Production of quality cut *Ornithogalum thyrsoides* flowers starting from *in vitro* propagated propagules

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Introduction

In vitro propagation has largely been concerned with the mass multiplication of true-to-the type plants; however, it also been utilized as a tool for genetic engineering, to produce secondary metabolites, and selecting useful somaclonal variants (Ruffoni and Savona, 2013; Fennell and van Staden, 2004; Griesbach *et al.*, 1998). If tissue cultured plantlets can be forced directly to produce quality cut flowers of *Ornithogalum thyrsoides* hybrid 'Chesapeake Starlight', the bulb production and programming phase can be bypassed (Roh and Hong, 2007), as reported in interspecific *L. longiflorum* and *L. ×elegans* hybrids (Roh *et al.*, 1996) and difficulties forcing immature and dormant bulbs (Roh *et al.*, 2007; Roh and Suh, 2013) can also be resolved.

The objectives of this research were to study the production of *Ornithogalum thyrsoides* 'Chesapeake Starlight' to produce commercially acceptable quality cut flowers by forcing *in vitro* propagated plants in a clump in one year by optimizing the size of explants of 2 mm in length and 2, 4, or 6 mm in width (2 × 2, 2 × 4, or 2 × 6 mm). Also studied were forcing temperatures given for 30 or 60 days of each at 16.5°/16°C (16°C) or 18.5°/18°C (18°C) after transplanting the clumps without separating plantlets in pots filled with growing medium.

Materials and methods

In vitro propagation and general culture

Ornithogalum thyrsoides 'Chesapeake Starlight' was used in this evaluation. When 3 to 4 florets opened in an air-conditioned greenhouse maintained at 16.5°/16°C (07001700/17000700 HR), the mid-portion of leaf subtending the scape was collected and sterilized as described previously (Joung *et al.*, 2002). One mm of tissue along the margin of each leaf was excised and the remaining leaf tissue was cut into the various sizes of explants (2 × 2, 2 × 4, and 2 × 6 mm; length × width). Three explants of each size were placed per petri-dish (60 × 15 mm) filled with a Murashige and Skoog medium supplemented with 2.7μM NAA plus 6.6μM BA on Sept. 13 (fig. 1A) and cultured in a room maintained at 21°C under 21μE/m^{-2}·s^{-1} irradiance (0800 - 2000 HR) provided from cool white florescence tubes.

After acclimation of explants in a petri dish under a mist bench for 3 days (Fig. 1B), agar medium was cleaned from the root mass/explants. Explants as a clump (fig. 1C) were planted in 6.3 cm pots filled with ProMix BM (Premier Tech Horticulture, Premier Tech. Canada) and vermiculite (1:1 by volume) on Dec. 17. Clump of plantlets were transplanted into 6.3 cm square pots filled with ProMix BM on Feb. 4 and grown until Apr. 12 when they were transplanted in 10 cm pots. Pots were then divided into two groups to grow in the air-conditioned greenhouse maintained at 16.5°/16°C (16°C) or 18.5°/18°C (18°C) between Apr. 13 and May 12 (forcing period A), and each group was further divided into two groups to grow in a greenhouse maintained at 16°C or in 18°C between May 13 and June 12 (forcing period B). Then all plants were grown at 18°C until completion of the experiment in 4 months. There were 21 pots per treatment and all pots were completely randomized during two-temperature treatment periods.

Data collection and analysis

When 3 - 5 florets opened, the number of days to flowering was counted from Feb. 4 when clumps were transplanted in 6.3 cm pots. Data from the first and second scape produced from the first plantlet and

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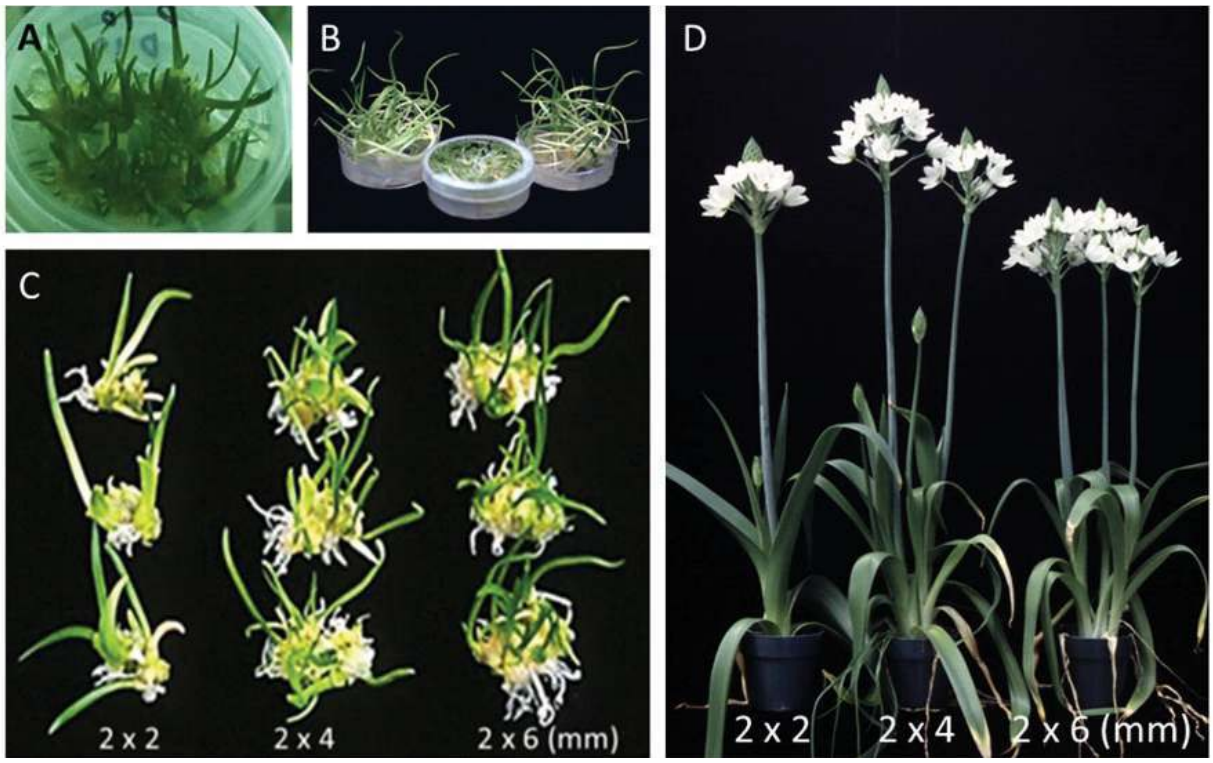

Fig.1 - Plantlet formation following placing three explants of *Ornithogalum thyrsoides* 'Chesapeake Starlight' per petri dish (A), in vitro propagated plantlets as a clump following acclimatization in petri dish (B), following acclimation (C), and at flowering in 10 cm pot (D) as affected by explant sizes of 2 × 2, 2 × 4, and 2 × 6 mm (D).

also from the second plantlet were recorded. Total number of scapes flowered from two plantlets from a clump was counted. Length of the scapes was measured to the tip of the inflorescence from the surface of the growing medium and stem diameter at one-half point of the scapes was recorded. The total number of scapes that flowered and of plantlets with 2 leaves without inflorescence (shoots) was recorded 30 days after flowering of the second scape of the second plantlet.

Data were analyzed using SAS Institute Inc. (2002) software with three variables; Explant size (variable A), forcing temperature between Apr. 13 and May 12 (forcing period A; variable B], and between May 13 and June 12 (forcing period B; variable C). Means were separated and significant differences were compared by Duncan's multiple range test at $P \leq 0.01$.

Results

Flowering of the first scape from the first plantlet (first scape/first plantlet) from a clump from a 2 × 2 mm explant was significantly accelerated, taking between 165 and 168 days with 100% flowering, which was earliest when forced at 18°C for 60 days during two forcing periods A and B from Apr. 13 to June 12 (fig. 2) regardless of the explant size and forcing period treatments given for two 30-day periods (data not presented). Scape length of first scape/first plantlet ranged from 44 cm (2 × 4 mm explant, and grown at 18°C for 60 days) to 48 cm (2 × 2 mm explant, and received 18°C from Apr. 13 to May 12 for 30 days (forcing period A) and 16°C from May 13 to June 12 for 30 days (forcing period B) (Table 1). Scape length was not affected by the size of explants or forcing temperatures given to each of two forcing periods (data not presented). The scape diameters, as significantly affected by explant size, forcing temp A and forcing temp B, and their interaction, were greater than 9.0 mm when 2 × 2 mm or 2 × 4 mm explants were forced at 16°C during forcing period A (tab. 1).

The second scape/first plantlet took between 197 and 201 days to flower, which was not significantly different regardless of the explant sizes, and forcing temperatures, and forcing periods, except that of clumps from 2 × 6 mm explants forced at 16°C during forcing temp B which took 219 days (fig. 2). More than 18 clumps produced plantlets with scapes that flowered when grown from a 2 × 6 mm explant, while less than 13 explants produced scapes from 2 × 2 mm explants (fig. 3).

Scapes were longer than 46.2 cm from plantlets from 2 × 2 mm or 2 × 4 mm explants, being affected

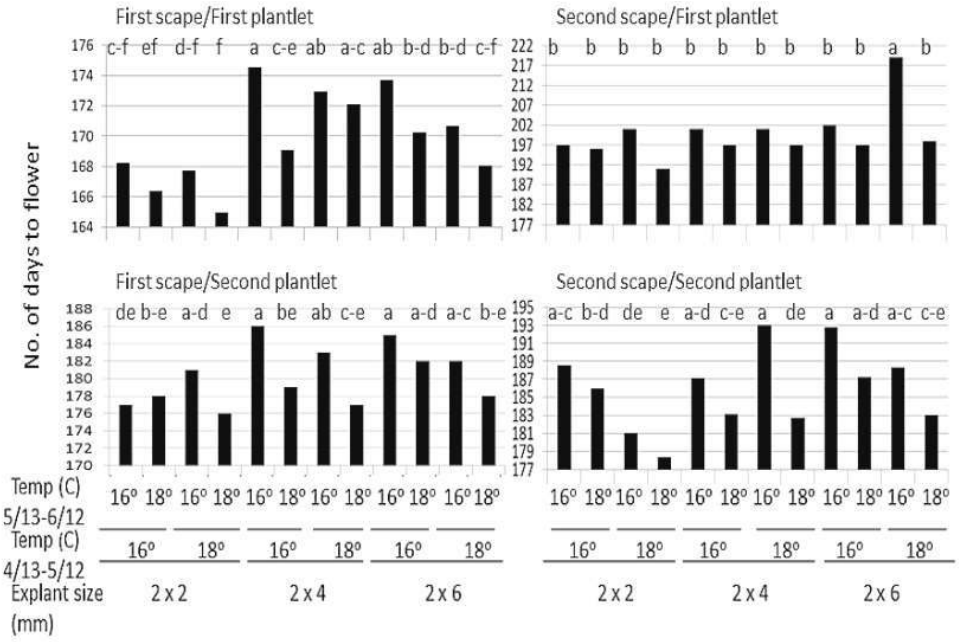

Fig. 2 - The number of days to flower from the first and second scapes developed from the first and second plantlets as influenced by explant size, forcing temperature from Apr. 13 to May 12 and from May 13 to June 12. Means with different letters are significantly different at $p \leq 0.01$.

significantly by explant size and forcing period B for explants grown at 18°C during forcing period A (tab. 1). Scape was significantly shorter when plantlets from 2 × 4 mm or 2 × 6 mm explants were grown at 18°C forcing period B. Scape diameter ranged from 7.2 mm to 8.7 mm, the diameter being significantly greater than 8.3 mm when grown from 2 × 2 mm explants at 16°C or 18°C during either of forcing period A or forcing period B (tab. 1).

The first scape/second plantlet took between 177 and 186 days to flower and produced 18 – 19 flowering scapes from 2 × 6 mm explants (fig. 2, 3). Scape lengths varied from 40.3 cm from 2 × 6 mm explants grown at 16°C forcing period A and at 18°C forcing period B to 45 cm from 2 × 2 mm explants grown at 18°C forcing period A and at 16°C forcing period B (tab. 1). The number of explants producing a second scape/second plantlet was less than 9, except for 13

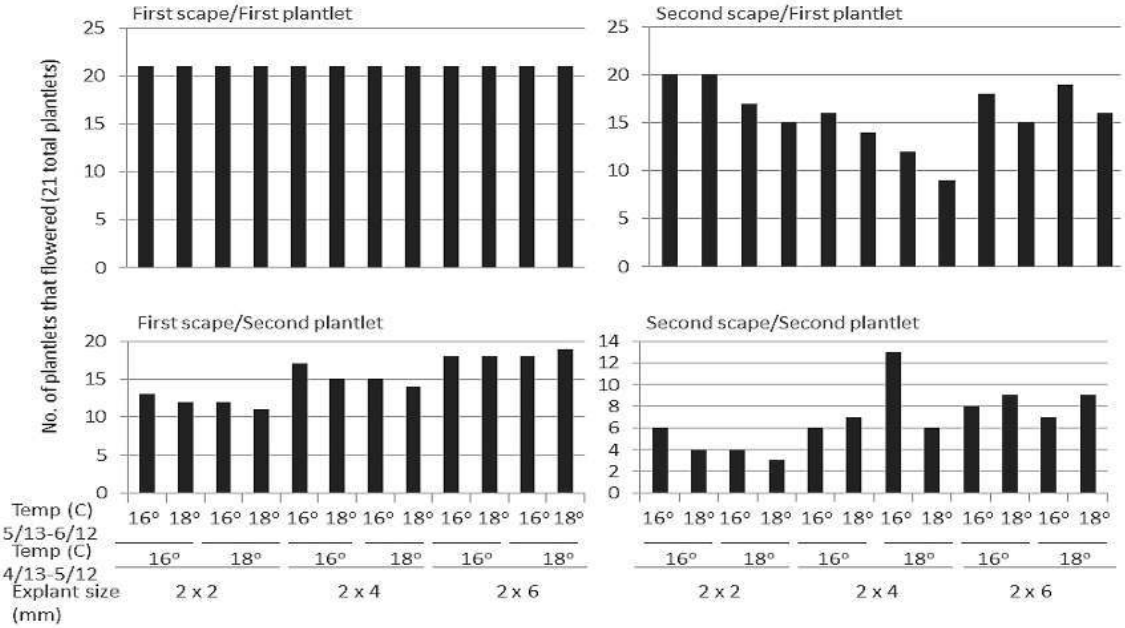

Fig. 3 - The number of plants (sample size = 21) that produced first and second scapes from the first and second plantlets as influenced by explant size, forcing temperature from Apr. 13 to May 12 and from May 13 to June 12.

Tab. 1 - The effect of explant size and greenhouse temperatures on flowering and development of *in vitro* tissue cultured propagules of *Ornithogalum thyrsoides* hybrid.

Ex-plant size (mm)	Night temp. (oC)		Order of plantlets and scapes from a plantlet that produced inflorescence [1]							Total no. of scapes flowered from explant (clump)
			First plantlet			Second plantlet				
			First scape [2]	Second scape		First scape		Second scape		
	apr-13	mag-13	diam. (mm)	length (cm)	diam. (mm)	length (cm)	diam. (mm)	length (cm)	diam. (mm)	
	5-dic	6-dic								
2 × 2	16	16	9.1 a	45.5 ab	8.7 a	42.9 b-d	7.8 a	41.8 bc	6.7 a	1.9 b-d
2 × 2	16	18	9.0 ab	43.0 b-d	8.3 ab	41.1 de	7.3 ab	39.5 cd	6.5 b	1.8 b-d
2 × 2	18	16	8.6 b-d	46.2 a	8.5 b	45.0 a	7.1 bc	41.0 b-d	6.3 bc	1.8 cd
2 × 2	18	18	8.2 de	41.6 cd	7.7 c-f	43.7 ab	6.6 c	38.3 d	6.0 a-c	1.7 d
2 × 4	16	16	8.7 a-c	41.0 d	7.9 bc	43.2 a-d	6.9 bc	45.5 a	6.8 a	2.3 ab
2 × 4	16	18	9.1 a	42.3 cd	7.8 b-d	42 b-e	7.1 bc	40.6 cd	6.1 bc	2.0 a-d
2 × 4	18	16	8.1 e	46.4 a	7.9 bc	43.3 a-c	6.8 bc	44.2 ab	6.5 b	2.2 a-c
2 × 4	18	18	8.1 e	42.7 cd	7.3 ef	42.1 b-e	6.9 ab	39.4 cd	6.1 bc	2.0 a-d
2 × 6	16	16	8.6 cd	44.0 a-c	7.9 bc	43.0 a-d	7.4 ab	41.7 bc	5.7 c	2.5 a
2 × 6	16	18	8.1 e	41.0 d	7.4 d-f	40.3 e	6.6 c	38.2 d	5.7 bc	2.5 a
2 × 6	18	16	8.3 c-e	43.4 b-d	7.7 c-e	42.6 b-d	7.0 bc	41.0 b-d	6.1 bc	2.2 a-c
2 × 6	18	18	7.9 e	40.7 d	7.2 f	41.2 c-e	6.7 c	37.8 d	6.0 bc	2.4 a
Level of significance [4]										
Explant size (ES) [5]			**	**	**	*	*	**	**	**
FP A; 4/13-5/12			**	ns	**	ns	**	ns	ns	ns
FP B; 5/13-6/12			*	**	**	**	**	**	**	ns
ES × FP A			*	**	ns	ns	*	ns	ns	ns
ES × FP B			**	ns	ns	ns	**	ns	ns	ns
FP A × FP B			ns	*	*	ns	ns	ns	ns	ns
ES × FP A × FP B			ns	ns	ns	ns	ns	ns	ns	ns

[1] Order of plantlets formed from a clump that produced scape that flowered.
[2] Two scapes that flowered from one plantlet. Scape lengths of the first scape from the first plantlet that varied from 43.9 cm to 47.9 cm were not significantly affected by explant size, forcing temperature, and forcing period.
[3] The number of days to flower (FL) was counted when plantlets were planted in 6.3 cm pot. The number of days to flower followed by Duncan's multiple range test at $P<0.01$, F-test .Means in a column with the same letters are not significantly different from each other.
[4] ns, *, **: non-significant, significant at $P\leq0.05$ and $P\leq0.01$, respectively.
[5] Explant size (ES), forcing period A (FP A) and forcing period B (FP B)

from explants 2 × 4 mm grown at 18°C during forcing period A (fig. 3).

The second scape/second plantlet took between 178 days and 193 days to flower (fig. 2) with scape lengths ranging between 37.8 cm and 45.5 cm, and diameter ranged between 5.7 mm and 6.8 mm (tab. 1). Length and diameter of scapes were significantly taller and wider as explant size was smaller (2 × 4 mm) when forced at 16°C for 60 days (forcing period A and forcing period B) (tab. 1).

The total number of plantlets that produced scapes was significantly higher from 2 × 6 mm explants and ranged from 2.2 to 2.5 scapes per clump, compared with 2 × 2 mm explants which produced less than 1.9 plantlets. The number of scapes ranged from 2.0 to 2.3 from 2 × 4 mm explants (tab. 1).

Discussion

Separating each plantlet from a clump regenerated from an explant is time consuming, and if strong scapes with many florets can be produced without separating plantlets, it will be beneficial to save labor. Further, programming one- or two-year old bulbs that requires longer than 8 months to break dormancy and to induce maturity from immature bulbs can be avoided (Luria *et al.*, 2002; Roh and Hong, 2007; Roh and Joung, 2004; Roh *et al.*, 2007). Although there are many reports on multiplication, *in vitro* flowering (Ziv and Naor, 2006), and morphogenesis of geophytes such as *Ornithogalum* (Halaban et al, 1965; Hussey, 1976), no reports cover the production of commercially acceptable cut flowers of *Ornithogalum* and few describe it in any geophytes, except in *Lilium longiflorum* and *L.* ×*elegans* interspecific hybrid lily (Roh *et al.*, 1996; Suh *et al.*, 2013).

Preparing the proper explant size is the first step (stage 1) (Debergh and Reed, 2012) for in vitro propagation to produce the optimum number of plantlets that can flower on several long and strong scapes. This research has mainly concentrated on stages 2 and 3 that involve proliferation and plants with scapes from different size of explants. It would be desirable to produce long (>40cm) and thick (>7mm) scapes from the first two or three plantlets and two scapes per plantlet with >90% flowering as the criteria based on commercially acceptable quality in this study. These standards are: scape length of >40cm and 100% flowering percentage with 2 scapes when one year old *O. thyrsoides* bulbs are forced (Roh *et al.*, 2007; Roh and Hong, 2007).

Further, one of the unique features of this work is that forcing tissue cultured propagules starting from leaf culture does not present dormancy/immaturity physiological problems, perhaps due to a lack of bulb formation. Even in vitro flowering of *Ornithogalum arabicum* L. was possible in buds from flowering sized bulbs (Halaban *et al.*, 1965). Forcing starting from bulbs involves immaturity that lowers flowering percentage and affects the number of days to flower. It takes longer than 293 days from potting bulbs or longer than 169 days after leaf emergence and flowering. Moreover, as low as 23 - 48% flowering occurs from bulbs that did not receive a 30°C inductive temperature treatment. On the other hand, >168 days are required with >78% flowering after 6 weeks of an inductive treatment (Roh *et al.*, 2007). Flowering of the first scape of the first plantlet (first scape/first plantlet) from a 2 × 2 mm explant takes between 165 and 168 days with 100% flowering when cultured on a MS medium supplemented with 2.7µM NAA plus 6.6µM BA. This is comparable to 168 days with 100% flowering using mature bulbs following 3 weeks of 30°C treatment given to bulbs (Roh *et al.*, 2007).

The second scape/first plantlet that takes between 197 and 201 days is comparable to flowering from immature bulbs and 75% flowering of the second scape/first plantlet is also comparable to 78% from immature bulbs. Scape length longer than 44 cm from in vitro propagated plantlets is also comparable to 46 cm from mature bulbs, although the length depends on the bulb programming treatments on bulbs ranging from 42 cm to 66 cm (Roh and Hong, 2007; Roh *et al.*, 2007). Scape length of immature bulbs was 41 cm with less than 78% flowering suggesting that forcing in vitro propagated propagules does not involve physiological dormancy to induce flowering (Roh *et al.*, 2007).

Further, scape diameter thicker than 7.7 mm in most treatments supports the hypothesis that quality cut flowers can be produced starting from in vitro propagated plantlets. Although the number of plantlets that produced flowering from a first scape/second plantlet and from a second scape scape/second plantlet is low, the days to flowering and number of flowering scapes is comparable to that from mature or immature treated bulbs (Suh *et al.*, 2013). This clearly suggests that forcing directly from in vitro propagated propagules can overall shorten the total production of quality *Ornithogalum* cut flowers by bypassing the bulb production phase that typically takes one full year.

Forcing plantlets at 16°C during forcing period A and forcing period B for 30 days each period produces long scapes from 2 × 4 mm explants. Forcing

temperatures tested in this experiment (16° and 18°C) are probably comparable to the suggested forcing temperatures (17°-18°C) considered best for forcing time and plant quality (Lee and Miller, 2015). Moreover, 2 × 6 mm explants could be cultured for potted plant production since more explants and more scapes per clumps produced 2.2 to 2.5 scapes per clumps (fig. 1D). This was more than those from 2 × 2 mm and 2 × 4 mm explants, which are acceptable although they are slightly shorter. Forcing experiments using 2 × 2 mm and 2 × 4 mm explants under various concentrations of NAA and BA will soon be reported.

Conclusion

Flowering of *O. thyrsoide*s 'Chesapeake Starlight' takes between 165 days (first scape, 2 × 2 mm explant forced at 18°C) and 201 days (second scape, 2 × 4 mm explants at 18° and 16°C) from the first plantlet when explants were cultured transplanted into pots without separating plantlets from the clump. Forcing plantlets at 16°C for 60 days produced longer scapes from 2 × 4 mm explants for the first and second scape of the first plantlet with 100% and 78% flowering, respectively. Flowering of the first scape from the second plantlet that produced an inflorescence takes 177 to 183 days. Three to four flowering scapes longer than 44 cm (first scape/first plantlet) are produced from 2 × 4 mm or 2 × 6 mm explants. Scape diameter greater than 6.6 to 7.7 mm in most treatments for the second scape of the first plantlets or the first scape of the second plantlet is strong enough to support many florets. This clearly demonstrates that forcing directly from in vitro propagated propagules can overall shorten the total production of quality *Ornithogalum* cut flowers bypassing the one-year bulb production phase and also avoid bulb maturity that affects the speed of flowering. Although a 2 × 2 mm explant can be used, due to a low number of scapes that flowered, it is concluded that 2 × 4 mm explants are considered more suitable for cut flower production.

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Abstract

The objective of this study is to investigate the feasibility to produce quality cut flowers of *Ornithogalum thyrsoides* hybrid 'Chesapeake Starlight' starting from tissue cultured propagules in one year. Three different sizes of explant [2 × 2, 2 × 4, and 2 × 6 mm (length × width)] were cultured on a Murashige and Skoog medium supplemented with 2.7µM NAA and 6.6µM BA. Plantlets were not separated from clumps following acclimation and one clump was transplanted per 10 cm pot. Plants were forced in air-conditioned greenhouses maintained at 16.5°/16°C (16°C) or 18.5°/18°C (day/night) (18°C) for 30 days or 60 days. The first plantlet produced two scapes with inflorescence that took between 165 days (first scape, 2 × 2 mm explants forced at 18°C) and 201 days (second scape, 2 × 4 mm explants at 18°C and 16°C), indicating that 2 × 2 mm and 2 × 4 mm explants were considered as suitable explant sizes. Flowering percentages for the first and second scape of the first plantlet from 2 × 4 mm explants forced at 16°C for 60 days were 100% and 78%, respectively. Flowering from the first scape of the second plantlet took 177 to 183 days. Three to four flowering scapes longer than 44 cm (first scape of the first plantlet) or 38 cm were produced from 2 × 2 mm or 2 × 4 mm explants with scape diameter greater than 7.7 and 6.6 mm in most treatments and scapes were strong enough to support florets. This demonstrates that in vitro propagated *Ornithogalum* propagules can be forced directly from clumps of plantlets in less than a year, thus shortening the total production time to produce cut flowers with an acceptable quality.

Key words: explant size, flowering, forcing, plant growth regulator, tissue culture propagation

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