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

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ABSTRACT

Sternbergia fischeriana (Herb.) Rupr.is an important bulbous geophyte with attractive golden yellow flowers. The bulbs are very difficult to multiply under natural conditions and take about 5 yr to mature, flower and set seeds that permits very slow rate of multiplication. There is need to develop new bulblet multiplication techniques for these using alternative methods. The study aimed to improve a method for rapid multiplication of the plants using 0.5, 1.0, and 1.5 cm long bulb explants with two, three, four, and five scale attached by a thin base plate segment and cultured them on 1.0 × Murashige and Skoog (MS) medium containing 1.0, 2.0, 3.0, 4.0 and 5.0 mg l^{-1} 2,4-D or 0.5, 2.5, 4.5, 6.5, and 8.5 mg l^{-1} 6-benzyl amino purine (BAP) with or without 0.2 mg l^{-1} α-naphthalene acetic acid (NAA) added with 30.0 g l^{-1} sucrose and 6.2 g l^{-1} agar as gelling agent. Each culture was incubated both at 15 and 24 °C in the growth chamber. The results clearly demonstrated that any concentration of 2,4-D was ineffective to induce bulblet regeneration under 15 and 24 °C on any type of explant. Variable results were obtained on all 12 types of explants on 1.0 × MS medium containing variants of BAP with and without 0.20 mg l^{-1} NAA at both temperatures. Maximum number of 4.97 bulblets^{-1} 0.5 cm long two scale bulb explant was obtained on 1.0 × MS medium containing 8.50 mg l^{-1} BAP plus 0.20 mg l^{-1} NAA. The rooting was achieved on 1 × MS medium containing 0.75 mg l^{-1} NAA. The rooting was affected by the size of bulblet. The maximum bulblet regeneration was recorded on 0.47 cm diameter bulblets. Optimum number of 0.47 cm diametered bulblets registered 3.73 cm long, 4.33 roots bulblet^{-1}. These bulblets were successfully acclimatized under greenhouse conditions. The results are very important for micropropagation of *S. fischeriana.*

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Key words: *Sterbergia fischeriana*, bulbous & ornamental plant, *in vitro* micropropagation

Running Title: BULBLET REGENERATION FROM *STERNBERGIA FISCHERIANA*

INTRODUCTION

Sternbergia species family Amaryllidacea are popularly found in the Mediterranean region, Central Europe and 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 a popular commodity in Turkish ornamental cut flower industry (Arslan et al. 2002; Zencirkiran, 2002, Mirici et al. 2005). *S. fischeriana* is also 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 favorable environmental conditions, these plants take more than 3 yr to mature, flower and set seeds. The bulbs multiply very slowly and add only 1-2 offset bulblets in a 3-yr period (Arslan et al. 2002); that inhibits their large scale multiplication (Arslan et al. 2002; Zencirkiran, 2002; Zencirkiran and Tumsavas, 2006). This suggests their low probabilities for use in ornamental or pharmaceutical industry unless alternative techniques to propagate them are developed. Micro propagation of *S. fischeriana* bulblets could serve as a possible alternative method to strengthen their number 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 the 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 of bulb scales on regeneration. This suggests a need to develop an improved micropropagation method to broaden the scope of *S. fischeriana* proliferation. In accordance with this, the 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 sterilization of bulbs

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Genetically mixed 2-3 cm diameter bulbs of *S. fischeriana* (n=60) were 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, they 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 d in dark. Thereafter, the bulbs were peeled off to select healthy and disease free material to minimize contamination during sterilization. The bulbs were surface sterilised with 100.0% (v/v) domestic bleach [Ace - Istanbul, Turkey, containing 5% (v/v) NaOCl] for 10.0, 15.0, 20.0, 25.0, and 30.0 min followed by rinsing with sterilized bidistilled water for 5 × 5 min.

Each bulb was cultured on 35 ml of 1.0 × Murashige and Skoog medium (Murashige and Skoog, 1962) 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 d to determine optimum duration of time to treat the explants with commercial bleach for surface sterilization. Subsequently, these bulbs were sliced longitudinally to obtain (i) two scale bulb explants (3 types of 0.5, 1.0, and 1.5 cm long and 0.4 - 0.5 cm wide each, 12 explants $bulblet^{-1}$), (ii) three scale bulb explants (3 types of 0.5, 1.0, and 1.5 cm long and 0.4 - 0.5 cm wide each, eight explants $bulblet^{-1}$), (iii) four scale bulb explants (3 types of 0.5, 1.0, and 1.5 cm long and 0.4 - 0.5 cm wide each, four explants $bulblet^{-1}$) and (iv) five scale bulb explants (3 types of 0.5, 1.0, and 1.5 cm long and 0.4 - 0.5 cm wide each, four explants $bulblet^{-1}$) attached by a thin segment at the base plate.

Above mentioned four types of bulb scale explants were cultured on 1.0 × MS bulblet medium containing 0.5, 2.5, 4.5, 6.5, and 8.5 mg l^{-1} 6-benzyl aminopurine (BAP) with or without 0.20 mg l^{-1} naphthaleneacetic acid (NAA) or 1.0, 2.0, 3.0, 4.0, 5.0 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) supplemented with 30.0 g l^{-1} sucrose. Each type of explant were cultured on each type of mass proliferation medium solidified with 6.20 g l^{-1} agar incubated at 15° ±1°C and 24° ± 1°C in sterile Magenta GA^7 Vessels under Philips-day light lamps (35 µmol m^{-2} s^{-1}TLD 36 W/54, Hungary) with 16 h light photoperiod in growth chamber (Fitotron SGC 120; Epinal Way, Loughborough, UK).

The developing bulblets were rooted on 1.0 × MS medium by adding 0.75 mg l^{-1} NAA, 30.0 g l^{-1} sucrose (w/v) and solidified with 6.20 g l^{-1} agar (w/v) for 28 d in Magenta GA^7 vessels using Philips-day light lamps TLD 36 W/54, Hungary (35 µmol m^{-2} s^{-1}) observing 16 h light photoperiod in Fitotron growth chamber.

The pH of each micropropagation culture medium was adjusted to 5.6 - 5.8 with 0.1 M KOH or 0.1 M HCl before autoclaving at 121 ºC, 117.679 kPa for 20 min.

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All rooted bulblets were taken out of the culture vessels and the agar sticking to residual roots was gently removed using flowing tap water. Subsequently, the bulbs were transferred to 4.5 l pots filled with 3 l peat moss. These plants were acclimatized at 21° ± 1°C in Fitotron growth chambers using Philips-day light lamps TLD 36 W/54 under light density of 35 µmol m^2 s^{-1}, 16 h light photoperiod and 80% humidity.

Statistical analysis

The regenerating bulblets on 4 types of explant with three different lengths (0.5, 1.0 and 1.5 cm each) and regeneration medium were scored for bulblet induction %, mean number of explants $bulblet^{-1}$, rooting %, Mean number of roots $explant^{-1}$ and root length after 60 d in each case. Each treatment used 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 one-way ANOVA using the F-test in "IBM® - SPSS® Statistics Version 20" for Windows (http://www-01.ibm.com/support/ docview.wss?uid=swg24029274). Means were compared selecting Duncans Multiple Range Test at $P \leq 0.05$ or $P \leq 0.01$.

RESULTS AND DISCUSSION

Surface sterilization

Treatment period in minutes with 100.0 % commercial bleach affected surface sterilization of the peeled off bulbs at significant level ($P < 0.05$). It was noted that 10.0 min of sterilization period was ineffective to sterilize bulbs (data not shown in tabulated form). Surface sterilization for 15.0, 20.0, 25.0, and 30.0 min was equally effective for complete sterilization of *S. fischeriana* bulbs. Comparing the periods for surface sterilization, each increase in the concentration of bleach testified increased damaging effects on skin of the bulb tissues. Therefore to minimise damage to experimental material in subsequent experiments, all bulbs were surface sterilised using 100.0% (v/v) commercial bleach for 15.0 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 different 2,4-D concentrations on 0.5, 1.0 and 1.5 cm long 2.0, 3.0, 4.0, and 5.0 scale explants at 15° ± 1°C

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Four types of 0.5, 1.0 and 1.5 cm long 2, 3, 4, and 5 scale bulb explants cultured on 5 different concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) induced increase in length on the explants at15° ± 1°C; which was ineffective to induce any regeneration on the explants. No shoot or bulblet regeneration except piled up white colored fluffy growths at margins of explants (Figure 1a) was recorded on any of the explant even after 60 d culture. Therefore, the experiment was terminated without obtaining any bulblet regeneration.

Bulblet regeneration using different 2,4-D concentrations on 0.5, 1.0 and 1.5 cm long 2.0, 3.0, 4.0, and 5.0 scale explants at 24° ± 1°C

A continuous culture of 0.50 (Figure 1b) and 1.0, 1.5 cm long two, three, four or five bulb scale explants for 60 d resulted in increased scale length, with discursive induction of one or two bulblets as offshoots near base plates and green transformation of scale tips at 24° ± 1°C on 6.5 and 8.5 mg l^{-1} BAP plus 0.2 mg l^{-1} NAA (two combinations only) after 60 d. Some scales on the explants showed up in upright or twisted upright position (Figure 1c).

Bulblet regeneration using different BAP plus 0.2 mg l^{-1} NAA concentrations on 0.5, 1.0 and 1.5 cm long 2.0, 3.0, 4.0, and 5.0 scale explants at 15° ± 1°C

Slow swelling and elongation was noted on all types of explants soon after culture that continued for about 28-30 d of culture. It was followed by inception of micro bulblets on incubated explants in variable way. The bulblet regeneration data recorded after 60 d of culture testified bulblet regeneration percentage and induction of bulblets (in number) explant^{-1} changed significantly ($P< 0.01$) among treatments, on each concentration of BAP with and without 0.2 mg l^{-1} NAA depending on the length and number of scales on the explant.

No bulblet induction was noted on 0.5 cm long two scale bulb explants using 1.0 × MS medium having 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 rest of the 0.5 cm long two scale bulb scales ranged 6.67 to 53.33% (Table 1).

No bulblets were recorded on two scale bulb explants on MS medium having 4.5 mg l^{-1} BAP plus 0.2 mg l^{-1} NAA. Rest of the 1 cm long two scale bulb explants had bulblet induction percentage range of 6.67 - 93.33%.

Bulblet induction on 1.5 cm long two scale explants ranged from 6.67 to 66.67% (Table 1). Maximum bulblet regeneration percentage was noted on 1.0 × MS medium containing 4.5 mg l^{-1} BAP on 1cm long two scale bulb explants.

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Number of bulblets explant^{-1} changed in a range of 0.00 - 2.67 (Figure 1d), 0.00 - 2.86 (Figure 1e) and 0.00 - 2.83 (Figure 1f) on 0.5, 1.0 and 1.50 cm long two bulb scale explants respectively.

Precarious and desultory induction of one or two 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 plant growth regulators.

Bulblet regeneration using different BAP plus 0.2 mg l^{-1} NAA concentrations on 0.5, 1.0 and 1.5 cm long 2.0, 3.0, 4.0, and 5.0 scale explants 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. The experiment further detected swellings on explants after 18 - 19 d and onset of micro bulblet initials after 22-24 d of culture. The bulblet regeneration data recorded after 60 d testified bulblet induction range of 41.7% - 75.0%, 50.0% - 66.7% and 25.0% - 66.7% on 0.5, 1, and 1.5 cm long two scale bulb explants of *S. fischeriana*, respectively (Table 2). Maximum bulblet regeneration (75%) was visualized 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 d of initiating culture.

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The mean number of bulblets explant^{-1} changed 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 conceived explant^{-1} had a range of 1.00 - 4.97 (Figure 1g), 1.72 - 2.33 and 1.00 - 2.33 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.

Erratic, unstable and inconsistent bulblet regeneration one or two bulblets 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.

Rooting

Thriving, healthy and sturdy 0.38 - 1.00 cm diametered bulbs rooted on 1.0 × MS medium testified the roles of bulblet diameter in rooting. No rooting was recorded on 0.38 and 0.42 cm diameter bulblets (Table 3). The rooting percentage on 0.47 - 1.00 cm diameter bulblets

ranged 33% to 100%. Cent percent (100%) rooting was registered on 0.47, 0.76, 0.93, and 1 cm bulblet diameters. Rooting percentage of 66.7% and 33.0% was noted on bulblets with diameter of 0.50 and 0.57 cm.

The results testified influence of bulblet diameter on number and length of roots per explant^{-1} that changed from 1.00 - 4.33 with root length range of 0.33 to 4.91 cm. The maximum number of 4.33 roots bulblet^{-1} and the longest (4.91 cm) roots were noted on 0.47 cm diameter bulblets (Figure 1h). It was followed by 1 cm diameter bulblets that bore 1.75 roots bulblet^{-1} with root length of 4.73 cm.

These bulblets were potted in plastic culture vessels holding peat moss in growth chamber for acclimatization. No problem was observed during acclimatization. All plants showed profuse development in the pots and flowered (Figure 1h).

DISCUSSION

Propagation technique is of particular importance in *S. ficheriana*, since multiplication of the plant by seeds or bulblets using traditional techniques are very slow.

The present study compared the effect of number of bulb scales and length of explants on bulblet regeneration from 12 different types of bulb scale explants of *S. fischeriana*. It was thought 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.

It is assumed that the variants of 2,4-D, BAP with or without NAA, length of explants (0.5, 1, and 1.5 cm), number of bulb scales and type or combinations of plant growth regulators along with incubation temperature may have changeable impact on regeneration and induction of bulblets.

In accordance with the hypothesis, the experimental results demonstrated that any concentration of 2,4-D was not appropriate for regeneration at 15 and 24 °C. Contrarily, when variable concentrations of BAP with and without NAA were compared at 15 and 24 °C; the results showed that 3, 4, and 5 scale explants of any length were also not good explants for regeneration of bulblets; as they induced erratic and unstable regeneration of one or two bulblets on these explants irrespective of culture at 15° ± 1° or 24° ± 1°C. It is thought that explants with more than two scales, and longer than 0.5 cm induced negative competition for nutrients result in no or negligible induction of bulblet meristems that only resulted in increased length on explants without regeneration. Regeneration on 0.5 cm long two scale bulb explants was discernible irrespective of the variation in temperature. It was also noted

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234 that MS medium including 0.5 - 8.5 mg l^{-1} BAP with or without 0.2 mg l^{-1} NAA were suitable
235 for regeneration variably at 15° ± 1°C. The number of regenerants never increased beyond
236 2.86 bulblets $explant^{-1}$.
237 At 24 °C, bulblet regeneration was registered only on 1.0 × MS medium containing 4.5,
238 6.5, and 8.5 mg l^{-1} BAP plus 0.2 mg l^{-1} NAA. All other concentrations of BAP with or
239 without NAA induced no regeneration. The results clearly indicated that the type of explant
240 and concentrations of BAP with or without NAA in the culture medium strongly influenced
241 the bulblet regeneration in agreement with Basalma et al. (2008). It appeared that thee
242 competence of regeneration was strongly influenced by the type of the explant, temperature
243 and growth regulator used in the study in acceptance to the results of McDaniel (1984) and
244 Christianson and Warnick (1985). Khawar et al. (2005) also emphasize that morphological
245 totality and choice of plant growth regulators strongly impacts induction of shoot
246 regeneration.
247 Previous studies report a single report about in vitro proliferation of *S. fischeriana* using
248 immature zygotic embryos and two scale explants by Mirici et al. (2005). The researchers did
249 not report length of the explants. The researchers agree that the type of explant and
250 concentrations of plant growth regulators induce variability on bulblet regeneration and their
251 frequency. They recorded maximum number of 2.6 bulblets $explant^{-1}$ on 1.0 × MS medium
252 containing 2 mg l^{-1} BAP plus 0.5 mg l^{-1} NAA at 24°C. The results of this study do not verify
253 the results of Mirici et al. (2005). This study reports maximum number of 2.86 bulblets per 1
254 cm long two scale bulb $explant^{-1}$ at 15° ± 1°C on MS medium including 2.5 mg l^{-1} BAP - 0.2
255 mg l^{-1} NAA and 4.67 bulblets 0.5 cm long two scale bulb $explant^{-1}$ on 1.0 × MS medium
256 containing 8 mg l^{-1} BAP plus 0.2 mg l^{-1} NAA at 24° ± 1°C. The results of this study show
257 positive effect of culture at 24° ± 1°C on bulblet regeneration. This suggests that the two scale
258 explants are negatively sensitive to 15° ± 1°C temperature, where regeneration was inhibited.
259 This might be due to differences in behaviour of explants due to metabolic differences in
260 regulation of plant growth regulators at two temperatures. The results of this study are
261 improvement over the previous study. The results further showed that the length of two scale
262 bulb explants were inversely proportional to the bulblet regeneration; such that maximum
263 mean number of bulblets $explant^{-1}$ decreased with each increase in the length of two scale
264 bulb explants. The results also underline the role of changing regeneration behavior of the
265 explants at different temperatures even on same plant growth regulators and combinations in
266 the culture medium. Presence of NAA with BAP was necessary to regenerate both at 15 and

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24 °C. The results corroborate and verify findings 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. There is also inhibition in the reactive oxygen species (ROS) activity.

The BAP plus NAA regenerated bulblets were easily rooted on 1.0 × MS medium in confirmation to Nayak and Sen (1995) and Ozel and Khawar (2007). They rooted bulblets of *Ornithogalum umbellatum* and *Muscari macrocarpum* on 1 × MS medium. No abnormality was recorded in the rooted and acclimatized bulblets. The bulblets diameter played decisive role in rooting. It was affirmed that bublet diameter affected 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 ≤ 0.47 cm diameter were physiologically mature to induce roots.

CONCLUSIONS

In conclusion, this protocol suggests possibility of inducing 60 new bulblets (4.97 bulblets × 12 two scale explants from a single bulb = 59.64 bulblets) from a single bulb. This is not possible under natural conditions where a bulb rarely induce 2-3 offshoot bulbs explant^{-1}. These results further approve that *in vitro* regenerated bulblets could prove efficient micropropagating unit of *S. fischeriana*.

Knowledge about selection of explants suitable for bulblet induction, and rooting of *S. fischeriana* is very important and will help in overcoming the problems related to *in vitro* production by offering possible alternatives for this valuable plant species. This propagation method is very important for *in vitro* commercial propagation of *S. fisheriana.*

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Kommentar [I35]: I think this literature is not really related to your results

Table 1. Effects of different concentrations of 6-benzyl amino purine (BAP) with and without 0.2 mg l^{-1} naphthaleneacetic acid (NAA) on percentage of *Sternbergia fischeriana* bulblet induction and mean number of bulblets explant^{-1} at 15° ± 1°C

BAP	NAA	Bulblet induction			Mean number of bulblets explant^{-1}		
		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
—— mg l^{-1} —		———————— % ————————			——— nr bulblet explant^{-1} ——		
0.5	0.0	20.00d*	40.00c	53.33b	1.00c	1.33b	1.00c
0.5	0.2	46.67b	26.67d	66.67a	1.22b	1.17bc	2.83a
2.5	0.0	0.00e	13.33	46.67c	0.00d	1.00c	1.44b
2.5	0.2	53.33a	66.67b	33.33bc	2.67a	2.86a	1.44b
4.5	0.0	6.67e	93.33a	6.67e	0.33d	1.40b	0.33d
4.5	0.2	0.00e	0.00e	20.00d	0.00d	0.00d	0.67d
6.5	0.0	0.00e	6.67e	26.67d	0.00d	0.33d	1.00c
6.5	0.2	33.33c	20.00d	40.00c	1.33b	1.44b	1.17c
8.5	0.0	0.00e	20.00d	20.00d	0.00d	0.67c	0.56d
8.5	0.2	33.33c	26.67d	46.67c	1.00c	1.00c	1.17c

*Means shown by different small letters in a column are significantly different at 0.05 level of significance using Tukey's test.

Kommentar [I36]: Because it is true for all variants, you should mention it in the Table description above

Kommentar [I37]: Why here Tukey test and in Table 2 LSD?

Table 2. Effects of different concentrations of 6-benzyl amino purine (BAP) with and without 0.2 mg l^{-1} naphthaleneacetic acid (NAA) on percentage of *Sternbergia fischeriana* bulblet induction and mean number of bulblets explant^{-1} at 24° ± 1°C

BAP	NAA	Bulblet induction			Mean number of bulblets explant^{-1}		
		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
— mg l^{-1} —		———— % ————			——— nr bulblets explant^{-1} ———		
0.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.20	0.00	0.00	0.00	0.00	0.00	0.00
2.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2.5	0.20	0.00	0.00	0.00	0.00	0.00	0.00
4.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4.5	0.20	58.33a*	50.00c	25.00b	1.00c	1.72b	1.00b
6.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6.5	0.20	41.67b	58.33b	66.67a	2.06b	2.06ab	2.00a
8.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8.5	0.20	75.00a	66.67a	66.67a	4.97a	2.33a	2.00a

*Means shown by different small letters in a column are significantly different at 0.05 level of significance using LSD test.

Kommentar [I38]: See Table 1

372 **Table 3.** Results of mean bulblet diameter, percentage of rooting, mean number of roots
373 bulblet^{-1} and root length of *Sternbergia fisheriana* bulblets regenerated on 1.0 × MS medium
374 having 0.75 mg l^{-1} naphthaleneacetic acid (NAA) in Murashige and Skoog (MS) medium

Mean bulblet diameter	Rooting	Mean number of roots explant^{-1}	Root length
——— cm ———	——— % ———	— nr root explant^{-1}	——— cm ———
0.38	0.00d*	0.00d	0.00e
0.42	0.00d	0.00d	0.00e
0.47	100.0a	4.33a	3.73a
0.50	66.7b	1.50bc	1.13c
0.57	33.0c	1.50bc	0.33d
0.76	100.0a	1.00c	1.12c
0.93	100.0a	2.00b	0.50d
1.00	100.0a	1.75b	4.73b
Mean 0.62	62.5	1.51	1.47

375 *Means shown by different letters in a column are significantly different at 0.05 level of
376 significance using Duncan test.

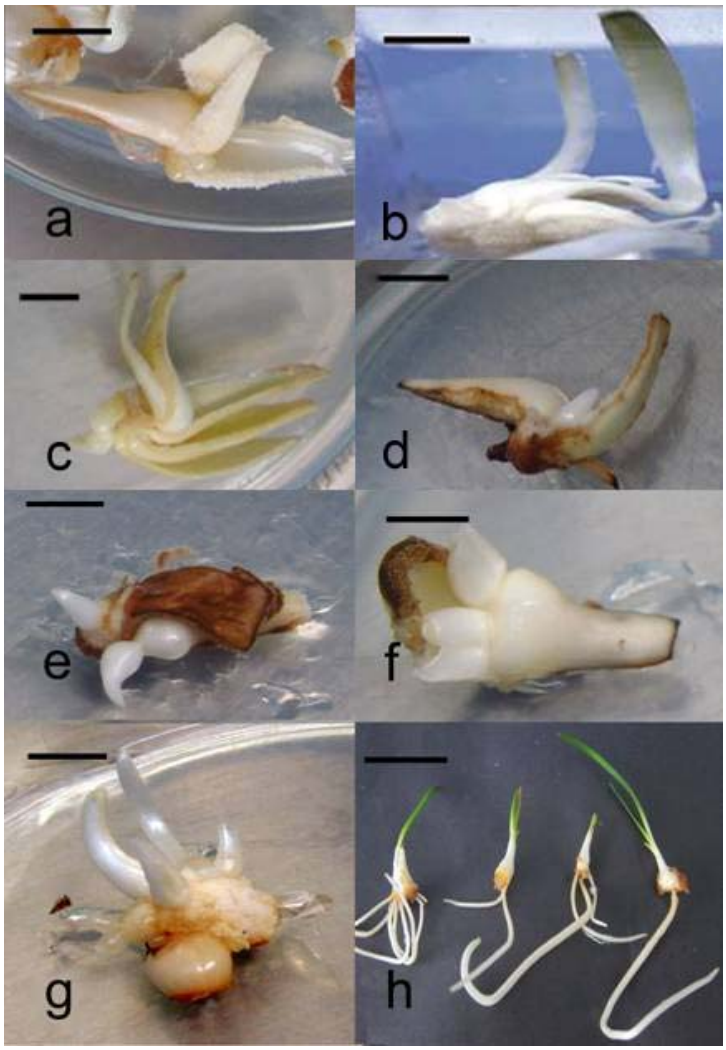

Figure 1. Bulblet regeneration from *Sternbergia fischeriana* bulb scale explants (a) piled up white colored fluffy growths at the margins of the explants on 1.0 × Murashige and Skoog (MS) medium containing any concentration of 2,4-D at 24 °C (b) increase in length of 1.5 cm long explants showing raising of 1 or 2 scales in upright (c) 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) 1 cm long two scale bulb explants (f) 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 0.19 mg l^{-1} mg l^{-1} naphthalene acetic acid (NAA).

Bar of Figures 1a-1d = 0.4 cm; Figures 1e-1g = 0.3 cm; Figure 1h = 4 cm.