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IN VITRO BULBLET REGENERATION FROM *SCILLA SIBERICA* HAW. SUBSP. ARMENA (GROSSH.) MORDAK PEDUNCLE

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Abstract

Attractive blue-flowering *Scilla siberica* subsp. *armena* bulbs multiply very slowly in 4-5 years under natural conditions. Therefore, this study aimed to accelerate multiplication by devising a strategy for an efficient *in vitro* bulblet regeneration system using peduncle and bulb scale explants on MS medium containing 0.25, 0.50, 1.00, and 2.00 mg l^{-1} TDZ, plus 0.10 or 0.20 mg l^{-1} 2,4-D (8 combinations). Bulb scale explants were difficult to culture due to high fungal infection and were, therefore, discarded. The peduncle explants induced direct bulblet regeneration after swelling on explants. Maximum mean number of bulblets and bulb diameter was acheived on MS medium containing 1.00 mg l^{-1} TDZ + 0.20 mg l^{-1} 2,4-D. The regenerated bulblets were isolated from peduncle explants and cultured on MS medium containing 40 g l^{-1} sucrose, where they grew in diameter and rooted.

Key words: Bulb diameter, endangered, mass propagation, TDZ, 2,4-D

INTRODUCTION

Bulbous geophytes including *Scilla* L. are commonly used for ornamental, aromatic, and medicinal (including folk medicine systems) purposes since ancient times (Satil et al. 2006, Ozel et al. 2010). *Scilla* is represented in Turkey by 14 taxa of glabrous bulbous perennial plants (Davis 1984). Most species of the genus except *Scilla siberica* Haw. subsp. *armena* (Grossh.) Mordak are commonly used as garden plants, and the bulbs of most of them are commercially available in Turkey or elsewhere.

S. siberica subsp. *armena* is perennial blue-flowering bulbous plant with vegetation period from February to May and a long period of dormancy (Mirek et al. 2002). It is used as forage plant by local people. It is polinated by bees for collection of nectar. *S. siberica* subsp. *armena* is an Irano-Turanian element with its geographical distribution encompassing Turkey, Georgia, and Armenia (Mordak 1984). It grows on open northern mountainous slopes of Bingol province in South Eastern Turkey after melting of snow, close to rivers or streams. Major prevailing threats to the plant include goat grazing and trampling by picknickers, who pluck the plants for fun.

Previous studies on chemical composition of different *Scilla* species revealed triterpenoids (Mimaki et al. 1999), stilbenoids (Bangani et al. 1999), cardiac glycosides (Kamano and Pettit 1974), polyhydroxylated alkaloids as well as homoisoflavanones, which are anti-angiogenic, antibacterial and inhibit the growth and sporogenesis of some microorganisms *in vitro* (Silayo et al. 1999, Lee et al. 2002, Mutanyatta et al. 2003, Shim et al. 2004, Yeo et al. 2006).

Despite its importance, very little work has been done generally on propagation, and specifically on tissue culture and micropropagation of *S. siberica* subsp. *armena* (Hussey 1977, Deumling and Clermont 1989). Therefore, this study aimed to develop and optimise a bulblet regeneration protocol using peduncle explants.

MATERIALS AND METHODS

Plant material collection

The plant material was collected from the moist land lying between Yelesen and Şaban villages (Bingol

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province, South Eastern Turkey) on northern slopes at 1400-1500 m altitude. The identification was based on "The Flora of Turkey and East Aegean Islands" (Davis 1984). Voucher specimens were deposited at the herbarium of the Department of Biology, Hacettepe University Ankara and the Department of Park and Garden Plants of Bingol University, Turkey.

The peduncles from the plants were washed under running tap water for 25 min to remove all adhering contaminants. Thereafter, both 5-6 g bulbs and peduncles of these plantlets were treated with 35% H_2O_2 solution for 30 min under aseptic conditions followed by 5 × 5 min rinsing with sterilized distilled water. Thereafter, ~ 0.5 cm long peduncle explants and twin bulb scales were excised aseptically. They were cultured on Petri dishes on 0.65% (w/v) plant agar (Duchefa) solidified MS medium (Murashige and Skoog 1962) containing 0.25, 0.50, 1.00 or 2.00 mg l^{-1} (1-Phenyl-3-(1,2,3,-thiadiazol-5-yl)urea (Thidiazuron; TDZ) plus 0.10 or 0.20 mg l^{-1} 2,4-Dichlorophenoxyacetic acid (2,4-D) (8 combinations) supplemented with 3% sucrose to regenerate bulblets. Plant growth regulator (PGR)-free MS medium was used as a control. All cultures were maintained under 16 h light photoperiod (35 μmol m^{-2} s^{-1}) in Aralab versatile growth chamber (Praha, Czech Republic) at 24 ± 1°C. Well developed bulblets on peduncle explants were rooted on MS medium supplemented with 40 g l^{-1} sucrose. All media were autoclaved for 20 min at 121°C (118 kPa nominal steam pressure). The pH of all media was adjusted to 5.7 ± 0.1 with 1 N NaOH or 1 N HCl.

Each treatment contained 60 explants divided into 6 replications with equal numbers of explants. The experimental data were subjected to one-way Analysis of variance and Post- hoc Tukey's *b* test using IBM SPSS version 20 for Windows. All values given in percentage were arcsine transformed before analysis following Snedecor and Cochran (1989).

RESULTS

All bulb scales contained *Fusarium* fungal contamination and were, therefore, discarded. Furthermore, different concentrations of TDZ and 2,4-D for bulblet regeneration induced swellings (not callusing) on peduncle explants of *S. siberica* subsp. *armena* before regeneration after 17-20 days of culture initiation. All combinations and rates of TDZ + 2,4-D induced swelling variably. It ranged from 70.3 ± 0.5% to 100.0 ± 0.0% (Table 1) with no swelling on control treatments (Table 1).

The maximum swelling was obtained on MS medium containing 0.25 mg l^{-1} TDZ + 0.20 mg l^{-1} 2,4-D followed very closely by MS medium containing 0.25 mg l^{-1} TDZ + 0.10 mg l^{-1} 2,4-D and 2.00 mg l^{-1} TDZ + 0.10 mg l^{-1} 2,4-D.

All peduncle explants regenerated bulblet on MS medium four months after culture establishment. The results showed that the combinations and concentrations of TDZ and 2,4-D sharply affected bulblet regeneration percentage. Bulblet regeneration ranged between 41.7 ± 0.6% to 100.0 ± 0.0% (Table 1). Furthermore, equal to or over 61.3 ± 0.7% bulblet regeneration was noted on 6 treatments. The highest bulblet regeneration percentage was obtained on MS medium containing 1.00 mg l^{-1} TDZ + 0.10 mg l^{-1} 2,4-D (Fig. 1A) followed very closely by MS medium containing 2.00 mg l^{-1} TDZ + 0.10 mg l^{-1} 2,4-D. Regeneration on the rest of the culture media was sharply reduced and never exceeded 74.7 ± 0.2%. Bulblet regeneration showed irregular patterns of growth on MS medium containing TDZ + 0.10 or 0.20 mg l^{-1} 2,4-D and was not parallel to explant swelling percentage (Table 1).

The results further showed that combinations and rates of plant growth regulators used in the study also affected the mean number of bulblets. The peduncle explants were found very suitable for bulblet regeneration

Table 1. Effect of different concentrations of TDZ + 2,4-D on MS medium on bulblet regeneration from peduncle explants of *S. siberica* subsp. *armena*.

Treatments		Explant swelling (%)	Bulblet regeneration (%)	Mean number of bulblets	Mean bulblet diameter (cm)
TDZ (mg l^{-1})	2,4-D (mg l^{-1})				
MS medium (control)		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
0.25	0.10	97.9 ± 1.0 b	61.3 ± 0.7 e	9.9 ± 0.2 g	0.1 ± 0.0 c
0.50	0.10	87.7 ± 0.9 c	74.7 ± 0.2 c	11.0 ± 0.4 f	0.1 ± 0.0 c
1.00	0.10	73.7 ± 0.5 e	100.0 ± 0.5 a	17.9 ± 0.5 d	0.1 ± 0.0 c
2.00	0.10	97.7 ± 0.6 b	92.9 ± 0.8 b	13.0 ± 0.7 e	0.1 ± 0.0 c
0.25	0.20	100.0 ± 0.0 a	41.7 ± 0.6 g	19.7 ± 0.4 c	0.2 ± 0.1 a
0.50	0.20	78.3 ± 0.9 d	54.3 ± 0.4 f	23.3 ± 0.5 b	0.1 ± 0.1 b
1.00	0.20	70.3 ± 0.5 f	61.7 ± 0.6 e	37.0 ± 0.4 a	0.2 ± 0.1 a
2.00	0.20	93.3 ± 0.9 b	67.3 ± 0.6 d	24.0 ± 0.6 b	0.1 ± 0.1 b

Means ± standard error within a column followed by different letters are significantly different according Tukey's *b* test at 0.01 level of significance.

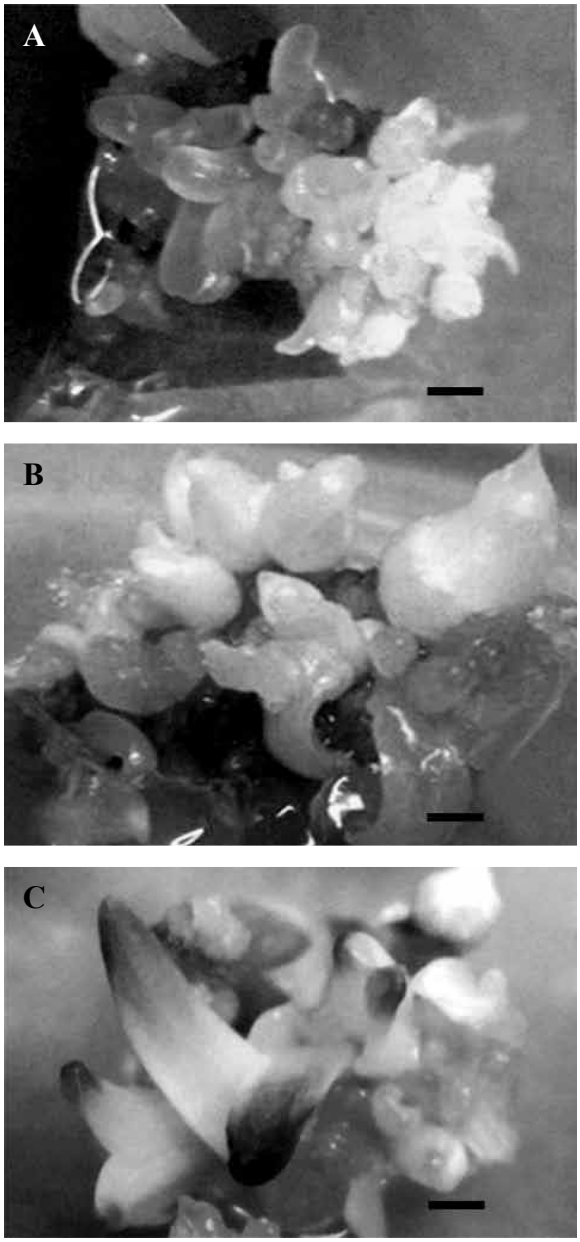

Fig. 1. Bulblet regeneration on peduncle explant of *Scilla*. A) Using MS medium containing 1.00 mg l^{-1} TDZ + 0.20 mg l^{-1} 2,4-D, B) Developing and growing bulblets, C) Development of green leaves on bulblets on MS medium containing 1.00 mg l^{-1} TDZ + 0.20 mg l^{-1} 2,4-D (Bars A, B, C = 0.3 cm).

and the bulblets were induced directly on these explants.

The mean number of bulblets ranged between 9.9 ± 0.2 and 37.0 ± 0.4. Maximum number of bulblets (37.0 ± 0.4) was recorded on MS medium containing 1.00 mg l^{-1} TDZ + 0.20 mg l^{-1} 2,4-D. It was followed by a significantly reduced number of 24.0 ± 0.6 and 23.3 ± 0.5 bulblets on MS medium containing 2.00 mg l^{-1} TDZ + 0.20 mg l^{-1} 2,4-D and 0.50 mg l^{-1} TDZ + 0.20 mg l^{-1} 2,4-D, respectively (Table 1). Any variant of TDZ + 0.20 mg l^{-1} 2,4-D had visible positive impacts on bulblet induction. The maximum number of bulblets in respective groups was induced by 1.00 mg l^{-1} TDZ + 0.10 or 0.20 mg l^{-1} 2,4-D (Table 1). Mean number of bulblets showed a linear increase for bulblet regeneration on MS medium containing 0.25 to 1.00 mg l^{-1} TDZ + 0.10 or 0.20 mg l^{-1} 2,4-D. It was followed by a sharp reduction in bulblet induction on MS medium containing 2.00 mg l^{-1} TDZ + 0.10 mg l^{-1} 2,4-D or 2.00 mg l^{-1} TDZ + 0.20 mg l^{-1} 2,4-D, in respective groups. The explants regenerated shoots after development of bulblets (Fig. 1B,C).

Mean bulblet diameter varied in the range 0.1-0.2 cm (Table 1). The maximum mean bulblet diameter (0.2 cm) was recorded on MS medium containing 1.00 mg l^{-1} TDZ + 0.20 mg l^{-1} 2,4-D. Bulblet diameter equal to 0.1 cm or above was recorded in 6 treatments.

A positive increase in bulblet diameter was recorded on MS medium containing 40 g l^{-1} sucrose at 4°C, where bulblet diameter of 0.5-0.6 cm was induced within nine weeks.

DISCUSSION

Bulb scales are the most common explants used for *in vitro* propagation of geophytes (Nasircilar et al. 2011, Kizil et al. 2014, Ozel et al. 2015), but peduncle explant is not commonly used for *in vitro* propagation of geophytes (George and Tripepi 2004, Nhut et al. 2012). The use of peduncle explant is advantageous since bulbs as a source of explants are often associated with heavy bacterial and destructive fungal contaminations (Langens-Gerrits et al. 1998, Ziv and Lilien-Kipnis 2000). It is assumed that as the bulbs were collected from moist soils, they may have become an habitat optimum of fusarium, which was proven by the heavy infestation when they were grown on a medium containing sucrose.

No contamination was observed on cultured peduncles, which can serve as an excellent source to obtain contamination-free explants. In micropropagation protocols, the multiplication phase is perhaps the most important part in terms of practical use of tissue culture. The results of this study showed that use of peduncles for the micropropagation of *S. siberica* subsp. *armena* may have great value. It was shown that ~ 0.5 cm long peduncle explants has capacity to induce average of 9.87 to 37 new bulblets. No previous study was found about the regeneration of *S. siberica* subsp. *armena* for comparison. Previous studies on other bulbous plants (Malabadi and Van Staden 2004, Suh et al. 2005, İpek et al. 2009) and other species like *Scutellaria orientalis* (Ozdemir et al. 2015) recorded that the media containing TDZ induce positive effects on regeneration capacity. Also TDZ did not effectively promote bulblet regeneration in *Muscari aucheri* (Uranbey 2010) and *Muscari azureum* (Uranbey et al. 2010). Results of the present study differ from these studies, but are in agreement with Huetteman and Preece (1993) who reported

that TDZ increased bulblet regeneration. The contrasting results may be related with different endogenous PGRs in explants obtained from different types of geophyte species used in the above mentioned reports.

In the present study high bulb regeneration was obtained with media using 1.00 mg l^{-1} TDZ + 0.20 mg l^{-1} 2,4-D. Similarly Nasircilar et al. (2011) reported positive increase in mean number of bulb regeneration when TDZ was used with NAA. Our results not in agreement with this finding and showed efficient bulblet regeneration from peduncle explants using TDZ + 2,4-D.

Efficient bulblet regeneration has been reported for many geophytes such as *Ornithogalum oligophyllum* (Ozel and Khawar 2007), *O. ulophyllum* (Ozel et al. 2008), and *Fritillaria thunbergii* (Paek and Murthy 2002) from various explants. However, the mean number of regenerants in all these studies remained much lower than that obtained in the present study. A part from bulb scales, a range of explants including stem nodes, leaves, mature seeds, and thin cell layers have also been used for *in vitro* bulblet production in geophytes (Ozel and Khawar 2007).

The bulblets rooted without treatment with auxin in contradiction to previous studies where auxins were recommended for rooting of *in vitro* cultured plants (Yildirim 2013, Ozdemir et al. 2014).

The commercial production of disease-free new uniform lines of bulbous geophytes is generally inhibited by low propagation rates under natural conditions. Micropropagation can help in solving this problem.

The findings of the study confirm that the peduncles can be used successfully as explants and can form an alternative source for regeneration without destruction of stock plant and positively evaluate potential of expediently isolated explants from inflorescence stems for an efficient micropropagation.

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