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Microbulb and plantlet formation of a native bulbous flower, *Lilium monodelphum* M. Bieb, *var. Armenum,* through tissue culture propagation

F.N. AZERİ [a,*], G. Öztürk [b]

[a] *Gümüşhane University Kelkit Aydın Doğan Vocational School, Ahmet Yesevi neighborhood, Aybuke Street, no 6-B 29600 Kelkit, Gümüşhane, Turkey*
[b] *Ege University Faculty of Agriculture Department of Field Crops, 35100 Bornova, İzmir, Turkey*

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ABSTRACT

The plant *Lilium monadelphum* M. Bieb. var. *armenum* (Mıscz. Ex Grossh) Davis et Henderson recognized as the caucasian lily grows in the Eastern Black Sea Region and is a perennial bulbous species belonging to the Liliaceae family. Its flowers, bulbs, and volatile oils are used in the perfume industry, as a folk medicine to treat eczema and abscess therapy, and as a tranquilizer among regional people respectively. Hence, it is commonly collected from the wild and could become endangered. In this study, we explored the optimal hormone treatment for efficient and rapid induction and production of this species in tissue culture. Explants taken from bulbs were cultured in Murashige and Skoog medium (MS medium, 1962) and modified with TDZ (thidiazuran) (0.5; 1.0; 2.0; 3.0; 5.0 mg/L), after which adventitious buds used for explants during the second stage were subcultured 4 different media supplemented with different concentrations of PAC (paclobutrazol) (0.1; 0.5; 1.0; 2.0 mg/L) to induce microbulbs formation. Microbulbs were cultured in a rooting medium containing IBA (0.5;1.0 mg/L). The highest bud regeneration was observed in medium containing 3.0 mg/L TDZ, 0.25 mg/L NAA and 0.1 mg/L GA3; the highest microbulb formation was observed in medium containing 2.0 mg/L PAC, 0.2 mg/L NAA, and 0.1 mg/L GA3. Besides, plant development from the microbulb was successful for roots in a medium containing 0.5 mg/I IBA and all of the plantlets obtained in tissue culture survived when transferred to the soil.

1. Introduction

The genus Lilium, which belongs to the Liliaceae family consists of about 100 species distributed in the Northern Hemisphere. Species in this genus have remarkable ornamental value worldwide because they produce colorful attractive flowers. Also, because some species contain aromatics and other useful substances, they are of interest in the perfume and medicine industries. In northern, Turkey which is part of the Euro-Siberian phytogeographical region, this genus is represented by eight species. Six of these have restricted geographical ranges in this region, and their individual population is small with a scattered distribution [1;,2] In recent years, the already limited of Lilium species have decli,ned due to excessive collection in the wild for their forementioned benefits, and attractive appearance. One of these species, *Lilium monadelphum* M.Bieb var. *armenum*, is examined in this report. Known colloquially as the Zigana lily (Caucasian lily). İt is distributed in northeast Anatolia [3]. Zigana lily is a perennial bulbous plant with yellow flowers [4], which make them a major cut flower crop. Its flowers have ornamental value. It also has medicinal characteristics and is commonly used in folk medicine. It contains tannin and mucilage and is effective against eczema. Lily bulbs are used as blisters and ointment. Its volatile oil has sedative effects [5], and its pressed bulbs are used as a medicine [6]. However, this species is listed as endangered on the IUCN Red List of Threatened Species [2] and is considered to be at high risk of extinction in the wild. In recent years, collecting wild species from their natural habitats and exporting them has been forbidden. To replenish the number of these wild species, they should be propagated as cultivated crops. In vitro techniques and novel propagation systems, have been used for the production of several plant species and are superior to traditional production techniques. The purpose of micropropagation techniques using tissue cultures is to rapidly propagate endangered species, such as *Lilium monadelphum*. Such techniques can rapidly increase plant numbers and achieve mass production. Other advantages of micropropagation over traditional plant propagation are that it requires very little material, does not damage the environment, can be conducted in a small production area, may be used to produce species that are difficult to propagate, can produce plants throughout the entire year, and can utilize any part of the plant for production. The previous investigations have indicated the possibility of using tissue culture techniques to commercially produce Lilium species [7; ,8;,9;,10]. However,

* Corresponding author.

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no study has analyzed the production of *Lilium monodelphum*. Bieb var. *armenum*.

2. Materials and methods

Plant material was collected from natural habitats in Konacık-Ozkurtun, Gumushane. Lily bulbs were maintained in the refrigerator (+4 C^0) for 2 to 2.5 months, wrapped in wet paper, and stored in plastic bags. Before applying culture practices, bulb scales were separated., washed under tap water, and maintained in the 70% ethyl alcohol and 25% NaOCl solution for 10 min for surface sterilization. Then they were washed by shaking three times in distilled water, after which all of the cultures were incubated in a dark light cycle of 8 8 h:16 16 h under 1600 lux light. The average temperature was maintained at 20-22 °C. The culture process included three stages. First, adventitious buds were induced. Explants taken from the bulb scales were latitudinally cut inoculated adaxial side upward, and cultured in five different media, all of which included thidiazuran(TDZ) to induce regeneration: MS medium [11] with media to induce regeneration 0.25 mg/L NAA, 100 mg/L inositol, 0.4 mg/L Thiamine, 0.1 mg/L gibberellic acid (GA3) and TDZ at 0.5, 1, 2, 3, and 5 mg/L (denoted T1-T5, respectively). GA3 was used because it can break dormancy in some bulbous plants, such as hyacinth [12], garlic [13;,14], and Lilium genus [15–17]. Explant that formed adventitious buds were observed after 35 days.

In the second stage, adventitious microbulbs were proliferated. Buds explants obtained from first stage scales were subcultured in four different nutrient media to induce microbulb formation: MS containing 0.2 mg/L NAA, 0.1 mg/L GA3, and paclobutrazol (PAC) at 0.1, 0.5, 1, and2 mg/L (denoted P1-P4, respectively). Eight weeks later, the number of newly formed microbulbs was recorded. In the third stage, microbulbs developed in PAC medium were subcultured in a rooting medium containing 0.1 mg/L IBA. Developed plantlets were observed and measured. The data obtained were analyzed using the statical procedure described by Steel et al [18], and the means were compared and grouped using the LSD test at the p=0.05 level.

3. Result and discussion

Medium T4 (containing 3 mg/L TDZ, 0.25 mg/L NAA, and 0.1 mg/L GA3) produced the largest mean number of buds, at 11.67. this was significantly more than the mean numbers produced by the other media tested (Table 1). The means of the T1 and T2 media were 5.6 and 7.13, respectively, and were not significantly different. The mean of theT3 medium was 7.87 which was significantly different from those of T1 and T2. The mean bud number obtained in this study was comparatively higher than the value (5.7) reported for 2 mg/L TDZ medium. Sharma et al. [19] reported that MS basal medium supplemented with 0.004 mg/L TDZ was most effective for the induction of bud formation. In oriental lily cultivars, however, their mean value of 5.91 was still much lower than our results for T4 (11.67). The microbulb numbers developed on the unroot seedling plants grown at media containing various PAC (Paclobutrazol) are shown in Table 2. İt could be observed in Table 2 that the medium P4 (2.0 mg/l PAC + 0.2 mg/l NAA + 0.1 mg/l GA3) had the highest number of bulblet number (15.83). These means were significantly different from the means of the other media. The result of the ANOVA for the root and the seedling development of the microbulbs cultured at the rooting media containing IBA (0.5 mg/l) are shown in Table 3. The significant F values shown in this table indicate that there is a significant variation for root number and seedling number. Therefore, the means were compared by the LSD test and shown in Table 4.

It could be seen in Table 4 that the R2 and R3 media had higher means than the R1 media for root number and seedling number. The difference was significant. Therefore, it could be concluded that the R2 and R3 media could be used effectively to increase bulblet of *Lilium monodelphum* Bieb var. *armenum* in vitro.

TDZ is a potent cytokinin for plant tissue culture. The cumulative TDZ concentration increases the encouragement of explants for forming shoot bud [20]. Hare and Van Staden [21] reported that TDZ has the capacity to inhibit the action of cytokinin oxidase, which in turn may increase the level of endogenous cytokinins. They also reported that TDZ concentration which higher than 2,2727 27 μM, encourages the shoot buds, and lower than 2,27 ensures fewer. It has been previously reported that TDZ plays an active role in direct multiple shoots and somatic embryo induction [22]. The concentration of TDZ in vitro significantly affects regeneration response depending on plant orientation, explant, and genotype. Kumar et al. [23] reported that TDZ increased the number of explants having shoot bud induction and the value reached the highest level in the medium enriched with 9 μM TDZ. They also stated that the effectiveness of TDZ does not differ between Jatropha curcas genotypes. In our study, we concluded that 3 mg (13.6 micromolar) TDZ stimulates direct adventitious bud formation from bulb explants of *Lilium monodelphum armenum* more than other concentrations which we use, 1, 2, and 5 mg/L. Contrary to this positive effect, the medium where it was added at a rate of 5 5 g/L caused fewer shoot buds than in control. Youssef et al. obtained the highest mean value of direct shoot regeneration (5,7) from the leaf explants of the Lilium orientalis cv starfighter plant from the medium supplemented with 0.5 mg / L TDZ and 10 mg / L 2,4-D. We recorded the highest mean number of adventitious buds as 11.67 in the medium supplemented with 3 mg / L TDZ, 0.25 mg/L NAA, and 0.1 mg/L GA3 using bulb scales as explants. In this case, in our opinion, the bulb explants and higher TDZ value were more beneficial. The addition of a low concentration of auxin to the medium together with a high concentration of cytokinin affects cell division and provides regeneration in vitro [24]. Therefore, the addition of NAA to the medium with cytokinin at low concentrations during the growth phase brings to a successful conclusion. Nowakowska et al. [25] in their study on the regeneration of the Daphne mezereum plant, observed that the MS medium to which cytokinin and 0,1 mg/L NAA were added together

Table 1
Means number of adventitious buds per bulb scale explant developed from media containing different doses of TDZ.

Medium no	Combination and Concentration (mg/L)	Means number of adventitious buds
T1	0.5 mg/l TDZ+ 0.25 mg/l NAA + 0.1 mg/lGA_3	5.60 b
T2	1.0 mg/l TDZ + 0.25 mg/l NAA + 0.1 mg/GA_3	7.13 b
T3	2.0 mg/l TDZ + 0.25 mg/l NAA + 0.1 mg/GA_3	7.87 ab
T4	3.0 mg/l TDZ + 0.25 mg/l NAA + 0.1 mg/GA_3	**11.67 a**
T5	5.0 mg/l TDZ + 0.25 mg/l NAA + 0.1 mg/GA_3	5.06 b

a LSD (P≤ 0.05): 4.145
a F-Value: 4.206*
a * significant at the P≤ 0.05 probability level

Table 2
The means of the microbulb obtained from the different PAC containing media.

Medium no	Concentration (mg/l)	Mean microbulb number
P1	0.1 mg/l PAC + 0.2mg/l NAA + 0.1 mg/l GA_3	6.00 b
P2	0.5 mg/l PAC + 0.2mg/l NAA + 0.1 mg/l GA_3	7.33 b
P3	1.0 mg/l PAC + 0.2mg/l NAA + 0.1 mg/l GA_3	8.67 b
P4	2.0 mg/l PAC + 0.2mg/l NAA + 0.1 mg/l GA_3	**15.83 a**

a LSD (P≤ 0.05): 5.151
a F-Value: 8.698*
a*significant at the P≤ 0.05 probability level

Table 3
The result of the ANOVA for root number and seedling number of microbulbs cultured at the media containing IBA.

Characteristics	Mean squares Medium	Error	F -value
Root number	16.871	1.364	12.365*
Seedling number	14.710	2.083	7.06*

a * significant at the P 0.05 probability level

Table 4
The means of the root number and seedling number of microbulbs cultured at media containing IBA (0.5 mg/l).

Medium Cod number	Concentration	Characteristics Root number	Seedling number
R1	0.0 mg/l (Control)	1.733 b	2.433 b
R2	0.5 mg/l IBA	**6.400 a**	**6.533** a
R3	1.0 mg/l IBA	5.933 a	5.933 a
LSD P≤ 0.05		2.648	3.272

serves the best efficiency especially on the number of shoots. Besides, GA3 which stimulates internode elongation as well as differentiation of wood and cambium is overutilized in studies as a growth regulator [26]. Previously, it has been reported that adding 0.1 mg / L GA3 to the medium together with cytokinin and auxin provides the longest shoots [25]. Similarly, the addition of 0.25 mg/L NAA and 0.1 mg/L GA3 to the medium with TDZ yielded beneficial results in our study. For adventitious buds regeneration of Lilium monodelphum and other similar species in tissue culture, we can recommend adding 3 mg / l TDZ, 0.25mg NAA, and 0.1 mg GA3 to the medium.

It is known that Paclobutrazol (PAC) play an important role in enhancing the accumulation of soluble carbohydrates and starch [27]. The treatments with paclobutrazol led to a shift in the partitioning of assimilates from the leaves to the storage organs and roots and increased chlorophyll and carbohydrates in all parts of seedlings [28]. Wang et al. [29] reported that paclobutrazol-treated Lilium plantlets showed better bulb formation, resulting in a greater percentage of survival ex vitro. In a study conducted to facilitate of Lilium liquid cultures, 3.4 4 μM (approximately 0.9-1 mg) PAC gave successful results. It was also concluded that 3 and 6 mg/L PAC significantly increased bulb formation in value of 2.99 and 2.84 (bulblets/explant), respectively as compared to other treatments [30]. In our study, 2 mg PAC we added to the medium together with 0.2 mg/L NAA and 0.1/L mg GA3 displayed the highest mean microbulb number value of 15.83. Also, the best medium for root generation was a medium containing 0.5 mg / L IBA. All plants acclimated to in vivo could also be grown in a healthy way.

4. Conclusion

If there were no plants, we would not have survived here, on earth. Being near plants make us feel good. For centuries, we have used plants from the natural world as medicine, for nutrients, and as a way of escapism from the stressors of daily life. we owe them a lot. But today, the existence of these delicate looking creatures is under threat due to various factors. Often some of us try to protect them from danger and extinction in various ways. One of these ways is to successfully reproduce endangered, threatened, and rare species in vitro. Many researchers are at work in determining the optimum breeding conditions. Only the visuality of the species belonging to the Lilium genus is enough to want them to be reproduced. Not only its visuality but also its use in medicine attracts people to itself. Thereby in our study, we focused on the in vitro reproduction of *Lilium monadelphum var. armenum* which is one of these beauties. The addition of 2 mg/L PAC to the culture medium in the formation of microbulb and 3 mg/L TDZ in adventitious buds regeneration yielded positive results in the propagation of *Lilium monadelphum var. armenum*. Consequently, the result of our study is a successful prescription for in vitro micropropagation of Lilium species. We wish it to provide a reference for the production of other beautiful Lilium species.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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