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Physiological status, rooting and bulb yield of leaf cuttings of *Eucomis comosa* (Houtt.) H.R. Wehrh. 'Sparkling Burgundy' as affected by chitosan

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Abstract: Breeding efforts within the *Eucomis* genus has resulted in the global availability of attractive cultivars with decorative leaves and inflorescences. A cultivar that is particularly valuable and attractive, but rarely cultivated due to its low propagation rate is 'Sparkling Burgundy'. In our research, we considered it advisable to check the possibility of intensifying the reproduction of the investigated cultivar by means of leaf cuttings. This study, conducted in the years 2016–2018, involved leaf cuttings collected from plants growing in an unheated polytunnel. The cuttings were soaked, watered or sprayed with 0.4% chitosan with a molecular weight of 7 000 or 10 000 g/mol. The control plants were not treated with chitosan. During the rooting period that followed, the cuttings were assessed for their chlorophyll a fluorescence and the intensity of their greenness, and for their root growth and bulb yield after five months. Chitosan applied during rooting improved some chlorophyll fluorescence parameters and the greenness index of the cuttings. Moreover, the compound with a molecular weight of 7 000 g/mol more efficiently increased the number and length of the roots and the number and weight of the adventitious bulbs than that with a molecular weight of 10 000 g/mol. Soaking the cuttings prior to rooting was more effective in terms of the number, length and weight of the produced roots and the number, diameter and weight of the daughter bulbs than watering or spraying.

Keywords: adventitious rooting; senescence; bulb reproduction; cutting necrosis

The species of the *Eucomis* genus have a low propagation rate (Cheesman et al. 2010; Duncan 2011; Masondo et al. 2014). During a vegetative season, *Eucomis autumnalis* usually produces from one to five adventitious bulbs (Taylor, van Standen 2001; Knippels 2012), *Eucomis bicolor* usually produces from one to four bulbs (Nndwambi et al. 2013), and *Eucomis comosa* usually produces from one to three bulbs (Carlson, Dole 2015). For bulbous plants with such a low propagation rate, it is necessary to develop methods that enable the mass production of planting material over a short period of time (Kariuki 2008, Ndhlala et al. 2012). According to Nndwambi et al. 2013, propagation via leaf cuttings seems to be an effective method with *Eucomis bicolor* and *Eucomis vandermerwei*.

In agriculture and horticulture, chitosan is used primarily to stimulate immunity (Hadwiger et al. 2002; Niekraszewicz et al. 2012) and to activate quicker plant defence responses to pathogen attacks (Bautista-Baños et al. 2006; Limpanavech et al. 2008; Coqueiro, di Piero 2011). Although chitosan is not present in plants, it shows high biological activity in their tissues by stimulating numerous physiological and biochemical processes (Nge et al. 2006). It also improves the plant tolerance

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to adverse environmental conditions, e.g., drought (Yang at al. 2009; Lizárraga-Paulín et al. 2011; Mahdavi et al. 2011). The effects of chitosan on plants depend on the species (Kananont et al. 2010; Obsuwan et al. 2010), cultivar (Uddin et al. 2004), plant development phase (Mondal et al. 2012), the application method (Algam et al. 2010), concentration (Sheikha, AL-Malki 2011; Mondal et al. 2012), or the number (El-Tanahy et al. 2012), and duration of treatments (Kaczmarek-Cichosz 2011).

Considering the reasons presented above, we decided to investigate the possibility of increasing the bulb propagation rate of *E. comosa* 'Sparkling Burgundy' via leaf cuttings and to examine the effects of chitosan at different molecular weights on the rooting of the cuttings and bulb initiation.

MATERIAL AND METHODS

Experimental design. The study was conducted in the years 2016–2018 in a greenhouse of the West Pomeranian University of Technology in Szczecin, Poland (53°25'N, 14°32'E). The plants from which the leaves were sourced were grown on beds in an unheated polytunnel. Two weeks before planting the bulbs, the soil nutrient deficiencies were replenished by applying Azofoska fertiliser INCO-Veritas S.A. (N 13.6%, P_2O_5 6.4%, K_2O 19.1%, MgO 4.5%, B 0.045%, Cu 0.180%, Fe 0.17%, Mn 0.27%, Mo 0.040%, Zn 0.045%) at 60 g/m^2. A top dressing with the same fertiliser at 50 g/m^2 was also supplied in the last ten days of June. The bulbs were first treated with a 1.5% Kaptan 50 WP suspension, and then planted each year on the 15th of April at 25 × 30 cm spacing and a depth of 12 cm. The leaves, for further experiments, were collected each year on the 13th of July from the non-flowering plants. The cuttings were harvested from two to four external, fully developed and healthy leaves without any signs of pest infestation. The leaves were removed at their base adjacent to the bulb, washed of the remaining substrate and dried. Then, with a sharp knife, they were cut crosswise into 10 cm long fragments. Only the cuttings from the central part of the leaves were intended for further experiments.

The experiments were established in a propagator equipped with a heating system that kept the substrate temperature 2 °C above the air temperature. The experiments were carried out in a greenhouse multiplier equipped with a heating system. The air temperature was maintained at 20–22 °C during the day and 15 °C at night. The light intensity depended on the prevailing weather conditions. During the rooting of the cuttings, the maximum light intensity ranged between 140.5 and 186.7 μmol/m^2/s. The length of the lighting period at that time ranged from 16 hours in July to 8 hours in December. The rooting substrate was composed of Kronen high peat (fraction 0–30 mm) and Perligran perlite (fraction 2–6 mm) mixed in a 1:1 volume ratio. Boxes with dimensions of 15 × 30 × 13 cm were filled with the substrate 24 h before planting the cuttings and sterilised with 0.15% Previcur Energy 840 SL (Bayer, Poland). Three cm deep grooves were made in the prepared substrate, the cuttings were placed there, and surrounded with the substrate. The substrate was gently compressed around the cuttings to keep them upright. A single box contained ten cuttings.

The study used chitosan with a molecular weight of 7 000 or 10 000 g/mol that was obtained by the controlled free radical degradation of chitin from shrimp shells carried out in the Department of Packaging and Biopolymers of the West Pomeranian University of Technology in Szczecin, Poland. A chloride form (0.4%) was used to dissolve the compound directly in tap water. The chitosan solution was applied by soaking, watering or spraying the cuttings. Soaking consisted of immersing the cuttings at a depth of 1 cm for 10 minutes and then planting the cuttings in the substrate. In the variants with chitosan spraying, the treatment was performed with a Kwazar hand pressure sprayer. The seedlings were covered with the chitosan solution on both sides in such a way as to thoroughly moisten their entire surface and prevent the solution from dripping down onto the substrate. In the variants where the chitosan was applied by watering, an appropriate amount was measured into a beaker, and then the solution was poured onto the substrate on both sides of the cutting. The treatments involving spraying or watering were repeated every two weeks and 3 ml of the solution was used per cutting each time. The chitosan was used for a total of 11 treatments. The control cuttings were not treated with chitosan.

The experiment compared eight variants created by accounting for the chitosan molecular weight (2) and application method (4). All the variants included three repetitions of ten cuttings, so a total of 240 cuttings were assessed.

Induction of chlorophyll a fluorescence. In the rooting period, the parameters of the chloro-

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phyll *a* fluorescence induction were measured twice – during the third and fifth month of the culture. The cuttings were shaded for 20 minutes before the measurements with ready-made clips. The measurements were performed with a HANDY–PEA spectrofluorometer (Hansatech®) and involved five cuttings. The readings were taken from the central parts of the cuttings and used to determine and calculate the following parameters:

- T_{FM} – time the chlorophyll fluorescence increased from the beginning of the measurement until maximum (FM) (Lichtenthaler et al. 2005);
- F_O – initial fluorescence resulting from the loss of excitation energy during its transmission in the energy (Baker, Rosenquist 2004);
- F_M – maximum fluorescence determined after dark adaptation (Kalaji, Łoboda 2010);
- $F_V = F_M - F_O$ – variable fluorescence determined after dark adaptation (Kalaji, Łoboda 2010);
- F_V/F_M – maximum potential efficiency of the photochemical reaction in PSII determined following dark adaptation (Angelini et al. 2001);
- *PI* (Performance Index) – vitality index of PSII describing a general condition of this system: P – ABS = (RC/ABS) [ΦPo/(1 – ΦPo)] [Ψo/(1 – Ψo)] where ΦPo is the maximum quantum yield of the primary photochemistry, $(F_M - F_O)/F_M$, and Ψo is the probability that a trapped exciton moves an electron further than QA = 1 – F_{V2ms} (RC/ABS) is the fraction of the active reaction centres of PSII relative to the total light absorbing chlorophyll (Stirbet, Govindjee 2011).

The minimum fluorescence (F_O) was measured during 60 minutes of dark adaptation of the leaves using weak light modulation < 0.15 mmol/m^2/s. The maximum fluorescence (F_M) was measured in the same leaves after 0.8 s and being saturated with a white light pulse (> 5 500 mmol/m^2/s).

According to Druege (2020), the physiological parameters of cuttings help to assess the effectiveness of their internal processes that affect their viability and root formation at their bases, and to adjust environmental factors for optimal cutting efficiency.

Greenness index of the cuttings. A SPAD-502 chlorophyll meter (Minolta, Japan) was used to determine the leaf greenness index of the cuttings in the third and fifth month of their rooting. The index is expressed in SPAD units (Soil Plant Analysis Development), which correlates to the chlorophyll content (Gregorczyk et al. 1998) and is further dependent on the plant nutritional status. The measurements performed on five cuttings included a spot of 6 mm^2 located in their central part. The index was determined based on reading the standardised units of the chlorophyll content in the plant assimilation organs.

Percentage of necrotic seedlings. The rooting cuttings were visually assessed every two days, and all those that showed signs of damage were removed.

Root parameters. Usually after three months of rooting, the adventitious bulbs formed at the base of the cuttings that produced leaves, and, after the next two months, the cuttings did not dry up and the new leaves continued their growth. For all the study years, all the cuttings were removed from the boxes on the 9th of December before they dried up, rinsed in running water to remove any substrate residue and their roots were subjected to biometric measurements. The measurements involved the length (from the cutting base to the root tip), number (all the developed roots were counted), and weight (all the developed roots were weighed).

Bulb yield. At the end of the experiments, we also assessed the yield of the adventitious bulbs. In the case of bulbs that developed leaves, the leaves were first removed and then we determined the number of all the formed bulbs, their diameter at the widest point and their weight.

Statistical analysis. The results on the chlorophyll *a* fluorescence parameters and the greenness index were statistically verified using a three-way analysis of variance, while the data on the roots and bulb yield were processed using a two-way analysis of variance, in a completely randomised design for the individual years of the study and as a three-year summary. The mean results were compared using Tukey's test, at a significance level of α = 0.05. All the statistical analyses were performed with FR-ANALWAR software (prof. F. Rudnicki), and the data on the percentage of the necrotic cuttings were based on the average values.

RESULTS AND DISCUSSION

Śmigielska, Jerzy (2013) and Kapczyńska (2019) claim that leaf cuttings of *Eucomis bicolor* (Knippels 2000) and *Eucomis vandermerwei* (Duncan 2011) should be kept in the substrate until they dry up, as this is when the adventitious bulbs form at the base of the leaf cuttings. This information was not confirmed in our study on *E. comosa* 'Sparkling Bur-

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gundy'. Irrespective of the experimental conditions, the adventitious bulbs formed at the base of the leaf cuttings and started to produce leaves after, on average, three months of rooting. The cuttings did not dry up for the next six months, and the newly developed leaves continued to grow. Most likely, it was caused by the increased photosynthesis efficiency (Table 1), which, according to Peng et al. (1991), results in faster vegetative growth and leads to increased biomass production.

Induction of chlorophyll a fluorescence. Chitosan intensifies the plant physiological processes (Pichyangkura, Chadchawan 2015). In their experiments, Dzung et al. (2011) showed that spraying Coffea sp. with the compound improved plant photosynthesis efficiency. Our study confirmed this finding, as we found a positive effect of chitosan on the maximum chlorophyll fluorescence (T_{FM}), but no significant changes in the maximum fluorescence (F_M), variable fluorescence (F_V), and maximum potential efficiency of the photochemical reaction (F_V/F_M) (Table 1). According to Mondal et al. (2012), a 100–125 mg foliar application of chitosan did not affect the photosynthesis in *Abelmoschus esculentus*. Our study demonstrated a significant effect of watering with chitosan on increasing the time of the chlorophyll fluorescence, initial fluorescence and vitality index. At the end of the rooting period, the cuttings also

Table 1. Parameters of the chlorophyll a fluorescence induction in the *Eucomis comosa* 'Sparkling Burgundy' leaf cuttings depending on the chitosan molecular weight and application method and measurement date (mean for the years 2016–2018)

Parameter	Chitosan molecular weight (g/mol) (A)	Application method (B)				Measurement date (C)		Mean
		control	soaking	watering	spraying	I	II	
T_{FM}	7 000	312.4	374.3	420.3	381.0	313.7	430.3	372.0
	10 000	314.2	331.9	419.4	365.6	301.4	414.2	357.8
	mean	313.3	353.1	419.9	373.3	307.5	327.4	364.9
	$LSD_{0.05}$ – A: 12.12, B: 42.50, C: 14.22, B×A: n.s., C×A: n.s., C(B): 47.09, B(C): 62.25, A×B×C: n.s.							
F_O	7 000	616.4	595.5	636.2	593.2	608.7	612.0	610.3
	10 000	611.2	605.5	618.1	590.9	608.5	604.3	606.4
	mean	613.8	600.5	627.1	592.1	608.6	608.1	608.4
	$LSD_{0.05}$ – A: n.s., B: 14.68, C: n.s., B(A): 9.72, A(B): 7.34, C×A: n.s., C(B): 14.27, B(C): 18.87, A×B×C: n.s.							
F_M	7 000	2 735.6	3 108.0	2 931.4	2 885.0	3 009.7	2 820.4	2 915.0
	10 000	2 855.7	2 740.5	3 055.9	2 852.4	2 978.3	2 773.9	2 876.1
	mean	2 795.6	2 924.3	2 993.7	2 868.8	2 994.0	2 797.2	2 895.6
	$LSD_{0.05}$ – A: n.s., B: n.s., C: 81.06, B(A): 180.59, A(B): 136.50, C×A: n.s., C(B): 203.99, B(C): 269.65, A×B×C: n.s.							
F_V	7 000	2 129.9	2 512.5	2 296.9	2 225.1	2 401.8	2 180.4	2 291.1
	10 000	2 244.9	2 193.2	2 542.7	2 260.8	2 380.2	2 240.6	2 310.4
	mean	2 187.4	2 352.9	2 419.8	2 243.0	2 391.0	2 210.5	2 300.8
	$LSD_{0.05}$ – A: n.s., B: n.s., C: 99.05, B(A): 236.36, A(B): 178.66, C×A: n.s., C(B): 247.98, B(C): 327.80, A×B×C: n.s.							
F_V/F_M	7 000	0.77	0.79	0.78	0.78	0.79	0.77	0.78
	10 000	0.78	0.78	0.80	0.79	0.79	0.78	0.79
	mean	0.77	0.79	0.79	0.79	0.79	0.78	0.78
	$LSD_{0.05}$ – A: n.s., B: n.s., C: 0.009, B×A: n.s., C×A: n.s., C(B): 0.023, B(C): 0.031, A×B×C: n.s.							
PI	7 000	0.67	0.65	0.78	0.58	0.67	0.66	0.67
	10 000	0.66	0.66	0.81	0.60	0.69	0.67	0.68
	mean	0.66	0.66	0.80	0.59	0.68	0.67	0.68
	$LSD_{0.05}$ – A: n.s., B: 0.079, C: n.s., B×A: n.s., C×A: n.s., C×B: n.s., A×B×C: n.s.							

I – the third month of rooting; II – the fifth month of rooting; n.s. – not a significant difference; C(B) – NIR value for the date of measurement depending on the chitosan application method

T_{FM} – maximum chlorophyll fluorescence; F_O – initial fluorescence; F_M – maximum fluorescence; F_V – variable fluorescence; F_V/F_M – maximum potential efficiency of the photochemical reaction; *PI* – performance index

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showed enhanced T_{FM}, while an increase in F_M, F_V and F_V/F_M was already visible in the third month of rooting. Rinaudo (2006), Yang et al. (2009) and Salachna and Zawadzińska (2014b) reported that chitosan induced plant defence mechanisms under stress. Our experiments did not corroborate this information, and our results are consistent with the data published by Reigossa and Weiss (2001) and Kalaji and Łoboda (2010), who claimed that prolonging the time of reaching the maximum level of chlorophyll fluorescence depended on the influence of stress factors on the investigated plant. A significant effect of the chitosan molecular weight (7 000 g/mol of chitosan) was only detected for T_{FM}, where the time from the beginning of the measurement to the maximum level of chlorophyll fluorescence was slightly longer than for 10 000 g/mol of chitosan. As far as the chlorophyll fluorescence parameters were concerned, the highest standard deviation was found for F_V (90.9), and the lowest was found for F_V/F_M (0.01).

Greenness index of the cuttings. Our experiments demonstrated a positive influence of the chitosan on the intensity of the green colour in the rooted cuttings of *E. comosa* 'Sparkling Burgundy (Table 2). Similar findings were published for *Ornithogalum saundersiae* by Salachna and Zawadzińska (2014a) and Salachna et al. (2015a), and for *Eucomis autumnalis* by Salachna et al. (2015b), who reported on the chitosan-mediated enhanced leaf greenness index. The differences in the chitosan molecular weight did not have any significant effects on the intensity of the green colour of the cuttings. According to Salachna et al. (2008), this parameter, to a considerable degree, depends on the chitosan molecular weight. Żurawik (2013) described different effects of the chitosan molecular weight of 10 000 g/mol in *Freesia* 'Silver Beach' for its different application methods, and reported more intense leaf greenness in plants watered or sprayed with the chitosan solution. Contrary to that, in our study, the cuttings with the most intense green colouration were those soaked in chitosan rather than those watered or sprayed with the compound. The SPAD greenness index of the leaf cuttings was significantly, i.e., by 3.2 times, higher in the fifth month of rooting than in the third month of rooting. According to Druege (2020), the chlorophyll content in the leaves determines their ageing process. Most probably, the observed increase in the index evoked by the chitosan application was caused by the enhanced chlorophyll content, which seems consistent with the findings of Sheikha and Al-Malki (2011). Regardless of the chitosan molecular weight, the mean values of the leaf greenness differed by 2.67 SPAD for the individual methods of its application.

Percentage of necrotic seedlings. The use of chitosan declined the percentage of necrotic cuttings (Figure 1) in an application dependent manner. Irrespective of the chitosan molecular weight, the watering decreased the percentage of necrotic cuttings by 55.7%, and spraying decreased it by 72.7% in comparison with the control. Soaking the cuttings was the most effective, as it reduced the percentage of necroses by 85.9%. The lower number of necrotic seedlings following the chitosan treatment was also probably due to their improved tolerance to adverse environmental conditions, as claimed by Górnik et al. (2008). In our study, the chitosan with a molecular weight of 7 000 g/mol was more effective in limiting the cutting's death.

Root parameters. Irrespective of its molecular weight, the 0.4% chitosan enhanced the number of adventitious roots forming at the base of the Eucomis comosa 'Sparkling Burgundy' leaf cuttings (Table 3). The increase over the control was considerable and ranged from 40.9% to 246.7%. These results were concurrent with those reported by Hasegawa and Kanechika (2005), who claimed that a substrate supplementation with 1% chitosan boosted the number of roots developing in Arisaema ternatipartitum. Similarly, soaking two-scale cuttings

Table 2. Greenness index (SPAD) in the *Eucomis comosa* 'Sparkling Burgundy' leaf cuttings depending on the chitosan molecular weight, application method, and measurement date (mean for the years 2016–2018)

Chitosan molecular weight (g/mol) (A)	Application method (B)				Measurement date (C)		Mean
	control	soaking	watering	spraying	I	II	
7 000	30.1	37.2	34.7	34.5	32.4	35.9	34.1
10 000	31.1	38.0	33.6	35.6	33.1	36.0	34.6
Mean	30.6	38.1	34.2	35.1	32.7	35.9	34.4

$LSD_{0.05}$ – A: n.s., B: 2.61, C: 1.27, B×A: n.s., C×A: n.s., C×B: n.s., A×B×C: n.s.

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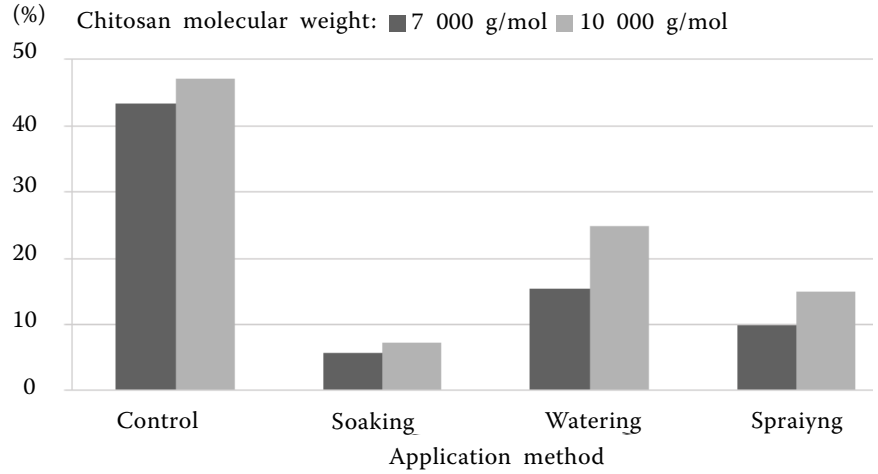

Figure 1. Percentace (%) of *Eucomis comosa* 'Sparkling Burgundy' damaged cuttings depending on the chitosan molecular weight, application method, and measurement date (mean for the years 2016–2018)

of *Eucharis* × *grandiflora* in 1% chitosan solution increased the number of adventitious roots (Salachna, Skierkowska 2010). In our research, the effect of chitosan depended on the method of its application, which is consistent with the report of Żurawik (2013). According to this author, watering and spraying were the most conducive to the development of the root system in Freesia hybrida 'Silver Beach'. Our study yielded contrary results, as the cuttings soaked in chitosan developed more roots than those watered or sprayed with its solution, and the differences reached 127.5% and 145.9%, respectively. The treatment with chitosan at a molecular weight of 7 000 g/mol resulted in developing 26.9% more roots than with chitosan at a molecular weight of 10 000 g/mol. Salachna and Skierkowska (2010) reported that two-scale cuttings of *Eucharis* × *grandiflora* produced the longest roots after soaking in 1% chitosan. Our experiments confirmed these findings, but the length of the roots depended on the chitosan molecular weight and its application method. The roots were 33.6% longer at the base of the cuttings treated with 7 000 g/mol than 10 000 g/mol of chitosan. The roots were also longer in the cuttings soaked in chitosan than in the watered, sprayed or control ones. The weight of the root system developed by the *Eucomis comosa* 'Sparkling Burgundy' leaf cuttings depended only on chitosan application method, as it was the greatest in the soaking variant than in the remaining ones. For the number of developed roots, the individual application methods produced great differences, with the standard deviation reaching 10 roots. For the root length and weight, the mean values differed by 7.52 cm and 0.51 g on average, respectively, and the standard deviation was 1.07 bulbs for the bulb number.

Table 3. Parameters of the roots developed in the *Eucomis comosa* 'Sparkling Burgundy' leaf cuttings depending on the chitosan molecular weight, application method, and measurement date (mean for the years 2016–2018)

Trait	Chitosan molecular weight (g/mol) (A)	Application method (B)				Mean
		control	soaking	watering	spraying	
Number (pcs)	7 000	10.4	42.8	17.9	14.8	21.7
	10 000	10.5	30.0	14.1	13.8	17.1
	mean	10.5	36.4	16.0	14.8	19.4
	$LSD_{0.05}$ – A: 1.64, B: 3.88, B(A): 3.63, A(B): 1.36					
Length (cm)	7 000	6.8	32.4	10.4	9.3	14.7
	10 000	7.8	19.3	8.4	8.5	11.0
	mean	7.3	25.8	9.4	8.9	12.8
	$LSD_{0.05}$ – A: 0.87, B: 2.58, B(A): 3.00, A(B): 1.13					
Mass (g)	7 000	0.64	1.93	0.89	0.95	1.10
	10 000	0.79	2.01	0.71	0.83	1.08
	mean	0.71	1.97	0.80	0.89	1.09
	$LSD_{0.05}$ – A: n.s., B: 0.242, B×A: n.s.					

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Bulb yield. Our study showed a positive effect of the chitosan on the yield of the adventitious bulbs developing on the Eucomis comosa 'Sparkling Burgundy' leaf cuttings (Table 4). The same relationship was described by Żurawik (2013) in Freesia hybrida 'Silver Beach'. Żurawik et al. (2017) concluded that the effects of chitosan depended on the methods of its application. This conclusion was also confirmed in our study, as we achieved the greatest bulb yield for the cuttings soaked in the chitosan solution. Our outcome is also concurrent with that of Ramos-Garcia et al. (2009), who reported that soaking bulbs of Gladiolus sp. 'Blanca Borrego' in a 1.5% solution of Biorent preparation increased the number of daughter bulbs. In our study, the number of bulbs was 11.5% greater after treatment with chitosan at a molecular weight of 7 000 g/mol than 10 000 g/mol. This confirmed the findings of Salachna et al. (2008) that the increasing bulb yield in Freesia hybrida depended on the molecular weight of this compound. Żurawik (2013) reported a positive effect of 10 000 g/mol of chitosan on the weight of Freesia hybrida 'Silver Beach' bulbs, which was confirmed in our experiments consisting in rooting the Eucomis comosa 'Sparkling Burgundy' leaf cuttings. The differences versus the control were considerable and ranged from 11.5% to 139.0%. However, this effect depended on the application method. The soaked cuttings developed bulbs heavier by 44.1% and 50.7% than those watered or sprayed. Startek et al. (2005) also demonstrated that the chitosan application method determined the daughter bulb weight rate increase in Freesia hybrida 'Popey'. The treatment with the chitosan with a molecular weight of 7 000 g/mol resulted in developing 82.9% heavier bulbs than with the chitosan with a molecular weight of 10 000 g/mol. Our findings confirmed those reported by Salachna et al. (2008), who showed a correlation between the chitosan effect and its molecular weight. In our experiments, the cuttings treated with chitosan, irrespective of its molecular weight, produced bulbs with a greater diameter than the non-treated ones. The differences versus the control were considerable and ranged from 26.7% to 91.1%. Soaking the cuttings in the chitosan solution prior to rooting was the most effective application method.

CONCLUSION

The propagation of *E. comosa* 'Sparkling Burgundy' by leaf cuttings seems a successful method for the reproduction of planting material. Treatment of the leaf cuttings with chitosan improved their tolerance to adverse environmental conditions during rooting by prolonging the time of chlorophyll fluorescence growth and reducing the percentage of necrotic cuttings. Irrespective of its application method, chitosan also enhanced the intensity of the cutting greenness index. Chitosan of molecular weight 7 000 g/mol exerted more beneficial effects on root and bulb parameters than that of 10 000 g/mol. Of the compared application methods, watering had the most favourable

Table 4. Bulb yield produced by the *Eucomis comosa* 'Sparkling Burgundy' leaf cuttings depending on the chitosan molecular weight, application method, and measurement date (mean for the years 2016–2018)

Trait	Chitosan molecular weight (g/mol) (A)	Application method (B)				Mean
		control	soaking	watering	spraying	
Number (pcs)	7 000	3.12	6.11	3.71	3.29	4.06
	10 000	2.97	5.23	3.38	2.97	3.64
	mean	3.05	5.67	3.55	3.13	3.85
	$LSD_{0.05}$ – A: 0.78, B: 1.059, B×A: n.s.					
Mass (g)	7 000	0.43	1.23	0.65	0.69	0.75
	10 000	0.38	0.73	0.64	0.67	0.61
	mean	0.41	0.98	0.65	0.68	0.68
	$LSD_{0.05}$ – A: 0.068, B: 0.200, B(A): 0.225, A(B): 0.085					
Diameter (cm)	7 000	0.38	1.01	0.76	0.47	0.66
	10 000	0.52	0.71	0.62	0.67	0.63
	mean	0.45	0.86	0.69	0.57	0.64
	$LSD_{0.05}$ – A: n.s., B: 0.105, B(A): 0.122, A(B): 0.046					

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effects on physiological parameters of the cuttings during rooting, while the parameters of roots and bulb formation attained their peaks following soaking the cuttings before rooting.

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