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Full Length Research Paper

Growth and photosynthetic responses of *Lycoris haywardii* Traub to watering frequencies

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To seek a way to solve the slow growth of bulbs of *Lycoris*, a pot experiment with three replications was conducted to evaluate the effects of watering frequency on growth, net photosynthetic rate and bulb biomass of *Lycoris haywardii* under greenhouse condition. The results revealed that leaf number, leaf length and fresh bulb weight was increased by 67, 41 and 323% respectively, under the irrigation frequency of once a month, which were significantly greater than that of other treatments. And leaf number increase rate was positively correlated (r=0.97, *P*<0.01) with the irrigation frequency, so was fresh bulb increase rate (r=0.98, *P*<0.01). However, no significant differences were observed with regard to bulb number in all the treatments. The species with a good ability of drought resistance can get by under irrigation frequency of once in every three months. The maximum net photosynthetic rate reached 18.0 μmol/m^2/s or higher, and the light saturation point was near or higher than 2000 μmol/m^2/s, therefore, a full-light management was recommended for the cultivation of *L. haywardii*. It took about a week to recover the normal photosynthetic ability after a severe drought stress. On the contrary, short-term drought stress had no more negative effect on net photosynthetic rate but remarkable light compensation effect. Higher temperature (under 22°C) was favourable for photosynthesis. Cultivation management in dormancy stage was necessary for increasing bulb yield of *Lycoris.*

Key words: Watering interval, drought resistance, photosynthesis, biomass, cultivation.

INTRODUCTION

Lycoris haywardii, a species of Amaryllidaceae, is a perennial herb with bulbs. Its leaves appear from early October to early May of the following year. Its perianth is reddish violet, a little lighter than that of *L. sprengeri*, and changes to ink-blue at the apex (Hsu et al., 1994). *Lycoris* spp. have a great ornamental value for their beautiful flowers and attractive foliage and important medicinal value in bulbs (Liu et al., 2012; Zhang and Cao, 2001). Species of *Lycoris* have a low reproduction rate, low field yield for bulb production and no technique for effective cultivation and it will take about five years to produce flowers from seedlings (Zhang and Cao, 2001). Slow growth of bulbs is a major problem in cultivation, which has not been solved effectively yet. It needs further exploratory cultural practices. We have conducted a series of studies on that problem, however, results revealed that fertilization does not increase bulb yield (Bao et al., 2012b). Species of *Lycoris* have nutrient strategies of slowly growing (Bao et al., 2012a; Chapin, 1980) and distinct eco-mechanism for retranslocation and conversion of nutrients (Bao et al., 2012a). In addition to fertilizer application, watering management is also important for cultivation. But *Lycoris* is usually regarded as a plant resistant to drought (Qin et al., 2003; Zhang

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Table 1. Soil Relative Water Content (SRWC) of the sand at different stages in early 2012.

Test date	SRWC (%)				
yyyy-mm-dd	A1	A2	A3	A4	A6
2012-01-03	61.9	52.3	2.7	2.6	2.4
2012-01-31	82.1	29.4	60.8	1.2	1.1
2012-02-28	75.2	67.0	32.9	46.4	2.3
2012-04-01	33.5	14.6	6.5	8.3	1.7
2012-04-30	20.5	17.6	13.4	2.7	17.1

and Cao, 2001). Furthermore, species of *Lycoris* are in dormant stage in hot summer when plants need more water management. As a result, very little attention is paid to the water management for *Lycoris* spp. Although there are many studies on the water effect on plant growth (Flagella et al., 2002; Rawson and Turner, 1983; Shan et al., 2010; Su et al., 2007; Xia et al., 2001), very limited information is available about the relationship between water and growth of *L. haywardii*. In order to develop a reasonable watering regime and evaluate the influence of irrigation on growth of *L. haywardi*, the effect of different watering levels on growth was comparatively studied.

MATERIALS AND METHODS

This study was conducted in a greenhouse at Hangzhou Botanical Garden (120°16'E, 30°15'N), Zhejiang Province, China. Seventy-five bulbs were selected from the Garden's nursery, which were as uniform in weight (≈15.5 g each bulb in wet weight), and every five bulbs were planted in each pot (20 cm in height, 20 cm in top width and 60 cm in top length) on 16 August 2010 with river sand as a medium, which had a total nitrogen content of 0.2 g/kg, a total phosphorus content of 0.07 g/kg, a total potassium content of 4.5 g/kg, an organic matter content of 2.4 g/kg, and a pH of 5.6.

With a randomized complete block design, the experiment was performed with one single factor at five levels (or five treatments) of watering intervals, viz. A1 (once every 1 month), A2 (once every 2 months), A3 (once every 3 months), A4 (once every 4 months), and A6 (once every 6 months) and three replications. The amount of water applied each time was 3.5 L per pot to ensure sand drenched. The first watering date was on 25 September 2010. Afterwards re-watering was implemented at the end or the beginning of a month according to the design until the last watering date on 30 April 2012.

Alive leaf length and number of each clump were first recorded on 9 March 2011 when leaves almost reached the stage of most vigorous vegetative growth in the first growing season after planting, followed by the second recording on 6 May, 2011 at the late stage of leaves. The following ten recordings were conducted in the second growing season from autumn of 2011 to spring of 2012, about once every 3 w during the leaf stage of all plants. The bulbs were harvested on 4 June, 2012. Samples were dried in an oven at 85°C for 48 h after cleaning.

The net photosynthesis rate (Pn) was measured using a portable gas exchange system Li-6400XT (Li-COR，USA) at the middle-upper part of the second leaf from bottom of a randomly selected plant in each pot based on an open air path, natural atmospheric temperature and CO_2 concentration in the greenhouse. Air flowing speed and photosynthetic photon flux (*PPF*) were set at 500 μmol/s and 800 μmol/m^2/s, respectively. Totally 17 measurements were carried out from January to April 2012, just prior to re-watering and 3, 6 and 14 days after re-watering. Besides, a sand sample in 10 cm depth was taken just before re-watering to be dried in an oven at 105°C for 24 h for the measurement of soil relative water content (SRWC). Dates for soil-sampled were shown in Table 1.

Light response curves were developed using the following *PPF*: 2000, 1800, 1600, 1400，1200, 1000, 800, 600, 400, 200, 100, 50 and 0 μmol/m^2/s at the middle-upper part of the second leaf from the bottom of a randomly selected plant for each treatment. Before recording, the system was adjusted for baseline for 30 min at 2000 μmol/m^2/s.

Data were analyzed using MS Excel 2003 and SPSS 16.0. Statistical significance was determined at $P \leq 0.05$. Differences between means were compared according to an LSD test.

RESULTS AND DISCUSSION

Watering interval and soil relative water content

SRWC is a ratio of soil water content to field saturated moisture capacity (Shan et al., 2010; Shangguan, 1997). SRWC of the medium just before re-watering at the end or the beginning of a month was shown in Table 1. The scope of SWRC of A1 was 20.5 to 82.1%. It indicated that treatment A1 could keep a higher SWRC in winter, but in early spring the interval might be too long. It was obvious that the longer a re-watering interval, the lower the SRWC. We estimated that it would be necessary to water when SRWC reached about 50%.

Impact of watering frequency on leaf number

The dynamic change in average leaf count per clump for each treatment was showed in Figure 1. It is obvious that the leaf number of treatment A1 was the highest, and A6 was the lowest. Analysis of variance (ANOVA) indicated that there was no significant difference among A1, A2 and A3, but there was significant difference between the group of A1, A2, A3 and the group of A4, A6 on 9 March 2011. Leaf number showed 3 levels on 16 November 2011 and 27 February 2012 in the second growing season, in which A1 had significantly more leaves than

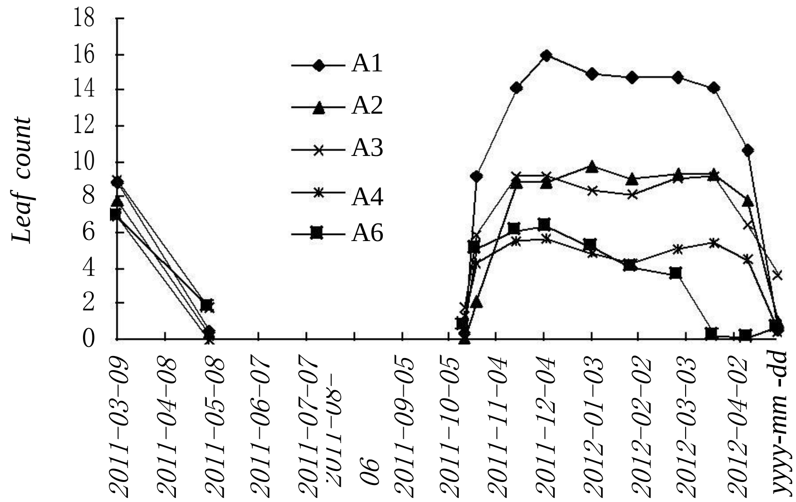

Figure 1. Dynamic changes in average leaf count per clump in different treatments.

A2 and A3, and the latter two also had significantly more leaves than A4 and A6. The ratio of the leaf number on 27 February, 2012 to that on 9 March, 2011 indicated the extent to which leaf number increased. The ratio of A1, A2, A3, A4 and A6 was 1.67, 1.19, 1.01, 0.75 and 0.52, respectively. The leaf number of treatments A1, A2 and A3 was increased by 67, 19 and 1% respectively. However, that of A4 and A6 was decreased by 25 and 48%, respectively. If watering frequencies were quantified (that is, A1, A2, A3, A4 and A6 were valued as 1, 1/2, 1/3, 1/4 and 1/6, respectively), it was known from regression analysis that the increase rate of the leaf number was significantly in positively linear correlation with watering frequency (correlation coefficient r = 0.97. *P* = 0.008), which indicated the higher the irrigation frequency, the more leaf number. The linear regression was shown as following:

$$Y = 127.487X - 54.572.$$

Here independent variable X was watering frequency on a monthly basis, and Y was the percentage of increased leaf number

It was illustrated that *L. haywardii* is strong in resistance to drought, because the leaf number of A3 was not decreased obviously one year after planting (Ratio = 1.01).

The difference in leaf number was not significant between A1 and A2 or A3 on 9 March, 2011. However, in the second growing season after dormancy (samples from 16 November, 2011) the difference was significant, indicating watering in the dormancy period was necessary. It may be the reason that bud differentiation requires certain moisture, for No Leaf Period (dormancy period) is the main period of leaf bud differentiation in *Lycoris* (Li and Zhou, 2005). Moist soil can ensure the bud differentiation, and generate more leaves.

Impact of watering frequency on leaf length

The dynamic change in leaf length of five treatments is shown in Figure 2. A1 had the longest average leaf length. In general, the impact on leaf length caused by water deficit was not obvious except in A6. In case of water deficit without replenishment for more than 4 months (A6), leaves started to wither from tip, but watering brought about new leaf emerging. Based on the analysis of variance of data from representative 5 December, 2011 and 27 February, 2012, leaf length in A1 was significantly greater than that in other four treatments, among which the difference were not significant with the data from 5 December 2011. Although leaf length in A1 was still significantly greater than that in other four treatments with the data from 27 February 2012, the leaf length in A2, A3 and A4 was significantly greater than that in A6. Compared with that on 9 March 2011, the leaf length of A1 on 21 March, 2012 was increased by 41%.

Dynamic change in net photosynthetic rate (Pn) and impact of watering frequency on Pn

The maximum of Pn in A1 was in January, and the minimum was in April (Figure 3). As a whole, Pn decreased over time with rising temperature and decreasing growth potential. However, Pn was positively correlated with air temperature before March when the air temperature in the leaf chamber (rTair) was below 15°C, and photosynthesis might be prone to inhibition when rTair was about higher than 22°C, for water deficit would take place if temperature is too high (Wang, 1997). It indicated that properly increasing air temperature in winter could increase photosynthesis, which was in line with that of *Lycoris aurea* (Bao et al., 2012b). We

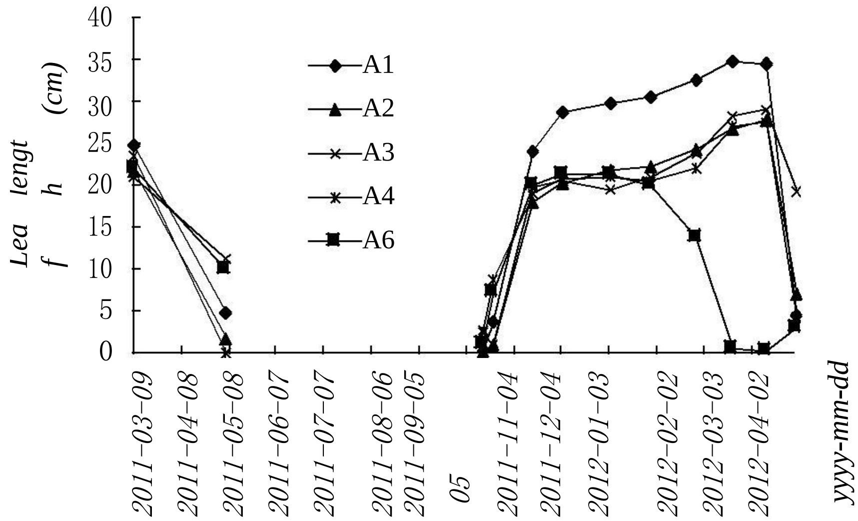

Figure 2. Dynamic changes in average leaf length per clump in different treatments.

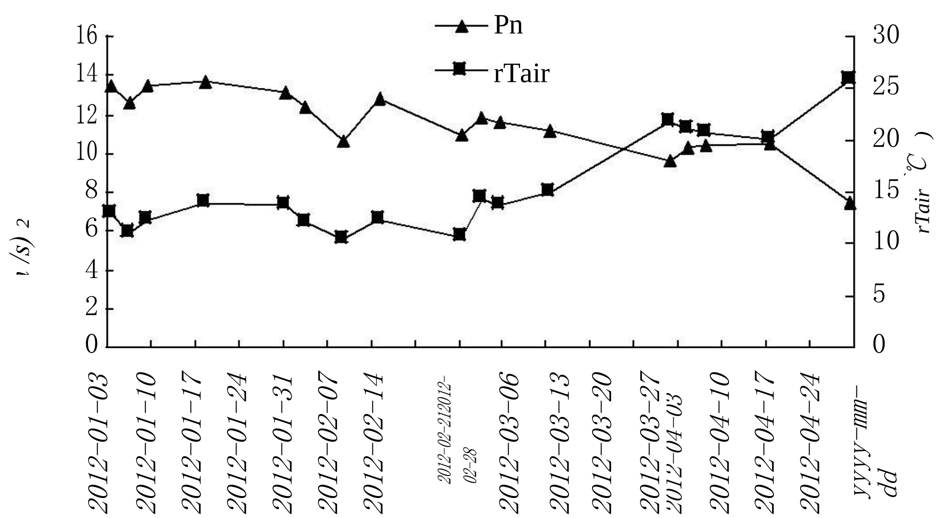

Figure 3. Dynamic changes in average Pn and rTair in Treatment A1. Pn: Net photosynthetic rate. rTair: Air temperature in the leaf chamber.

speculated that the best environmental temperature for photosynthesis should be about 22°C. And It would be better to cultivate this species in greenhouse to avoid low temperature at the leaf stage.

According to measurement, the total average Pn for A1, A2, A3, A4 and A6 was 11.55, 11.72, 11.28, 6.71 and 1.64 μmol/m^2/s, respectively. Impact of A2 on Pn was not obvious, but when watering interval was over 2 months, the effect was outstanding, such as that of A3 even in winter, and Pn appeared to be negative, but recovered to normal within six days after watering (Table 2). Pn could recover to normal photosynthesis even in treatment A4. It demonstrated that *L. haywardii* was in strong tolerance of drought and could maintain alive when relative lack of water and recovered photosynthesis after watering. As for A6, because leaves died, few new leaves appeared a week after watering, and the leaf was so long that it could be used for measurement of Pn half a month later when Pn reached a normal level again. On 30 April, 2012, RSWC of A1, A2, A3, A4 and A6 was 20.5, 17.6, 13.4, 2.7 and 17.1%, respectively, and the corresponding Pn was 7.48, 9.41, 10.34, 0.04 and 10.11 μmol/m^2/s. It indicated that *L. haywardi*i had a good physiological capability for photosynthetic recovery, with which resistance to drought such as intensity of drought tolerance, recovery rate and recovery extent after watering was stronger than that of other plants, such as wheat, maize, *Leymus chinensis,* and sunflower (Liang et al., 2009; Lin et al., 2008;

Table 2. Re-watering effect on Pn recovery in five treatments from January to April 2012[z].

Days before or after re-watering	Pn (µmol/m²/s)				
	A1	**A2**	**A3**	**A4**	**A6**
The day just before re-watering	10.95	8.57	-0.09	0.33	(NL)
3 days after re-watering	11.81	11.49	10.57	7.86	(NL)
6 days after re-watering	11.52	11.77	13.61	13.04	(LTS)
14 days after re-watering	12.08	10.71	13.55	12.23	11.86

[z] Pn: Net photosynthetic rate; NL: No leaf; LTS: The leaf was too short to measure Pn

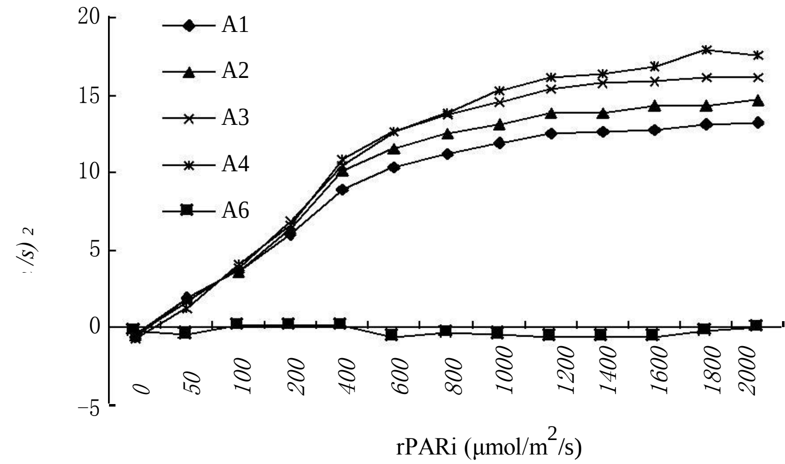

Figure 4. Light response curves of treatments on 14 February 2012. Pn: Net photosynthetic rate. rPARi: active photosynthetic radiation.

Shangguan, 1997; Yagoub et al., 2010).

Watering interval and light response

Treatment A6 had had no watering for 4 months on 14 February 2012, and the leaf was in wilting status, and total Pn was negative (Figure 4.). Photosynthesis of other treatments was normal (watered after 6 weeks for A3 and watered after 2 weeks for the others). The normal curve shape was like a parabola in line with that of *L. aurea*, *L. chinensis, L. longituba* and *L. sprengeri* (Bao et al., 2012b; Liu et al., 2012; Meng et al., 2008). The photosynthetic ability had no positive correlation with watering frequency. According to the average, photosynthetic capacity was in the following order: A4>A3>A2>A1. Lower watering frequency had a higher utilization of irradiance. That meant photosynthetic capacity was increased by watering after drought stress because of the light compensatory effect (Liu et al., 2004) We could infer that *L. haywardii* was heliophilous because of its light resistance. Light-inhibition phenomenon had not appeared at the active photosynthetic radiation (rPARi) level of 2000 µmol/m²/s, so full-light management was suggested in cultivation in certain conditions if temperature was not too high. The maximum of Pn in this test was 18.0 µmol/m²/s (from treatment A4).

Impact of watering frequency on bulb count and biomass

Results from ANOVA revealed that there was no significant difference in bulb number, but significant difference for dry bulb weight (Table 3). The ratio of the present fresh weight (PFW) to the initial fresh weight (IFW) represents an increase in biomass. Treatments A1, A2 and A3 were increased by 323, 164 and 47% in fresh bulb weight, respectively. However, that of A4 and A6 was decreased by 21 and 52% in fresh bulb weight, respectively. This indicated that fresh biomass was also positively correlated with watering frequency if quantifiedly regressed with watering frequency as leaf number ($r=0.98$, $P=0.003$). Watering once every 3 months could be regarded as a critical interval value. It could be seen that the wet bulb weight still increased at this watering frequency, and stopped increasing when the interval was longer than 3 months for bulbs became lighter and lighter. Additionally, the single average bulb biomass had a significant difference among treatments. The weight of a single bulb was negatively correlated

Table 3. Average fresh bulb weight ratio, bulb number and dry bulb weight and multiple comparison[y].

Treatment	PFW/IFW[x]	Bulb number	Dry bulb weight (g)
A1	4.23	8.7[a]	109.87[a]
A2	2.64	7.0[a]	70.67[b]
A3	1.47	8.7[a]	38.03[c]
A4	0.79	5.7[a]	23.82[d]
A6	0.48	7.3[a]	8.44[e]

[y]Different letters behind values in the same column indicate significantly different ($P \le 0.05$). [x]PFW: Present fresh weight. IFW: Initial fresh weight.

with the watering interval. It is partly because the bulb gradually sacrificed the outside epimatium to maintain the survival of the inner short shoot, partly because the bulb tended to split into smaller new bulbs to survive under dry stress.

Our study revealed that within a certain range the more watering frequency, the more leaf number, leaf length and bulb biomass, which demonstrated the importance of water to plant (Yagoub et al., 2010). It was necessary to strengthen watering management even in no leaf period for *Lycoris* although *L. haywardii* is a drought-resisting species because of high water content in bulb. Since temperature was low in winter and evaporation was weak, watering once a month could ensure plant growth. But in early autumn or early spring, when temperature occurs to be a little higher, watering frequency should be higher. Roughly speaking, timely watering should be carried out when SRWC reached about 50%. While in a high temperature situation dry stress should be avoided to ensure leaf bud differentiation and leaf sprout. Since the minimum watering interval was once a month in this study, further study was needed to determine if there exists a shorter and better watering pattern.

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