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An Efficient *in vitro* Propagation Protocol of Pot Calla lily (*Zantedeschia* spp cv. Orania and Sunclub) via Tuber Production

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ABSTRACT

Zantedeschia spp is an important flower in the ornamental plants market. Due to the high demand for this plant in the horticultural industry, it is indispensable to introduce an *in vitro* protocol for its mass propagation. For this aim, the tubers of calla lily were disinfected in a hot water bath with different temperatures (30, 35, 40, 45, and 50 ºC) and duration (30 or 35 min). Then explants were cultured on MS medium with different combinations of 6-Benzylaminopurine (BAP) and Kinetin (Kin). Based on the obtained results, the highest disinfection percentage (more than 90%) was obtained at 45 ºC for 35 min. Also, the highest proliferation rate (with an average of 15.33 and 14.32 in cv. Orania and cv. Sunclub, respectively) was observed in 2.5 mg L^{-1} BAP + 1.5 mg L^{-1} Kin. The highest rooting percentage (100% in both cultivars) and root number per explant (with an average of 4.00 and 3.03 in cv. Orania and Sunclub, respectively) was obtained in 0.5 mg L^{-1} Indole-3-acetic acid (IAA) + 0.1 mg L^{-1} Kin, but the highest root length (with an average of 120.0 and 106.6 mm in cv. Orania and Sunclub, respectively) was observed in 1.0 mg L^{-1} IAA + 0.1 mg L^{-1} Kin. In MS medium + 2.0 mg L^{-1} Indole-3-butyric acid (IBA) + 4% Sucrose, the highest number of tubers (with an average of 6.66 and 5.21 in cv. Orania and Sunclub, respectively) was formed. The highest fresh and dry weights (with an average of 948.33 and 851.33 mg in cv. Orania and Sunclub, respectively) of tuber were obtained in 2.0 mg L^{-1} IBA + 6% sucrose. The rooted and tuberous plants were adapted in the greenhouse successfully.

Introduction

Ornamental plants are an essential section of the horticulture industry that has a significant impact on the international horticultural market (Azadi et al., 2016). *Zantedeschia* genus is one of the most important and favorite bulbous ornamental plants worldwide. The colored calla

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lily is very attractive and popular in the market (Wei et al*.,* 2017), with a high turnover of 19.2 million euros in the Netherlands (Hubner 2017). Calla lily usually propagates by seed culture and dividing the tuber. Sexual reproduction with seeds in this flower takes about 2 to 3 years and shows a variation in offspring (Zhang et al*.,* 2011). On the other hand, the distribution of the divided tuber needs high-level of skills and leads to the development and spread of bacterial soft rot disease, caused by the *Erwinia carotovora*. Therefore, the use of an alternative, efficient, and fast method, such as *in vitro* culture in calla lily, is essential (Chang et al*.,* 2003). Micropropagation is a suitable method for the rapid production of healthy, free of pests and diseases and uniformity plants (Deloire et al*.,* 1995). The *in vitro* culture method has a high commercial ability because it provides rapid growth of plants and the production of high-quality plants. This method is a suitable tool for achieving goals that are challenging in a conventional way (Cardoso and Teixeira da Silva, 2012). Micropropagation through shoot primordial in calla lily was reported by Chang et al. (2003) in *Z. albomaculata*, Koech et al. (2005) in *Z. albomaculata* 'Black Magic', Kozak and Stelmaszczuk (2009) in *Zantedeschia aethiopica* 'Green Goddess, and Kulpa (2016) in *Zantedeschia rehmannii*. Traditionally, *in vitro* shoots are transferred to a rooting medium and then planted into a high-humidity environment for acclimatization. This step is necessary because *in vitro* plantlets are not autotrophic (McCartan et al*.,* 2004), often lack a functional cuticle, and have impaired stomatal functioning (Asayesh et al., 2017, 2021). It is possible to reduce or eliminate this problem by inducing shoots to form a storage organ *in vitro*. *In vitro* tuber production is more advantageous than the rooting stage. Those advantages include: 1) elimination of *in vitro* rooting, 2) increased survival rates, and 3) prevention of hyperhydricity that can result from high multiplication rates (Kim and De Hertogh, 1997). Furthermore, these organs are usually resilient and can be stored or planted when desired. Chen et al. (2000) studied the tuber formation of *Zantedeschia elliottiana* Spreng cv. Super Gold using three size ranges (7–10, 4–7, and <4 mm shoot diameter) of *in vitro* plantlets in *ex vitro* conditions. For this purpose, *in vitro* plantlets were acclimated in pots with soil beds. Results showed that larger *in vitro* plantlets (7-10 mm shoot diameter) could produce the highest number, dry weight, and tuber growth. Also, Jao et al. (2005) studied the effect of light treatments on tuber formation of *Zantedeschia jucunda* 'Black Magic' using plantlets *in vitro* in the greenhouse. The natural rate (*ex vitro*) of tuber formation is low in calla lily; therefore, conventional tuber propagation techniques take a long time. There are several reports of the production of storage organ in some of the bulbous ornamental plants via tissue culture such as *Gladiolus primulinus* (Sinha and Roy, 2002), *Narcissus asturiensis* (Santos et al*.,* 2002), *Lilium ledebourii* (Azadi and Khosh-Khui, 2007), and *Tulipa tarda* Stapf. (Małgorzata and Bach, 2014), but to the best of our knowledge, production of tuber via tissue culture was not reported in calla lily.

In this study, we developed an efficient *in vitro* propagation protocol in *Zantedeschia* spp cv. Orania and Sunclub for rapid and high-quality production. Moreover, to the best of our knowledge *in vitro* tuber productions were introduced for the first time in calla lily *in vitro* culture.

Materials and Methods

Plant materials and treatments

The tubers of two cultivars of pot calla lily (*Zantedeschia* spp cv. Orania and Sunclub) at the end of the dormancy period (end of summer) were prepared from a greenhouse. The tubers were cut into small parts (2 cm^2 diameter) and washed thoroughly under running water. Mother tuber as explant is shown in Fig. 1A, F.

Small parts of tuber as explants were sterilized with a hot water bath in different

temperatures including 30, 35, 40, 45, and 50 °C for 30 or 35 min. Then, these explants were treated with 70% ethanol for 30 s, and 1% sodium hypochlorite solution (NaOCl) for 10 min and eventually rinsed three times with sterilized distilled water under laminar air flow cabinet. These explants were placed on the Murashige and Skoog (MS) medium without plant growth regulators (PGRs) for 30 days to establishment of explants.

The developed shoots derived from the buds were transferred to the shoot proliferation medium supplemented with 6-Benzylaminopurine (BAP) (0, 1.0, 1.5, 2.0, 2.5, and 3.0 mg L^{-1}), Kinetin (Kin) (0, 1.0, and 1.5 mg L^{-1}) and 3% sucrose (PGRs combinations shown in Table 2). The shoot number and length were calculated after 30 days. In the present study, after the proliferation stage, half of the plants were planted in the medium for root induction, and half of them entered the tuber production stage.

After 30 days the *in vitro* shoots (at least 60-80 mm) were transferred to root induction medium containing Indole-3-acetic acid (IAA) (0, 0.25, 0.5 and 1.0 mg L^{-1}) and Kin (0, 0.05 and 0.1 mg L^{-1}). The rooting percentage, number and length of root were calculated after 30 days.

To produce the tubers, the plantlets were placed on a MS medium containing different concentrations of Indole-3-butyric acid (IBA) (0, 0.5, 1 and 2 mg L^{-1}) and sucrose (0, 3, and 4 and 6%).

The rooted and tuberous plantlets were removed from the culture medium and agar. Then they were transferred to a pot containing sterile cocopeat: perlite (1:1) in the greenhouse (26 ± 2 °C), separately which on top of them was a plastic cover for moisture retention. Irrigation was performed at two days intervals. After two weeks, the plastic cover was gradually removed. Adapted plantlets were transferred to bigger pots.

For all media, 7 g L^{-1} agar was used, and the pH was adjusted to 5.8. In this study, all hormones, sucrose, and plant agar were prepared from Duchefa Biochemic B.V. Haarlem, The Netherlands. Growth chamber conditions were 16h photoperiod (60 μmol m^{-2} s^{-1}) under cool white fluorescent lamps with a temperature of 26±2 °C for maintenance of media.

Statistical analysis

The experiment was conducted in a completely randomized design with three replications. Data analyzed by Duncan's test by using SAS 9.1 program.

Results

Effect of hot water bath treatments on disinfection of explants

The results showed that increasing the temperature from 30 to 45 °C reduced the infections in tubers of both cultivars, and the highest amount of disinfection was obtained in 45 °C for 35 min (91.66% and 93.33% in cv. Orania and Sunclub, respectively) (Fig. 1 B, G). The lack of positive effect of higher temperatures can be due to the negative effect of high temperature on the survival of explants, therefore, the lowest survival was observed at 50 °C (00.00% in both cultivars) (Table 1).

Effects of PGRs on proliferation

The response of explants was different on various combinations of BAP and Kin. Based on our results, the highest rate of shoot proliferation (with an average number of 15.33 and 14.32 in cv. Orania and cv. Sunclub, respectively) was observed in the MS medium supplemented with BAP (2.5 mg L^{-1}) + Kin (1.5 mg L^{-1}) (Table 2). Our results showed that the rate of proliferation improved by five times compared to previous researches (Fig. 1 C, H). The highest shoot lengths (with an average of 94.00 mm and 95.20 mm in cv. Orania and Sunclub, respectively) were obtained in medium supplemented with BAP (3 mg L^{-1}) + Kin (1.5 mg L^{-1}) (Table 2).

Table 1. Effect of hot water bath on disinfection and survival of *Zantedeschia* spp cv. Orania and Sunclub tubers after 30 days

Hot water bath		Disinfection [%]		Survival [%]	
Temperature [˚C]	Time [min]	Orania	Sunclub	Orania	Sunclub
30	30	8.30 ± 0.50 g	11.66 ± 0.66 g	96.66 ± 3.3 a	93.33 ± 2.33 a
30	35	18.33 ± 0.57 f	21.66 ± 1.77 f	93.33 ± 2.84 a	90.00 ± 2.96 a
35	30	30.00 ± 0.88 e	31.66 ± 1.09 e	96.66 ± 2.0 a	93.33 ± 1.23 a
35	35	35.00 ± 1.20 de	38.33 ± 1.00 d	93.33 ± 2.51 a	90.00 ± 2.08 a
40	30	38.33 ± 1.58 cd	45.00 ± 1.15 c	83.33 ± 2.90 b	73.33 ± 3.17 b
40	35	43.33 ± 1.52 c	48.33 ± 1.06 bc	76.66 ± 3.0 b	73.33 ± 2.18 b
45	30	50.00 ± 0.10 b	53.33 ± 1.33 b	66.67 ± 1.92 c	60.00 ± 2.72 c
45	35	91.66 ± 1.45 a	93.33 ± 1.76 a	66.67 ± 1.52 c	60.00 ± 2.00 c
50	30	00.00 h	00.00 h	00.00 e	00.00 e
50	35	00.00 h	00.00 h	00.00 e	00.00 e

In each column values with the same letters are not significantly different at 5% level using Duncan's multiple range tests. ± Standard Error (SE).

Table 2. Effect of different concentrations of 6-Benzylaminopurine (BAP) and Kinetin (Kin) on proliferation of *Zantedeschia* spp cv. Orania and Sunclub after 30 days

PGRs [mg L^{-1}]		Average shoot number/explant		Average shoot length [mm] /explant	
BAP	Kin	Orania	Sunclub	Orania	Sunclub
0.0	0.0	0.0 h	0.0 i	0.0 j	0.0 j
1.0	0.0	2.62 ± 0.33 g	3.66 ± 0.33 h	43.9 ± 0.20 i	49.5 ± 0.23 i
1.5	0.0	3.67 ± 0.33 g	4.02 ± 0.8 h	56.6 ± 0.35 hi	62.3 ± 0.31 gh
2.0	0.0	5.33 ± 0.33 f	6.33 ± 0.31 g	60.0 ± 0.06 gh	65.1 ± 0.09 gh
2.5	0.0	8.33 ± 0.66 cd	9.00 ± 0.33 c-e	78.2 ± 0.57 c-f	83.8 ± 0.56 b-d
3.0	0.0	9.00 ± 0.01 cd	8.33 ± 0.34 d-f	72.7 ± 0.65 d-g	76.8 ± 0.60 d-f
1.0	1.0	3.00 ± 0.57 g	3.66 ± 0.57 h	56.0 ± 0.26 hi	60.4 ± 0.24 h
1.5	1.0	5.64 ± 0.66 f	6.33 ± 0.52 g	67.0 ± 0.30 e-h	72.6 ± 0.31 e-g
2.0	1.0	5.66 ± 0.34 f	7.02 ± 0.32 fg	71.5 ± 0.07 d-g	76.7 ± 0.08 d-f
2.5	1.0	9.00 ± 0.56 cd	10.00 ± 0.66 cd	86.6 ± 0.56 a-c	90.1 ± 0.42 a-c
3.0	1.0	9.33 ± 0.32 c	10.33 ± 0.88 c	83.8 ± 0.65 a-d	86.0 ± 0.42 a-d
1.0	1.5	3.32 ± 0.012 g	4.32 ± 0.61 h	64.6 ± 0.26 f-h	70.1 ± 0.25 f-h
1.5	1.5	6.67 ± 0.61 ef	8.00 ± 0.51 e-g	75.3 ± 0.31 c-f	80.9 ± 0.25 c-e
2.0	1.5	7.66 ± 0.62 de	9.03 ± 0.57 c-e	80.0 ± 0.05 b-e	84.8 ± 0.06 a-d
2.5	1.5	15.33 ± 0.87 a	14.32 ± 0.33 a	92.0 ± 0.64 ab	92.8 ± 0.31 ab
3.0	1.5	11.66 ± 0.31 b	12.34 ± 0.34 b	94.0 ± 0.47 a	95.2 ± 0.21 a

In each column values with the same letters are not significantly different at 5% level using Duncan's multiple range tests. ± Standard Error (SE).

Effects of PGRs on root characteristics

In all treatments root successfully produced (Fig. 1 D, I); however, concentrations of IAA significantly affect root characteristics. The maximum rooting percentage (100% in both cultivars), root number (with an average number of 4.00 and 3.03 in cv. Orania and Sunclub, respectively) were obtained in MS medium + 0.5 mg L^{-1} IAA + 0.1 mg L^{-1} Kin. The highest root length (with an average of 120.0 and 106.6 mm in cv. Orania and Sunclub, respectively) was observed on the MS medium supplemented with 1.0 mg L^{-1} IBA + 0.1 mg L^{-1} Kin (Table 3).

Effects of PGRs on tuber production

After the proliferation stage, half of the plantlets were transferred to the tuber production medium. After 40-50 days, the tubers were formed from the base of the plantlets (Fig. 1 E, J). The results showed that sucrose and PGRs concentrations were effective in tuber formation. The highest number of tubers (with an average of 6.66 and 5.21 in cv. Orania and Sunclub, respectively) was obtained in MS medium supplemented with 2 mg L^{-1} IBA + 4% Sucrose. But the highest fresh (with an average of 2705 and 2443 mg in cv. Orania and Sunclub, respectively) and dry weight (with an average of

948.33 and 851.33 mg in cv. Orania and Sunclub, respectively) of tubers were detected in MS medium supplemented with 2 mg L^{-1} IBA + 6% sucrose (Table 4).

Table 3. Effect of Indole-3-acetic acid (IAA) and Kinetin (Kin) on root characteristics of *Zantedeschia* spp cv. Orania and Sunclub after 30 days

PGRs [mg L^{-1}]		Rooting [%]		Average root number/explant		Average root length [mm] /explant	
IAA	Kin	Orania	Sunclub	Orania	Sunclub	Orania	Sunclub
0.0	0.0	30 ± 0.23 e	20 ± 0.14 e	2.00 ± 0.33 d	10 ± 0.57 e	33.3 ± 2.4 c	23.3 ± 1.17 d
0.25	0.0	50 ± 0.31d	50 ± 0.28 d	2.00 ± 0.33 d	1.33 ± 0.33 d	40.0 ± 1.3 c	32.0 ± 2.02 cd
0.50	0.0	80 ± 0.42 c	50 ± 0.33 d	3.26 ± 0.87 bc	2.00 ± 0.32 cd	58.0 ± 1.84 bc	43.3 ± 0.66 b-d
1.00	0.0	85 ± 0.23 bc	80 ± 0.13 c	3.00 ± 0.57 c	2.66 ± 0.86 bc	60.2 ± 3.38 a-c	54.3 ± 1.51 a-c
0.25	0.05	90 ± 0.10 b	90 ± 0.34 b	2.66 ± 0.01	1.69 ± 0.33 d	69.0 ± 3.03a-c	62.3 ± 2.84 cd
0.50	0.05	90 ± 0.17 b	90 ± 0.56 b	3.66 ± 0.33 ab	3.00 ± 0.88 ab	73.0 ± 3.18 a-c	70.0 ± 3.19 a-c
1.00	0.05	93 ± 0.20 ab	95 ± 0.68 ab	3.30 ± 0.33 b	3.13 ± 0.98 ab	83.3 ± 3.17 ab	73.3 ± 3.74 ab
0.25	0.10	93 ± 0.10 ab	95 ± 0.96 ab	2.66 ± 0.88	1.66 ± 0.31 d	97.9 ± 2.88 a-c	81.3 ± 2.18cd
0.50	0.10	100 ± 0.0 a	100 ± 0.0 a	4.00 ± 0.57 a	3.03 ± 0.66 a	103.6 ± 2.40 a-c	100.0 ± 2.21 a-c
1.00	0.10	100 ± 0.0 a	95 ± 0.87 ab	3.66 ± 0.03 ab	2.64 ± 0.57 bc	120.0 ± 3.17 a	106.6 ± 3.18 a

In each column values with the same letters are not significantly different at 5% level using Duncan's multiple range tests. ± Standard Error (SE).

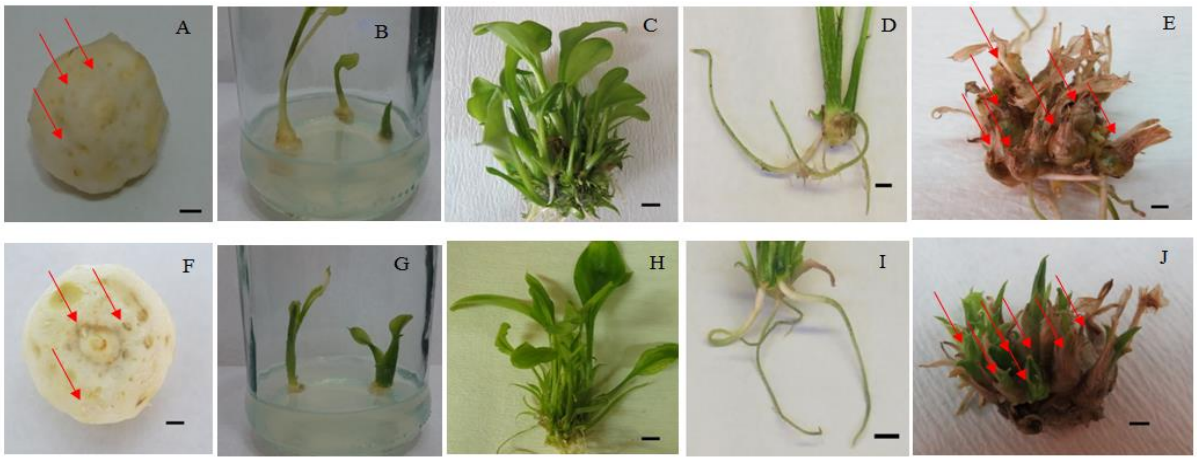

Fig. 1. Different stages of micropropagation in *Zantedeschia* spp: Calla lily mother tuber. Red arrows show some of the buds (A, F), explants cultured in MS medium (B, G), proliferation (C, H), rooted plants (D, I), and tuber production. Red arrows show some of the tubers (E, J), A-E) cv. Orania, F-J) cv. Sunclub. Bar = 10 mm

Table 4. Effect of Indole-3-butyric acid (IBA) and sucrose on tuber characteristics of *Zantedeschia* spp cv. Orania and Sunclub after 40-50 days

Treatments		Tuber number		Average fresh weight [mg]		Average dry weight [mg]	
IBA [mg L^{-1}]	Sucrose [%]	Orania	Sunclub	Orania	Sunclub	Orania	Sunclub
0.0	0	0.0 f	0.0 f	0.0 d	0.0 d	0.0 e	0.0 g
0.5	3	0.66 ± 0.01 ef	0.30 ± 0.2 ef	632.0 ± 32.1 c	596.3 ± 63.1 c	100.0 ± 12.4 e	65.67 ± 22.5 fg
1.0	3	2.0 ± 0.09 d	1.31 ± 0.33 d	1891.0 ± 21.5 b	1784.2 ± 21.5 b	296.67 ± 26.03 d	193.31 ± 45.7 ef
2.0	3	1.66 ± 0.33 de	1.00 ± 0.23 de	1834.3 ± 79.3 b	1856.0 ± 31.7 ab	462.67 ± 51.7 cd	358.00 ± 26.6 cd
0.5	4	2.33 ± 0.56 d	1.33 ± 0.13 d	1877.3 ± 63.1 b	1813.0 ± 25.1 ab	322.0 ± 23.0 d	217.67 ± 31.8 d-f
1.0	4	4.66 ± 0.66 bc	3.67 ± 0.32 b	2183.3 ± 92.7 ab	2019.5 ± 31.8 ab	530. 0 ± 30.5 c	427.33 ± 31.8 c
2.0	4	6.66 ± 0.33 a	5.21 ± 0.66 a	2250.3 ± 28.8 ab	2209.7 ± 92.7 ab	721.67 ± 95.05 b	619.32 ± 94.08 b
0.5	6	3.66 ± 0.12 c	2.64 ± 0.57 c	2260.0 ± 96.3 ab	1953.0 ± 49.3 ab	368.0 ± 31.9 cd	264.67 ± 33.1 de
1.0	6	5.33 ± 0.41 b	4.00 ± 0.56 b	2310.0 ± 55.07 a	2199.8 ± 58.1 ab	713.0 ± 60.5 b	607.00 ± 56.4 b
2.0	6	6.06 ± 0.12 a	4.96 ± 0.66 a	2705.3 ± 92.92 a	2443.0 ± 65.5 a	948.33 ± 51.6 a	851.33 ± 64.9 a

In each column values with the same letters are not significantly different at 5% level using Duncan's multiple range tests. ± Standard Error (SE).

Hardening and acclimatization

Transferring *in vitro* propagated plantlets to the *ex vitro* environment is a critical part of the propagation process. If this stage is successful, financial losses can be minimized. However, substantial numbers of micropropagated plants do not survive the transfer from the *in vitro* environment to the greenhouse. Plantlets grow under very special *in vitro* conditions. For example, the use of closed vessels creates high humidity and limits the inflow of CO_2 and outflow of gaseous plant products from the vessels. *In vitro* plants use sucrose as energy sources constantly which leads to decreased water potential of the medium and increases the risk of bacterial and fungal contamination. After transfer to the greenhouse conditions, the plantlets have to recover themselves from these properties (Pospisilova et al., 1999). Because the greenhouse has considerably lower relative humidity, higher light levels and septic conditions that are hazardous to micropropagated plants compared to the *in vitro* environment (Janick 2007). In the present study, the rooted and tuberous plants were transferred to controlled conditions in the greenhouse, separately (Fig. 2). The infection to pathogens and disease was lower in tuberous plants than rooted plants in the greenhouse (less than 5%), following that the survival percentage of tuberous plants was higher than rooted plants after two weeks in the greenhouse (data not shown).

Fig. 2. Adaptation of *in vitro* rooted plants (Top) and tuberous plants (Bottom) of Calla lily in greenhouse condition

Discussion

The primary disinfection of explants is the first and most important step in *in vitro* culture (Bairu and Kane, 2011). *In vitro* pathogens are very serious and most significant problems in the micropropagation process. The contamination of the explants in the culture medium causes plant tissue culture to fail at the first stage (Altan et al., 2010). In general, the success of the plant tissue culture protocols is based on explants disinfection, and therefore, determining the concentration of disinfectants and the duration of disinfection concerning the type of explant is crucial (Habiba et al., 2002). The critical factor for the successful cultivation of Araceae family plants is the elimination of infections, which are caused by the activity of the endogenous bacteria in *Zantedeschia* tuber (Kritzinger et al., 1998). Elimination of these internal contaminants can be challenging (Chang et al., 2003). In this research, using a hot water bath (45 ºC for 35 min) and soaking in hypochlorite sodium (1% concentration for 10 min), efficient disinfection was detected in more than 90% of the explants. The advantage of hot water bath treatment is the absence of chemical wastes and the elimination of internal pathogens in comparison with chemical treatments (Langens-Gerrits et al., 1997).

The effectiveness of BAP for the multiplication of calla lily has been confirmed by Chang et al. (2003) in *Z. albomaculata* (with an average number of 3.8), Koech et al. (2005) from *Z. albomaculata* 'Black Magic' (with an average number of 2.5), Kozak and Stelmaszczuk (2009) in *Zantedeschia aethiopica* 'Green Goddess (with an average number of 2.4), and Kulpa (2016) in *Zantedeschia rehmannii* (with an average number of 4.13). In some reports, the highest rates of multiplication was obtained with different concentrations of 2IP (Ebrahim, 2004; El-Shamy et al., 2009).

The lack of proper rooting affects the adaptation of plantlets negatively (Hazarika et al., 2006). It has been proved that a high concentration of auxins has an inhibitor effect and decreases the number of roots (Baker and Wetzstein, 2004). It seems that calla lily has a suitable rooting potential with the proper level of internal auxin, and shoots can be rooted in the control medium (non-PGR medium), But we increased the root number and length by using IAA + Kin, successfully.

The importance of *in vitro* bulb or tuber induction has been emphasized in previous reports (Askari et al., 2018). Sucrose is an essential nutrient that is added to the medium for the induction of tuber in *in vitro* conditions, and it is an appropriate carbon source that is easily absorbed by the plant and converted into starch (Yu et al., 2000). The use of low concentrations of sucrose is not sufficient for the formation and growth of the tuber. On the other hand, high sucrose concentration increases the osmotic pressure of the medium and causes imbalances in pH and finally reduces the yield (Altindal and Karadogan, 2010). Therefore, it is essential to select a suitable concentration of sucrose to produce the tuber. A positive effect of carbohydrates on the growth of bulblets has been reported in lilium (Mojtahedi et al., 2014)

Conclusion

Increasing production and reducing injury and disease are crucial goals in the flower and ornamental industry. Mass propagation of plants through tissue culture has adopted at a commercial level that used for the commercial production of desired varieties to compete in the international markets. In this study, an efficient protocol for micropropagation of calla lily was introduced. The combination of 2.5 mg L^{-1} BAP + 1.5 mg L^{-1} Kin produced the highest proliferation rate. Moreover, for the first time *in vitro* tuber production of call lily was reported here. The highest number of tuber production was observed in MS medium supplemented with 2 mg L^{-1} IBA + 4% Sucrose. *In vitro* tuber production has

particular advantages, including the potential of high replication rate (at least six tubers per cultured tuber) that make production completely economical, and storage tuber can be easily used, transported and stored. Moreover, they do not need an adaptation after translocation to the soil.

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Conflict of Interest

The authors indicate no conflict of interest for this work.

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