Alkaloid Accumulation in Different Parts and Ages of *Lycoris chinensis*

Hong-mei Mu[a,b], Ren Wang[a,*], Xiao-dan Li[a], Yu-mei Jiang[a], Feng Peng[a], and Bing Xia[a,*]

[a] Institute of Botany, Jiangsu Province & Chinese Academy of Sciences, Jiangsu Province Key Laboratory for Plant Ex-Situ Conservation (Nanjing Botanical Garden, Mem. Sun Yat-sen), Nanjing, 210014, China.
E-mail: jswangren@yahoo.com.cn or njbgxiab@yahoo.com.cn
[b] College of Agriculture, Liaocheng University, Liaocheng, 252059, China

* Authors for correspondence and reprint requests

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The galanthamine, lycorine, and lycoramine content of *Lycoris chinensis* was researched during development from young to old plants, *i.e.* in seeds, ten-day-old seedlings, three-month-old seedlings, one-year-old seedlings, and perennial seedlings. Notably the alkaloid level reduced to its lowest content 10 days after seed germinating. Then the accumulation of galanthamine tended to increase with age, reaching a higher value in perennial seedlings. The production pattern of lycorine and lycoramine was found similar to that of galanthamine. Different plant organs were also evaluated for their galanthamine, lycorine, and lycoramine contents. Mature seeds had the highest content of galanthamine (671.33 μg/g DW). Kernels, seed capsules, and root-hairs were the main repository sites for galanthamine, lycorine, and lycoramine. The leaves were the least productive organs.

Key words: Lycoris chinensis, Galanthamine, Alkaloid Accumulation

Introduction

There is one ordinarily shared matter namely that plants have evolved many compliant mechanisms to deal with their invariably transforming environment during the course of ontogenetic evolution and growth. In this relation, a great number of secondary metabolites, such as alkaloids and phenylpropanoids, are developed, accommodated and had relation to primary metabolic development (Nessler, 1994). By reason of the pharmaceutical value of many secondary metabolites, more and more medical and biological scientists have paid attention to them (St-Pierre *et al.*, 1999). The form and accumulation of useful components in plants, not only correlate with the heredity gene, but also are related with specific developmental control (St-Pierre *et al.*, 1999). Previous studies indicated that alkaloid biosynthesis and production are connected with the development of plants (De Luca *et al.*, 1986; Rafael *et al.*, 2001).

The life-span of plants is different, from only one year to more than 10 years, or even over thousand years. Thereby the secondary metabolites may change differently based on the distinct phase of growth. Different tissues and organs in medicinal plants also show different rules of accumulation of secondary metabolites. So the accumulation of alkaloids may vary in different tissues and organs of officinal plants.

Galanthamine is an important drug for the symptomatic treatment of senile dementia or Alzheimer's disease (AD) used all over the world (Diop *et al.*, 2006). Lycorine has calmness, acesodyne, and anticancer functions in medical use. Lycoramine [(–)-1,2-dihydrogalanthamine] is another galanthamine-type alkaloid with medicinal activity similar to galanthamine (Qian, 1992).

Seed, seed germination, and the following life-span are important phases of growth and development during which many of the particular processes needed for plant development take place (Larkins and Vasisleds, 1997). Previous studies with alkaloids illustrated that their biosynthesis and accumulation are not random processes, but associated with a particular growth or developmental stage, controlling the expression of pathways inside organs, inside specific cells, or inside organelles in those cells (De Luca and St-Pierre, 2000; Lattanzioa *et al.*, 2009).

Furthermore, alkaloid biosynthesis cultures appear to be coordinated with cytodifferentiation

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in cell suspension (Sellés *et al.*, 1999). Laurain-Mattar (2008) has pointed out that low or no accumulation of alkaloids in plant cell cultures can be due to an deficient level of cell differentiation. Thus there remain many questions, for example, whether the accumulation of galanthamine changes with the plant development. It seems interesting to evaluate the metabolic dynamics of galanthamine during growth. The levels of lycorine and lycoramine, and the correlation and transformation among these alkaloids have been investigated in the present study.

It is very meaningful to master the connection of plant growth and development with the accumulation of galanthamine, lycorine, and lycoramine. It will help to improve the quality of the secondary metabolites.

Material and Methods

The materials used were raised in an experimental field at the Jiangsu Province Key Laboratory for Medicinal Plants, Nanjing, Jiangsu Province, P. R. China. In November 2008, mature seeds were collected and stored at 4 °C. The seeds for measurement of the alkaloids content were oven-dried at 45 °C for about 3 d, and then pulverized separately and stored at about 0 °C. The seeds for germination were kept in the same site with the materials until March 2009. The germinating seedlings with different developmental levels were collected and oven-dried at 45 °C until constant weight, then stored in dry environment to investigate changes of the alkaloids. The different organs of the seedlings were harvested in May 2009.

Alkaloid extraction was according to Colque *et al.* (2004). The oven-dried seeds and leaves, root-hairs, and bud powders (100 mg each) were dissolved in 10 ml methanol (analytical grade) for 24 h at room temperature, with three sonications (30 min each) at regular intervals. The methanolic extracts were centrifuged at 4000 rpm for 20 min, and then filtered through a 0.22-μm pore filter (Millipore) before HPLC analysis. The quantitative amounts of the alkaloids galanthamine, lycorine, and lycoramine were measured by HPLC analysis according to Li *et al.* (2003). The separation was performed on a Kromasil C18 reverse-phase column (5 μm, 4.6 mm × 150 mm). The mobile phase was formed of acetonitrile/water (20:80), supplemented 2.67 ml di-*n*-butylamine in 800 ml water, adjusted to pH 9.0 ± 0.05 with phosphoric acid. The flow rate was 1.0 ml/min. UV detection was at 280 nm. The chromatography was conducted at room temperature, 20 μl were injected. The identification and quantification of the three alkaloids were completed according to retention times and absorbance spectra of external standard samples of galanthamine, lycorine, and lycoramine (Fujian Like Bio-pharmaceutical Technology Co., Ltd., batch No. 061210-2, purity ≥ 98.0%).

Results

Galanthamine, lycorine, and lycoramine changes during different times of life of Lycoris chinensis

Galanthamine, lycorine, lycoramine, and total alkaloids levels were quantified during the development process of seeds, 10-day-old seedlings, 3-month-old seedlings, and 1-year-old seedlings (Figs. 1, 2). It is notable that the mature seeds had the highest content of galanthamine (671.33 μg/g DW) which is about more than 10.88 times that of 10-day-old seedlings. This alkaloid level reduced to its lowest content 10 days after seed germination. Then the accumulation of galanthamine tended to increase with age, reaching a higher value in perennial seedlings which was lower than in mature seeds.

The accumulation and variation patterns of lycorine, lycoramine, and total alkaloids levels in these different phases exhibited evident similarity with those of galanthamine. The level of lycorine

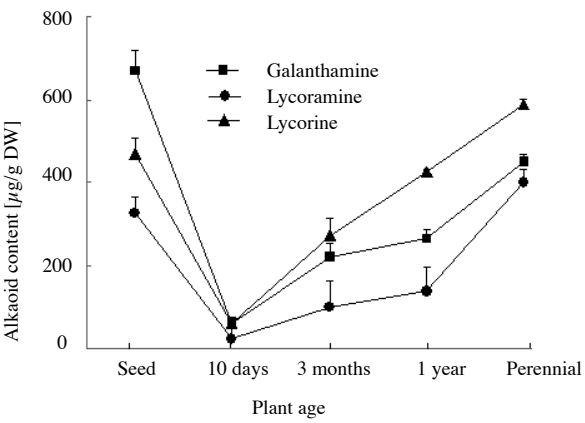

Fig. 1. Galanthamine, lycorine, and lycoramine content dynamics in *Lycoris chinensis* during different life times. Each value is the mean of three replicate plants ± SE.

reached its climax in mature seeds (587.48 μg/g DW) which is 9.71 times that of 10-day-old seedlings. Fig. 1 shows that in mature seeds the content of lycoramine is highest at 399.85 μg/g DW, which is 17.19 times that of 10-day-old seedlings. The amounts of galanthamine, lycorine, and lycoramine accumulated at different ages followed the order: seeds > perennial seedlings > seedlings of 1 year > seedlings of 3 months > seedlings of 10 days.

Galanthamine, lycorine, and lycoramine changes in different organs of Lycoris chinensis

Fig. 3 shows the galanthamine, lycorine, and lycoramine contents in the kernels of seeds, seed capsules, root-hairs, bulbs, and leaves of *Lycoris chinensis*. It is notable that the mature kernels of seeds have the highest content of galanthamine (671.33 μg/g DW) which is about more than 5.20 times that of leaves. The amounts of galanthamine accumulated in different organs followed the order: kernels of seeds > seed capsules > root-hairs > bulbs > leaves. The climax accumulation of lycoramine is in the capsules of seeds (383.62 μg/g DW), which is about 3.54 times that of leaves. The production of lycoramine in different organs followed the order: seed capsules > kernels of seeds > root-hairs > leaves > bulbs. The largest production of lycorine took place in the root-hairs (505.85 μg/g DW), which is approximately 3.06 times greater than in the leaves. The profile changes of lycorine accumulated in different organs followed the order: root-hairs > kernels of seeds > seed capsules > bulbs > leaves.

The highest accumulation of total alkaloids was in the seed capsules (4.98 mg/g DW), which is about 2.00 times greater than in the leaves. The production of total alkaloids in different organs followed the order: seed capsules > kernels of seeds > root-hairs > bulbs > leaves.

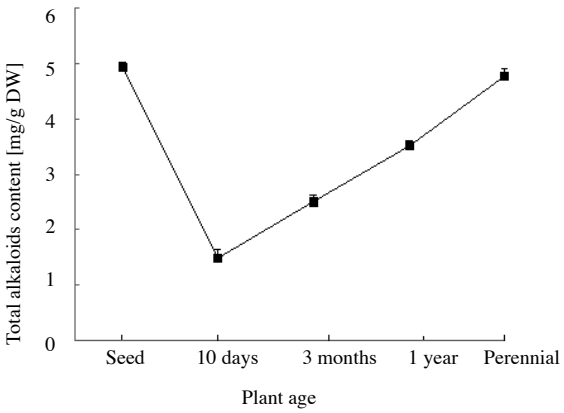

Fig. 2. Total alkaloids content dynamics in *Lycoris chinensis* during different life times. Each value is the mean of three replicate plants ± SE.

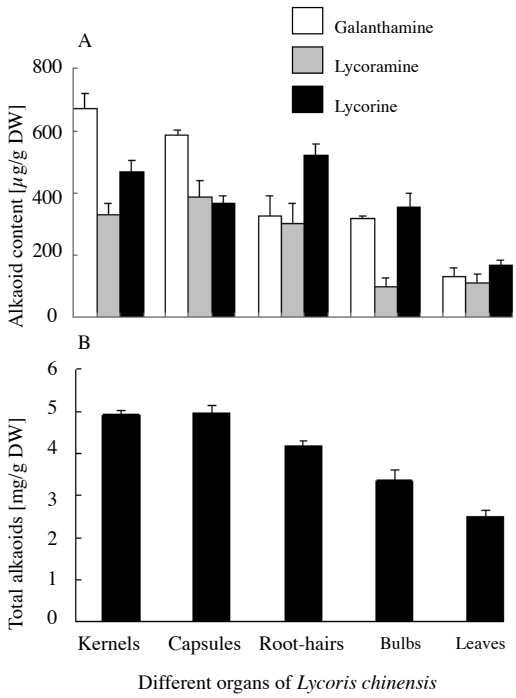

Fig. 3. (A) Galanthamine, lycorine, and lycoramine content in different organs of *Lycoris chinensis*. (B) Total alkaloids content in different organs of *Lycoris chinensis*.

Discussion

The results show that metabolic properties of galanthamine, lycorine, lycoramine, and total alkaloids are under firm developmental regulation and tissue-specific localization. The contents of these compounds increased with the ages of the seedlings (Figs. 1, 2). The 10-day-old seedlings displayed the lowest levels of galanthamine, lycorine, lycoramine, and total alkaloids. On the other hand, the mature seeds and perennial seedlings surprisingly had higher contents of these alkaloids, indicating that a more advanced metabolism is active at these ages. This rule is similar in some medicinal plants such as ginseng. Shi *et al.* (2007) have

Table I. Galanthamine content of different species under different culture conditions.

Species	Extract	Galanthamine content (0.01‰ DW)	Researcher
Narcissus confusus	Seed-derived explants	61	Sellés *et al.* (1997)
Narcissus confusus	Different cytodifferentiation phase *in vitro* cultures	0.143–0.003	Sellés *et al.* (1999)
Narcissus confusus	Shoot-clumps with elicitors	800–400	Colque *et al.* (2004)
Leucojum aestivum	*In vitro* bulblets developed on hairy roots	10.3–51.3	Diop *et al.* (2006)
Leucojum aestivum	Embryogenic calli with different regulators	73–0	Diop *et al.* (2006)
Leucojum aestivum	*In vitro* bulblets with different regulators	6.79–1.14	Diop *et al.* (2007)
Lycoris chinensis	Seed capsules, kernels of seeds, root-hairs, bulbs, leaves	67.1–12.9	Present study

found that the content of ginsenosides in *Panax ginseng* root-hairs increases with age. Our studies illustrated that alkaloid biosynthesis is not a random process, but is highly ordered with respect to plant development and controlling the expression of pathways within organs.

One interesting phenomenon observed in the present investigations is that the kernels of seeds, seed capsules, and root-hairs contain more alkaloids than bulbs and leaves. Our study proved that kernels of seeds and especially seed capsules and root-hairs are major storage sites for alkaloid accumulation. The black seed coat of *Lycoris chinensis* contains noticeable alkaloids. This is very similar with the black seed of soybean which has been proved to contain anthocyanins, isoflavonoids, and other phenylpropanoids (Dhaubhadel *et al.*, 2003). Whether the black seed coat of *Lycoris chinensis* programmes the synthesis of alkaloids needs more research.

Generally speaking, the bulbs and leaves are not very important as accumulative organs. For the sake of effective protection of important organs from external stresses, plants commonly allocate resources of alkaloids, terpenoids and other secondary metabolites in important organs, which are vulnerable to insect and herbivore attacks (Bryant and Julkunen-Tiitto, 1995).

At present the content of secondary metabolites is often limited to one species. Table I shows the content of galanthamine in different species. At present people traditionally use *Leucojum aestivum* and *Narcissus confusus* as resource of galanthamine. In the present study, we proved *Lycoris chinensis*; especially the seeds have a large amount of alkaloids. Geneticists have the noticeable hypothesis that plant populations hold out a different reservoir of secondary metabolites able to fit the changing selective pressures of their environment and that it is possible to enhance the generation of qualitative and quantitative variations in secondary chemistry (Lewinsohn and Gijzen, 2009). Our research may be useful to breeding a species with higher content of alkaloids.

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