25 and  $50 \mu\text{M}$ . The highest number of axillary shoots was observed on MS medium supplemented with BA with a concentration of 25 and  $50 \mu\text{M}$ . The treatment with BA  $25 \mu\text{M}$  shows an increase in the formation of rhizomes in the cultures. The rooting was observed in all media used in the experiments. The highest rooting percentage was found in MS medium supplemented with BA of concentration 5 or  $10 \mu\text{M}$ .

#### **In vitro establishment of calla lily through shoot tips**

The addition of auxins to the MS medium supplemented with  $8.87\text{mM}$  BA slightly enhanced multiple shoot formation in the explants. Multiplication of six cultivars of *Zantedeschia* genus comprising different flower types and colors were tested and achieved using only one regeneration medium (MS +  $8.87 \text{ mM}$  BA +  $2.46\text{mM}$  IBA). Different MS medium strength, air temperature ( $15, 20, 25,$  and  $30^\circ\text{C}$ ) and light quality fluorescent, red + blue, red and blue light provided by an LED (light-emitting diode) system were used (without phytohormone) to stimulate in vitro shoot and root development. Half strength MS or MS and cultures maintained at  $25^\circ\text{C}$  were found to be equally suitable for shoot tip culture of *Zantedeschia albomaculata*. Shoot elongation, as well as fresh and dry weight, was significantly increased when cultures were kept under red or blue light (Chang et al., 2002). Multiplication of six cultivars of *Zantedeschia* genus comprising different species, flower types and colors were tested using only one regeneration medium (MS +  $8.87\text{mM}$  BA +  $2.46 \text{ mM}$  IBA). All the cultivars responded well and regeneration was observed among all the cultivars tested in the present experiments. However, the number of new shoots developed per explant and their fresh weight at 25d varied in different cultivars. Such differential responses among cultivars have already been reported in various plant species (Ahroni et al., 1997; Zuker et al.,

1997; Chakrabarty et al., 1999). However, the shoots that regenerated from shoot tip explants on this medium had small leaves and a slow growth rate. Bulblets and shoots of *Narcissus* rooted only when the culture medium contained MS salts at half strength (Seabrook et al., 1976) and the response worsened with increasing MS salt levels. The promoting effect of diluted mineral salt solution on rooting is probably due to a reduced nitrogen level (Sriskandarajah et al., 1990). Cultures maintained at 25°C showed the highest growth rate as evidenced by the highest fresh weight, dry weight, leaf area, root number, and chlorophyll content. Chang et al., 2003 reported that the micropropagation of calla lily (*Zantedeschia albomaculata*) via in vitro shoot tip proliferation onto Murashige and Skoog (MS) medium supplemented with different plant growth regulator concentrations and combinations of the four cytokinins tested, 6-benzyl adenine (BA) and thidiazuron (TDZ) were found to be more effective. An effective concentration of BA (8.87 µM) or TDZ (4.54 µM) produced an average of 3.8 and 3.2 shoots per explants but with the increasing concentrations of cytokinins often leads to lower proliferation rate and slow growth. Addition of auxins to the MS medium supplemented with 8.87µM BA slightly increasing multiplication of shoot formation in the explants. Multiplication of six cultivars of *Zantedeschia* genus comprising different flower types and colors were tested and developed using only one regeneration medium (MS + 8.87 µM BA + 2.46µM IBA). Half strength MS or MS medium cultures maintained at 25°C were found to be suitable for the shoot tip culture of *Z. albomaculata*.

#### **In vitro establishment of calla lily through the leaf**

Micropropagation and histological analysis of Calla lily by transferring the leaf, nodal and root (1 cm<sup>2</sup>) explants to Murashige and Skoog medium (MS) supplemented with 6-benzyl adenine (BA) (0.0, 0.25, 0.5, 1.0, 1.5, 2.0, 4.0

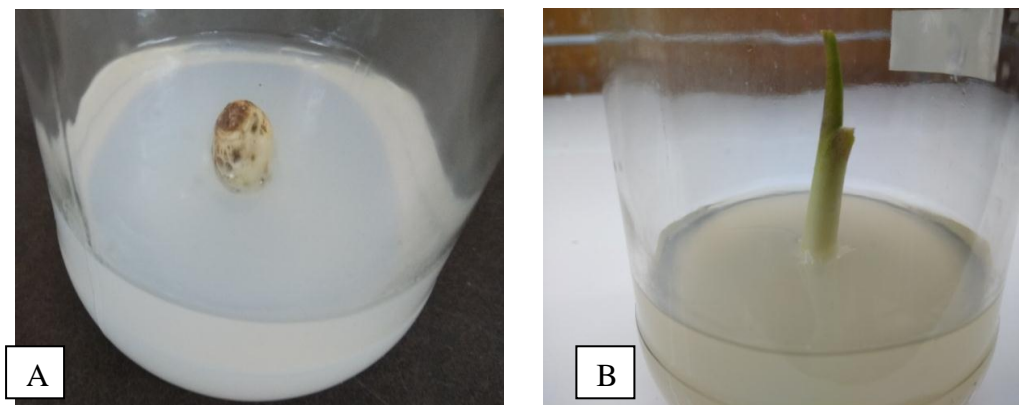
and 6.0 mg/L) combined with 0.1mg/L naphthalene-1-acetic acid (NAA), 3% sucrose and 0.7% agar. CTT dye was used for quantifying cell viability of callus from nodal explants. Plantlets were individualized and inoculated with different concentrations of NAA and indol-3-butyric acid (IBA) (0.0, 1.0, 2.0 and 3.0 mg/L) for in vitro rooting. The highest shoot recovery occurred with 4.0 mg/L BA combined with 0.1 mg/L NAA. The highest percentage of rooted shoots (85%) was found in the culture medium with 2.0 mg/L IBA. The acclimatization of rooted shoots in Plantmax<sup>®</sup> was successfully accomplished and 100% survival of plant material was observed during this stage. Our results demonstrate that micropropagation of calla lily is successful using nodal explants (Nery et al. 2018). The medium used for explants was based on Murashige and Skoog medium. Plantlets regenerated from explants were examined by electron microscopy. Plantlets from basal parts of the petals and of basal pieces of leaves used as explants resulted in the best virus elimination. In conclusion, tissue culture is applicable for virus elimination in lily cultivars and hybrids but the success depends on the type of explants. The medium used for explants was based on Murashige and Skoog (1962; MS) medium: twice MS with addition of 1 mg/L 6-benzyl amino purine, 1 mg/L naphthaleneacetic acid for basal pieces of the petals by Takayama and Misawa (1982); for bulbils from stem – with addition of 5 mg/L naphthaleneacetic acid, 0.5 mg/L kinetin, 30 g/L sucrose and 10 g/L agar; MS with addition of 5 mg/L 6-benzyl amino purine, 0.1 mg/L naphthaleneacetic acid for bulblets from scale by Jakobsone and Andersone (1997) and MS with addition of 0.1 mg/L 6-benzyl amino purine, 1 mg/L naphthaleneacetic acid for the basal part of leaves as explants by Niimi and Onozava (1979). All mother material and plantlets were examined by electron microscopy (Robinson et al. 1987) and DAS ELISA (Hagita, 1989).

Vered et al., 2004 studied the hormonal control of inflorescence development in plantlets of calla lily (*Zantedeschia* spp.) grown in vitro. Hormonal control of flower induction and inflorescence development in vitro was studied in photo periodically day-neutral calla lily (*Zantedeschia* spp., colored cultivars). The effect of gibberellins (GA<sub>3</sub>, 5.8–2900 μM) and the cytokinin (BA, 0.4–13.3 μM) on inflorescence development were studied in plantlets developed from plant tissue culture. Plantlets were kept in GA<sub>3</sub> and BA solutions before replanting in new media. GA<sub>3</sub> is essential for the shift from the vegetative to the reproductive stage. Inflorescence development from the apical bud was observed after 30–50 days in GA<sub>3</sub> treated plantlets grown in vitro, while BA did not show an effect on flower induction. But in the presence of GA<sub>3</sub>, BA at concentrations up to 4.4 μM shows enhanced inflorescence differentiation. The results show that inflorescence developed

in *Zantedeschia* plantlets in plant tissue culture can act as an efficient model to study the role of GA<sub>3</sub> and other factors in the flowering process of day-neutral plants that do not require external signals for flower induction under natural conditions. Jonytiene et al., 2017 studied that Factors affecting *Zantedeschia* Spreng dedifferentiation in vitro. callus induction on somatic tissues of the arum lily, the most effective MS medium supplemented with 0.5 mg/L IAA + 2.0 mg/L BAP (leaf explants), 2.0 mg/L IAA + 2.0 mg/L BAP (spathe explants), and 1.0 mg/L IAA + 2.0 mg/L BAP (petiole explants) was used. Somatic tissues of the calla lily formed callus most intensively in the MS medium fortified with 1.0 mg/L IBA + 2.0 mg/L BAP (leaf explants), 1.0 mg/L IAA + 2.0 mg/L BAP (petiole explants), and 2.0 mg/L KIN (spathe explants). Petiole segments explants formed callus more closely than isolated tissues of leaves and spathe.

**Table 1: In vitro micropropagation of calla lily**

Type of explants	Shooting media	Rooting media	References
<b>Bulbils from Stem</b>	5 mg/L BAP	0.1 mg/L NAA	Jakobsone and Andersone, 1997
<b>Leaf</b>	0.1 mg/L BAP	1 mg/L NAA	Niimi and Onozava, 1979
<b>Leaf</b>	1 mg/L BAP	1 mg/L NAA	Takayama and Misawa, 1982
<b>Leaf</b>	0.1 mg/l BAP	1 mg/l NAA	Niimi and Onozava, 1979
<b>Leaf</b>	0.5 mg/L IAA + 2.0 mg/L BAP	–	Jonytiene et al., 2017
<b>Nodal</b>	4.0 mg L <sup>-1</sup> BA + 0.1 mg L <sup>-1</sup> NAA	2.0 mg L <sup>-1</sup> IBA	Nery et al., 2018
<b>Petiole</b>	1.0 mg/L IAA + 2.0 mg/L BAP	–	Jonytiene et al., 2017
<b>Shoots</b>	25 Mm BA	BA 5 or 10 Mm BA	Kozak et al., 2009
<b>Shoots</b>	8:87 mM BA + 2:46mM IBA	–	chang et al., 2003
<b>Spathe</b>	2.0 mg/L KIN	–	Jonytiene et al., 2017
<b>Tubers</b>	2.5 mg dm <sup>-3</sup> BAP	0.1 mg dm <sup>-3</sup> IBA	Danuta Kulpa, 2016
<b>Tubers</b>	10 mg/L GA <sub>3</sub>	51.13 – 56.5 g/L sucrose	Ribeiro et al., 2009



**Fig. 2: Calla lily tuber on MS medium supplemented with different growth regulators (A) Shoot establishment (B)**

### CONCLUSION

Calla lily is a commercially important crop for cut flower production as well as medicinal property gaining worldwide. Lack of quality planting material is a major drawback in large-scale cultivation of calla lily. Tissue culture techniques proved to be boon for the production of high quantity and quality planting material for farmers. At present, direct regeneration of plantlets via adventitious shoot and tuber explants is considered as a preferred method for calla lily plant regeneration. Future research emphasized on the development of protocols for direct regeneration of shoot buds from leaf explants, protocols for regeneration through somatic embryo-genesis need to be improved to develop as it can help in producing true to type and homozygous plants with improved quality and development of improved genotypes with a high medicinal value content, higher biomass production, wider adaptability, viable seed production.

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