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Research Paper

Regulated root zone water temperatures and soilless media improve bulb yield and offset production of hydroponically cultivated *Amaryllis belladonna* L.

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ABSTRACT

The protracted natural bulb growth rate and offset generation constrain the timely expansion of bulbs in *Amaryllis belladonna*. An 18-month experiment was conducted to determine the efficacy of different root zone water temperatures and soilless media on the bulb yield and offset production of *A. belladonna* cultivated in a deep water culture hydroponic system. The study comprised dormant juvenile bulbs that were planted in plastic cavity trays filled with soilless media and suspended in heated water reservoirs at different temperatures (16 °C control, 22 °C, 28 °C, and 34 °C). The results found that the autonomous analysis of water temperatures, as opposed to soilless media and the combination, was significant. Warm root zone water temperatures ranging from 16 °C to 28 °C resulted in comparable aerial and subsurface morphological growth and development, promoting the vegetative proliferation of larger juvenile mother bulbs and offset induction. However, an excessively high temperature of 34 °C proved injurious and depleting. The results infer that the intrinsic processes for 'bulbing' were synchronized under inducive extrinsic hydroponic stimuli. A water temperature of 22 °C is recommended for long-term, sustainable production of simultaneously generating optimum reclaimable bulbs and offset generation to abridge the timeous cultivation of continuous A. belladonna vegetative bulb stock. A water temperature of 22 °C is recommended for long-term, sustainable production of simultaneously generating optimum bulbs and offset generation to abridge the timeous cultivation of continuous A. belladonna vegetative bulb stock.

1. Introduction

Geophytes are a diverse classification of flora, and their collective multiplicity is intensifying owing to ubiquitous market changes, competitive demands (Anderson 2006, 2019; Slezák et al., 2020) and the breeding of new cultivars with specialized and desired features (Marasek-Ciolakowska et al., 2021). Important scheduling ramifications to advance economic profitability are affected by selected bulb species, tubers and novel cultivars or hybrids with prolonged juvenile phases ranging between 8 and 25 years (Marasek-Ciolakowska et al., 2021). Thus, understanding and insight into a geophytic species' behavioural traits are critical to its advancement and provide tools for forecasting and implementing innovative strategies to determine responses under competitive and stringent conditions (Thompson et al., 2011; Kamenetsky-Goldstein, 2019; Slezák et al., 2020; Marković et al., 2021). Present geophytic research initiatives include those centred on vegetative propagation (Marković et al., 2021), a progressive decrease of the juvenile period, early selection of desired features (Anderson 2019; Marasek-Ciolakowska et al., 2021) and the procurement of indigenous and underutilized species (Reinten and Van Wyk, 2018; De Pascale and Romano, 2019; Darras, 2021).

Global ornamental cultivation practices have greatly revolutionized sophisticated agricultural practices to alleviate adverse conditions during the last three decades (Darras 2020, 2021). The regulated regimen of hydroponic technology allows for the precise management and use of long-term environmental conditions and inert soilless media transversely over multiple latitudinal regions. This results in widespread year-round production and enhanced crop yields per unit area (Wahome et al., 2010; Karagöz et al., 2022). Although the initial hydroponic investment is habitually capital-intensive and necessitates skilled

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personnel and technical expertise, the benefits of quantity and quality assurance, ubiquity, space efficiency, and economic outputs outweigh the costs (Karagöz et al., 2022; Velazquez-Gonzalez et al., 2022). Likewise, sustainability is realized in a species' life cycle assessment (LCA) with improved cultivation techniques (Darras 2020, 2021). Optimization of various cereal, condiment, flower, fodder, fruit, medicinal, ornamental, and vegetable crop yields in hydroponic cultivation has been extensively investigated (Fussy and Papenbrock, 2022; Velazquez-Gonzalez et al., 2022). However, geophytic optimization has primarily been used to promote flower-forcing events with little evidence of augmenting vegetative (asexual) plant material. This includes but is not limited to *Crocus, Hippeastrum, Hyacinthus, Leucojum, Lilium, Narcissus* and *Tulipa* species flower-forcing (Miller 2012; Schroeder et al., 2020). In contrast to market quality collections of iterative breeding and flower forcing, hydroponic cultivation may promote the vegetative proliferation of certain geophytic dispositions in readily available cultivars, hybrids, heritage, imperilled, and underutilized species (Wilmot and Laubscher, 2023).

Notwithstanding, in field and greenhouse conditions, the above-ground and subsurface thermoperiodic temperatures are imperative in maintaining a plant species' cardinal range while minimizing potential harm from greater or sub-optimal variations affecting the biochemical rate of many morphological and physiological activities of the rhythmic cycles of geophytic cultivation (Du Toit et al., 2002; Yan et al., 2013; Proietti et al., 2022). These activities include but are not limited to, water and nutrient absorption, translocation, transpiration, chlorophyll production and photosynthesis, metabolite accumulation and synthesis (Seiler, 1998; Zobayed et al., 2005; Sakamoto and Suzuki, 2015). Among several approaches, the modification of climatic factors is most prevalent. As such, before planting or cultivating a new crop, growers should determine the species' optimal production temperatures that govern plant development (Carlson and Dole, 2015). Substantial research has been undertaken on manipulating root zone temperatures to retain actively developing plant crops outside their precedented life cycle to sustain market needs (Wang et al., 2016; Balliu et al., 2021; Proietti et al., 2022).

Amaryllis belladonna L. (Amaryllidaceae) is an autumn flowering hysteranthous endemic geophyte to the Western Cape, South Africa's fynbos biome (Manning et al., 2002; Duncan et al., 2020). The species' adaptability, tenacity and attractive beauty have permitted it to be naturalised in several Mediterranean locations, displaying the transient, pink-shaded florets on a seasonal single-harvest inflorescence (Adams 2001; Reinten et al., 2011; Wilmot and Laubscher, 2019a; Gul et al., 2020). Irrevocably, emphasis and differentiation are placed on its economic significance as containerized flowering potted plants or as fragrant cut flowers lasting up to a week or longer in floral arrangements (Theron and De Hertogh, 2001; Reinten et al., 2011; Barnhoorn, 2013; Gul et al., 2020; Darras, 2021). The species has likewise garnered scientific attention and use within the pharmacological arena because of its isolated alkaloid constituents, some of which exhibit anti-bacterial, anti-fungal, anti-neoplastic, and anti-parasitic properties (Pettit et al., 1984; Evidente et al., 2004; Nair et al., 2013; Chavarro et al., 2020; Evidente, 2023). The hysteranthous species' perennial bulb growth, development and maturation are contingent upon the subterranean storage organs to amass sufficient carbohydrates from seasonal winter aerial growth through summer dormancy to flowering under inductive environmental stimuli (Dafni et al., 1981; Howard and Cellinese, 2020). However, encountered in many other geophytes and typified in Amaryllidaceae species (Manning et al., 2002; Duncan et al., 2020), *A. belladonna* has a prolonged natural juvenile bulb growth rate that necessitates several years to attain a competent adult reproductive flowering size and minimized replication in which the mother bulb divides, inducing offsets (daughter bulbs) (Theron and De Hertogh, 2001; Duncan, 2010). Moreover, premature offset detachment further hinders future development (Duncan, 2010). These traits debilitate the potential and timeous expansion of *A. belladonna* to produce a competent flowering size and continual quality bulb stock material for local and export floriculture and horticultural endeavours.

Considering the preceding, it is plausible to abridge the life cycle of *A. belladonna* and enhance the species' growth and multiplication in a cost-effective, eco-friendly, and timely manner. There has been no research using hydroponic technology to improve the shortcomings of phenotypic vegetative features in the *A. belladonna* species. As a result, the ideal method for increasing the species from the mother bulb material remains unknown as seed propagation produces varied genetic offspring, and traditional bulb cutting is time-consuming and costly (Duncan, 2010; Veeraballi et al., 2017). Therefore, to establish a novel method of inducing *A. belladonna* bulb offsets while simultaneously advancing the mother bulb's protracted juvenile phase, the efficacy of regulated root zone water temperatures and soilless media for *A. belladonna* bulbs cultivated in a deep water culture (DWC) hydroponic system was investigated.

2. Materials and methods

2.1. Experimental location

Experimentation was carried out from April 2020 to October 2022 (eighteen months) in the Cape Peninsula University of Technology's Department of Horticultural Sciences Research Greenhouse in Bellville, Cape Town, South Africa; 33°55′45″ S, 18°38′31″ E. The ventilated greenhouse was outfitted with a thermostatically controlled Envirowatch system (Envirowatch Solutions, Escourt, South Africa), fans, heaters, evaporative cooling pads, and clear polycarbonate roof sheeting to maintain a controlled and regulated greenhouse environment. Ambient greenhouse temperatures ranged between 16 °C and 28 °C during the day and 9 °C and 14 °C at night, with relative humidity (RH) ranging from 59 to 86 %. The average daily photosynthetic photon flux density (PPFD) was 420 mol/m^2/s, with an optimal light level of 1020 mol/m^2/s recorded under natural light conditions.

2.2. Plant material and preparation

Eighty dormant juvenile *A. belladonna* bulbs (10–14 cm circumference, 3–5 cm diameter) were sourced from a commercial flower bulb company (Hadeco® (Pty) Ltd., Maraisburg, South Africa). The bulb and contractile roots were cleaned of any unnecessary debris. After 5 min of immersion in a 0.1 % biocidal solution (Sporekill™, ICA International Chemicals (Pty) Ltd., Stellenbosch, South Africa) (active ingredient: didecyldimethylammonium chloride), the bulbs were removed and air-dried. Before planting and experimental treatments, the bulbs were stored in breathable crates in a darkened and ventilated room maintained at an ambient temperature of 18 ± 2 °C and RH of 40–60 % for a week.

2.3. Experimental design and hydroponic setup

The experiment was carried out in a split-plot design with four specified heated water treatments, a control of 16 °C, 22 °C, 28 °C and 34 °C as the main plots, combined with one of two soilless growing media, LECA® (4–10 mm lightweight expanded clay aggregate) and pre-rinsed silica sand (Consol®, grade 6/17) as the sub-plots (Table 1 and Fig. 1). Four identical and separately closed deep water culture (DWC) hydroponic systems were constructed. Each system comprised a 68 L black low-density polyethene (LDPE) plastic rough tote reservoir (620 × 410 × 425 cm, 2.33 kg) (Addis 98310BK, Builders Warehouse, Boksburg, South Africa) placed at ground level. A thermostatically adjustable submersible aquarium glass heater (Eheim, Plochinger Str. 54, 733,779 Deizisau, Germany) was installed to maintain each system's specified heated water temperatures. In addition, an electromagnetic air compressor (BOYU ACQ-003, Jungle Aquatics, Gauteng, South Africa) linked to two air stones (50 mm) was fitted in each system to increase the

Table 1
Outline of the split-plot design consisting of eight treatment combinations of heated root zone water temperatures (main plot) and soilless media (sub-plot) in four separate closed deep water culture (DWC) hydroponic systems.

	Leca clay (LC)		Silica sand (SS)	
DWC system 16 °C (control)	SGT 1	16 °C + LC	SGT 2	16 °C + SS
DWC system 22 °C	SGT 3	22 °C + LC	SGT 4	22 °C + SS
DWC system 28 °C	SGT 5	28 °C + LC	SGT 6	28 °C + SS
DWC system 34 °C	SGT 7	34 °C + LC	SGT 8	34 °C + SS

DWC = deep water culture system; SGT = suspended growing tray.

Fig. 1. Visual representation of the split-plot hydroponic experimental setup in the greenhouse of juvenile *Amaryllis belladonna* bulbs grown in four identical and separately closed deep water culture hydroponic systems with different root zone water temperatures and soilless media (Photo: C. Wilmot).

aeration of the static nutrient solutions, bubbling air at a rate of 50 L/min. Unlike shallower hydroponic systems, where temperature variations are difficult to mediate, large-volume closed deep water culture (DWC) systems permit error-buffering of solution temperatures in addition to the efficient use of water and fertilizers, reduced waste management contamination and increased recovery (Inden and Torres 2004; Viljoen et al. 2021; Velazquez-Gonzalez et al. 2022). Bulbs were randomly allocated and planted into individual growing cavities (250 mL) of a plastic cavity tray with one of two different soilless media, leca clay or sand, ensuring the bulb neck was visible at the surface (Duncan 2010). Leca clay has several advantages, including its lightweight and porous character, capacity to maintain a neutral pH and thermal insulation of the roots (Boudaghpour and Nasir 2008). Sand, despite its high density, poor water retention, and sensitivity to salt build-up, provides the most sustainable and inexpensive plant-supporting medium with good porosity and a low carbon footprint (Olle et al., 2012; Fussy and Papenbrock, 2022). The plastic cavity trays were suspended in each tote reservoir and held by 10 mm wooden dowels to ensure the root zone depth (lower portion of the growing tray) remained immersed in the heated nutrient solutions and absent from the bulb basal plates (Fig. 1). Ten bulb replicates (n = 10) for each treatment combination were planted at a density of 20 bulbs per 0.2542 m^2 in the plastic cavity trays suspended in the reservoirs.

2.4. Nutrient solution preparation and monitoring

A hydroponically formulated granular fertilizer, Nutrifeed™, was prepared by dissolving 10 g/10 L of tap water in a 1:1000 (*w/v*) ratio for each treatment reservoir. The hydro-soluble fertilizer's chemical composition included macro- and micronutrients of N (65 g/kg), P (27 g/kg), K (130 g/kg), Ca (70 g/kg), Cu (20 mg/kg), Fe (1500 mg/kg), Mo (10 mg/kg), Mg (22 g/kg), Mn (240 mg/kg), S (75 mg/kg), B (240 mg/kg), Zn (50 mg/kg) (Starke Ayres Pty, Ltd., Gauteng, South Africa). The pH and electrical conductivity (EC) were maintained during the treatment period using a calibrated hand-held digital pH meter (HM Digital PS pH-200) and EC and temperature meter (PS COM-100) (HM Digital Inc., Culver City, CA, USA 90,230). The nutritional solution was kept at a pH between 5.5 and 6.5 and an EC between 0.5 and 1.2 mS/cm. The pH was adjusted using either sodium hydroxide (NaOH) to raise the pH or hydrochloric acid (HCl) to reduce the pH. Additional tap water or nutrient solution was added when the EC fell below or above the required range. To further exclude changes in the nutrient solution due to plant absorption and evapotranspiration, volumes were monitored and maintained at a constant 60 L level in the reservoirs (Velazquez-Gonzalez et al., 2022). A waterproof data logger (HOBO MX 2201, Onset, Bourne, Massachusetts, USA) was used to record the water temperatures in all reservoirs at 60-minute intervals. To maintain an aseptic environment, Sporekill™, a biocidal solution containing the active ingredient Didecyldimethylammonium Chloride (ICA International Chemicals (Pty) Ltd., Stellenbosch, South Africa), was added (100 mL/100 L) to each tote reservoir at the commencement of treatments and at each interval when the solutions were renewed. In minimizing nutrient concentration build-up and phytotoxicity and to guarantee optimal solution productivity, planting trays were flushed, reservoirs were cleaned and sanitized, and aqueous nutrient solutions were replenished monthly (Fussy and Papenbrock, 2022; Velazquez-Gonzalez et al., 2022).

2.5. Data collection

2.5.1. Determination of the morphological parameters

Phenotypic data were recorded before, during and after post-harvest as indicators of bulb growth, development and regeneration using an electronic laboratory scale (Radwag® PS 4500.R2, Radwag Waagen, Hilden, Germany) with a 0.001 g readability, a standard soft cloth metric tape measure (Empisal EMT-001, Builders Warehouse, Boksburg, South Africa) (1500× 12 mm) and a standard metric retractable metal tape measure (Stanley Power Lock®, Builders Warehouse, Boksburg, South Africa) (450 × 25 mm).

Before planting, pre-treatment measurements of the individual mother bulb circumference and fresh weight were recorded to ensure consistency and reliability using the abovementioned measuring apparatus. Various observations and transitional measurements of the number of leaves, leaf length, width, and area were measured 24 weeks after initial leaf growth (2020-planting season and 2021-consecutive planted growth season) to determine the seasonal resumptive vegetative leaf development. Leaf length was measured as the length of the longest leaf from the base of the leaf, where it emerged from the mother bulb to the leaf apex. Leaf width was measured at the broadest mid-section of the same leaf. Leaf area was calculated using the Montgomery equation (ME) described by Yu et al. (2020) and Shi et al. (2021).

$$A_{leaf} = \alpha L_{leaf} \times W_{leaf}$$

where A = area; L = length; W = width and α = Montgomery parameter

The treatment period was concluded, and the bulbs were harvested after leaf senescence to allow for natural nutrient reabsorption after the second growth season before bulb dormancy (Duncan, 2010; Barnhoorn, 2013). Before overnight air drying, the roots were gently rinsed with water to eliminate any soilless medium. The post-harvest mother bulb, offsets and roots were separated. The mother bulb circumference, fresh weight, number of offsets, offset fresh weight, and total root fresh weight were measured and recorded using the above measuring apparatus. In addition, calculations were made of the coefficients of mother bulb circumference (the ratio of the post-harvest bulb circumference to pre-plant circumference), mother bulb weight (the ratio of the post-harvest bulb weight to pre-plant weight) and the offset weight (the proportion of the post-harvest offset weight and the pre-plant mother bulb weight). The coefficients were calculated to determine the overall synthesis of bulb growth and development, a practical and supporting selection tool for achieving quantifiable performance standards, variability, and production objectives.

2.6. Statistical analysis

Phenotypic data were analyzed using the Minitab 17 statistical analysis software (Minitab LLC, Pennsylvania State University, USA). The results were statistically evaluated using a two-way analysis of variance (ANOVA) for the variables, root zone water temperatures (4 levels) and soilless media (2 levels). Data are presented as mean values with standard errors (S.E.s). Tukey's LSD test further separated mean values at a $p \leq 0.05$ significance level.

3. Results

3.1. Pre-plant and post-harvest mother bulb circumference

The pre-plant mother bulb circumferential response in the elevated water temperatures was significant, according to the analytical findings of factors; however, the post-harvest response was significantly impacted ($p \leq 0.05$) by the interaction of water temperature and soilless media as well as their independent comparative assessments (Table 2). The 16 °C–LC treatment had the largest circumference post-harvest, although it was statistically comparable to all other treatment combinations. Greater circumferential dimensions were found in all temperature-treated bulbs other than those grown at 34 °C, where the final circumference was statistically suppressed (Table 2). Relative to the comparative reduction of 1.2 cm between the post- harvest and pre-plant circumferential dimensions in the warmest water treatment at 34 °C, a considerably marked improvement of between 6.4–7.1 cm was observed in the three lower heated temperature treatments. Compared to silica sand (16.1 cm), the post-harvest bulb circumference cultivated in Leca clay (17.5 cm) was significantly higher (Table 2).

3.2. Pre-plant and post-harvest mother bulb fresh weight

In evaluating the factorial analysis of variance of soilless media and interaction with heated water temperatures, the responses of the pre-plant and post-harvest fresh weights of mother bulbs were not statistically different ($p > 0.05$). However, the water temperature responses indicated otherwise (Table 2). Statistically, similar post-harvest fresh weights for the lower three temperature treatments were found, with the bulbs grown in the 16 °C treatment exhibiting the highest weight value. In comparison to the pre-plant fresh weights, all temperature-treated mother bulbs increased the post-harvest fresh weight by 62.7–78.6 g, except for those cultivated at 34 °C, where the final comparative weight was suppressed, and an incremental increase of 0.9 g was measured (Table 2).

3.3. Post-harvest root fresh weight

The total fresh root mass from bulbs grown at different water temperatures differed significantly ($p \leq 0.05$) compared to soilless media and the variable interaction, which were found to be unaffected (Table 2). The 22 °C heated water treatment produced the maximum root fresh weight (20.1 g) with the least variability compared to the weights (17.2 g and 14.0 g) in the 16°C and 28 °C water temperatures, respectively. In bulbs suspended in the 34 °C treatment, the accumulated root fresh weight (4.1 g) was severely inhibited and proved fatal (Table 2).

3.4. Number of bulb offsets

As shown in Table 3, water temperature comparisons exhibited significant differences in the number of bulb offsets initiated at the 95 % confidence level; however, soilless media and the variable interaction indicated otherwise. As depicted in Fig. 2, the bulbs suspended in the 22 °C heated water stimulated the production of the most offsets from the mother bulb (2.3) yet, were not significantly different from those produced (2.1 and 1.3) in the 16 °C and 28 °C treatments, respectively. At least one bulb offset was initiated from the mother bulb in each heated water treatment, apart from those in the warmest treatment of 34 °C, which did not evince such an ability (Fig. 4).

3.5. Post-harvest bulb offset fresh weight

The post-harvest fresh weight of bulb offsets grown at various water temperatures differed significantly ($p \leq 0.05$); however, no significant variations were identified in soilless media and their interaction (Fig. 2). The 22 °C water treatment showed the highest accumulated fresh weight (49.8 g) yet did not differ from the 16 °C or 28 °C treatments (38.3 and 26.4 g) (Fig. 2). The offset weight measured in the 34 °C heated water was negligible (Fig. 2).

3.6. Mother bulb circumference, weight, and offset weight coefficients

The coefficients were used to assess the bulbs' total vegetative productive proliferation and marketability (Fig. 3). The circumferential coefficient of mother bulbs was considerably improved ($p < 0.05$) by water temperature and the factorial interaction with soilless media. However, the coefficient found in soilless media was unaffected (Fig. 3). The mother bulbs grown in water temperatures of 16 °C, 22 °C, and 28 °C showed a similar circumference coefficient variability (1.6–1.7). In comparison, those grown under the same conditions at 34 °C showed a significant ($p < 0.05$) decline (0.9). Compared with the interaction with soilless media, the three lower-heated water treatments exhibited the most significant difference in the circumference coefficient. Based on the results in Fig 3, the mother bulb weight coefficient was significantly enhanced ($p < 0.05$) by the autonomous analysis of water temperature; however, the response was unaffected ($p > 0.05$) by the soilless media and the interaction of variables. The mother bulb weight coefficient largely reflected the improved statistical differences in the post-harvest fresh weight. The 28 °C water temperature produced the largest and nearly four times greater bulb weight coefficient (3.9). However, it was not statistically better than the coefficients (3.5 and 3.6) in the 16 °C and 22 °C treatments (Fig. 3). Bulbs in the 34 °C treatment maintained their weight coefficient (1.1) over the study period. The independent analysis of water temperature significantly increased the offset weight coefficient ($p < 0.05$); however, it was not influenced by soilless media or the interaction of variables (Fig 3). The 22 °C heated water yielded the highest offset weight coefficient (2.0), nearly double that of all other treatments (Fig. 3).

3.7. Number of leaves

The number of leaves in the leafing phases was counted in the 2020 (planting) and 2021 (consecutive planted) seasons. The number of leaves of the mother bulbs treated to varied water temperatures was significant ($p \leq 0.05$). In contrast, soilless media and the variable interaction did not affect the number of emerging bulb leaves each season ($p > 0.05$). In 2020 and 2021, the highest mean number of leaves (10.1 and 9.1) appeared in bulbs grown in the 16 °C water temperature; however, minimal statistical variability was observed compared to the 22 °C and 28 °C treatments. The lowest leaf counts (7.9 and 5.1) were initiated in the warmest water treatment at 34 °C. Noticeable comparative differences in the 2021 cultivation season were found as the quantification of bulb foliage presented differently. They were negatively influenced by all heated water treatments, with an average reduction of 1–2.8 fewer leaves than in the previous season (Table 3).

3.8. Leaf length, leaf width, and leaf area

The resumptive mother bulb foliage parameters of leaf length, width and area were evaluated following 24 weeks in the 2020 planting and 2021 sequential vegetative growth seasons. The independent evaluation

Table 2
Effects of root zone water temperatures and soilless media on the characteristics of pre-plant, post-harvest mother bulb circumference and fresh weight and post-harvest root fresh weight of *Amaryllis belladonna* bulbs grown in a deep water culture hydroponic system

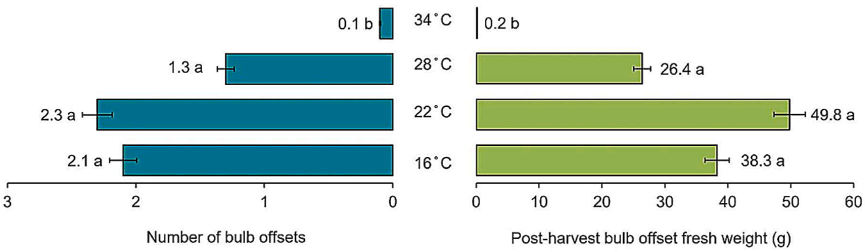
Treatment	Mother bulb and root characteristics				
	Mother bulb				Roots
	Pre-plant circumference (cm)	Post-harvest circumference (cm)	Pre-plant fresh weight (g)	Post-harvest fresh weight (g)	Post-harvest fresh weight (g)
Water Temperature					
16 °C (Control)	12.4 ± 0.24 a	19.2 ± 0.59 a	33.4 ± 1.84 ab	112.0 ± 9.16 a	17.2 ± 0.63 a
22 °C	10.9 ± 0.30 b	18.0 ± 0.66 a	25.4 ± 1.30 b	88.1 ± 8.32 a	20.1 ± 2.91 a
28 °C	11.8 ± 0.32 ab	18.2 ± 0.50 a	29.5 ± 2.66 ab	105.7 ± 8.01 a	14.0 ± 1.48 a
34 °C	12.9 ± 0.39 a	11.7 ± 0.66 b	34.4 ± 2.69 a	35.3 ± 3.79 b	4.1 ± 0.53 b
Soilless Media					
Leca clay (LC)	12.3 ± 0.26 a	17.5 ± 0.50 a	32.2 ± 1.64 a	88.0 ± 6.77 a	14.0 ± 1.63 a
Silica sand (SS)	11.7 ± 0.23 a	16.1 ± 0.74 b	29.1 ± 1.60 a	82.6 ± 7.54 a	13.7 ± 1.40 a
Water Temperature × Soilless Media					
16 °C × LC	12.6 ± 0.35 ab	19.4 ± 0.76 a	34.5 ± 2.61 a	108.1 ± 15.10 a	17.4 ± 1.12 a
× SS	12.1 ± 0.32 ab	19.0 ± 0.93 a	32.2 ± 2.69 a	116.0 ± 11.10 a	17.1 ± 0.66 a
22 °C × LC	10.8 ± 0.43 b	18.8 ± 0.91 a	27.4 ± 2.26 a	89.5 ± 12.30 ab	19.7 ± 5.15 a
× SS	11.1 ± 0.44 b	17.3 ± 0.94 a	23.3 ± 1.01 a	86.8 ± 11.80 ab	20.5 ± 3.01 a
28 °C × LC	12.3 ± 0.50 ab	18.0 ± 0.68 a	33.0 ± 4.87 a	108.9 ± 9.77 a	13.8 ± 2.54 ab
× SS	11.2 ± 0.33 b	18.4 ± 0.78 a	26.0 ± 1.84 a	102.5 ± 13.10 a	14.2 ± 1.69 ab
34 °C × LC	13.3 ± 0.45 a	13.7 ± 0.46 b	33.7 ± 2.72 a	45.4 ± 4.94 bc	5.6 ± 0.59 bc
× SS	12.4 ± 0.63 ab	9.7 ± 0.83 c	35.1 ± 4.80 a	25.1 ± 3.68 c	2.6 ± 0.60 c
Two-way ANOVA–F statistic					
Water temperature	7.13 *	36.8 *	3.47 *	20.48 *	16.38 *
Soilless media	3.18 ns	5.79 *	1.89 ns	0.49 ns	0.05 ns
Water temperature × Soilless media	1.05 ns	2.99 *	0.63 ns	0.57 ns	0.26 ns

Mean values ± standard error (S.E.) in the same column with a different letter(s) are significantly different at $p \leq 0.05$ (*) based on Tukey's.
Least significant difference test; ns = not significant.

Table 3
Effects of root zone water temperatures and soilless media on leaf characteristics of the number of leaves, leaf length, width and area of *Amaryllis belladonna* bulbs that were grown in a deep water culture hydroponic system following 24 weeks of the 2020 and 2021 leafing seasons

Treatment		Leaf characteristics							
		2020 season				2021 season			
		Number of leaves (n)	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)	Number of leaves (n)	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)
Water temperature									
16 °C (Control)		10.1 ± 0.29 a	42.8 ± 1.14 a	1.9 ± 0.06 a	80.8 ± 3.14 a	9.1 ± 0.34 a	55.6 ± 1.55 a	2.6 ± 0.07 a	143.5 ± 5.74 a
22 °C		9.6 ± 0.62 a	43.1 ± 1.49 a	1.8 ± 0.07 ab	77.2 ± 4.69 a	8.9 ± 0.50 a	56.6 ± 1.68 a	2.5 ± 0.12 a	140.8 ± 9.21 a
28 °C		10.1 ± 0.45 a	36.2 ± 2.72 a	1.9 ± 0.06 a	68.7 ± 5.22 a	7.8 ± 0.62 a	49.9 ± 1.67 b	2.2 ± 0.15 a	111.9 ± 9.42 b
34 °C		7.9 ± 0.30 b	19.5 ± 1.62 b	1.6 ± 0.07 b	32.3 ± 3.37 b	5.1 ± 0.32 b	44.0 ± 1.03 c	1.8 ± 0.07 b	79.9 ± 3.79 c
Soilless media									
Leca clay (LC)		9.2 ± 0.35 a	34.5 ± 1.90 a	1.8 ± 0.05 a	65.5 ± 4.35 a	7.7 ± 0.35 a	50.8 ± 1.39 a	2.3 ± 0.08 a	120.4 ± 6.51 a
Silica sand (SS)		9.6 ± 0.32 a	36.3 ± 2.09 a	1.8 ± 0.05 a	64.0 ± 4.16 a	7.7 ± 0.50 a	51.2 ± 1.25 a	2.2 ± 1.0 a	117.7 ± 6.74 a
Water temperature × Soilless media									
16 °C	× LC	10.1 ± 0.31 ab	41.6 ± 1.24 a	2.0 ± 0.06 a	80.9 ± 2.09 a	8.8 ± 0.51 a	55.2 ±2.34 ab	2.6 ± 0.11 a	143.0 ± 10.20 a
	× SS	10.1 ± 0.50 ab	44.0 ± 1.89 a	1.8 ± 0.10 a	80.8 ± 6.10 a	9.4 ± 0.45 a	56.0 ± 2.15 ab	2.6 ± 0.08 a	143.9 ± 5.82 a
22 °C	× LC	8.4 ± 0.96 ab	41.5 ± 2.50 a	1.8 ± 0.11 a	76.2 ± 8.13 a	8.4 ±0.78 a	56.1 ± 2.76 ab	2.4 ± 0.17 a	138.7 ± 15.0 a
	× SS	10.7 ± 0.65 a	44.6 ± 1.62 a	1.8 ± 0.08 a	78.2 ± 5.16 a	9.4 ± 0.64 a	57.1 ± 2.06 a	2.5 ± 0.17 a	142.9 ± 11.50 a
28 °C	× LC	10.4 ± 0.60 ab	34.4 ± 3.81 a	1.9 ± 0.12 a	64.6 ± 7.20 a	8.3 ± 0.54 a	47.5 ± 2.69 bc	2.3 ± 0.18 a	112.1 ± 12.70 ab
	× SS	9.8 ± 0.68 ab	38.0 ± 4.01 a	1.9 ± 0.06 a	72.8 ± 7.72 a	7.2 ± 1.11 ab	51.9 ± 1.84 abc	2.1 ± 0.24 a	111.7 ± 14.60 ab
34 °C	× LC	7.8 ± 0.51 b	20.5 ± 2.75 b	1.6 ± 0.08 a	34.2 ± 5.50 b	5.4 ± 0.40 b	44.2 ± 1.35 c	2.0 ± 0.09 a	87.60 ± 4.62 b
	× SS	7.9 ± 0.35 b	18.4 ± 1.81 b	1.6 ± 0.12 a	30.3 ± 4.10 b	4.8 ± 0.51 b	43.7 ± 1.64 c	1.6 ± 0.07 a	72.2 ± 5.11 b
Two-way ANOVA–F statistic									
Water temperature		6.24 *	35.36 *	4.65 *	27.01 *	15.81 *	14.85 *	10.25 *	14.44 *
Soilless media		1.09 ns	0.90 ns	0.42 ns	0.13 ns	0.0 ns	0.88 ns	1.27 ns	0.13 ns
Water temperature × Soilless media		2.25 ns	0.49 ns	0.37 ns	0.35 ns	1.14 ns	0.49 ns	0.83 ns	0.33 ns

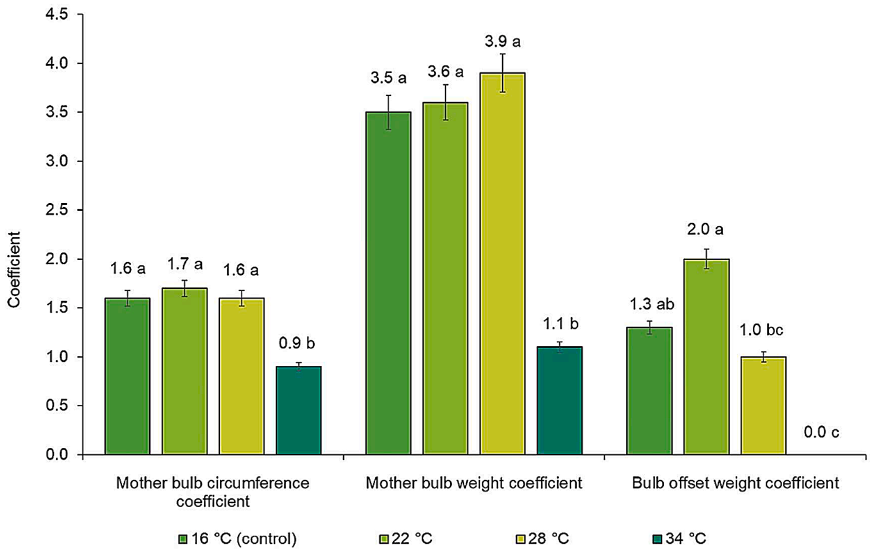
Mean values ± standard error (S.E.) in the same column with a different letter(s) are significantly different at $p \leq 0.05$ (*) based on Tukey's least significant difference test; ns = not significant.



Two-way ANOVA–*F*-statistic

Treatments	Number of bulb offset (n)	Post-harvest bulb offset fresh weight (g)
Water temperature	12.37 *	10.41 *
Soilless media	0.79 ns	0.18 ns
Water temperature × Soilless media	0.52 ns	0.05 ns

Fig. 2. Effect of root zone water temperatures on the number of bulb offsets and the post-harvest bulb offset fresh weight of *Amaryllis belladonna* grown in a DWC hydroponic system. Bars represented by mean values followed by a different letter are significantly different at $p \leq 0.05$ according to Tukey's least significant difference (L.S.D.), (*) indicates significance at $p \leq 0.05$; ns = not significant.



Two-way ANOVA–*F*-statistic

Treatments	Mother bulb circumference coefficient	Mother bulb weight coefficient	Offset weight coefficient
Water temperature	44.3 *	18.13 *	9.16*
Soilless media	1.13 ns	0.55 ns	0.32 ns
Water temperature × Soilless media	3.89 *	0.92 ns	0.05 ns

Fig. 3. Coefficient outcomes of *A. belladonna* mother bulb circumference, mother bulb weight, and bulb offset weight in response to root zone water temperatures in a DWC hydroponic system. The bars represented by the mean values followed by a different letter(s) are significantly different at $p \leq 0.05$ according to Tukey's least significant difference (L.S.D.), (*) indicates significance at $p \leq 0.05$; ns = not significant.<AuQuery: Please check,Part designations in figure legends should be in normal typeface (not bold face).>

of water temperature significantly affected the leaf length ($p < 0.05$). In contrast, the soilless media and the variable interaction had no significance over the two years of cultivation (Table 3). In the 2020 and 2021 seasons, statistically similar trends in the extension of bulb leaf blades subjected to temperatures of 16 °C and 22 °C were observed. A notable decline was found as temperatures rose, with the 34 °C water-treated bulbs displaying the shortest leaves. The resumptive increase in leaf length in the second year compared to the first ranged between 13.5 and 24.5 cm across all warm water treatments. The expansive leaf width in bulbs showcased similar significant tendencies ($p \leq 0.05$) observed from the autonomous and interactive analysis of the leaf blade extension cultivated in the two seasons (Table 3). Compared to the 2020 season, visibly broader leaves in the 2021 treatments were observed, with an increased width between 0.2 and 0.7 cm. The findings of the overall leaf area found that the 16 °C treatment had the highest surface area, but it was not statistically different from the 22 °C and 28 °C treatments in both seasons (Table 3).

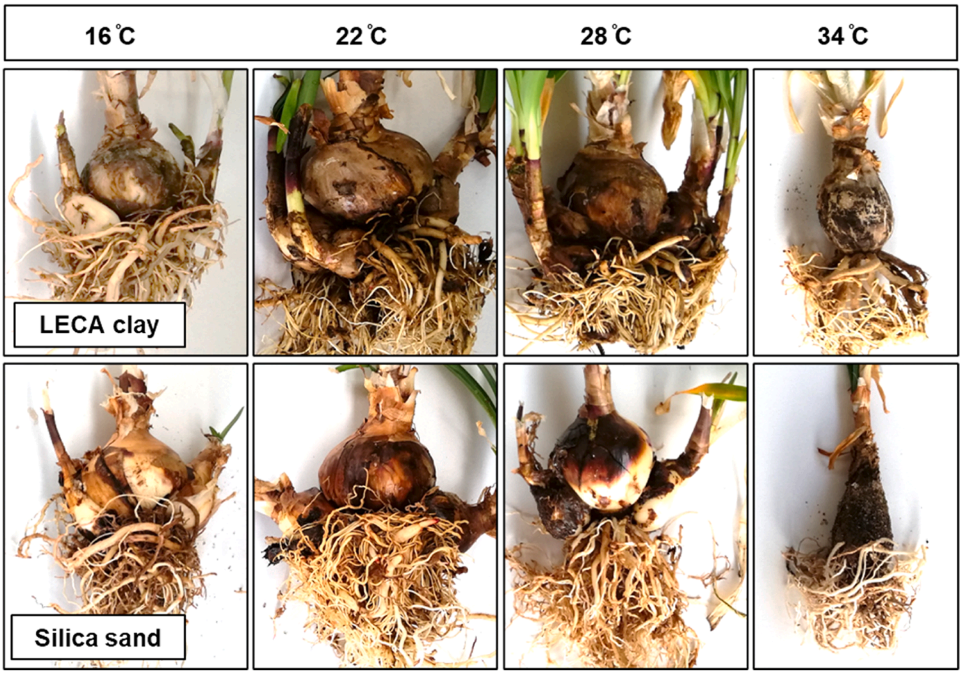


Fig. 4. Observable effects of root zone temperatures and soilless media on the mother bulb size, offset production, and root architecture of hydroponically grown *Amaryllis belladonna* bulbs (Photographic diagram: C. Wilmot).

4. Discussion

The all-encompassing findings established that the independent treatment of heated water expressively altered the morphological growth and development responses of *A. belladonna* bulbs. However, soilless media and the combined interaction were insignificant. Warm root zone water temperatures of 16–28 °C were beneficial and comparable; however, a temperature of 34 °C, which was too high, was damaging and depleting. These responses corroborate the enhanced and subdued physiological activity and metrics from the subsurface root zone temperature variations in hydroponically produced crops. *Valerianella locusta,* an annual herb plant grown at low (15 °C) and high (25 °C) root zone temperatures, produced plants that were smaller than those grown at 20 °C (Costa et al. 2011). Similarly, He et al. (2013) found temperatures of 20 °C over sub-optimal and high temperature-stress responses favoured aeroponic cultivated Lactuca sativa growth and development. *Streptocarpus formosus* responded more positively to lower root zone water temperatures of 18 and 22 °C than to temperatures of 26 and 34 °C (Viljoen et al., 2021). Similarly, temperatures between 26 and 30 °C were more advantageous to *Ornithogalum longibracteatum* bulbs than 34 °C (Nxawe et al., 2011). At root zone temperatures ranging from 11 to 34 °C, six Cucurbitaceae species responded inversely for biomass, photosynthesis, and stomatal conductance (Zhang et al., 2008).

Increased performance yields may also be ascribed to the year-round continual availability and accessibility of water and nutrients, which are absorbed by the actively searching contractile bulb roots through perpetual immersion in an oxygen-rich hydroponic nutrient solution (Trejo-Téllez and Gómez-Merino, 2012; Barnhoorn, 2013). Similar findings of greater vegetative development and water-holding capacity were found in hydroponically grown Oriental lily hybrids (Ryota et al., 2002) and *Eucomis autumnalis* (Ndhlala et al., 2012). Asker (2015) found that growing larger Asiatic hybrid lily bulblets and daughter bulbs in an NFT hydroponic system was advantageous. Long-term solution temperatures influence the amount and accessibility of dissolved oxygen, with high temperatures having been demonstrated to diminish oxygen solubility, impede plant performance and root growth, and increase the risk of pests and disease (Gruda, 2005; Falah et al., 2010; Thakulla et al., 2021). In this study, prolonged root exposure of *A. belladonna* bulbs to 34 °C resulted in hypoxic stress response observations of poor root formation, rotting, and blackening, bulb growth suppression, weight loss, increased respiration, and root ethylene production due to reduced root-oxygen solubility and nutrient absorption (Shaw et al., 2013; Sakamoto and Suzuki, 2015; Balliu et al., 2021). Apart from the boundaries of restricted root volumes in the hollow plastic cavity trays (Shi et al., 2007; Balliu et al., 2021), root-oxygen assimilation was optimal at lower temperatures ranging from 16 to 28 °C. The dense root architecture, increased biomass, density, and surface area of mother bulbs and offsets for adequate nutrient absorption evidenced this. These important properties determine the definitive operation of the species' contractile root system (Duncan, 2010). Similarly, to the root performance yields, lower temperatures demonstrated improved aerial growth, development, and parameter metric observations. In contrast, the 34 °C treatment showed poor yields in addition to delayed leaf development at the start of the 2020 and 2021 leafing phases, lower levels of leaf turgor, and early leaf fading. These observations may account for the lack of oxygen in the root zones causing epinastic leaf curvature, stomatal closure, a slowing of leaf growth, turgor and yellowing, all of which are shoot adaptations to compensate for the root system's debilitation and poor shoot-root communication (Jackson, 2002; Shaw et al., 2013). Although leaf number may indicate a transition from juvenile to reproductive maturity (Langens-Gerrits et al., 2003), all treatments had fewer leaves in the second cultivation season than before. According to Hartsema and Leupen (1942), this may relate to the planting of the bulbs in the previous season, affecting the leaf formation and resulting in decline. However, the lower temperature treatment differentials were notably counterbalanced by the greater leaf length and width parameter values in the second season. The enlarged surface area is suggested to have maximized the photosynthetic activity to amass sufficient photosynthates while reducing the barriers to obtaining nutrients and water within the warm water hydroponic system (Nxawe et al., 2009; Anderson, 2019).

Soilless media is currently one of the fastest-growing areas, offering unlimited potential within the horticultural sector (Balliu et al., 2021; Karagöz et al., 2022;). *Fritillaria imperialis* growing in a soilless culture (sand medium) (Kahraman and Özzambak, 2006) and *Gladiolus* *grandiflorus* in a hydroponic bag culture (Wahome et al., 2010) procured the highest vegetative growth, bulblet and flower formation and quality attributes. Soilless media had no significant impact on the study's morphological bulb traits; however, the findings suggest that different soilless substrates could be evaluated, or the media substituted entirely with reusable LDPE plastic aqua prick trays (Schroeder et al., 2020). This suggestion is under careful evaluation in support of input resource factors, cultivation and LCA (Darras, 2020; Fussy and Papenbrock, 2022) associated with the *A. belladonna* species.

In elucidating the study's coefficients, the juvenile *A. belladonna* mother bulb circumference and weight coefficients were comparable and significant at 16 °C, 22 °C, and 28 °C. However, the weight coefficient indicated a more significant dynamism than the circumference. The largest induced offset weight coefficient was found at 22 °C due to more significant offset generation. Thus, at lower heated root zone water temperatures, particularly at 22 °C, juvenile mother bulbs simultaneously generated larger, heavier bulbs and maximum offset capacity without early separation. According to Khodorova and Boitel-Conti (2013), Kapczyńska (2014) and Manimaran et al. (2017), bulb size impacts output quality, yield, and flowering productivity during natural production since bulbs function as a source and sink of internal carbohydrate reserves to reach competency. It is suggested in this study, and supported by Miller (1992), Thompson et al. (2011), Wu et al. (2012) and Andrade-Rodríguez et al. (2015), that the variables related to bulb root zone temperatures improved the efficiency of carbohydrate absorption and mobilization, leading to higher yields. Intriguingly, the 22 °C water treatment marginally lowered the mother bulb weight; however, this was compensated for by a greater offset weight than the 16 °C and 28 °C treatments. These findings infer that the vegetative processes under the role of phytohormones and inductively mediated extrinsic hydroponic conditions were competing and synchronized, governing the intrinsic provision of accumulated biomass and 'bulbing' (Thompson et al., 2011; Atif et al., 2020). Furthermore, offsets are suggested as possible sinks for regulating the allocation of excess assimilates in the developing *A. belladonna* bulb, owing to the insensitive juvenility, meristem inability, and unresponsiveness to inductive cues that promote flowering (Levy and Dean, 1998; Proietti et al., 2022). Similarly, under favourable external circumstances, Thompson et al. (2011) found improved seasonal corm and cormlet production responses in unsuccessfully flowered *Watsonia* species.

Offset induction was neither vast, vigorous, nor uniform; however, visual observations of up to 5 formed offsets suggested that some *A. belladonna* bulbs, which were not homogenous, were more proliferous, signifying a potentially robust genetic disposition (Chandel et al., 2023). According to Kharrazi et al. (2017), offset division in species of *Hippeastrum* is promoted in those bulbs that produce a minimum of 3 bulblets per year. However, in most cases, propagation is accomplished by other conventional techniques. Notching, sectioning, scooping, twin scaling, and, more recently, tissue culture depletes the mother bulb, which cannot be reused (Barnhoorn, 2013; Hartmann et al., 2017). Moreover, conventional vegetative methods are not always economically practical due to the slow replication rate, ineffective techniques, meticulous labour practices, stringent hygiene standards, and survival rates that rely on fresh bulb development and sprouting (Kharrazi et al., 2017; Kapczyńska, 2019). In contrast, this study demonstrated that hydroponic cultivation promoted the vegetative multiplication of *A. belladonna* through offset induction and development without premature separation while establishing the size and longevity of the reclaimable clonal juvenile mother bulb.

This study supports the fundamental role of subterranean temperature stimuli in geophytic cultivation. Moreover, it reveals that plant species may navigate environmental and seasonal variations as a climate-smart functional and survival strategy by innovative or passive tactical adaptions to strengthen asexual propagation viability at different periods (Atif et al., 2020). The enhanced hydroponic performance activity from juvenile *A. belladonna* bulbs indicates a favourable multimodal propagation and cultivation technique that promotes extended growth beyond dormancy, which is crucial for economic efficiency. It may be a precursor to supporting growers and retailers with intriguing and remedial opportunities in fulfilling long-term commercial horticultural demands and conservation efforts of small, incompetent bulbs. Similarly, this study established that with systematic and thorough research and the timely adoption of treatment routines, greater cultivation product outcomes can be calibrated in the *A. belladonna* species, as previously published in Wilmot and Laubscher (2019b), Gul et al. (2020) and Wilmot et al. (2023, 2024).

5. Conclusion

In summary, the independent treatment of heated root zone water temperatures other than soilless media and the collective interaction significantly increased the *Amaryllis belladonna* vegetative bulb production under deep water hydroponic cultivation. Warm water temperatures of 16 °C–28 °C resulted in comparable and optimistic quantitative and qualitative growth and development of the aerial and subsurface organs. However, a high temperature of 34 °C impacted negatively through increased injury and depletion. This study established that juvenile mother bulb growth and offset generation were promoted via competing and simultaneous assimilation processes in response to inducive exogenous controlled hydroponic stimuli. The authors recommend a 22 °C water temperature optimal for contemporaneously proliferating larger, heavier juvenile mother bulbs and offset capacity while minimizing energy resource inputs (intensity and duration) for *A. belladonna*'s long-term ecoculture, production and LCA. The authors advocate further research to unravel the practicality and viability of various geophytes, bulb sizes, and soilless substrates that may advance definitive protocols and agronomic efficiency for continuous duplication of vegetative bulb stock, ultimately leading to flower production goals.

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Institutional review board statement

This study was approved by the Office of the Chairperson, Research Ethics Committee, Faculty of Applied Sciences, Cape Peninsula University of Technology. Reference no: 205054196/06/2020.

CRediT authorship contribution statement

Carolyn M. Wilmot: Writing – original draft, Investigation, Formal analysis, Conceptualization. **Muhali O. Jimoh:** Writing – review & editing, Supervision, Methodology, Formal analysis. **Charles P. Laubscher:** Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest in this manuscript.

Data availability

Data associated with the current study are presented in the manuscript.

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