

# Optimization of *Ornithogalum Saundersiae* Baker Propagation by Twin Scale Cuttings with the Use of Biopolymers

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**Abstract:** *Ornithogalum saundersiae* Baker, commonly known as Giant Chinchinchee, is an interesting bulbous plant with horticultural and medicinal potential. For increasing production of *O. saundersiae* planting material can be used rapid propagation by twin scaling. The aim of the study was to investigate the effect of parent bulb circumference, twin scale cutting weight and the type of biopolymer coating on the yield of *O. saundersiae* bulblets. Propagules were encapsulated in three polymer mixtures: 1% gellan and 0.5% chitosan; 1% iota-carrageenan and 0.5% chitosan; 1% xanthan and 0.5% chitosan. Chitosan had a molecular weight (Mw) of 48 000 g·mol<sup>-1</sup> and degree of deacetylation (DD) 85%. The twin scale cuttings were mixed with perlite and peat 1:1 (v/v) and stored for 100 days at 22-24°C and relative humidity of 70-80%. The highest number of bulblets was produced by the parent bulbs 22-24 cm in circumference. The bulblets derived from the twin scale cuttings weighing 2.1-4.1 g were characterized by greater fresh weight, the greatest circumference and they produced the highest number of adventitious roots. Encapsulation of twin scale cuttings in gellan and chitosan or in iota-carrageenan and chitosan, resulted in the bulblets with more roots and greater weight and circumference, as compared to the control ones and the bulblets obtained from scales treated with xanthan and chitosan.

**Keywords:** Bulblets, carrageenan, chitosan, Giant Chinchinchee, gellan.

## INTRODUCTION

*Ornithogalum saundersiae* Baker is an original bulbous plant belonging to Asparagaceae family, naturally occurring in southern Africa in the Eastern Transvaal, Natal and Swaziland. On the industrial scale *O. saundersiae* is grown mainly in field [1] and under covers [2] used for cut flowers but it is also an attractive garden and potted plant [3]. The bulbs of *O. saundersiae* are valuable raw materials in pharmacological studies, because they contain biologically active compounds exhibiting strong cytotoxic activity against various tumor cell lines [4]. A primary method of *O. saundersiae* propagation involve adventitious bulblets, forming in small numbers around a parent bulb, and seed planting, which is a much more time-consuming approach. An intensive method of vegetative propagation of *O. saundersiae* is the use of scale cuttings, applicable both *in vivo* [5] and *in vitro* [6]. Organogenesis of the bulbs forming on the scales depends on many factors, including temperature, light, humidity, position of the scales in the bulb, and a type of medium [5]. Plant bulbs are composed of scales of different sizes and the resulting scale cuttings differ in weight, which may probably affect the regeneration process and bulblet yield. This problem has not been addressed so far in the process of intensive propagation of *O. saundersiae* via scale cuttings. One

way of increasing the yield of adventitious bulblets formed on scale cuttings is using synthetic plant hormones [7]. An alternative solution may be the use of environmentally friendly algae extracts [8] and polymers stimulating plant growth, development and resistance, such as carrageenan [9] or chitosan [10]. Additionally, biopolymers can be used to form hydrogel coatings on the surface of plant organs to protect them against adverse influence of such external factors as UV radiation, humidity and temperature fluctuations [11].

The aim of this study was to evaluate the effect of parent bulb circumference, twin scale cutting weight and the type of biopolymer coating on the quantity and quality of *O. saundersiae* bulblets.

## MATERIALS AND METHODS

### Plant Material

The plant material included *O. saundersiae* bulbs derived from a culture maintained at the Department of Horticulture, West Pomeranian University of Technology in Szczecin (53° 25' N, 14° 32' E). The bulbs were treated for 30 min with a fungicide suspension containing 0.7% (w/v) Topsin M 500 SC (Nippon Soda, Tokio, Japan, active ingredient: thiophanate-methyl) and 1% (w/v) Kaptan 50 WP (Organika-Azot Chemical Company, Jaworzno, Poland, active ingredient: Captan). The twin scale cuttings were prepared on 15<sup>th</sup> March 2012 from healthy round bulbs. All roots and dry scales covering the bulbs were

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removed and the upper one third of each bulb was cut off. Then the bulbs were disinfected for 30 seconds in 70% (v/v) ethanol and cut longitudinally into 6 segments. Twin scale cuttings, connected by a fragment of a basal plate, were excised with a scalpel from each segment. Only the outer five twin-scales were used from each segment. The cuttings were disinfected for 10 seconds in 70% (v/v) ethanol.

### Effect of the Parent Bulb Size on the Number and Weight of Bulblets

The twin scale cuttings were prepared from parent bulbs with a circumference of 16-18 cm, 18-20 cm, 20-22 cm, and 22-24 cm, 20 bulbs per variant. The cuttings derived from each parent bulb were counted and weighed with RADWAG PS 200/2000/C/2 electronic scales (0.001 g accuracy). Then, they were mixed with perlite and peat 1:1 (v/v) and placed in plastic bags with a volume of 2 dm<sup>3</sup>. The peat had a pH 3.7 and contained: N-NO<sub>3</sub> 19.5, P 79.2, K 12.7, Ca 90.5, Mg 37.4 and Cl 40.0 (mg·dm<sup>-3</sup>). Bags with cuttings were stored in the dark, at a temperature of 22-24 °C and relative humidity of 70-80 %. After 100 days of incubation, the number and fresh weight of the bulblets formed on the twin scale cuttings were determined.

### Effect of Twin Scale Cutting Weight on the Yield and Quality of Bulblets

Thirty parent bulbs with a circumference of 20-22 cm were used to prepare twin scale cuttings that were subsequently sorted into four groups weighing 0.5-1.0 g; 1.1-2.0 g; 2.1-3.0 g, and 3.1-4.0 g. The sorted cuttings were placed by 10 in plastic bags with a capacity of 0.75 dm<sup>3</sup> filled with peat and perlite 1:1 (v/v). After 100 days of incubation in the dark, at a temperature of 22-24 °C and relative humidity of 70-80 %, the number, fresh weight and circumference of the bulblets and the number and length of adventitious roots were determined.

### The Effect of Polymer Encapsulation of Twin Scale Cuttings on the Yield and Quality of Bulblets

The twin scale cuttings obtained from the parent bulbs with a circumference of 20-22 cm were encapsulated in three polymer mixtures: (I) 1% (w/v) gellan and 0.5 % (w/v) chitosan; (II) 1% (w/v) iota-carrageenan and 0.5 % (w/v) chitosan; (III) 1% (w/v) xanthan and 0.5 % (w/v) chitosan. The encapsulation was performed as described by Startek *et al.* [12]. The polymers were purchased from Sigma-Aldrich (USA).

Chitosan used for the encapsulation had a molecular weight ( $M_w$ ) of 48 000 g·mol<sup>-1</sup> and mean degree of deacetylation (DD) 85%. The chitosan was obtained through a free radical degradation [13], and its parameters were determined by HPLC (SmartLine Knauer, Germany; Tessek Separon Hema-Bio 40 column, the Czech Republic) at the Center of Bioimmobilisation and Innovative Packaging Materials of West Pomeranian University of Technology in Szczecin. In each variant 40 cuttings were encapsulated, and after drying they were placed by 10 in plastic bags with a capacity of 0.75 dm<sup>3</sup> filled with peat and perlite 1:1 (v/v). The controls were twin scale non-encapsulated cuttings. Bulblet yield was evaluated after 100 days of incubation in the dark, at a temperature of 22-24 °C and relative humidity of 70-80 %.

### Statistical Analysis

The experiment was designed as fully randomized and univariate study. The mean values were calculated using the analysis of variance ANOVA using the Statistica 10.0 software (Statsoft, Poland), and the significance of differences between means was tested using Tukey's test at a significance level  $P \leq 0.05$

## RESULTS AND DISCUSSION

The number and weight of twin scale cuttings obtained from a single *O. saundersiae* parent bulb significantly depended on the bulb circumference (Table 1). The greatest number of twin scale cuttings was obtained from the bulbs with a circumference of 22-24 cm, and the lowest from those of 16-18 cm in circumference. The weight of twin scale cuttings obtained from the largest parent bulbs was over two times greater than that of the cuttings prepared from the parent bulbs of smaller circumference. After 100 days of incubation, the number and weight of *O. saundersiae* bulblets formed on the twin scale cuttings was different and depended on the circumference of the parent bulbs (Table 1). The greatest number of bulblets from a single parent bulb was obtained from the parent bulbs with a circumference of 22-24 cm. The parent bulbs with a circumference of 16-18 cm yielded the lowest number of the bulblets. The bulblets originating from the parent bulbs with a circumference of 20-22 cm and 22-24 cm were characterized by the highest fresh weight (mean 2.69 g), and those obtained from the bulbs with a circumference of 16-18 cm had the lowest fresh weight (0.82 g). Similar results to those reported in this paper were obtained in a study on the

**Table 1: The Effect of the Parent Bulb Size on the Yield of Twin Scale Cuttings and Bulblets of *Ornithogalum saundersiae* Baker**

Circumference of parent bulb (cm)	Number of cuttings per parent bulb	Fresh weight of cutting (g)	Number of bulblets per parent bulb	Fresh weight of bulblet (g)
16-18	28.5 ± 1.09 d	1.75 ± 0.09 d	8.10 ± 0.07 d	0.82 ± 0.03 c
18-20	32.0 ± 0.10 c	2.00 ± 0.15 c	9.80 ± 1.02 c	1.18 ± 0.08 b
20-22	39.0 ± 3.07 b	2.95 ± 0.44 b	22.1 ± 2.57 b	2.41 ± 0.21 a
22-24	52.8 ± 4.60 a	3.70 ± 0.20 a	35.6 ± 4.60 a	2.97 ± 0.02 a

Values represent the means of four replications ± standard deviations. Mean values followed by the same letter in each column are not significantly at  $P \leq 0.05$  by Tukey's multiple range test.

propagation of *Hippeastrum hybridum* 'Appleblossom' via twin scale cuttings [14]. The higher the diameter of the parent bulbs, the greater was the number of the bulblets. In *Stenbergia lutea* (L.) Ker-Gawl ex Sprengel, propagated in a vegetative way through chipping techniques, the circumference of the parent bulbs affected the number, length and weight of the bulblets [15].

The weight of the twin scale cutting significantly affected the number and quality of *O. saundersiae* bulblets (Table 2). The twin scale cuttings with a weight of 3.1-4.0 g yielded over two times more bulblets than the smallest cuttings. Fresh weight of the bulblets obtained from twin scale cuttings with the weight larger than 2.1 g was over three times higher compared to the weight of the bulblets formed on twin scale cuttings weighing from 0.5 to 2.0 g. The bulblets obtained from twin scale cuttings weighing from 2.1 to 4.0 g had the greatest circumference (mean 2.61 cm), while those obtained from the cuttings weighing 0.5-1.0 g were characterized by the smallest circumference (1.21 cm). The bulblets obtained from the twin scale cuttings weighing 2.1-3.0 g and 3.1-4.0 g produced the highest number of adventitious roots (mean 2.38). The adventitious roots were over two times less abundant in the bulblets grown from the twin scale cuttings weighing 0.5-1.0 g. Longer adventitious roots were

found in the bulblets obtained from the twin scale cuttings weighing from 2.1 to 4.0 g, and shorter ones were observed in the bulblets formed from the cuttings weighing 0.5 to 2.0 g. The relationship between the weight of a cutting and bulblets yield was investigated in earlier studies in *Hymenocallis narcissiflora* (Jacq.) J.F. Macbr. [16] and in *Eucharis × grandiflora* Planch. et Linden [17]. In both geophytes, good quality bulblets were obtained from twin scale cuttings weighing at least 2 g. This agrees with results of our experiment.

The properties of biopolymers, particularly their biodegradability and non-toxicity, make them a safe tool for treating seeds [18] and encapsulating bulbs, tubers, and other plant organs [19]. Chitosan and its derivatives are successfully used in plant production to stimulate plant growth and resistance. It was reported that soaking freesia [10] and gladiolus [20] corms in chitosan solution increased the yield of inflorescences and propagation rate. Encapsulation of the cuttings in biopolymers significantly affected the bulblets quality of *O. saundersiae*, but not their number (Table 3). It was found that the bulblets obtained from the twin scale cuttings encapsulated in gellan and chitosan or in iota-carrageenan and chitosan, had the greatest fresh weight, circumference and number of adventitious roots, as compared to the bulblets obtained in variant with xanthan and the control ones. Chitosan was found

**Table 2: The Effect of Twin Scale Cutting Weight on the Yield and Quality of Bulblets of *Ornithogalum saundersiae* Baker**

Cutting weight (g)	Number of bulblets per cutting	Bulblet fresh weight (g)	Bulblet circumference (cm)	Number of bulblet roots	Root length (cm)
0.5- 1.0	0.75 ± 0.02 c	0.24 ± 0.31 b	1.21 ± 0.28 c	1.05 ± 0.02 c	1.07 ± 0.01 b
1.1-2.0	0.86 ± 0.03 bc	0.52 ± 0.14 b	2.11 ± 0.13 b	1.59 ± 0.06 b	1.20 ± 0.02 b
2.1-3.0	1.30 ± 0.11 ab	1.08 ± 0.06 a	2.53 ± 0.08 a	2.36 ± 0.09 a	3.13 ± 0.08 a
3.1-4.0	1.61 ± 0.09 a	1.27 ± 0.19 a	2.69 ± 0.08 a	2.40 ± 0.22 a	2.27 ± 0.08 a

Values represent the means of four replications ± standard deviations. Mean values followed by the same letter in each column are not significantly at  $P \leq 0.05$  by Tukey's multiple range test.

**Table 3: The Effect of Encapsulation of Twin Scale Cuttings in Biopolymers on the Bulblet Yield of *Ornithogalum saundersiae* Baker**

Coating variants	Number of bulblets per cutting	Bulblet fresh weight (g)	Bulblet circumference (cm)	Number of bulblet roots	Root length (cm)
control (without coating)	1.12 ± 0.02 a	0.73 ± 0.07 b	1.90 ± 0.27 b	5.28 ± 0.73 b	2.50 ± 0.26 b
gellan and chitosan	1.23 ± 0.04 a	1.52 ± 0.12 a	3.02 ± 0.55 a	8.00 ± 0.60 a	10.4 ± 2.21 a
iota-carrageenan and chitosan	1.16 ± 0.11 a	1.33 ± 0.07 a	2.95 ± 0.12 a	8.81 ± 0.24 a	2.03 ± 0.10 b
xanthan and chitosan	1.24 ± 0.11 a	0.83 ± 0.10 b	2.04 ± 0.11 b	4.55 ± 0.26 b	2.18 ± 0.88 b

Values represent the means of four replications ± standard deviations. Mean values followed by the same letter in each column are not significantly at  $P \leq 0.05$  by Tukey's multiple range test.

to stimulate the development of the root system in *Ficus microcarpa* L. [21] and in *Dendrobium* 'White Pancy Lip' [22]. Also, treating twin-scales of *E. grandiflora* with chitosan resulted in bulblets with a higher number of adventitious roots [17]. A similar positive effect of chitosan on the root system was observed by Chamnanmanoontham *et al.* [23] in growing of rice (*Oryza sativa* L.). The seedling treated with oligomeric chitosan with an 80% DD applied at 40 mg·dm<sup>-3</sup> significantly enhanced the root fresh weights. Another group of polymers that are becoming increasingly important in the plant cultivation are carrageenans derived from red algae. A growth-inducing effect of carrageenan was described in such species as *Cicer arietinum* L. [24], *Eucalyptus globulus* Labill. [25], or *Zea mays* L. [24]. Carrageenan's effects on plant growth has also been shown in tobacco 'Burley' [26]. The foliar spraying of oligo-carrageenans, mainly oligo-carrageenan iota, on tobacco stimulated of photosynthesis, basal metabolism and cell cycle in plant. The present study showed that the adventitious roots were significantly longer in the bulblets derived from the cuttings encapsulated in gellan and chitosan (Table 3). Similar results were observed in microcuttings of *Pyrus pyrifolia* (Burm F.) Nakai.I., which grew roots more effectively in a gellan-containing medium [27].

## CONCLUSION

The yield of *O. saundersiae* bulblets propagated via outer twin scale cuttings depended on the size of the parent bulbs, cutting weight, and the use of polymer coatings. The highest number of bulblets was produced by the parent bulbs 22-24 cm in circumference. Bulbs of good quality were obtained from the twin scale

cuttings weighing at least 2 g. Encapsulation of the cuttings in iota-carrageenan and gellan resulted in the formation of bulblets with greater weight and circumference. Moreover, both biopolymers had a stimulating effect on the bulblet root system, which may improve the bulblet growth and shorten the time of growing up to the commercial size.

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## REFERENCES

- [1] Kariuki W, Kako S. Growth and flowering of *Ornithogalum saundersiae* Bak. Sci Hort 1999; 8: 57-70. [http://dx.doi.org/10.1016/S0304-4238\(98\)00233-7](http://dx.doi.org/10.1016/S0304-4238(98)00233-7)
- [2] Salachna P. Effect of bulbs size on the inflorescences and bulbs yield of *Ornithogalum saundersiae* Baker grown in an unheated plastic tunnel. Folia Pomer Univ Technol Stetin Agric Aliment Pisc Zootech 2014; 312: 153-158.
- [3] Salachna P, Zawadzińska A. The effects of flurprimidol concentrations and application methods on *Ornithogalum saundersiae* Bak. grown as a pot plant. Afr J Agric Res 2013; 8: 6625-6628.
- [4] Chin YW, Yoon KD, Kim J. Cytotoxic anticancer candidates from terrestrial plants. Antic Ag Med Chem 2009; 9: 913-942. <http://dx.doi.org/10.2174/187152009789124664>
- [5] Kariuki W. Rapid multiplication of *Ornithogalum saundersiae* Bak. through bulblet production *in vivo*. Acta Hort 2008; 766: 135-142.
- [6] Kariuki W. Plant regeneration on bulb scales of *Ornithogalum saundersiae* Bak. *in vitro*. Acta Hort 2007; 764: 231-238.
- [7] Zhang W, Song L, Teixeira da Silva JA, Sun H. Effects of temperature, plant growth regulators and substrates and changes in carbohydrate content during bulblet formation by twin scale propagation in *Hippeastrum vittatum* 'Red lion'. Sci Hort 2013; 160: 230-237. <http://dx.doi.org/10.1016/j.scienta.2013.06.001>
- [8] Salachna P, Zawadzińska A, Piechocki R, Wilas J. Propagation of arabian star flower (*Ornithogalum arabicum*

- L.) by twin scales using seaweed extracts. *Folia Pomer Univ Technol Stetin Agric Aliment Pisc Zootech* 2014; 310: 105-112.
- [9] González A, Moenne F, Gómez M, Sáez CA, Contreras RA, Moenne A. Oligo-carrageenan kappa increases NADPH, ascorbate and glutathione syntheses and TRR/TRX activities enhancing photosynthesis, basal metabolism, and growth in *Eucalyptus* trees. *Front. Plant Sci* 2014; 5: 512. <http://dx.doi.org/10.3389/fpls.2014.00512>
- [10] Salachna P, Zawadzińska A. Effect of chitosan on plant growth, flowering and corms yield of potted freesia. *J Ecol Eng* 2014; 15: 97-102.
- [11] Bartkowiak A, Startek L, Salachna P, Kamińska M, Mazurkiewicz – Zapałowicz K. The influence of new method of seed and corm coating on polyelectrolyte complex. Proceedings of XIIth International Workshop on Bioencapsulation, Faculty of Pharmacy; 2004: Victoria, Spain: p. 347-349.
- [12] Startek L, Bartkowiak A, Salachna P, Kamińska M, Mazurkiewicz – Zapałowicz K. The influence of new method of corm coating on freesia growth, development and health. *Acta Hort* 2005; 673: 611-616.
- [13] Bartkowiak A. Binary polyelectrolyte micro-capsules based on natural polysaccharides. PS Szczecin; 2001.
- [14] Gushing P, Klingaman G. Effects of benzyladenine, temperature, and bulb diameter on twin-scale propagated *Amaryllis*. *HortScience* 1995; 30: 836
- [15] Seyidoglu N, Zencirkiran M. Vegetative propagation of *Sternbergia lutea* (L.) Ker-Gawl. ex Sprengel (Winter Daffodil) by chipping techniques. *J Biol Sci* 2008; 8: 966-969. <http://dx.doi.org/10.3923/jbs.2008.966.969>
- [16] Salachna P, Startek L, Żurawik P. Possibility of lily spider (*Hymenocallis* Salisb.) bulbs reproduction. *Zesz Prob Post Nauk Roln* 2003; 491: 223-227.
- [17] Salachna P, Skierkowska M. Effects of the weight of twin-scale cutting and chitosan on the yield of adventitious bulbs of *Eucharis × grandiflora* Planch. et Linden. *Zesz Prob Post Nauk Roln* 2010; 551: 321-326.
- [18] Chookhongkha N, Sopondilok T, Photchanachai S. Effect of chitosan and chitosan nanoparticles on fungal growth and chilli seed quality. *Acta Hort* 2013; 973: 231-238.
- [19] Bartkowiak A, Startek L, Salachna P, Żurawik P. Method of hydrogel coating formation on the surface of plant organs. Poland patent PL 197101. 2008 Feb.
- [20] Ramos-Garcia M, Ortega-Centeno S, Hernandez-Lauzardo AN, Alia-Tejagal I, Bosquez-Molina E, Bautista-Baños S. Response of gladiolus (*Gladiolus* spp) plants after exposure corms to chitosan and hot water treatments. *Sci Hortic* 2009; 121: 480-484. <http://dx.doi.org/10.1016/j.scienta.2009.03.002>
- [21] Gamlath M, Abeywickrama K, Wickramarachchi S. Root growth promotion of *Ficus* species during air-layering. *Cey J Sci* 2010; 39: 45-51.
- [22] Obsuwan K, Sawangsri K, Ukong S, Uthairatanakij A. Effects of chitosan concentration on *in vitro* growth of dendrobium hybrid seedlings. *Acta Hort* 2010; 878: 289-294.
- [23] Chamnanmanoontham N, Pongprayoon W, Pichayangkura R, Roytrakul S, Chadchawan S. Chitosan enhances rice seedling growth *via* gene expression network between nucleus and chloroplast. *Plant Growth Regul* 2014. <http://dx.doi.org/10.1007/s10725-014-9935-7>
- [24] Bi F, Iqbal S, Arman M, Ali A, Hassan M. Carrageenan as an elicitor of induced secondary metabolites and its effect on various growth characters of chickpea and maize plants. *J Saudi Chem Soc* 2011; 15: 269-273. <http://dx.doi.org/10.1016/j.jscs.2010.10.003>
- [25] González A, Contreras RA, Moenne A. Oligo-carrageenans enhance growth and contents of cellulose, essential oils and polyphenolic compounds in *Eucalyptus globulus* trees. *Molecules* 2013; 18: 8740-8751. <http://dx.doi.org/10.3390/molecules18088740>
- [26] Castro J, Vera J, González A, Moenne A. Oligo-carrageenans stimulate growth by enhancing photosynthesis, basal metabolism, and cell cycle in tobacco plants (var. Burley). *J Plant Growth Regul* 2012; 31: 173-185. <http://dx.doi.org/10.1007/s00344-011-9229-5>
- [27] Tetsumura T, Ishimura A, Aikou Y, Eguchi N, Kai Y, Tashiro K, Honsho C. Vermiculite-containing and gellan gum-solidified medium improves rooting of microcuttings of Japanese pear cultivars. *Acta Hort* 2011; 923: 59-64.

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