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The influence of fungi of the *Trichoderma* genus on the flowering of *Freesia refracta* Klatt 'Argentea' in winter

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Abstract: The flowering and quality of *Freesia refracta* Klatt 'Argentea' were assessed after the application of fungi of the *Trichoderma* genus and assimilation lighting. The assimilation lighting accelerated the flowering of the *Freesia refracta* 'Argentea' plants by 3–4 weeks. The fungi of the *Trichoderma* genus accelerated the flowering of the *Freesia refracta* 'Argentea' plants with light deficit by about one week. The assimilation lighting resulted in the development of shorter main inflorescence shoots regardless of the fact whether the plants had been treated with the fungi of the *Trichoderma* genus or not. The assimilation lighting and the fungi of the *Trichoderma* genus stimulated the development of lateral inflorescence shoots in the 'Argentea' cultivar. The fungi of the *Trichoderma* genus stimulated the development of flowers in the 'Argentea' cultivar. This effect was particularly noticeable when the plants were exposed to the assimilation lighting. The assimilation lighting stimulated the uptake of potassium in the 'Argentea' cultivar. The fungi of the *Trichoderma* genus stimulated the uptake of phosphorus and calcium in the plants underexposed to light. They also stimulated the uptake of potassium in the plants exposed to the assimilation lighting. The assimilation lighting stimulated the uptake of microelements. The fungi of the *Trichoderma* genus stimulated the uptake of iron, manganese and zinc both in the plants exposed to the assimilation lighting and those underexposed to light. The assimilation lighting combined with the treatment with the fungi of the *Trichoderma* genus stimulated the uptake of copper.

Keywords: geophytes; *Trichoderma*; flowering; micro- and macro-elements

Trichoderma are saprophytic fungi used for the biological protection of plants (Whipps 2001; Benitez et al. 2004; Ghazanfar et al. 2018). These fungi grow fast and quickly reproduce. They can survive under unfavourable conditions. They stimulate the growth of plants and their defence mechanisms (Benitez et al. 2004). It is possible to stimulate plant growth with fungi of the *Trichoderma* genus because they enable plants to absorb more nutrients and they stimulate the production of vitamins and growth regulators (Yedidia et al. 2001). *Trichoderma* fungi are particularly important for the biological protection of plants grown under cover and in the ground, because they limit the development of diseases caused by soil pathogens, which are transferred with the seeds and in the air (Elad 2000; Mancini, Romanazzi 2013).

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The interaction process between plants and fungi of the *Trichoderma* genus has been relatively poorly investigated. This study assesses the influence of fungi of the *Trichoderma* genus (*T. hamatum, T. harzianum* and *T. viride*) on the flowering and nutrition of *Freesia refracta* 'Argentea' plants exposed to light and those with a light deficit.

MATERIAL AND METHODS

An experiment on *Freesia refracta* Klatt 'Argentea' plants was conducted at the Department of Phytopathology and Seed Science, Poznań University of Life Sciences, Poland over two seasons in both 2015/2016 and 2016/2017. The flowering and quality of the *Freesia refracta* Klatt 'Argentea' plants were assessed after the application of the fungi of the *Trichoderma* genus and the assimilation lighting.

Freesia refracta tubers with a circumference of 10–11 cm were prepared at a temperature of 28 °C for 14 weeks. Next, on 1 August 2015 and 2016, they were planted in containers with a capacity of 17 dm^3, which had been filled with a peat substrate (pH 6.2). Six tubers were planted in each container and placed on tables in a greenhouse. The variants of plants used in the experiment were either exposed or underexposed to light and treated or untreated with *Trichoderma*.

The plants were grown in a greenhouse, where the air temperature was 14–16 °C. In both research years, the assimilation lighting started at the beginning of December. Sodium lamps with a quantum irradiance intensity of 25–30 $\mu mol/m^2/s^1$ were used in the experiment so that the day was extended to 16 hours.

Two weeks after the tubers had been planted, after the roots had developed, a suspension of fungi of the *Trichoderma* genus (*T. hamatum*, *T. harzianum* and *T. viride*) was applied to the substrate. 50 ml of the suspension was applied to each plant.

An inoculum of fungi of the *Trichoderma* genus (*T. hamatum*, *T. harzianum*, and *T. viride*) was prepared in sterile, plastic Petri dishes with a diameter of 90 mm. A PDA medium of 16 ml was poured into each dish. When it solidified, a 5 mm disc of the nutrient medium overgrown with the mycelium of an appropriate isolate was placed in the central part of the plate. The disc had been cut from the circumference of a 10-day-old culture. Next, the cultures were incubated at 20 °C for three weeks. Distilled water (20 ml) was poured onto the sporulating cultures, and the resulting suspension was poured into a flask.

The experiment consisted of eight treatments, each of them with 3 replicates and 6 plants.

During the growing season, the plants were regularly watered. Once a week, they were fertilised with an aqueous solution of a 0.2% multicomponent Peters Professional fertiliser (20; 10; 20). Each time, 0.5 dm^3 of the solution was applied into one container.

At the harvest maturity stage, when the lower flower in the inflorescence was open and the bottom two buds were coloured, the earliness of the flowering season was determined by means of the weighted average of the days from planting the tubers to the flowering, the number of the 1st and 2nd order shoots in the inflorescence, the length of the main shoot and the length of the inflorescence, as well as the number of flowers in the inflorescence. The content of the macro-elements (nitrogen, phosphorus, potassium, calcium, magnesium) and micronutrients (iron, manganese, zinc, copper, boron) in the leaves was also measured.

The tops of leaves (10-cm long) were collected for the chemical analyses in each treatment. They were dried at a temperature of 45–50 °C and ground. Then, they were mineralised in concentrated sulphuric acid to measure the total nitrogen, phosphorus, potassium, calcium and magnesium content. The following methods were used to measure the content of the nutrients: total N – the Kjeldahl distillation method with a Parnas-Wagner apparatus (Chemland, Stargard Szczeciński, Poland), P – the colorimetric method with ammonium molybdate (developed by Schillak), K, Ca, and Mg – atomic absorption spectrometry (AAS). The leaves were mineralised in a mixture of nitric and perchloric acids (3 : 1, v : v) to measure the total iron, manganese, zinc, boron and copper content (Kamińska et al. 1972). After the mineralisation, the content of Fe, Mn, Zn, B and Cu was measured with the AAS method (with a Carl Zeiss Jena apparatus; pAAS-3N, Analityk Jena, Jena, Germany).

The results were analysed statistically with a two-factor analysis of variance. The assimilation lighting was the first-order factor, and the *Trichoderma* fungi were the second-order factor (the mean values from the two years were taken into account). The averages were grouped by means of Duncan's test at a significance level of $\alpha = 0.05$.

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RESULTS

32% of the fragments of the *Freesia refracta* 'Argentea' roots belonging to the plants underexposed to light and 33% of the roots of the plants exposed to light were colonised by fungi of the *Trichoderma* genus (Figure 1).

The comparison of the earliness of the flowering showed that the plants exposed to light were the first to start flowering irrespective of the fact whether they had been treated with the *Trichoderma* fungi or not. These combinations began flowering 30 and 23 days earlier, respectively, than the combinations where the plants had not been illuminated during the cultivation. The plants that had been underexposed to light and not treated with the *Trichoderma* fungi were the last to start flowering. However, the plants that had been underexposed to light, but treated with the fungi began flowering 9 days earlier, on average (Figure 2A).

The comparison of the length of the main inflorescence shoot of the ‚Argentea' cultivar showed that regardless of the treatment with the *Trichoderma* fungi, the first-order inflorescence shoots of the plants exposed to light were significantly shorter, i.e., 11.5 and 10.0 cm, respectively (Figure 2B).

The experiment showed that the plants underexposed to light had significantly fewer lateral inflorescence branches than those exposed to light. However, the treatment with the *Trichoderma* fungi did not affect this parameter. The plants exposed to light and treated with the *Trichoderma* fungi significantly had the largest number of lateral branches of inflorescence shoots (Figure 2C).

The number of flowers in the inflorescence significantly depended on the assimilation lighting and treatment with the *Trichoderma* fungi (Figure 2D). The inflorescences of the plants underexposed to light and not treated with the *Trichoderma* fungi significantly had the fewest flowers. There were significantly more flowers in the inflorescences of the plants underexposed to light and treated with the *Trichoderma* fungi, and in the combination where the plants had been exposed to light, but had not been treated with the fungi. The largest significant numbers of flowers were found in the inflorescences of the plants exposed to light and treated with the *Trichoderma* fungi.

The comparison of the content of macro-elements in the leaves of the 'Argentea' cultivar showed that the treatment with the *Trichoderma* fungi significantly increased the content of the phosphorus and calcium both in the plants exposed to light and in the underexposed ones. The content of these elements was also significantly greater in the plants that had been treated with the fungi (Table 1). The highest significant phosphorus content was found in the leaves of the plants exposed to light and treated with the *Trichoderma* fungi. The calcium content was similar both in the plants exposed and underexposed to light, which had been treated with the *Trichoderma* fungi.

The lowest significant potassium content was found in the plants underexposed to light, regardless of the treatment with the *Trichoderma* fungi. The assimilation lighting positively affected the uptake of this element. However, the highest significant content of this element was found in the leaves of the plants that had been exposed to light and treated with the *Trichoderma* fungi (Table 1).

Neither the assimilation lighting nor the treatment with the *Trichoderma* fungi affected the nitrogen and magnesium uptake by the 'Argentea' cultivar (Table 1).

The comparison of the content of the microelements in the leaves of the 'Argentea' cultivar showed

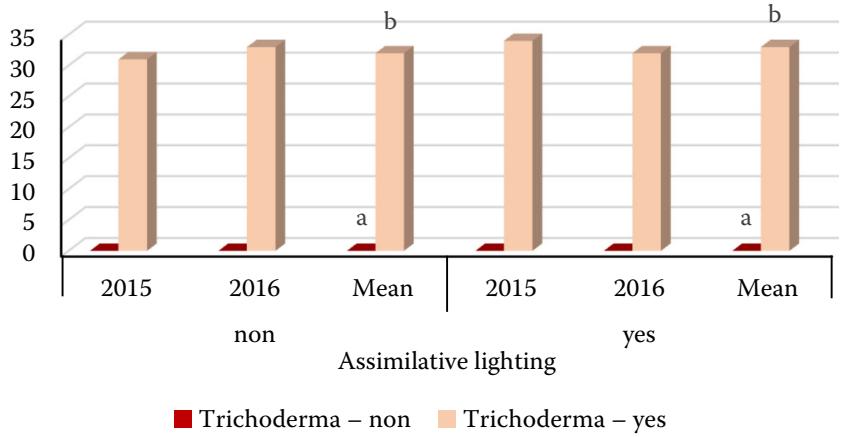

Figure 1. Effect of the supplementary lighting on the root colonisation percentage of the inoculated *Freesia refracta* 'Argentea' plants

The means followed by the same letter do not differ significantly at $\alpha = 0.05$

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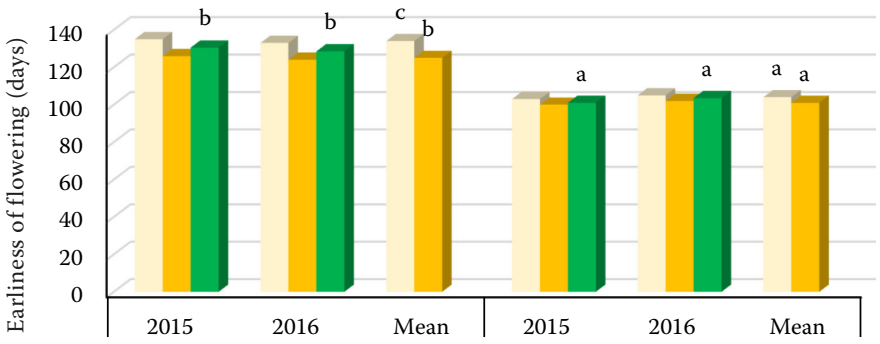
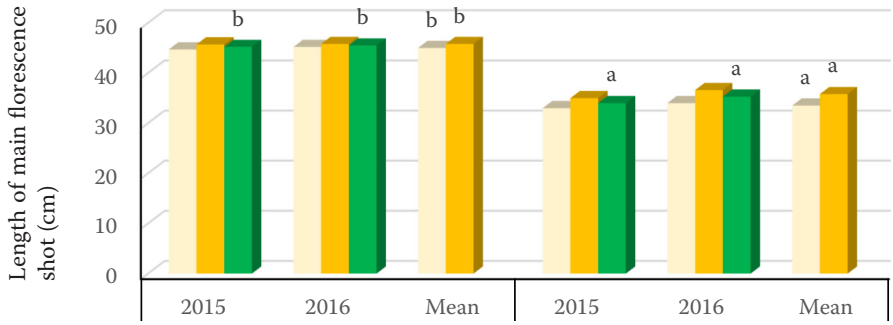
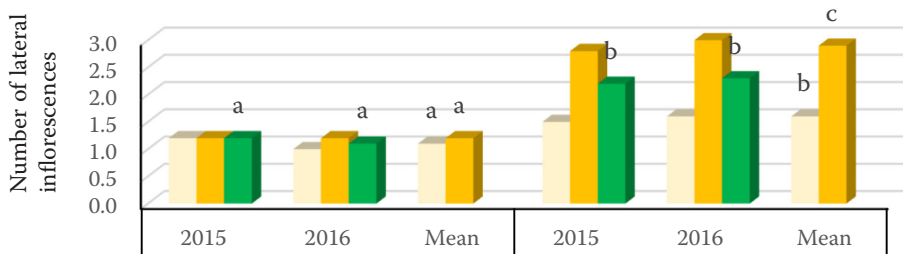
(A) Earliness of flowering (days)



(B) Lenght of main floerescence shot



(C) Number of lateral influorescence



(D) Number of the flowers in the main inflorescence

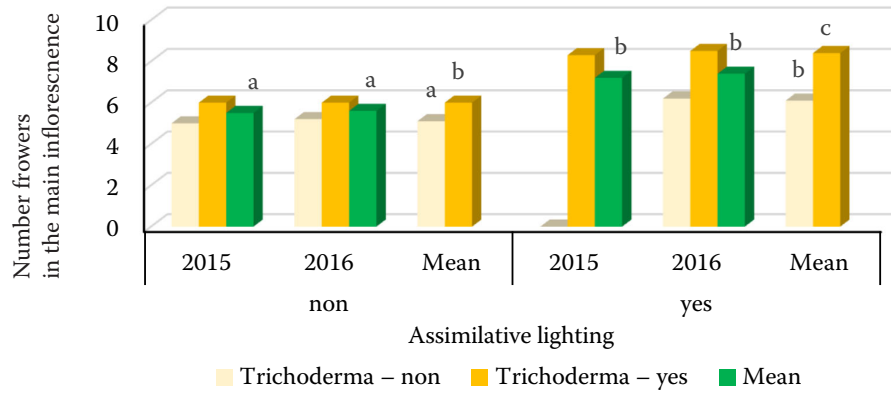


Figure 2. Earliness of the flowering (A) and quality (B, C, D) of the inflorescence of the *Freesia refracta* 'Argentea' plants after the application of the supplementary lighting and the fungus of the genus *Trichoderma*

The means followed by the same letter do not differ significantly at α = 0.05

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Table 1. Effect on the content of macro-elements in the leaves of the *Freesia refracta* 'Argentea' plants after the application of the supplementary lighting and the fungus of the genus *Trichoderma* (% d.w.)

Trichoderma spp.	Assimilative lighting					
	non			yes		
	2015	2016	mean	2015	2016	Mean
Nitrogen						
Non	2.50	2.46	2.48[a]	2.70	2.84	2.67[a]
Yes	2.48	2.46	2.48[a]	2.68	2.60	2.64[a]
Mean	2.49[a]	2.46[a]		2.69[a]	2.62[a]	
Phosphorus						
Non	0.45	0.47	0.46[a]	0.46	0.48	0.47[a]
Yes	0.78	0.68	0.73[c]	0.84	0.80	0.82[d]
Mean	0.62[b]	0.58[b]		0.65[b]	0.64[b]	
Potassinum						
Non	4.47	4.49	4.48[a]	5.27	5.37	5.32[b]
Yes	4.95	4.80	4.88[a]	5.47	7.20	6.34[c]
Mean	4.71[a]	4.65[a]		5.37[b]	6.29[c]	
Magnesium						
Non	0.18	0.19	0.19[a]	0.12	0.15	0.14[a]
Yes	0.22	0.16	0.19[a]	0.12	0.17	0.15[a]
Mean	0.20[a]	0.18[a]		0.12[a]	0.16[a]	
Calcium						
Non	1.16	1.12	1.14[a]	1.18	1.14	1.16[a]
Yes	1.33	1.30	1.32[c]	1.28	1.30	1.29[c]
Mean	1.25[b]	1.21[b]		1.23[b]	1.22[b]	

The means followed by the same letter do not differ significantly at $\alpha = 0.05$

that the assimilation lighting stimulated their uptake (Table 2). The *Trichoderma* fungi stimulated the manganese, zinc and iron uptake both in the plants exposed to light and in the underexposed ones. The assimilation lighting combined with the treatment with the *Trichoderma* fungi stimulated the copper uptake (Table 2).

DISCUSSION

The research showed that 32% of the fragments of the *Freesia refracta* ‚Argentea' roots of the plants underexposed to light and 33% of the roots of the plants exposed to light were colonised by the fungi of the *Trichoderma* genus. It is most likely that the good result was caused by the appropriate fungal injection. The fungal suspension was applied to the substrate placed directly above the tubers, because *Trichoderma* are aerobic organisms, which best develop in the surface layers of the substrate (Kosicka et al. 2014). Apart from that, as Benitez et al. (2004) indicated, the fungi of the *Trichoderma* genus tend to sporulate faster when they have greater access to visible light. Humidity is a very important factor affecting the proper development of these fungi. According to Das et al. (2008), the highest metabolic activity of these fungi can be observed when the humidity of the environment is about 80%.

Due to the moderate insolation deficit in autumn and winter in a temperate climate zone, artificial sources of light are used in greenhouse plantations. This intensifies the photosynthesis and, in consequence, it accelerates the growth and increases the yield of the plants (Marcelis et al. 2002; Heuvelink et al. 2006; Klamkowski et al. 2012). *Freesia* is a photoperiodically neutral plant, but it is very sensitive to light intensity. A high light intensity is particularly important during the emergence of the inflorescence, because the inflorescence shoots become rigid and the leaves grow vertically (Startek et al. 2005). According to Lee and Hwang (2014), a green LED lighting advanced the time of flowering and a metal halide lamp was good for the cut-flower quality in *Freesias*. Darras et al. (2019) showed that forcing with UV-C irradiation reduced the flowering time by 10.4 days and increased

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Table 2. Effect on the content of the microelements in the leaves of the *Freesia refracta* 'Argentea' plants after the application of the supplementary lighting and the fungus of the genus *Trichoderma* (mg/kg in D.W.)

Trichoderma spp.	Assimilative lighting					
	non			yes		
	2015	2016	mean	2015	2016	mean
Manganese						
Non	33.30	38.50	35.9[a]	44.3	43.7	44.0[b]
Yes	63.60	58.40	61.00[e]	59.9	60.1	60.00[e]
Mean	48.45[c]	48.45[c]		52.1[d]	51.9[d]	
Copper						
Non	5.59	7.48	6.54[a]	7.63	9.61	8.62[c]
Yes	6.02	7.60	6.81[a]	17.42	16.0	16.71[e]
Mean	5.81[a]	7.54[b]		12.53[d]	12.81[d]	
Zinc						
Non	34.4	33.0	33.7[a]	46.1	50.00	48.01[b]
Yes	62.8	65.4	64.1[d]	68.8	62.20	65.50[d]
Mean	48.60[b]	49.2[b]		57.45[c]	56.10[c]	
Iron						
Non	79.40	80.6	80.00[a]	97.10	111.80	104.45[b]
Yes	140.90	120.4	130.65[c]	127.70	157.10	142.40[d]
Mean	110.15[b]	100.50[b]		112.40[b]	134.45[c]	
Boron						
Non	23.70	23.70	23.70[a]	26.4	26.10	26.25[b]
Yes	24.40	22.70	23.55[a]	27.40	27.50	27.45[b]
Mean	24.05[a]	23.20[a]		26.90[b]	26.80[b]	

The means followed by the same superscript letter do not differ significantly at $\alpha = 0.05$

the lateral branching and number of flowers by up to 22 and 30%, respectively, in *Freesia*.

The research showed that the assimilation lighting accelerated the flowering of *Freesia refracta* 'Argentea' plants by 3-4 weeks in winter. This finding met our expectations and confirmed the results of earlier studies (Kawata 1973; Gilbertson-Ferriss, Wilkins 1978). Apart from that, the experiment showed that the treatment of the 'Argentea' plants underexposed to light with the *Trichoderma* fungi accelerated their flowering only by about one week. According to Brotman et al. (2013), the fungi of the *Trichoderma* genus may improve the growth of plants exposed to abiotic stress, such as a light deficit, because they reduce harmful, elevated levels of ethylene and exhibit increased an antioxidative capacity. However, although the fungi of the *Trichoderma* genus are capable of relieving abiotic stress, knowledge about the mechanisms controlling various stress factors in plants is still insufficient (Mastouri et al. 2010).

The research showed that the assimilation lighting of the 'Argentea' cultivar resulted in shorter main inflorescence shoots growing from the tubers, regardless of the treatment with the *Trichoderma* fungi. However, it is noteworthy that the elongation of the inflorescence shoots were inhibited, because the quality of the shoots improved – they were stiff, did not bend and the inflorescence was properly shaped. Apart from that, the experiment showed that the treatment with the *Trichoderma* fungi stimulated the development of flowers. It was particularly noticeable in the plants exposed to the assimilation lighting. The results of this study are in line with the findings of the research conducted by Startek et al. (2005). The authors indicated that the illumination of *Freesias* during the entire cultivation period or only during the development of the buds accelerated the flowering and shortened the length of the inflorescence shoots. However, the assimilation lighting may also reduce the number of inflorescences. In our study, we did not observe that the assimilation lighting and fungi of the *Trichoderma* genus stimulated the development of lateral inflorescence shoots of the 'Argentea' cultivar.

The uptake of nutrients during cultivation depends on the lighting conditions. Light intensifies the ion

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transport in plants – it opens the stomata, activates the water transpiration current and, thus, the passive ion transport . Light also influences the active ion uptake from the substrate, because the assimilation products formed as a result of photosynthesis are respiratory substrates for the roots, and the energy which they release increases the active ion uptake from the substrate (Kujawski 2005). Our research showed that the assimilation lighting of the 'Argenta' plants stimulated their potassium uptake. This effect was particularly noticeable when the illumination was combined with the treatment with the *Trichoderma* fungi. The fungal treatment compensated for the light deficit in the variant where the plants were underexposed to light. Thanks to the treatment, the plants' phosphorus and calcium uptake was intensified. The phosphorus in the *Freesias* significantly affected the days to flowering, spike persistency, corm diameter, corm weight (Khan et al. 2012). Yedidia et al. (2001) also observed higher phosphorus levels after applying fungi from the *Trichoderma* genus. The authors found a significantly higher phosphorus content in 10- and 28-day-old *Cucumis* seedlings after applying the T-203 *Trichoderma harzianum* strain. The researchers observed that the elevated uptake of nutrients resulted in the better development of the root system, which allowed the plants to access a larger volume of the substrate. In consequence, the plants won the competition for nutrients versus the other plants, which had a less developed root system, or when there were small amounts of mineral compounds. According to Altomare et al. (1999), phosphorus compounds can be dissolved and stored in a biomass of fungi of the *Trichoderma* genus, and then they can be released in an available form near the roots after the lysis of the mycelium. Alpa et al. (2015) reported that an AMF (arbuscular mycorrhizal fungi) in combination with *Trichoderma viride* enhances the nutrient alimentation, especially for P, leading to an improved rhizospheric condition in the soil, influencing the physiological and biochemical properties of *Helianthus annuus*.

Micronutrients play a key role in the plants' metabolic and physiological processes. It is noteworthy that they have a greater influence on the yield quality (Khosa et al. 2011) rather than its volume (Lahijie 2012). Proteins are composed of micronutrients, which play a role in catalytic functions. Our experiment showed that the fungi of the *Trichoderma* genus stimulated the iron, manganese, and zinc uptake both by the 'Argentea' plants exposed to light and those with a light deficit. The results of our study are in line with the observations made by Yedidia et al. (2001), who studied the uptake of these micronutrients in *Cucumis* seedlings treated with the fungi of the *Trichoderma* genus. They found that the iron, copper and zinc content had increased both in the roots and in the aerial parts of the plants. According to Benitez et al. (2004), *Trichoderma* fungi are capable of having a quick uptake of elements even if they only occur in trace amounts. For example, iron is chelated by these fungi. *Trichoderma* fungi have siderophores, which inhibit the growth and development of other fungi. The authors stressed the fact that *Trichoderma* fungi aggressively competed with other microorganisms for space and nutrients and this competition was beneficial to the plants. Yedidia et al. (2001) observed that the copper content in the *Cucumis* plants increased when they had been treated with the fungi of the *Trichoderma* genus. In our study, the copper uptake was better in the variant where the plants had been exposed to the assimilation lighting and treated with the fungi of the *Trichoderma* genus.

CONCLUSION

The assimilation lighting accelerated the flowering of the *Freesia refracta* 'Argentea' plants by 3–4 weeks. The fungi of the *Trichoderma* genus accelerated the flowering of the *Freesia refracta* 'Argentea' plants with a light deficit by about one week. The assimilation lighting resulted in the development of shorter main inflorescence shoots regardless of the fact whether the plants had been treated with the fungi of the *Trichoderma* genus or not. The assimilation lighting and the fungi of the *Trichoderma* genus stimulated the development of the lateral inflorescence shoots in the 'Argentea' cultivar. The fungi of the *Trichoderma* genus stimulated the development of flowers in the 'Argentea' cultivar. This effect was particularly noticeable when the plants were exposed to the assimilation lighting. The assimilation lighting stimulated the potassium uptake in the 'Argentea' cultivar. The fungi of the *Trichoderma* genus stimulated the phosphorus and calcium uptake in the plants underexposed to light. They also stimulated the potassium uptake in the plants exposed to the assimilation lighting. The assimilation lighting stimulated the uptake of microelements. The fungi of the *Trichoderma* genus stimulated the iron, manganese, and zinc uptake both in the plants exposed to the assimilation lighting and the ones underex-

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posed to light. The assimilation lighting combined with the treatment of the fungi of the *Trichoderma* genus stimulated the copper uptake.

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