Electronic Letters on Science & Engineering 18(2) (2022)
Available online at dergipark.org.tr/else

Impact of Growth Regulators on Rapid Clonal Propagation of *Rotala rotundifolia* (Buch-Ham. ex Roxb) in Liquid Culture Medium

Muhammet DOĞAN[1*]

[1,2] Department of Nutrition and Dietetics, Faculty of Health Sciences, Karamanoglu Mehmetbey University, Turkey
mtdogan1@gmail.com, ORCID: 0000-0003-3138-5903

Abstract: *Rotala rotundifolia* (Buch-Ham. ex Roxb) is water plant used for medicinal and ornamental purposes. The aim of the work was to investigate the impacts of different growth regulators on the *in vitro* clonal propagation of *R. rotundifolia*. All assays were performed in liquid Murashige and Skoog (MS) media (without agar). Nodal explants were transferred to liquid MS nutrient media. Different combinations of Gibberellic Acid (GA_3) + Thidiazuron (TDZ) and Kinetin (KIN) + Gibberellic Acid (GA_3) were added to the nutrient solutions as growth regulators. The best results in terms of shoot numbers and shoot lengths were determined in nutrient solution including 0.25 mg/L GA_3 + 0.25 mg/L TDZ and 0.25 mg/L KIN + 0.25 mg/L GA_3. When both hormone combinations were compared, higher shoot numbers were found in nutrient solutions including GA_3+TDZ. On the other hand, longer shoots were obtained in nutrient solutions including KIN+GA_3 After the propagation trials, the plants formed roots in the media with 0.25 mg/L indole-3-butyric acid (IBA). The plants that have been produced have been successfully adapted to the aquarium.

Keywords: in vitro propagation, medicinal plant, MS medium, nodal explant, tissue culture

Sıvı Kültür Ortamında Büyüme Düzenleyicilerinin *Rotala rotundifolia* (Buch-Ham. Ex Roxb)'nın Hızlı Klonal Çoğaltımı Üzerine Etkisi

Özet: *Rotala rotundifolia* (Buch-Ham. ex Roxb) tıbbi ve süs amaçlı kullanılan önemli bir su bitkisidir. Bu çalışmanın amacı farklı büyüme düzenleyicilerinin *R. rotundifolia*'nın *in vitro* klonal çoğaltımı üzerine farklı büyüme düzenleyicilerinin etkilerini araştırmaktır. Tüm dememeler sıvı Murashige ve Skoog (MS) ortamında yürütülmüştür (agarsız). Boğum eksplantları sıvı besin ortamlarına aktarılmıştır. Besin solüsyonlarına büyüme düzenleyici olarak farklı kombinasyonlarda Gibberellik asit (GA_3) + Thidiazuron (TDZ) ve Kinetin (KIN) + Gibberellik asit (GA_3) eklenmiştir. Sürgün sayısı ve uzunlukları açısından en iyi sonuçlar 0.25 mg/L GA_3 + 0.25 mg/L TDZ ve 0.25 mg/L KIN + 0.25 mg/L GA_3 içeren besin solüsyonunda belirlenmiştir. Her iki hormon kombinasyonları karşılaştırıldığında, daha fazla sürgün sayıları GA_3+TDZ içeren besin solüsyonlarında bulunmuştur. Buna karşın daha uzun sürgünler KIN+GA_3 içeren besin solüsyonlarında elde edilmiştir. Çoğaltım denemelerinden sonra bitkiler 0.25 mg/L indol-3-bütirik asit (IBA)'lı ortamda kök oluşturdu. Üretimi tamamlanan bitkiler akvaryuma başarıyla adapte edilmiştir.

Anahtar Kelimeler: in vitro çoğaltım, tıbbi bitki, MS ortamı, boğum eksplant, doku kültürü

Reference to this paper should be made as follows (bu makaleye aşağıdaki şekilde atıfta bulunulmalı):
Dogan, M., 'Impact of Growth Regulators on Rapid Clonal Propagation of *Rotala rotundifolia* (Buch-Ham. ex Roxb) in Liquid Culture Medium', Elec Lett Sci Eng, vol. 18(2) , (2022), 79-86

1. Introduction

Tissue culture allows to obtain a large number of plants or herbal products from a small part of the plant called explant (root, stem, leaf, shoot tip, etc.). Propagation studies are carried out in a sterile environment under laboratory conditions [1]. The basis of this biotechnological production method depends on the totipotency ability of plant cells [2]. Murashige and Skoog [3] salts (MS) are generally used in the preparation of nutrient media [4,5].

Tissue culture has significant advantages for large-scale propagation of plants, providing virus- and bacteria-free plants, production of important bioactive compounds, and plant cloning. Also, this technique is important for the propagation of plants that are difficult to reproduce or take a long time to grow [1]. Thanks to this technique, agricultural plants such as *Ipomoea batatas* (L.) Lam [6] and *Zea mays* L. [7], horticultural crops such as *Prunus avium* L. [8] and *Prunus armeniaca* L. [9], medicinal and aromatic plants such as *Vaccinium vitis-idaea* L. [10] and *Bacopa monnieri* L. Pennell [11], and ornamental plants such as *Pogostemon erectus (*Dalzell) Kuntze [12] ve *Gerbera jamesonii* Bolus ex Hooker F. [13] have been successfully propagated

Rotala rotundifolia (Buch-Ham. Ex Roxb) is water plant. It is also known as a medicinal herb due to its therapeutic features. It is also preferred as an ornamental plant in aquariums [14]. Growth regulators are added to the nutrient medium for multiple shoots in tissue culture studies. The concentration, combination and variety of growth regulators is an important parameter that directly affects shoot regeneration from explants [15,16]. Therefore, for efficient production optimization, it is necessary to determine the type of growth regulators and to know the ratios to be used. In the work, the impacts of different growth regulators on the *in vitro* clonal propagation of commercially important *R. rotundifolia* were investigated.

2. Materials and Method

The plants used in the experiments were brought from Konya (Turkey). Sterilization of the plants was achieved with 20% hydrogen peroxide (H_2O_2) after 10 min. Nodal segments was used as explant in multiplication experiments. The explants were placed in MS [3] media fortified with 3% sucrose and different combinations of Gibberellic Acid (GA_3) + Thidiazuron (TDZ) and Kinetin (KIN) + Gibberellic Acid (GA_3) (Table 1).

The nutrient solutions were prepared without agar. The pH value of the nutrient solutions was set to 5.7±1 and autoclaved (120 °C, 20 min). The nutrient solutions were poured into magenta dishes and the nodal explants were added in magentas. Magentas were kept in 16 hours of illumination and 24 °C temperature-adjusted conditions. The trials ended in the eighth week.

After the clonal propagation process, the shoots were placed to MS food media with 0.25 mg/L indole-3-butyric acid (IBA). *In vitro* rooting took four weeks. Produced plants were planted in the aquarium environment to adapt to *ex vitro* conditions. The conditions of the aquarium environment are set at 23°C and 16 h light.

Applications were carried out with 6 repetitions. Data were analyzed in SPSS 16 for Windows. Duncan test was preferred for post hoc.

Table 1. Growth regulators used in *in vitro* cloning studies

Abbreviation	GA_3 (mg/L)	TDZ (mg/L)
GT1	0.05	0.25
GT2	0.25	0.25
GT3	0.50	0.25
GT4	0.75	0.25
GT5	1.00	0.25
Abbreviation	**KIN (mg/L)**	**GA_3 (mg/L)**
KG1	0.05	0.25
KG2	0.25	0.25
KG3	0.50	0.25
KG4	0.75	0.25
KG5	1.00	0.25

3. Results and Discussion

3.1. GA_3 and TDZ combination trials

The explants were placed in liquid media including doses of GA_3 (0.05-1.00 mg/L) and TDZ (0.25 mg/L). *In vitro* shoots started to be observed clearly after the fourth week in the culture medium. The trial was stopped after eight weeks and multiple shoot formations were noted in the nodal explants (Figure 1a, b and c). Data of regenerated shoots were taken and statistically analyzed (Table 2).

GA_3 is an important growth regulator used in tissue culture studies. Tissue culture studies with GA_3 have also been performed previously on *Daphne mezereum* L. 'Alba' [17], *Argania spinosa* (L.) Skeels [18], and *Pterocarpus marsupium* Roxb. [19].

In the current trials, the nodal explants were placed in nutrient solutions. The nodal explants are a widely used explant in clonal multiplication studies. Similarly, the nodal explants were preferred in clonal propagation of *Vernonia amygdalina* Delile [20], *Limnophila aromatica* (Lamk.) Merr. [21] and *Lycium barbarum* L. [16].

Figure 1. Multiple shoots of *R. rotundifolia* in nutrient solution with GA_3+TDZ; (**a, b and c**) Eight weeks later, multiple shoot formations in nutrient solution with 0.25 mg/L GA_3 + 0.25 mg/L TDZ.

Table 2. Impacts of GA_3+TDZ doses on *in vitro* multiplication of *R. rotundifolia*.

	Hormone combinations (mg/L)		Regeneration (%)	Num. of shoots	Shoot extent (cm)
	GA_3	TDZ			
GT1	0.05	0.25	88.89^{a}*	8.22^{bc}**	1.47^{b}**
GT2	0.25	0.25	100^{a}	13.55^{a}	1.83^{a}
GT3	0.50	0.25	83.33^{ab}	9.72^{b}	1.58^{ab}
GT4	0.75	0.25	66.66^{b}	7.05^{bc}	1.29^{b}
GT5	1.00	0.25	66.66^{b}	6.33^{c}	1.25^{b}

** Different letters indicate significant at $p<0.01$ level.
* Different letters indicate significant at $p<0.05$ level.

As can be seen from Table 2, shoot regeneration rates in MS media varied between 66.66-100%. 100% shoot growth was obtained in GT2 nutrient solution. Low shoot percentages (66.66%) were determined in GT4 and GT5 nutrient solutions. Shoot formations in the solutions were recorded inversely with GA_3 value. As the GA_3 value increased, shoot emergence from explants was suppressed.

The shoot counts in the nutrient solutions including different GA_3+TDZ combinations showed statistically significant differences ($p<0.01$). The best value in shoot numbers (13.55) was obtained in GT2 solution. On the other hand, the least number of shoots (6.33) was determined in GT5 nutrient solution. The use of GA_3 more than 0.25 mg/L in nutrient solutions adversely affected the number of shoots.

Shoot lengths ranged from 1.25-1.83 cm. The length of the shoots decreased with increasing GA_3 value. When the length values are compared, the highest result was obtained in GT2 (1.83 cm). On the other hand, the lowest length value was found in GT5 (1.25 cm).

3.2. KIN and GA_3 combination trials

The nodal explants of *R. rotundifolia* were exposed to different doses of KIN + GA_3. Propagation trials were completed in the eighth week of culture. Proliferating and elongating shoots were recorded (Figure 2 a, b and c). Data were evaluated statistically (Table 3).

KIN is an essential growth regulator used in tissue culture experiments. Due to its effects, it is preferred in clonal propagation experiments. Similarly, KIN was applied in multiplication of *Solanum melongena* L. Cv. Bulat Putih [22] and *Musa paradisiaca* L. [23].

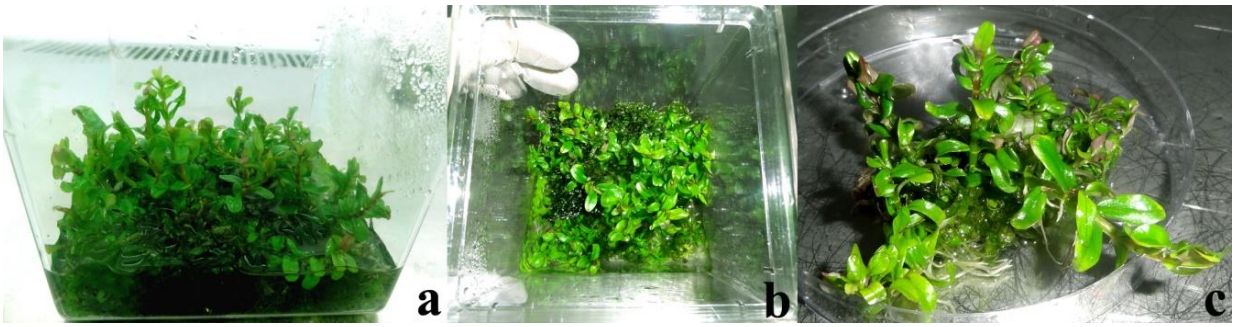

Figure 2. Multiple shoots of *R. rotundifolia* in nutrient solution with KIN+GA_3; (**a, b and c**) Eight weeks later, multiple shoot formations in nutrient solution with 0.25 mg/L KIN + 0.25 mg/L GA_3.

Table 3. Impacts of KIN+GA_3 doses on *in vitro* multiplication of *R. rotundifolia*.

	Hormone combinations (mg/L)		Regeneration (%)	Num. of shoots	Shoot extent (cm)
	KIN	**GA_3**			
KG1	0.05	0.25	94.44^{a}	4.62^{b}	2.25^{a}
KG2	0.25	0.25	100^{a}	8.39^{a}	2.47^{a}
KG3	0.50	0.25	88.89^{a}	5.81^{b}	1.67^{b}
KG4	0.75	0.25	83.33^{a}	4.29^{b}	0.98^{c}
KG5	1.00	0.25	77.77^{a}	4.05^{b}	0.83^{c}

Different letters indicate significant at $p<0.01$ level.

As seen in Table 3, regeneration frequencies in nutrient solutions varied between 77.77-100%. 100% regeneration was achieved in KG2 nutrient solution. 77.77% regeneration was detected in KG5 nutrient solution.

The shoot number data in the nutrient solutions were statistically significant (p<0.01). In general, as the KIN doses increased, the number of shoots decreased. High number of shoots were reached in KG2 nutrient solution (8.39). A small number of shoots (4.05) were reached in KG5 nutrient solution.

The length value varied between 0.83-2.47 cm. The best length value was achieved in the KG2 solution (2.47 cm), followed by the KG1 solution (2.25 cm). Equal use of KIN and GA_3 showed positive results for shoot lengths. On the other hand, the nutrient solutions including high levels of KIN caused the shoots to be short.

Growing plants grown in liquid cultures were rooted in solid nutrient media including 0.25 mg/L IBA. After the rooting trials were completed, the plantlets were placed in the aquarium. As a result, acclimatization of the plants to the aquatic environment was achieved. Similarly, acclimatization of *in vitro* grown plants to aquatic conditions has been previously reported by many researchers [24,25].

4. Conclusions

As a result, *in vitro* clonal propagation of *R. rotundifolia* were successfully achieved in GA_3+TDZ and KIN+GA_3 nutrient solutions. In GA_3+TDZ trials, the most productive shoot number and length were obtained in GT2 nutrient solution. In KIN+GA_3 trials, the highest shoot number and length were reached in KG2. When GA_3+TDZ and KIN+GA_3 growth regulators were compared, higher shoot numbers were found in nutrient solutions with GA_3+TDZ. When shoot lengths were compared, nutrient solutions with KIN+GA_3 provided longer shoots.

Acknowledgements

This work was supported by the Scientific and Technological Research Council of Turkey (TUBITAK) under Grant (Project no: 2130190).

References

[1] Oseni, O. M., Pande, V., Nailwal, T. K., “A review on plant tissue culture, a technique for propagation and conservation of endangered plant species”, International Journal of Current Microbiology and Applied Sciences, 7(7): 3778-3786, 2018.

[2] Bidabadi, S. S., Jain, S. M., “Cellular, molecular, and physiological aspects of *in vitro* plant regeneration”, Plants, 9(6): 702, 2020.

[3] Murashige, T., Skoog, F., “A revised medium for rapid growth and bioassays with tobacco tissue cultures”, Physiologia Plantarum, 15: 473-497, 1962.

[4] Phillips, G. C., Garda, M., “Plant tissue culture media and practices: an overview”, *In Vitro* Cellular & Developmental Biology-Plant, 55(3): 242-257, 2019.

[5] Akin, M., Eyduran, S. P., Eyduran, E., Reed, B. M., “Analysis of macro nutrient related growth responses using multivariate adaptive regression splines”, Plant Cell, Tissue and Organ Culture (PCTOC), 140(3): 661-670, 2020.

[6] Abubakar, A. S., Yahaya, S. U., Shaibu, A. S., Ibrahim, H., Ibrahim, A. K., Lawan, Z. M., Isa, A. M., “*In vitro* propagation of sweet potato (*Ipomoea batatas* (L.) Lam.) cultivars”, Agricultural Science Digest-A Research Journal, 38(1): 17-21, 2018.

[7] Jing, C. H. E. N., "Efficient somatic embryogenesis and plant regeneration through callus initiation from seedling-derived leaf materials of maize (*Zea mays* L.)", Agricultural Biotechnology, 8(3): 2164-4993, 2019.

[8] Zong, X., Chen, Q., Nagaty, M. A., Kang, Y., Lang, G., Song, G. Q., "Adventitious shoot regeneration and *Agrobacterium tumefaciens*-mediated transformation of leaf explants of sweet cherry (*Prunus avium* L.)", The Journal of Horticultural Science and Biotechnology, 94(2): 229-236, 2019.

[9] Pérez-Caselles, C., Faize, L., Burgos, L., Alburquerque, N., "Improving adventitious shoot regeneration and transient *Agrobacterium*-mediated transformation of apricot (*Prunus armeniaca* L.) hypocotyl sections", Agronomy, 11(7): 1338, 2021.

[10] Debnath, S. C., Arigundam, U., "*In vitro* Propagation strategies of medicinally important berry crop, lingonberry (*Vaccinium vitis-idaea* L.)", Agronomy, 10(5): 744, 2020.

[11] Dogan, M., Emsen, B., "Anti-cytotoxic-genotoxic influences of *in vitro* propagated *Bacopa monnieri* L. Pennell in cultured human lymphocytes", Eurasian Journal of Biological and Chemical Sciences, 1(2): 48-53, 2018.

[12] Dogan, M., "*In vitro* rapid propagation of an aquatic plant *Pogostemon erectus* (Dalzell) Kuntze", Anatolian Journal of Botany, 3(1): 1-6, 2019.

[13] Talla, S. K., Madam, E., Manga, S., Aileni, M., Mamidala, P., "Efficient TDZ-induced regeneration from capitulum explants of *Gerbera jamesonii* Bolus ex Hooker F.-an ornamental plant with high aesthetic value", Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology, 153(5): 679-685, 2019.

[14] Yang, Y., Zhang, X., Xiao, J., Chu, S., Huang, Z., Nutrient removal by *Rotala rotundifolia*: a superior candidate for ecosystem remediation at low temperatures", RSC Advances, 10(49): 29139-29146, 2020.

[15] Chen, Y. M., Huang, J. Z., Hou, T. W., Pan, I. C., "Effects of light intensity and plant growth regulators on callus proliferation and shoot regeneration in the ornamental succulent Haworthia", Botanical studies, 60(1): 1-8, 2019.

[16] Karakas, F. P., "Efficient plant regeneration and callus induction from nodal and hypocotyl explants of goji berry (*Lycium barbarum* L.) and comparison of phenolic profiles in calli formed under different combinations of plant growth regulators", Plant Physiology and Biochemistry, 146: 384-391, 2020.

[17] Nowakowska, K., Pacholczak, A., Tepper, W., "The effect of selected growth regulators and culture media on regeneration of *Daphne mezereum* L.'Alba', Rendiconti Lincei. Scienze Fisiche e Naturali, 30(1): 197-205, 2019.

[18] Amghar, I., Diria, G., Boumlik, I., Gaboun, F., Iraqi, D., Labhilili, M., ... Abdelwahd, R., "An efficient regeneration pathway through adventitious organogenesis for the endangered *Argania spinosa* (L.) Skeels", Vegetos, 1-13, 2021.

[19] Ahmad, A., Ahmad, N., Anis, M., Alatar, A. A., Abdel-Salam, E. M., Qahtan, A. A., Faisal, M., "Gibberellic acid and thidiazuron promote micropropagation of an endangered woody tree (*Pterocarpus marsupium* Roxb.) using *in vitro* seedlings", Plant Cell, Tissue and Organ Culture (PCTOC), 144(2): 449-462, 2021.

[20] Mei-Yin, C., Sani, H., "*In vitro* plantlet regeneration from nodal explant and callus induction of *Vernonia amygdalina* Delile", Journal of Plant Sciences, 6(1): 1-6, 2018.

[21] Dogan, M., "*In vitro* shoot regeneration of *Limnophila aromatica* (Lamk.) Merr. from nodal and internodal explants", Journal of the Institute of Science and Technology, 8: 77-84, 2018.

[22] Foo, P. C., Lee, Z. H., Chin, C. K., Subramaniam, S., Chew, B. L., "Shoot induction in white eggplant (*Solanum melongena* L. Cv. Bulat Putih) using 6-benzylaminopurine and kinetin", Tropical Life Sciences Research, 29(2): 119, 2018.

[23] Shukla, S., Prasad, Y., Yadav, A. K., Dixit, P., Patel, B., Yadav, V., "Effect of different auxins and cytokinins in callus induction and shoot regeneration in banana (*Musa paradisiaca* L.) variety Udhayam under in vitro condition", International Journal of Chemical Studies, 6(4): 2041-2045, 2018.

[24] Unal, S., Turkmen, G., Yagmur, B., Bayraktar, M., Gurel, A., "Improved *in vitro* propagation and direct acclimatization of *Cryptocoryne wendtii* in aquarium in the presence of aquarium fish *Puntius tetrazona* (Bleeker)", Indian Journal of Experimental Biology, 57: 330-337, 2019.

[25] Haque, S. M., Ghosh, B., "A submerged culture system for rapid micropropagation of the commercially important aquarium plant,'Amazon sword'(*Echinodorus* 'Indian Red')", In Vitro Cellular & Developmental Biology-Plant, 55(1): 81-87, 2019.