

Article

Changes in Growth and Physiological Parameters of \times Amarine Following an Exogenous Application of Gibberellic Acid and Methyl Jasmonate

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Abstract: \times Amarine hybrids are attractive ornamental geophytes grown for cut flower production. Their cultivation is limited due to lesser flowering percentages and lesser bulb weight gain. To optimize the growth and propagation of geophytes, plant growth regulators (PGRs) are used, but so far none have been tested in \times Amarine. We investigated the effect of gibberellic acid (GA₃; 50, 100, and 200 mg dm⁻³) and methyl jasmonate (MeJA; 100, 500, and 1000 μmol dm⁻³) on growth, flowering, bulb yield, and select physiological parameters of \times A. tubergenii “Zwanenburg”. PGRs were applied as foliar sprays on the 70th and 77th day after planting. GA₃ treatment at 200 mg dm⁻³ exhibited the greatest leaf number, leaf length, bulb weight, daughter bulb number, CO₂ assimilation intensity, greenness index, total sugars, and total protein content in bulbs. GA₃ application at 100 and 200 mg dm⁻³ accelerated flowering and at 50 and 100 mg dm⁻³ significantly increased the bulb flowering percentage. MeJA at all tested concentrations prolonged anthesis time and reduced the bulb flowering percentage. GA₃ at all concentrations and MeJA at 500 and 1000 μmol dm⁻³ stimulated daughter bulbs formation. GA₃, especially at 200 mg dm⁻³ can improve anthesis and increase \times A. tubergenii “Zwanenburg” bulb yield.

Keywords: cut flower; bulb propagation; phytohormones; GA₃; MeJA; gas exchange

1. Introduction

Ornamental plant production is an important horticultural branch. The expansion of the selection with previously unknown species, their hybrids, and new varieties is a key factor for the development of this sector [1]. An important part of flower production is the cultivation and reproduction of bulbous plants [2]. There are more than 800 different botanical genera of ornamental geophytes on the market. Their number systematically increases due to species introduction from natural sites and extensive breeding programs [3,4]. Plants newly introduced to the flower market lack appropriate cultivation technologies and methods of species propagation. Therefore, research is needed to effectively encourage producers to start growing previously unknown plants.

Among bulbous plants, considerable success was achieved in the breeding of Amaryllidaceae interspecific and intergeneric hybrids [5], using for crosses South African species from the genera

Amaryllis L., *Brunsvigia* Heister, *Clivia* Lindl., *Cyrtanthus* W. Aiton, *Haemanthus* L., and *Nerine* Herb. These plants are cultivated for their attractive flowers [6] and as a source of valuable alkaloids with therapeutic effects [7]. Breeding resulted in the nothogenus \times *Amarine tubergenii* Sealy, a hybrid between *Amaryllis belladonna* L. and *Nerine bowdenii* Watson [8]. Inflorescence of \times *A. tubergenii* sets on a long leafless stem and consists of helicoid cymes inflorescence, each with several pink florets (Figure 1a,b). The leaves are dark green, ensiform, form a rosette, and grow directly from bottle-shaped perennial bulbs covered with brown scales (Figure 1c,d). \times *A. tubergenii* inflorescences demonstrate very good post-harvest durability and are a desirable commodity on the cut flower market [9]. As a result of further intergeneric crosses, many \times *Amarine* varieties were obtained, differing in floret color, size, and flowering time. Research is lacking on \times *Amarine* cultivation, which is an obstacle to the wider spread of this prospective ornamental plant.

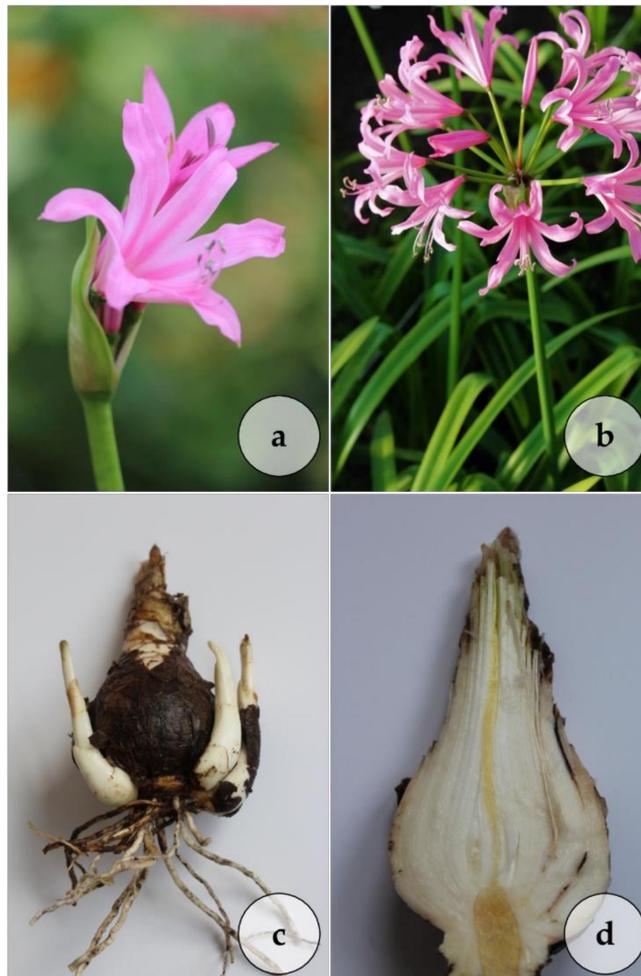


Figure 1. An inflorescence of \times *A. tubergenii* (a) and in full anthesis (b). Perennial bulb of \times *A. tubergenii* with daughter bulbs (c) and a longitudinal section through a dormant bulb (d).

A. belladonna and *N. bowdenii* species, from which \times *Amarine* was obtained, differ in the duration of dormancy and growth and development stages. In *A. belladonna*, the foliage emerges after anthesis (a hysteranthous growth habit), while in *N. bowdenii* foliage emerges before anthesis (a synanthous growth habit) [10]. In the Northern Hemisphere, after a dormancy period, \times *Amarine* bulbs first grow leaves in the spring, followed by floral stems in late summer and autumn. After anthesis, the plants become dormant. During this time, the bulbs should be exposed to decreased temperatures for flower primordia initiation. A serious problem in \times *A. tubergenii* cultivation is decreased flowering percentages commonly observed in *N. bowdenii* [11], a parent plant of the hybrid. The reasons for nonflowering in

N. bowdenii are complex and result from many independent factors, such as inadequate temperature during bulb dormancy and plant growth, undersized bulbs, or insufficient carbohydrate content [12]. The flowering of $\times A. tubergenii$ hybrids are irregular and extended in time (unpublished data), which limits their widespread use as a cut-flower crop.

In ornamental plant cultivation, plant growth regulators (PGRs) are used. They are active at exceptionally decreased concentrations and participate in the regulation of growth, anthesis, propagation, and physiological and metabolic processes [13,14]. Gibberellins are one of the best known natural phytohormones widely used in horticulture to terminate dormancy [15], stimulate floret formation and development [16], and accelerate or delay plant anthesis [17]. Gibberellins are responsible for stem elongation [18], stimulation of cell division and development of lateral buds [19], and also intensify photosynthesis and respiration [20]. Conversely, jasmonates, including methyl jasmonate (MeJA), are a fairly recently discovered phytohormone class [21]. MeJA is involved in regulating germination [22], morphogenesis [23], and aging [24], as well as the plant response to environmental stresses [25]. The available data from studies on the influence of MeJA on plant growth present divergent results. MeJA shows both growth-stimulating and growth-inhibiting effects [26,27], it can speed up or inhibit anthesis [28,29], and increase or decrease bulbing [30,31].

As there is no information on the use of PGRs in $\times A. tubergenii$ cultivation, we assessed the effect of gibberellic acid (GA_3) and MeJA at different concentrations on $\times A. tubergenii$ morphological traits, anthesis, and bulb yield. To obtain information on potential physiological changes induced by GA_3 or MeJA, the study also examined select gas exchange and chlorophyll fluorescence parameters and determined total sugars and total protein content in bulbs. We hypothesized that the applied regulators influenced the growth and physiological condition of $\times A. tubergenii$ plants.

2. Materials and Methods

2.1. Experimental Location, Plant Materials, and Growth Conditions

The experiment was conducted in an unheated plastic house (25 m in length, 9 m in width, and 4.7 m in total height), covered with a double layer of UV-resistant foil, located at the West Pomeranian University of Technology in Szczecin (53°25' N, 14°32' E, 25 m a.s.l., sub-zone 7a USDA).

Dormant $\times A. tubergenii$ "Zwanenburg" bulbs with a 12–14 cm circumference and an average fresh weight of 39.0 g, imported from the Netherlands by Ogrodnictwo Wiśniewski Jacek Junior (Góraszka, Poland), were stored for 3 weeks in dark at 5–8 °C until planting. Before planting, sorted for disease-free bulbs were treated for 30 min in a fungicide mixture containing 0.7% (w/v) Topsin M 500 SC (Nippon Soda, Tokyo, Japan, active ingredient: thiophanate-methyl) and 1% (w/v) Kaptan 50 WP (Organika-Azot Chemical Company, Jaworzno, Poland, active ingredient: Captan). On 14 April, the bulbs were planted individually into black round PVC pots with 15 cm diameter and a 1.5 dm³ capacity, filled with deacidified peat (Kronen, Cerkwica, Poland) (pH 6.3; 16 mg dm⁻³ N-NO₃, 42 mg dm⁻³ P, 19 mg dm⁻³ K, 1550 mg dm⁻³ Ca, 101 mg dm⁻³ Mg and 27 mg dm⁻³ Cl) mixed with Hydrocomplex fertilizer (Yara International ASA, Oslo, Norway) containing 12% N, 11% P₂O₅, 18% K₂O, 2.7% MgO, 8% S, 0.015% B, 0.2% Fe, 0.02% Mn, and 0.02% Zn at a dose of 3 g dm⁻³. The pots were placed in 60 × 40 × 19 cm plastic boxes, six pots per box, which were placed in a tunnel on white non-woven fabric. The air temperature was regulated with air vents, which opened automatically when the temperature exceeded 22 °C. The average monthly maximum/minimum air temperature and average relative humidity (RH) in the plastic house were respectively: April 22.7 °C/6.8 °C, 70.9% RH; May 25.0 °C/6.9 °C, 76.9% RH; June 27.3 °C/13.3 °C, 70.6% RH; July 32.5 °C/17.6 °C, 69.4% RH; August 25.6 °C/14.7 °C, 78.5% RH; September 25.9 °C/13.0 °C, 80.4% RH; October 18.6 °C/7.1 °C, 90.2% RH; and November 14.9 °C/5.5 °C, 95.7% RH. The plants were cultivated until 15 November under natural day/night conditions (without shade nets or artificial lighting). The photosynthetic photon flux density (PPFD) in the plastic house on a sunny day ranged from 420 to 1151 μmol m⁻² s⁻¹ (as per Radiometer-Fotometr RF-100, Sonopan, Białystok, Poland).

2.2. Experimental Design

On the 70th and 77th day after planting we used two growth regulators from Sigma-Aldrich Chemie GmbH (Schnelldorf, Germany): gibberellic acid (GA₃) at 50, 100, and 200 mg dm⁻³, and methyl jasmonate (MeJA) at 100, 500, and 1000 µmol dm⁻³. The plants were sprayed in the afternoon with an aqueous solution of GA₃ or MeJA, using about 15 cm³ solution per plant. The control plants were sprayed with distilled water. Ethanol (0.04%, v/v) was used as a solvent. Directly after spraying, a plastic bag was placed on each plant and removed after two hours. Each experimental variant included 18 plants, six plants per repetition, in a random block system (Figure 2).



Figure 2. ×*A. tuberosum* “Zwanenburg” plants on the 70th day after planting.

2.3. Determination of Plant Growth Parameters

The number of days from bulb planting to the beginning of anthesis was recorded when the first florets in the inflorescence opened. In this phase, we determined the stem length, leaf number, and length and width of the longest leaf. The flowering bulb number (%) was determined in relation to the bulb number planted. When the inflorescences were fully developed, we counted the florets in each. Once the cultivation was complete, we removed the plants from the pots and determined the bulb fresh weight and the daughter bulb number per single planted bulb.

2.4. Determination of Gas Exchange Rate, Chlorophyll Fluorescence, and Leaf Relative Chlorophyll Content

The parameters of the gas exchange rate, including CO₂ assimilation intensity (A), transpiration (E), stomatal conductance for water (g_s), and CO₂ concentration in the intercellular spaces of the assimilatory parenchyma (c_i) were measured with a TPS-2 (PP Systems) portable gas analyzer (with standard settings), equipped with a PLC4 measuring chamber operating in an open system. Based on CO₂ assimilation intensity and transpiration, the photosynthetic water-use efficiency (ω_W) was calculated as a ratio of assimilation intensity to transpiration [32].

Chlorophyll fluorescence parameters were recorded using a Handy PEA (Hansatech) spectrofluorometer, based on the standard apparatus procedure. Leaves were shaded for 20 min prior to the measurement with a leaf clip (4 mm in diameter). The following parameters of chlorophyll fluorescence induction were measured and calculated using the spectrofluorometer: initial fluorescence excitation energy loss index in power antennas (F₀); maximum fluorescence after reduction of acceptors in photosystem II (PSII) and after dark adaptation (F_M); variable fluorescence, determined after dark adaptation, a parameter dependent on the maximum quantum yield of PSII (F_V = F_M – F₀); maximum potential photochemical reaction efficiency in PSII determined after dark adaptation and after reduction

of acceptors in PS II (F_V/F_M); PSII vitality index for the overall viability of this system (P I); the surface area above the chlorophyll fluorescence curve and between the F_0 and F_M points proportional to the size of the reduced plastoquinone acceptors in PS II (Area) [33].

Leaf relative chlorophyll content (greenness index) on the Soil and Plant Analysis Development (SPAD) scale was measured with the Chlorophyll Meter SPAD 502 (Konica-Minolta cooperation, Ltd., Osaka, Japan).

Non-destructive measurements of the gas exchange rate, chlorophyll fluorescence, and SPAD were carried out on the 91st cultivation day from 09:00 AM to 12:00 PM in the middle part of the adaxial leaf blades. The measurements involved two fully expanded leaves with a length of 48–50 cm and width of 2.0–2.2 cm in two matched for size plants from each repetition. The conditions in the tunnel during the analyses were: temperature 20–22 °C, relative air humidity 70–75%, natural light (PPFD 450 $\mu\text{mol m}^{-2} \text{s}^{-1}$), air CO_2 concentration 600 $\mu\text{mol mol}^{-1}$.

2.5. Determination of Total Sugars and Total Protein Content in Bulbs

Once the cultivation was complete, four bulbs from each experimental variant were cleaned from the covering scales and roots. The analyses involved bulbs of similar fresh weight. The bulbs were cut longitudinally into four segments with a knife, and then middle scales were cut out from each segment and analyzed. Samples (250–300 g) were taken for the determination of total sugars and total protein. Concentrations of both reducing and invert sugars were determined by extraction with diluted ethanol, clarification of extracts with Carrez solutions, and titration with sodium thiosulphate solution in the presence of Luff-Schoorl reagent according to PN-R-64784:1994 standard. Total protein content was calculated based on nitrogen content determination according to the Kjeldahl method using a mineralization block, copper catalyst, and steam distillation into boric acid according to PN-EN ISO 5983-2:2009 standard. Content determination of the tested components was performed in three repetitions and presented as a percentage of fresh weight.

2.6. Data Analysis

The experimental results were statistically analyzed with the one-way ANOVA using Statistica 13.3 (TIBCO Software Inc. Statsoft, Kraków, Poland). To ensure the normality of data distribution, the plant flowering percentages were subjected to the Bliss transformation ($\arcsin(\sqrt{X})$) and the analysis of variance. The confidence intervals were calculated based on Tukey's HSD test ($p \leq 0.05$).

3. Results

3.1. Effects of Foliar Application of GA_3 and MeJA Solutions on Growth, Flowering, and Bulb Yield

Treatments with exogenous PGRs significantly affected the leaf number and their length, but not the stem length and floret number per inflorescence (Table 1). Plants treated with GA_3 at all applied concentrations and MeJA at 500 and 1000 $\mu\text{mol dm}^{-3}$ formed significantly more leaves as compared with the control. The plants sprayed with GA_3 at 200 mg dm^{-3} produced the greatest leaf number, which were also the longest.

In all cases, one bulb produced only one inflorescence. Anthesis time and bulb flowering percentage depended on the phytohormone type and concentration (Table 2). Plants treated with 100 and 200 mg dm^{-3} GA_3 were the first to start anthesis, while those sprayed with 50 and 100 mg dm^{-3} GA_3 showed the greatest flowering percentage. MeJA treatment delayed flowering and significantly reduced the flowering plant number, regardless of the concentration applied.

PGR treatments and their concentrations influenced bulb yield (Table 3). Spraying with GA_3 solutions at all tested concentrations and MeJA at 1000 $\mu\text{mol dm}^{-3}$ significantly increased the fresh bulb weight as compared with the control plants. Plants treated with 100 and 200 mg dm^{-3} GA_3 exhibited the greatest fresh bulb weight. Greater PGR concentrations (GA_3 at 100 and 200 mg dm^{-3} ;

MeJA at 500 and 1000 $\mu\text{mol dm}^{-3}$) significantly increased the daughter bulb number in comparison with untreated plants.

Table 1. Effects of GA₃ and MeJA on morphological traits of *×Amarine tubergenii* “Zwanenburg” at the beginning of anthesis.

Treatment		Leaves (no.)	Leaf Length (cm)	Stem Length (cm)	Florets (no.)
GA ₃	Control	9.00 ± 0.50 ^{d,*}	49.9 ± 1.49 ^{bc}	72.5 ± 4.39 ^a	7.97 ± 0.64 ^a
	50 mg dm ⁻³	11.2 ± 0.76 ^{bc}	51.8 ± 1.07 ^{bc}	72.5 ± 0.95 ^a	8.33 ± 0.76 ^a
	100 mg dm ⁻³	12.3 ± 0.25 ^{ba}	53.8 ± 1.97 ^b	74.0 ± 4.52 ^a	8.33 ± 0.76 ^a
	200 mg dm ⁻³	12.8 ± 0.29 ^a	58.1 ± 2.28 ^a	75.6 ± 4.15 ^a	7.97 ± 0.64 ^a
MeJA	100 $\mu\text{mol dm}^{-3}$	9.00 ± 0.50 ^d	49.1 ± 1.50 ^c	72.3 ± 2.78 ^a	7.87 ± 0.15 ^a
	500 $\mu\text{mol dm}^{-3}$	10.5 ± 0.50 ^c	51.1 ± 0.75 ^{bc}	71.6 ± 3.45 ^a	7.58 ± 0.38 ^a
	1000 $\mu\text{mol dm}^{-3}$	11.5 ± 0.50 ^{abc}	50.4 ± 0.80 ^{bc}	73.5 ± 4.20 ^a	7.50 ± 0.43 ^a

* Means over each column not marked with the same letter are significantly different at $p \leq 0.05$. Data are expressed as mean and standard deviation (\pm SD).

Table 2. Effects of GA₃ and MeJA on anthesis time and the bulb flowering percentage of *×Amarine tubergenii* “Zwanenburg”.

Treatment		Time to Anthesis (d)	Bulbs Flowering (%)
GA ₃	Control	188 ± 1.00 ^{b*}	46.5 ± 8.85 ^{bc}
	50 mg dm ⁻³	185 ± 2.65 ^{bc}	82.2 ± 16.3 ^a
	100 mg dm ⁻³	177 ± 2.65 ^c	82.2 ± 16.3 ^a
	200 mg dm ⁻³	176 ± 1.00 ^c	65.6 ± 4.21 ^{ab}
MeJA	100 $\mu\text{mol dm}^{-3}$	206 ± 5.51 ^a	30.4 ± 6.66 ^c
	500 $\mu\text{mol dm}^{-3}$	206 ± 5.03 ^a	22.8 ± 8.28 ^c
	1000 $\mu\text{mol dm}^{-3}$	203 ± 5.86 ^a	28.0 ± 7.68 ^c

* Means over each column not marked with the same letter are significantly different at $p \leq 0.05$. Data are expressed as mean and standard deviation (\pm SD).

Table 3. Effects of GA₃ and MeJA on *×Amarine tubergenii* “Zwanenburg” bulb yield.

Treatment		Bulb Weight (g)	Daughter Bulbs (no.)
GA ₃	Control	97.7 ± 1.46 ^{c*}	1.28 ± 0.03 ^b
	50 mg dm ⁻³	107 ± 3.61 ^b	1.78 ± 0.20 ^{ab}
	100 mg dm ⁻³	128 ± 5.51 ^a	2.16 ± 0.15 ^a
	200 mg dm ⁻³	134 ± 4.26 ^a	2.30 ± 0.30 ^a
MeJA	100 $\mu\text{mol dm}^{-3}$	99.1 ± 2.80 ^c	1.35 ± 0.13 ^b
	500 $\mu\text{mol dm}^{-3}$	100 ± 3.61 ^c	2.27 ± 0.25 ^a
	1000 $\mu\text{mol dm}^{-3}$	112 ± 4.63 ^b	2.27 ± 0.25 ^a

* Means over each column not marked with the same letter are significantly different at $p \leq 0.05$. Data are expressed as mean and standard deviation (\pm SD).

3.2. Effects of Foliar Application of GA₃ and MeJA Solutions on Gas Exchange Rate

Table 4 presents the data on leaf gas exchange parameters depending on the plant hormone and its concentration. The greatest CO₂ assimilation intensity (A) was found in plants sprayed with 200 mg dm⁻³ GA₃ solution. This parameter in the other variants did not differ significantly from the control. GA₃ or MeJA application resulted in a significant increase in the intensity of transpiration (E), as compared with untreated plants, except for 1000 $\mu\text{mol dm}^{-3}$ MeJA. The greatest E was recorded in plants sprayed with 100 $\mu\text{mol dm}^{-3}$ MeJA. The increase was more than twofold vs. the control. Plants treated with 50 mg dm⁻³ GA₃ exhibited the greatest photosynthetic water-use efficiency (ω_w). The ω_w for the other treatments did not differ significantly from the control. Exogenous application of GA₃ and MeJA at all tested concentrations resulted in a significant increase in water stomatal conductance

(g_s). Compared to untreated plants, the greatest, more than fourfold increase in g_s was recorded after the application of 100 and 500 $\mu\text{mol dm}^{-3}$ MeJA. Both phytohormones triggered a significant increase in CO_2 concentration in the intercellular spaces (c_i), particularly noticeable in the plants treated with 1000 $\mu\text{mol dm}^{-3}$ MeJA.

Table 4. Effects of GA_3 and MeJA on CO_2 assimilation intensity (A), transpiration (E), photosynthetic water-use efficiency (ω_w), stomatal conductance (g_s), and CO_2 concentration (c_i) of *×A. tubergenii* “Zwanenburg”.

Treatment		A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	E ($\text{mmol m}^{-2} \text{s}^{-1}$)	ω_w (mmol mol^{-1})	g_s ($\text{mol m}^{-2} \text{s}^{-1}$)	c_i ($\mu\text{mol mol}^{-1}$)
GA ₃	Control	6.67 ± 0.41 ^{bc*}	0.82 ± 0.21 ^d	4.67 ± 1.14 ^{bc}	0.10 ± 0.02 ^d	409 ± 40.3 ^c
	50 mg dm^{-3}	7.40 ± 0.60 ^{bc}	1.49 ± 0.12 ^{bc}	6.99 ± 0.60 ^a	0.28 ± 0.03 ^b	449 ± 4.12 ^b
	100 mg dm^{-3}	7.75 ± 0.65 ^{ab}	1.41 ± 0.26 ^c	5.75 ± 0.98 ^{ab}	0.31 ± 0.07 ^b	455 ± 9.66 ^{ab}
MeJA	200 mg dm^{-3}	8.99 ± 0.84 ^a	1.44 ± 0.15 ^c	5.40 ± 0.65 ^{bc}	0.34 ± 0.07 ^b	470 ± 9.35 ^{ab}
	100 $\mu\text{mol dm}^{-3}$	6.32 ± 0.27 ^c	1.75 ± 0.09 ^a	4.32 ± 0.21 ^c	0.43 ± 0.05 ^a	444 ± 6.00 ^b
	500 $\mu\text{mol dm}^{-3}$	6.16 ± 0.52 ^c	1.72 ± 0.07 ^{ab}	4.16 ± 0.40 ^c	0.44 ± 0.04 ^a	468 ± 6.57 ^{ab}
	1000 $\mu\text{mol dm}^{-3}$	7.10 ± 0.77 ^{bc}	1.05 ± 0.14 ^d	5.10 ± 1.48 ^{bc}	0.21 ± 0.04 ^c	482 ± 21.1 ^a

* Means over each column not marked with the same letter are significantly different at $p \leq 0.05$. Data are expressed as mean and standard deviation (\pm SD).

3.3. Effects of Foliar Application of GA_3 and MeJA Solutions on Chlorophyll Fluorescence and SPAD

In comparison with the control plants, GA_3 and MeJA did not significantly affect the chlorophyll fluorescence parameters (F_v/F_m , PI, and Area), but caused changes in the leaf greenness (SPAD index) (Table 5). Plants treated with 200 mg dm^{-3} GA_3 showed the greatest SPAD values. Similarly, an increased greenness index was observed in plants treated with 50 and 100 mg dm^{-3} GA_3 and 1000 $\mu\text{mol dm}^{-3}$ MeJA.

Table 5. Effects of GA_3 and MeJA on chlorophyll fluorescence parameters (F_v/F_m , PI, Area) and leaf greenness index (SPAD) in *×A. tubergenii* “Zwanenburg” leaves.

Treatment		F_v/F_m	PI	Area	SPAD
GA ₃	Control	0.79 ± 0.03 ^{ab*}	1.72 ± 0.39 ^{ab}	49,428 ± 8097 ^{ab}	52.2 ± 1.40 ^c
	50 mg dm^{-3}	0.77 ± 0.03 ^{ab}	1.71 ± 0.30 ^{ab}	40,656 ± 6448 ^b	56.9 ± 0.67 ^b
	100 mg dm^{-3}	0.78 ± 0.02 ^{ab}	1.66 ± 0.18 ^b	47,401 ± 7553 ^{ab}	58.5 ± 1.35 ^b
MeJA	200 mg dm^{-3}	0.82 ± 0.04 ^a	2.02 ± 0.20 ^a	59,420 ± 13,999 ^a	66.0 ± 2.48 ^a
	100 $\mu\text{mol dm}^{-3}$	0.77 ± 0.05 ^{ab}	1.73 ± 0.08 ^{ab}	41,702 ± 6330 ^b	51.8 ± 0.40 ^c
	500 $\mu\text{mol dm}^{-3}$	0.73 ± 0.04 ^b	1.66 ± 0.06 ^b	47,593 ± 7939 ^{ab}	55.0 ± 1.50 ^{bc}
	1000 $\mu\text{mol dm}^{-3}$	0.76 ± 0.05 ^{ab}	1.76 ± 0.12 ^{ab}	37,339 ± 14,819 ^b	59.5 ± 2.35 ^b

* Means over each column not marked with the same letter are significantly different at $p \leq 0.05$. Data are expressed as mean and standard deviation (\pm SD).

3.4. Effects of Foliar Application of GA_3 and MeJA Solutions on Total Sugars and Total Protein Content

GA_3 and MeJA application significantly increased bulb total sugar content (Figure 3a) at all concentrations. The greatest total sugars content was found in plants treated with 100 and 200 mg dm^{-3} GA_3 and 1000 $\mu\text{mol dm}^{-3}$ MeJA. GA_3 and MeJA at the greatest concentrations also increased the bulb total protein content (Figure 3b).

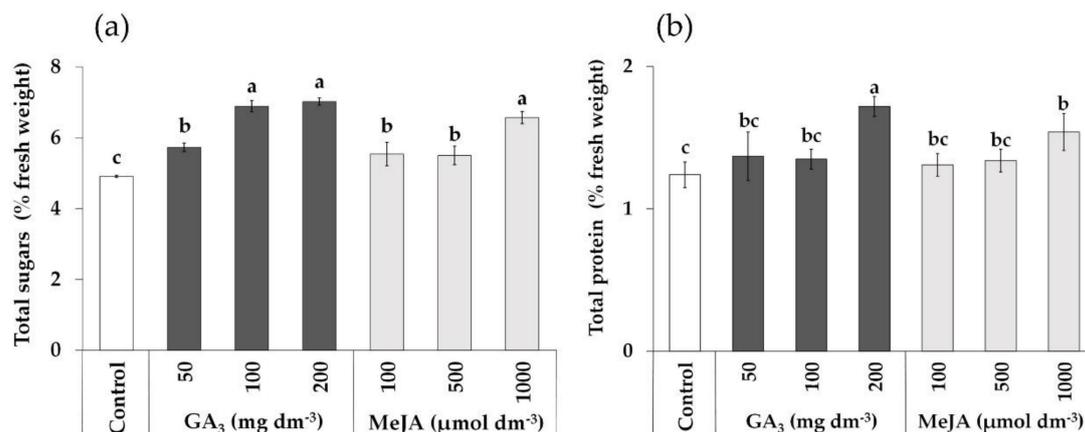


Figure 3. Effects of foliar application of GA₃ and MeJA solutions on total sugar (a) and total protein (b) content in the bulbs of *xA. tubergenii* "Zwanenburg". Data are mean \pm SD (n = 3). Different letters above the error bars indicate significant differences for $p < 0.05$.

4. Discussion

4.1. Effect of GA₃ and MeJA on Growth, Flowering, and Bulb Yield

To improve ornamental geophyte quality, various plant growth and development regulators are used in horticultural practice [34,35]. *xAmarine tubergenii* "Zwanenburg" treated with GA₃ (50, 100, and 200 mg dm⁻³) and MeJA (500 and 1000 μmol dm⁻³) exhibited a significantly increased leaf number (Table 1). Moreover, at the greatest GA₃ concentration, the leaves were much longer. More leaves after GA₃ application were observed by Ramzan et al. [36] in *Tulipa gesneriana* L. The activity of gibberellin family growth regulators involves the stimulation of plant cell mitotic division [37], which can lead to intense growth and production of more leaves. Diallo et al. [38] showed a stimulating effect of MeJA on leaf count in *Triticum aestivum* L. The researchers suggested that the beneficial effect of MeJA on the leaf count may be because MeJA maintained plants in the vegetative phase longer, so that plants continued their intense growth instead of proceeding to the generative phase. Similarly, we showed MeJA treatment delayed anthesis time (Table 2).

Neither of the phytohormones used in this study affected the inflorescence morphology, including the floret number (Table 1). The lack of PGR effects on the floret number may stem from the fact that both phytohormones were applied after the inflorescence initiation inside of the bulb. In *Nerine bowdenii* the time between flower primordia initiation and anthesis is 2-3 years, and in *Amaryllis belladonna* over a year [6,10].

Foliar treatment with both GA₃ and MeJA strongly influenced the anthesis time and the flowering percentage, but GA₃ worked differently than MeJA (Table 2). The plants sprayed with GA₃ at 100 and 200 mg dm⁻³ began anthesis faster. Earlier flowering following GA₃ application at 150–300 ppm was also observed in *A. belladonna* [39]. However, in *Nerine flexuosa* GA₃ bulb-dip treatment at 100 mg/L did not accelerate flowering [40]. In *Nerine*, many inflorescences did not elongate and became desiccated, thus no flowering occurred [41]. It may be assumed that in *xA. tubergenii* "Zwanenburg" GA₃ stimulates the final stages of stem elongation and anthesis. GA₃ treatment at 50 and 100 mg dm⁻³ not only accelerated anthesis, but also positively affected the inflorescence yield by increasing the bulb flowering percentage (Table 2). The increased number of *xA. tubergenii* "Zwanenburg" flowering plants following GA₃ treatment is probably because of the faster execution of the flowering phase. Our results are highly practical, as GA₃ makes more plants flower at an earlier stage, which allows for harvesting the inflorescences of field or unheated tunnel-grown plants before the autumn frosts.

MeJA, regardless of its concentration, delayed flowering and decreased the number of flowering *xA. tubergenii* "Zwanenburg" plants (Table 2). Similarly, Zhai et al. [42] reported an inhibitory effect of jasmonic acid on *Arabidopsis thaliana* flowering. Maciejewska and Kopcewicz [28] observed that

treatment with MeJA reduced the flower bud number in *Pharbitis nil* (L.) Roth. Conversely, 50 μM MeJA application in *Brassica napus* L. moved the flowering time forward and increased the flower number [29]. Jasmonates control individual plant ontogenesis phases, but their exact mode of action in the regulation of growth and flowering induction is not known.

A common problem in bulbous plant propagation is their low propagation rate, which can be increased using PGRs [43]. Bulb yield analysis in *×A. tubergenii* “Zwanenburg” clearly showed that GA_3 increases the bulb weight and daughter bulb number (Table 3). In *Allium karataviense* Regel GA_3 spraying increased the bulb weight and total bulb yield [44]. *Allium sativum* L. treatment with GA_3 positively affected axillary bud formation, clove number per bulb, bulb weight, and bulb volume [45]. The stimulating effect of GA_3 on bulbing may be because gibberellins enhance gene expression correlated with cell elongation necessary for cell development and differentiation, and induced lateral bud formation [46].

MeJA at concentrations of 500 and 1000 $\mu\text{mol dm}^{-3}$ had a similarly stimulating effect on the propagation rate as GA_3 application. Moreover, bulbs obtained from plants treated with the greatest MeJA dose displayed significantly greater weight (Table 3). In vitro jasmonates application increased the bulb number and improved bulblet quality parameters in *A. sativum* [47] and *Narcissus triandrus* L. [48]. Nojiri et al. [49] postulated that both jasmonic acid and MeJA are bulbing hormones, as they both stimulate bulbing through microtubules disruption.

4.2. Effect of GA_3 and MeJA on Physiological Parameters

Numerous studies have shown that the course and intensity of the most important plant physiological processes can be regulated with exogenous growth regulators [50,51]. In our study, GA_3 regardless of its concentration enhanced transpiration, stomatal conductance for water, CO_2 concentration in the intercellular spaces of the assimilatory parenchyma, and SPAD in *×A. tubergenii* “Zwanenburg” (Tables 4 and 5). A significant increase in CO_2 assimilation intensity was found only in plants sprayed with GA_3 solution at 200 mg dm^{-3} . These plant leaves showed the greatest chlorophyll fluorescence and SPAD index values. It can be assumed that as a result of increased photosynthesis, plants treated with GA_3 in the greatest dose produced the greatest leaf number and daughter bulbs, and had the longest leaves and bulbs with the greatest fresh weight. Increased photosynthetic efficiency results in faster vegetative growth and leads to increased biomass production [52].

MeJA applied in all tested concentrations increased the stomatal conductance and leaf CO_2 concentration without affecting CO_2 assimilation intensity and assessed fluorescence parameters (Table 4). Moreover, MeJA application at 100 and 500 $\mu\text{mol dm}^{-3}$ increased transpiration, and at 1000 dm^{-3} enhanced leaf greenness (SPAD; Table 5). Our results partially confirm those of Ahmadi et al. [53], who found that spraying *B. napus* with 100 μM MeJA significantly increased the CO_2 compensation point and respiration rate. A positive effect of MeJA on photosynthetic parameters was observed in two *Prunus dulcis* Mill. rootstocks [54]. The effect of exogenous MeJA on the plant photosynthetic apparatus is complex and depends on many factors, including species, concentration, and environmental conditions [21,23,25].

Geophytes accumulate reserve substances in their bulbs that determine the correct course of dormancy, growth, and flowering [55]. Both PGRs significantly increased total sugar content in *×A. tubergenii* “Zwanenburg” bulbs, especially when applied at 100 and 200 mg dm^{-3} (GA_3) and 1000 $\mu\text{mol dm}^{-3}$ (MeJA) (Figure 3a). Moreover, bulbs obtained from plants treated with GA_3 or MeJA in the greatest concentrations featured significantly more total protein (Figure 3b). Wakchaure et al. [56] found an increased total soluble sugar content and total protein content in *Allium cepa* L. bulbs treated with GA_3 . Jasmonates promote starch accumulation in tubers, as it was apparent in *Solanum tuberosum* L. subsp. *tuberosum* [57]. In *×A. tubergenii* “Zwanenburg”, the greatest total sugars content and total bulb protein was found in plants sprayed with GA_3 at 200 mg dm^{-3} (Figure 3). This could have been related to increased CO_2 assimilation intensity, and as a result, better bulb supplementation with photosynthesis products. Plant bulbs treated with GA_3 at the greatest dose showed the greatest fresh

weight (Table 3). Most probably, increased bulb weight and a greater nutrient content, especially sugars, improves plant flowering in the subsequent growing season. In the case of *N. bowdenii*, the parent species of the nothogenus \times *Amarine*, inflorescence quality and flowering percentage is related to bulb size and carbohydrate content in fleshy leaf bases and fleshy scales [11,12]. Decreased sugar level in bulbs during gynoecium development is implicated in inflorescence abortion [58]. Therefore, it seems advisable to conduct further research on the residual impact of PGRs on the growth and anthesis of \times *A. tubergenii*. We intend to observe bulb performance in the following year, and to dissect them to observe flower primordia and their development after external PGR application, and monitor how it affects floral initiation and development.

5. Conclusions

We examined the possibility of using PGRs in the cultivation of \times *A. tubergenii*, an ornamental geophyte with great floricultural potential. Plant growth and physiological condition depended on the PGR type and its concentration. The influence of GA₃ and MeJA on anthesis time was opposite, as GA₃ accelerated and MeJA delayed the beginning of anthesis. Additionally, GA₃ increased and MeJA decreased the bulb flowering percentage. All GA₃ concentrations and MeJA at 500 and 1000 $\mu\text{mol dm}^{-3}$ stimulated daughter bulb formation. Among all treatments, GA₃ at 200 mg dm^{-3} seemed to most favorably affect the leaf number, their length, bulb weight, daughter bulb number, photosynthesis rate, greenness, total sugar, and total protein content in bulbs. This treatment could be used as a method for improving \times *A. tubergenii* “Zwanenburg” plant growth, anthesis, and bulb yield in commercial production.

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