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DIRECT BULBLET REGENERATION FROM *STERNBERGIA FISCHERIANA* (HERB.) RUPR. BULB SCALE EXPLANTS

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Abstract

Attractive golden yellow flowered *Sternbergia fischeriana* (Herb.) Rupr. multiplies very slow under natural conditions. The study reports multiplication of plants using 0.5, 1.0, and 1.5 cm long bulb scale explants with two, three, four, and five scales attached by a thin base plate segment. Any concentration of 2,4-D 1.0, 2.0, 3.0, 4.0, and 5.0 mg l^{-1} in MS medium was ineffective to induce bulblet regeneration on any explant at 15° ± 1°C. Discursive induction of one or two 0.1 cm diameter bulblets was noted at 24 ± 1°C on 0.5 cm long two-scale explants. Variable regeneration was observed on 0.5, 1.0, and 1.5 cm long two-scale explants on MS basal medium containing combination of BAP and 0.2 mg l^{-1} NAA. Maximum number of 5.0 ± 0.5 bulblets per 0.5 cm long two-scale bulb explant was obtained on MS medium containing 8.5 mg l^{-1} BAP plus 0.20 mg l^{-1} NAA. The rooting as affected by the size of bulblet was achieved on MS medium containing 0.75 mg l^{-1} NAA. The highest rooting was recorded on 0.47 cm diameter bulblets with 4.3 ± 0.9 roots per bulblet and 3.7 ± 0.4 cm long roots.

Key words: bulbous plant, *in vitro* micropropagation, rooting

INTRODUCTION

Sternbergia species (Amaryllidaceae) occur naturally in the Mediterranean region, Central Europe, Central and Western Asia including Turkey, and Northern Iran (Davis et al. 1984, Parmaksiz and Khawar 2006).

Sternbergia fischeriana (Herb.) Rupr. with attractive beautiful golden yellow flowers that open during early spring to autumn (Zencirkiran and Tumsavas 2006) is very popular in Turkish ornamental cut flower industry (Arslan et al. 2002, Zencirkiran 2002, Mirici et al. 2005). It also has high potential as garden and pot plant, where it can be used in rock gardens and as border plant. *S. fischeriana* is very rich in tazettin, lycorin, belladin, galanthamin, etc., with known antitumor, antiviral, antimicrobial, anticholinesterase and antileukaemial activities (Gabrielsen et al. 1992, Weniger et al. 1995, Barthelmes et al. 2001, Baxendale et al. 2002). Under favourable environmental conditions, these plants take more than 3 years to mature, flower and set seeds. The bulbs multiply very slowly and add only 1-2 offset bulblets in a 3-year period (Arslan et al. 2002), which inhibits their large-scale multiplication (Arslan et al. 2002, Zencirkiran 2002, Zencirkiran and Tumsavas 2006). Unless alternative faster propagation techniques are developed, there is limited probability of using the species in ornamental or pharmaceutical industry. Micropropagation of *S. fischeriana* bulblets could serve as a possible alternative method to strengthen their mass and easy commercial propagation.

In vitro micropropagation has been reported previously for many geophytes including *Lilium longiflorum* Thunb. (Nhut 1998, Nhut et al. 2002), *Lilium nepalense* D. Don (Wawrosch et al. 2001), *Fritillaria thunbergii* Miq. (Paek and Murthy 2002), and *Lilium candidum* L. (Khawar et al. 2005, Sevimay et al. 2005) from a range of explants. There are only two reports on tissue culture of *Sternbergia* species (Mirici et al. 2005, Parmaksiz and Khawar 2006), the former reports regeneration of *S. fischeriana* through immature zygotic embryos, two- or four-scale explants and the later describes *Sternbergia candida* regeneration using immature seeds. No report describes the effects of the length and number of bulb

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scales on regeneration. This suggests a need to develop an improved micropropagation method to broaden the scope of *S. fischeriana* proliferation. Therefore, the present study aimed to develop an efficient mass proliferation system of *S. fischeriana* using 0.5, 1.0, and 1.5 cm long two-, three-, four- and five-scale bulb explants.

MATERIALS AND METHODS

Surface disinfection of bulbs and experiments

The study made use of 2 - 3 cm diameter bulbs of *S. fischeriana* collected from the experimental fields of the Field Crops Department, Dicle University (37°56' N, 40°17' E; 696 m a.s.l.), Diyarbakir, Turkey, during 2010. After removing attached roots, bulbs were washed in slow flowing tap water to get rid of adhering soil and dirt. They were dried over blotting papers at room temperature (25° ± 1°C) for 3 h followed by storage at 4° ± 1°C for 30 days in dark. Thereafter, the bulbs were peeled off to select healthy and disease-free material to minimize contamination during disinfection. The bulbs were surface disinfected with 100.0% (v/v) domestic bleach (Ace - Istanbul, Turkey, containing 5% (v/v) NaOCl) for 10, 15, 20, 25, and 30 min followed by rinsing with sterilized bidistilled water for 5 × 5 min.

Each bulb was cultured on 35 ml of MS (Murashige and Skoog 1962) basal medium by adding 30.0 g l^{-1} (w/v) sucrose that was solidified with 6.2 g l^{-1} (w/v) agar (Duchefa, Haarlem, The Netherlands) for 7 days to determine optimum duration of time to treat the explants with commercial bleach for surface disinfection. Subsequently, these bulbs were sliced longitudinally to obtain (i) 0.5, 1.0, and 1.5 cm long two-scale bulb explants (12 explants per bulblet), (ii) 0.5, 1.0, and 1.5 cm long three-scale bulb explants (eight explants per bulblet), (iii) 0.5, 1.0, and 1.5 cm long four scale bulb explants (four explants per bulblet) and (iv) 0.5, 1.0, and 1.5 cm long five scale bulb explants (four explants per bulblet) attached by a thin segment at the base plate. All explants were 0.4 - 0.5 cm wide.

These 4 × 3 = 12 type of explants were cultured on MS medium containing variants (1.0, 2.0, 3.0, 4.0, and 5.0 mg l^{-1}) of 2,4-dichlorophenoxyacetic acid (2,4-D) or variants (0.5, 2.5, 4.5, 6.5, and 8.5 mg l^{-1}) of 6-benzylaminopurine (BAP) with or without 0.20 mg l^{-1} α-naphthalene acetic acid (NAA). Each culture medium was supplemented with 30.0 g l^{-1} sucrose.

Each type of explant was cultured on each type of mass proliferation medium solidified with 6.2 g l^{-1} agar. They were further incubated at 15° ± 1°C and 24° ± 1°C in sterile Magenta GA^7 vessels for 60 days.

The effect of bulblet diameter on their rooting was studied. Developing bulblets were rooted on MS basal medium supplemented with 0.75 mg l^{-1} NAA, 30.0 g l^{-1} sucrose (w/v) and solidified with 6.2 g l^{-1} agar (w/v) for 28 days in Magenta GA^7 vessels. The results were evaluated after 60 days.

The pH of each culture medium was adjusted to 5.6 - 5.8 with 0.1 M KOH or 0.1 M HCl before autoclaving at 121ºC, 117.7 kPa for 20 min.

All cultures were grown in Fitotron growth chamber (Fitotron SGC 120; Epinal Way, Loughborough, UK) with 16 h of cool white fluorescent light (Philips lamps TLD 36 W/54, Hungary) at a photon flux density of 35 µmol m^{-2} s^{-1} per day.

Statistical analysis

The data for bulblet induction measured and compared the effects of plant growth regulator levels or their combinations on bulblet regeneration from 12 bulb types. Data for rooting compared the effects of bulb size on rooting (%), mean number of roots, and root length (mm). Each treatment in the regeneration and rooting experiment contained 60 explants divided into six replicate groups. Arcsine transformation was performed for all experimental data taken in percentages before subjecting them to statistical analysis (Snedecor and Cochran 1967). Data of regenerating bulblets were analyzed by univariate analysis and data of rooting experiment was analysed by one way ANOVA using "IBM® - SPSS® Statistics Version 20 for Windows. Means were compared selecting Duncan's Multiple Range Test at $p < 0.05$ or $p < 0.01$.

RESULTS AND DISCUSSION

Surface disinfection

Before an explant is cultured onto a regeneration medium, it must be surface disinfected against external threats like fungi, bacteria, yeast etc., which can cause considerable losses during carrying out of micropropagation experiments and result in reduced regeneration (Kane 2003). These microorganisms also compete adversely with plant tissue cultures for nutrients (Oyebanji et al. 2009). Duration of treatment period with 100.0% commercial bleach affected surface disinfection of the peeled off bulbs significantly ($p < 0.05$). It was noted that 10 min of disinfection period was ineffective to disinfect bulbs with considerable development of fungus (data not shown). Surface disinfection for 15, 20, 25, and 30 min was equally effective to achieve complete disinfection of *S. fischeriana* bulbs. However, comparing the periods of surface disinfection, each increase in concentration of bleach resulted in increased damaging effects on skin of the *S. fischeriana* bulb tissues. It might be due to strong oxidizing action of HOCl ions from diluted sodium hypochlorite causing damage to bulb tissues or cells in agreement with Nakagarwara et al. (1998) and Tirawat et al. (2013).

Surface disinfection must be performed by selecting the minimum concentration of disinfectant and duration of time to minimize losses of explants (Mathias et al.

1987), as the living material must not lose biological activity and should result in effective elimination of microorganisms causing contamination (Oyebanji et al. 2009). Therefore, to minimise damage and phytotoxicity to experimental material in subsequent experiments, all bulbs were surface disinfected using 100.0% (v/v) commercial bleach (or 5% sodium hypochlorite) for 15 min.

All bulbs or bulb scales that showed any fungal or bacterial contamination at any stage of the experiment were eliminated in the autoclave to avoid spread and growth of undesired contaminating microorganisms.

Bulblet regeneration using 2,4-D at 15°C or 24°C ± 1°C

Irrespective of the 5 variants of 2,4-D used in the experiment, the 12 types of bulb scales used in the experiment behaved similarly by inducing variable swelling and slow but continuous elongation at 15° ± 1°C. First signs of development on these explants were observed after 7 to 12 days of culture. The 12 types of explants failed to induce regeneration except piled up shoot initials on margins of explant cut edges (Fig. 1A). No shoot or bulblet regeneration was recorded on any of the explant even after 60 days culture and the experiment was terminated without achieving bulblet regeneration.

Increased scale length with discursive induction of one or two 0.1 cm diameter bulblets as offshoot bulblets were observed near base plates of 0.5 cm long (Fig. 1B) two-scale explants at 24 ± 1°C (data not shown).

It is assumed that the interaction among variants of 2,4-D, length of explants (0.5, 1.0, and 1.5 cm) and number of bulb scales (2, 3, 4 or 5) along with two different incubation temperatures had completely negative effect on regeneration at 15 ± 1°C and partially variable impact on regeneration and induction of bulblets at 24 ± 1°C. Both experiments were finished without accomplishing remarkable success.

Bulblet regeneration using BAP with or without NAA at 15° ± 1°C

The BAP plus NAA study also compared the effects of 12 different types of *S. fischeriana* bulb scale explants (made of 0.5, 1.0, and 1.5 cm long two, three, four and five bulb scales attached by a thin segment at the base plate). We hypothesized that selection of an appropriate length and number of bulb scales may play an important role in successful setting up of cultures under *in vitro* conditions in line with Huang et al. (1990 a, b). The idea was to test whether *S. fischeriana* bulblet regeneration on different number of scales was significantly different. Information on the particular number and bulb sizes of scales that will produce good quality bulb yield in *S. fischeriana* is not documented in the literature. Such information is necessary to allow selection of the right sizes of explants for micropropagation.

Swelling and slow elongation was noted on all type of 0.5, 1.0, and 1.5 cm long explants cultured at 15°C using variants of BAP with or without 0.2 mg l^{-1} NAA. Variable number of micro bulblets was recorded on 0.5, 1.0, and 1.5 cm long two sale explants only. The data recorded after 60 days of culture showed that bulblet regeneration percentage and mean number of induced bulblets varied significantly ($p < 0.01$) among treatments depending on the type of explant (bearing different lengths and number of scales).

No bulblet induction was noted on 0.5 cm long two-scale bulb explants using MS medium containing 4 combinations of BAP and NAA (2.5 mg l^{-1} BAP, 4.5 mg l^{-1} BAP plus 0.2 mg l^{-1} NAA, 6.5 mg l^{-1} BAP and 8.5 mg l^{-1} BAP). Bulblet induction on 0.5 cm long two-scale bulb scales on the remaining BAP plus NAA treatments ranged from 6.7 ± 0.9 to 53.3 ± 4.1% (Table 1). Maximum bulb induction on 0.5 cm long bulb scales was noted on MS medium containing 2.5 mg l^{-1} BAP plus 0.2 mg l^{-1} NAA (Table 1).

No bulblets were induced on 1 cm long two-scale bulb explants on MS medium containing 4.5 mg l^{-1} BAP plus 0.2 mg l^{-1} NAA (Table 1). Regeneration on the other combinations of BAP and NAA treatments showed bulblet induction range from 6.7 ± 0.4% to 93.3 ± 6.1%. Maximum bulb induction on 1.0 cm long bulb scales was noted on MS medium containing 4.5 mg l^{-1} BAP.

Bulblet induction on 1.5 cm long two scale explants ranged from 6.7 ± 3.1% to 66.7 ± 1.1 % (Table 1). Maximum bulblet regeneration percentage was recorded on MS medium containing 4.5 mg l^{-1} BAP.

Excluding non-regenerative cultures, mean number of bulblets varied in a range of 0.3 ± 0.1 - 2.7 ± 0.2 (Fig. 1D), 0.3 ± 0.1 - 2.9 ± 0.1 (Fig. 1E) and 0.6 ± 0.1 - 2.3 ± 0.2 (Fig. 1F) on 0.5, 1.0, and 1.5 cm long two bulb scale explants, respectively (Table 1). Maximum number of bulblets per 0.5 cm and 1 cm long two-scale explants was noted on MS medium containing 2.5 mg l^{-1} BAP plus 0.2 mg l^{-1} NAA. Maximum number of bulblets per 1.5 cm long two-scale explants was noted on MS medium containing 0.5 mg l^{-1} BAP plus 0.2 mg l^{-1} NAA. Furthermore, green transformation of scale tips were also noted on 6.5 and 8.5 mg l^{-1} BAP plus 0.2 mg l^{-1} NAA (two combinations only). Some scales on the explants showed up in upright or twisted upright position (Fig. 1C) without inducing any bulblet. The results indicated that use of 0.5 or 2.5 mg l^{-1} BAP without 0.2 mg l^{-1} NAA was inhibitory both for bulblet induction percentage and man number of bulblets per 0.5 cm and 1.5 cm long bulb scales. Whereas, 1 cm long bulb scale explants showed partial or significant inhibition for both parameters when 0.5 and 2.5 mg l^{-1} BAP was used singly. At 4.5 and 8.5 mg l^{-1} BAP with 0.2 mg l^{-1} NAA,

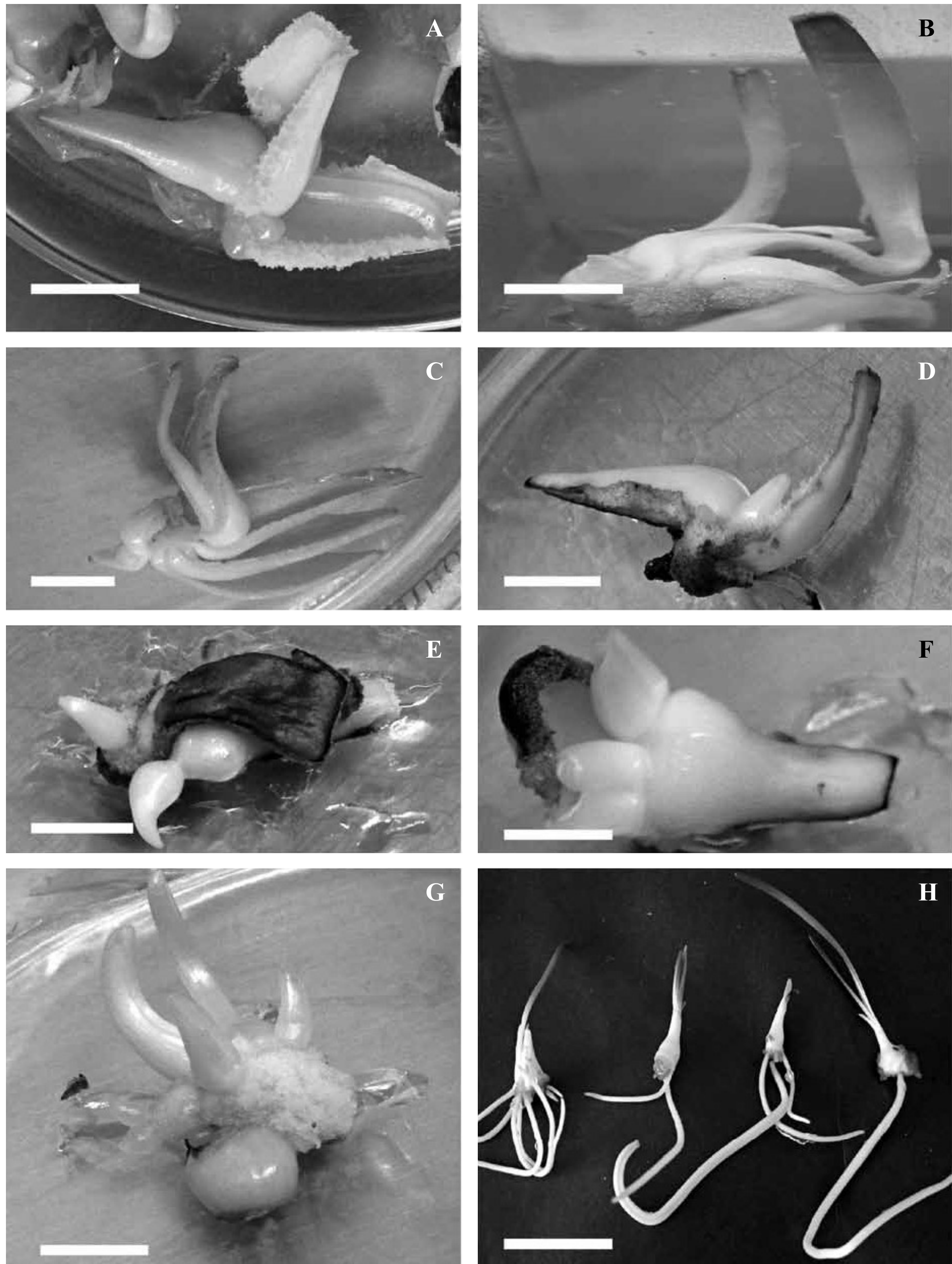

Fig. 1. Bulblet regeneration from *Sternbergia fischeriana* bulb scale explants A) Piled up white colored fluffy growths at the margins of the explants on MS basal medium containing any concentration of 2,4-D at 24°C, B) Increase in length of 1.5 cm long explants showing raising of 1 or 2 scales in upright, C) Increase in length of scales on 1 cm long explants showing increase in length of scales with the development of chlorophyllated shoot tips, D) Growing bulblets on 0.5 cm long two scale bulb explants, E) Regeneration on 1 cm long two scale bulb explants, F) Regeneration on 1.5 cm long two scale bulb explants, G) Growing bulblets on 0.5 cm long two scale bulb explants, H) Rooting of bulblets on 0.75 mg l^{-1} NAA. Bar of figures: A-D = 0.4 cm, E-G = 0.3 cm, H = 4 cm.

Table 1. Effects of different concentrations of BAP with or without 0.2 mg l^{-1} NAA on *Sternbergia fischeriana* bulblet induction.

BAP (mg l^{-1})	NAA (mg l^{-1})	Bulblet induction (%)			Mean number of bulblets		
		0.5 cm long two bulb scales	1 cm long two bulb scales	1.5 cm long two bulb scales	0.5 cm long two bulb scales	1 cm long two bulb scales	1.5 cm long two bulb scales
0.5	0.0	20.0 ± 2.9 d	40.0 ± 3.3 c	53.3 ± 2.6 b	1.0 ± 0.1 c	1.3 ± 0.2 b	1.0 ± 0.1 c
0.5	0.2	46.7 ± 3.9 b	26.7 ± 2.5 d	66.7 ± 1.1 a	1.2 ± 0.3 b	1.2 ± 0.3 bc	2.8 ± 0.2 a
2.5	0.0	0.0 ± 0.0 e	13.3 ± 1.4 e	46.7 ± 1.8 c	0.0 ± 0.0 d	1.0 ± 0.1 c	1.4 ± 0.1 b
2.5	0.2	53.3 ± 4.1 a	66.7 ± 4.5 b	33.3 ± 2.2 d	2.7 ± 0.2 a	2.9 ± 0.1 a	1.4 ± 0.1 b
4.5	0.0	6.7 ± 0.9 e	93.3 ± 6.1 a	6.7 ± 3.1 f	0.3 ± 0.1 d	1.4 ± 0.4 b	0.3 ± 0.1 d
4.5	0.2	0. 0 ± 0.0 e	0.0 ± 0.0 e	20.0 ± 0.6 e	0.0 ± 0.0 d	0.0 ± 0.0 d	0.7 ± 0.3 d
6.5	0.0	0.0 ± 0.0 e	6.7 ± 0.4 e	26.7 ± 0.3 e	0.0 ± 0.0 d	0.3 ± 0.1 d	1.0 ± 0.2 c
6.5	0.2	33.3 ± 0.8 c	20.0 ± 2.1 d	40.0 ± 2.7 c	1.3 ± 0.3 b	1.4 ± 0.3 b	1.2 ± 0.2 c
8.5	0.0	0.0 ± 0.0 e	20.0 ± 2.5 d	20.0 ± 1.9 e	0.0 ± 0.0 d	0.7 ± 0.1 c	0.6 ± 0.1 d
8.5	0.2	33.3 ± 1.7 c	26.7 ± 2.9 d	46.7 ± 2.4 c	1.0 ± 0.1 c	1.0 ± 0.1 c	1.7 ± 0.2 c

Means ± standard error within a column followed by the same letter are not significantly different according Tukey's multiple range test at $p \leq 0.05$.

and 6 mg l^{-1} BAP without 0.2 mg l^{-1} NAA, the results showed full or partial inhibition in both parameters.

Precarious and desultory induction of one or two 0.1 cm diameter bulblets was noted on three, four and five bulb scale explants of 0.5, 1.0, and 1.5 cm length on all concentrations of BAP-NAA (results not shown).

Matsuo and Van Tuyl (1986) reported that outer bulb scales in Easter lily show high regeneration capacity, while inner scales show progressively lower bulblet regeneration potential. In line with this study it was assumed that explants with more than two scales induced negative competition for nutrients due to variable physiological maturity of inner or outer scales. This resulted in no or negligible induction of bulblet meristems and increased length of explants without any regeneration. It was also presumed that young thin inner scales might have secreted some compounds in the regeneration medium that had influenced regeneration negatively on adjacent thick outer scales and resulted in no regeneration. Huang et al. (1990 a, b) reported that the thickness and length of outer scales affected the rate of bulblet formation and subsequent leaf development. They also found that vascular bundles of the proturbances are initiated on the abaxial surface of the inner scale connected with the vascular system of the outer scale and not with these vascular bundles of inner scale in orchid plants. The results of our study are also in partial agreement with Addai and Scott (2011), who compared small and large bulbs and found that large bulblets tend to have higher vegetative growth and development, because they have relatively higher reserves and volume of scales.

Bulblet regeneration using BAP with or without NAA at 24° ± 1°C

No bulblet regeneration was registered on any length of explants cultured on 0.5, 2.5, 4.5, 6.5, and 8.5 mg l^{-1} BAP or 0.5, and 2.5 mg l^{-1} BAP plus 0.2 mg l^{-1} NAA (Table 2). This is in agreement with Karatas et al. (2013), who reported that BAP with NAA inhibited shoot regeneration more as compared to BAP used singly in the culture medium on all internode explants of *Bacopa monnieri* at 24°C. The results of this experiment further detected swellings on explants after 18 - 19 days and onset of micro bulblet initials after 22 - 24 days of culture. The bulblet regeneration data recorded after 60 days showed bulblet induction range of 41.7 ± 1.5% - 75.0 ± 4.2%, 50.0 ± 3.5% - 66.7 ± 4.7% and 25.0 ± 2.7% - 66.7 ± 8.7% on 0.5, 1, and 1.5 cm long two scale bulb explants of *S. fischeriana*, respectively (Table 2). The results clearly demonstrated that 0.5, 2.5 and 4.5 mg l^{-1} BAP with or without 0.2 mg l^{-1} BAP was completely inhibitory or non regenerative. Simailarly, 6.5 and 8.5 mg l^{-1} BAP without 0.2 mg l^{-1} NAA in the regeneration medium was strongly inhibiotory or non regenerative.

Bulblet regeneration was registered only on MS medium containing 4.5, 6.5, and 8.5 mg l^{-1} BAP plus 0.2 mg l^{-1} NAA (Table 2). All other concentrations of BAP with or without NAA induced no regeneration. The results clearly indicated that the type of explant and concentrations of BAP with or without NAA in the culture medium strongly influenced the bulblet regeneration, which is in agreement with Basalma et al. (2008). Lower concentrations of BAP (0.5 and 2.5

Table 2. Effects of different concentrations of BAP with or without 0.2 mg l^{-1} NAA on *Sternbergia fischeriana* bulblet induction at 24° ± 1°C.

BAP (mg l^{-1})	NAA (mg l^{-1})	Bulblet induction (%)			Mean number of bulblets		
		Explants					
		0.5 cm long two bulb scales	1 cm long two bulb scales	1.5 cm long two bulb scales	0.5 cm long two bulb scales	1 cm long two bulb scales	1.5 cm long two bulb scales
0.5	0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
0.5	0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
2.5	0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
2.5	0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
4.5	0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
4.5	0.2	58.3 ± 1.2 a	50.0 ± 3.5 c	25.0 ± 2.7 b	1.0 ± 0.2 c	1.7 ± 1.5 b	1.0 ± 0.2 b
6.5	0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
6.5	0.2	41.7 ± 1.5 b	58.3 ± 2.3 b	66.7 ± 7.2 a	2.1 ± 0.3 b	2.1 ± 1.4 ab	2.0 ± 0.5 a
8.5	0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
8.5	0.2	75.0 ± 4.2 a	66.7 ± 4.7 a	66.7 ± 8.7 a	5.0 ± 0.5 a	2.3 ± 2.6 a	2.3 ± 0.4 a

Means ± standard error within a column followed by the same letter are not significantly different according LSD multiple range test at $p \leq 0.05$.

mg l^{-1}) with or without 0.2 mg l^{-1} NAA were completely inhibitory and non regenerative. Higher concentrations of BAP (4.5, 6.5, and 8.5 mg l^{-1}) were non generative in the absence of 0.2 mg l^{-1} NAA. These concentrations showed full or partial regeneration of bulblets when the treatment also embodied 0.2 mg l^{-1} NAA. Maximum bulblet regeneration was noted on MS medium containing 8.5 mg l^{-1} BAP plus 0.2 mg l^{-1} NAA on 0.5 cm long two scale explants. The developing bulblets displayed green shoots after 45 - 50 days of initiating culture.

The mean number of bulblets varied significantly ($p < 0.01$) on each regeneration medium depending on length of the explant and concentration of BAP with or without 0.2 mg l^{-1} NAA. Excluding non-regenerative cultures, mean number of bulblets was within ranges of 1.00 ± 0.2 – 5.0 ± 0.5 (Fig. 1G), 1.7 ± 1.5 - 2.3 ± 2.6, and 1.0 ± 0.2 - 2.3 ± 0.4 on 0.5 cm, 1.0 cm, and 1.5 cm long two-scale bulb explants, respectively. Maximum bulblet induction in each case was noted on MS medium including 8.5 mg l^{-1} BAP plus 0.2 mg l^{-1} NAA (Table 2).

Erratic, unstable and inconsistent bulblet regeneration of one or two bulblets of 0.1 cm diameter was noted on three, four and five bulb scale explants of 0.5, 1.0, and 1.5 cm length, each on all concentrations of plant growth regulators (data not shown). It seems that the competence of regeneration was strongly influenced by the explant type, temperature, and growth regulator used in the study, in acceptance to the results of McDaniel (1984), Christianson and Warnick (1985), and Khawar et al. (2005) who emphasized that morphological integrity of explants and choice of plant growth regulators strongly impacts induction of shoot regeneration.

Mirici et al. (2005) reported that the type of explant and concentrations of plant growth regulators (PGRs) induce variability on bulblet regeneration and their frequency in *S. fischeriana*. They recorded maximum number of 2.6 bulblets per two-scale explant on MS medium containing 2.0 mg l^{-1} BAP plus 0.5 mg l^{-1} NAA at 24°C. We report 5.0 bulblets per 0.5 cm long two-scale bulb explant on MS medium containing 8 mg l^{-1} BAP plus 0.2 mg l^{-1} NAA at 24° ± 1°C. The results indicate positive effect of culture at 24° ± 1°C on bulblet regeneration and suggest that differences in behaviour of explants at two temperatures might be due to metabolic differences in the functions of plant growth regulators. The findings concord with the results of Sonoike et al. (1995), who suggested that low temperatures result in decrease in membrane fluidity, diffusion rates of molecules, and chemical enzyme reaction rates.

The results of this study represent an improvement in comparison to the previous study of Mirici et al. (2005). The results show that presence of BAP in combination with NAA was necessary to induce regeneration from explants at both 15 and 24 °C.

Rooting

Thriving, healthy and sturdy 0.38 - 1.0 cm diameter bulbs were cultured on MS medium containing 0.75 mg l^{-1} NAA for rooting. The results confirmed the role of bulblet diameter in rooting. No rooting was recorded on 0.38 and 0.42 cm diameter bulblets (Table 3). The rooting on 0.47 - 1.0 cm diameter bulblets ranged from 33% to 100%. Highest rooting (100 ± 0.0%) was registered at 0.47, 0.76, 0.93, and 1 cm bulblet diameters.

Table 3. Effect of mean bulblet diameter on the rooting of *Sternbergia fisheriana.*

Mean bulblet diameter (cm)	Rooting (%)	Mean number of roots	Root length (cm)
0.38	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 e
0.42	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 e
0.47	100.0 ± 0.0 a	4.3 ± 0.9 a	3.7 ± 0.4 a
0.50	66.7 ± 4.5 b	1.5 ± 0.1 bc	1.1 ± 0.1 c
0.57	33.0 ± 1.8 c	1.5 ± 0.1 bc	0.3 ± 0.1 d
0.76	100.0 ± 0.0 a	1.0 ± 0.2 c	1.1 ± 0.1 c
0.93	100.0 ± 0.0 a	2.0 ± 0.3 b	0.5 ± 0.1 d
1.00	100.0 ± 0.0 a	1.8 ± 0.5 b	4.7 ± 1.2 b

Means ± standard error within a column followed by the same letter are not significantly different aaccording Duncan's test multiple range test at $p \leq 0.05$.

Rooting of 66.7 ± 4.5% and 33.0 ± 1.8% was noted on bulblets with diameter of 0.50 and 0.57 cm.

The results also confirm influence of bulblet diameter on mean number and length of roots that ranged from 1.0 ± 0.2 to 4.3 ± 0.9 with root length range of 0.3 ± 0.1 cm to 4.7 ± 1.2 cm. The maximum mean number of 4.3 roots was recorded on 0.47 cm diameter bulblets (Fig. 1H). The longest (4.7 ± 1.2 cm) roots were recorded on 1 cm diameter bulblets. The BAP plus NAA regenerated bulblets of *Lilium lancifolium* were easily rooted on MS medium supplemented with NAA (Sun et al. 2013). Ozel and Khawar (2007) reports *in vitro* rooting of *Ornithogalum oligophyllum* on PGRs-free MS medium only. No abnormality was recorded in the rooted and acclimatized bulblets. The bulblets diameter played decisive role in rooting and no rooting was registered on 0.38 and 0.42 cm diametered bulblets. This could be due to their physiological immaturity in relation to bulb diameter. The bulblets with $\geq$ 0.47 cm diameter were physiologically mature to induce roots.

In conclusion, this protocol suggests possibility of inducing 60 new bulblets (5.0 bulblets × 12 two scale explants from a single bulb = 60.0 bulblets) from a single bulb. The present investigation confirms possibilities of rapid and increased micropropagation of *S. fischeriana*. Purposely extension of this study will help in rapid unrestricted multiplication of this plant throughout the year. This is not possible under natural conditions where a bulb rarely induce 2 - 3 offshoot bulbs per explant. The results suggest possible alternatives for *in vitro* multiplication of *S. fisheriana* and could be useful for its commercial propagation.

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