Journal of Ornamental Plants
https://sanad.iau.ir/en/Journal/jornamental
ISSN (Print): 2821-0093 ISSN (Online): 2783-5219

Research Article
Volume 15, Number 1: 37-46, March, 2025
https://doi.org/10.71645/jop.2025.140311161198569

Enhancing Callus Growth in Calla Lily (*Zantedeschia* 'Sun Club') through Fipexide, Activated Charcoal, and Ascorbic Acid in *In Vitro* Cultivation

Hassan Abedini Aboksari[1], Pejman Azadi[2*], Mohammad Hossein Azimi[3*], Sepideh Kalatejari[1], Azam Borzouei[4]

[1]Department of Horticultural Science and Agronomy, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2]Department of Genetic Engineering and Biosafety, Agricultural Biotechnology Research Institute of Iran (ABRII), Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran
[3]Ornamental Plants Research Center (OPRC), Horticultural Sciences Research Institute (HSRI), Agricultural Research, Education and Extension Organization (AREEO), Mahallat, Iran
[4]Agriculture Research School, Nuclear Science and Technology Research Institute, PO Box 31485-498, Karaj, Iran

Received: 04 February 2025 **Accepted:** 27 March 2025

***Corresponding author's email:** azadip22@gmail.com, m.h.azimi58@gmail.com

The potted calla lily (*Zantedeschia* spp.) is a popular ornamental plant in the global flower market. The most important problem of calla lily in indirect organogenesis is callus production, low durability and growth of callus due to the phenolic and alkaloidal compounds present. For this purpose, an experiment was conducted to investigate the effect of fipexide (FPX) compared with activated charcoal and ascorbic acid in the culture medium to improve the quality of the callus of the potted calla lily (*Zantedeschia* 'Sun Club'). FPX is one of the chemical compounds used in the pharmaceutical industry. Recently, for *in vitro* conditions FPX was used in the culture media to improve the quality of different stages of plant growth. A factorial experiment in the form of a completely randomized design including FPX at concentrations of 0, 15 and 30 µmol L^{-1}, activated charcoal (0 and 1 g L^{-1}) and ascorbic acid at concentrations of 0 and 2 g L^{-1}, including 12 treatments, 3 replications, 12 samples for each treatment and in the collection with 192 callus samples were implemented. In this research, callus diameter, callus fresh weight, callus growth index and healthy callus percentage were evaluated. The results showed that, FPX treatment, especially at a concentration of 15 µmol L^{-1} with activated charcoal, had the greatest effect on the examined traits such as callus diameter (15.98 mm), healthy callus percentage (90%) compared to the control samples (with an average of 7.36 mm and 54 % respectively). Totally, FPX can be used as an effective and alternative compound in the callus growth medium to increase quality and performance.

Keywords: Crown calli, Geophyte, Ornamental plant, Pot plant, Plant tissue culture.

INTRODUCTION

Callus is used as an important tool for some genetic studies, for example, mutation and gene transfer (Efferth, 2018). Today, many plant researches have focused on callus production under *in vitro* conditions, which is influenced by nutrients and plant growth regulators (Nalousi *et al*., 2019; Yu *et al.*, 2021; Chutipaijit and Sutjaritvorakul, 2018). However, the use of the organogenesis method requires callus from many plants cultured in vitro, which can be associated with browning of the explant and production of phenolic compounds (Corduk and Cuneyt Aki, 2011). Browning and possible death of plant tissues during the initial stages of plant tissue culture remains one of the persistent problems (Gerema and Emiru, 2021). Browning occurs through high activities of polyphenol oxidase, peroxidase, and other enzymes that are triggered for their defense in response to wounding (Onuoha *et al*., 2011). When cells are wounded during cutting, with temperature fluctuations and aging of the sample, the browning reaction begins depending on the plant sample's nature. One of the key factors in callus production, preservation, and use is maintaining color stability and callus quality (Pan and Staden, 1999; Onay *et al*., 1996). Browning in tissue culture samples may be dependent on species, cultivar, developmental stage, physiological condition, tissue type, explant size, and age (Mohd Din *et al*., 2016; Ozyigit, 2009). This may decrease the propagation rate, regeneration rate, and viability of plant samples (Parthasarathy *et al*., 2007).

One of the significant problems in producing callus from some plant species involves the browning effect caused by exudation of phenolic compounds into the culture medium, followed by the deterioration and poor quality of the sample (Karolina *et al*., 2024). This could be part of the plant response due to factors such as plant variety, injury, or other factors like physiological aging or fluctuation in environmental conditions (Dias *et al.*, 2016).

In some plant species like calla lily, tissue culture callus samples are highly sensitive and produce a significant amount of phenolic compounds (Nery *et al.*, 2015; Kulpa, 2016; Xuan *et al.*, 2023). Over the past few years, the potted calla lily (*Zantedeschia* spp.) has been one of the most popular tuberous flowers in demand throughout the world. It possesses many cultivars and plenty of colors. It is grown and traded as one of the most popular ornamental plants in several regions all over the world (Xuan *et al*., 2023).

To reduce browning, substances like activated charcoal, ascorbic acid, PVP (polyvinylpyrrolidone) and others are used to absorb the released compounds or prevent quality degradation of callus samples due to their antioxidant properties (Thomas, 2008). Activated charcoal is a tasteless material with an excellent porous system and large internal surface areas that eliminate all non-carbon impurities. This chemical is used for the absorption of phenolic substances that could cause browning of the explants and the culture medium (Huang *et al.*, 2007). Other chemicals that might be used to maintain quality and prevent excessive phenolic compounds secretion from the callus are antioxidants such as ascorbic acid (Giri *et al.*, 2012).

Numerous reports have demonstrated the use of activated charcoal and ascorbic acid in in vitro conditions to preserve the quality of plant samples against damage caused by phenolic compounds produced by callus (Thomas, 2008; Chaabani *et al.*, 2015; Fitriana *et al*., 2019; Chutipaijit and Sutjaritvorakul, 2018). Fipexide is a phytochemical compound which has also served use in the pharmaceutical industry in the treatment of patients who have been suffering from dementia and Alzheimer disease (Missale *et al*., 1983). Recent studies have revealed that this compound induces callus growth and maintains its quality. Nakano *et al.* (2018) reported that FPX acts as a new bioactive compound in the formation and growth of callus in plants. Similarly, Yoshiki *et al.* (2022) conducted a study on the ornamental plant *Matthiola incana*, reporting that the use of FPX in the culture medium enhanced callus formation and quality.

Given the high production rate of phenolic compounds in calla lily, which may influence callus production and quality under in vitro conditions, this study aimed to investigate the effects of FPX—a novel compound—on callus-related indicators and quality. Additionally, we examined the role of ascorbic acid and activated charcoal in relation to the quantitative and qualitative performance of callus in the ornamental potted calla lily (*Zantedeschia* 'Sun Club') under controlled *in vitro* conditions.

MATERIALS AND METHODS

The experiment was conducted in the Plant Tissue Culture Laboratory, National Ornamental Plant Research Institute of Iran, Mahallat city. Plant materials used in this study were the ornamental pot calla lily (*Zantedeschia* spp. cv 'Sun Club'). Freshly formed callus masses, derived from tissue-cultured microtubers of the calla lily plant with a diameter of 6 mm and of similar size, were used for the experiment.

For the experiment, different treatments were applied as outlined in table 1 using the MS medium (Murashige and Skoog, 1962). The treatments included different concentrations of activated charcoal (A0 and 1 g L^{-1}), ascorbic acid (0 and 2 g L^{-1}) and fipexide (0, 15, and 30 µM L^{-1} immediately after inoculation, the treated callus samples were transferred to a growth chamber with 16 hours of light (60 µmol m^{-2} s^{-1}) provided by white fluorescent lamps at a temperature of 23 ± 2°C for 14 days.

Table 1. Different concentrations of fipexide, ascorbic and activated charcoal on the callus parameters of calla lily (*Zantedeschia* 'Sun Club').

Treatments code	Activated charcoal (g/L)*	Ascorbic acid (g/L)	Fipexide (µmol/L)
T_1	-	-	-
T_2	-	-	15
T_3	-	-	30
T_4	-	2	-
T_5	-	2	15
T_6	-	2	30
T_7	1	-	-
T_8	1	-	15
T_9	1	-	30
T_{10}	1	2	-
T_{11}	1	2	15
T_{12}	1	2	30

*1 mol activated charcoal (AC)= 12.01 g, 1 mol ascorbic acid (AA)= 176.124 g, 1 mol fipexide (F)= 388.85 g

In this experiment, the measured traits included callus diameter, fresh callus weight, callus growth index, callus survival percentage, and callus regeneration percentage. Callus diameter was measured using a digital caliper, fresh callus weight using a digital scale, and the callus growth index and healthy callus percentage were calculated using the following formulas.

$$\text{Formula (1): Cell growth index} = \frac{\text{Callus diameter at the beginning of the experiment} - \text{The final diameter of the callus}}{\text{Callus diameter at the beginning of the experiment}} \times 100$$

$$\text{Formula (2): Percentage of healthy callus} = \frac{\text{Number of healthy calli}}{\text{The number of calluses at the begining of the expripent}} \times 100$$

This study was conducted as a factorial experiment based on a completely randomized design with a total of 12 treatments, each with 3 replications and 12 samples per replication. Statistical analysis of the data was performed using SAS 9.4 software, and the mean comparisons were done using Duncan's multiple range test. The charts were drawn using Excel software.

RESULTS

The effects of different treatments on the measured traits were evaluated after 14 days. Based on the results, it appears that among the three examined factors, the treatments containing FPX were the most effective on the measured indices.

Callus diameter

According to the results presented in Fig. 1 and 6, the treatment with 15 µM L^{-1} FPX combined with 1 g L^{-1} activated charcoal ($AC_1.AA_0.F_1$) had the greatest effect on callus size, with an average diameter of 15.98 mm, compared to the other treatments. However, no significant difference was observed between the $AC_1.AA_0.F_1$ and the $AC_1.AA_0.F_2$ treatment (15 µM L^{-1} FPX + 1 g L^{-1} activated charcoal), which shared the same average diameter of 15.98 mm. Furthermore, the application of 2 g L^{-1} ascorbic acid alone ($AC_0.AA_1.F_0$) gave a higher value in terms of callus diameter, with an average of 9.86 mm, compared to the application of 1 g L^{-1} activated charcoal ($AC_1.AA_0.F_0$), which gave a mean of 6.87 mm in the culture medium. However, in combination with FPX, the factor of activated charcoal ($AC_1.AA_0.F_1$= 15.98 and 15.68= $AC_1.AA_0.F_2$) had a higher effect on this trait compared to ascorbic acid (11.75= $AC_0.AA_1.F_1$ and 11.73= $AC_0.AA_1.F_2$). Also, in those two levels (15 and 30 µM L^{-1}) of application of FPX alone in the culture medium ($AC_0.AA_0.F_1$ and $AC_0.AA_0.F_2$), it is more effective on callus diameter compared to 2 g L^{-1} ascorbic acid ($AC_0.AA_1.F_0$) or 1 g L^{-1} activated charcoal ($AC_1.AA_0.F_0$) alone (11.75= $AC_0.AA_0.F_1$ and 11.53= $AC_0.AA_0.F_2$). Moreover, the use of all three factors in the culture medium, at both levels of FPX (12.86= $AC_1.AA_1.F_1$ and 13.12= $AC_1.AA_1.F_2$), had a greater effect compared to the use of ascorbic acid with different levels of FPX ($AC_0.AA_0.F_1$ and $AC_0.AA_0.F_2$), but less effect compared to the use of 1 g L^{-1} activated charcoal with both levels (15 and 30 µM L^{-1}) of FPX ($AC_0.AA_0.F_1$ and $AC_0.AA_0.F_2$).

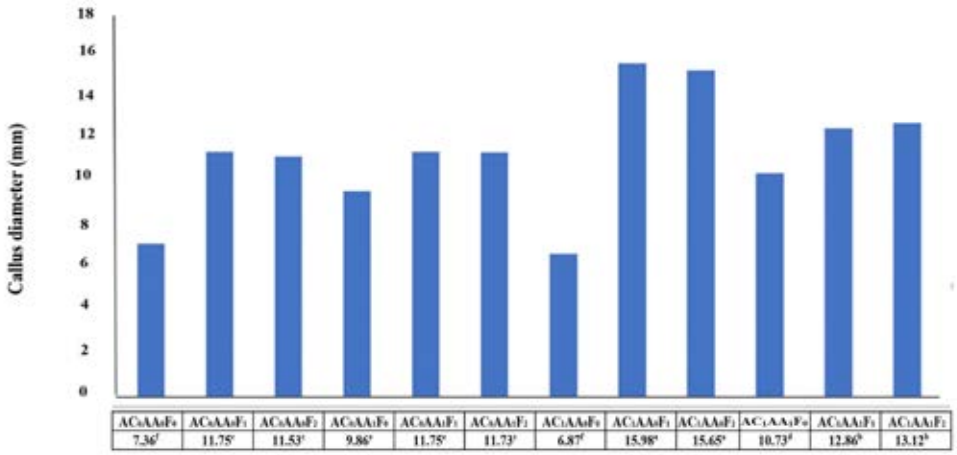

Fig. 1. The effect of different treatments of fipexide, ascorbic acid and actived charcoal on the callus diameter of calla lily (*Zantedeschia* 'Sun Club').

Callus weight

Based on the result, the treatment with 15 Mm L^{-1} FPX in combination with 1 g L^{-1} activated charcoal was the highest effect for callus weight with an average of 284 mg as compared with other treatments. Similar to callus diameter, FPX treatments at both concentrations combined with 1 g L^{-1} activated charcoal ($AC_1.AA_0.F_1$= 284 mg and $AC_1.AA_0.F_2$= 276 mg) had a more significant effect than when used with 2 g L^{-1} ascorbic acid ($AC_0.AA_1.F_1$= 223 mg and $AC_0.AA_1.F_2$=219 mg) in the culture medium. Even when all three factors were used in the culture medium ($AC_1.AA_0.F_1$= 284 mg and $AC_1.AA_0.F_2$= 276 mg), this resulted in higher callus weight (Fig. 2).

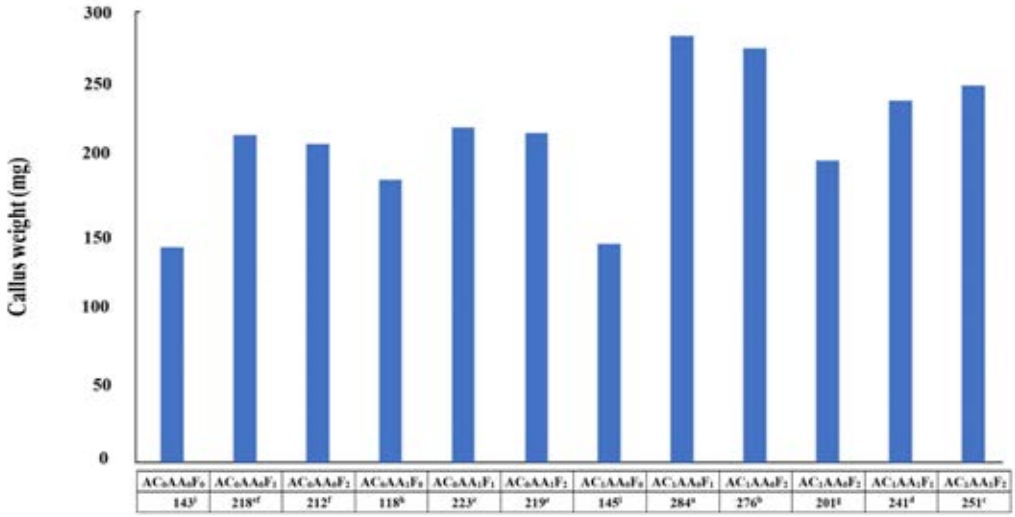

Fig. 2. The effect of different treatments of fipexide, ascorbic acid and actived charcoal on the callus weight of calla lily (*Zantedeschia* 'Sun Club').

Callus growth index

The term cell growth index has been used to determine the magnitude of callus growth from the initiation of the culture up to the end of the experiment. Based on the analyzed results, it appears that the $AC_1.AA_0.F_1$ treatment had a strong correlation with the evaluated traits, as all traits showed significant relationships with each other. According to the results in Fig. 3 and 6, treatments that included FPX (15 and 30 µM L^{-1}) showed a notable performance for this trait compared to other treatments. Treatments $AC_1.AA_0.F_1$ and $AC_1.AA_0.F_2$ demonstrated significant increases in the growth index, with averages of 136 and 130, respectively, compared to the control treatment ($AC_1.AA_0.F_2$= 19).

Percentage of healthy callus

Maintaining health without the necrosis of callus is among the main factors for callus quality. This factor has a direct impact on the division and multiplication of callus and sample regeneration, enhancing the chances of success in callus production and propagation projects, whatever the aim may be. The treatment with 15 µM L^{-1} FPX along with 1 g L^{-1} activated charcoal ($AC_1.AA_0.F_2$=90%) had the most significant effect on the health and quality of the callus. The most interesting finding within this experiment was the significant increase in callus quality while using various FPX concentrations (15 and 30 µM L^{-1}) in comparison with the control treatment (54%). According to the obtained results, it can be assumed that FPX not only reduced necrosis and increased the lifespan of callus but also had the higher effect on callus growth and size (Fig. 4 and 5).

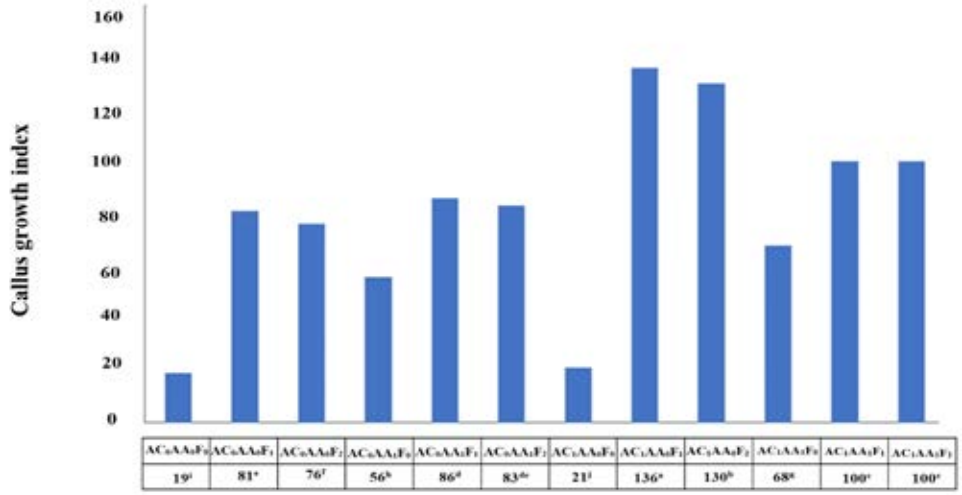

Fig. 3. The effect of different treatments of fipexide, ascorbic acid and actived charcoal on the callus growth index of calla lily (*Zantedeschia* spp. cv 'Sun Club').

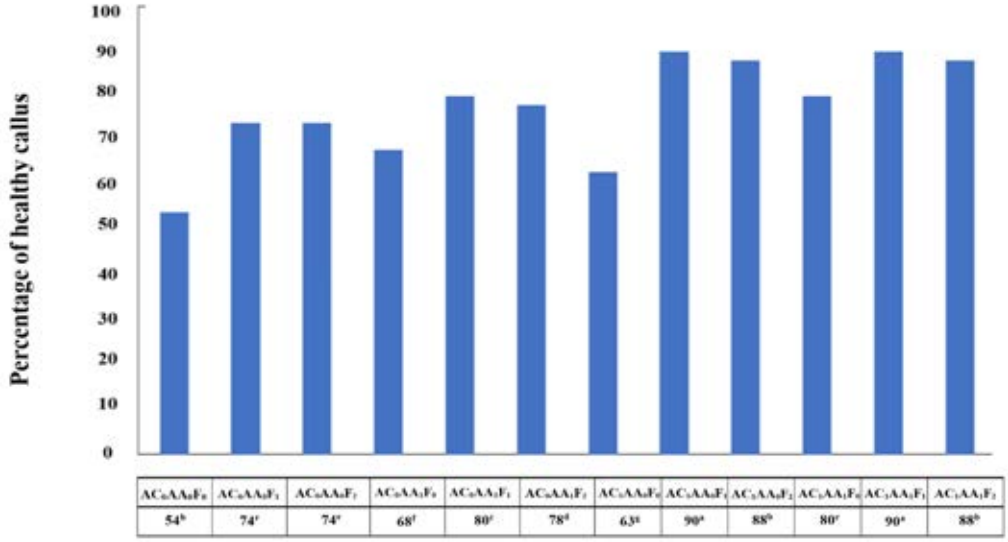

Fig. 4. The effect of different treatments of fipexide, ascorbic acid and actived charcoal on the percentage of healthy callus of calla lily (*Zantedeschia* 'Sun Club').

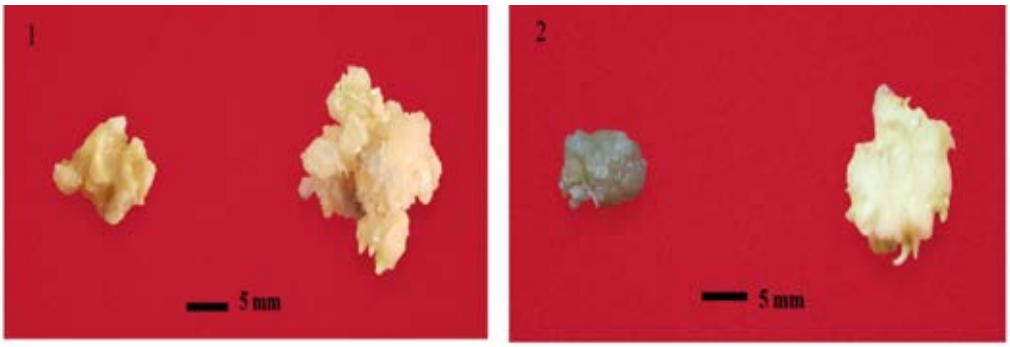

Fig. 5. (1). Callus size increase in $C_1A_0F_1$ treatment containing FPX (15 Mm L^{-1}) and activated charcoal (2 g L^{-1}) compared to the control. (2): Appearance of health and severity of necrosis of calluses, with $C_1A_0F_1$ treatment compared to control.

DISCUSSION

Based on previous research, there are numerous reports on the application of compounds like activated charcoal and ascorbic acid on the quality and growth of callus. As the results showed, ascorbic acid and activated charcoal treatments decreased browning and improved the quality of callus to some extent compared with the control; however, this difference was not significant. Due to its antioxidant properties, ascorbic acid can significantly reduce substances that negatively affect callus quality and the culture medium. Supplementation of ascorbic acid into the culture medium was reported by Amente and Chimdessa (2021) to reduce browning of callus quickly and improve their coloration. However, this activity is ceased after some time because of the loss of antioxidant activity of ascorbic acid (Huang *et al*., 2007). Elmore *et al.* (1990) did identify ascorbic acid as one of the antioxidant chemicals responsible for inhibiting the browning of plant tissues in tissue culture. Using activated charcoal in the culture medium increased callus growth and quality compared to the control, although its effects were less pronounced compared to ascorbic acid across all traits.

A striking observation of the results showed that the use of activated charcoal in combination with ascorbic acid in the culture medium proved more effective than the use of ascorbic acid alone. In addition, the treatment consisting of the interaction of activated charcoal with FPX proved more effective with regard to the characteristics evaluated in callus. Activated charcoal has a positive effect on growth as a result of its being able to adsorb phenolic compounds present in the culture medium (Chutipaijit and Sutjaritvorakul, 2018).

In a study conducted on date palm callus under tissue culture conditions, it was found that the application of activated charcoal in the culture medium, by reducing callus browning, created favorable conditions for the growth and survival of callus, and when combined with other growth-promoting substances, it had better performance on callus growth and quality (Fitriana *et al*., 2019). Activated charcoal exerts an indirect stimulatory effect by providing a better cultural condition, thus enhancing the bioactivity of other active agents in the medium (Chutipaijit and Sutjaritvorakul, 2018). However, it should be noted that in high concentrations, activated charcoal can absorb not only phenolic compounds but also other active substances in the culture medium, which may render them unavailable to the plant samples. Therefore, using the minimum effective amount of activated charcoal in the medium, along with other growth-promoting supplements, can help achieve the highest callus quality (Sakularat *et al*., 2015).

Moreover, using these compounds in high concentrations may have the opposite effect and exacerbate callus browning. Thus, selecting the optimal minimum concentration of compounds in the culture medium is crucial to achieving maximum quality (Thomas, 2008). A study showed that the combination of activated charcoal with ascorbic acid in the culture medium had a higher effect in inhibiting phenolic compounds in the culture medium and promoting the growth of plant samples compared to using each of these compounds alone (Nisyawati and Kariyana, 2013; Priyanka and Alok, 2015).

In the present work, FPX has been used as an additive for both enhancement of growth and improvement in quality of callus. Nakano *et al*. (2018) stated that FPX is an effective compound that enhances the intake of active agents in plants, thus enhancing callus induction and proliferation during tissue culture. Additionally, the researchers noted that the physiological, biochemical, morphological, and even gene expression mechanisms of plant samples in callus production with FPX differ significantly from those of plant hormones, and further studies are needed to investigate its effects on plants. Our research showed that FPX is effective in enhancing callus growth and quality of calla lily *in vitro*, acting as an effective elicitor for induction and development. Identification of FPX as an inducing molecule can add significant

understanding and also open ways for further studies related to plant science. Yoshiki *et al*. (2022) introduced FPX as a compound effective in callus growth, induction, and quality, which leads to faster callus formation and growth compared to plant hormones. It was reported by the researchers that applying FPX at a concentration of 15 µmol L^{-1} showed higher efficacy in callus growth for plants such as soybeans, tomatoes, and Matthiola incana. Furthermore, the authors indicated that higher concentrations of FPX would inhibit or reduce growth and quality-related parameters, and the degree of effect depended on plant species (Nakano *et al*., 2018; Yoshiki *et al*., 2022).

CONCLUSION

In this study investigated the effects of FPX—a novel compound—on callus-related indicators and quality. Additionally, we examined the role of ascorbic acid and activated charcoal in relation to the quantitative and qualitative performance of callus in the ornamental potted calla lily (*Zantedeschia* 'Sun Club') under controlled *in vitro* conditions. In summary, combining FPX with substances such as activated charcoal, which aids in absorbing phenolic compounds in the growth medium, can be helpful in managing problems with callus growth and browning in calla lily. The reduced effectiveness of the mixture with activated charcoal, ascorbic acid, and FPX might be because of the higher levels of these substances in the mixture, could have a lesser effect. Moreover, carrying out additional studies on this substance in plant research and determining the best concentration to enhance its effectiveness in the growth medium for different plant growth phases like callogenesis, regeneration, rooting, and gene transformation based on the specific challenges of the plant in tissue culture procedures could be beneficial for addressing hurdles in all *in vitro* culture related investigations. Our findings pave the way for significant advancements in the improvement of calla lily through the application of *in vitro* mutagenesis, genetic engineering, and genome editing techniques. By harnessing these innovative methodologies, we anticipate the development of more robust and diverse cultivars that are better suited to both commercial cultivation and ornamental use.

ACKNOWLEDGMENT

This study was supported by Ornamental Plants Research Center (OPRC).

Literature Cited

Amente, G. and Chimdessa, E. 2021. Control of browning in plant tissue culture: A review. Journal of Scientific Agriculture, 5: 67-71.

Chaabani, G., Tabart, J., Kevers, C., Dommes, J., Khan, M. I., Zaoui, S. and Karray-Bouraoui, N. 2015. Effects of 2,4-dichlorophenoxyacetic acid combined to 6-benzylaminopurine on callus induction, total phenolic and ascorbic acid production, and antioxidant activities in leaf tissue cultures of *Crataegus azarolus* L. var. Aronia. Acta Physiologiae Plantarum, 37(2): 28-37.

Chutipaijit, S. and Sutjaritvorakul, T. 2018. Application of activated charcoal and nanocarbon to callus induction and plant regeneration in aromatic rice (*Oryza sativa* L.). Chemical Speciation and Bioavailability, 30(1): 1–8.

Corduk, O. and Cuneyt Aki, N. 2011. Inhibition of browning problem during micropropagation of sideritis trojana bornm an endemic medicinal herb of Turkey. Romanian Biotechnological Letters, 16 (6): 6760-6765.

Dias, M.I., Sousa, M.J., Alves, R.C. and Ferreira, I.C.F.R. 2016. Exploring plant tissue culture to improve the production of phenolic compounds: A review. Industrial Crops and Products, 82: 9–22.

Efferth, T. 2018. Biotechnology applications of plant callus cultures. Engineering, 5: 50-59.

Elmore, H., Samples, B., Sharma, S. and Harrison, M. 1990. Influence of cultural and physiochemical factors on ascorbate stability in plant tissue culture media. Plant Cell, Tissue and Organ Culture, 20: 131-135.

Fitriana, D., Prihastanti, E., Nurchayati, Y. and Hastuti, R.B. 2019. Effect of combination explant difference leaf part and concentration of active charcoal on callus initiation mangrove (*Rhizophora apiculata* BI) by *in-vitro*. Journal of Physics Conference Series, 1217(1): 012166.

Gerema, A. and Emiru, Ch. 2021. Control of browning in plant tissue culture: A review. Journal of Scientific Agriculture, 5: 67-71.

Giri, L., Dhyani, P., Rawat, S., Bhatt, I. D., Nandi, S. K., Rawal, R. S. and Pande, V. 2012. *In vitro* production of phenolic compounds and antioxidant activity in callus suspension cultures of *Habenaria edgeworthii*: A rare Himalayan medicinal orchid. Industrial Crops and Products, 39: 1–6.

Huang, Y., Li, N., Ma, Y., Du, F., Li, F., He, X., Lin, X., Gao, H. and Chen, Y. 2007. The influence of single-walled carbon nanotube structure on the electromagnetic interference shielding efficiency of its epoxy composites. Carbon, 45 (8): 1614-1621.

Karolina, J., Pawel, K., Marta, S., Aleksandra, K., Magdalena, A., Malinowska, S.G. and Agnieszka S. 2024. Phenolic compound profiling and antioxidant potential of different types of Schisandra Henryi *in vitro* cultures. Applied Microbiology and Biotechnology, 108 (322): 108-322.

Kulpa, D. 2016. Micropropagation of calla lily (*Zantedeschia rehmannii*). Folia Horticulturae, 28(2): 181–186.

Missale, C, Pasinetti, G, Govoni, S, Spano, P.F. and Trabucchi, M. 1983. Fipexide: A new drug for the regulation of dopaminergic system at the macromolecular level. Bollettino Chimico Farmaceutico, 122 (2): 79–85.

Mohd Din, A. R. J., Iliyas Ahmad, F., Wagiran, A., Abd Samad, A., Rahmat, Z. and Sarmidi, M. R. 2016. Improvement of efficient *in vitro* regeneration potential of mature callus induced from Malaysian upland rice seed (*Oryza sativa* cv. Panderas). Saudi Journal of Biological Sciences, 23(1): S69–S77.

Murashige, T. and Skoog. F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Plant Physiology, 15 (3): 473-479.

Nakano, T., Tanaka, S., Ohtani, M., Yamagami, A., Takeno, S., Hara, N. and Shinozaki, K. 2018. FPX is a novel chemical inducer that promotes callus formation and shoot regeneration in plants. Plant and Cell Physiology, 59(8): 1555–1567.

Nalousi, A. M., Hatamzadeh, A., Azadi, P., Mohsenpour, M. and Samizadeh Lahiji, H. 2019. A procedure for indirect shoot organogenesis of *Polianthes tuberosa* L. and analysis of genetic stability using ISSR markers in regenerated plants. Scientia Horticulturae, 244: 315–321.

Nery, F. C., Goulart, V. L. A., Viol, M. A., Paiva, P. D. O., Paiva, R., Prudente, D. O. and Nery, M. C. 2015. *In vitro* geramination and chemical composition of *Zantedeschia aethiopica* callus. Acta Horticulturae, 1083: 189–196.

Nisyawati, N. and Kariyana K. 2013. Effect of ascorbic acid, activated charcoal and light duration on shoot regeneration of banana cultivar barangan (*Musa acuminata* L.) in vitro culture. International Journal of Research and Reviews in Applied Sciences, 15(1): 13-17.

Onay, A., Jeffree, C.E. and Yeoman, M.M. 1996. Plant regeneration from encapsulated embryoids and an embryogenic mass of pistachio. Plant Cell Reports, 15: 723–726.

Onuoha, I.C., Chinonye, J.E. and Chibuikem, I.N.U. 2011. *In vitro* prevention of browning in plantain culture. Online Journal of Biological Sciences, 11 (1): 13-17.

Ozyigit, L.L. 2009. In vitro shoot development from three different nodes of cotton (*Gossypium hirsutum* L.). Notulae Botanicae Horti Agrobotanici, 37(1): 74-78.

Pan, M.J. and Staden, J.V. 1999. Effect of activated charcoal, autoclaving and culture media on sucrose hydrolysis. Journal of Plant Growth Regulation, 29(3): 135-141.

Parthasarathy, V.A., Anitha K. and Rajesh, M. K. 2007. Biotechnology of coconut. Journal of Horticultural Sciences, 2(1): 1-12.

Priyanka, D. and Alok, K.S. 2015. To study the effect of activated charcoal, ascorbic acid and light duration on *in vitro* micropropagation of *Aloe vera* L. International Journal of Innovative Research in Science, Engineering and Technology, 4(5): 3131-3138.

Sakularat, S., Tiwa, R. and Sorapong, B. 2015. Influence of different type of culture media and activated charcoal on callus induction and shoot multiplication of *Cardamine lyrata*. Journal of Agricultural Technology, 11(8): 1697-1704.

Thomas, T. D. 2008. The role of activated charcoal in plant tissue culture. Biotechnology Advances, 26 (6): 618–631.

Xuan, S., Xue, W., Bijaya, Sh. S., Yin, J., Di, W., Rongxin, G., Guojun, Zh., Wenting X. and Zunzheng, W. 2023. Tissue culture of calla lily (*Zantedeschia spreng*): An updated review on the present scenario and future prospects. International Journal of Social Robotics, 92(8): 2413-2428.

Yoshiki, T., Kaho Y., Kentaro, K., Yukiko, A. and Takashi, N. 2022. Establishment of an efficient transformation method of garden stock (*Matthiola incana*) using a callus formation chemical induce. Plant Biotechnology, 39: 273–280.

Yu, Y., Liu, D., Liu, C., Yan, Z., Yang, X. and Feng, G. 2021. *In vitro* regeneration of *Phaseolus vulgaris* L. via direct and indirect organogenesis. Plant Biotechnology Reports, 15: 279–288.

How to cite this article:

Abedini Aboksari, H., Azadi, P., Azimi, M.H., Kalatejari, S. and Borzouei, A. (2025). Enhancing Callus Growth in Calla Lily (*Zantedeschia* 'Sun Club') through Fipexide, Activated Charcoal, and Ascorbic Acid in *In Vitro* Cultivation. Journal of Ornamental Plants, 15(1), 37-46.

https://sanad.iau.ir/en/Journal/jornamental/Article/1198569