

# EFFECTS OF COLD STORAGE DURATION AND ROOT ZONE TEMPERATURE ON FLOWERING OF AMARYLLIS (*HIPPEASTRUM STRIATUM* ‘BLOSSOM PEACOCK’)

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## Abstract

Cold storage and subsequent growth temperature are essential for flower bud differentiation, vegetative growth and flowering for bulbous plants. Amaryllis (*Hippeastrum striatum* ‘Blossom Peacock’), a perennial herbaceous bulbous plant, must undergo vernalization before blossom. In the present study, *H. striatum* ‘Blossom Peacock’ were stored at 7°C for 30 and 45 days and then planted on the seedbeds with root zone temperatures of 22, 25, and 28°C. We found that no significant differences in flowering between 30 and 45 days of cold storage. Three constant root zone temperatures accelerated flowering 18 to 32 days but did not change the anthesis duration. The leaf size and the scale length at 28°C root zone temperature were decreased by 29% compared to the control. The concentrations of nitrogen and phosphorus in the roots at 22°C were 7% and 34% higher than those in the control. The root zone temperature of 25°C increased the length, volume, and weight of the roots most and accelerated the flowering more obviously. The results suggest that 30 days cold storage is enough for the cultivar of ‘Blossom Peacock’ and different root zone temperatures have different effects on growth and flowering, which can be selected according to the market demand.

**Key words:** Flower quality, Emergence time, Nutrient, Root order, Storage temperature.

## Introduction

Amaryllis (*Hippeastrum striatum* (Lam.) H.E. Moore) is a flowering perennial herbaceous bulbous plant that is popularly known as fresh cut flower. It has many cultivars with different flower types and colors and is widely used as pot plant or planted in gardens as well. Amaryllis is one of the top 20 popular flowers in the world (Azimi & Alavijeh, 2020). It is native to Brazil and has been introduced into many countries due to its high ornamental properties.

As the important ornamental flower and fresh cut flower, it is expected to bloom exactly in some special festivals, even at any time of the whole year. Controlling flowering time and flower scale length by appropriate methods can improve its commercial value.

Flowering and growth are regulated by a variety of factors, such as cultivation management, growth environment, hormones, nutritional status, etc. Jamil *et al.* (2015) reported that growth regulators such as IAA, Ethrel, GA<sub>3</sub> increased the number of bulblets and flowers, promoted early flowering, and harvested bigger flowers of *H. hybridum*. Among abiotic factors (light, moisture, temperature, etc.), temperature plays the most important role in controlling the growth and flowering of Amaryllis (Khorodova & Boitel-Conti, 2013; Trang *et al.*, 2018). Ephrath *et al.*, (2001) found that 27°C was optimal for leaf area development and 22°C was optimal for bulb development of *H. hybridum* cv. Red Lion. Amaryllis must complete vernalization to gain the ability to flower. After *H. hybridum* ‘Cam Tu’ was stored at 4°C with coir fiber wrapping for 6 weeks, pedicel and flower scape were increased and the emergence time of flower buds was shortened. However, the number of florets per scape and the longevity of a flower were not significantly affected (Trang *et al.*, 2018).

Vegetative growth is the basis of reproductive growth. Roots absorb enough water and nutrients to provide aboveground growth and development. So far, the growth

and function of Amaryllis roots have not been widely studied, especially the research on root order has not been reported. Zakizadeh *et al.*, (2013) found that suitable concentrations of NAA and 2-iP increased the number and length of *H. johnsonii* roots. However, the types and properties of roots were not described in detail. Classification by root order was often used in woody plants (Fitter, 1982; Pregitzer *et al.*, 2002; Guo *et al.*, 2004; King *et al.*, 2021). Amaryllis is a perennial herbaceous plant that shows obvious root hierarchy. Different root branching order performs different functions, absorption and/or transportation. The first two order roots mainly serve absorptive function while the roots higher than the fourth order are mainly in charge of transportation (Pregitzer *et al.*, 2002; McCormack *et al.*, 2015; Li *et al.*, 2022). Thus, it is necessary to explore the root order of Amaryllis to elucidate its functions on the growth and flowering of the aboveground part.

Naturally, *H. striatum* flowers in April and May depending on cultivars in the research area, Shanghai, China. We chose *H. striatum* ‘Blossom Peacock’ cultivar that was pure white with red-edged petals and red highlights throughout each petal in the present study. The bulbs of *H. striatum* ‘Blossom Peacock’ were subjected to low temperature induction at 7°C in the fridge for 30 and 45 days to acquire the ability to flower and then were transferred to temperature-controlled seedbeds (22°C, 25°C, and 28°C) to finish subsequent additional vegetative growth before flowering. The objective of the present study was to confirm if 30 days storage is feasible and to find the optimal subsequent growth temperature that affects growth and flowering.

## Material and Methods

**Experimental materials and design:** The experiment was conducted in the greenhouse of Shanghai Institute of Technology, Shanghai, China in 2017. The average annual temperature is 16.0°C and average annual precipitation is

1200 mm in Shanghai with the highest air temperature occurring in July and August and the lowest temperature occurring in January.

It has been proved that the shortest exposure time for *H. striatum* to blossom between 5 and 10°C was 30 days (Cao *et al.*, 2012; Su *et al.*, 2014). However, the quality and quantity of flowers of some cultivars were markedly affected. The response of flowering and growth to cold treatment duration might be cultivar specific. In the present study, 30 days and 45 days were selected for the cultivar of 'Blossom Peacock'.

Eighty-five-year-old plants with flowering-sized bulbs (6.5-7.5 cm diameter) of *H. striatum* 'Blossom Peacock' were collected and planted on November 15, 2017. Two weeks prior to storage, all plants were stopped from watering and then the whole pot plants with leaves were sealed with black plastic bags and stored in the refrigerator at 7°C for 30 days and 45 days. After cold storage treatment, all roots and leaves 8 cm above the bulbs were removed. If the leaves were completely cut off, the remaining leaves in the bulbs would cause the bulbs to rot. Our previous results showed that keeping about 8 cm long leaves above the bulbs could successfully prevent the decay of bulbs. The bulbs were respectively planted in the temperature-controlled seedbeds where the soil temperature was controlled at 22°C, 25°C, 28°C, and room temperature on December 15 and December 30. The room temperature was controlled by the air conditioner at a constant temperature of 18°C. There were 3 raised beds where the cables were buried at 5 cm depth to control the target temperatures. Soil temperature was continuously and automatically monitored using a heat controller (Huoyu, China). The media was Wosong cultivation substrate containing peat, coconut husk, and lime (organic matter 90%, total nitrogen 1.0%, P<sub>2</sub>O<sub>5</sub> 0.2%, K<sub>2</sub>O 0.2%, pH 5.5-6.5, EC<2.0 dS/m). Leaves began to grow in January and flower buds began to appear in February 2018. Since the emergence time of the first leaf and the first flower opening were different under different treatment conditions, the emergence time of leaf and flowering was calculated according to the time of low temperature storage (Nov. 15th).

**Morphology and nutrient assessment:** Only the morphological characteristics and nutrients of roots subjected to low-temperature storage for 30 days were determined since no differences in flowering between 30 days and 45 days. Root sampling was conducted in April 2018. All roots were carefully removed from the basal stem with a sharp knife one by one, placed in deionized water, and gently washed soil from the roots. After cleaning, each root network was kept moist and dissected into branch orders. Distal roots were classified as the first order, and then second order, and so on (Guo *et al.*, 2004). The root diameter was measured at the top of a section which connected higher-level root order using a digital caliper. The root length was determined using a ruler. Different order roots were mixed for the measurement of volume, biomass, and nutrient and then dried at 60°C for at least 24 hours to a constant mass. The leaf width and thickness were measured in the middle of the leaves.

## Statistical analysis

Statistical analysis was performed using IBM SPSS 24. Two-way ANOVA method was used to analyze the effects of storage duration and subsequent growth temperature on flower properties. The difference between any two parameters was compared by LSD multiple comparisons at the level of 0.05.

## Results

**The statistical results of storage duration and root zone temperature:** There was no significant difference in leaf emergence and flowering between 30 days and 45 days of cold storage at 7°C. However, the subsequent root zone temperature (22°C, 25°C, 28°C) significantly affected the emergence of the first leaf and flower, the number and length of scale. The interaction of low-temperature storage period and growth temperature was only observed on the first leaf emergence (Table 1).

**The effects of root zone temperature on flowering:** The flowering time of plants that were treated by 22°C, 25°C, and 28°C was accelerated ranging from 18 to 32 days compared to the control plants ( $p<0.05$ ). The first flower opened the earliest at 25°C and 28°C, 12 days earlier than that at 22°C for plants experiencing 30 days of cold storage ( $p<0.05$ ) and 8 days earlier for 45 days of cold storage ( $p>0.05$ ) (Table 2). The anthesis duration was not affected by growth temperature, ranging from 21 to 28 days (Table 2). The number of scales was one or two for all treatments. Each scale had two to five florets, averaging 3.6 (Table 2). Most of the scales had 3 or 4 flowers. The length of scale at 28°C was significantly lower than the others, average 29% lower than the control ( $p<0.05$ ) (Table 2). The first leaf of plants grown at 25°C appeared the earliest while appeared the latest at 22°C, accelerating leaf emergence by about 2 days compared to the control (Table 2).

**The effects of root zone temperature on leaf size:** The root zone temperature of 28°C significantly decreased leaf length by 14%, leaf width by 7%, and leaf thickness by 11% compared to the control, while no difference in leaf size was found among 22°C, 25°C, and the control (Table 3).

**The effects of root zone temperature on root orders:** No significant difference in root number was observed except that the second order roots at 22°C were decreased by 43% compared to the control (Fig. 1A). Root zone temperature of 22°C, 25°C, and 28°C significantly increased the length of the second, third, and fourth order roots, with descending order of 25°C, 22°C, and 28°C (Fig. 1B). On average, the length of the second, third, and fourth order roots were increased by 23%, 14%, and 27% compared to the control across the three temperatures ( $p<0.05$ ). There was no significant difference in root diameter between all levels of root order: 0.927, 1.037, 1.233, and 3.156 mm for the first, second, third, and fourth order roots (Fig. 1C).

**Table 1. Two-way ANOVA statistical results of cold storage duration (D) (30 days and 45 days at 7 °C) and the subsequent root zone temperatures (T) (22 °C, 25 °C, 28 °C) on the flowering and leaf emergence.**

	D	T	D×T
Number of scale	ns	*	ns
Length of scale (cm)	ns	***	ns
Number of florets per scale	ns	ns	ns
Time of first flower opening (d)	ns	***	ns
Anthesis duration (d)	ns	ns	ns
Time of first leaf emergence (d)	ns	***	*

Levels of significance are indicated as: \**p*< 0.05, \*\**p*< 0.01, \*\*\**p*< 0.001, ns *p*> 0.05

**Table 2. The flower and leaf properties of plants that were stored at 7 °C for 30 and 45 days and then were planted at three root zone temperatures of 22°C, 25°C, 28°C. The control temperature referred to the room temperature of 18 °C (Mean ± SE, n=10).**

	22°C	25°C	28°C	Control
<b>30 days at 7°C</b>				
Time of first flower opening (d)	108.8 ± 1.209 b	99.3 ± 2.61 c	95.1 ± 2.137 c	126.7 ± 2.533 a
Anthesis duration (d)	22.3 ± 2.380 a	28.5 ± 1.898 a	22.2 ± 2.548 a	28.2 ± 3.058 a
No. of scape	1.4 ± 0.163 a	1.6 ± 0.163 a	1.6 ± 0.163 a	1.8 ± 0.133 a
Length of flower scape (cm)	50.1 ± 1.936 a	47.6 ± 2.246 a	34.8 ± 2.181 b	52.3 ± 3.431 a
No. of florets per scape	3.9 ± 0.097 a	3.8 ± 0.144 ab	3.5 ± 0.194 ab	3.4 ± 0.209 b
Time of first leaf emergence (d)	50.2 ± 0.722 a	48.5 ± 0.401 b	50.0 ± 0.471 ab	/
<b>45 days at 7°C</b>				
Time of first flower opening (d)	108.0 ± 3.785 ab	100.4 ± 3.367 b	100.5 ± 4.655 b	126.7 ± 2.533 a
Anthesis duration (d)	21.1 ± 2.879 a	21.6 ± 1.863 a	25.0 ± 3.069 a	28.2 ± 3.058 a
No. of scape	1.3 ± 0.213 b	1.3 ± 0.152 b	1.7 ± 0.152 ab	1.8 ± 0.133 a
Length of flower scape (cm)	49.8 ± 1.255 a	47.3 ± 2.615 a	39.7 ± 1.533 b	52.3 ± 3.431 a
No. of florets per scape	3.6 ± 0.212 a	3.7 ± 0.224 a	3.4 ± 0.190 a	3.4 ± 0.209 a
Time of first leaf (d)	50.3 ± 0.366 a	48.8 ± 0.200 b	48.0 ± 0.365 b	/

Different lower-case letters in the same row indicated a significant difference at the level of 0.05 among treatments

**Table 3. The leaf length, width, and thickness of plants grown at three root zone temperatures (22, 25, 28°C). The control temperature referred to room temperature of 18 °C (Mean ± SE, n=10).**

	22°C	25°C	28°C	Control
Leaf length (cm)	59.9 ± 1.167 a	59.8 ± 1.191 a	50.8 ± 1.276 b	59.1 ± 1.854 a
Leaf width (cm)	5.8 ± 0.111 a	5.8 ± 0.088 a	5.5 ± 0.075 b	5.9 ± 0.056 a
Leaf thickness (mm)	1.2 ± 0.032 a	1.1 ± 0.047 ab	1.1 ± 0.040 b	1.2 ± 0.028 a

Different lower-case letters in the same row indicated a significant difference at the level of 0.05 among treatments

**The effects of growth temperature on root volume and weight:** The root volume at 25°C was 29%, 46%, and 126% higher than that at 22°C, 28°C, and the control (*p*<0.05) (Fig. 2). The difference in the root volume was mainly attributed to the length of the second, third, and fourth order roots. Root volume was positively correlated to the number and length of roots. The root dry weight showed the same trend as the root volume (Fig. 2). The root dry weight at 25°C was 33% higher than that at 22°C and 28°C, and 58% higher than the control (*p*<0.05), while no significant differences in the root volume and dry weight between 22°C and 28°C.

**The effects of root zone temperature on root nutrient:** The nitrogen (N) concentration in the roots at 22°C was 7% higher than the other treatments (Fig. 3A). The phosphorus (P) concentration at 22°C was 17%, 25%, and 34% higher than that at 25°C, 28°C, and the control, while no significant difference between 25°C and 28°C (Fig. 3B). No significant difference in the potassium (K) concentration was observed between the treatments and

the control, averaging 0.04% (Fig. 3C).

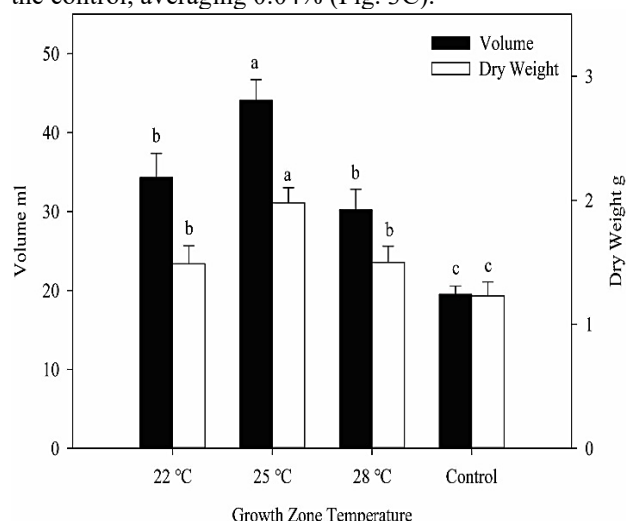


Fig. 2. The root volume and dry weight of all roots grown at 22°C, 25°C, 28°C root zone temperatures and in the control room temperature of 18°C (Mean ± SE, n=10).

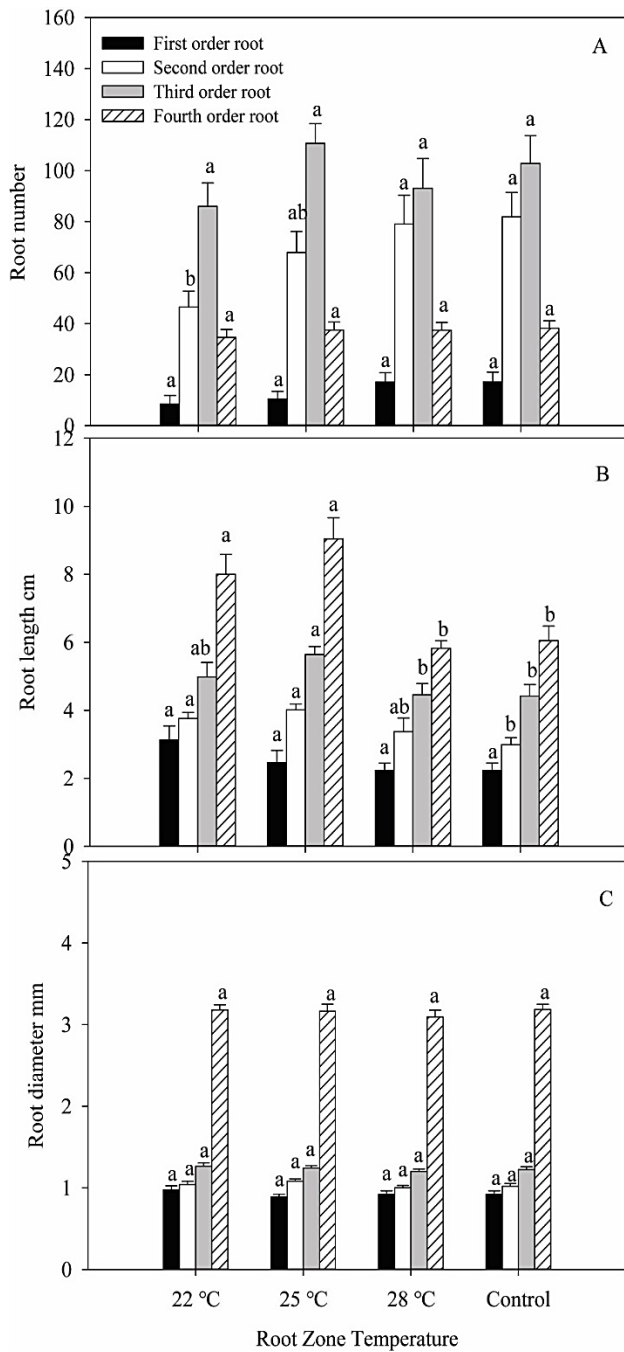


Fig. 1. The number (A), length (B), and diameter (C) of different root orders grown at 22°C, 25°C, 28°C root zone temperatures and in the control room temperature of 18°C after 30 days cold storage (Mean ± SE, n=10).

**Discussion**

Flower induction is mainly triggered by photoperiod and temperature (Corbesier & Coupland, 2006; Horvath, 2009). For most geophytes, including *Hippeastrum*, the most important abiotic factor controlling flowering is thermoperiodicity rather than photonasty (Bose *et al.*, 1980; Ephrath *et al.*, 2001; Khodorova & Boitel-Conti, 2013; Trang *et al.*, 2018). Amaryllis don't have natural dormancy and can flower at all seasons under a suitable condition (Ijira & Ogata, 1997). The flower induction of Amaryllis is also related to the size of bulbs (Bose *et al.*, 1983). The

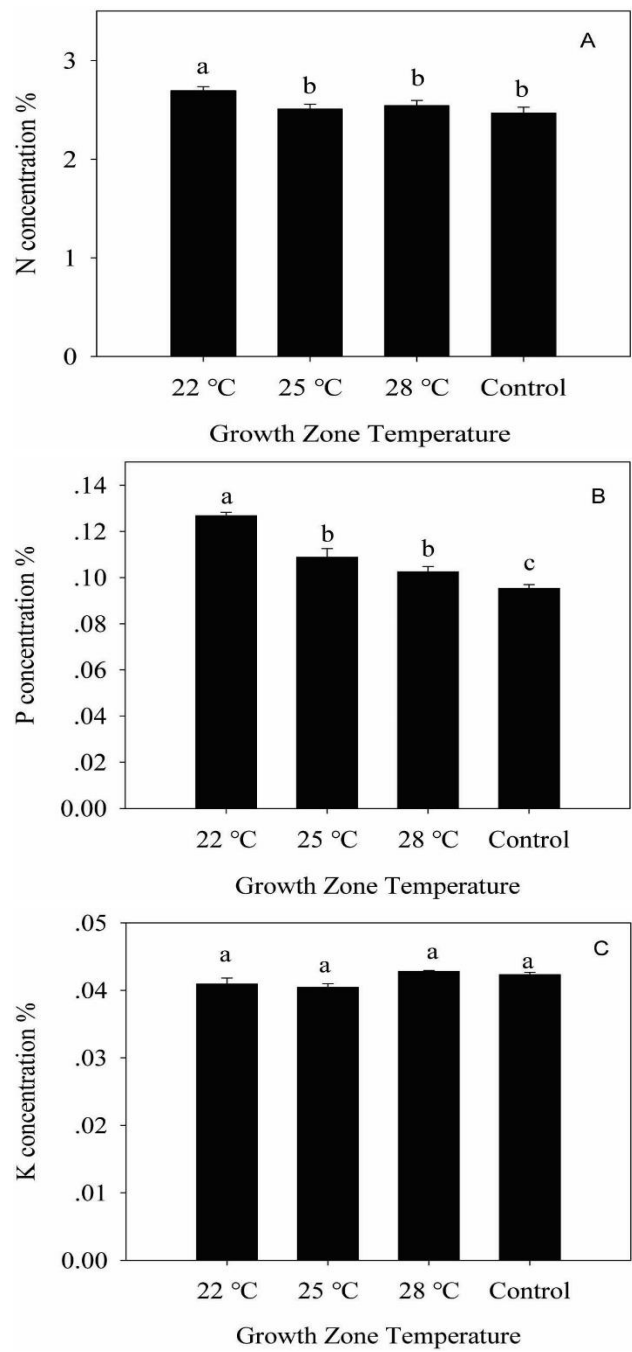


Fig. 3. The nitrogen (N) (A), phosphorus (P) (B), and potassium (K) (C) concentrations of roots grown at 22°C, 25°C, 28°C root zone temperatures and in the control room temperature of 18°C (Mean ± SE, n=10).

bulbs need a period of low temperature (4-12°C) to finish vernalization after flower bud differentiation is finished (Song *et al.*, 2009; Khodorova & Boitel-Conti, 2013), below 10°C being better for most cultivars. The bulbs would suffer from freezing injury or flowering would be inhibited if the temperature is too low (Song *et al.*, 2009). In our study, 7°C is an ideal temperature to store for *H. striatum* 'Blossom Peacock' (the temperature is based on a series of previous experiments, data not shown). For example, when they were stored at 3°C, the flower amount significantly decreased. Cold storage duration is another important factor affecting flowering. We found no

differences in flowering characteristics and the time of the first leaf emergence between 30 days and 45 days of cold storage (Table 1). Trang *et al.*, (2018) also reported that treatment time didn't significantly change the number and length of flower scape and the number of florets per scape for *H. hybridum* cultivar 'Cam Tu'. To reduce costs and bloom as soon as possible, 30 days of low temperature storage is preferred. If gardeners want to delay flowering, such as for some festivals, storing longer is also an option. However, the shortest or the longest storage time might be cultivar specific, and there may be an interaction between storage temperature and storage duration.

Naturally, flowering initiation occurs in April or May (late spring) for different cultivars in the study area, based on the vegetative development in last summer and autumn (approx. by mid-December). Amaryllis typically produces one or two scales up to 550 mm in height which contains two to five (mostly four) large trumpet-shaped flowers (Wang *et al.*, 2018). Scale length is an important feature in evaluating flower quality of Amaryllis, which is most influenced by storage duration and temperature (Warrington *et al.*, 2011) and other factors, such as cultivar and season (Karavadia & Dhaduk, 2002). The root zone temperature changed the length of scale although storage duration did not significantly affect it for the cultivar of 'Blossom Peacock' in the present study. Fresh cut flowers prefer a long scale, while dwarf flowers look more beautiful for potted flowers. Based on our results, higher root zone temperature (28°C) significantly decreased the length of scale.

Whether as fresh cut flowers or potted flowers, longer anthesis is always welcomed and plays an important role in pollen dispersal and reproductive success (Zhao *et al.*, 2020). The duration of flower anthesis is influenced by many factors, like planting environment, light, post-harvest handling conditions, growth regulators, nutrient level, etc. (Khalaj & kanani, 2018; Azimi, 2020). The flowering duration ranged from 10 to 20 days for the cultivars of 'Moscow', 'OPRC-202', and 'OPRC-206'. And the number of florets per branch was between 3.3 and 5.3 (Azimi & Alavijeh, 2020). Consistent with the previous results (Trang *et al.*, 2018; Wang *et al.*, 2018; Azimi & Alavijeh, 2020), the number of florets in one scale for *H. striatum* 'Blossom Peacock' varied from 2 to 5, averaging 3.6 (Table 2). The average duration of flowering was 24.6 days. Gardeners and florists are more interested in longer anthesis but root zone temperature did not change it, which might be decided by genetic characteristics. After undergoing 45 days low temperature storage, subsequent growth temperatures didn't significantly affect the floret number per scale, while 22°C increased it by 15% for plants treated for 30 days. The results indicate that low temperature accumulation may affect the number of flowers, but the mechanism is not clear. Future research may focus on the interaction between temperature and treatment duration to obtain the shortest and longest storage time for specific cultivars.

*Nerine sarniensis*, in the same family as Amaryllis, was stored at 3°C and 30°C after vernalization. Soil temperature accounted for 92% of the variation in the flower bud emergence and first flower opening (Warrington *et al.*, 2011). Our results showed that 22°C, 25°C, and 28°C significantly accelerated flower opening, in which 25°C and 28°C were more obvious (Table 2). After vernalization, bulbs uptake enough water and nutrients for aboveground growth and flower maturation. The strong root system can ensure faster

leaf growth and earlier flowering.

According to the theory of Pregitzer *et al.*, (2002) and McCormack *et al.*, (2015), lower root orders (first and second order roots) have greater absorptive ability which can provide necessary mineral nutrients to leaf growth and flowering, whereas higher root orders, like fourth order roots, mainly transport water and nutrients for the aboveground part. The roots of Amaryllis show obvious root order structure although they are different from the woody root system. We expected that there were differences in root orders, including number and length among three root zone temperatures, especially in the lower root orders. The differences in root orders would further lead to differences in nutrient uptake, growth, and flowering. Only the length of the second, third and fourth order roots at 25°C and 22°C was significantly increased compared to the control (Fig. 1), resulting in higher root volume and dry weight (Fig. 2). In addition, we found that the first leaf emergence and the first flower opening occurred earlier and the scale was longer for the plants grown at 25°C. The root arrangement of Amaryllis is completely different from woody plants with taproot. The roots of Amaryllis grow around the basal stem, most of which have four levels of root order. The boundaries and functions between lower and higher root orders are not absolute. The longer second, third, and fourth order roots of Amaryllis can increase water and nutrient absorption and transportation.

The length of roots and flower scape and leaf size were the smallest at 28°C for *H. striatum* 'Blossom Peacock' (Fig. 1; Tables 2, 3). The optimum temperature for root elongation was 27-30°C for *Opuntia ficus-indica*, while 20°C for *Pinus resinosa*, generally ranging from 18°C to 28°C for some ornamentals (Andersen *et al.*, 1986; Drennan & Nobel, 1998). When *H. hybridum* 'Star of Holland' was planted in the growth chambers maintained at 17°C/12°C, 24°C/17°C, and 30°C/24°C day/night temperature, the total number of roots, average root length, and weight were the highest at 30°C/24°C and the smallest at 17°C/12°C (Ijiri & Ogata, 1997). However, the cultivar of 'Apple Blossom' grown in tropical areas showed opposite results that 11°C root zone temperature increased the root length compared to the control at 23°C (Inkham *et al.*, 2020).

Compared to the control, 22°C root zone temperature accelerated flowering, increased root length and volume although they were not as high as 25°C. The N and P concentrations at 22°C were significantly higher than those at 25°C, 28°C and the control, and only 22 increased the floret number per scale. Root uptake capacity is related to root temperature and nutrient supply of soil. High temperature can result in a reduction in uptake capacity, such as 27°C for *Agropyron desertorum* (Clarkson *et al.*, 1992; BassiriRad *et al.*, 1993). Different species and cultivars have different sensitivity to temperature. Bingham & Cumbus (1991) reported that K uptake capacity was not sensitive to root zone temperature, which was consistent with our results.

## Conclusion

The constant root zone temperatures of 22°C, 25°C, and 28°C accelerated flowering although anthesis duration did not change. Low temperature storage at 7°C for 30 days can successfully finish vernalization for the cultivar of

'Blossom Peacock'. We can't say which temperature is the most favorable, but controlling root zone temperatures can be applied for different commercial purposes. We conclude that 22°C increases the floret number per scale and root nutrients; 25°C more obviously accelerates flowering and leaf emergence, and improves root growth; 28 °C decreases the length of scale which is better for pot plants.

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