Pak. J. Bot., 49(4): 1415-1421, 2017.

BIOCHEMICAL AND PHYSIOLOGICAL RESPONSES OF *LYCORIS SPRENGERI* BULBLETS (AMARYLLIDACEAE) TO EXOGENOUSLY APPLIED N-(2-CHLORO-4-PYRIDYL)-N1-PHENYLUREA (CPPU)

ZIMING REN[1], YIPING XIA[1*], LINFANG SHE[2], YUMIAN XIAO[1], DONG ZHANG[1] AND XUESI LV[1]

[1]Physiology & Molecular Biology Laboratory of Ornamental Plants, Department of Horticulture, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou (310058), China,[2]Nanjing Horticulture Technology Extension Station
[*]Corresponding author's email: ypxia@zju.edu.cn

Abstract

Bulblets of *Lycoris sprengeri* (Amaryllidaceae) were obtained by cutting. Sixconcentrations of N-(2-chloro-4-pyridyl)-N1-phenylurea (CPPU) solutions were sprayed on leaves from one-year-old bulblets during their green period. Fresh weight, diameter,carbohydrate content, activity of starch metabolism-related enzymes and levels of endogenous hormones of bulblets were determined. The effects of CPPU treatment on bulblet development and biochemical and physiological indices of *L. sprengeri* were analyzed using the determined values. The results showed that CPPU treatment at an appropriate concentration promoted the enlargement of *L. sprengeri* bulblets; the optimal concentration was 7.5 mg L^{-1}. Bulblet growth showed a significant positive correlation with starch content and the activities of soluble starch synthase (SSS) and starch-bound starch synthase (GBSS). Bulblet growth showed anextremely significant positive correlation with the ratio of endogenous gibberellic acid/abscisic acid (GA/ABA). The GA/ABA ratio showed a significant positive correlation with the activities of (α+β)-amylase and GBSS. The exogenous application of CPPU promoted the synthesis and accumulation of starch in the bulblets of *L. sprengeri* and the activities of starch metabolism-related enzymes; an increase in the endogenous GA/ABA ratio had a synergistic effect.

Key words: CPPU, Bulblet, Starch metabolism, Endogenous hormones.

Introduction

The genus *Lycoris* (Amaryllidaceae) consists of more than 20 species that are widely distributed in China, Japan, North Korea, Laos, and Burma (China Flora, 1985 –Chinese Academy of Sciences). *Lycoris* species have been termed "Magic lilies" in Western countries because they never show flowers and leaves simultaneously during their entire life cycle (Adams, 1976). *L. sprengeri* is unique to China and originated in the coastal areas of East China and Taiwan. *L. sprengeri*, which has a beautiful flower shape and an elegant floral color, is usually considered an ornamental plant (Xu, 1989). This plant is grown in flower beds, flower borders, and on the edges of forests. *L. sprengeri* is planted in combined pots, and its flowers are cut for ornamental purposes. *L. sprengeri* has been extensively applied in urban landscaping and greening (Ji, 2002). However, *L. sprengeri* has smaller bulbs than other *Lycoris* species (Zhang *et al.*, 2002). Under natural conditions, *L. sprengeri* exhibits slow bulb growth and a low reproduction rate. Zhang *et al.* (2002) reported cutting propagation for *L. sprengeri* by cutting bulbs but obtained low propagation coefficients ranging from 2.37 to 3.48. The average diameter and fresh weight of two-year-old bulblets from *L. sprengeri* were significantly lower than those from *L. aurea*, *L. radiata*, and *L. chinensis*. Approximately 4.4% of two-year-old bulblets reached the qualification standards.

N-(2-chloro-4-pyridyl)-N1-phenylurea (CPPU) is a cytokinin-like substance with 10- to 100-fold higher levels of biological activity than 6-benzylaminopurine (Zhang & Chang, 2010). Significant increases in the number of lily cells and cell layers, as well as bulb growth and development, were observed by applying CPPU (Watanabe, 1989). In our previous studies, Xiao *et al.* (2013) demonstrated that 5 mg L^{-1} CPPU treatment on *L. radiata* bulblets accelerated nutrient metabolism, starch accumulation and bulblet enlargement. Work by She *et al.* (2014) demonstrated an optimal CPPU concentration of 1.0 mg l^{-1} CPPU for *L. aurea*. Accordingly, bulblets of *L. sprengeri* from artificial propagation were used as materials in the current study. The effects of CPPU treatment on the development and biochemical and physiological indices of *L. sprengeri* bulblets were quantified. We correlated bulb growth with the activities of starch metabolism-related enzymes and levels of endogenous hormones. Key factors affecting the enlargement of *L. sprengeri* bulblets were screened. The results of this study serve as an experimental basis for chemically induced methods to accelerate the growth of bulblets from the genus *Lycoris*.

Materials and Methods

The experiment was conducted in a bulb and perennial root germplasm resource garden at Zhejiang University from March 2013 to July 2013. The experimental materials included *L. sprengeri* bulblets from bulb cuttings in June 2012 (average fresh weight: 0.63 g; average diameter: 8.81 mm). Two weeks before the experiment, bulblets with two to three leaves, consistent growth status, and no pests or diseases were planted in pots, with five bulblets per pot. Beginning on March 27, 2013, five concentrations of CPPU were applied three times to the leaves of the entire plants (Table 1) once every week. Three replicates were established. The pots were sheltered from rain, and conventional management procedures were adopted. When all leaves had fallen (after 12 weeks of treatment), the underground bulblets were dug out of the ground to determine morphological, biochemical, and physiological indices.Sampling was performed once before treatment (March 27, 2013). The bulblets were sampled 12 weeks after leaf spray of different concentrations of CPPU, labeled C0 to C5. The bulblet samples were treated as follows: mud was washed off; aged brown scales were removed; pictures were taken; roots were cut; bulbs were wiped clean with water; fresh weight and diameter were measured; bulbs were cut into pieces with scissors and mixed evenly; 0.5 g of each sample was wrapped in tinfoil, frozen in liquid nitrogen for 30 min, and stored in an ultra-low temperature freezer at -75°C.

From the washed bulblets, a 1 cm^2 piece of the bulb was cut from the second layer of fresh scales, counting from outside to inside. The samples were fixed in formalin/acetic acid/alcohol, stained in periodic acid-Schiff (PAS) reagent and observed under a microscope for starch granule distribution (Hu & Xu, 1990). The sucrose, total soluble sugar, and starch content in bulblets were measured using the anthrone colorimetric method (Zhang, 2001). The activities of amylases were determined using 3,5-dinitrosalicylic acid colorimetry (Li, 2000). The activity of starch synthase was measured using Fangmin's method with some modifications (Zheng *et al.*, 2012). The levels of endogenous hormones were determined using the ELISA method. The ELISA test kit was provided by the Center of Crop Chemical Control in China Agricultural University.

The data were statistically analyzed using Excel 2007 and SPSS 18.0. Duncan's test was performed using one-way ANOVA. Bivariate correlation analysis was performed based on data from C0 to C5 before and after treatment.

Results

Bulblet growth: Twelve weeks after CPPU treatment at six different concentrations, the fresh weight of the bulblets in C4 treatment was significantly higher than that in C0; the bulblet diameter after C1 treatment was significantly larger than in C0. No other obvious changes were observed in other treatments in comparison with C0 (Table 2).

Carbohydrate content in *L. sprengeri* bulblets: The starch concentration in *L. sprengeri* bulblets was significantly higher (1.55 times) in C4 than in C0 12 weeks after CPPU treatment; other treatments showed no such difference from C0. C5 significantly decreased the total soluble sugar content in the bulblets, with no other significant changes in other CPPU treatments. Changes in sucrose content were similar to those in total soluble sugar content for all treatments compared to C0 (Table 3).

The microstructure of the bulb scales was observed after PAS staining, and uneven starch granule size distributions were observed in the scales. Most starch granules were small and round. In each cell of the bulb scale in the C0 treatment, the average number of starch granules was 5.6. Under 400x magnification, the average number was approximately 96.3. The average number of starch granules in each cell of the scales in C4 was 7.7. Under 400x magnification, the average number was 124.7(Fig. 1).

Activities of starch metabolism related enzymes in *L. sprengeri* bulblets: The β-amylase activities in bulblets in the CPPU treatments significantly increased compared to the control. The highest activity for β-amylase was found in C4, which was 7.25-fold higher than C0. The tendency of activity changes in (α+β)-amylase was consistent with that of β-amylase (Fig. 2).

After CPPU treatment for 12 weeks, the activities of AGPase and starch-bound starch synthase (GBSS) in *L. sprengeri* bulblets in C3 was significantly higher than in the control. The activities of SSS in *L. sprengeri* bulblets in C1 were significantly higher than in the control.However, AGPase activity slightly decreased in C5. No significant changes were found for other CPPU treatments (Fig. 3).

Table 1. Concentrations of CPPU for treatment.

Treatment	C0	C1	C2	C3	C4	C5
Concentration (mg L^{-1})	0	1.0	2.0	5.0	7.5	10.0

C represents CPPU

Table 2. Bulblet diameter and fresh weight of *L. sprengeri* bulblets 12 weeks after CPPU treatment.

Treatment	Diameter (mm)	Fresh weight (g)
C0	10.21 ^{bc}	1.35 ^{bc}
C1	11.45 ^{a}	1.75^{ab}
C2	9.91 ^{c}	1.31 ^{c}
C3	11.29 ^{ab}	1.66 ^{abc}
C4	11.18 ^{ab}	1.77 ^{a}
C5	10.49 ^{abc}	1.44 ^{abc}

Values are mean ± SD. Different letters in each column indicate significant differences (p≤0.05) according to Duncan's Multiple Range Test

Table 3. Carbohydrate contents in bulblets of *L. sprengeri*12 weeks after CPPU treatment.

Treatment	Sucrose content (mg g^{-1} FW)	Content of total soluble sugar(mg g^{-1} FW)	Starch content (mg g^{-1} FW)
C0	59.00 ± 5.8^{ab}	83.239 ± 6.7^{ab}	84.93 ± 4.6^{b}
C1	68.34 ± 9.0^{a}	82.847 ± 8.8^{ab}	89.29 ± 13.7^{b}
C2	64.34 ± 0.8^{ab}	84.651 ± 4.6^{ab}	100.27 ± 4.5^{b}
C3	68.73 ± 2.1^{a}	86.298 ± 3.4^{ab}	104.82 ± 3.8^{b}
C4	62.46 ± 2.9^{ab}	91.082 ± 5.1^{a}	131.33 ± 2.6^{b}
C5	47.08 ± 8.0^{b}	63.396 ± 3.7^{c}	84.14 ± 7.9^{b}

Values are mean ± SD. Different letters in each column indicating significant differences (p≤0.05) according to Duncan's Multiple Range Test

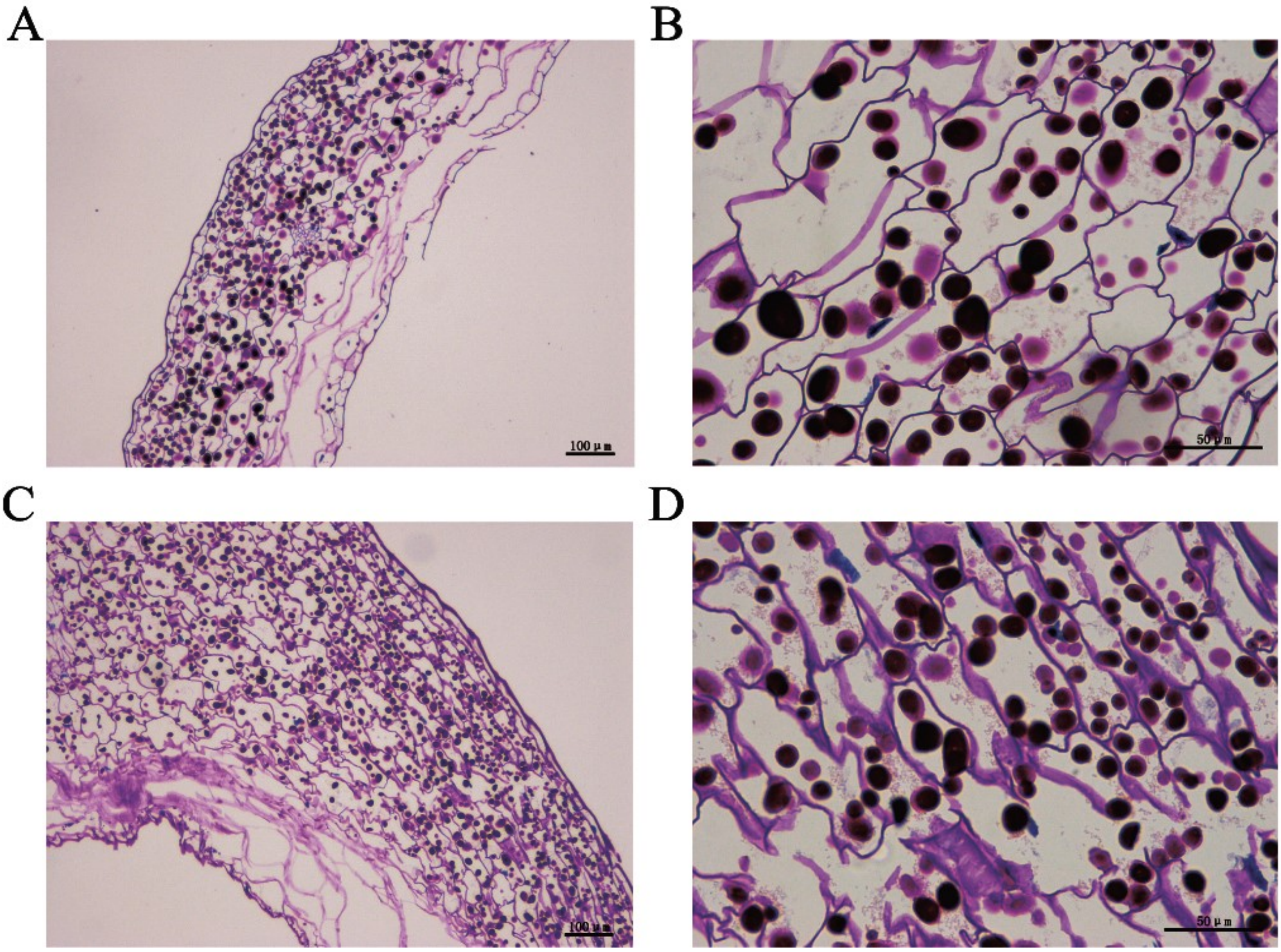

Fig. 1. Starch granule distribution in *L. sprengeri* bulblets.
A, B: *L. sprengeri* bulblets after 0mg/L CPPU treatment; C, D: *L. sprengeri* bulblets after 7.5mg/LCPPU treatment A, C (100×); B, D (400×).

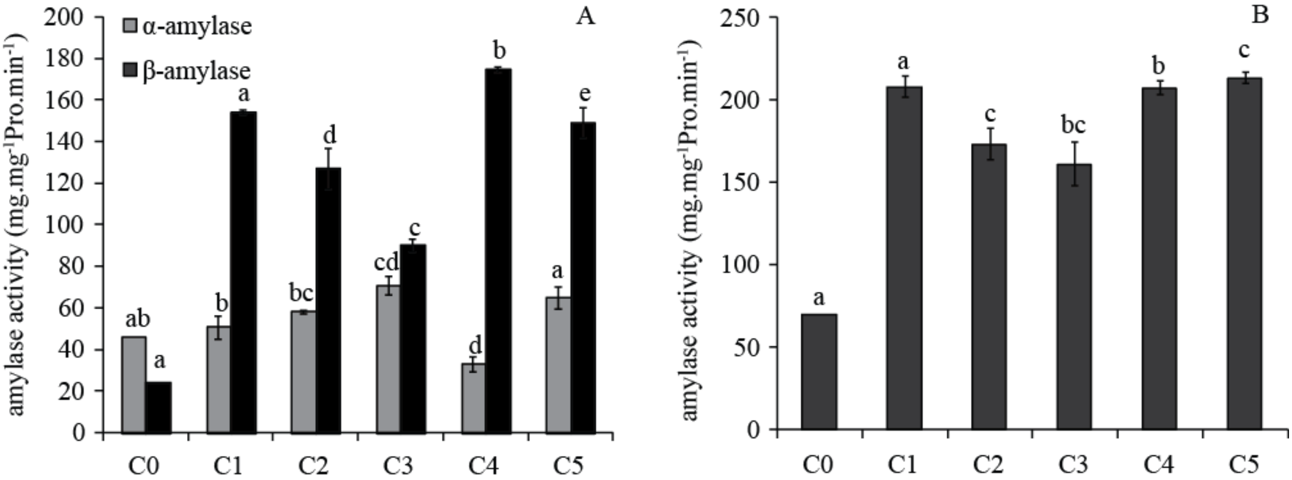

Fig. 2. Amylase enzyme activity in *L. sprengeri* bulblets 12 weeks after CPPU treatment.
A, B: Amylase enzyme activity in *L. sprengeri* bulblets
Each value represents mean ± SD. Lines with different letters indicate significant differences (p≤0.05) according to Duncan's Multiple Range Test.

Endogenous hormone levels in *L. sprengeri* bulblets: 12 weeks after CPPU treatment, the zeatin riboside (ZR) levels in the bulblets of C1 to C5 were significantly higher than in the control, with the ZR level of C4 reaching 1.50-fold that of the control. The indole acetic acid (IAA) levels in the bulblets of C2, C3, and C5 significantly increased in comparison with the control; the IAA level of C5 was 1.39-fold higher than the control. Twelve weeks after CPPU treatment, the gibberellic acid (GA), abscisic acid (ABA), and jasmonic acid (JA) levels in the bulblets of each treatment significantly decreased in comparison with those of C0. The GA level of C2 was 0.62-fold that of C0. The ABA and JA levels of C3 were 0.48 and 0.64 times that of C0, respectively (Table 4).

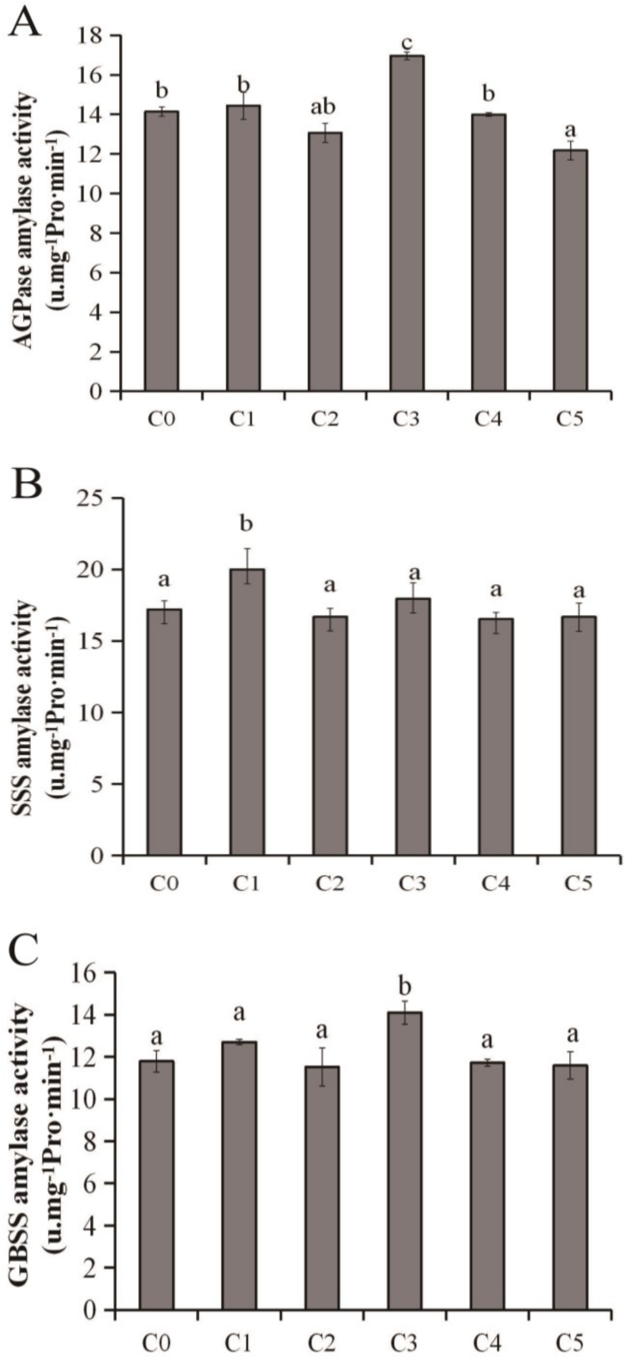

Fig. 3. Starch-synthesizing enzyme activity in *L. sprengeri* bulblets 12 weeks after CPPU treatment.

A, B, C: Starch-synthesizing enzyme activity in *L. sprengeri* bulblets. Each value represents mean ± SD. Lines with different letters indicate significant differences ($p<0.05$) according to Duncan's Multiple Range Test

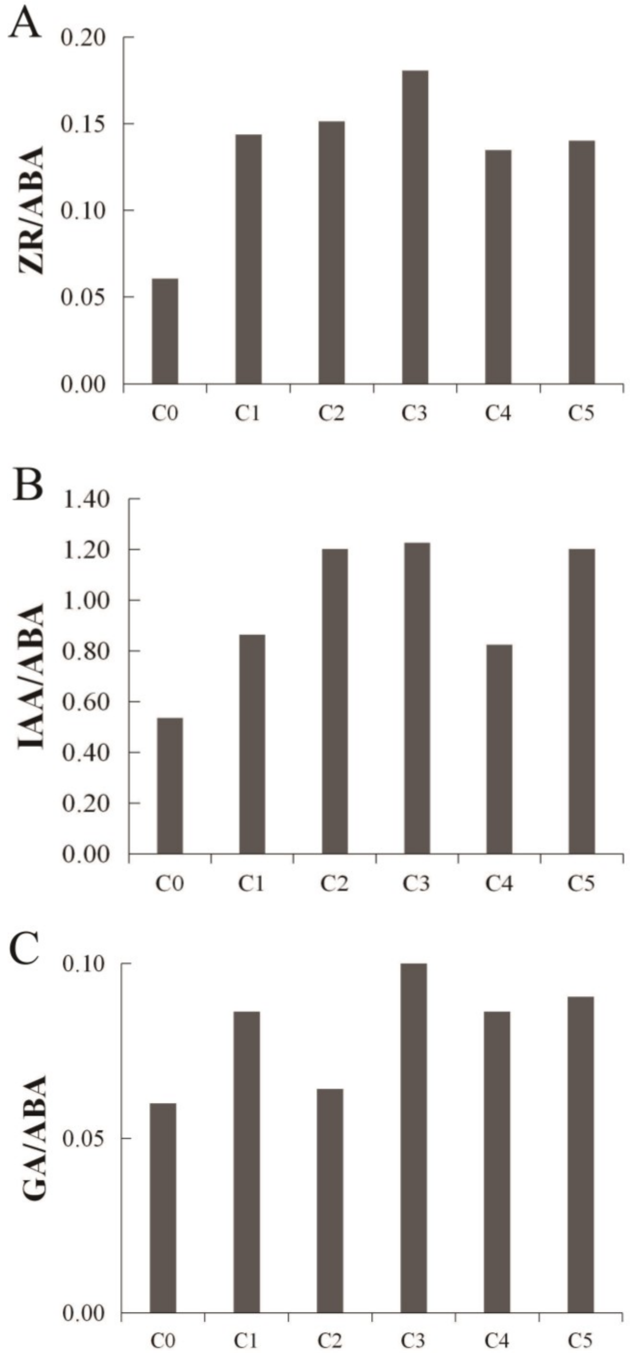

Fig. 4. Ratios of endogenous hormones in *L. sprengeri* bulblets 12 weeks after CPPU treatments.

Each value represents mean ± SD. Lines with different letters indicate significant differences ($p \leq 0.05$) according to Duncan's Multiple Range Test.

Table 4. Levels of endogenous hormones in the bulblet of *L. sprengeri* 12 weeks after CPPU treatment.

Treatment	GA ($mg\ g^{-1}$ FW)	ABA ($mg\ g^{-1}$ FW)	IAA ($mg\ g^{-1}$ FW)	JA ($mg\ g^{-1}$ FW)	ZR ($mg\ g^{-1}$ FW)
C0	4.53 ± 0.04^{a}	75.49 ± 0.35^{a}	40.53 ± 0.72^{de}	47.75 ± 0.72^{a}	4.58 ± 0.04^{c}
C1	3.91 ± 0.01^{d}	45.37 ± 0.47^{c}	39.20 ± 0.14^{e}	44.84 ± 0.55^{b}	6.52 ± 0.03^{b}
C2	2.83 ± 0.01^{f}	44.17 ± 1.34^{c}	53.04 ± 0.68^{b}	$34. 59 \pm 0.23^{c}$	6.69 ± 0.02^{ab}
C3	3.76 ± 0.03^{e}	36.41 ± 0.474^{a}	44.67 ± 0.50^{c}	30.33 ± 0.43^{e}	6.58 ± 0.06^{b}
C4	4.39 ± 0.03^{b}	50.91 ± 0.68^{b}	41.97 ± 0.55^{d}	30.77 ± 0.29^{de}	6.87 ± 0.07^{a}
C5	4.23 ± 0.02^{c}	46.74 ± 0.25^{c}	56.17 ± 0.56^{a}	32.24 ± 0.70^{d}	6.55 ± 0.09^{b}

Values are mean ± SD. Different letters in each column mean significant differences ($p \leq 0.05$) according to Duncan's Multiple Range Test

Table 5. Correlation between bulblet development and physiological and biochemical indices of *L. Sprengeri*.

Index	Fresh weight	Diameter	Sucrose	Total soluble sugar	Starch	α-amylase	β-amylase	(α+β)-amylase	AGPase	SSS	GBSS	ZR/ ABA	IAA/ ABA	GA/ ABA
Fresh weight	1													
Diameter	**0.974****	1												
Sucrose	0.678	0.681	1											
Total soluble sugar	0.534	0.490	**0.858***	1										
Starch content	**0.822***	0.725	0.618	0.722	1									
α-amylase	0.197	0.231	0.145	-0.265	-0.041	1								
β-amylase	0.723	0.656	0.305	0.177	0.675	0.089	1							
(α+β)-amylase	0.751	0.707	0.316	0.102	0.628	0.280	**0.978****	1						
AGPase	0.593	0.679	**0.791***	0.655	0.475	0.338	0.017	0.105	1					
SSS	**0.830***	**0.850***	**0.867***	0.608	0.569	0.423	0.331	0.414	**0.836***	1				
GBSS	**0.790***	**0.845***	0.754	0.472	0.542	0.585	0.313	0.438	**0.895****	**0.942****	1			
ZR/ABA	0.731	0.72	0.586	0.296	0.647	0.619	0.734	**0.824***	0.510	0.659	0.750	1		
IAA/ABA	0.506	0.457	0.303	0.003	0.453	**0.793***	0.608	0.722	0.267	0.453	0.577	**0.909****	1	
GA/ABA	**0.884****	**0.909****	0.486	0.243	0.668	0.518	0.671	**0.773***	0.614	0.738	**0.851***	**0.855***	0.718	1

Note: * and ** represent significance at the 0.05 and 0.01 levels, respectively

The ratios of ZR/ABA, IAA/ABA, and GA/ABA were calculatedto reveal the variation trends for endogenous hormones. These ratios in C1 to C5 significantly increased in comparison with the control 12 weeks after CPPU treatment. All differences reached significance levels, except for the GA/ABA ratio in C2(Fig. 4).

Correlation analysis between bulblet growth of *L. sprengeri* and the physiological and biochemical indices: The results indicated that the bulb growth of *L. sprengeri* showed an extremely significant positive correlation with the endogenous GA/ABA ratio. A significantly positive correlation was also found between the contents of starch and the activities of soluble starch synthase (SSS) and GBSS. A significantly positive correlation was observed between the sucrose content in the bulblets and the content of total soluble sugar together with the activities of starch synthase(AGPase, SSS). The activities of α-amylase and (α+β)-amylase in the bulblets showed a significantly positive correlation with the ratios of ZR/ABA, IAA/ABA, and GA/ABA. The activity of GBSS exhibited a significantly positive correlation with GA/ABA ratio. The activity of β-amylase demonstrated an extremely significant correlation with the activity of (α+β)-amylase. The activity of GBSS showed an extremely significantly positive correlation with the activity of AGPase and SSS; the activity of SSS and AGPase also showed a significantly positive correlation (Table 5).

Discussion

Effects on growth and carbohydrate content in bulblets: Applying CPPU at an appropriate concentration can facilitate the enlargement of *L. sprengeri* bulblets. The fresh weight and starch content in the bulblets of C4 were 1.31-fold and 1.55-fold higher than the control, respectively. The optimal concentration of CPPU was 7.5 mg L^{-1} in the current study. The microscopic observation of the bulb scales of C4 showed that most starch granules in the cell lumen were small and round 12 weeks after CPPU treatments, indicating high starch metabolism at this stage. In general, smaller starch granules in *Lycoris* species correspond to higher metabolic activities because newly synthesized starch granules are immediately degraded into soluble sugar to be used in plant growth (Wang, 2011).

Similar research has shown that the exogenous application of plant growth regulators affects the starch metabolism of underground bulblets. CPPU is currently used in various crops (Zhao, 2012), vegetables (Wang, 2001; Jin, 2010), and fruit trees (Fang & Wei, 2009; Hou *et al.*, 2012) because it promotes cell division and elongation, bulb enlargement and yield. At the early stage of potato tuber formation, the exogenous application of cytokinins could increase the starch synthesis ability in plants by 25% to 50% (Borzenkova *et al.*, 1998). The addition of IAA into MS increases starch content and starch granule size in potato in vitro (Gukasyan *et al.*, 2005). The CCC treatment of potato promoted the transfer of photosynthetic products to the tubers of potato, thereby promoting tuber growth and increasing yield (Wang *et al.*, 2009). In the current study, the starch content in *L.*

sprengeri bulblets after 7.5 mg/L CPPU treatment was 1.55-fold higher than the control, indicating that an appropriate concentration of CPPU treatment could accelerate starch accumulation in the bulblets and promote bulb enlargement. The exogenous application of CPPU provides a possible chemical method to accelerate the growth and development of *Lycoris* bulbs.

Effects on the biochemical indices of bulblets: The activities of starch synthase in *L. sprengeri* bulblets were increased after CPPU treatment. The activities of AGPase and GBSS after 5mg/L CPPU treatment significantly increased compared with that of the control treatment, similar to that observed for hybrid rice treated with CPPU (Tang *et al.*, 2002). The CPPU treatment significantly increased the activity of amylase (approximately 2.3- to 3.1-fold) compared with the control. Thus, starch metabolism in the bulblets was active. As an amylase enzyme, β-amylase exhibited the highest activity, indicating that β-amylase is the most important starch decomposition enzyme in *L. sprengeri* bulblets. This result is consistent with the results of CPPU application in *L. radiata* (Chang *et al.*, 2013). This finding can also be confirmed from an enzymological perspective; β-amylase is the key enzyme in the bulb growth of *Lycoris*, as indicated by transcriptomics (Chang *et al.*, 2013).

While studying of the effects of CPPU on endogenous hormone levels in *L. sprengeri* bulblets, the most prominent results were an increase in ZR level and decrease in ABA level. ZR, an important component of cytokinin, promotes cell division. Wang *et al.* (2005) demonstrated for potato that the ZR level has a significantly positive correlation with tuber yield. At the early stage of carrot fleshy root enlargement (seedling age: 45 d to 75 d) (Yang *et al.*, 2011) and the rhizome formation period of lotus (Xu, 2002), ZR promotes rapid cell division. The exogenous application of CPPU promoted bulblet growth by increasing the level of ZR, as reported for lily treated with salicylic acid (SA) (Fang & Wei, 2009).

Of all plant hormones, endogenous GA has the most definite inhibitory effect on tuber formation (Guo *et al.*, 1991). GA delays the bulb formation of onions (Kato, 1965), inhibits the tuber formation of potatoes (Quan *et al.*, 2001), and hinders the bulblet enlargement of *Gradiolus grandavensis* (Qian & Yi, 2006). Our results also demonstrated that the endogenous GA levels in the *L. sprengeri* bulbs in each treatment were lower than in the control after exogenous CPPU treatment at five concentrations (C1 to C5). This finding indicated that CPPU promoted bulblet development by decreasing GA levels, as had been found for other bulbs.

Endogenous ABA inhibits growth and accelerates aging. Many researchers have confirmed the ability of ABA to inhibit garlic bulb enlargement (Li & Zhou, 2002) and the lotus rhizome enlargement (Xu, 2002). In this study, ABA levels in the bulblets of *L. sprengeri* significantly decreased in C1 to C5, which is similar to our previous study of CPPU application in *L. radiata* (Xiao *et al.*, 2013). Promoting *L. sprengeri* bulb growth by decreasing ABA levels is a biochemical pathway for exogenous CPPU application.

Correlation analysis of hormone balance and physiological and biochemical indices: The balance of endogenous hormones acts as a regulator for the occurrence and growth of bulblets. At the early stage of garlic bulb formation, the GA/ABA ratio decreases and the IAA/ABA ratio increases (Liu *et al.*, 1997). Xia *et al.* (2005) suggested that the GA/ABA ratio affects the accumulation and distribution of carbohydrates in tulip bulbs. These researchers suggested that lower GA/ABA ratios contribute to the transport of carbohydrates. In the current study, the ratios of endogenous IAA/ABA, ZR/ABA, and GA/ABA in *L. sprengeri* bulblets were all higher than those of the control 12 weeks after CPPU treatment. This finding is consistent with the results for SA-treated lily (Fang & Wei, 2009). For *L. sprengeri*, the GA/ABA ratios in all CPPU treatments were higher than in the control, which was opposite of changes for tulip bulbs but similar to changes for *L. radiata* (Xiao *et al.*, 2013). Further study is needed to determine whether this finding is caused by the difference in growth stages resulting from the different species and seedlings under investigation.

The indices for bulblet growth, carbohydrate content, activities of starch metabolism-related enzymes and the ratios of endogenous hormone levels were subjected to correlation analysis (Table 5). A significantly positive correlation was found between bulblet growth and starch content and between SSS and GBSS activities. This study is the first to correlate the activities of starch synthase and starch accumulation with the bulblet enlargement of *Lycoris*. A similar finding was reported for chesnut fruit growth (Xie *et al.*, 2012).

Bulb growth showed an extremely significantly positive correlation with the endogenous GA/ABA ratio in *L. sprengeri*. This finding indicated that a higher GA/ABA ratio was beneficial to bulb growth. This result differs from that obtained for tulip bulbs (Xia *et al.*, 2005) and lily (Zheng, 2011). In *L. sprengeri* bulblets, the GA/ABA ratio showed a significantly positive correlation with the activities of (α+β)-amylase and GBSS. Thus, the high GA/ABA ratio was related to the high starch metabolism level of *L. sprengeri* bulblets, which may be the mechanism for the promotion of *L. sprengeri* bulb growth.

The activity of β-amylase in the bulb showed an extremely significantly positive correlation with that of (α+β)-amylase. This result is similar to the findings of Chang *et al.* (2013). GBSS activity showed an extremely significantly positive correlation with the activities of AGPase and SSS, whereas the activities of SSS and AGPase were significantly positively correlated. This finding suggested that the three starch synthases were synergistic. In subsequent experiments, comparison of gene expression levels in *L. sprengeri* before and after CPPU will provide greater insight into the screening of key genes related to bulblet development.

Acknowledgements

This work was supported by the Science and Technology Project of Zhejiang Province (2012C12909).

References

Adams, P. 1976. Lycoris-surprise lilies. *Pacific Horticulture*, 37: 23-29.

Borzenkova, R., E. Sobyanina, A. Pozdeeva and M. Yashkov. 1998. Effect of phytohormones on starch-synthesizing capacity in growing potato tubers. *Russ. J. Plant Physiol.*, 45(4): 472-480.

Chang, L., Y.M. Xiao, L.F. She and Y.P. Xia. 2013. Analysis of gene expression and enzyme activities related to starch metabolism in *Lycoris sprengeri* bulbs of different sizes. *Sci. Hort.*, 161: 118-124.

China Flora, 1985.*The Flora of China*. 16th Edition. Council of Flora of China Editors from the Chinese Academy of Sciences. Science Press, Beijing, P.R. China. 20 pp. (In Chinese.)

Fang, L. and Z.Y. Wei. 2009. Effects of salicylic acid on bulb development and its relation to endogenous hormone contents in two species of lily. *Plant Physiol. Comm.*, 45(11): 1085-1088.

Gukasyan, I.A., S.A. Golyanovskaya, E.V. Grishunina, T.N. Konstantinova, N.P. Aksenova and G.A. Romanov. 2005. Effect of rol transgenes, IAA, and kinetin on starch content and the size of starch granules in tubers of *In vitro* potato plants. *Russ. J. Plant Physiol.*, 52(6): 809-813.

Guo, D.P., Z.T. Ying and G.A. Shan. 1991. Plant hormones andpotato tuber formation. *Plant Physiol. Comm.*, 27: 130-133. (In Chinese.)

Hou, Y.R., B.G. Wang, X.Y. Feng, Y. Yang, L. Shi, W.S. Li and J.H. Wang. 2012. Residues of plant growth regulators in fruit and regulation on fruit quality in summer black grape. *J. Fruit Sci.*, 29: 36-41.

Hu, S. and L.Y. Xu. 1990. A cytochemical technique for demonstration of lipids, polysaccharides and protein bodies in thick resin sections. *J. Integr. Plant Biol.*, 32: 841-846.

Ji, C.F. 2002. Development and utilization of *Lycoris* resources. *Chinese Wild Plant Resources*, 21: 14. (In Chinese).

Jin, H. 2010. *The Effect of CPPU on Growth Development and Endogenous Hormones in Cucumber*. Shandong Agricultural University.

Kato, T. 1965. Physiological studies on bulb formation and dormancy in the onion plant. V. The relation between the metabolism of gibberellin and nucleic acid and bulbing phenomenon. *J. Jpn. Soc. Food Sci.*, 34: 305-307.

Li, C.X. and X. Zhou. 2002. Effect of methyl jasomonate on bulb expansion and endogenous plant hormone in *Allium statium* L. *Life Sci. Res.*, 6(2): 183-185.

Li, H.S. 2000. *Principle and Technology of Plant Physiological and Biochemical Experiments*. Higher Education Press, Beijing.

Liu, G.Q., S.J. Li and X.P. Zhang. 1997. Effect of temperatureand photoperiod on garlic plantlet bulbing and endohormonevariation *In vitro*. *Acta Horticulturae Sinica*, 24: 165-169. (InChinese.)

Qian, S.L. and M.F. Yi. 2006. Analysis on the changes of endogenous hormones with gladiolus cormels during different growth and development stages. *J. Agric. Univ. Hebei*, 29: 9-12.

Quan, F., A.X. Zhang and X.W. Cao. 2001. The role of plant hormones in potato tuber formation and development process. *Chinese Potato*. 16: 29-32.

Quan, F., A.X. Zhang and X.W. Cao. 2002. Role of plant hormones in potato tuber formation and development process. *Chinese Potato*, 16: 29-32. (In Chinese).

She, L., Y. Xia, L. Chang, Y. Xiao, Z. Ren and L. Zhang. 2014. Biochemical and physiological responses of bulblets of *Lycoris aurea* to exogenously applied N-(2-chloro-4-pyridyl)-N1-phenylurea. *J. Hort. Sci. Biotechnol.*, 89(5): 549-556.

Tang, X.R., Z.W. Tan, Z.L. Li and T.Q. Yu. 2002. Effects of CPPU and PP333 on three starch synthase activity and grain quality of hybrid rice. *Hybrid Rice*, 17: 44-46.

Wang, H., H. Li, F. Liu and L. Xiao. 2009. Chlorocholine chloride application effects on photosynthetic capacity and photoassimilates partitioning in potato (*Solanum tuberosum* L.). *Sci. Hort.*, 119(2): 113-116.

Wang, Q. 2001. Effect of CPPU on growth and endogenous phytohormonne contents of balsmpear (*Momordica charantia* L.) fruit. *J. of Zhejiang Uni. Agri. & Life Sci.,* 27(5): 513-517.

Wang, Q., L. Zhang and Z. Wang. 2005. Formation and thickening of tuberous roots in relation to the endogenous hormone concentrations in sweet potato. *Sci. Agri. Sin.*, 38(12): 2414-2420.

Wang, X.J. 2011. *Studies on Accumulation of Starch and Growth of* Lycoris Chinensis Traub *Bulbs*. Nanjing Forestry University.

Watanabe, H. 1989. The use of growth regulators applied to stem cuttings of lilies. Bull. Nara *Agri. Exp. Stn*., 20: 67-71.

Xia, Y.P., C.H. Huang, H.J. Zheng and X.C. Gao. 2005. Advances in researches on bulb development of *Lilium* spp. and its physiological mechanisms. *Acta Hortic. Sin.*, 32(5): 947-953.

Xiao, Y.M., L.F. She, L. Chang and Y.P. Xia. 2013. Effect ofthree plant growth regulators on the bulb development of *Lycoris radiata*. *Acta Agriculturae Nucleatae Sinica*, 27: 1409-1415. (In Chinese).

Xie, P., S.J. Guo, H. Xiong, G.H. Li and W.J. Lü. 2012. Changes in sugar, starch, some enzymes involved and their relationships during the development of Chinese chestnut. *Acta Hort. Sin.*, 39(12): 2369-2376.

Xu, C. 2002. *Studies on Physiological and Biochemical ChangesDuring the Formation of Swollen Lotus Rhizomes*. M.S. Thesis.Yangzhou University, Yangzhou, Jiangsu, P. R. China. 12 pp. (In Chinese).

Xu, Z.T. 1989. The future of study and cultivation on the emerging bulb flowers - *Lycoris* and *Nerine*. *Taiwan Flower Art*, 49: 8-11. (In Chinese).

Yang, Y.G., S. Zhang and Y.L. Li. 2011. Endogenous hormone content in relation to thickening of carrot fleshy root during summer season in plateau region. *Chin. J. Eco-Agric.*, 19(2): 342-346.

Zhang, G.F. and K.Z. Chang. 2010. Introduction on the applied research of CPPU. *Enterprise Sci. & Technol. & Develop.*, 23: 22-24.

Zhang, L., G. Wang and F. Cao. 2002. Studies on vegetative propagation in the genus *Lycoris*. *J. Nanjing For.Univ.*, 26(4): 1-5.

Zhang, Z.L. 2001. *Experiments in Plant Physiology*. HigherEducation Press, Beijing, P. R. China. 35 pp. (In Chinese).

Zhao, F. 2012. *Effect of CPPU on Some Physiology-Biochemistry Traits and Epigenetics in the* Triticum aestivum *L*. Agricultural University of Hebei.

Zheng, R.R. 2011. *Investigation on the Influence of Plant Growth Retardants on Plant Growth and Bulb Carbohydrate Accumulation in* Lilium *Oriental Hybrids 'Sorbonne*. Zhejiang University.

Zheng, R.R., Y. Wu and Y.P. Xia. 2012. Chlorocholine chloride and paclobutrazol treatments promote carbohydrate accumulation in bulbs of lilium oriental hybrids 'Sorbonne'. *Journal of Zhejiang University-Science B (Biomedicine & Biotechnology)*, 13(2): 136-144.

(Received for publication 16 June 2016)