RESEARCH ARTICLE

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Effects of different explant and growth regulator combinations on in vitro propagation of Poinsettia (*Euphorbia pulcherrima* Willd.)

Lolav Rajab Hasan ALMZORI[1] **Bekir Erol AK**[1] **Rafail TOMA**[2]
Ibrahim Halil HATIPOGLU[1] **Heydem EKINCI**[1]

[1] Department of Horticulture, Faculty of Agriculture, Harran University, Şanlıurfa, Türkiye
[2] Department of Horticulture, University of Duhok, Duhok, Iraq

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Correspondence:
İbrahim Halil HATİPOĞLU
E-mail: ibrahimhhatipoglu@gmail.com

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Abstract
The aim of the research is to develop a suitable micropropagation protocol for the Poinsettia (*Euphorbia pulcherrima* Willd.) plant, which is used as an ornamental plant. In the sterilization step, mercuric chloride together with sodium hypochlorite is approved as the best option. Thus, contamination of explants is eliminated. At the shoot propagation stage, BA is absolutely necessary as all doses of BA increased shoot propagation compared to the control. The highest number of shoots per explant (2.62 shoots/explant) and the longest shoots (2.16 cm) were obtained from 1.0 mg.L^{-1} BA medium. The highest number of leaves (20.41 leaves/explant) was obtained from 2.0 mg.L^{-1} BA medium. Kinetin was also effective when adding different concentrations to the culture medium compared to the control. In general, BA is more effective than Kinetin in these parameters. Kinetin, on the other hand, performed much better than BA in terms of shoot number. On the other hand, the highest rooting percentage (58.2%) was obtained from the addition of 0.3 mg.L^{-1} NAA. The highest root number (5.10 roots/explant) was obtained by adding 0.1 mg.L^{-1} NAA. Good performance was found in the acclimatization phase with plantlets transferred to the soil with a high survival rate reaching 100%. Most of the plantlets started growing well. The plantlets grew well and did not show morphological abnormalities. In addition, a successful plant regeneration was achieved by adding 1.0 mg.L^{-1} BA and 0.5 mg.L^{-1} NAA on the callus produced in leaf disc explants and a very good organogenesis was determined in terms of roots and shoots.
Keywords: Poinsettia, *Euphorbia pulcherrima* Willd., *in vitro*, Micropropagation, Regeneration

INTRODUCTION

The Euphorbiaceae family is a family of taxa with high economic value (Mwine et al. 2011). Poinsettia (*Euphorbia pulcherrima* Willd) belongs to this family and belongs to the genus Euphorbia, which consists of about 2000 species. Poinsettia plant is the most popular ornamental plant in North America, Australia and various European countries (Castellanos, 2010). Their colors range from red to white, from yellow to orange-red (Bidarigh and Azarpour, 2013). Increasing its popularity as an interesting plant, these magnificent flowers can stay fresh and intact for 3-4 months. Its origin is Central America. It is considered one of the fastest growing plants (Toma and Almizory, 2012).

Poinsettia plant is one of the widely grown indoor ornamental plants (Clarke et al., 2008). In Iraqi ecological conditions, poinsettia is considered as an outdoor landscape plant. Euphorbia species are usually propagated by cuttings. Propagation by seeds is difficult, as the seeds lose their viability during storage. Ordinary reproduction is often done with cuts that focus on a period before the

most extensive sale time. The development of new *in vitro* regeneration procedures is likely to play a decisive role in successful production systems (Pickens et al., 2005; Trejo, 2020). In this context, it is of great importance to develop an effective micropropagation protocol *in vitro* and in sterile conditions. İn *vitro* micropropagation is a culture technique that has gained great popularity today. Tissue culture is also very important for the preservation of genetic material. With this technique, clone plants can be reproduced, endangered plants can be protected, gene sources can be protected and virus-free plants can be obtained (Babaoğlu et al., 2001).

In the research, it is aimed to determine the micropropagation methods of poinsettia in detail. With this study, it was aimed to develop a suitable sterilization and environment protocol for this species, to facilitate mass production by applying various concentrations of different growth regulators, to create a market for important landscape plants, and to test the adaptation of *in vitro* plants to external conditions. In addition, it is thought that the data obtained will be a basis for researches to quickly switch to ornamental plant production and to ensure success in production.

MATERIALS AND METHODS

This study was carried out in the plant tissue culture laboratory of the Department of Horticulture, Faculty of Agricultural Engineering Sciences, Duhok University, Northern Iraq Region, in 2021 and 2022.

The plant material was collected from healthy plants grown in local nurseries in the city of Duhok. Different explants were tested, including apical buds, axillary buds and leaves (1-2 cm). In this study, regeneration shoot tip and nodal explants of Poinsettia plant, which is an important ornamental plant, were examined, the effect of different plant growth regulators and concentrations on obtaining adventitious shoots from different explants was investigated, thus an effective protocol for *in vitro* was created. Shoots about 1.5 cm long were washed under tap water with the addition of detergent every 10 minutes for about 1 hour and then rinsed. In addition, ascorbic acid and citric acid (100 and 150 mg) solutions were prepared and then the explants were kept in this solution for 30 minutes.

Explants were immersed in 1.5%, 2.0% and 2.5% v/v sodium hypochlorite (NaOCl) solution for surface disinfection under a laminar air flow cabinet for fifteen minutes and then washed three times with sterile distilled water. Another sterile material was used to disinfect at 0.15% for 7 and 10 minutes using mercury chloride ($HgCL_2$). In this way, the sterilization protocol was determined. MS (Murashige and Skoog, 1962) was used as the basic medium. Microshoots that responded well at this stage were tested at BA 0, 0.5, 1, 1.5, and 2 $mg.L^{-1}$ and kinetin 0, 0.5, 1, 1.5, and 2 $mg.L^{-1}$ and transferred to the propagation stage. For shoot propagation experiments, three explants were subcultured in each culture dish and five vials were used for each treatment. The cultures were then incubated in the growth chamber at below 25+2°C and exposed to 1000 lux lighting for 16 hours per day. The number of leaves per explant, the number of shoots per explant, and the mean shoot length were recorded 4 weeks after the first culture (Ogras et al., 2022).

In the rooting step, auxins (IBA and NAA) were used at 0, 0.1, 0.2, and 0.3 $mg.L^{-1}$ using half MS medium. For root promotion, the bottles were sealed with aluminum foil. After six weeks in subculture, rooting parameters such as percentage rooting, number of roots per explant, and mean root length were recorded. Rooted plantlets were transferred to outdoor conditions and sprayed with Benlate fungicide (0.1%). It was then transferred to pots containing sterilized peat moss. The plants were sprayed with a nutrient solution containing ¼ MS salts. Plants were transferred to normal greenhouse conditions after 8-10 days. For the callus induction experiment, leaf discs of about 1 cm^2 were taken from the plants grown *in vitro* from the initial stage. Three explants were taken into each culture dish and five petri dishes were used for each replicate. After 8 weeks in culture, parameters such as percent callus induction, callus weight and callus consistency were recorded.

Planting was done in nutrient media in culture tubes, with 3 explants in each jar. The experiment was set up in 3 replicates for each explant type and nutrient medium, with 10 jars per replicate. It is designed as Completely Random Design (CRD). Comparison was made according to Duncan's multiple range test ($P < 0.05$) using a computerized SPSS and JMP program.

RESULTS AND DISCUSSION

Micro shoots consisting of apical bud explants were selected for the determination of developmental stages. This is because in the initial stage apical buds are much better than axillary buds. The results showed that the addition of BA was highly beneficial for raising the values of the amplification parameters compared to the control treatment. However, no critical difference was noted between the different concentrations of added BA. The highest number of shoots per explant (2.62 shoots/explant) and the longest shoots (2.16 cm) were obtained from the addition of 1.0 $mg.L^{-1}$ BA. The highest number of leaves (20.41 leaves/explant) was recorded from the addition of 2.0 $mg.L^{-1}$ BA (Table 1).

On the other hand, Table 2 shows that Kinetin is also effective when adding different concentrations to the culture medium compared to the control. Addition of 1.5 $mg.L^{-1}$ Kinetin produced the highest number of shoots and leaves (3.63 shoots/explant and 18.03 leaves/explants), respectively. Whereas, control treatment gave the longest shoots reaching 2.03 cm compared to 0.58 cm recorded for 1.5 $mg.L^{-1}$ kinetin. In general, BA was more effective than kinetin in terms of leaf number and

Table 1. Effect of BA Concentrations on poinsettia multiplication stage after four weeks in culture MS medium

Treatments (BA mgL^{-1})	Number of Shoots/Explant	Number of Leaves/Explant	Length of Shoots (cm)
0.0	1.05 ± 0.12 b	7.66 ± 0.48 c	2.03 ± 0.24 ab
0.5	2.35 ± 0.28 a	18.86 ± 0.80 b	2.13 ± 0.24 a
1.0	2.62 ± 0.25 a	20.30 ± 0.66 a	2.16 ± 0.16 a
1.5	2.36 ± 0.28 a	18.25 ± 0.17 b	1.66 ± 0.15 b
2.0	2.35 ± 0.24 a	20.41 ± 0.76 a	2.13 ± 0.33 a
LSD≤.05	0.13	0.49	0.14

Table 2. Effect of Kinetin Concentrations on poinsettia multiplication stage after four weeks in culture MS medium

Treatments (Kinetin mgL^{-1})	Number of Shoots/Explant	Number of Leaves/Explant	Length of Shoots (cm)
0.0	1.05 ± 0.12 d	7.66 ± 0.48 d	2.03 ± 0.24 a
0.5	2.13 ± 0.21 c	15.08 ± 0.60 c	1.18 ± 0.24 bc
1.0	2.96 ± 0.22 b	16.40 ± 0.66 b	0.86 ± 0.14 cd
1.5	3.63 ± 0.38 a	18.05 ± 0.19 a	0.58 ± 0.25 d
2.0	3.08 ± 0.27 b	15.28 ± 0.36 c	1.63 ± 0.37 ab
LSD≤.05	0.18	0.36	0.15

average shoot length. However, kinetin performed much better than BA in terms of shoot number (Figure 1).

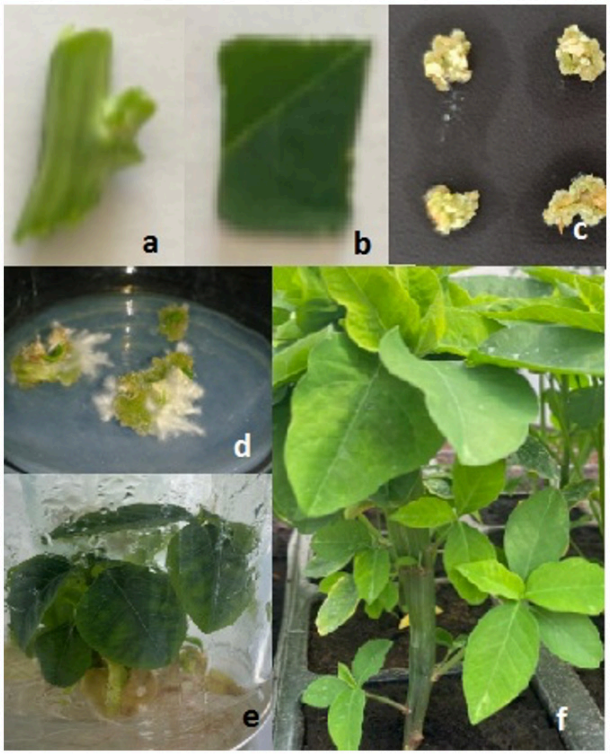

Figure 1. Tissue culture stages (a; auxiliary bud, b;Leaf discs for callus indication, c; callus, d; indirect organogenesis of roots, e; plants grown *in vitro*, f; after one week from transplating in greenhouse).

The best poinsettia microshoots produced in the shoot propagation stage were transported to the root formation stage by testing different concentrations of IBA and NAA at 0.0, 0.1, 0.2 and 0.3 mg.L^{-1}. (Table 3 and Figure 2). Addition of 0.2 mg.L^{-1} IBA was higher than the remaining treatments. It was critically higher than the control, which produced only 2.18 cm, as it reached the highest rooting percentage (63.1%), the highest root number (2.20 roots/explant) and the longest roots reaching 5.50 cm.

Figure 2. Root formation stage (A; effect of different IBA concentrations, B; rooted microshoots treated with 0.2 mgL^{-1} IBA, C; effect of different NAA concentrations, D; rooted microshoots treated with 0.3 mgL^{-1} NAA)

Explants were followed for root, callus and shoot formation. The shoot-forming explants were subcultured and grown. The shoots that reached a sufficient level were cleaned and taken into the rooting medium. Plantlets that grew and developed in the rooting medium were potted and new plants were obtained (a high percentage of plant survival reaching 90%). These plants performed very well under greenhouse conditions and showed no growth abnormalities or any physiological disturbances.

Table 3. Effect of different IBA concentrations on rooting parameters

Treatments (IBA ppm)	Percentage (%)	Number of Roots (pcs)	Length of Roots (mm)
0.0	58.50 ± 2.12 b	1.20 ± 0.12 c	2.18 ± 0.44 c
0.1	44.30 ± 1.44 d	1.80 ± 0.17 b	3.39 ± 0.12 b
0.2	63.10 ± 1.65 a	2.20 ± 0.32 a	5.50 ± 0.32 a
0.3	53.40 ± 2.12 c	1.55 ± 0.14 bc	3.61 ± 0.32 b
LSD≤.05	0.69	0.18	0.26

Table 4. Effect of different NAA concentrations on rooting parameters

Treatments (NAA ppm)	Percentage (%)	Number of Roots (pcs)	Length of Roots (mm)
0.0	58.50 ± 2.12 a	1.20 ± 0.12 b	2.18 ± 0.44 b
0.1	44.20 ± 2.44 b	5.10 ± 1.15 a	4.55 ± 0.70 a
0.2	33.00 ± 0.92 c	4.42 ± 1.05 a	4.53 ± 0.52 a
0.3	58.20 ± 2.25 a	4.43 ± 0.42 a	5.20 ± 1.12 a
LSD≤.05	1.37	0.99	0.61

Table 5. Callus induction in leaf disc explants affected by combinations of BA and NAA

BA+ NAA (mg.L^{-1})	Callus Induction (%)	Weight of Callus (g)	Remarks
0.0+ 0.0	0	0 c	-
0.0+ 0.5	0	0 c	-
0.0+ 1.0	0	0 c	-
0.0+ 2.0	0	0 c	-
0.0+ 4.0	5.6 c	0.58 b	Green, compact
1.0+ 0.0	0	0 c	-
1.0+ 0.5	6.6 c	1.16 b	Green, compact
1.0+ 1.0	6.0 c	0.65 b	Yellow, friable
1.0+ 2.0	6.6 c	0.5 b	Yellow, friable
1.0+ 4.0	6.6 c	1.2 b	Yellow, friable
2.0+ 0.0	0	0 c	-
2.0+ 0.5	93.33 a	2.6 a	Yellow, friable
2.0+ 1.0	60 b	1.49 b	Green, yellow, compact
2.0+ 2.0	66.66 b	1.4 b	Yellow, friable
2.0+ 4.0	93.33 a	2.57 a	Yellow, green, compact
3.0+ 0.0	0	0 c	-
3.0+ 0.5	60 b	2.31 a	Green, compact
3.0+ 1.0	80 ab	2.56 a	Yellow, compact
3.0+ 2.0	40 bc	2.52 a	Green, compact
3.0+ 4.0	60 b	2.63 a	Yellow, friable

In general, good callus induction was obtained from culture of leaf discs. Table 5 shows that the addition of BA and NAA at 2.0 mg.L^{-1} + 0.5 mg.L^{-1} NAA and 2.0 mg.L^{-1} + 4.0 mg.L^{-1} are the best combined treatments, creating and giving 93.33% callus induction. The consistency of the callus was very good, it had a brown to green color and a compact texture. This formed callus was selected for the next regeneration experiment. As mentioned above, the best callus produced from 2.0 mg.L^{-1} BA+ 4.0 mg.L^{-1} NAA was transferred to culture medium containing 1.0 mg.L^{-1} BA and 0.5 mg.L^{-1} NAA.

Toma and Al-Mizory (2012) have been very successful in acclimatizing poinsettia plantlets with a high survival rate of up to 90%. After several months of acclimatization, their plants did not show any abnormal features or any physiological problems.

Perera and Trader (2008) produced red poinsettia callus by grafting their explants into culture medium containing only BA and a combination of BA and IAA. No callus was formed from the culture medium without PGR. The combination between BA and IAA produced better red calluses than the use of BA alone. The best callus size (1.27 mm) and the highest number of bud calluses (1.29) were recorded with the half stem cut application. Many important points should be considered when sterilizing explants.

Sodium hypochlorite is very widely used in tissue culture for various herbaceous and woody plants (Perera and Trader 2010), but a high rate of contamination (90%) was incurred when using different concentrations of NaOCl (1.5, 2.0 and 2.5%) This problem forced to use mercuric chloride (HgCl2) at 0.15% for 10 minutes. This

sterilant was very effective in preventing contamination by obtaining 100% healthy and clean cultures. These results are consistent with similar literature (Toma and Al-Mizory, 2012; Rangel-Estrada et al., 2015).

At the shoot propagation stage, both BA and kinetin were tested as cytokinins, and BA was more effective than kinetin in terms of leaf number and average shoot length. However, kinetin performed much better than BA in terms of shoot number. The results for callus induction in leaf disc explants showed that callus could be successfully induced while finding the best combination between auxins and cytokinins. Plant growth regulators are the key solution for callus induction in plant tissue culture, especially for moderate levels of auxins and cytokinins (George, 2008). Finally, for plant regeneration from the produced corn, it is very important to find the best combination between cytokinins and auxins to ensure organogenesis. In the present study, the corn produced was of the embryogenic callus type, which was able to regenerate roots and shoots after treatment with 1.0 $mg.L^{-1}$ BA and 0.5 $mg.L^{-1}$ NAA. This phenomenon proves the main role of auxins and cytokinins in cell division and differentiation (Pickens et al., 2005).

CONCLUSION

In conclusion, although the response of shoot propagation parameters varies according to cytokinin types and levels, the addition of cytokinins is essential for better shoot propagation of the poinsettia plant. Indole butyric acid (IBA) proved to be a better choice than naphthalene acetic acid (NAA) to initiate the rooting process in poinsettia microshoots. Although the acclimatization step is considered the most laborious step during micropropagation protocols, a high survival rate of poinsettia plantlets can be achieved if the grower follows the acclimatization steps very carefully. Browning of the plant material occurred as the proportions of sterilants used and the residence time of the plant material in the sterilant increased. Finding a better combination of auxins and cytokinins as well as a suitable explant is still key to inducing embryogenic callus in poinsettia explants. Indirect organogenesis on poinsettia callus can be achieved after obtaining an embryogenic callus by appropriate exogenous addition of plant growth regulators to the culture medium.

COMPLIANCE WITH ETHICAL STANDARDS

This research article complies with research and publishing ethics.

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

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Ethics committee approval is not required.

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Data availability

Not applicable.

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REFERENCES

Babaoğlu, M., Gürel, E. & Özcan S. (2001). Bitki Biyoteknolojisi I. Doku Kültürü Uygulamaları. Selçuk Basımevi, 374.

Bidarigh, S. & Azarpour, E. (2013). Study effect of MS medium and shoot tip, Biotechnology,17(2), 137-147.

Castellanos, M., Power, J.B. & Davey, M.R. (2010). Micropropagation of poinsettia by organogenesis. In Protocols for *in vitro* propagation of ornamental plants (pp. 67-75). Humana Press. https://doi.org/10.1007/978-1-60327-114-1_7

Clarke, J.L., Spetz, C., Hauglien, S., Xıng, S., Dees, M.W., Moe, R. & Blystad, D.R. (2008). Agrobacterium tumefaciens-mediated transformation of poinsettia,Euphorbia pulcherrima, with virus-derived hairpin RNA constructs confersresistance to Poinsettia mosaic virus. Plant Cell Reports,27(6), 1027-1038. https://doi.org/10.1007/s00299-008-0526-9

George, M.W. (2008). The elements of library investigation. Princeton University Press.

Mwine, T.J. & Van Damme, P. (2011). Evaluation of pesticidal properties ofEuphorbia tirucalli L.(Euphorbiaceae) against selected pests. Afrika Focus, 24(1), 119-121. https://doi.org/10.1163/2031356X-02401014

Ogras, T.T., Tahtasakal, E. & Ozturk, S. (2022). *In vitro* production of tropane alkaloids from *Brugmansia suaveolens*. Int. J. Sec. Metabolite, Vol. 9, No. X, (2022) pp. 1-13. https://doi.org/10.21448/ijsm.934222

Perera, D. & Trader, B.W. (2008). Poinsettia (Euphorbia pulcherrima) *in vitro* propagation. In Combined Proceedings International Plant PropagatorsSociety (Vol. 58, pp. 578-582).

Perera, D. & Trader, B.W. (2010). Poinsettia 'Prestige™ Red'(Euphorbiapulcherrima) *in vitro* propagation. Hort. Science, 45(7), 1126-1128. https://doi.org/10.21273/HORTSCI.45.7.1126

Pickens, K.A., Cheng, Z.M. & Trigiano, R.N. (2005). Axillary bud proliferation and organogenesis of Euphorbia pulcherrima Winter Rose ™. İn *vitro* Cell DevBio-Plant 41: 770–774. https://doi.org/10.1079/IVP2005706

Rangel-Estrada, S.E., Canul-Ku, J., Osuna-Canizalez, F.D.J., Garcia-Perez, F., Rosario-Montes, P.D., Vences-Hemandez, Á.S.B. & Hemandez-Meneses, E. (2015). İn *vitro* regeneration of poinsettia hybrids via organogenesis. Revista Mexicana de Ciencias Agrícolas, 6(7), 1571-1585.

Toma, R.S. & Al-Mizory, L.S. (2012). İn *vitro* micropropagation of poinsettia (Euphorbia pulcherrima Willd.). Journal of Kirkuk University For Agricultural Sciences, 3(1).

Trejo, L. (2020). The wild poinsettia: origins of the most beautiful Euphorbia. Chronica Horticulturae, 60 (4): 28-32.