Journal of Ecological Engineering
Volume 15, No. 3, July 2014, pp. 97–102
DOI: 10.12911/22998993.1110223

Research Article

EFFECT OF CHITOSAN ON PLANT GROWTH, FLOWERING AND CORMS YIELD OF POTTED FREESIA

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Received: 2014.01.07
Accepted: 2014.06.06
Published: 2014.07.01

ABSTRACT

The research was aimed at determining the influence of chitosan with different molecular weights on the growth and yield of flowers and corms of 'Gompey' freesia cultivated in pots. Freesia corms were soaked for 20 minutes in 0.5% chitosan solutions with low molecular weight (2 kDa), medium molecular weight (50 kDa) and high molecular weight (970 kDa). The average deacetylation level of the chitosans used was 85%. The plants were cultivated under controlled conditions (18/16 °C day/night, relative air humidity of 60%, quantum irradiance of 90 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a light/dark cycle of 14/8 hours). The research results obtained have shown that chitosan is used as a biostimulator in the cultivation of potted freesia. Regardless of the molecular weight of the compound, the chitosan-treated plants had more leaves and shoots, flowered earlier, formed more flowers and corms. The application of medium- and high-molecular-weight chitosan resulted in higher plants with a higher relative chlorophyll content (SPAD). The highest increase in the corm weight was observed as a result of treating plants with high-molecular-weight chitosan. No chitosan influence on the length of the main inflorescence shoot or the inflorescence length was observed.

Keywords: Freesia, ecological polymer, plant-growth enhancer, molecular weight.

INTRODUCTION

Chitosan is a biopolymer, a chitin derivative, a compound which is completely safe for the environment. This compound is characterized by unique properties, such as bioactivity and biocompatibility [Dias et al. 2013]. The results from the literature show that, when used in plants, chitosan can increase the yield [Mondal et al. 2012], reduce transpiration [Dzung et al. 2011] and induce a range of metabolic changes as a result of which, plants become more resistant to viral, bacterial and fungal infections [Al-Hetar et al. 2011]. Moreover, plants treated with chitosan may be less prone to stress evoked by unfavourable conditions, such as drought, salinity, low or high temperature [Lizarraga-Pauli et al. 2011, Jabeen and Ahmad 2013, Pongprayoon et al. 2013]. Chitosan stimulates vital processes of plants on every level of biological organization, from single cells and tissues, through physiological and biochemical processes, to changes on the molecular level related to expression of genes [Limpanavech et al. 2008, Hadwiger 2013, Nguyen Van et al. 2013].

On an industrial scale, chitosan is obtained as a result of chemical or enzymatic chitin deacetylation. Chitin occurs mostly in crustacean armours. Chitosan parameters are mostly determined by three parameters: morphological structure, the degree of deacetylation and the molecular weight [Aranaz et al. 2009]. Some of the aforementioned properties of chitosan may specifically stimulate plant reactions and the impact on microorganisms and the molecular weight seems to be one of the most important factors affecting the biological activity of this biopolymer [Kulikov et al. 2006, Li et al. 2011].

Freesia (*Freesia* Eckl. ex Klatt) is one of the ornamental plants, which are of the greatest economic importance grown for cut flowers. On

Dutch markets, in 2012 almost 300 million cut freesia flowers of the value of 50 million euro were sold and a sales growth of 6.5% in comparison to 2011 was observed [FloraHolland 2012]. As a result of growth, the assortment of freesia cultivars is extended to include new genetically low cultivars which are becoming more and more popular as pot plants [Ehrich et al. 2009]. Basic and still insufficiently solved problems in freesia production include improvement of cultivation methods, corm reproduction and health of plants [Vaira et al. 2009, Ruamrungsri et al. 2011].

There is no information on the use of chitosan in the cultivation of pot freesia, which is why the research was aimed at establishing how this polymer with various molecular weight affects the growth, flowering and health of freesia plants.

MATERIALS AND METHODS

The material included freesia corms (5-6 cm in circumference) of the 'Gompey' cultivar, which belongs to the Easy Pot group of cultivars characterised by genetically low height. Before planting, the corms were stored for 14 weeks at 28–30 °C and the relative humidity of 85%.

Freesia corms were soaked for 20 minutes in 0.5% chitosan solutions with low molecular weight (2 kDa), medium molecular weight (50 kDa) and high molecular weight (970 kDa). Corms soaked in water were the control. Biopolymers were purchased from Center of Bioimmobilisation and Innovative Packaging Materials at West Pomeranian University of Technology in Szczecin, Poland. Native chitosan supplied by Yuhuan Ocean Biochemical Co. Ltd. (China). The products have been obtained using the free-radical degradation process [Bartkowiak 2001]. The chitosan have been purified (filtration), and characterized using HPLC method (SmartLine Knauer, Germany; Tessek Separon HEMA-BIO 40 column – Tessek, The Tschech Republik). The average deacetylation degree of the products was 85%.

After drying, the single corms were planted in pots with a diameter of 12-cm in peat substrate with a pH value of 6.5–6.8. Directly before planting, the slow-release fertiliser Osmocote Plus 5–6 (15N-10P-12K plus micronutrients) was added to the substrate, at a dose of 5.0 $g \cdot dm^{-3}$ of the substrate. The plants were placed under controlled conditions in the phytotron at 18 °C during the day and 16 °C at night, relative air humidity of 60%, quantum irradiance of 90 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a light/dark cycle of 14/8 hours. The plants were cultivated in the phytotron for 16 weeks in two cycles.

At full blossom of the plants, when half of the main inflorescences were developed, the following parameters were defined: the height of plants, the number of shoots and leaves, the number of inflorescence shoots, the length of main inflorescence stems, the length of main inflorescence. The relative chlorophyll content in the leaves was measured using the Chlorophyll Meter SPAD 505 (Minolta, Japan). This chlorophyll meter measures the leaf transmittance at approximately 650 nm and 940 nm. Three fully developed leaves of each plant were used for the measurement. After the cultivation period was over, the corms were taken out of the pots and after being slightly dried, they were counted and weighed to calculate the coefficients of increase in weight and number.

Tests for the occurrence of freesia mosaic virus (FMV) were performed in three samples for each corm variant by means of the DAS-ELISA serological method [Clark and Adams 1977], with a set of γ-globulins and γ-globulin conjugates containing alkaline phosphatase [Kamińska 1991]. Corm samples were homogenised with the extract buffer at a 1:10 ratio. The results of the reaction were defined using a Multiscan II spectrophotometer (Labysystems) with a 405 nm filter. The result was considered to be positive if the absorbance value (A_{405}) was at least twice as high as the average value A_{405} for the negative control.

The experiment was conducted in a complete randomisation system as a one-factor experiment. Each experimental variant consisted of 40 corms, 10 in each replication. The obtained feature values as the average of two cycles of cultivation were statistically verified by means of a variance analysis method (ANOVA) and the differences between the means were assessed using Tukey's test at the significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

The research showed that soaking 'Gompey' freesia corms in a chitosan solution before planting had a stimulating effect on the plant height, the number of shoots and leaves and the relative chlorophyll content (Table 1). On the basis of statistical analysis, it was found that as a re-

sult of chitosan use with medium- and high-molecular-weights, plants were by 16.8% higher as compared with the control. The chitosan-treated plants had more shoots and leaves, by respectively 45.9% and 35.1%. It was not confirmed that the number of freesia shoots or leaves depend on the molecular weight of the biopolymer used. A similar positive influence of chitosan on plant vegetative features was observed in multiple genera from family *Orchidaceae*, such as *Cymbidium* [Nahar et al. 2012] or *Dendrobium* [Tantasawat et al. 2010]. The research by Lee et al. [2005] showed a positive chitosan influence on soy seedling growth and the stimulating action of chitosan was directly proportional to the molecular weight of the compound used in the experiment. The authors quoted above suggest that soaking seeds in a chitosan solution with a high molecular weight (>1,000 kDa) has the most beneficial effect on the growth of soy seedlings. On the other hand, in the research by Luan et al. [2005], the stimulating effect of low-molecular-weight chitosan (16 kDa) was shown. It was added *in vitro* to the medium culture, which increased the height of shoots and fresh biomass *Chrysanthemum morifolium*, *Limonium latifolium*, *Eustoma grandiflora* and *Fragaria ananasa*. The observed discrepancies could result from various application methods and chitosan concentrations and conditions for plant cultivation.

As a result of chitosan treatment with medium and high molecular weight, the relative chlorophyll content in the leaves increased significantly by 13.4%, as compared with the control plants (Table 1). An increase in the chlorophyll content in plants, as a result of chitosan used, has been confirmed by numerous authors. Dzung et al. [2011] report that as a result of spraying of coffee seedlings with chitosan solutions of the molecular weight of 2 kDa, the content of chlorophylls and carotenoids in leaves increased by 15.36% in plants grown in the field and by 46.38–73.5% in plants grown in the greenhouse in comparison to the control. The increase of the chlorophyll content as a result of application of chitosan may be caused by plants' enhanced uptake of nutrients, which occurred in the studies by Nguyen Van et al. (2013) on coffee seedlings. The authors demonstrated that after spraying the seedlings with chitosan of the molecular weight of 600 kDa three times, an increase of the content of nitrate, phosphorus and potassium in leaves by respectively 9.8–27.4%, 17.3–30.4% and 30–45% was observed. The experiment also proved that seedlings sprayed with a 10–50 ppm chitosan solution were characterized by increased intensity of net photosynthesis.

Soaking freesia corms in chitosan solutions before planting accelerated the plant flowering (by 11 days on average) and increased the number of inflorescences per plant (1.75 on average) (Table 2). It was not found that the number of days until flowering and the number of inflorescences per plant depend on the chitosan molecu-

Table 1. Effect of molecular weight of chitosan on the growth of freesia 'Gompey'

Molecular weight of chitosan	Plant high (cm)	No. of shoots	No. of leaves	Relative chlorophyll content (SPAD)
Control	51.2 ^{b}	1.12 ^{b}	5.23 ^{b}	65.7 ^{b}
Low (2 kDa)	54.2 ^{ab}	1.55 ^{a}	7.50 ^{a}	71.7 ^{ab}
Medium (50 kDa)	58.5 ^{a}	1.58 ^{a}	7.25 ^{a}	74.3 ^{a}
High (970 kDa)	61.1 ^{a}	1.41 ^{a}	8.14 ^{a}	74.5 ^{a}

Comments: Values followed by different letters are significantly different according to Tukey's multiple range test at $P < 0.05$.

Table 2. Effect of molecular weight of chitosan on the flowering of freesia 'Gompey'

Molecular weight of chitosan	No. of days to flowering	No. of inflorescence shoot	Length of inflorescence shoot (cm)	Length of inflorescence (cm)
Control	75.7 ^{a}	3.75 ^{b}	15.1^{a}	6.15^{a}
Low (2 kDa)	64.0 ^{b}	5.75 ^{a}	15.2^{a}	6.47^{a}
Medium (50 kDa)	65.5 ^{b}	5.50 ^{a}	14.7^{a}	6.57^{a}
High (970 kDa)	64.7 ^{b}	5.25 ^{a}	16.3^{a}	6.25^{a}

Comments: Values followed by different letters are significantly different according to Tukey's multiple range test at $P < 0.05$.

lar weight. The results obtained are confirmed by the results of the research by Ohta et al. [1999], where it was shown that lisianthus (*Eustoma grandiflora*) cultivated in a substrate with addition of chitosan flowered 15 days earlier than the control plants. Earlier flowering resulting from chitosan application was also found in the following species: *Exacum affine*, *Lobelia erinus*, *Mimulus×hybridus*, *Sinningia speciosa* and *Torenia fournieri* [Ohta et al. 2004]. A stimulating effect of chitosan on the number of flowers was observed in plants such as lisianthus [Ohta et al. 1999], gerbera [Wanichpongpan et al. 2001] and gladioli [Ramos-Garcia et al. 2009]. Also Limpanavech et al. [2008], applying chitosan of various physicochemical properties in growing of *Dendrobium* 'Eiskul', found that plants treated with chitosan of the molecular weight of 45 kDa and deacetylation degree of >90% flowered earlier and produced more inflorescences. The research results showed that chitosan did not affect the length of the inflorescence shoot and the length of the 'Gompey' freesia inflorescence (Table 2). According to Win et al. [2005], spraying *Dendrobium* 'Missteen' plants with chitosan significantly increased the length of the inflorescence but did not affect the size of flowers.

The stimulating effect of chitosan on the increase in the weight and number of 'Gompey' freesia corms was confirmed statistically (Table 3). The use of high-molecular-weight chitosan made it possible to obtain the highest coefficient of corm weigh increase by 31.6% higher than in control plants. Regardless of the chitosan molecular weight, an increase in the number of corms in chitosan-treated plants was on average 40.4% higher, as compared to the plants which were not treated with chitosan. Also, Ramos-Garcia et al. [2009] found that the number of gladiolus corms increased as a result of using the chitosan-containing Biorend® preparation. A similar positive effect of chitosan on the yield of potato minituber was observed by Asghari-Zakaria et al. [2009] in *in vitro* cultures. Plants grown on a medium with the addition of 500 $mg \cdot dm^{-1}$ chitosan produced 3.33 minituber on average, while plants that were not treated with chitosan formed 2.44 minituber. Hasegawa et al. [2005] reported that corms with an increased diameter and height are obtained as a result of *Arisaema ternatipartitum* cultivation in a substrate with an addition of chitosan.

In this study, serological tests on 'Gompey' freesia corms rendered positive results for the FMV in all the experimental variants (Table 3). No influence of the chitosan molecular weight on the degree of freesia corm infection was found. Research on the bean mosaic virus [Kulikov et al. 2006] showed that the highest antiviral activity of chitosan was exhibited by the fractions with molecular weight 2.2 and 1.2 kDa but chitosan monomers did not exhibit any antiviral activity. Pospieszny and Atabekov [1989] reported that chitosan may exhibit antiviral activity in plants depending on the species and environmental conditions. As freesia's infections with viruses and phytoplasmas are still a serious problem [Vaira et al., 2009], it is necessary to conduct further, detailed studies involving different concentrations and methods of chitosan's applications, for example spraying or drenching plants.

CONCLUSIONS

The presented research results have shown chitosan usefulness as a biostimulator in the cultivation freesia 'Gompey' as a pot plant. As a result of soaking corms in a chitosan solution before planting the plants were higher, had more leaves and shoots, flowered earlier, had more flowers and corms. It was observed that among the chitosan types tested in the experiments, the applica-

Table 3. Effect of molecular weight of chitosan on corms yield of freesia 'Gompey'

Molecular weight of chitosan	Coefficient of corm weight increase	Coefficient of corm number increase	Value at 405 nm (detection of FMV)*
Control	2.28 ^{c}	4.87 ^{c}	0.16 ^{a} (positive)
Low (2 kDa)	2.94 ^{ab}	6.62 ^{ab}	0.15 ^{a} (positive)
Medium (50 kDa)	2.70 ^{b}	6.87 ^{ab}	0.17 ^{a} (positive)
High (970 kDa)	3.00 ^{a}	7.02 ^{a}	0.15 ^{a} (positive)

Comments: Values followed by different letters are significantly different according to Tukey's multiple range test at $P < 0.05$.

Explanations: *buffer (negative control) = 0.07; absorbance values above 0.14 were considered positive outcomes (samples containing FMV-infected).

tion of medium- and high-molecular-weight chitosan resulted in higher plants with leaves contained more chlorophyll. Soaking freesia corms with chitosan, especially high-molecular-weight, seems to be an effective way to enhance of coefficient of corm weigh increase.

Acknowledgements

The investigation is being conducted in the frame of own research Nr 2PO6R07227 financed by Polish Ministry of Science and Higher Education.

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