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In vitro Shoot Regeneration of *Lysimachia nummularia* L. in Solid and Liquid Culture Medium

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Abstract

Lysimachia nummularia L. is a perennial medicinal and aromatic plant. In this study, the effects of solid and liquid culture media, and plant growth regulators on micropropagation of *L. nummularia* were investigated. Nodes were used as an explant. Different combinations of thidiazuron (TDZ: 0.05-0.80 mg L^{-1}) and indole-3-butyric acid (IBA: 0.10 mg L^{-1}) were used as growth regulators in Murashige and Skoog (MS) basal medium. In solid and liquid media experiments, the highest number of shoots was obtained from MS nutrient media supplemented with 0.40 mg L^{-1} TDZ + 0.10 mg L^{-1} IBA. The shoot length was higher in both liquid and solid culture medium supplemented with 0.05 mg L^{-1} TDZ + 0.10 mg L^{-1} IBA. The best results for number of shoots and regeneration ratio were determined in the solid nutrient media according to results . On the other hand, the best results for shoot length were found in the liquid culture media. Increasing doses of TDZ adversely affected shoot length in both culture media. The regenerated plants were successfully acclimatized to aquatic conditions. The results of this study may help the large-scale propagation of *L. nummularia* by tissue culture.

Key Words: *In vitro* propagation, MS medium, Node Explant, Tissue culture

1. Introduction

Medicinal plants have a great value in the treatment of diseases, nutrition and cosmetics industry since ancient times (Solomou et al., 2016). They are used commercially in many industrial sectors today. In addition, studies on medicinal and aromatic plants are carried out in different disciplines such as production of medicinal plants and the extraction of herbal products from them (Kala, 2015).

Lysimachia nummularia L. is a perennial plant belonging to the *Primulaceae*. This herb is known as Creeping Jenny or Moneywort. It is a plant native to Eurasia. It

can live in aquatic or humid environmental conditions (Kodela and Jobson, 2016). *L. nummularia* has been preferred since ancient times for the cure of many disease symptoms. It has also been reported to have antimicrobial properties (Podolak et al., 2013).

Plant tissue culture applications allow the production of many economically valuable plants, especially medicinal and aromatic plants. Recently, it was reported that many medicinal plants such as *Broussonetia papyrifera* L'Hér. Ex Vent (Lin et al., 2021), *Eupatorium glandulosum* L. (Nithya and Kamalam, 2021) and *Mondia whitei* (Hook. F.) Skeels (Patricia et al., 2021) produced by tissue culture.

There are a few studies on the production of *L. nummularia* by tissue culture. (Dogan, 2018; Dogan, 2019a). It is important to ensure optimization in the production of plants via tissue culture methods. Therefore, in this study, the effects of liquid and solid nutrient media and plant growth regulators on the *in vitro* propogation of *L. nummularia* were investigated.

2. Material and Methods

L. nummularia was purchased from aquarium store. Sterilization of the plants was achieved using bleach (5.7% active chlorine-NaOCI-ACE) for 10 min. After rinsing 3 times for 5 min, the node explants were placed to MS (Murashige and Skoog 1962) medium without plant growth regulators.

4.4 g L^{-1} MS basal salts (Duchefa), 30 g L^{-1} sucrose (Duchefa) and 6.5 g L^{-1} agar (Duchefa) were added to the solid MS medium. 4.4 30 g L^{-1} MS basal salts (Duchefa) and 30 g L^{-1} sucrose (Duchefa) were added to the liquid MS medium (no agar). Thidiazuron (TDZ:0.05-0.80 mg L^{-1}) and indole-3-butyric acid (IBA:0.10 mg L^{-1}) were added in both solid and liquid nutrient media at different combinations as growth regulators (Table 1). Propagation studies were completed at the end of eight weeks.

The pH of the culture media was adjusted to 5.7±0.1 via 1N NaOH and 1N HCl and then sterilized in an autoclave (120 °C for 20 min). The sterile nutrient media were poured into sterile plastic petri dishes. The node explants were placed in the petri dishes. These culture dishes were kept under white light emitting diodes (1500 lux) in a photoperiod of 16 hours light and 8 hours dark. The room temperature was set to 24°C.

The rooting experiments were not performed because dense roots were formed in the *in vitro* propagation experiments. For this reason, the stage of acclimatization of the regenerated plants to external conditions was started.

The MS nutrient medium on the regenerated plants was carefully removed. The plants were then placed to an aquatic environment (aquarium conditions were set at 24°C temperature and 16 hours of light).

Experiments were carried out in petri dishes with 6 replications. Data were reviewed with SPSS 21 for the Windows and Duncan was used for Post Hoc tests.

Table 1. The growth regulators used for *in vitro* shoot regeneration

TDZ (mg L^{-1})	IBA (mg L^{-1})
0.05	0.10
0.10	0.10
0.20	0.10
0.40	0.10
0.80	0.10

3. Results and Discussion

3.1. Solid media experiments

The node explants were cultured in solid medium supplemented with TDZ (0.05-0.80 mg L^{-1}) + IBA (0.10 mg L^{-1}). After two weeks from beginning of the culture callus formations and three weeks later shoot formations were observed in the MS media. After eight weeks, multiple shoots were obtained (Figure 1a and b) from cultures.

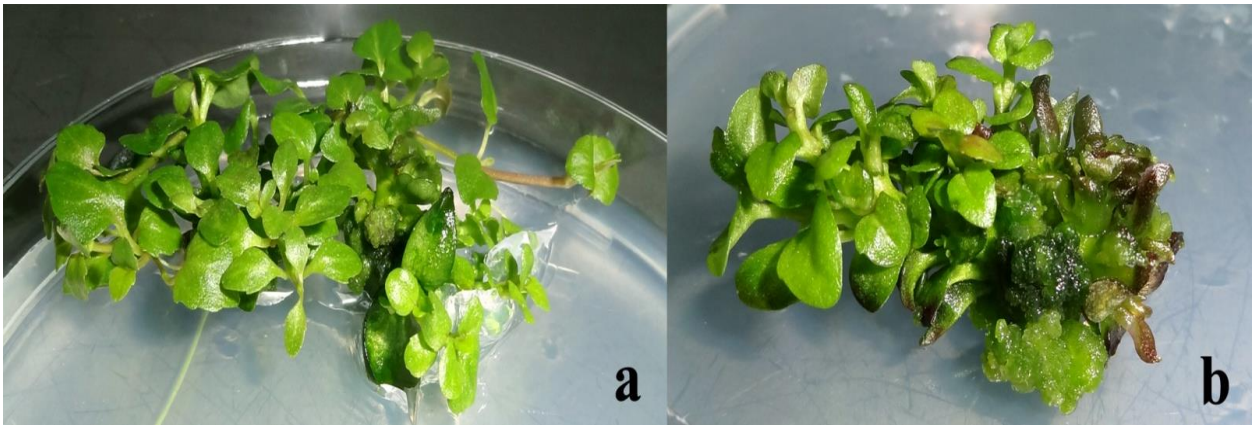

Figure 1. Shoot formations in the solid MS medium supplemented with IBA + TDZ. **(a)** Multiple shoot formations in the solid medium supplemented with 0.20 mg L^{-1} TDZ + 0.10 mg L^{-1} IBA **(b)** 0.40 mg L^{-1} TDZ + 0.10 mg L^{-1} IBA

Table 2. The effect of liquid medium supplemented with IBA + TDZ on *in vitro* shoot formation.

Growth regulators (mg L^{-1})		Regeneracy (%)	Average shoot numbers	Shoot lengths (cm)
TDZ	**IBA**			
0.05	**0.10**	100^{a}	5.39^{d}	1.28^{a}
0.10	**0.10**	77.78^{a}	9.94^{bc}	1.24^{ab}
0.20	**0.10**	72.22^{a}	12.30^{ab}	1.03^{ab}
0.40	**0.10**	94.44^{a}	15.16^{a}	0.95^{ab}
0.80	**0.10**	94.44^{a}	8.38^{cd}	0.81^{b}

Values shown with different letters in the same column are important at the $p<0.01$ level.

As seen on Table 2, shoot regeneration rate was recorded between 72.22 and 100%. 100% regeneration was achieved in the MS medium supplemented with 0.05 mg L^{-1} TDZ.

The number of shoots in MS medium supplemented with TDZ and IBA were recorded as statistically significant at the $p<0.01$ level. The best result in terms of shoot number (15.16 shoots/explant) was achieved in MS medium including 0.40 mg L^{-1} TDZ + 0.10 mg L^{-1} IBA, while the least poor result (5.39 shoots/explant) was obtained in MS medium with 0.05 mg L^{-1} TDZ + 0.10 mg L^{-1} IBA. The least count of shoots was determined at the lowest rates of TDZ (0.05 mg L^{-1}).

Shoot lengths were generally short in MS media including TDZ and IBA. The longest shoot (1.28 cm) was obtained in MS medium with 0.05 mg L^{-1} TDZ + 0.10 mg L^{-1} IBA, while the shortest shoot (0.81 cm) was reached on the MS medium with 0.80 mg L^{-1} TDZ + 0.10 mg L^{-1} IBA. Increasing TDZ dose negatively affected shoot lengths.

3.2. Liquid media experiments

The node explants were placed in liquid nutrient medium including 0.05-80 mg L^{-1} TDZ + 0.10 mg L^{-1} IBA. Multiple shoot formations were observed in the second week (Figure 2 a, b and c). The experiment was terminated in the eighth week of culture. Data were analyzed statistically (Table 3).

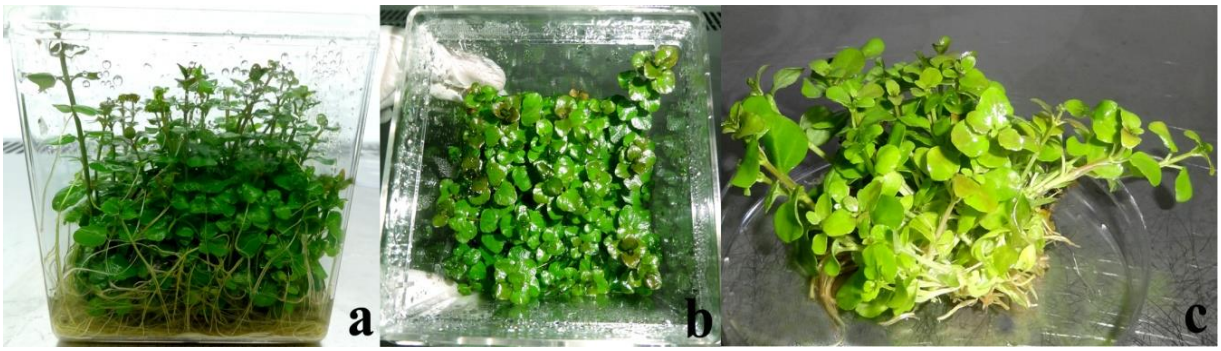

Figure 2. Shoot formations in the in liquid MS medium with IBA + TDZ. After eighth week of culture **(a**, **b**, **c)** Multiple shoot formation in MS medium including 0.40 mg L^{-1} TDZ + 0.10 mg L^{-1} IBA

Table 3. The effect of liquid medium with IBA + TDZ on *in vitro* shoot formations.

Growth regulators (mg L^{-1})		Regeneracy (%)	Average shoot counts	Shoot lengths (cm)
TDZ	**IBA**			
0.05	**0.10**	66.66[a]	3.88[c]	3.53[a]
0.10	**0.10**	66.66[a]	7.16[bc]	3.42[a]
0.20	**0.10**	88.89[a]	10.05[ab]	2.25[ab]
0.40	**0.10**	83.33[a]	12.33[a]	1.94[b]
0.80	**0.10**	72.22[a]	5.84[c]	1.79[b]

Values shown with different letters in the same column are important at the $p<0.01$ level.

Regeneration values in liquid culture medium ranged from 66,66 to 88,89% (Table 3). 88.89% regeneration was reached in MS medium including 0.20 mg L^{-1} TDZ + 0.10 mg L^{-1} IBA. The least regeneration was reached in nutrient medium including 0.05 and 0.10 mg L^{-1} TDZ + 0.10 mg L^{-1} IBA.

The count of shoots per explant in liquid MS nutrient media was recorded between 3.88-12.33 and was recorded to be statistically important ($p<0.01$). The most count of shoots (12.33) was achieved in MS medium including 0.40 mg L^{-1} TDZ + 0.10 mg L^{-1} IBA. Whereas, the lowest shoot count (3.88) was obtained in MS medium including 0.05 mg L^{-1} TDZ + 0.10 mg L^{-1} IBA.

Shoot longs were between 1.79 and 3.53 cm. While the longest shoot (3.53 cm) was obtained in MS medium with 0.05 mg L^{-1} TDZ + 0.10 mg L^{-1} IBA, the shortest shoot (1.79 cm) was recorded in MS medium with 0.80 mg L^{-1} TDZ + 0.10 mg L^{-1} IBA.

Since root formations were obtained in liquid and solid propagation media, *in vitro* rooting study was not carried out. The regenerated plants were transferred to aquariums. In the fourth week, the plantlets were acclimatized to *ex vitro* conditions**.**

4. Discussion

Propagation of plants by tissue culture provides many advantages. For this reason, the transition from traditional production to biotechnological production has accelerated. In this study, *in vitro* production of *L. nummularia* in solid and liquid MS media was investigated.

Preparation of the nutrient medium as liquid or solid/semi-solid is a parameter that affects the regeneration value of explants (Moniruzzaman et al., 2021; Ab Aziz et al., 2021). Similarly, solid/semi-solid and liquid nutrient media trials have been carried out for *Urginea altissima* (Lf) Baker (Baskaran et al., 2018), *Dendrocalamus strictus* (Khare et al., 2021), *Ficus carica* L. (Moniruzzaman et al., 2021), *Begonia pavonina* (Ab Aziz et al., 2021) and *Ananas comosus* (L) Merr (Hamad et al., 2021) before.

In the current study, trials were carried out with different combinations of TDZ + IBA. TDZ is widely used in tissue culture studies. In recent years, tissue culture studies including TDZ have been carried out in some species such as *Ceratophyllum demersum* L. (Emsen and Dogan, 2018), *Pterocarpus marsupium* Roxb. (Tippani and Thammidala, 2021) and *Plumbago europaea* L. (Beigmohamadi et al., 2021).

When compared in terms of shoot regeneration values, higher regeneration values were obtained in solid nutrient medium. These results showed that solid media increased the regeneration abilities of explants compared to liquid media. When the average shoot numbers were compared, the best results were reached in nutrient medium including 0.40 mg L^{-1} TDZ + 0.10 mg L^{-1} IBA in both nutrient media. The most shoot counts in solid and liquid nutrient media were determined as 15.16 and 12.33 shoots/explant, respectively. These results revealed that solid nutrient medium is much more efficient than liquid nutrient medium.

Similarly, Gupta et al. (2020) transferred the nodal explants of *Tylophora indica* (Burm. f) Merrill to liquid and solid MS nutrient medium including different doses of BAP for *in vitro* propagation. They found the best results in terms of shoot number in solid media (13.00 ± 0.25). On the other hand, Yimam (2018) conducted a study for *in vitro* shoot regeneration of *Plectranthus edulis* in liquid and solid nutrient media. More shoots were obtained in the liquid environment than in the solid medium. The greatest number of shoots in liquid and solid nutrient media was recorded as 6.07 and 5.85, respectively. Shekhawat et al. (2015) investigated the *in vitro* micropropagation of *Morinda citrifolia* L. in liquid and semisolid (with agar) MS environment including different doses of BAP, Kinetin and IAA. The most count of shoots was obtained in 2.0 mg L^{-1} BAP + 1.0 mg L^{-1} Kinetin in liquid and semi-solid environments, respectively, as 11.4 ± 0.47 and 10.6 ± 0.17. The number of regenerated shoots of *Ajuga multiflora* Bunge was found to be higher in liquid medium compared to solid medium (Sivanesan et al., 2016).

When the shoot lengths were compared, it was seen that the liquid medium had higher length values than the solid medium. The maximum length was determined as 3.53 cm in liquid nutrient medium and 1.28 cm in solid nutrient medium. Shoots almost 3 times longer were obtained in liquid nutrient medium. This may be due to the fact that the liquid medium is more favourable for the elongation of the plant. Since *L. nummularia* is an aquatic plant, it may have given longer shoots in liquid

medium. Similarly, Rezali et al. (2017) conducted experiments at different MS levels (1/2, 1/4 and Full) and in liquid and solid media for *in vitro* multiproduction of *Typhonium flagelliforme*. The longest shoots at all MS levels were obtained in liquid nutrient medium. On the other hand, Yimam (2018) obtained longer shoots in solid medium than in liquid medium. Shekhawat et al. (2015) compared the lengths of regenerated shoots of *M. citrifolia* in semi-solid and liquid media and determined the longest shoot in solid MS medium.

Considering the effect of TDZ on shoot lengths, shoot lengths decreased with increasing TDZ concentration in both culture media. Similarly, the negative effects of TDZ on shoot length values have been reported in *Rauvolfia tetraphylla* (L.) (Hussain et al., 2018), *C. demersum* (Dogan, 2019b).

4. Conclusion

In this study, *in vitro* shoot regeneration of *L. nummularia* was investigated in solid and liquid nutrient media. Multiple shoots were obtained successfully in both culture media. The best results in terms of shoot number and regeneration values were determined in solid MS medium. The best results for shoot lengths were determined in liquid MS medium. In addition, the negative effects of high values of TDZ on shoot length were also recorded. These results may help the efficient production of *L. nummularia*, a medicinal and aromatic plant, by tissue culture. In addition, this study may contribute to the production of secondary compounds by the multiple productions of *L. nummularia*.

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Author Contribution

Muhammet DOĞAN designed the experiments and wrote the paper.

Conflicts of Interest

No potential conflict of interest was reported by the author.

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