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Yield and Quality of Inflorescences in the *Zantedeschia albomaculata* (Hook.) Baill. 'Albomaculata' after the Treatment with AMF and GA_3

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Abstract: This study was conducted to assess the influence of gibberellic acid (GA_3) and arbuscular mycorrhizal fungi (AMF) on the flowering and quality of *Zantedeschia albomaculata* (Hook.) Baill 'Albomaculata' plants. Before planting, the rhizomes were soaked in water or an aqueous solution of GA_3 at a concentration of 150 mg dm^{-3} for 30 min. A mixture of AMF was applied to the rhizomes a week after planting. The AMF treatment increased the yield of inflorescences of the 'Albomaculata' cultivar by 100%. AMF and GA_3 had a favourable effect on the quality of inflorescences, expressed by the length of peduncles, whereas AMF individually positively affected the length of the spathes. AMF and GA_3 had no effect on the level of macroelements in calla lily leaves, with the exception of calcium (Ca). The leaves of mycorrhized plants had a high content of sodium (Na) and micronutrients, except for iron (Fe). The results of the study showed that GA_3 could be replaced by mycorrhizal inoculation when applied to Zantedeschia plants.

Keywords: *Zantedeschia albomaculata* 'Albomaculata'; yield; quality; micro and macroelements; mycorrhization; GA_3

Citation: Andrzejak, R.; Janowska, B. Yield and Quality of Inflorescences in the *Zantedeschia albomaculata* (Hook.) Baill. 'Albomaculata' after the Treatment with AMF and GA_3. *Agronomy* **2021**, *11*, 644. https://doi.org/10.3390/agronomy11040644

Academic Editors: Matteo Caser and John P. Thompson

Received: 27 January 2021
Accepted: 25 March 2021
Published: 27 March 2021

1. Introduction

Calla lilies—*Zantedeschia*—are African plants of the Araceae family. They are mostly grown for cut flowers and they rank thirteenth in the flower trade in The Netherlands. Due to unsatisfactory flower harvests mainly because of the high prices of rhizomes produced abroad, Calla lilies are not so popular among Polish producers. Even though the flower yield can be increased with the application of gibberellic acid (GA_3) [1–6], the risk of contamination via fertilizer solution, such as *Pectobacterium carotovorum* subsp. *carotovorum* [7], a pathogen bacteria associated with soft rot, is high and could seriously limit the production.

Therefore, it is advisable to seek other methods to improve the flowering of *Zantedeschia* cultivars as efficiently as with GA_3. According to Matysiak [8], arbuscular mycorrhizal fungi (AMF) stimulates flowering, because it produces growth regulators, including gibberellin. AMF are in symbiotic relationships with most wild and cultivated terrestrial plants except species belonging to the *Brassicaceae* and *Chenopodiaceae* families [9]. Mycorrhizal fungi facilitate the uptake of micro- and macronutrients in exchange for photosynthetic products [9].

In addition, AMF facilitates the completion of biogeochemical cycles, increases plant tolerance to biotic and abiotic stresses [10], and increases the phytochemical content of health-promoting compounds [11,12]. Fungal spores germinate at the right humidity, temperature, and pH. However, their vitality is limited [13]. The morphogenesis of fungal

spores takes place when they encounter host plants [14,15]. Fungal hyphae penetrate the root cells and form arbuscules, through which nutrients can be exchanged between the fungi and the plant [16,17]. AMF acquires nutrients for plants, in particular phosphorus in poor environments. In return, the fungi receive the necessary carbohydrates [18].

In this study, we assessed the effects of AMF and GA_3 on the flowering and quality of *Zantedeschia albomaculata* 'Albomaculata' plants. The content of micro- and macronutrients in the leaves was assessed. The assumption was that mycorrhizal fungi would establish a symbiotic relationship with plants and, thus, increase the flower yield and intensify the uptake of nutrients.

2. Material and Methods

2.1. Plants

This study was conducted to assess the influence of gibberellic acid (GA_3) and arbuscular mycorrhizal fungi (AMF) on the flowering and quality of *Zantedeschia albomaculata* (Hook.) Baill. 'Albomaculata' plants. For three consecutive years of research, on 16 May, rhizomes of more than 20 cm in circumference, with leaf buds of 0.5–1.5 cm in length, were planted into 20-cm pots with a substrate consisting of peat (pH 6.2) enriched with a slow-release fertilizer Osmocote Plus (3–4 M) (15 + 11 + 13 ± 2 MgO + microelements) at an amount of 3 g·dm^{-3} and mixed with fresh, shredded pine bark at a 3:1 ratio (v/v).

Before planting, the rhizomes were soaked in water (control plants) or an aqueous solution of gibberellic acid (GA_3) at a concentration of 150 mg·dm^{-3} for 30 min. Earlier research by Janowska and Andrzejak [5], Janowska [6], Janowska and Schroeter [19], and Kozłowska et al. [20] showed that this concentration of GA_3 was optimal for the 'Albomaculata' cultivar.

The plants were also treated with a commercial product containing a mixture of AMF: *Rhizophagus aggregatus* (N.C. Schenck and G.S. Sm.), *Funneliformis mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler, *Rhizophagus intraradices* (N.C. Schenck and G.S. Sm.) C. Walker and A. Schüßler, *Rhizophagus clarus* (T.H. Nicolson and N.C. Schenck) C. Walker and A. Schüßler, *Claroideoglomus etunicatum* (W.N. Becker and Gerd.) C. Walker and A. Schüßler, and *Gigaspora margerita* W.N. Becker and I.R. Hall. One week after the planting, the fungi were applied in the form of spores at an amount of 100 propagation units per plant—2 g of the product per plant. Each treatment involved five plants in three replications.

2.2. Cultivation

The plants were grown in a greenhouse. Fertilization started in the fifth week of cultivation. We applied 0.2% solutions of Peters Professional (20:20:20) or Brown Superba (14–10–25 + microelements) fertilizers every 10–14 days. At the beginning of vegetation, when the leaves were fully developed, 0.2% calcium saltpetre was applied foliarly once.

The lengths of the peduncles and spathes were measured, and the yield of inflorescences developing from a single rhizome was determined.

2.3. Chemical Analysis

Leaves were dried at a temperature of 45–50 °C and then ground. To determine the total content of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg), these leaves were mineralized in concentrated sulphuric acid (H_2SO_4). The following methods were used to measure the content of the nutrients: total N—by Kjeldahl digestion with distillation in a Parnas—Wagner apparatus, P—the colorimetric method employing ammonium molybdate (after Schillak), and K, Ca, and Mg—atomic absorption spectrometry (AAS).

To determine the total iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu), the leaves were mineralized in a mixture of nitric (HNO_3) and perchloric acids ($HClO_4$) (3:1, v/v), and for sodium (Na), in concentrated sulphuric acid (H_2SO_4) [21]. After the mineralization, the Na, Fe, Mn, Zn, and Cu contents were determined using the AAS method (on a Carl Zeiss Jena apparatus).

2.4. Root Colonisation

The method described by Phillips and Hayman [22] was used to stain the root mycorrhizas. Root colonization was expressed as the percentage of colonized root lengths versus observed root lengths. The soil hyphal length was measured with the method described by Bethlenfalvay and Ames [23].

2.5. Data Analysis

Three-way analysis of variance and the Statistica 8.0 software were used for statistical analysis of the results. The means were grouped with the the Newman–Keuls test.

3. Results and Discussion

3.1. Root Colonisation

In the three consecutive years of the study the AMF colonization, measured as the percentage of root lengths colonized by hyphae, amounted to 31.2%, 32.0%, and 30.7% in the plants not treated with GA_3, whereas, in those treated with GA_3, the AMF colonization was 29.9%, 31.1%, and 30.4%. The results of the study showed that GA_3 did not affect the root colonization by AMF. According to Eloise et al. [24], endogenous levels of gibberellins (GA) influence the formation of arbuscules in mycorrhizated pea roots. The authors suggested that GA suppresses the formation of arbuscules in pea roots; however, exogenous treatment does not enable distinction between occurrences taking place in the rhizosphere and those inside the root cells.

3.2. Yield and Quality of Flowers

The yield of flowers significantly depended on mycorrhization only. When the plants were treated with AMF, their average yield of flowers was 100% greater than that of the non—mycorrhized plants (Table 1). The comparison of interactions showed that the most flowers developed on the mycorrhized plants, irrespective of whether or not their rhizomes had been soaked in GA_3. The soaking of rhizomes in GA_3 without mycorrhiza boosted the yield of flowers; however, their number was significantly smaller compared with the mycorrhized plants.

The soaking of rhizomes in GA_3 is increasingly popular in large-scale production because it improves the flowering of *Zantedeschia* cultivars [1–6,25]. The study showed that GA_3 could be replaced with mycorrhiza, which proved to be more effective than the soaking of rhizomes in GA_3. Janowska et al. [26] observed that mycorrhization increased the yield of flowers of *Zantedeschia albomaculta* 'Albomaculata' cultivated in a greenhouse. According to Janowska et al. [27], the mycorrhization of *Syningia speciosa* cultivars affected only the number of flower buds. After mycorrhization, both the 'Defiance' and 'Blanche de Meru' cultivars yielded significantly more buds (66.7% and 57%, respectively) compared with the control plants.

The addition of mycorrhizal fungi to the substrate stimulated flower bud induction, likely because mycorrhizal fungi produce growth regulators, including those from the gibberellin group [7,8]. According to Nowak [28], mycorrhization had no effect on the abundance of *Callistephus chinensis* flowers. Lovato et al. [29] noted that mycorrhized miniature roses and chrysanthemums were better-branched, and, as a result, they flowered more abundantly. According to Janowska and Andrzejak [30], *Tagetes patula* 'Yellow Boy' plants treated with mycorrhiza produced more inflorescence buds, whereas *Salvia splendens* 'Saluti Red' developed more flowers per inflorescence.

The study showed that mycorrhization and GA_3 had favourable effects on the quality of flowers, expressed by the length of peduncles. Regarding the spathes, only the symbiosis with fungi was found to be effective (Table 1). The length of peduncles and spathes in calla lilies is largely a cultivar-related trait. GA_3 treatment has been shown to modify it in certain cultivars, e.g., 'Black Eyed Beauty', 'Cameo', and 'Treasure' as was observed by Janowska and Zakrzewski [31]. On the other hand, Janowska and Krause [25], found that

GA_3 was detrimental to the quality of inflorescences, finding that double and triple spathes tended to develop.

Table 1. The yield and quality of the inflorescences of *Zantedeschia albomaculata* 'Albomaculata' plants after the treatment with AMF and GA_3. The means followed by the same letter do not differ significantly at $\alpha = 0.05$.

Year	Mycorrhization	GA_3 (150 mg·mg^{-3})		Mean for Mycorrhization
		No	Yes	
Yield of flowers				
I	no	2.2 a	3.5 b	2.8 a
II		1.9 a	4.0 b	
III		2.1 a	3.0 b	
I	yes	5.8 c	5.0 c	5.6 b
II		6.2 c	5.8 c	
III		5.4 c	5.2 c	
Mean for GA_3		3.9 a	4.4 a	
Flower peduncle length (cm)				
I	no	36.0 a	40.7 b	39.0 a
II		38.0 a	41.3 b	
III		37.8 a	40.0 b	
I	yes	43.2 c	45.5 d	44.3 b
II		44.0 c	43.7 c	
III		43.2 c	46.0 d	
Mean for GA_3		40.4 a	42.9 b	
Spathe length (cm)				
I	no	10.0 a	10.0 a	10.3 a
II		10.4 a	10.6 a	
III		10.2 a	10.4 a	
I	yes	12.2 b	11.5 b	11.7 b
II		12.0 b	12.0 b	
III		11.6 b	11.0 b	
Mean for GA_3		11.0 a	10.9 a	

This effect was not observed in our study, either in the mycorrhized plants or those treated with GA_3. According to Janowska et al. [26], *Zantedeschia albomaculata* inflorescences had longer peduncles after AMF treatment. Nowak and Nowak [32] found that AMF and carbon dioxide (CO_2) enrichment increased the number of leaves as well as the fresh and dry weights of *Osteospermum ecklonis* shoots.

3.3. Content of Macroelements

AMF and GA_3 had no effect on the content of macroelements in the leaves of the 'Albomaculata' cultivar, with the exception of Ca. Ca is a structurally and functionally essential element in plant physiology. The majority of plant Ca can be found in the cell walls and in the in the vacuoles; however, it is also a key component regulating the functions of the plasma membrane. Ca additionally controls the activity of various key metabolic enzymes [33]. There was a significantly higher Ca content in the leaves of the plants treated with AMF and GA_3. The leaves of the non-mycorrhized plants whose rhizomes had been soaked in the GA_3 had a lower Ca content (Table 2).

Table 2. The effect of AMF and GA_3 (% DW) on the content of macroelements in the leaves of the *Zantedeschia albomaculata* 'Albomaculata' plants. The means followed by the same letter do not differ significantly at $\alpha = 0.05$.

Year	Mycorrhization	GA_3 (150 mg·mg^{-3})		Mean for Mycorrhization
		No	Yes	
		N		
I	no	5.19 a	4.79 a	4.93 a
II		4.90 a	4.70 a	
III		5.00 a	4.99 a	
I	yes	4.77 a	5.11 a	4.92 a
II		4.91 a	4.99 a	
III		4.71 a	5.00 a	
Mean for GA_3		4.91 a	4.93 a	
		P		
I	no	0.43 a	0.42 a	0.43 a
II		0.44 a	0.40 a	
III		0.45 a	0.44 a	
I	yes	0.42 a	0.42 a	0.41 a
II		0.39 a	0.41 a	
III		0.43 a	0.39 a	
Mean for GA_3		0.43 a	0.41 a	
		K		
I	no	3.91 a	3.96 a	3.91 a
II		3.87 a	4.00 a	
III		3.84 a	3.86 a	
I	yes	3.68 a	4.15 a	3.88 a
II		3.76 a	3.97 a	
III		3.80 a	3.92 a	
Mean for GA_3		3.81 a	3.98 a	
		Ca		
I	no	0.76 b	0.66 a	0.70 a
II		0.75 b	0.64 a	
III		0.76 b	0.62 a	
I	yes	0.73 b	0.85 c	0.80 b
II		0.75 b	0.90 c	
III		0.71 b	0.87 c	
Mean for GA_3		0.74 a	0.76 a	
		Mg		
I	no	0.22 a	0.24 a	0.23 a
II		0.25 a	0.22 a	
III		0.24 a	0.26 a	
I	yes	0.25 a	0.30 a	0.28 a
II		0.27 a	0.26 a	
III		0.24 a	0.29 a	
Mean for GA_3		0.25 a	026 a	

Janowska et al. [26] found that the leaves of mycorrhized *Zantedeschia albomaculata* 'Albomaculata' plants contained: N—5.35–5.55% d.w., P—0.44% and 0.53% DW, K—3.89% and 4.41% DW, Ca—0.78% and 0.80% d.w., and Mg—0.22% and 0.23% DW. The mycorrhization had no effect on the content of Ca and Mg in *Zantedeschia* leaves. Nowak [28], observed a slightly elevated P level in the leaves of mycorrhized *Callistephus chinensis* plants.

However, the mycorrhization did not affect the N, K, or Ca content in this species. Available scientific publications do not provide information how GA_3 affects the levels of

macroelements in *Zantedeschia albomaculata* leaves. Janowska et al. [34] noted that all GA_3 treatments stimulated the Ca uptake in *Gladiolus hybridus* 'Black Velvet'; however, they had no effect on the uptake of other macronutrients. Another study conducted by the same authors [35] showed that benzyladenine (BA) applied at 100–600 mg·dm^{-3} stimulated the Ca uptake in this cultivar but had no effect on the uptake of other macronutrients.

3.4. Content of Microelements

Mycorrhization had a favourable effect on the content of the Na and other microelements, with the exception of Fe (Table 3). Micronutrients as essential components for plant life were discovered in the 1920s and 1930s. Their role is limited to the regulation of biochemical processes taking place in plants during the growing season. This means that plants have a low demand for these components [36]. Microelements have a direct and indirect influence on the flowering and quality of flowers in ornamental plants.

Table 3. Effect of the of AMF and GA_3 (mg·kg^{-1} in DW) on the content of microelements and sodium in the leaves of the *Zantedeschia albomaculata* 'Albomaculata'. Means followed by the same letter do not differ significantly at $\alpha = 0.05$.

Year	Mycorrhization	GA_3 (150 mg·dm^{-3})		Mean for Mycorrhization
		No	Yes	
		Fe		
I	non	43.68 a	42.20 a	43.14 a
II	non	43.56 a	43.42 a	43.14 a
III	non	42.99 a	43.01 a	43.14 a
I	yes	43.88 a	42.75 a	43.36 a
II	yes	43.85 a	43.21 a	43.36 a
III	yes	42.89 a	43.56 a	43.36 a
Mean for GA_3		43.48 a	43.03 a	
		Mn		
I	non	61.00 a	60.15 a	60.66 a
II	non	60.88 a	61.26 a	60.66 a
III	non	59.79 a	60.88 a	60.66 a
I	yes	70.53 c	65.15 b	67.62 b
II	yes	69.73 c	64.83 b	67.62 b
III	yes	69.99 c	65.51 b	67.62 b
Mean for GA_3		65.32 a	62.96 a	
		Zn		
I	non	31.60 a	30.05 a	30.85 a
II	non	30.99 a	31.16 a	30.85 a
III	non	30.56 a	30.78 a	30.85 a
I	yes	35.13 b	34.28 b	35.04 b
II	yes	34.87 b	35.56 b	35.04 b
III	yes	35.15 b	35.26 b	35.04 b
Mean for GA_3		33.05 a	32.84 a	
		Cu		
I	non	6.73 a	6.91 a	6.54 a
II	non	6.36 a	6.71 a	6.54 a
III	non	6.16 a	6.36 a	6.54 a
I	yes	7.24 b	7.10 b	7.25 b
II	yes	7.31 b	7.53 b	7.25 b
III	yes	7.41 a	7.29 b	7.25 b
Mean for GA_3		6.80 a	6.98 a	

Table 3. *Cont.*

Year	Mycorrhization	GA_3 (150 mg·dm^{-3})		Mean for Mycorrhization
		No	Yes	
		Na		
I	non	0.05 a	0.04 a	0.05 a
II		0.05 a	0.06 a	
III		0.04 a	0.05 a	
I	yes	0.05 a	0.06 a	0.05 a
II		0.05 a	0.04 a	
III		0.05 a	0.05 a	
Mean for GA_3		0.05 a	0.05 a	

The accumulation of microelements, except for Zn, in *Zantedeschia* leaves is a favourable occurrence, as it may result in more abundant inflorescences of higher quality. Janowska et al. [34] found that all GA_3 treatments increased the Mn content but did not affect the Cu content in the leaves *of Gladiolus hybridus* 'Black Velvet'. GA_3 concentrated at 600 mg·dm^{-3} stimulated the uptake of Fe and B, but lower GA_3 concentrations inhibited the uptake of both microelements.

The Zn uptake was stimulated at 100 mg·dm^{-3}; however, it was inhibited at a higher concentration. Janowska et al. [35] observed that another growth regulator—BA—applied at 600 mg·dm^{-3}, stimulated the uptake of Mn and Zn in *G. hybridus* 'Black Velvet', whereas, at concentrations ranging from 100 to 600 mg·dm^{-3}, it stimulated the B uptake but inhibited the Cu uptake. Walker et al. [37] observed a similar phenomenon in *Betula lenta* leaves. Janowska et al. [26] found that mycorrization only affected the Mn content in *Zantedeschia albomaculata* leaves but had no effect on the content of Na and other microelements. Nowak [28] noted elevated levels of Mg in the leaves of *Callistephus chinensis* after the treatment with mycorrhizal fungi.

4. Conclusions

AMF increased the yield of *Zantedeschia albomaculata* 'Albomaculata' inflorescences by 100%.AMF and GA_3 had a favourable effect on the quality of inflorescences, expressed by the length of peduncles, whereas mycorrhization on its own positively affected the length of the spathes. AMF and GA_3 had no effect on the level of macroelements in calla lily leaves, with the exception of calcium. The leaves of mycorrhized plants had a high content of Na and micronutrients, except for Fe. The results of the study showed that GA_3 could be replaced by mycorrhiza when applied to *Zantedeschia* plants.

Author Contributions: Conceptualization, Methodology, Formal Analysis, Writing, R.A. and B.J. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The publication was co-financed within the framework of Ministry of Science and Higher Education programme as "Regional Initiative Excellence" in years 2019–2022, Project No. 005/RID/2018/19, financing amount 12 000 000 PLN.

Conflicts of Interest: The authors declare no conflict of interest.

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