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The Influence of Bulb Storage Regimes on the Growth and Flowering of Hippeastrum (*Hippeastrum hybridum* Hort.)

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Abstract

Hippeastrum hybridum cultivar 'Cam Tu' bulbs were subjected to ten different treatment regimens to evaluate the effect of temperature (4°C or 25°C), duration (4, 6, or 8 weeks), and wrapping materials (coir fiber or newspaper) on their growth and flowering. The data revealed that the storage treatments had significant effects on several growth and flowering characteristics of *H. hybridum* cv. 'Cam Tu'. In terms of vegetative characteristics, bulbs stored at 4°C with coir fiber wrapping for 6 weeks (T9) had the largest leaf size (a length of 39.46 cm, a width of 4.39 cm), the longest flower scape (48.64 cm in length), and the longest pedicel (4.75 cm in length). Bulbs of the T9 treatment also showed the shortest time to flower bud emergence (62.24 days) and first flower opening (80.43 days), and the date of 70% first flower fully opening was January 19, 2018. Both of the two thermal treatment regimens shortened flowering time (80.43-103.16 days) compared to the control (132.46 days). However, the number of bulblets per plant, number of leaves per plant, number of florets per scape, floret diameter, longevity of a flower, and longevity of a flower scape were not statistically impacted by the treatments.

Keywords

Hippeastrum hybridum, Amaryllis, temperature, wrapping materials, flowering time

Introduction

Hippeastrum Herb. (Amaryllidaceae) is an important genus comprised of about 60 to 70 species and more than 300 cultivars (Liberty, 1976; Read, 2004). Amaryllis plants (belonging to the genus *Hippeastrum*) are primarily indigenous to Central and South America and easily grow in tropical and subtropical regions (Okubo, 1993). *Hippeastrum hybridum* Hort. is one of the most

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well-known bulbous ornamental plants with an attractive color, large size, and lily-like flowers on their long stalks. It can be planted in beds, edging, and borders along paths or sides, in pots, or indoors. Naturally, in Vietnam, amaryllis often flowers from the middle of March until the end of April (Ho, 2000; Do, 2007). However, the main market demands for amaryllis flowers are special events or vacations from the end of December to the middle of February, such as, Christmas, New Year's Day, Lunar New Year, and Valentine's Day. Therefore, if the flowering time of amaryllis can be controlled to bloom exactly in these times, the commercial value of amaryllis could be increased.

Amaryllis plants are geophytes that have an underground organ, and are classified as a bulbous plant. According to a review by Khodorova and Boitel-Conti (2013), among several environmental factors, temperature is considered to play a predominant role in controlling growth and flowering in geophyte bulbs. Most bulbs require a "warm-cold-warm" sequence to complete their annual cycle. Hartsema (1961) clearly demonstrated that the optimal temperature for flowering processes of flower bulbs can range from -2^{o}C to 34^{o}C depending on the bulb species, growing season, and cultivar. The mature amaryllis bulb initiates flowers while growing vegetatively. Its initiation of a flowering scape within the bulb is not photoperiodically controlled. A study on *Hippeastrum hybridum* cv. 'Red Lion' by Ephrath *et al.* (2001) revealed that temperature had a strong effect on bulb and leaf development. A temperature of 27^{o}C was optimal for leaf area development while a temperature of 22^{o}C was optimal for bulb development. In addition, Roberts *et al.* (1983) also showed that the rate of leaf and flower bud development, and stem elongation on the primary (mother) axis of *Lilium longiflorum* (a bulbous plant) were directly proportional to the range of temperatures used (6-24^{o}C) in their experiment. In addition, the data also demonstrated that fairly short periods of chilling (12-18^{o}C) were sufficient for hastening the sprouting of mature bulbs (anthesis + 80 days).

After a critical bulb size is reached, the flower meristem of most geophytes is induced and differentiated at the end of summer. Dormancy is broken in autumn, resulting in shoot and flower bud growth that continues throughout the winter. This period of autumn-winter cooling seems to be extremely important for flower development, as its absence leads to slow shoot growth and severe flowering disorders (Khodorova and Boitel-Conti, 2013). Therefore, the flowering time of amaryllis could be manipulated by applying an appropriate thermal regime.

Amaryllis bulb packing systems (peat, wood chips, poly bag, and houtwol) also have an effect on leaf length and flowering time. Indeed, Dutch-grown Hippeastrrum bulbs ('Apple Blossom' and 'Red Lion') that were stored in houtwol, a type of excelsior, for 84 days at 48^{o}F (9^{o}C) had the longest leaves and the earliest flowering time (De Hertogh and Gallitano, 1998).

The main goal of this study is to evaluate the effects of bulb treatment regimens (temperature, duration, and wrapping materials) on the growth and flowering of *Hippeastrum hybridum* cv. 'Cam Tu'. The results could be used as the fundamental knowledge to control the expected flowering time of amaryllis.

Materials and Methods

Plant materials, study site, and time

H. hybridum cultivar 'Cam Tu', a kind of popular domestic amaryllis, planted at the campus of Vietnam National University of Agriculture was collected. Three-year-old plants with flowering-sized bulbs (6.5-7.0 cm diameter) of the *H. hybridum* cv. 'Cam Tu' were selected and then substrate and leaves were removed for the treatments. Experiments were conducted at the plastic house in the Faculty of Agronomy, Vietnam National University of Agriculture in Gia Lam, Hanoi (N 21^{o}, E 105.93^{o}) from September 2017 to March 2018.

Experimental design and measurements of parameters

H. hybridum cv. 'Cam Tu' bulbs were randomly assigned to one of ten treatments comprised of two temperature regimes of either 4°C or 25°C, at durations of 4, 6, or 8 weeks. In the low temperature (4°C) treatment, bulbs were stored in two kinds of materials (newspaper or coconut coir fiber). The 4°C storage conditions were maintained in a laboratory refrigerator, and the 25°C treatment conditions were maintained in an air conditioner controlled room with a relative humidity of 70%.

On completion of each treatment, all bulbs were treated with a fungicide and then immediately replanted into (16x18 cm) plastic pots with a substrate containing alluvial soil, black rice husks ash, and coconut fiber (coir fiber) under the rate (1:1:1 by volume). Bulbs of all the treatments were replanted in a randomized complete block design (RCBD), with three replications, each containing 30 bulbs. In this experiment, a total of 900 bulbs were used for the ten treatments.

The growth characteristics of the plants were recorded by measuring parameters including the leaf length, leaf width, number of leaves per plant, and number of bulblets per plant. The number of bulblets per plant was counted after the flower fading stage. At anthesis (the period of blossom), flower quality was assessed by recording the flower stalk (scape) length, pedicel length, floret diameter (the maximum diameter when the floret was fully opened), number of florets per scape, and number of scapes per plant. Flowering duration was observed by days to flower bud emergence, days to first flower opening, longevity of a flower, longevity of a flower scape, and date of 70% first flower fully opening.

Data analysis

All data collected were processed by Microsoft Excel version 2014 and analyzed by the statistical software IRRISTART 5.0. The means were separated on the basis of the least significant difference (LSD) test at the 5% probability level.

Results and Discussion

The effect of bulb storage regimes on growth of *H. hybridum* cv. 'Cam Tu'

Leaf size and number of leaves per plant

The main source of assimilates used for the development of the various organs of the plant is the leaves. Long leaves are desirable for flowering potted amaryllis plants. They

Table 1. The effect of bulb storage regimes on the growth of *H. hybridum* cv. 'Cam Tu'

Treatments	Leaf length (cm)	Leaf width (cm)	Number of leaves/plant	Number of bulblets/plant
Control (T1)	32.21	3.69	6.27	0.15
25°C-stored-treatment for				
4 weeks (T2)	33.29	3.76	6.26	0.14
6 weeks (T3)	33.28	3.71	6.27	0.15
8 weeks (T4)	33.77	3.73	6.32	0.13
4°C-stored-treatment and				
wrapped in newspaper for				
4 weeks (T5)	36.20	3.86	6.43	0.16
6 weeks (T6)	36.45	3.97	6.47	0.13
8 weeks (T7)	36.27	3.94	6.36	0.12
wrapped with coir fiber for				
4 weeks (T8)	37.63	4.22	6.38	0.15
6 weeks (T9)	39.46	4.39	6.45	0.11
8 weeks (T10)	37.55	4.09	6.45	0.13
$LSD_{0.05}$	*1.36*	*0.26*	*0.49*	*0.06*
CV%	*2.20*	*3.90*	*4.50*	*24.70*

contribute to the aesthetic value of forced potted plants and increase photosynthesis, which is necessary for satisfactory reflowering of the bulb. Leaf size was strongly affected by the temperature regime (Table 1). Significant differences in leaf size were found between the two thermal regimes, with the biggest leaves in the 4°C treatments. There were slight statistical differences in leaf size between wrapping materials. Among the 4°C storage treatments, the leaves of the bulbs wrapped in newspaper were smaller than those of the bulbs wrapped with coir fiber. The structure of coir fiber is similar to that of houtwol, and the larger leaf sizes may have been due to the increased aeration of this wrapping system, which De Hertogh and Gallitano (1998) demonstrated, is beneficial for amaryllis. It also proposed that the houtwol packing system for bulb storage retained and promoted regrowth of the old basal roots, increased secondary roots, and helped the plants grow long leaves (De Hertogh and Gallitano, 1998). Furthermore, the treatment duration also affected leaf size in all of the 4°C coir fiber wrapping treatments. Indeed, bulbs stored at 4°C with coir fiber packing for 6 weeks had the biggest leaf sizes with averages of 39.46 cm in length and 4.39 cm in width, compared with the shortest leaves of the control plants (an average length of 32.21 cm and width of 3.69 cm).

The data in Table 1 show slight increases in the number leaves of the low (4°C) temperature treatments in comparison with that of the control (increases of 0.09 to 0.19 leaves/plant). However, the differences have had no statistical significance due to the $LSD_{0.05}$ = 0.49. Indeed, these data indicated that the thermal regime, storage duration, and wrapping material had no statistical effect on the number of leaves per plant.

Number of bulblets per plant

The average number of bulblets per plant of the ten treatments ranged from 0.12 to 0.16 (Table 1). There were no statistical differences in the number of bulblets among the storage treatments. These results indicated that storage temperature, duration, and wrapping material had no statistical impact on the number of bulblets per plant.

The effect of bulb storage regimes on the flowering characteristics of H. hybridum cv. 'Cam Tu'

Flower scape and pedicel length

Flower scape and pedicel length of amaryllis plants were measured when the first flower fully opened. It was observed that the flower scape and pedicel length were significantly influenced by the different temperature treatments (Table 2). The longest flower scape (48.64 cm) and pedicel (4.75 cm) were recorded from bulbs stored at 4°C and wrapped with coir fiber for 6 weeks (T9), while the shortest (41.48 cm scape and 4.12 cm pedicel) were produced by the control bulbs (T1). These differences were statistically significant. However, there were no statistical differences in flower scape and pedicel lengths of bulbs among the different 4°C treatment durations (4, 6, or 8 weeks) when bulbs were wrapped in coir fiber. These results are in agreement with the previously shown optimal conditions for stem elongation and anthesis of geophytic plants which includes a several-week period of lower temperatures (4-9°C), while the absence of a low-temperature treatment leads to slow shoot growth (Khodorova and Boitel-Conti, 2013). The impacts of bulb treatment temperature on flower scape length were also revealed in a study by Warrington *et al.* (2011) on *Nerine sarniensis* (a geophytic plant). The results demonstrated that flower scape length increased in the 3°C treatment bulbs compared with stems from the control bulbs, while the stem length decreased in the 30°C treatments (Warrington *et al.*, 2011). The paper also proposed that the production of auxin and gibberellin is affected by the surrounding temperature and probably plays one of the leading roles in geophyte growth regulation. For bulbous plants, it has been reported that the amount of GA correlates directly with the shoot elongation rate and the presence/absence of a cold treatment. Similarly, auxin has been reported to be the main hormonal factor involved in the induction of tulip flower scape elongation (Khodorova and Boitel-Conti, 2013).

Moreover, low-temperature storage (5°C) leads to a selective expression of the aquaporin

Table 2. The effect of bulb storage regimes on the flowering characteristics of *H. hybridum* cv. 'Cam Tu'

Treatments	Flower scape length (cm)	Pedicel length (cm)	Floret diameter (cm)	Number of flower scapes/plant	Number of florets/scape
Control (T1)	41.48	4.12	14.09	1.02	3.14
25°C-stored-treatment for					
4 weeks (T2)	43.15	4.20	14.19	1.03	3.19
6 weeks (T3)	43.47	4.19	14.12	1.00	3.11
8 weeks (T4)	42.18	4.22	14.11	1.01	3.10
4°C-stored-treatment and					
wrapped in newspaper for					
4 weeks (T5)	45.05	4.52	14.16	1.01	3.14
6 weeks (T6)	46.22	4.47	14.15	1.05	3.15
8 weeks (T7)	45.32	4.61	14.18	1.04	3.16
wrapped with coir fiber for					
4 weeks (T8)	48.37	4.69	14.14	1.03	3.19
6 weeks (T9)	48.64	4.75	14.17	1.06	3.17
8 weeks (T10)	47.44	4.71	14.18	1.00	3.14
$LSD_{0.05}$	*1.94*	*0.31*	*0.24*	*0.05*	*0.16*
CV%	*2.5*	*4.1*	*1.0*	*2.7*	*2.9*

γTIP gene in flower scapes after planting. Since aquaporins facilitate water transport during cell enlargement and contribute greatly to cell growth, the absence of γTIP gene expression might also play a role in water deficiency in buds and the inhibition of flower scape growth in plants stored at ambient temperatures (Khodorova and Boitel-Conti, 2013).

Furthermore, the aeration ability of the fiber coir packing system (similar to the houtwol packing system) may prevent a buildup of excess moisture and increase old root regrowth (De Hertogh and Gallitano, 1998). Both of these packing systems might promote the growth of organs via the elongation of scapes and pedicels.

Floret diameter, number of florets per scape, and number of flower scapes per plant

Bulbs stored at 25°C and wrapped with coir for 4 weeks showed the largest floret diameters (14.19 cm) but this value was statistically similar to the diameters of florets in the other treatments (Table 2). The T4 plants also produced the narrowest florets (14.01 cm), however, no statistical variations in the diameters of amaryllis florets were observed due to the effects of all the different treatments.

In addition, the thermal treatments did not have an influence on the number of amaryllis florets per scape. The maximum number of florets per scape (3.19) was recorded in the T2 and T8 treatments, while T4 produced the minimum (3.10), which were not statistically different (Table 1). These results were in agreement with the report of De Hertogh and Gallitano (1998) who found that there were no significant effects of the packing system on floret diameter or the number of florets per scape. The study of Warrington *et al.* (2011) on *Nerine sarniensis* had similar results in that the effects of temperature on floret number were minor.

However, a slight statistical variation in the number of flower scapes per amaryllis plant was recorded due to the influence of the different treatments (Table 2). Bulbs stored at 4°C in coir wrapping for 6 weeks (T9) had the highest the number of flower scapes per plant (1.06) while treatments T10 and T3 had the lowest number of flower scapes per plant (1.00). Overall, the effect of the treatments on the number of flower scapes per plant was minor.

The effect of bulb storage regimes on flowering duration of H. hybridum cv. 'Cam Tu'

Days to flower bud emergence

The number of days to flower scape emergence of amaryllis was significantly influenced by the different treatments (Table 3).

The results showed that the earliest flower scape emergence commenced after 62.24 days for bulbs stored at 4°C in coir fiber wrapping for 6 weeks (T9), while the latest bulbs emerged after 102.14 days in the control plants. The data also demonstrated that the 4°C-stored bulbs emerged nearly 10 days earlier than the 25°C-stored bulbs. The report of Khodorova and Boitel-Conti (2013) also concluded that a lower temperature tends to favor the induction and organogenesis of flower buds. During storage, it has been recorded that a low temperature (5°C) induces water transfer from lateral scales to central ones and also enhances the subsequent transfer of water from the basal plate and scales to the developing bud. Water transport to the bud seems to be inhibited in some way when the storage temperature is too high (Khodorova and Boitel-Conti, 2013).

Days to first flower opening

Different treatments were found to significantly influence the number of days to first flower opening of amaryllis (Table 3). The results showed that the number of days to first flower opening was the shortest (80.43 days) in plants stored at 4°C with coir fiber wrapping for 6 weeks (T9). The control plants took the longest period (132.46 days) for their first flower to open. A study by Fernandez *et al.* (2009) on *Iris xiphium* (a geophytic plant) bulbs also revealed that bulbs stored at a low temperature (9°C) flowered 18 days earlier than those stored at 20°C. Besides, plants grown from bulbs in the houtwol system (similar to coir fiber) flowered the earliest, due to houtwol retaining and promoting the regrowth of old basal roots that lead to early flowering (De Hertogh and Gallitano, 1998). In the 4°C-stored treatments with coir fiber wrapping, there were no statistical differences in the first flower opening period among the treatment durations (4, 6, or 8 weeks).

Longevity of a flower and longevity of a flower scape

The maximum longevity of a flower (6.72 days) was recorded in T9 (4°C-stored-treatment with newspaper wrapping for 6 weeks) while the minimum (6.51 days) was in the control (T1) (Table 3). However, there were no statistical differences in flower longevity among treatments.

Table 3. The effect of bulb storage regimes on the flowering duration of *H. hybridum* cv. 'Cam Tu'

Treatments	Days to flower bud emergence	Days to first flower opening	Longevity of a flower (days)	Longevity of a flower scape (days)	Date of 70% first flowers fully opening
Control (T1)	102.14	132.46	6.53	9.68	Feb 26
25°C-stored-treatment for					
4 weeks (T2)	77.19	103.16	6.47	9.70	Jan 28
6 weeks (T3)	76.30	100.33	6.67	9.79	Feb 7
8 weeks (T4)	77.85	103.01	6.38	9.78	Feb 22
4°C-stored-treatment and					
wrapped in newspaper for					
4 weeks (T5)	68.08	88.97	6.52	9.57	Dec 13
6 weeks (T6)	65.16	86.10	6.72	9.67	Jan 24
8 weeks (T7)	66.82	88.77	6.51	9.61	Feb 8
wrapped in coir fiber for					
4 weeks (T8)	64.64	84.24	6.67	9.83	Dec 8
6 weeks (T9)	62.24	80.43	6.47	9.82	Jan 19
8 weeks (T10)	63.24	81.72	6.44	9.75	Feb 1
$LSD_{0.05}$	*2.40*	*3.17*	*0.47*	*0.39*	
CV%	*1.90*	*1.90*	*4.20*	*2.30*	

The maximum longevity of a flower scape (9.83 days) was recorded in T8, while the minimum (9.57 days) was in T5. Similar to the longevity flowers, there were also no statistical variations of flower scape longevity among treatments. Indeed, storage temperature, duration, and wrapping material had no statistical effects on the longevity of flowers or scapes.

Date of 70% first flower fully opening

A significant variation in the date of 70% first flower fully opening of amaryllis was recorded due to the influence of different treatments (Table 3). Flowering time of these treatments was divided into several periods. The earliest period was the December 8-December 13 period found in the flowering of bulbs treated at 4^{o}C with newspaper wrappings for 4 weeks (T5) and coir fiber wrapping for 4 weeks (T8). The next set of treatments (T2, T3, T6, T7, T9, and T10) had scattered flowering dates from January 24 to February 8. The latest period was nearly natural flowering time (February 26-28).

Conclusions

In conclusion, it could be stated that the thermal regime, wrapping material, and storage duration have significant effects on several growth and flowering characteristics of H. hybridum cv. 'Cam Tu'. Indeed, bulbs stored at 4^{o}C with coir fiber wrapping for 6 weeks had enhanced leaf size growth (39.46 cm in length, 4.39 cm in width), flower scape length (48.64 cm), and pedicel length (4.75 cm), in addition to the earliest emergence of flower scapes (62.24 days) and flowering (80.43 days), with the date of 70% first flower fully opening occurring on January 19, 2018. All the treatments shortened the flowering time (from 80.43 to 103.16 days) compared to untreated bulbs (132.46 days). However, the storage regime, duration, and wrapping material had no effect on the number of bulblets per plant, number of leaves per plant, number of florets per scape, floret diameter, longevity of a flower, or longevity of a flower scape.

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