

Full Length Research Paper

# Photosynthetic characteristics of *Lycoris aurea* and monthly dynamics of alkaloid contents in its bulbs

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The leaf photosynthetic characteristics of *Lycoris aurea*, the monthly dynamics in lycorine and galantamine contents in its bulb and the correlation among the photosynthetic characteristics and the lycorine and galantamine during the annual growth period were studied by using LI-6400 portable photosynthetic measurement system, high performance liquid chromatography and SPSS13.0 software. The results show that the leaf net photosynthetic rate of *L. aurea* differs monthly with the maximum of  $12.05 \mu\text{mol m}^{-2} \text{s}^{-1}$  and minimum of  $7.07 \mu\text{mol m}^{-2} \text{s}^{-1}$  in December and April, respectively; the content of both lycorine and galantamine in *L. aurea* bulbs also alters monthly with maxima of about 0.53 and 0.09  $\text{mg g}^{-1}$ , corresponding to the largest accumulation periods of December and March, respectively; and minimum of 0.21 and 0.03  $\text{mg g}^{-1}$  in January respectively; and there is a certain correlation between the net photosynthetic rate and lycorine and galantamine contents. The lycorine content in bulbs of *L. aurea* is higher in winter with lower light illumination and relative humidity. Photosynthesis intensity and environmental factors affect accumulation of lycorine and galantamine in *L. aurea* bulbs and the optimum harvest seasons of the bulbs for extracting lycorine and galantamine are in December and March, respectively.

**Key words:** *Lycoris aurea*, photosynthetic characteristic, lycorine, galantamine, dynamic variation.

## INTRODUCTION

*Lycoris aurea*, also called Golen Magic Lily, is one of the important perennial herbs in the genus *Lycoris*. Its bulbs contain several kinds of alkaloids, among which lycorine and galantamine are major ingredients. Lycorine and galantamine can be used to treat rheumatoid arthritis and have a certain therapeutic effect on some cancer diseases. Especially, as an inhibitor of acetylcholinesterase, galantamine has a good therapeutic effect on Alzheimer's disease, and is a specific remedy for myasthenia gravis and poliomyelitis sequela. Hence, it has important medicinal value and broad application

prospect (Ma, 1998; Deng et al., 2004). Lycorine is toxic and it would be also important to distinguish applications of the investigated alkaloids in native medicine from those based on results of pharmacological science.

Alkaloids, a class of nitrogen-containing secondary metabolites widespread in the plant kingdom, are major ingredients of several medicinal plants. Their production and distribution usually have distinctive features in different species and genus, period of growth, organs and tissues. Their metabolism is a dynamic and complex process, not only regulated by their genetic background, growth and developmental process, but induced by environmental factors as well (Wang et al., 2007; He et al., 2002). *L. aurea* has unique growth characteristics: Its leaves wither from May till August, entering the summer dormancy; its floral stems and flowers start growing from August till September, and its leaves grow from September till October (Bao and Chen, 2000). In recent years, studies have reported that *L. aurea* is a good material for extraction of galantamine and other alkaloids

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**Abbreviations:**  $P_n$ , Net photosynthetic rate; PAR, photosynthetically active radiation;  $T_{air}$ , air temperature; RH, atmospheric relative humidity; RSD, relative standard deviation.

(Yang and Tan, 2003; Yuan et al., 2010). The net photosynthetic rate of *L. aurea* was related to ecological factors, among which atmospheric relative humidity and air temperature were the major ones (Quan et al., 2010). However, the dynamics of alkaloid content in its bulbs and their correlation with plant growth and development, as well as with the environmental factors have not yet been extensively studied. In this study, we measured the photosynthetic characteristics of *L. aurea* and monthly dynamics of lycorine and galantamine content in its bulbs, explored their maximum accumulation period in order to provide scientific evidences for efficient utilization of *L. aurea*. Meanwhile, we also explored the correlation between accumulation of lycorine and galantamine and its net photosynthetic rate and environmental factors in order to provide information for the standardization of *L. aurea* cultivation and studies on the relationship between photosynthesis and accumulation of plant secondary metabolites.

## MATERIALS AND METHODS

The plant material of *L. aurea* was cultivar in Huaihua University. The age of bulbs was 4 years. The bulbs with the same or similar sizes were selected and planted in the Botanical Garden of Huaihua University in August 2009, with planting space of 15 × 20 cm and grown under the same environmental conditions (natural light, soil fertility and others) and conventional management. Five bulbs of *L. aurea* were randomly picked up at mid month of each month from May 2010 to September 2010 when the leaves of the plant wither; they were washed, dried at 65°C and stored at -20°C for future use.

### Analysis of leaf photosynthetic rate of *L. aurea* and other environmental factors

Photosynthetic parameters such as leaf net photosynthetic rate and photosynthetic active radiation, air temperature, relative humidity and other major physiological and ecological factors were measured by using of *LI-6400* portable photosynthesis measurement system (*LI-cor*, USA) under an open flow gas exchange system. In detail, the same position of the leaves of five randomly selected *L. aurea* were used to measure leaf net photosynthetic rate during 9:00 to 11:00 am in three days of the mid month of each month from October 2010 to April 2011 when the plant was in nutrition period. Immediately after that the corresponding leaves were collected to extract chlorophyll and their bulbs were collected for future lycorine and galantamine determination. The treatment methods of the bulbs were the same as above. Data were expressed as mean of the five determinations.

### Pigment analysis

Leaf samples were extracted in 95% ethanol and measured by spectrophotometric method (*Ruili UV-2100*, Beijing, China) at the wavelengths of 665 and 649 nm. Contents of chlorophyll (Chl) *a* and *b* were calculated using the equations of Arnon (1949) and Bao and Leng (2005).

### Standard preparation

12.50 mg lycorine standard (purity ≥ 98%, Chemical Technology

Institute of Beijing Hengyuan) and 12.50 mg galantamine standard (purity ≥ 99%, Sigma-Aldrich co.) were dissolved in 25.0 ml methanol and filtered through 0.45 μm membranes as 500 μg ml<sup>-1</sup> methanol solution, respectively.

### Sample preparation

Bulbs collected at different months were dried at 65°C to constant weight, milled and filtered through a 60-mesh sifter. 2.0 g of the sieved sample was dissolved in 100 ml methanol in 80°C water bath, extracted using Soxhlet method for 3 h (Wu et al., 2007) and filtered. The filtrate was dried with a rotary evaporator. The residue was then dissolved in 10.0 ml of 2.0% hydrochloric acid and filtered. The filtrate was adjusted to pH 10.0 by adding ammonia and then extracted three times with 15.0 ml chloroform each. The chloroform solutions were combined and dried with a rotary evaporator. The residue was dissolved in methanol in 10.0 ml methanol, filtered through 0.45 μm membrane and used as sample solution.

### Selection of detecting wavelength

The obtained sample solutions as well as lycorine and galantamine standards were scanned from 200 to 600 nm on a DU-800 UV-Vis scanning spectrophotometer (Beckman, USA) using methanol as blank. All samples and standards showed the same absorption peak and maximum absorption value at 288 ± 2 nm. Thus, 288 nm was selected as detection wavelength.

### Conditions used in high-performance liquid chromatography

Contents of lycorine and galantamine in *L. aurea* bulbs were measured with a LC-20AT high-performance liquid chromatography system (Shimadzu, Japan). Because *L. aurea* bulb samples contain complex components, we first optimized the elution condition using a gradient mobile concentration and found that elution with a single mobile concentration could obtain a better separation. Therefore, the final chromatographic conditions used was as follows: 20 μl of samples or standards were injected onto the Agilent Eclipse XDB-C18 (150 mm × 4.6 mm, 5 μm) column at 25°C and eluted with mobile phase of 0.1% phosphoric acid : methanol of 65 : 35 at flow rate of 1.0 ml/min. The eluents were detected by absorption at 288 nm. The retention times were 6.178 and 10.805 min for lycorine and galantamine, respectively indicating a good separation.

### Precision and sample stability

Precision was examined by repeatedly injecting 20 μl of 100.0 μg ml<sup>-1</sup> lycorine or galantamine standard solutions and eluting according to the above chromatographic conditions for 5 times. Their peak areas were measured and average was used to calculate the relative standard deviation (RSD), which was 0.68 and 0.65%, respectively indicating a good precision.

Sample stability was measured by injecting 20 μl of samples pre-incubated at RT for 0, 2, 4, 6 and 8 h, respectively and eluting according to the above chromatographic conditions. Their peak areas were measured and averaged to calculate the relative standard deviation (RSD), which was 0.76%, indicating that samples were stable at least for 8 h.

### Linear regression and recovery rate

20 μl of lycorine and galantamine standard solutions at concentration of 20.0, 40.0, 60.0, 80.0 and 100.0 μg ml<sup>-1</sup> were

**Table 1.** Calibration curve, linear range and recovery of alkaloids.

Standard	Calibration curve	Correlation coefficient	Linear range ( $\mu\text{g ml}^{-1}$ )	Recovery (%)
Lycorine	$y=41652x-11406$	0.9995	20.0~100.0	98.85
Galantamine	$y=49171x-69194$	0.9991	20.0~100.0	98.97

\* x is content ( $\mu\text{g ml}^{-1}$ ), y is peak area.

injected into the column and separated under the above conditions, respectively. Their peak areas were measured and used to calculate the linear regression equation. In addition, three *L. aurea* samples were mixed with standards to determine recovery rate of the method. As listed in Table 1, the linear relationship and the recovery rate met the measurement requirements.

#### Measurement of alkaloid contents

20  $\mu\text{l}$  of sample solutions were separated as described above and the amounts of lycorine and galantamine were calculated using the peak area according to the linear regression equation.

#### Data processing and analysis

The data were analyzed with Microsoft Excel 2003 and SPSS13.0 software.

## RESULTS

### Comparison of photosynthetic characteristics of *L. aurea* leaves at different months

*L. aurea* grew rapidly since its leaves started growing from late September. When the leaves continued to mature, their net photosynthetic rate was also enhanced. As shown in Figure 1, with decrease of the photosynthetic active radiation and the air temperature, the leaf net photosynthetic rate and chlorophyll content of *L. aurea* gradually increased from October to December, reaching their peaks of  $12.05 \mu\text{mol m}^{-2} \text{s}^{-1}$  and  $2.18 \text{mg g}^{-1}$ , respectively and decreased from January to April reaching the minimum of  $7.07 \mu\text{mol m}^{-2} \text{s}^{-1}$  and  $1.01 \text{mg g}^{-1}$ , respectively. Its leaves wither from May till August. Correlation analysis showed that leaf net photosynthetic rate and chlorophyll content of *L. aurea* were significantly and positively correlated ( $p < 0.01$ ) with a correlation coefficient of 0.945.

The results indicate that *L. aurea* leaf net photosynthetic rate and chlorophyll content varied seasonally and along with their growth and development, showing a trend of first increase from October to December and then decreased from January to April. Similarly, photosynthetic active radiation showed a seasonal trend of first decrease from October to December and then increase from January to April, with a minimum in winter (January), together with relative humidity and air temperature.

### Monthly dynamic variations of alkaloid content in *L. aurea* bulbs

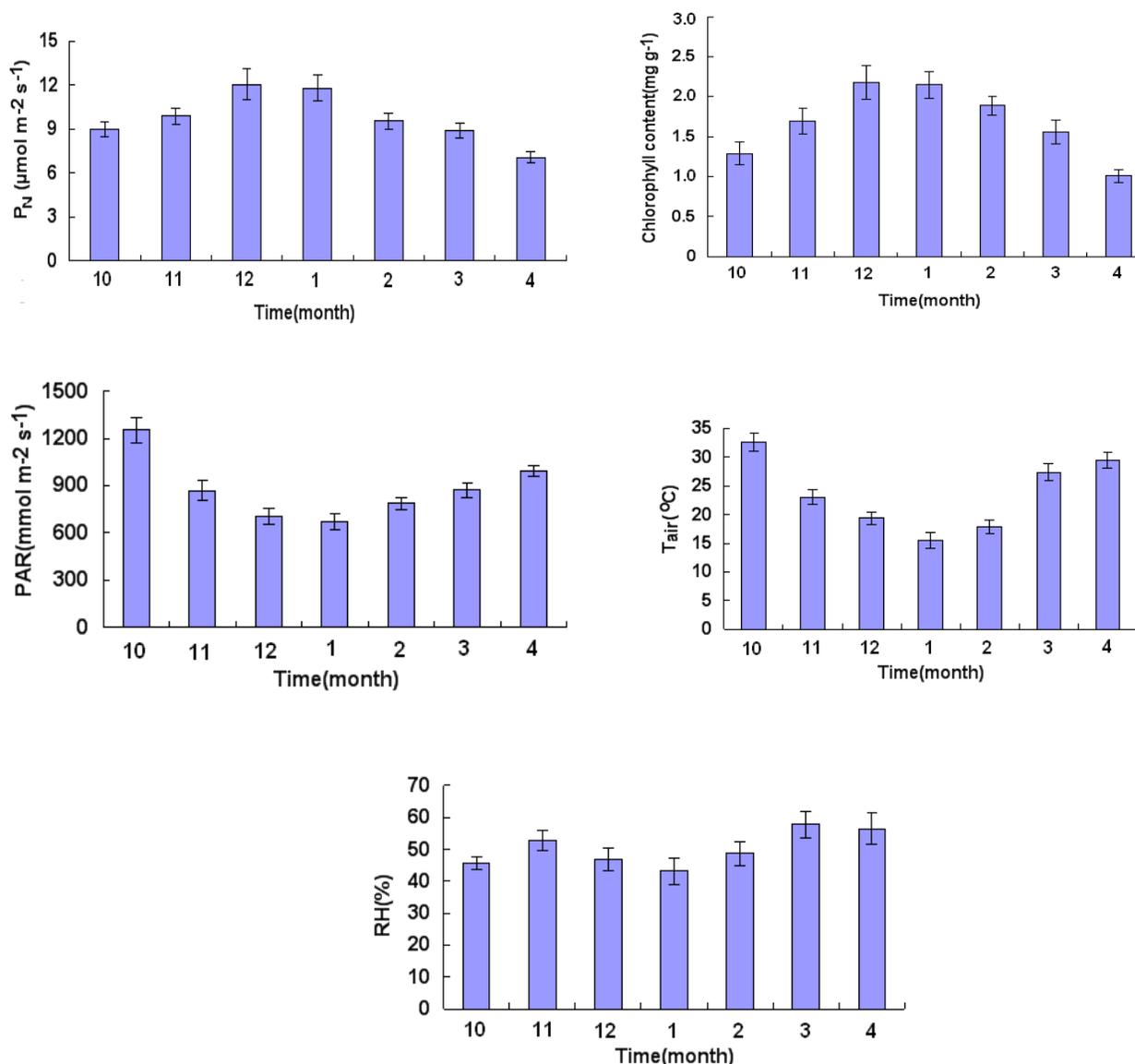
As shown in Figure 2, lycorine content in *L. aurea* bulbs showed a dynamic change: Lycorine content slowly decreased from May to September, but gradually increased to the maximum value of about  $0.53 \text{mg g}^{-1}$  from October to December, then sharply decreased to the minimum value of  $0.21 \text{mg g}^{-1}$  from December to January of the next year. From January to April, its content had an increasing trend with a small peak in April.

Galantamine content in *L. aurea* bulbs also varied dynamically: From May to January of the next year, galantamine content slowly decreased to the minimum value of about  $0.03 \text{mg g}^{-1}$ . From January to March, its content gradually increased to the maximum value of about  $0.09 \text{mg g}^{-1}$ , then decreased monthly. Taken together, the results indicated that lycorine and galantamine contents in *L. aurea* bulbs varied with plant growth and season change. Compared with the minimum values, the maximum values of lycorine and galantamine were 2.5 and 3.0 times, respectively.

### Correlation analysis of photosynthetic characteristics and alkaloid content in *L. aurea* bulbs

Correlation analysis showed (Table 2) that leaf net photosynthetic rate and lycorine content in *L. aurea* bulbs were positively correlated with a correlation coefficient of 0.365, indicating that the greater the leaf photosynthetic capacity of *L. aurea* in a certain growth and development period, the more the lycorine accumulation in its bulbs. In contrast, leaf net photosynthetic rate and galantamine content in *L. aurea* bulbs were negatively correlated, indicating that the greater the leaf photosynthetic capacity of *L. aurea* in a certain growth and development period, the lesser the galantamine accumulation in its bulbs. Taken together, the results indicated that different alkalines were differentially correlated with leaf net photosynthetic rate, which may be related to their synthetic and conversion mechanisms.

The lycorine content in *L. aurea* bulbs was negatively correlated with photosynthetic active radiation, air temperature and relative humidity, while galantamine content was positively correlated with photosynthetic active radiation, air temperature and relative humidity. Thus, the ecological and environmental factors may have impact on the accumulation of lycorine and galantamine contents



**Figure 1.** Comparison of photosynthetic parameters of *L. aurea* leaves at different months from October 2010 to April 2011.

in *L. aurea* bulbs.

**DISCUSSION**

**The best harvest period for alkaloids in *L. aurea* bulbs**

This study shows that in the annual growth cycle, the monthly dynamic variation pattern of lycorine and galantamine contents in *L. aurea* bulbs were different. Their highest accumulation period or the best harvest period, was December and March, respectively indicating that accumulation of different alkaloids obeyed different rules and had growth stage specificity. The maximal

accumulation period of secondary metabolite is different in plants planted in different areas, even when they were at same age and from same clone, due to differences in phenology such as latitude and climatic conditions. Therefore, the studies on rules of dynamics accumulation of plant secondary metabolites should consider its relationship with phenology (Li et al., 1999; Zhang et al., 2000; Dong et al., 2004). Considering that leaves of *L. aurea* in Hunan Province started growing in late September, the peak accumulation, that is, the best harvest time, of lycorine and galantamine in bulbs appeared 80 and 170 days after bud blossoms stage (phenology), respectively. Whether other related alkaloid accumulation in bulbs of *L. aurea* in other regions also has similar trends need to be further studied.

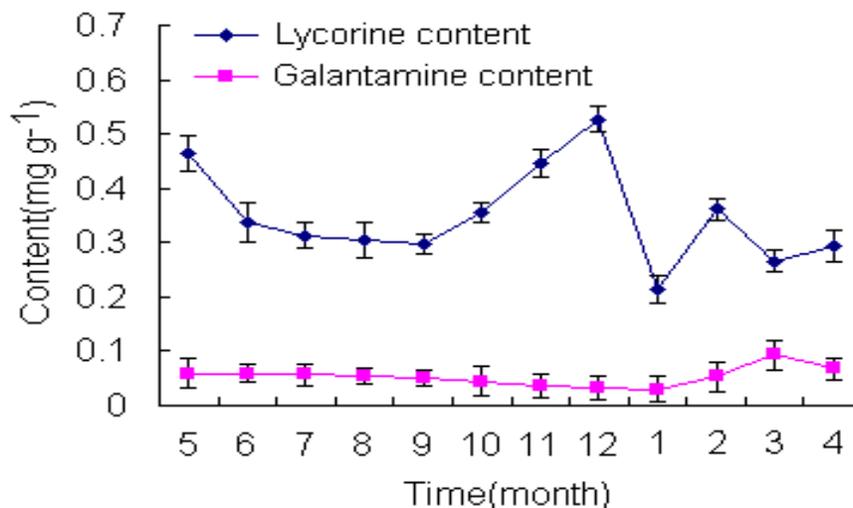


Figure 2. Monthly dynamics of lycorine and galantamine content in *L. aurea* bulbs.

Table 2. Correlation of photosynthetic characteristics and alkaloid content in *L. aurea* bulbs.

Influencing factor	Lycorine content	Galantamine content	$P_N$	PAR	Tair	RH
Lycorine content	-	-	-	-	-	-
Galantamine content	-0.339	-	-	-	-	-
$P_N$	0.365	-0.752	-	-	-	-
PAR	-0.063	0.256	-0.574	-	-	-
Tair	-0.089	0.518	-0.707	0.916**	-	-
RH	-0.117	0.817*	-0.768*	0.178	0.489	-

\* and \*\* mean significance at 0.05 and 0.01 level, respectively.

$P_N$ , Net photosynthetic rate; PAR, photosynthetically active radiation; Tair, air temperature; RH, atmospheric relative humidity.

### Relationship of alkaloid accumulation in *L. aurea* bulbs with photosynthesis and environmental factors

Green plants produce carbohydrates, proteins and other primary metabolites through photosynthesis. These products are not only the important compounds of plant, but also the precursors of plant secondary metabolites. For example, amino acids (phenylalanine, tyrosine and tryptophan) are the precursor of alkaloid and peptide antibiotics. Primary metabolism and secondary metabolism are closely related (Sun et al., 2009). Continuous synthesis and transformation of secondary metabolites are present in all cells growing rapidly (Dong et al., 2005). The synthetic pathways of plant secondary metabolites are present as different metabolic pathways. Their quantitative trait loci may be located in different chromosomes, and regulated by developmental processes. Therefore, they may be expressed at different developmental stages or induced by different factors (Winkel-Shirtey, 1999; Mc-Mullen et al., 1998). Indole-3-glycerol phosphate lyase (BX1), tyrosine/dopa

decarboxylase (TYDC), berberine bridge enzyme (BBE) etc, are possibly the rate-limiting enzymes in alkaloid biosynthesis in determining the biological synthesis and accumulation of alkaloids (Hashimoto et al., 1992). Our results showed that dormant *L. aurea* bulbs had very low alkaloids content. However, after its leaves started growing in September, with the rapid growth from October to December and increase in photosynthesis, lycorine content gradually rose to its annual peak, indicating a close relationship between *L. aurea* leaf photosynthesis and lycorine accumulation in its bulbs. In other words, during leaf growth period, the higher the photosynthetic capacity of *L. aurea* was, the more the lycorine was accumulated in *L. aurea* bulbs. The data also showed that the synthesis and transformation of secondary metabolites was closely related to plant growth and development. This phenomenon may be due to the fact that photosynthesis provides a lot of carbohydrates, proteins and other primary metabolites for the synthesis of alkaloids and other secondary metabolites, and may be also related to the activities of

some key enzymes in alkaloids synthesis. In contrast, weather conditions such as low temperature, fog and snow may affect photosynthesis and the activities of related rate-limiting enzymes, thus affecting the synthesis and accumulation of alkaloids and other secondary metabolites and possibly leading to the sharp decline in alkaloid contents in *L. aurea* bulbs in January. In addition, our result showed that  $T_{\text{air}}$  had a negative relation with  $P_{\text{N}}$ , which may be related to unique growth characteristics and development law of *L. aurea* and season, and so on.

The arid environment can promote alkaloid biosynthesis in plant (Ainouche et al., 1996; El-Shazly et al., 2005; Bustamante et al., 2006) and reduced light intensity can induce alkaloid accumulation and consequently enhance alkaloid content in plant tissues (Haeghele and Rowell, 1999; Salmore and Hunter, 2001). Our study shows that lycorine accumulation in *L. aurea* bulbs gradually increased after leaf started growing in fall, reaching its peak in December, which was possibly related to the decline in light intensity and environmental factors such as dry weather, low relative humidity, less rainfall and less soil moisture in winter. The data also showed that reduced light intensity and relatively dry growing environment were favorable to lycorine synthesis and accumulation. Our findings provide not only the methods for increased alkaloid production in *L. aurea*, but also guidance for alkaloids-related cultivation of *L. aurea*.

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