

IN VITRO MICROPROPAGATION OF *ZANTEDESCHIA ELLIOTTIANA* PLANTS

Hoda I.M. El-Gedawey*, M.F. Rida* and M.K. Gaber**

* Ornamental Plants and Landscape Gardening Res. Dept., Hort. Res. Inst.,
Agricultural Research Center, Alexandria, Egypt

** Plant Production Dept., Fac. of Agric., Saba Basha, Alexandria Univ., Egypt



Scientific J. Flowers &
Ornamental Plants,
9(1):1-11 (2022).

Received:

14/2/2022

Accepted:

6/3/2022

Corresponding author:

Magd El-Din F. Rida

magdeldinhamza2@yahoo.com

ABSTRACT: A set of experiments was done on *Zantedeschia elliottiana* tubers at Tissue Culture Laboratory, Faculty of Agric. Saba Basha, Alex. Univ., Egypt; during the years of 2021 and 2022 to achieve an optimal and reliable system for its micropropagation. At least one tuber eye derived from (1 cm²) tuber piece was used as initial explants. The explants were drenched in L-ascorbic acid solution (10 mg/l). The explants were cultured on initiation media (MS medium) complement with auxin: 1-naphthaleneacetic acid (NAA) and cytokinin: N6-benzyladenine (BA) [0 to 2 mg/l]. On multiplication stage, adventitious shoots were put in MS media complement with BA (0.5 to 5 mg/l), kinetin: KIN (0.5 to 5 mg/l), with NAA (0.5 to 2 mg/l). The effect of BA is more than KIN in multiplication stage, taller shoots and large number of adventitious shoots were produced after addition of BA to culture medium. Top efficient combination of the studied cytokinins and auxins on multiplication stage was obtained from the addition of 3 mg/l BA and NAA at 2.00 mg/l, which led to the highest increase in number of shoots, leaflets and root numbers/propagule. Rooting took place on MS media complement with auxin: indole-3-butyric (IBA) and NAA at (0.5 to 2 mg/l). The most plentiful and the longest roots were obtained from explants cultured on MS medium complement with IBA at 1.0 mg/l. The highest rooting percentage 100% was obtained after the addition of NAA at 0.5 mg/l to culture media. Acclimation percent reached up to 90% under greenhouse conditions.

Key words: *Zantedeschia elliottiana*, *in vitro* propagation, 1-naphthaleneacetic acid (NAA), N6-benzyladenine, indole-3-butyric acid (IBA), kinetin (KIN).

INTRODUCTION

Zantedeschia spp. (family Araceae) is herbaceous perennial ornamental plant. It is native to the quagmire or rough area of South Africa (Earl, 1957 and Letty, 1973). They have a unique flower spathe (external "petal" look like a tube) and pretty leaves. Moreover, *Zantedeschia* spp. are popular rhizomatous flower all over the world. It is classified into two sections; *Zantedeschia* section (white calla lily) and *Aestivea* section (colored calla lily) (Letty, 1973 and Singh *et al.*, 1996). Colored calla lilies

(*Zantedeschia* hybrids, are interspecies hybrids, mainly derived from *Z. elliottiana*, *Z. pentlandii*, *Z. albomaculata* and *Z. rehmannii* within the *Aestivea* section (Funnell, 1993). *Zantedeschia* cultivars are bred for cut flower production, pot production, landscape and garden use.

Zantedeschia elliottiana (golden or yellow calla) is characterized by its green dark leaves with white specks, heart-shaped, with a blade 28 to 60 cm long and 5 to 25 cm wide, supported by a spongy petiole that measures 30 cm to 100 cm in length. The

roots come from a tuberous rhizome, are fleshy, adventitious and branched. Its spathe has the shape of a yellow trumpet that can measure up to 12 cm in length (Larson, 1988).

Massive production of plant cells and extraction required product from cell cultures (high yielding), has become a highly desirable technology. With the advancement of this technology the production becomes more economic to produce low cost and high volume materials. Three broad classes of plant growth regulators mainly auxines, cytokinins and gibberellins are used in tissue culture. The growth differentiations and organogenesis of tissues become feasible only on the addition of one or more of these classes of hormones to a medium (Dipak and Soma, 2010).

The main commercial method for reproduction calla lily is tuber division. This method produces narrow scale of calla lily plants, which resulted in low propagation coefficient. Moreover, division propagation causes wounds which can be easily infected by several fungal and bacterial infections (Kumar and Dogra, 2020). Serious crop losses are caused by infected filed grown tuners such as *Erwinia* soft rot (Chen *et al.*, 2000). Tissue culture propagation is a quick way to produce pathogen-free plants. Many researches have been done on the *in vitro* micropropagation of *Zantedeschia* species (Ruiz *et al.* 1996; Kritzinger *et al.*, 1998; Fang *et al.*, 1999 and Ebrahim, 2004).

Development of a rapid, and quick way for *in vitro* propagation of *Zantedeschia elliotiana* plants is the aim of this study.

MATERIALS AND METHODS

Culture initiation:

Tubers of *Zantedeschia elliotiana* were brought from well-known commercial nursery to the Plant Tissue Culture Laboratory of the Plant Production Department of the Faculty of Agriculture, Saba Basha, Alexandria University during the years 2021 and 2022. The average

diameter of the used tubers was (2.5-3 cm). Dust or sand particles were eliminated from tubers by using running tap water for 30 minutes to wash them.

Subsequently, the tubers pre-sterilization was done in warm water (30 °C) with a few drops of detergent for 20 min and submerging in 70% (v/v) ethanol for 30 seconds followed by several washing with sterilized water. The tubers were surface sterilized using 10% of sodium hypochlorite for 20 minutes and then with 0.5 mg/l mercuric chloride for 5 min. Few drops of Tween-20, were also, added as a surfactant to sterilized water with gentle shaking under sterile conditions for 20 minutes. After that sterilized water was used to wash plant material for five times and get ready for culture.

Micropropagation initiation stage:

At least one tuber eye derived from (1 cm²) tuber piece was used as initial explants. The explants were drenched for 1 min in L-ascorbic acid solution (10 mg/l) before transplanting to the media (Panda and Hazra, 2010 and Kulpa, 2016).

The explants were cultured on MS medium (Murashige and Skoog, 1962) complemented by benzyladenine (BA) at four different concentrations (0.0, 0.5, 1.0 and 2.0) mg/l) in combination with the 1-naphthalene acetic acid (NAA) at three different concentrations (0.0, 1.00 and 2.00 mg/l)

Micropropagation multiplication stage:

Explant growth during multiplication stage under plant growth regulators effect was examined by two experiments. In the first experiment, newly adventitious shoots were placed on 16 kinds of proliferation media: MS medium, complemented with cytokinins: (BA at 0.5, 1.0, 2.5 and 5.0 mg/l), kinetin (KIN at 0.5, 1.0, 2.5 and 5.0 mg/l) in combination with or without NAA at 0.2 mg/l, beside the control treatment (hormone-free medium), to compare the effect between both cytokinins on the explant growth. In the second one,

adventitious shoots from the initiation stage were cultured in MS medium complemented with NAA at (0.5, 1.0 and 2.0 mg/l) and BA at (1.0, 3.0 and 5.0 mg/l). The multiplication stage was done through six weeks.

Micropropagation rooting stage:

In vitro proliferated shoots obtained from multiplication stage were transferred to rooting media: MS medium complemented with various concentrations of indole-3-butyric acid (IBA) and (NAA) at (0.0, 0.5, 1.0 and 2.0 mg/l). The rooting stage lasted six weeks.

Jars containing 30 ml of medium were used to culture the explants. The jars were placed, vertically. Each treatment was replicated five times with three explants (i.e. 15 explants/treatment) and incubated in a growth chamber at $23\pm 1^\circ\text{C}$ temperature under 16 hrs. daily light and 8 hrs. darkness. Illumination was done by a florescent light intensity of 2880 lux ($40\mu\text{ mol m}^{-2}\text{S}^{-1}\text{PPF}$). The cultures in all stages were cultured for 6 weeks on solidified MS medium.

Acclimatization stage:

The new-formed plantlets were moved out to the greenhouse for hardening. The potting mix used in this study was 1:1:1 (v/v/v) vermiculite: sand: peat moss. The transferred plants were monitored weekly for 6 weeks.

Experimental design and data analysis:

Data were arranged and statistically analyzed in a completely randomized design (C.R.D.) with five replications according to Gomez and Gomez (1984), however, survival percentage was transformed using annular transformation before analysis. The means of the studied treatments were compared by D.M.R. test at 5% level of probability according to Duncan (1955).

RESULTS AND DISCUSSION

A set of experiments were conducted to achieve an optimal and reliable system for micropropagation of *Zantedeschia elliottiana*

plants. The obtained results are presented and discussed in the following section.

Initiation stage:

Table (1) showed that different levels of NAA, BA and their combinations have a significant effect on the initiation stage characters of *Zantedeschia elliottiana* explants.

For the effect of augmenting the culture medium with NAA, Table (1) cleared that, adding NAA at 2.00 mg/l to culture medium was concomitant with the longest mean shoot length/propagule (5.94 cm; Fig., 1). The addition of NAA to the culture medium at 1.00 mg/l as sole source of PGR resulted in the highest mean number of roots (6.09/propagule).

On the other hand, Table (1) showed that supplementing the MS culture medium with 2.00 mg/l of BA resulted in the highest mean numbers of shoots and leaflet number/propagule with or without combination with NAA, but the highest shoots and leaflet/propagule value 3.40 and 10.66 respectively were recorded after application of 2.00 mg/l BA combined with NAA at 2.00 mg/l.

The obtained results can be explained by the mode of action of both applied growth regulators, whereas auxin exerts significant roles in plant micropropagation and composition of nutrient media. The growth of callus, cell suspensions and organs and regulation of morphogenesis direction can be promoted by auxin or a combination of auxins and cytokinins. At the cellular level, auxin control main processes such as cell division and cell elongations (George *et al.*, 2008). Also, play critical event in promoting rhizogenesis (Kim *et al.*, 2003).

Cytokinin, with or without auxin, participate in the regulation of the plant cell cycle (i.e. stimulation of cell division, break apical dominance, enhance axillary shoot proliferation, and adventitious, inhibition root formation). Also, the interaction between auxin and cytokinin represents important signal in the formation of cell

Table 1. Means of initiation stage characters of *Zantedeschia elliotiana* explants cultured *in vitro* for 45 days as affected by complementing the culture media by different levels of NAA, BA and their combinations (mg/l).

PGR (mg/l)		Shoot length (cm)	Shoots number/propagule	Leaflets number/propagule	Roots number/propagule
NAA	BA				
0.00	0.00	0.68 e	0.20 d	1.30 d	0.66 e
	0.50	1.52 d	1.70 bc	2.26 d	1.13 e
	1.00	1.46 d	1.86 bc	3.93 cd	1.00 e
	2.00	1.58 d	3.06 ab	6.66 b	0.53 e
1.00	0.00	4.01 bc	1.30 c	2.10 d	6.09 a
	0.50	3.76 bc	1.95 bc	2.59 d	3.78 c
	1.00	3.38 c	2.43 b	3.39 cd	4.04 c
	2.00	4.13 b	3.24 ab	9.39 ab	2.39 d
2.00	0.00	5.94 a	1.76 bc	1.73 d	4.96 b
	0.50	5.25 a	2.19 b	4.38 c	3.73 c
	1.00	3.33 c	2.86 ab	8.24 b	3.70 c
	2.00	3.53 bc	3.40 a	10.66 a	3.33 c

Values in the same column not followed by the same letters are significantly different at the 5% level of probability.

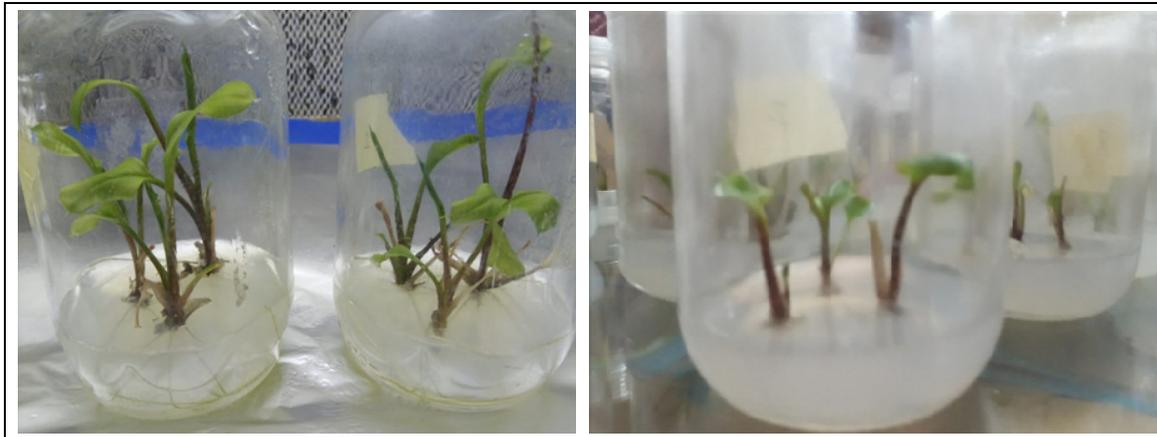


Fig. 1. Initiation stage of *Zantedeschia elliotiana* explants cultured on MS medium complemented with 2 mg/l NAA.

phenotype and maintenance of the process of cell division (Stickens *et al.*, 1996).

Skoog and Miller (1957) discovered the regulation of organogenesis *in vitro* by means of the auxin : cytokinin ratio. The relationship between auxin and cytokinin levels and morphogenetic response was recorded in several studies (e.g., Li *et al.*, 1994; Centeno *et al.*, 1996 and Leyser *et al.*, 1996).

Higher concentration of auxin is usually ineffective in shoot proliferation. Jerzy and

Pawlak-Anhalt, (2002) and Waseem *et al.* (2011) mentioned that the best growth regulator for initiating *Zantedeschia elliotiana* explants was BAP and IAA at the concentrations of 3 and 2 mg/dm³, respectively. Kulpa (2016) reported that there was a positive effect on the shoot length and number of adventitious shoots of *Zantedeschia rehmannii* after supplementing the culture media with 2.5 mg/dm³ BAP.

Multiplication stage:

Data presented in Table (2) declared that the effectiveness of BA significantly surpassed its counterpart of KIN in micropropagation of *Zantedeschia elliottiana* plant. The effect of BA is more than KIN in multiplication stage, taller shoots and large number of adventitious shoots were produced after the addition of BA to MS culture medium (Fig., 2).

Regarding shoot length, Table (2) cleared that the highest shoot length (4.27 and 3.55 cm) were obtained after the addition of 2.50 and 5.00 mg/l of BA in combination with NAA at 0.2 mg/l respectively. While, the highest number of shoots (4.60) of *Z. elliottiana* were obtained after the addition of 5.00 mg/l of BA in combination with NAA at 0.2 mg/l. Moreover, respecting the fresh weight of shoots, the highest significant weight (1.26 mg) was recorded after supplementing the culture medium with 2.50 mg/l BA in combination with NAA at 0.2 mg/l.

These results could be attributed to the mode of action of BA which is more effective than KIN and/or variations in their metabolism as reported earlier. Also, this variation may be due to the degree of cell sensitivity towards both tested cytokinins, which depends on the endogenous levels of growth regulators. Likewise, on other occasions, KIN was reported to be not suitable for *Zantedeschia* characters in the multiplication stage (Kulpa, 2016).

Results displayed in Table (3) showed that supplementing the culture medium with different levels of NAA and BA and their combinations resulted in significant effects on the characters of the multiplication stage of *Zantedeschia elliottiana* explants.

Table (3) cleared that applying NAA at 2.00 mg/l combined with BA at 1.00 mg/l caused the highest significant increase in the mean shoot length (6.92 cm). Also, Table (3) declared that augmenting the culture medium with the combination of NAA at 2.00 mg/l and BA at 3.00 mg/l led to the highest

increase in number of shoots/propagule (5.31), number of leaflets/propagule (16.18) and root numbers/propagule (5.80), respectively (Fig., 3)

Our previous results on the effects of auxin (NAA) and cytokinin (BA) and their combination on the multiplication stage of *Zantedeschia elliottiana* are in harmony with the results obtained by Chang *et al.* (2003) who reported that increasing BA concentration led to low shoot proliferation and stunted growth in *Zantedeschia albomaculata* and concentration of 5 mg/l BA resulted in the highest shoot multiplication of *Zantedeschia aethiopica*. In *Zantedeschia albomaculata* the highest mean number of shoots, fresh and dry weight was obtained after using low concentration of BA (2 mg/l). Furthermore, some studies revealed that the addition of IAA or IBA with BA enhanced shoot proliferation. Ruffoni and Savona (2005) who cleared that the highest multiplication rate can be obtained by using BA at lower concentration (4.0 mg/l) and exposure of *in vitro* explants to higher BA concentrations resulted in lower shoot length and fresh weight. Naor *et al.* (2005) who showed that low concentration of BA (3 mg/l) resulted in a substantial proliferation of axillary buds/shoot in some *Zantedeschia* cultivars. Furthermore, Nqobile *et al.* (2015) who found that supplementing MS medium with a combination of cytokinin (BA) at 4 mg/l and auxin (IBA) at 1 mg/l resulted in a significant increase in shoot length and fresh weight and the highest mean number of axillary shoots for *Zantedeschia aethiopica*.

Rooting stage:

Results in Table (4) showed that adding different levels of IBA or NAA to MS culture media resulted in significant effects on the studied characters of *Zantedeschia elliottiana* explants during the rooting stage. These effects are presented in Fig. (4).

For the effect of NAA, Table (4) demonstrated that the presence of NAA at 2.00 mg/l in a culture medium resulted in the highest significant shoot length (5.44 cm),

Table 2. Means of multiplication stage (shoot characters) of *Zantedeschia elliptiana* explants cultured *in vitro* for 45 days as affected by complementing the MS culture media by different levels of BA (mg/l) or KIN (mg/l) in combination with NAA at 0.2 mg/l.

NAA	PGR (mg/l)		Shoot length (cm)	Shoot numbers	Fresh weight of shoots (mg)
	BA	KIN			
0.00	0.00	0.00	1.55 cd	0.80 g	0.47 e
0.2	0.50	0.00	1.76 cd	2.16 e	0.77 c
0.2	1.00	0.00	2.95 bc	3.32 c	0.94 b
0.2	2.50	0.00	4.27 a	3.86 b	1.26 a
0.2	5.00	0.00	3.55 ab	4.60 a	1.04 b
0.2	0.00	0.50	1.30 d	1.47 f	0.32 f
0.2	0.00	1.00	1.95 cd	2.69 d	0.62 d
0.2	0.00	2.50	3.42 b	2.23 de	0.79 c
0.2	0.00	5.00	2.22 c	1.93 ef	0.65 cd

Values in the same column not followed by the same letters are significantly different at the 5% level of probability.

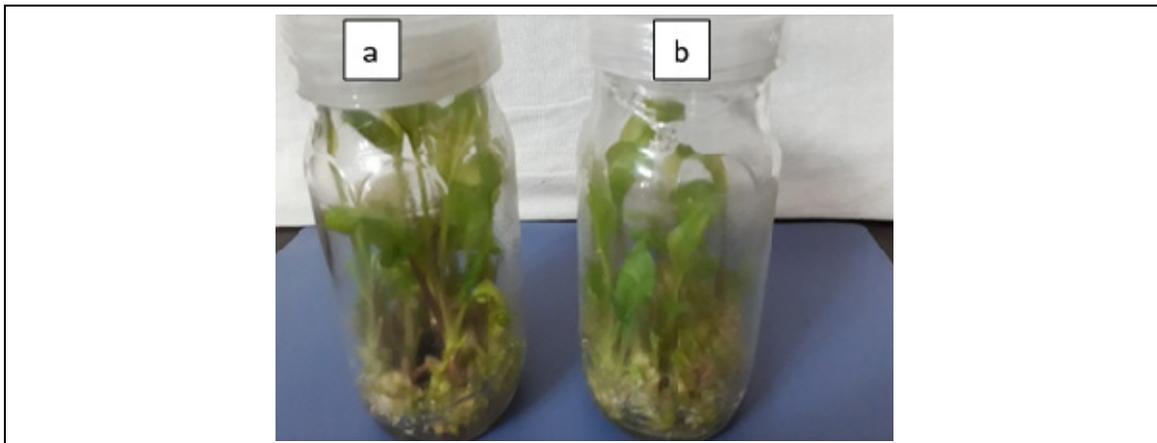


Fig. 2. *In vitro* comparison of the effect of supplementing MS medium with (a) BA (b) Kin at 2.5 mg/l in combination with NAA at 0.2 mg/l on the growth of performance of *Zantedeschia elliptiana* in Multiplication stage.

Table 3. Means of multiplication stage (shoots and roots characters) characters of *Zantedeschia elliptiana* explants cultured *in vitro* for 45 days as affected by different combination levels of NAA (mg/l) and BA (mg/l) in MS medium.

NAA	PGR (mg/l)		Shoot length (cm)	Number of Shoots/propagule	Number of leaflets/propagule	Root Numbers/propagule
	BA					
0.0	0.0		0.84 f	0.73 g	2.15 g	1.35 c
	1.00		2.09 e	2.11 f	6.56 ef	3.85 b
0.5	3.00		3.06 d	2.04 f	7.25 c	1.93 c
	5.00		3.05 d	2.54 e	8.89 d	1.51 c
1.0	1.00		2.45 e	1.90 f	5.70 f	4.56 b
	3.00		4.06 c	2.42 e	7.83 de	4.16 b
	5.00		3.27 d	3.58 c	10.52 c	3.88 b
2.0	1.00		6.92 a	3.23 d	13.23 b	5.53 a
	3.00		5.32 b	5.31 a	16.18 a	5.80 a
	5.00		3.19 d	4.34 b	15.93 a	4.22 b

Values in the same column not followed by the same letters are significantly different at the 5% level of probability.



Fig. 3. Multiplication stage of *Zantedeschia elliptiana* newly formed explants cultured on MS medium complemented with 3.0 mg/l BA+2.00mg/l NAA.

Table 4. Means of rooting stage characters of *Zantedeschia elliptiana* explants cultured *in vitro* for 45 days as affected by different levels of IBA or NAA (mg/l).

PGR (mg/l)		Shoot length (cm)	Weight of fresh roots/propagule (mg)	Root numbers/propagule	Root length (cm)	percentage of roots (%)
IBA	NAA					
0.00	0.00	1.16 d	0.37 d	0.26 d	0.30 c	16.67 c
0.50	0.00	2.39 c	0.59 c	3.53 c	3.46 b	60.0 b
1.00	0.00	3.12 bc	1.03 ab	6.49 ab	5.76 a	86.60 ab
2.00	0.00	4.06 b	0.93 b	4.76 bc	4.26 b	86.80 ab
0.00	0.50	2.13 cd	0.60 c	4.00 c	0.96 c	100.0 a
0.00	1.00	3.79 b	0.85 b	5.46 b	1.28 c	86.80 ab
0.00	2.00	5.44 a	1.11 a	7.00 a	0.30 c	93.40 a

Values in the same column not followed by the same letters are significantly different at the 5% level of probability.



Fig. 4. Rhizogenesis of *Zantedeschia elliptiana* micro-shoots in the multiplication stage, upon culture and after for 45 days on MS medium fortified with 1.00mg/l IBA.

the highest fresh weight of roots/propagule (1.11 mg) and the highest root numbers/propagule (7.00) with approximately the same level of significance of the application of IBA at 1.00 mg/L).

Respecting the effect of the addition of IBA to the medium, Table (4) cleared that supplementing the MS culture media with IBA at 1.00 mg resulted in the highest significant root length (5.76 cm). Also, the addition of IBA at 1.00 to the medium caused the highest significant increase in fresh weight of roots/propagule (1.03) and root numbers/propagule (6.49), with the same approximate level of significant of NAA application at 2.00 mg/l (Fig., 4).

For the effect of both auxins on the percentage of roots, Table (4) showed that IBA addition at 1.00 or 2.00 mg/l or any NAA studied levels (0.50, 1.00 and 2.00 mg/l) resulted in the highest significant increase with approximately no significant difference between them.

The obtained results are in agreement with those mentioned by Kulpa (2016) who mentioned that the longest and numerous roots were obtained after the addition of 0.1 mg/dm³ IBA to culture media of *Zantedeschia rehmannii*.

Acclimatization stage:

The *ex vitro* growth was done in pots containing a mixture media of 1:1:1 (v/v/v) vermiculite : sand: peatmoss. The survival rate was calculated and recorded 90% (Fig., 5).

Fig. (6) illustrated the successfully rooted and acclimatized *in vitro*-derived shoots of *Zantedeschia elliottiana* under greenhouse conditions after six weeks of acclimatization in the greenhouse. These results are in harmony with those obtained by Nqobile *et al.* (2015) who reported that acclimatization of *in vitro*-derived shoots of *Z. aethiopica* in a soil mixture containing 1:1 (v/v) vermiculite: sand recorded 94% of survival rate.

CONCLUSION

It could be concluded that there is a possibility to propagate *Zantedeschia elliottiana* by *in vitro* micropropagation by using at least one tuber eye derived from 1 cm² tuber piece as initial explants and drenching the explants for 1 min in L-ascorbic acid solution (10 mg/l) before transplanting to the culture media. In the initiation stage using MS media complement with 2.00 mg/l BA and NAA resulted in the highest mean numbers of shoots and leaflet/propagule. In proliferation stage augmenting the culture medium with a combination of NAA at 2.00 mg/l and BA at 3.00 mg/l led to the highest increase in number of shoots, leaflets and root numbers/propagule. Also, rooting characters can be improved by using MS media supplemented with 2.00 mg/l NAA to the culture medium. Moreover, 90% of obtained plantlets were successfully acclimatized under greenhouse conditions using pots with 1:1:1 (v/v/v) vermiculite: sand: peat moss.



Fig. 5. *Ex vitro* acclimatized *Z. elliottiana* tissue culture derived-plants.



Fig. 6. The successfully rooted and acclimatized *in vitro*-derived shoots of *Zantedeschia elliottiana* under greenhouse conditions after six weeks of acclimatization in the greenhouse.

REFERENCES

- Centeno, M.L.; Rodriguez, A.; Feito, I. and Fernandez, B. (1996). Relationship between endogenous auxin and cytokinin levels and morphogenic responses in *Actinidia deliciosa* tissue cultures. *Plant Cell Rep.* 16: 58-62.
- Chang, H.S.; Charkabarty, D.; Hahn, E.J. and Paek, K.Y. (2003). Micropropagation of calla lily (*Zantedeschia albomaculata*) via *in vitro* shoot tip proliferation. *In vitro Cellular and Developmental Biology-Plant*, 39: 129-134.
- Chen, J.J.; Liu, M.C. and Ho, Y.H. (2000). Size of *in vitro* plantlets affects subsequent tuber production of acclimated calla lily, *Horticulture Science*, 35:290-298.
- Dipak, K. and Soma, H. (2010). *Plant Breeding Biometry Biotechnology*. New Central Book Agency (P) Ltd. London, Delhi Kolkata, 411 p.
- Duncan, D.B. (1955). Multiple range and multiple F tests. *Biometrics*, 11: 1-42.
- Earl, G. (1957). Chromosome numbers and speciation in the genus *Zantedeschia*. *Plant life*, 13: 140-146.
- Ebrahim, M.K.H. (2004). Comparison, determination and optimizing the conditions required for rhizome and shoot formation and flowering of *in vitro* cultured calla explants. *Sci. Hort.*, 101:305-313.
- Fang, W.I.; Xion, G.I.; Qu, Y.H. and Qu, S.P. (1999). Tissue culture of coloured common calla lily. *J. South. Agric. Univ.*, 21: 423-426.
- Funnell, K.A. (1993). *Zantedeschia*. In: Hertogh, A.D. and LeNard, M. (eds.), *The Physiology of Flower Bulbs*, 1st Ed., Elsevier: Amsterdam, The Netherlands, pp. 683-703.
- George, E.F.; Hall, M.A. and Klerk, G.J.D. (2008). *Plant Propagation by Tissue Culture*, 3rd Edition. Springer, Dordrecht, The Netherlands, 501 p.
- Gomez, K. and Gomez, A.A. (1984). *Statistical procedures for Agricultural Research* (2nd Ed.). An International Rice Researcher Institute Book, A Wiley Interscience Publisher, New York., USA, 680 p.
- Jerzy, M. and Pawlak-Anhalt, A. (2002). *In vitro* regeneration of *Zantedeschia elliottiana* from *ex vivo* derived rhizome explants. *Zesz. Probl. Post. Nauk Roln.*, 483: 101-107.
- Kim, Y.S.; Han, E.J. and Paek, K.Y. (2003). Lateral root development and saponin accumulation as affected by IBA or NAA in adventitious root cultures of *Panax ginseng* CA Meyer. *In vitro Cell. Develop. Biol.*, 39: 245-249.
- Kritzinger, E.M.; Vuuren, R.J.V.; Woodward, B.; Rong, I.H.; Spreeth, M.H. and Siabbert, M.M. (1998). Elimination of external and internal contaminants in rhizomes of *Zantedeschia aethiopica* with

- commercial fungicides and antibiotics. *Plant Cell Tissue Organ Cult.*, 52: 61-65.
- Kulpa, D. (2016). Micropropagation of calla lily (*Zantedeschia rehmannii*). *Folia Hort.*, 28:181-186.
- Kumar, A., and Dogra, I. (2020). *In vitro* Micropropagation of Calla lily: An Overview, *Ind. J. Pure App. Biosci.*, 8:144-153.
- Larson, R.A. (1988). *Introducción a la floricultura*. Calypso. México, D.F., 551 p.
- Letty, C. (1973). The genus *Zantedeschia*. *Bothalia*, 11:5-26.
- Leyser, H.M.O.; Pickett, F.B.; Dharmasiri, S. and Estelle, M. (1996). Mutation in AXR3 gene of Arabidopsis result in altered auxin response including ectopic expression from the SAUR-ACI promoter. *Plant J.*, 10: 403-413.
- Li, Y.; Shi, X.Y.; Strabala, T.J.; Hagen, G. and Guilfoyle, T.J. (1994). Transgenic tobacco plants that overproduce cytokinins show increased tolerance to exogenous auxin and auxin transport inhibitors. *Plant Science*, 100: 9-14.
- Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Plant Physiology*, 15:473-497.
- Naor, V.; Kigel, J. and Ziv, M. (2005). The effect of gibberellin and cytokinin on floral development in *Zantedeschia* spp. *in vivo* and *in vitro*. *Acta Horticulturae*, 673: 255-263.
- Nqobile, P.H.; Mack, M.; Johannes, V.S. and Jeffrey, F.F. (2015). Micropropagation of *Zantedeschia aethiopica* (L.) Spreng.: towards its commercial use in the cut flower industry. *Propagation of Ornamental Plants*, 15:73-78.
- Panda, B.M. and Hazra, S. (2010). *In vitro* regeneration of *Semecarpus anacardium* L. from axenic seedling-derived nodal explants. *Trees*, 24:733-742.
- Ruffoni, B. and Savona, M. (2005). The temporary immersion system (TIS) for the improvement of micropropagation of ornamental plants. *Acta Horticulturae*, 683: 445-454.
- Ruiz, S.G.; Rosa, M.E. and Flores, O.C.E. (1996). *Zantedeschia aethiopica*, propagation by tissue culture. *J. Agr. Univ. Puerto-Rico.*, 80: 193-194.
- Singh, Y.; Wyk, A.E.V.; Baijnath, H. (1996). Taxonomic notes on the genus *Zantedeschia* Spreng. (Araceae) in Southern Africa. *S. Afr. J. Bot.*, 66:321-324.
- Skoog, F. and Miller, C.O. (1957). Chemical regulation of growth and organ formation in plant tissue cultured *in vitro*. *Symp. Soc. Exp. Biol.*, 11:118-130.
- Stickens, D.; Tao, W. and Verbelen, J.P. (1996). A single cell model system to study hormone signal transduction. *Plant Growth Regul.*, 18:149-154.
- Waseem, K.; Jelani, M.S.; Khan, M.S; Kiran, M. and Khan, G. (2011). Efficient *in vitro* regeneration of chrysanthemum (*Chrysanthemum morifolium* L.) plantlets from nodal segments. *Afri. J. Biotechn.*, 10:1477-1484.

الإكثار الدقيق لنبات الكلا الذهبي بتقنية زراعة الأنسجة

هدى اسماعيل محمد الجداوي*، مجد الدين فؤاد رضا*، محمد قدرى عبد الحفيظ جابر**
* فرع بحوث نباتات الزينة بأنطونيداس، الإسكندرية، قسم بحوث الزينة وتنسيق الحدائق، معهد بحوث البساتين، مركز
البحوث الزراعية، مصر
** قسم الإنتاج النباتي، كلية زراعة سايا باشا، جامعة الإسكندرية، مصر
أجريت مجموعة من التجارب على كورمات نبات الكلا الذهبي في معمل زراعة الأنسجة، كلية الزراعة، سايا باشا،
جامعة الإسكندرية، مصر خلال أعوام ٢٠٢١ - ٢٠٢٢ لإنتاج هذا النبات عن طريق الإكثار الدقيق. أنسجة صغيرة

(١ سم^٢) من كورمات الكلا تحتوي على عين واحدة على الأقل نعتت في حمض الأسكوربيك بتركيز ١٠ ملجم/لتر ثم زُرعت في بيئة البدء (بيئة موراشيخ وسكوج) مضاف إليها الأكسين NAA والسيتوكينين BA بتركيز (صفر إلى ٢ ملجم/لتر). في مرحلة التضاعف زرعت المجاميع الخضرية المتكونة في بيئة موراشيخ وسكوج مضافة إليها السيتوكينين BA بتركيز (٠,٥ إلى ٥ ملجم/لتر) و السيتوكينين KIN بتركيز (٠,٥ إلى ٥ ملجم/لتر) بالإضافة إلى الأكسين NAA بتركيز (صفر إلى ٢ ملجم/لتر). وجد أن BA كان أكثر كفاءة من KIN حيث أدى استخدامه إلى إنتاج نباتات أكثر طولاً وذات عدد أكبر من المجاميع الخضرية وأحسن توليفة من السيتوكينات والأكسينات في مرحلة التضاعف تم الحصول عليها من اضافة BA بتركيز ٣ ملجم/لتر و NAA بتركيز ٢ ملجم/لتر إلى بيئة موراشيخ وسكوج، حيث أدت إلى أعلى زيادة في عدد الافرع الخضرية، وبادئات الأوراق والجذور. أما مرحلة التجذير فقد تمت بإضافة الأكسين IBA و NAA إلى بيئة موراشيخ وسكوج بتركيز (٠,٥ إلى ٢ ملجم/لتر). أكبر زيادة في عدد الجذور وطولها تم الحصول عليها من بيئة موراشيخ وسكوج مضاف إليها IBA بتركيز ١ ملجم/لتر. وأعلى نسبة تجذير ١٠٠٪ تم الحصول عليها من اضافة NAA بتركيز ٠,٥ ملجم/لتر إلى بيئة موراشيخ وسكوج. ولقد نجحت عمليات اقلمة النباتات المتحصل عليها بنسبة ٩٠٪ تحت ظروف الصوب الزجاجية.