

Short Communication

Temporal physiological and biochemical changes in *Hippeastrum vittatum* ‘Red Lion’ bulbs stored at different temperatures

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Abstract: Starch and soluble sugar concentrations, α -amylase activity and soluble protein of *Hippeastrum vittatum* ‘Red Lion’ bulbs were assessed under different storage temperatures and storage periods. Bulbs were stored for 45 days at 20°, 12°, 8° or 4°C. Starch concentration decreased most at 4°C on the 45th day, changing from 29.7% to 10.9% in the exterior scales and from 33.0% to 13.0% in the interior scales. The α -amylase activity in the exterior scales, except at 4° and 8°C, decreased significantly between 0 and 15 days of storage, and then increased significantly from the 15th day until the end of the trial. The soluble sugar concentration increased most at 4°C: in the exterior scales it changed from 54.73 to 153.93 mg·g⁻¹ while in the interior scales it increased from 39.67 to 148.11 mg·g⁻¹. The soluble protein concentration in all treatments peaked on the 30th day at 8°C in the exterior scales (2.15 mg·g⁻¹) and at 12°C in the interior scales (2.17 mg·g⁻¹). Understanding these physiological and biochemical changes in the bulbs of *H. vittatum* after storage would serve as a reference for bulb dormancy mechanisms in future studies.

Keywords: *Hippeastrum vittatum*, ‘Red Lion’ bulbs, low-temperature sweetening, α -amylase activity, bulb dormancy

INTRODUCTION

Hippeastrum vittatum Herbert inhabits the lightly-forested Bolivian slopes at 610-1830 m, where conditions are cool and shady. It has 2-6 large, white-and-red striped, moderately fragrant blooms per scape, which is 90 cm tall with an intense green throat [1]. Due to its beautiful flowers and the continuous development of new varieties, *H. vittatum* can be used as an indoor potted ornamental, as a cut flower, or in landscape compositions as a flowering bush or border plant. Although the ornamental and economic value is high, China’s *H. vittatum* bulb self-sufficiency rate

is very low and the storage technology gap relative to advanced international levels is large while flowering and the quality of cut flowers are also unsatisfactory. Domestic and foreign research on forcing culture of *H. vittatum* bulbs focused more on the relationship between storage temperature, storage time and the effect of cultivation on plant characters (leaf length, flower bud number, etc.). For example, De Hertogh and Gallitano [2] found that highest quality plants were produced at 22/18°C with long days (3-hr night break with incandescent light). These plants reached the market stage in 25 days and had 15.4-cm-long leaves. They reached full flower in 32 days with 18.6-cm-long leaves, 43.6-cm-tall flower stalks, and 19.3-cm-diameter flowers. Lu et al. [3] showed that bulbs used for forcing culture need to be stored at 4-7°C for 45-60 days, that buds germinate at 22°C over 10-14 days, and that flower buds sprout above 25°C. The effect of temperature on *in vitro* bulb production has also been examined [4]. However, the physiological and biochemical changes that take place within *H. vittatum* bulbs have still not been reported even though the bulb is the central organ for the success of this ornamental. In order to provide a theoretical basis for bulb germination, flowering control of fresh cut flowers, and postharvest handling, this article studies the physiological and biochemical changes to *H. vittatum* bulbs at different storage temperatures and storage periods.

MATERIALS AND METHODS

The experiment was conducted from November 2011 to January 2012 and *H. vittatum* bulbs were harvested when leaves turned yellow. *H. vittatum* 'Red Lion', which has red, velvety blooms and a sturdy scape, is very popular in China and is used to decorate the Spring Festival. After removing the aerial parts, 45 bulbs (purchased from Binfen Horticultural Co., Beijing) that had tightly-held scales, no pests or bulb plate injury and that were 6 cm in diameter were selected. Older outer scales, dead roots and some devitalised tissues attached to the root disk surface were all removed. Bulbs were soaked and sterilised for 1 hr in 80% Carbendazim (disinfectant) (Lianhelichen Crop Science Chemical Co. Ltd, Jiangsu) diluted 800fold with distilled water, and were used for the experiment after having dried off.

Ten bulbs were used for each storage temperature, i.e. 20°, 12°, 8° and 4°C. Bulbs were placed at each temperature for 45 days. From November 26th 2011 onwards, physiological and biochemical indices were determined for each temperature treatment every 15 days. Five bulbs were set aside at room temperature (20±0.5°C). Before physiological and biochemical tests, three bulbs were randomly selected and scales were carefully removed. Since many differences exist between exterior and interior scales (first 2-3 layers from the outside to the inside and first 2-3 layers from the inside to the outside respectively), for example the inner scales are much more tender than the outer scales, they were analysed separately. Three bulbs per treatment were shredded and mixed evenly and each analysis was done in triplicate.

The anthrone method was used to determine soluble sugar [5, 6]. Starch content was determined by the method of Men and Liu [7] using perchloric acid. α -Amylase activity was determined by the 3,5-dinitrosalicylic acid colorimetric method [6]. Soluble protein content was determined by the method of Bradford [8] using Coomassie Brilliant Blue G-250 staining.

SPSS version 17 was used to analyse all biochemical indicators which changed over time at different storage temperatures. Duncan's multiple range test was used to calculate the significance ($P < 0.05$) between single factors while two-way ANOVA was applied to test the effects of between-subject factors.

RESULTS AND DISCUSSION

The change in carbohydrate concentration is one of the most sensitive physiological indicators of plant metabolism under low temperature conditions, and starch and soluble sugar play an important role in the maintenance of balance between carbohydrate supply and demand [9]. Generally, in the process of dormancy at low temperature, starch-degrading enzyme activity increases and starch is hydrolysed in bulbs with subsequent accumulation of soluble sugar [10, 11]. This is also intimately related to carbon and nitrogen metabolism [12]. In the present experiment, the soluble sugar concentration of both the inner and outer scales of *H. vittatum* increased during storage (Figure 1), a process commonly termed ‘low temperature sweetening’ [13]. This response is similar to that in lily bulbs [14, 15, 16]. As seen from Figure 1, the lower the storage temperature, the higher the soluble sugar concentration becomes. At the same temperature, as storage progresses from 0 to 45 days, starch content consistently declines (Figure 2) while soluble sugar concentration increases in both exterior and interior scales, suggesting that a longer experiment (exceeding 45 days) might reveal more information about the internal biochemical changes in *H. vittatum* bulb.

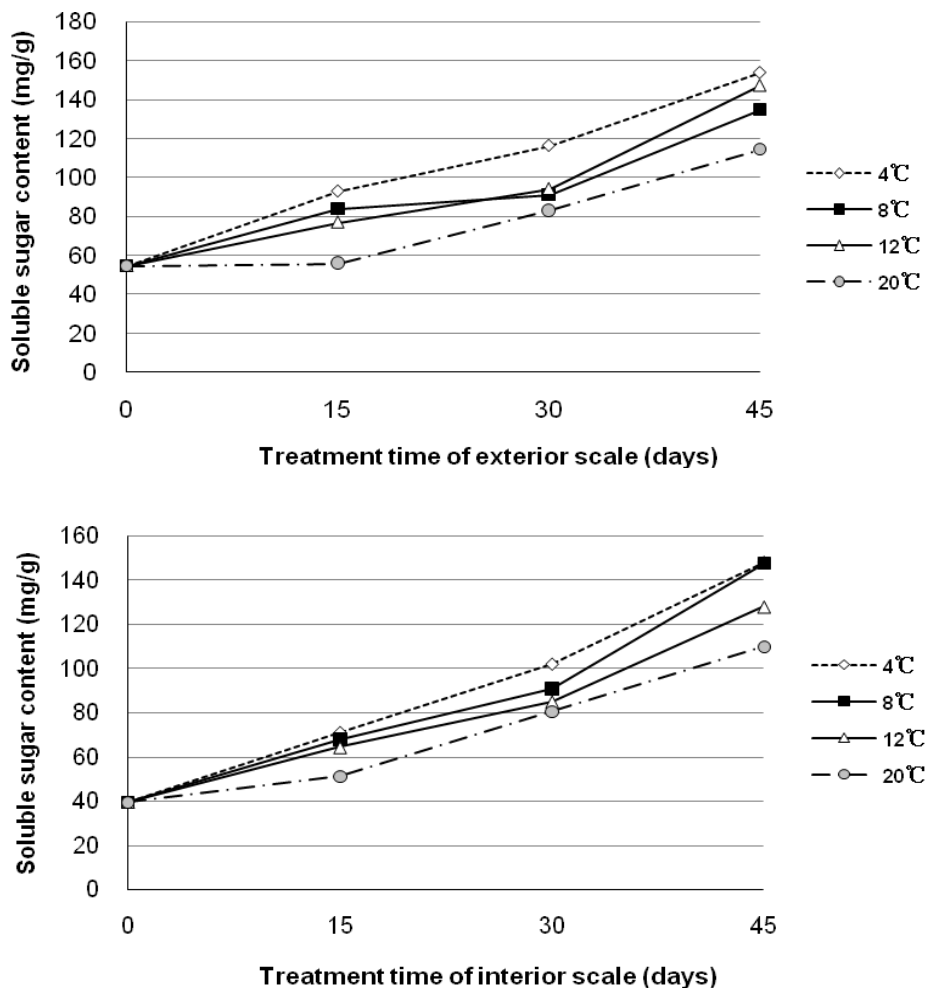


Figure 1. Changes in soluble sugar content of exterior scale and interior scale of *H. vittatum* ‘Red Lion’ bulb stored at different temperatures

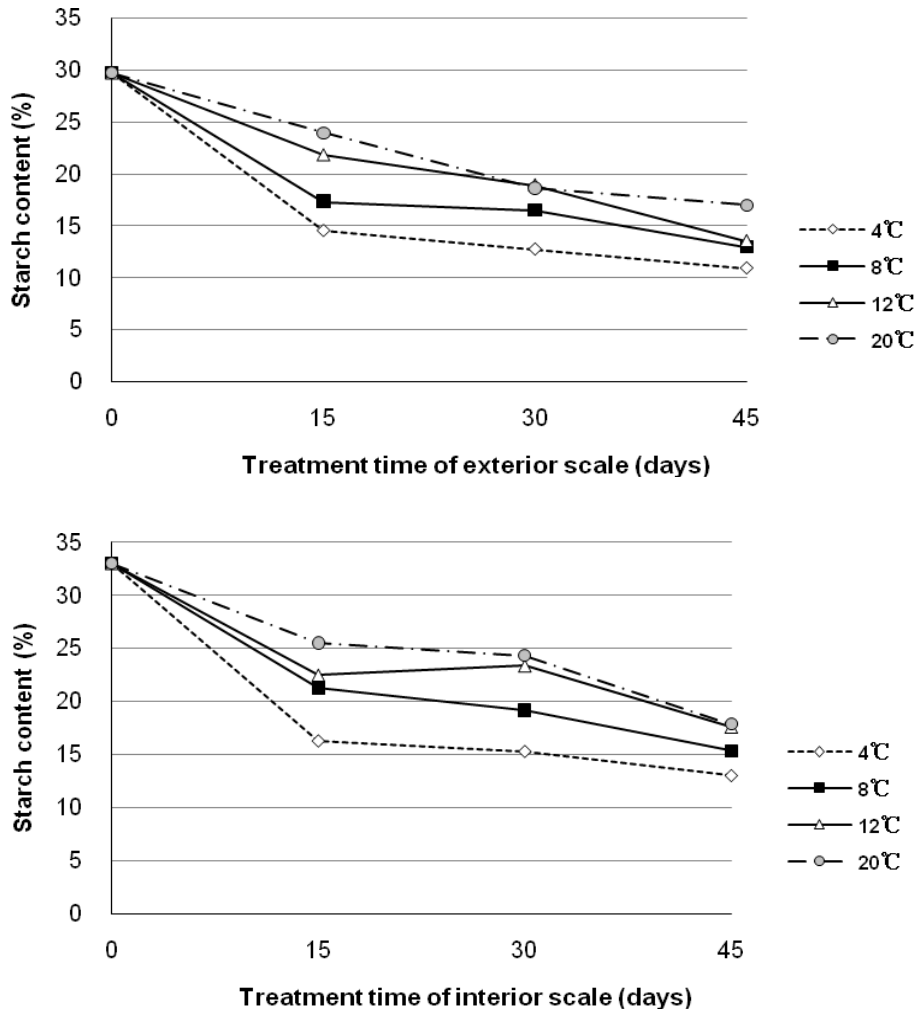


Figure 2. Changes in starch content of exterior scale and interior scale of *H. vittatum* 'Red Lion' bulb stored at different temperatures

Starch degradation and α -amylase activity are related within scale cells. In our experiments, α -amylase activity increases during 15-45 days after temperature treatment (DATT) (Figure 3) while starch content decreases steadily during 0-45 DATT at all temperatures (Figure 2). The discrepancy at 0-15 DATT may be due to the complexity of starch decomposition [17,18]. α -Amylase activity at 4°C increases consistently. Interestingly, most studies of forcing culture do not employ a storage temperature of less than 5°C. However, whether this would negatively impact bulb quality still requires further studies.

The soluble protein in most plants is involved in various metabolic enzymes, and its concentration is an important indicator to understanding plant metabolism. In this study, the concentrations of soluble protein at their highest levels at 30 DATT was almost the same for all temperature treatments, showing a constant metabolism of the *H. vittatum* bulb between 4° and 20°C (Figure 4).

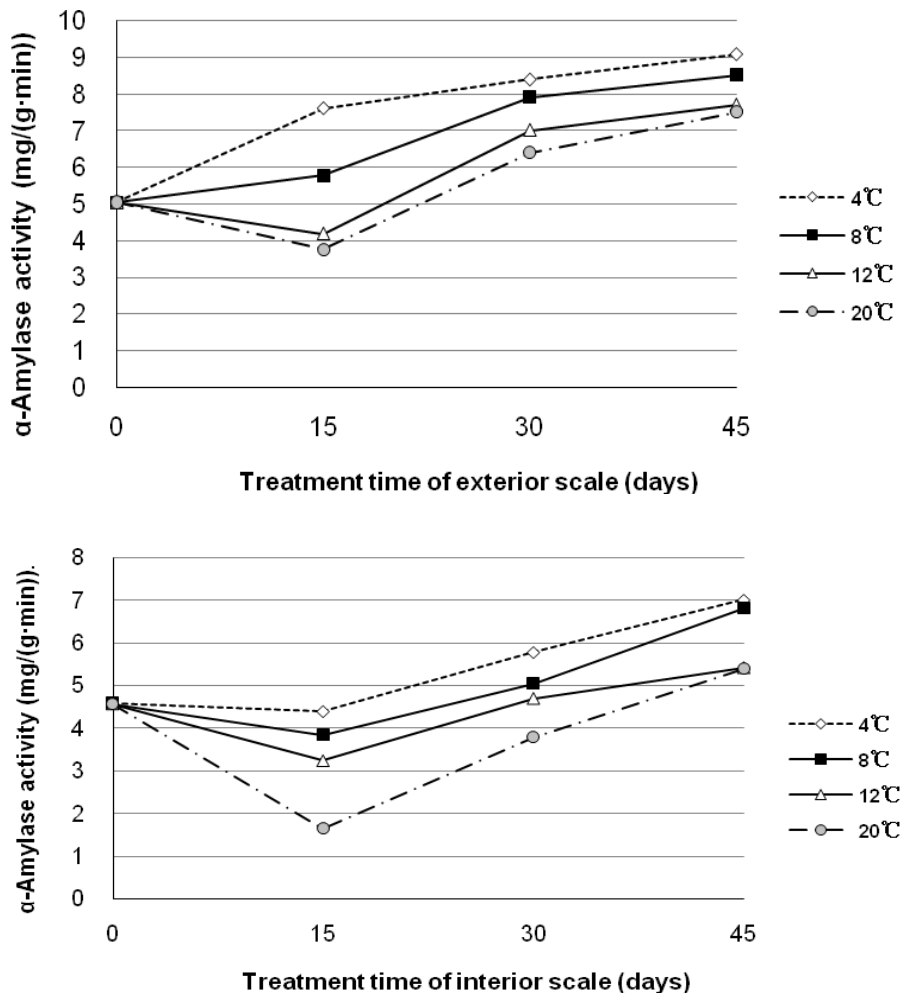


Figure 3. Changes in α -amylase activity in exterior scale and interior scale of *H. vittatum* 'Red Lion' bulb stored at different temperatures

Almost every study to date on *H. vittatum* bulb has focused on forcing culture and little is known about the internal mechanism of its dormancy. From the perspective of physiological and biochemical changes in bulbs, forcing culture can also be used to illustrate the internal changes in bulbs to some extent. The temperature of *H. vittatum* bulbs during the cultivation period between bud development and flowering ranges from 15° to 25°C [19]. When 'Red Peacock' bulbs were stored at 9-10°C for 6 weeks and then placed in the greenhouse for 2 weeks, 60% of flower buds sprouted, but only 30% sprouted when kept at 9-10°C for 8.5 weeks [20]. Read [1] claimed that 6 weeks at 13°C is sufficient for scapes to emerge when bulbs are cultivated at 18-19°C. Lu and Wang [21] noted that for most *Hippeastrum* varieties, bulbs stored at 12°C for 8-13 weeks could effectively break dormancy. When *H. vittatum* 'Red Lion' was propagated *in vitro* by twin scales, carbohydrate content throughout bulblet formation changed in response to temperature, plant growth regulators and substrate [4].

This experiment provides a theoretical basis for physiological and biochemical mechanisms of *H. vittatum* bulbs after dormancy and would thus allow for the improvement of post-harvest technology.

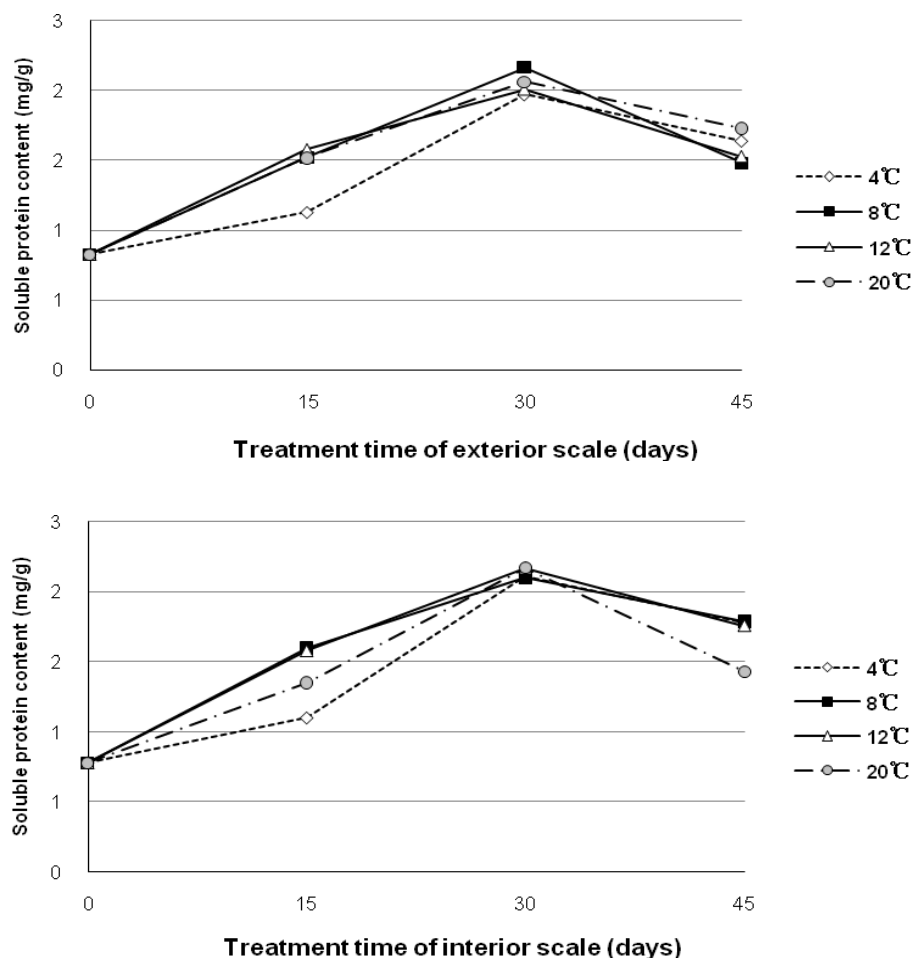


Figure 4. Changes in soluble protein content of exterior scale and interior scale of *H. vittatum* 'Red Lion' bulb stored at different temperatures

CONCLUSIONS

Temperature and storage time induce the biochemical changes in the bulb of *Hippeastrum vittatum* 'Red Lion'. Starch concentrations in both the interior and exterior scales decrease with storage time and the lowering of temperature. Except for 4° and 8°C, α -amylase activity both in the exterior and interior scales decreases significantly between 0 and 15 days of storage then increases significantly from 15 to 45 days. Soluble sugar concentrations in both the interior and exterior scales increase with storage time and the lowering of temperature. Soluble protein concentrations in both the interior and exterior scales peak at approximately the same level on day 30 regardless of temperature and storage time. There seems to be no significant difference in the degree of biochemical changes occurring in the interior and exterior scales of the *H. vittatum* 'Red Lion'.

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